PREDATOR INDUCED PHENOTYPIC PLASITICITY WITHIN AND BETWEEN GENERATIONS IN THE POND SNAIL *PHYSA ACUTA*

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Abstract:
Prey often induce changes in the phenotype of their prey through phenotypic plasticity. Phenotypes that change include life history traits, morphology, and behavior, and changes may be adaptive or non-adaptive. The goal of this dissertation was to test hypotheses about predator-induced phenotypic plasticity related to the timing of predation exposure, differences between maternal and paternal effects, the effects of resource availability on plasticity, and the effect of predation cues on mating behavior. To address these questions, I exposed pond snails (*Physa acuta*) to cues from predators (crayfish, *Procambarus* sp.), and measured aspects of their life history, morphology, and behavior. In chapter 1, I found evidence that the effects of predator cues on snails depend on when the snails are exposed. Snails exposed to predator cues early in life experienced a delay in reproduction, laid fewer eggs, and had reduced life expectancy. Interestingly, these effects remained the same whether the cues were removed post-reproduction or not, and suggests changes in life history may be maladaptive response to early life stress. In chapter 2, I found snails exposed to food restrictions or predator cues responded less to predation cues than control snails, as predicted by theory. I found some evidence of predator-induced parental effects in the offspring of predator exposed snails, but they did not fully match modelling predictions from the literature. In chapter 3, I found evidence that control snails are less likely to mate with predator exposed snails than with other control snails, but that if they did mate, the length of the mating was not affected. These results have implications for the interpretation of parental effect experiments. Overall, within generation plasticity was well predicted by existing theory, while transgenerational plasticity was more difficult to predict. This suggests that more mechanistic studies may be needed to fully understand transgenerational plasticity.

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CHAPTER I

LIFE EXPECTANCY AND REPRODUCTIVE OUTPUT REDUCED BY PREDATOR EXPOSURE EARLY, BUT NOT LATE, IN LIFE IN THE POND SNAIL *PHYSA ACUTA*

Abstract

Predation risk can affect life history by altering optimal life history strategies or by causing stress-related changes that impact life history. In this experiment, I exposed pond snails (*Physa acuta*) to predation cues at different points during their life and measured how predation exposure early and late in life affected growth, reproduction, and life expectancy. I found that exposure to predation early in life led to a delay in first reproduction, lower life expectancy, and lower fecundity throughout their life. Exposure to predation cues later in life had no effect on growth, life expectancy, or egg production. These results suggest that the effects of predation on life history are detrimental to fitness and more dependent on timing of predation exposure than the duration of predator exposure.

Introduction

When organisms are exposed to predation, they can alter their phenotype to avoid being killed. Predator-induced phenotypic plasticity is ubiquitous, and includes shifts in morphology, life history, and behavior. Phenotypic plasticity often occurs as a response to informational cues in the environment (Nettle and Bateson 2015, Pigliucci 2001). Prey can

detect conspecific alarm cues and predator kairomones and then produce altered phenotypes in response, including a fish resistant helmet morphology in daphnia (Agrawal et al. 1999) and a rounder, more crush resistant shell in snails (DeWitt et al. 2000, Appleton and Palmer 1988). Phenotypic plasticity of life history traits may also occur via non-informational mechanisms (Nettle and Bateson 2015). For example, the marine snail snail *Nucella lamellosa* develops a thicker shell in response to predation cues from crabs. However, this same response can be elicited in the absence of crabs by exposing the snail to food restriction suggesting that the response is a byproduct of reduced foraging rather than a direct response to predation risk (Bourdeau 2009). Bourdeau and Johansson (2012) suggest that this type of behaviorally mediated phenotypic plasticity may be common across a wide range of taxa.

Optimal life history strategies change in the face of predation risk, and organisms may shift their life history based on current cues in the environment. The presence of predators in an environment increases the risk of early death for prey. Therefore, many life history models predict that individuals exposed to predation should speed up their life history and shift reproductive effort to earlier in life so that they are able to maximize their reproduction before being eaten by a predator. This prediction has been validated for many organisms, including fish (Johnson and Belk 2000, Reznick and Endler 1982) and daphnia (Beckerman et al. 2007). However, predation pressure on different age classes can alter the optimal life history shift in response to predation (Law 1979). For example, if predation occurs mainly on smaller, less mature individuals, individuals may benefit by placing their full energy reserves into growth and delaying reproduction until they pass the size threshold allowing for relative safety from predation, particularly when there is high resource availability (Chase 1999).

Life history traits such as age at first reproduction, growth, lifespan, and fecundity often exhibit phenotypic plasticity in response to predation cues (e.g. Pietrzak et al. 2020, Pietrzak et al. 2015, Crowl and Covich 1990, Sheriff et al. 2009). This plasticity is often interpreted via the informational model (Nettle and Bateson 2015), and shifts and tradeoffs in life history traits are thought to be responses that increase fitness in an environment with predators. For example, Crowl and Covich (1990), suggest that their observation of delayed reproduction and increased growth in snails exposed to crayfish cues increased snail fitness by allowing them to grow to a point where they were less likely to be killed by a crayfish.

However phenotypic plasticity of life history may also occur as byproducts of costs associated with other anti-predator responses, or as a byproduct of a stress response. For example, an individual may respond to predation risk by reducing their foraging time or developing anti-predator morphologies. These responses may come with costs that reduce an animal's ability to grow and reproduce (Lima and Dill 1990, Sheriff et al. 2009). Many models of stress responses predict that repeated activation of stress responses leads to reduced energy reserves, a reduced ability to react to future stress events, and changes in life history traits such as reduced growth, lifespan, and reproduction (Romero and Wingfield 2015). In these cases, the changes in life history traits that occur when an animal is exposed to predation are not driven by an informational mechanism by which life history traits change to increase fitness under predation risk. Instead they are driven by a non-informational pathways in which the phenotypic change occurs as a result of a change in the animal's body condition. While reduced growth, survival, and fecundity are not adaptive, the overall anti-predator response is not necessarily maladaptive, as the benefit of increased probability of

surviving a predator outweighs the costs of reduced growth, life expectancy, or fecundity (Boonstra 2013, Werner and Arnholt 1993).

The effects of predation cues on life history may depend on the timing of exposure to the cues. Early predation cues may cause changes in development and growth, and most life history studies focus on predation risk early in life. For example, amphibians speed up hatching and development when they are exposed to predation cues as eggs. (Saenz et al. 2003), and reduce time to metamorphosis when exposed as tadpoles (Chivers et al. 1998). Later in life, predation cues may cause a decline in survival and reproductive output. For example, Auld and Houser (2015) found snails exposed to predation and control cues have similar reproductive output and survival early in life, but that the survival and reproductive output of predator exposed snails declined much faster as the snails aged. While the effects of early predation exposure on life history are well studied, there are fewer studies showing how the effects of predation cues on life history change when they are applied at different life stages (but see Luhring et al. 2019). Studying how the timing of predation cues affects phenotypic plasticity in life history can help disentangle life history responses that are a direct adaptive response to predation cues, tradeoffs with other life history traits, or byproducts of chronic stress activation.

In this chapter, I report the results of an experiment to explore how the presence and timing of predation risk affects aspects of life history, including growth, age at first reproduction, total reproductive output, investment in offspring, and life expectancy. In this experiment, I used a predator-prey system consisting of the pond snail *Physa acuta*, and predatory crayfish (*Procambarus* sp). *Physa acuta* is well suited to an experiment examining the effects of timing of predation risk on life history. The snails respond readily to predatory

cues from crayfish and other predators, making it easy to simulate the risk of predation in the lab, and they have a short generation time and rapid growth in the lab. The literature on the responses of *Physa* to predation is well developed with studies on the effects of predation on life history (Crowl and Covich 1990, Auld and Houser 2015) shell morphology (DeWitt et al. 2001, Gustafson et al. 2014), behavior (Turner et al. 1996, Beaty et al. 2016), and parental effects (Beaty et al. 2016, Goeppner et al. 2020, Laquet and Tariel 2016). The experiment consisted of two 5.5 week periods, during which snails were exposed to predation early in life, late in life, continuously, or not at all.

If phenotypic plasticity in the life history of *Physa* occurs as a direct adaptive response to predation cues, I expected to see the predator cue cause changes in life history phenotypes that would increase fitness when a predator is present. Based on Crowl and Covich (1990), larger snails may be less vulnerable to crayfish predation. If this is true, early life predator exposure should lead snails to increase growth and delay first reproduction. However, I have observed snails of all sizes consumed by crayfish (personal observation), and crayfish predation success is not fully dependent on size (Auld and Relyea 2011). Under this scenario, early life predator exposure should lead snails to increase their reproductive output early in life at the expense of growth, total lifetime reproductive output, and life expectancy. Because most growth and first reproduction occur early in life, I do not expect subsequent predation exposure late in life to affect these traits. I expect snails exposed to predation risk later in life to increase their current reproduction at the expense of life expectancy and later reproduction.

If phenotypic plasticity in the life history of *Physa* occurs as a maladaptive byproduct of stress, or tradeoffs with anti-predator behaviors or morphologies, I expected to see reduced

growth, delayed reproduction, reduced reproductive output, and reduced life expectancy. Some models of stress, such as the allostatic model (McEwen and Wingfield 2003) or cumulative stress model (Nederhof and Schmidt 2012) suggest the cost of stress builds up with repeated exposure. Likewise, many predation models assume an energetic cost to responding to predators (Lima and Dill 1990, Lima and Bednekoff 1999), which would cause costs to accumulate with longer exposures. Under these models, I expected to see that the duration of predator cue exposure had a larger effect on life history traits than the timing of predator cue exposure, and that continuously exposed snails would suffer more severe effects than early exposed or late exposed snails. I also expected to see the effects on reproductive output and lifespan to be reduced when the predator cue was removed between periods and increased when the cue was added between periods. Other models, such as silver spoon model, suggest that the timing of a stressor affects how the stressor alters life history (Taborsky 2017). Under these models, I would expect the timing of predation cue exposure to have a larger effect than the duration of predator cue exposure, with predator cues early in life having a larger effect than predator cues late in life. I also expect that removing a predator cue will not alleviate the effects of the early cue exposure on life expectancy and reproductive output.

Methods

Production of F_1 snails

On April 28, 2018 I collected ~ 40 wild F_0 snails from Sanborn Lake in Stillwater, Oklahoma (Google map coordinates: 36.15498997922014, -97.07793318507633). I brought them back to the lab and placed them in a 5.7L plastic shoebox filled with 3L of water. About

48 hours later, I placed 23 egg masses from the snails into individual 16oz (473 mL) deli cups. Eggs from twenty-two of the twenty-three egg masses hatched approximately one week later. I collected 5 snails from each hatched egg mass and placed each snail into an individual deli cup.

Experimental set-up

The experiment consisted of three five-week periods, 1) an early life period 1 – 5 weeks after hatching, 2) a middle life period 6-10 weeks after hatching, and 3) a late life period 11-15 weeks after hatching. For each group of five snails, I randomly assigned one to each of five treatments: 1) control snails (C) were exposed to control cue during all three periods, 2) continuous predator snails (CP) were exposed to predator cue during all three periods, 3) early predator snails were exposed to predator cue during the first period and control cue during later periods (EP), 4) middle predator snails were exposed to predator cue during the second period and control cue during other periods (MP), and 5) late predator snails were exposed to predator cue during the third period and control cue during other periods (LP). This process resulted in 110 snails from 22 lines, divided into the five treatment combinations. One snail in the MP treatment died before the first treatment was applied. I eliminated this snail from the sample, resulting in 109 total treatment snails.

Most of the EP and CP snails died prior to the third period. In addition, I was not able to collect egg production data from the third time period. As a result, I only examined data from the first two periods, and only considered the effects of period 1 and period 2 treatment on life expectancy. I treated LP snails as control snails because over the length of the considered data they had not been exposed to predator cue and combined them into the C

group. For life expectancy (the only metric that was measured during the third period), I only tested the effects of the early and middle life treatments and I counted the LP snails as control snails with their lifespan right censored at the end of period 2.

Production of predator cues

Snails respond to predator cues that are a combination of predator scents and alarm cues from crushed conspecifics (Crowl and Covich 1990). I generated such a predator cue following Beaty et al. (2016). I first crushed 1.5mg of snails in 200mL of dechlorinated water. I then fed 0.3g of snails to five crayfish placed in Pyrex bowls containing 600mL of water. After allowing the crayfish to feed for 1 hour, I removed 50mL of water from each bowl, and combined it with the 200mL of crushed snail water. I then strained the mixture through a coffee filter and froze it in 2mL aliquots. The remaining water from the crayfish was also frozen, and I combined 200mL of the crayfish water with 200mL of water containing 1.5g of crushed snails for future batches of predator cue. This was done to reduce the number of snails sacrificed to produce predator cues. I froze 2mL aliquots of dechlorinated water to act as control cues.

Husbandry, mating time, and cue application

The snails were transferred to deli cups filled with 300 ml of clean dechlorinated water, fed, and exposed to cues twice per week. Snails in predator treatments were exposed to 1 ml of predator cues and snails in control treatments received 1 ml of previously frozen dechlorinated water. At each water change, I checked each snail's old cup for egg masses, and if they were present I saved the egg masses in order to count the number of eggs in each

mass. For the first four weeks, snails were fed a ~10 mg of algae wafer. After 4 weeks, the amount of algae wafer provided was increased to ~30mg. At two weeks of age I started measuring the length of each snail every two weeks. Length was measured from the tip of the spire to the farthest point on the aperture using digital calipers, and was recorded to 0.01mm. I also recorded the date of first reproduction, and the date of death for each snail.

Snails preferentially outcross and plasticity and reproductive effort are both altered when snails are not able to mate (Escobar et al. 2011, Auld and Relyea 2010, Tsitrone et al. 2003). Thus, starting when the experimental snails reached three weeks of age, I began to expose them to mating partners once per week. The mating snails were hatched from the same egg masses as the experimental snails, but not separated by line. They were group housed in 6L shoebox bins, with 40 snails to a bin, and 5 total bins. I conducted full water changes on these snails once per week. I fed them ~0.2g algae wafer per bin per week for the first 3 weeks post hatching, and subsequently fed them ~0.4g per bin per week. At three weeks of age, I painted all of the mating snails with yellow nail polish to distinguish them from the treatment snails (Henry and Jarne 2007)

For the mating sessions, I set up ~30 clean deli cups with no cues and added three painted mating snails to each. I then added a treatment snail to each cup and left them undisturbed for 2 hours. Next, I removed the treatment snails and placed them back into individual deli cups. Mating snails were randomly moved between cups and another round of treatment snails were added to the cups. I repeated the procedure until all of the treatment snails had an opportunity to mate.

Measurements of age at first reproduction, lifespan, and egg counts

During each water change, I collected any egg masses present out of the cup. The first time I observed egg masses I recorded the date, and the age at first reproduction was calculated as the number of days between the time the snail hatched and when it laid its first egg masses. I counted the eggs collected at each water change by placing each egg mass on a petri dish and covering it with a glass cover slide, then counting the eggs under a dissecting scope. In the event that a snail laid more than 10 egg masses, I counted the first 10 and then multiplied the total number of egg masses by the average number of eggs per mass to estimate the total egg number. This only occurred twice during the experiment. If the snails hatched prior to counting, I counted all hatchlings, plus all unhatched eggs. I added up the total number of eggs counted per week to get the snail's egg production per week. To obtain egg production over the course of the period, I added up the total number of eggs laid during the period. When a snail was found dead in its cup, I recorded the date. The lifespan of the snail was calculated as the number of days between when the snail hatched and when it was recorded dead in the cup.

Data Analysis

I analyzed the effect of treatment on lifespan, age at first reproduction, egg production per period, egg size, and growth. I used survival analysis to test for the effects of predator cue exposure on the age of first reproduction. A majority of the snails began reproduction before the start of the second period, and there were too few that had not reproduced before period 2 to assess the effect of period 2 treatment on age at first reproduction (not reproducing by start of period 2= 7/22 EP, 4/22 CP, 4/44 C, and 3/22 MP).

As a result, I considered the effect of period 1 treatment on age at first reproduction only. Some snails did not reproduce before dying. I treated these individuals as right censored data points, with their date of death serving as the minimum age of first reproduction. I fit two Cox survival regressions to the age at first reproduction data, a null model and a model containing period 1 treatment using the package 'survive' in R. In both models, I included Line as a frailty term, a term that acts as a random effect for survival models (Balan and Putter 2020). I compared the null and period 1 treatment models using likelihood ratio tests (lrtest function, package lmtest, Zeilus and Hothorn 2002). In general, I compared models with likelihood ratio tests when I had two nested models, and with Akaike Information Criterion (AIC) scores when there were more than two models.

I analyzed the effects of predator exposure on lifespan using survival analysis. No snails died during period 1, and therefore all of the snails experienced a period 1 and period 2 treatment. The most complex model included treatment in period 1 interacting with treatment in period 2 with Line included as a random effect. I fit this model and all simpler models using a Cox regression in the package ‘survive’ in R. I included Line as a frailty term in all models, and compared them using AIC scores.

To determine the effect of period 1 treatment on growth up to week 6, I fit linear mixed models of size at week 6. The full model included treatment in period 1, with line as a random intercept effect. I compared the full model with treatment in period 1 to a null model containing only the random intercept of line using a likelihood ratio test. By week 10, a majority of snails receiving predator cues in period 1 were dead. I therefore only considered sizes up to week 8. To determine the effect of period 1 and period 2 treatment on growth between weeks 6 and 8, I first calculated growth for each snail by subtracting their length at

week 6 from their length at week 8. I then fit linear mixed models to the differences, with treatment in period 1, treatment in period 2, and their interaction in the full model. Line was included as a random intercept in all models, and I compared the models by AIC.

For egg counts, I totaled up the number of eggs laid during period 1 and the number laid during period 2. For eggs laid during period 1, I first fit a generalized linear mixed model with a negative binomial link function that included treatment in period 1 as a fixed effect and line as a random effect (fit with the R package glmmTMB). I then tested the model for zero-inflation using the "TestZeroInflation" function in the package DHARMa. The test showed evidence of zero inflation ($p= 0.048$). I therefore fit three zero-inflated models to the data. One included treatment in period 1 in both the zero-inflation and conditional portions, and line as a random intercept in the conditional portion. The second included treatment in period 1 in the zero-inflation portion, and line as a random intercept in the conditional portion. The third was a null model containing line as a random intercept in the conditional portion only, and no terms in the zero-inflation portion. I compared the evidence for the three models using AIC.

For egg production in period 2, I first fit a negative binomial model including treatment in period 1, treatment in period 2, and their interaction as fixed effects and line as a random intercept effect. I then tested the model for zero-inflation using the "testZeroInflation" function in DHARMa. The test did not reveal significant evidence of zero-inflation ($p=0.432$). I fit all simpler versions of the model and then compared them using AIC scores. To determine if egg production changed as a result of the change in treatments between periods 1 and 2, I calculated the difference in egg production between the last 2 weeks of period 1 and the first two weeks of period 2. I then compared linear mixed

models to determine if period 1 treatment, period 2 treatment, or their interaction affected the change in egg production, with line as a random effect in all models.

Results

Life expectancy and age at first reproduction

In my analysis of life expectancy, I fit a full Cox regression survival model with treatment in period 1, treatment in period 2, their interaction, and a frailty term for line. I then fit all of the simpler nested models and compared them with AIC. The best supported model included only the effects of period 1 treatment (Table 1.1, Figure 1.1). Snails exposed to predator cues during the first period (Treatments EP and CP) lived shorter lives than snails exposed to control cues during the first period (Treatments MP and C; Figure 1.1, Hazard ratio for model with predator cue in period 1 = 48.97, 95% Confidence interval = 19.16 - 121.1). There was not strong evidence that treatment during period 2 affected lifespan (Table 1, Figure 1).

For age at first reproduction, I fit the full model (including treatment in period 1 and a frailty term for Line) and a null model (containing only the frailty term for Line) using a Cox regression. There was significant support for including the period 1 treatment in the model of age at first reproduction (Likelihood ratio test, chi-square = 14.43 on 2.73 df, p=0.002). Snails that were exposed to predation cues during period 1 delayed reproduction, and fewer of them ultimately reproduced (Figure 1.2, Hazard ratio = 0.5075, 95% Confidence interval = 0.334 – 0.771). Given the low number of snails in each group that had not reproduced by the end of period 1, I did not attempt an analysis of how treatment in period 2 affected age at first reproduction (n = 7/22 EP, 4/22 CP, 4/44 C, and 3/22 MP).

Growth

Most growth occurred in the first 6 weeks of life. To assess the effect of treatment in period 1 on growth during the first 6 weeks of life, I used a Likelihood ratio test to compare a linear mixed model including treatment in period 1 and a random intercept of Line to a null model containing only the random intercept for Line. The test indicated that the model including treatment in period 1 was not significantly better than the null model (Likelihood ratio test chi-square = 2.30, df = 1, p = 0.13, Figure 1.3).

I assessed the effects of period 1 and period 2 treatment on growth between weeks 6 and 8 by fitting linear mixed models and comparing them with AIC. The full model included treatment in period 1, treatment in period 2, and their interaction, with Line as a random intercept effect. Between weeks 6 and 8 there was some weak evidence that period 1 predator treatment reduced growth (Table 1.2), but the effect size (-0.29mm, standard error 0.11 mm) was small, and the observed effects may reflect the deaths of snails exposed to predator cues in during period 1.

Egg production

For egg production in period 1, I assessed how the number of eggs produced during period 1 was affected by exposure to predator cues during period 1 by fitting zero-inflated negative binomial models including treatment in period 1 in the conditional and zero-inflation part, the zero-inflation part only, or neither. The model best supported by the data contained treatment in period 1 in both the conditional and zero-inflation sections of the model. The model suggests that snails that received the predator treatment during period 1 were more likely to produce 0 eggs than control snails (β=1.025, SE=0.55. Model prediction

of structural zeros: control in period 1 = 10.7% of individuals produced 0 eggs, predator period 1 = 25.1% of individuals produced 0 eggs). It also suggested snails exposed to predator cues in period 1 laid fewer eggs than control snails (Groups EP and CP, $\beta = -1.03$, standard error (SE)=0.195, the best model estimates an average of 551 eggs from snails with control cues period 1, and 188 eggs from snails receiving predator cues in period 1) (Table 1.3, Figure 1.4a). While I did not fit a model with treatment in period 1 in the conditional part and an intercept only in the zero-inflation part, a comparison of the two models with an intercept only in the conditional part suggests including treatment during period 1 in the zero-inflation part of the model was supported by the data (compare the bottom two models in Table 1.3).

For egg production in period 2, I fit negative binomial models assessing how the number of eggs laid in period 2 was affected by period 1 treatment, period 2 treatment, and their interaction. The model best supported by the data included treatment in period 1 only (Table 1.4). The model suggests that snails receiving predator cues in period 1 (Groups EP and CP) produced fewer eggs during period 2 than the snails that did not receive cues in period 1 (Groups C and MP) (β=-1.15, SE=0.216, prediction: predator cue in period 1 (EP and CP) = 305 eggs, control cue in period 1 (MP and CP) = 966 eggs, Figure 1.4b).

To assess whether period 1 treatment, period 2 treatment, or their interaction changed the snail's shift in egg production between the end of period 1 and the start of period 2, I fit linear mixed models of each snail's shift in egg production in the last two weeks of period 1 and the first two weeks of period 2 with treatment in period 1, treatment in period 2, and their interaction as fixed effects. The data best supported the null model that had none of the fixed effects, suggesting that there was no evidence that treatments affected the change in egg

production between the final weeks of period 1 and the first weeks of period 2 (Table 1.5, Figure 1.5).

Discussion

I found evidence that exposure to predation cues early in life caused the snails to have reduced life expectancy, delayed reproduction, and fewer eggs produced across their life regardless of whether or not the predation risk persisted later in life. The balance of evidence suggests that changes in lifespan, first reproduction, and egg production were byproducts of stress induced by predation risk rather than adaptive responses to the risk. While life expectancy was lower in snails exposed to predation cues early in life (Figure 1.1), I found no evidence that they reproduced earlier or increased their early life reproduction as would be predicted if the snails responded to the crayfish as a predator that can kill members of all size classes (Chase 1999, Pietrzak et al. 2015). Snails exposed to predator cues early in life delayed reproduction and laid fewer eggs (Figures 1.2, 1.4), but they do not appear to have increased their growth rate (Figure 1.3) as would be predicted if the snails were delaying reproduction to invest more energy in growth and reach a size threshold at which they are safe from predation (Chase 1999, Pietrzak et al. 2020). There was also no evidence of terminal investment when snails detect a predator in the environment, as snails who started experiencing predation cues in period 2 showed no evidence of increasing their reproductive investment after the predator exposure started (Figure 1.5). Overall, these results suggest that the level of exposure to predation cues that I imposed on snails during the early life period resulted in a stress response with long term consequences to life expectancy and reproduction rather than an adaptive shift in life history strategy in response to predation.

Reduced fitness due to chronic stress is a commonly observed phenomenon, and is thought to occur as a continuing response to an environmental stressor makes it challenging for an organism to maintain homeostasis (McEwen 2000). However, the duration of predation exposure did not seem to make a difference to the snail's reproduction or survival. Both the EP and MP snails received predator cues for the same duration of time, and yet only the EP snails that received it during period 1 suffered reduced lifespan and egg production relative to the control snails (Figures 1.1, 1.4). Furthermore, there was no difference between the lifespan and egg production between the EP snails that had the predation cues removed during period 2, and the CP snails for which the predation risk continued during period 2 (Figures 1.1, 1.4). These results suggest that it is the timing of exposure early in life that triggers changes in life expectancy and reproduction, and not the duration of exposure.

Although I did not quantify the mechanism of the stress response, the results were not consistent with the energy budget mechanism described in the allostasis model (McEwen and Wingfield 2003) or the energetic metrics used when modeling the costs of anti-predator responses (Lima and Dill 1990). If changes in the animals' energy budget were the mechanism, I would have expected the snails that were switched from the predation treatment in period 1 to the control treatment in period 2 to see an increase in their life expectancy and reproductive output with the removal of the stressor. The results are more consistent with models focused on the timing of a stressor rather than the energetic cost of responding to the stressor. The "silver spoon" model (Taborsky 2017) for example predicts animals that develop in adverse conditions suffer negative fitness consequences, similar to what I observed. A significant challenge in applying stress models to snails is that many stress models were developed in vertebrates and make assumptions about exposure to

glucocorticoids (Harris 2020). The stress response system of mollusks seems to be dependent on shorter-lived hormones such as norepinephrine, epinephrine, and dopamine (Ottaviani et al. 1992) and there may be important differences in the effects of prolonged stress between vertebrates and invertebrates. It is also not clear from this experiment if a repeated exposure was needed during the early life period. It is possible that the same effects of lifespan and fecundity may have been achieved from a single predation exposure directly after hatching.

My results were a mixture of consistency and inconsistency with previous results from this species. The observed life expectancy for control snails and late predator exposed snails was very similar to that observed by Auld et al. (2014), with increased mortality starting around 17 weeks (119 days) and a maximum lifespan of about 30 weeks (220 days) (Figure 1.1). Crowl and Covich (1990) observed the lifespan of non-predator exposed snails to be about 3-5 months (90-150 days) which also seems consistent with both our results and those of Auld et al. (2014). However, the life expectancy of our early exposed and continuously exposed snails was substantially shorter than estimates previously reported. Crowl and Covich (1990) found life expectancy of crayfish exposed snails to be about 11-14 months (330 – 420 days), which was much longer than the median ~60 day lifespan for early exposed snails in this study. Some of the discrepancy may be related to the exact timing of predation cues during the early parts of life. I exposed snails to predation cues directly out of the egg, whereas Crowl and Covich (1990) waited until the snails were 10 days old and about 2mm in length to start the cue exposure. This early period after hatching may be a critical period for cue exposure. Rundle et al. (2010) found evidence that predation cues alter the development of *Radix* snails prior to hatching, and it seems reasonable that such effects could extend to the period just after hatching.

Diet may play a role in determining life expectancy, as richer diets lead to faster reproduction and shorter life expectancy (Auld 2018). The diet of algae wafers that I fed to the snails was more nutrient rich than the lettuce Crowl and Covich (1990) fed their snails. More work is necessary to fully understand how diet interacts with predation exposure to affect lifespan. Another issue is mating. I exposed the snails to potential mates every week, whereas Crowl and Covich (1990) housed their snails in groups of 3, all receiving the same treatment. The increase in life expectancy and size observed by Crowl and Covich (1990) was highly consistent with the increase in life expectancy and size observed in snails with no access to mates (Tsitrone et al. 2003), which suggests their snails may have mated less frequently.

I surprisingly failed to observe any difference in length between control snails and those exposed to predation cues (Figure 1.3). Prior studies (Crowl and Covich 1990) have found evidence that *Physa* snails increase their size in the presence of predation cues, a response thought to protect the snails as crayfish preferentially prey on smaller snails (Crowl 1990). I provided the snails with a large amount of food (60mg algae per week), and this high food exposure may have allowed the snails to avoid the growth-reproduction tradeoff. This is supported by the fact that all of the snails, regardless of treatment, grew to an average of 9-10mm, which is as large as the predator exposed snails in Crowl and Covich (1990), and the fact high quality spirnula diets lead to faster growth and a larger size at first reproduction (Auld and Henkel 2014). It does however contradict theory suggesting delaying reproduction in favor of increased growth is more likely to occur in resource rich environments (Chase 1999).

Age at first reproduction was about 5-6 weeks old for most snails (Figure 2). This matches the age of reproduction found by Beaty et al. (2016), Goeppner et al. (2020), and Tistrone et al. (2003) but is far earlier than some other studies (Auld and Henkle 2014, Crowl and Covich 1990). The discrepancy may be diet related, as snails exposed to high quality diets reproduce earlier than those exposed to low quality diets (Auld and Henkle 2014). Temperature may be another important candidate to explain differences in the age of first reproduction and growth. A number of aspects of snail life history are mediated by temperature (Brackenbury and Appleton 1991), and it is likely that the timing of first reproduction, growth, and life expectancy is heavily influenced by temperature in wild snails. More work is needed to determine how diet and temperature interact with predation risk to affect age at first reproduction.

Overall, I found evidence that exposure to predation cues early in life, but not late in life can decrease life expectancy, increase time to first reproduction, and decrease total reproductive output. These results do not provide evidence for adaptive changes in life history in response to predation cues, but they do provide evidence for a long term effect of early life predator exposure in *Physa acuta,* and suggest that there may be a developmental window during which predation risk must occur to cause changes in life history.

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Figure 1.1. Life expectancy of snails in the control (blue, C), continuous predator (red, CP), early predator (purple, EP) and late predator (yellow, MP) treatments. N=109snails (44 control, 22 continuous predator, 22 early predator, 21 late predator). The vertical lines show the ends of period 1 and period 2.

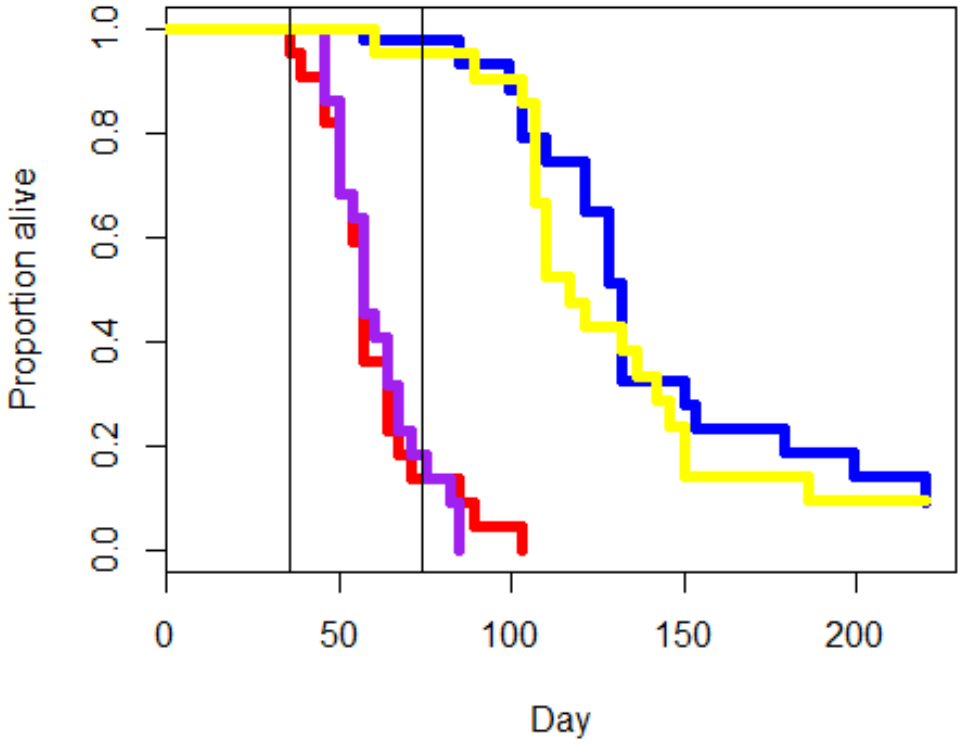


Figure 1.2. Proportion of snails who that had reproduced by time. Treatments shown include control (blue, C), continuous predator (red, CP), early predator (purple, EP) and late predator (yellow, MP) treatments. N=109 snails from 22 lines (44 control, 22 continuous predator, 22 early predator, 21 late predator). The vertical lines show the ends of period 1 and 2.

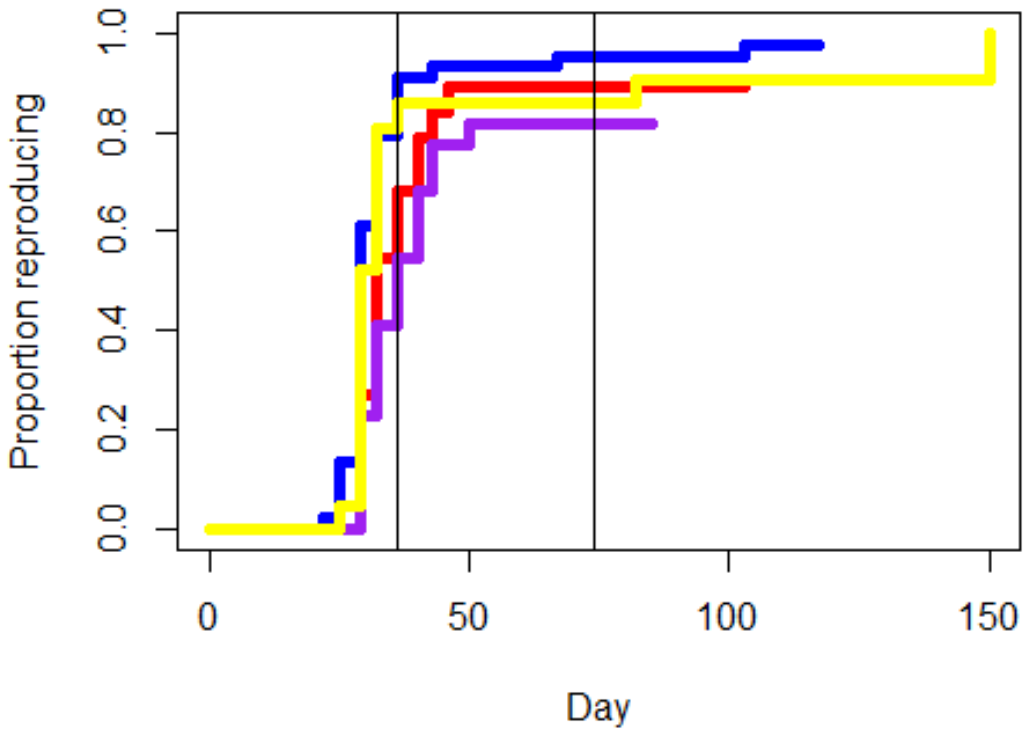


Figure 1.3. Mean size of live snails in each treatment by week. Error bars are standard deviation. Sizes past 8 weeks were removed because most of the snails in the CP and EP treatments died before the next measurement at week 10.

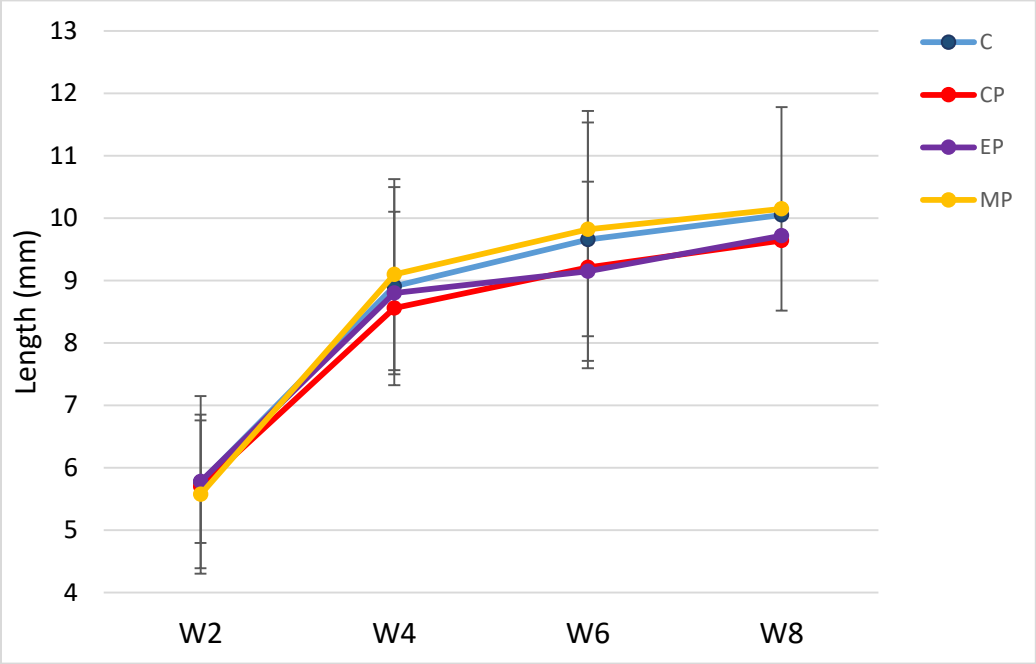
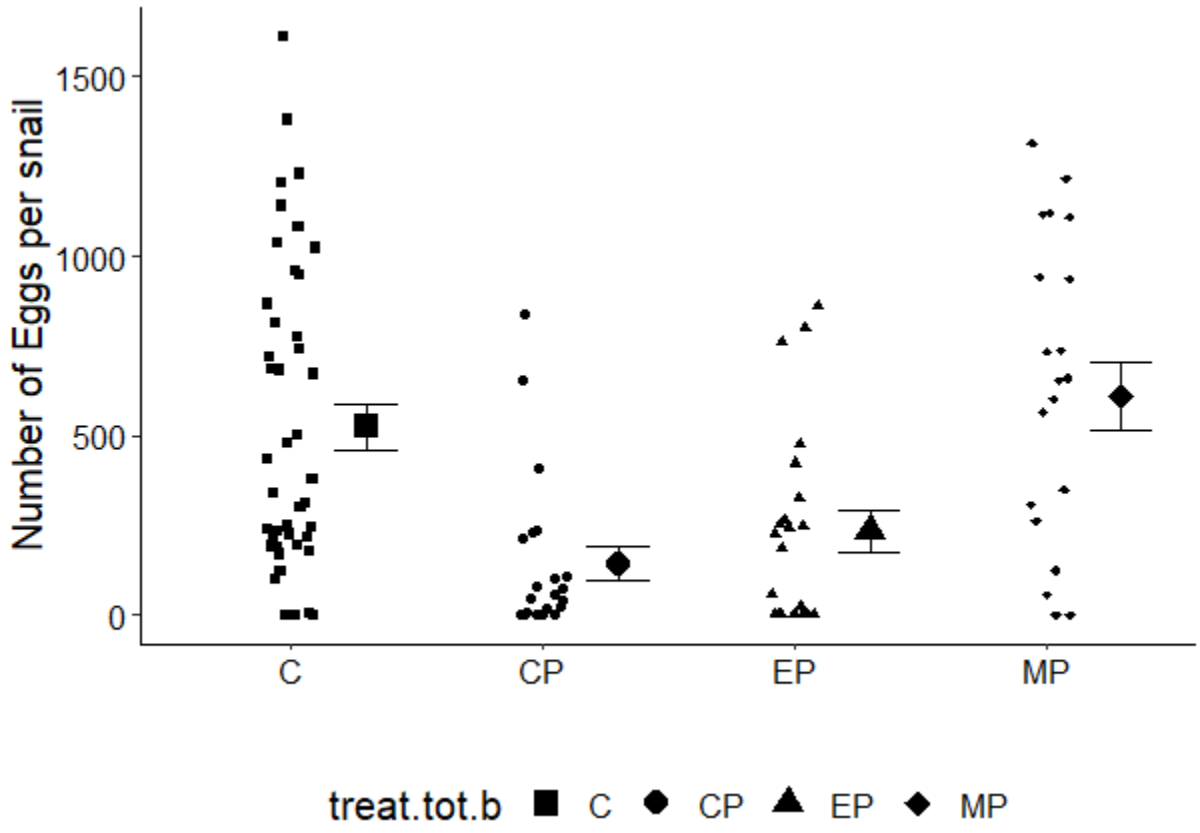
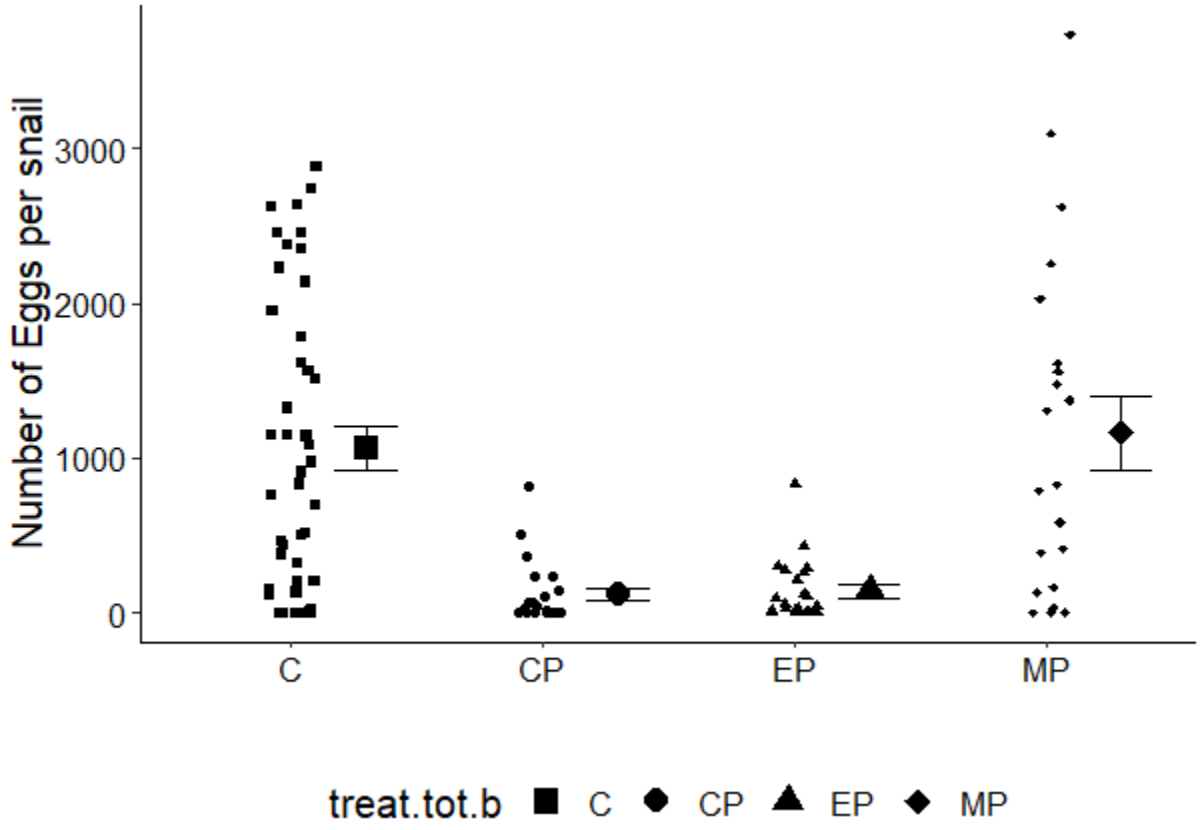


Figure 1.4. Total egg production by treatment for snails in A) period 1, B) period 2, and C) in total across both periods.

A



B


Number of Eggs per snail
3000
2000
1000
0
C
CP
EP
MP
treat.tot.b
C
CP
EP
MP

C

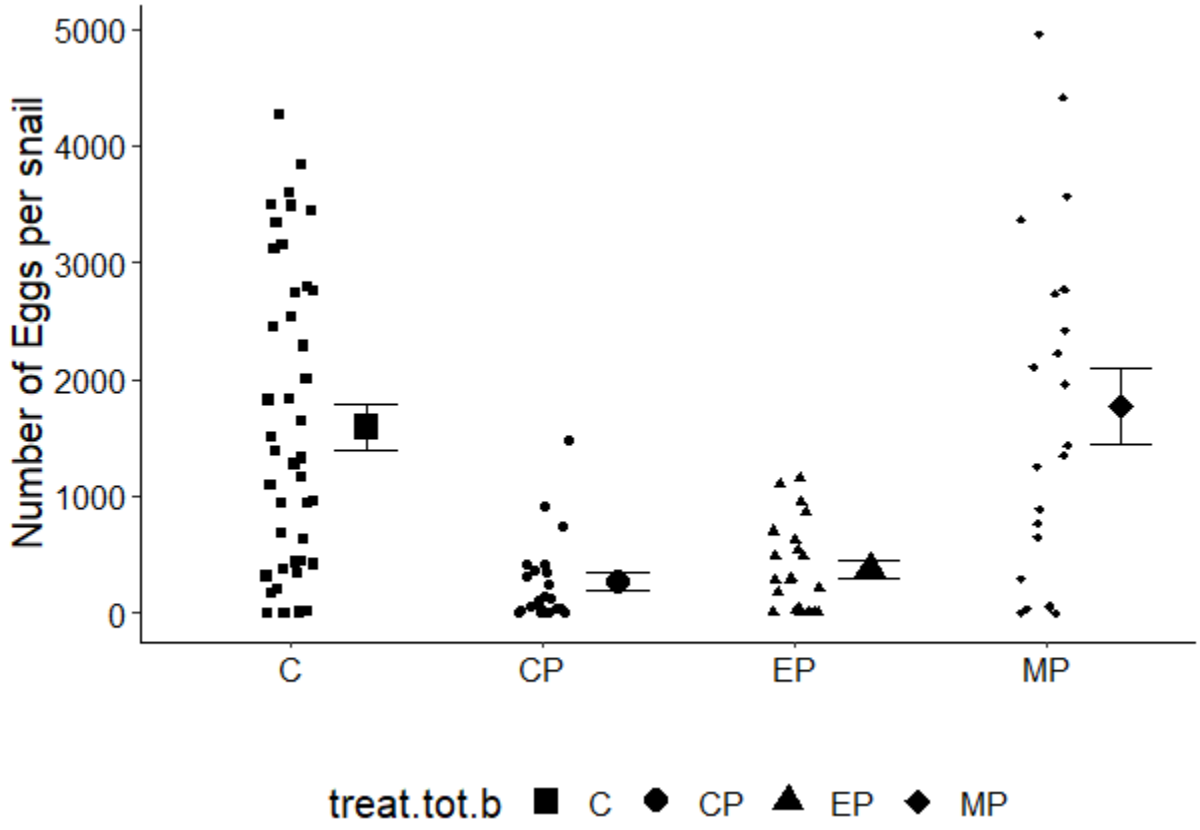

Number of Eggs per snail
5000
4000
3000
2000
1000
0
C
CP
EP
MP
treat.tot.b
C
CP
EP
MP

Figure 1.5. Mean egg production per snail per a half week in each of the four treatment groups. Error bars are standard error.

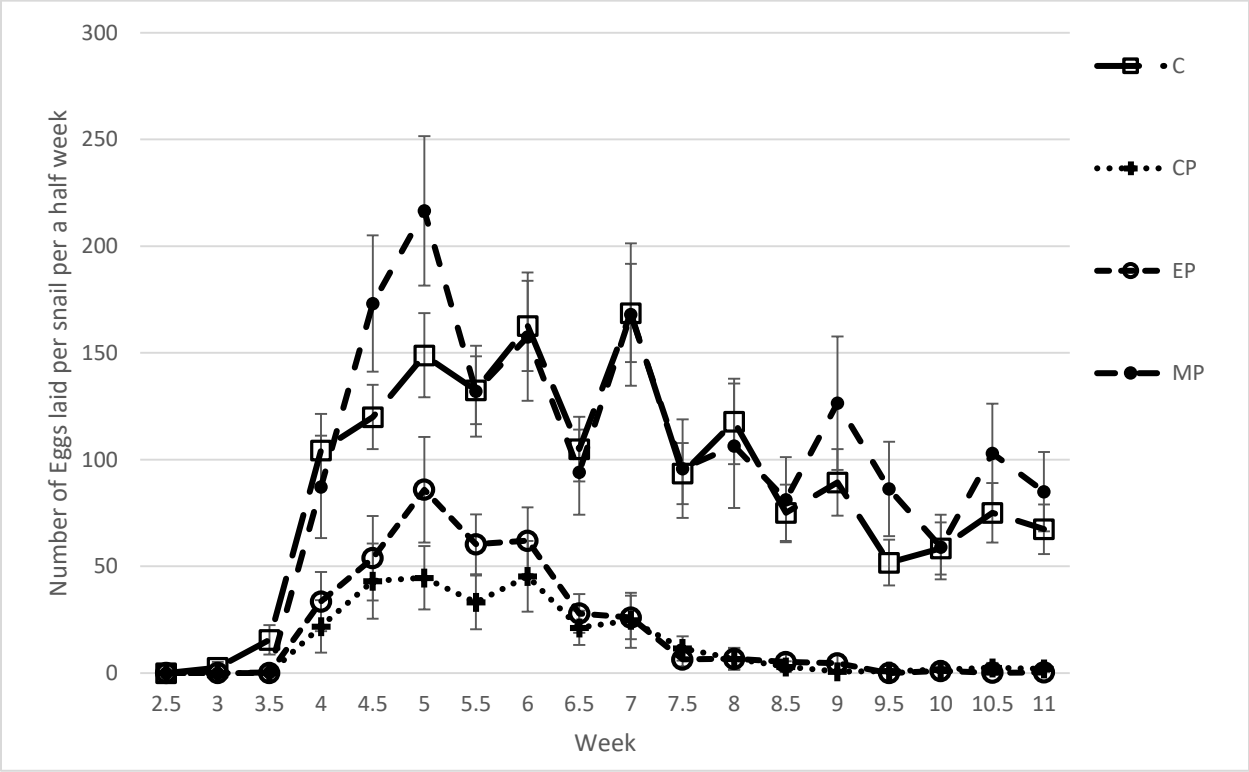


Table 1.1. Model selection results for life expectancy. Models were fit with a Cox regression

Model	ΔAIC	df	Weight
Surv ~ TreatP1 + Frailty(Line)	0	7.8	0.50
Surv ~ TreatP1 + TreatP2 + Frailty(Line)	0.9	10.6	0.36
Surv ~ TreatP1 * TreatP2 + Frailty(Line)	1.8	11.9	0.15
Surv ~ TreatP2 + Frailty(Line)	111.7	1	<0.001
Surv ~ 1 + Frailty(Line)	112.0	0	<0.001

Table 1.2. AICc table of linear mixed models describing effect of treatments on the change in snail size from week 6 to 8

Model	**Δ**AICc	df	Weight
Diff ~ TreatP1 + (1|Line)	0	4	0.50
Diff ~ TreatP1 + TreatP2 + (1|Line)	1.3	5	0.26
Diff ~ TreatP1 * TreatP2 + (1|Line)	2.1	6	0.17
Diff ~ 1 + (1|Line)	4.8	3	0.05
Diff ~ TreatP2 + (1|Line)	6.6	4	0.02

Table 1.3. AIC table for zero-inflated negative binomial models describing egg production during period 1

Model	**Δ**AIC	df	Weight
Eggs ~ TreatmentP1 + (1|Line) ZI ~ TreatmentP1	0.0	6	1
Eggs ~ (1|Line) ZI ~ TreatmentP1	28.1	5	<0.001
Eggs ~ (1|Line) ZI ~ 1	31.0	4	<0.001

Table 1.4. AIC table for negative binomial models describing egg production during period 2

Model	ΔAIC	df	Weight
EggsP2 ~ TreatP1 + (1|Line)	0.0	4	0.63
EggsP2 ~ TreatP1 + TreatP2 + (1|Line)	1.8	5	0.25
EggsP2 ~ TreatP1* TreatP2 + (1|Line)	3.3	6	0.12
EggsP2~ 1 + (1|Line)	27.2	3	<0.001
EggsP2 ~ TreatP2 + (1|Line)	27.2	4	<0.001

Table 1.5. AICc table for models describing shift in egg production between the final two weeks of period 1 and the first two weeks of period 2.

Model	ΔAICc	df	weight
Shift ~ 1 + (1|Line)	0	3	0.35
Shift ~ TreatP1 + (1|Line)	0.8	4	0.24
Shift ~ TreatP2 + (1|Line)	1.4	4	0.17
Shift ~ TreatP1 * TreatP2 + (1|Line)	1.7	6	0.15
Shift ~ TreatP1 + TreatP2 + (1|Line)	2.6	5	0.10

CHAPTER II

HOW DOES PHENOTYPIC PLASTICITY INDUCED BY PREDATION CUES CHANGE WITH RESOURCE AVAILABILITY?

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Abstract

Predators can not only induce phenotypic changes in their prey but also induce phenotypic change in the offspring of their prey. Questions remain about the mechanisms of predator-induced parental effects, and how they may be mediated by resources available to parents. In this experiment, I examined how trans-generational and within-generation plasticity may be mediated by available resources in freshwater snails (*Physa acuta*). I exposed 88 individually snails to a full factorial combination of predator exposures (exposure to crayfish cues or control cues) and food availability at reproduction. I then allowed these snails to mate with "mating" snails given no cues and standard food. I collected offspring from both the treatment snails and mating snails, and raised them with or without predator exposure. I found that in the F_1 "treatment" snails, low food snails had smaller soft tissue mass than high food snails, while predator exposed snails had larger soft tissue mass than control cue snails. Predator exposed F_1 snails had lighter than expected shells for their body size. Food restriction and predator exposure

reduced anti-predator behavior in the presence of predator cues. In the F_2 snails, I found weak evidence that maternal food restriction and predator exposure interacted to increase shell mass, and evidence that maternal food restriction and predator exposure interacted to increase shell mass, and evidence that maternal food restriction and paternal predator exposure reduced anti-predator behavior in the presence of predator cues. Maternal food restrictions, individual predator exposure, and paternal predator exposure also affected shell shape in the F_2 snails.

Introduction

Phenotypic plasticity occurs when the same genotype produces different phenotypes in different environments (Pigliucci 2001). The two main ways in which adaptive phenotypic plasticity can evolve (Nettle and Bateson 2015) are informational and somatic pathways. In the informational pathway, phenotypic plasticity evolves when a cue in the environment is correlated to the state of the environment, and the optimal phenotype varies with the state of the environment (Nettle and Bateson 2015). Predator-prey interactions often create conditions for phenotypic plasticity to evolve along this path. Predators produce cues that are correlated with their presence and optimal behaviors and phenotypes differ when predators are present or absent. Examples include Daphnia that develop helmet spines in the presence of fish cues (Agrawal et al. 1999), and snails that develop thicker shells when exposed to cues from predatory crabs (Trussell 1996, Appleton and Palmer 1988).

Theoretical models show how this informational pathway can lead to the evolution of trans-generational plasticity in addition to within-generation plasticity (Leimar et al. 2016, McNamara et al. 2015, Kuijper and Hoyle 2015). In these models,

transgenerational plasticity provides a fitness advantage by allowing offspring to develop phenotypes optimal to the environmental conditions they are likely to experience before they have the opportunity to experience their environments. The models suggest that transgenerational plasticity is favored when parental cues accurately predict the state of the offspring's environment, which generally occurs when parental cues provide accurate information about the parental environment and the parental environment has high autocorrelation with the offspring environment. Galloway and Etterson (2007) provided evidence that informational cues from American bellflowers (*Campanula americana*) shaped their offspring's phenotype in a way that enhanced its fitness when its environment matched the parental environment. American bellflowers can grow in sunny or shaded patches and they showed that the fitness, measured by seedling yield, of flowers grown in the same light environment as their parent was higher than of flowers grown in the other light environment. However, despite a few well-known examples, two meta-analyses have come to different conclusions about how common adaptive transgenerational plasticity is. Uller et al. (2013) found little support for transgenerational plasticity enhancing fitness while Yin et al. (2019) found transgenerational plasticity increasing fitness. A recent review of predator induced transgenerational plasticity also found examples of a wide range of fitness consequences, including adaptive, maladaptive, and no effect (Tariel et al. 2020).

One potential reason for this is that phenotypic plasticity can still evolve in situations where informational cues correlated with environmental states are not present (Ghalambor et al. 2007, Nettle and Bateson 2015). For example, a lower quality resource can cause a shift in life history traits such as growth and the timing of reproduction (Auld

and Henkle 2014). Transgenerational plasticity can also evolve in the absence of informational cues. Parental activities such as investment in offspring, the choice of where to place offspring, and the exposure of offspring to parental hormones during development can have long-lasting effects on offspring phenotype (Mousseau and Fox 1998). Often these activities affect offspring phenotype independently of the correlation between the parent cue and the offspring's environment. For example, a well provisioned parent or a parent undergoing terminal reproduction may place more resources into their eggs. This extra provisioning may cause the parent to produce larger more robust offspring, even when there is no correlation between the parent's environment and the offspring environment. In these situations, provisioning is not an informational cue as it does not predict the offspring environment (Nettle and Bateson 2015). Similarly snowshoe hares (*Lepus americanus*) produce smaller and more nervous offspring after exposure to predation risk (Sheriff et al. 2009), an event that seems to occur regardless of what type of environment the offspring are exposed to, making it questionable as to whether the parental effect evolved as a form of information transfer.

Distinguishing between transgenerational plasticity that relies on informational cues and transgenerational plasticity that does not can be difficult. For example, Storm and Lima (2010) found that when they exposed female crickets to wolf spiders, the crickets produced offspring that spent more time stationary and were less likely to be consumed by predators. They interpreted these results in an informational framework – female spiders exposed to spiders passed a cue, such as a hormone or epigenetic marker, to their offspring and this cue "prepped" them for an environment with spiders. An alternate explanation would be that the mothers themselves are more stressed upon

exposure to a predator, and the mother's stress response induces changes in their eggs regardless of whether the changes benefit the offspring. The key difference between the two explanations is that the first suggests the parental effect evolved because the maternal cue provided information to the offspring that allowed the offspring to develop a phenotype more suitable to surviving an environment with spiders. In the second, the parental effect evolved because it is adaptive for the mother to mount a stress response that help her avoid predation, and the effects on the offspring are an unavoidable byproduct.

Were the effect on offspring maladaptive, the offspring may be under selection to reduce their response to parental stress hormones, thus eliminating the parental effect. However, stress models such as the reactive scope model (Romero and Wingfield 2015) show how reducing sensitivity to a stress hormone can adversely affect an individual's ability to maintain homeostasis during stressful events. There are also empirical examples of non-adaptive transgenerational plasticity that may be explained by offspring exposure to maternal hormones. For example, three-spined sticklebacks deposit higher levels of cortisone into their eggs when they are exposed to predation cues (Giesing et al. 2010). This is a possible explanation for why the offspring of predator exposed fish exhibited reduced anti-predator behavior and increased vulnerability to predators (McGhee et al. 2012). It is therefore not justifiable to assume that selection can always eliminate fitness reducing responses to parental cues.

Further complicating matters, informational and non-informational parental effects are not mutually exclusive and the same developmental cue could be interpreted as an informational cue or a somatic cue depending on circumstance. For example, if egg

provisioning is correlated to resource availability in the parent's environment and the parent's environment is correlated to the offspring environment, then egg provisioning may provide the offspring with information about the resource state of the environment. Thus, egg provisioning could potentially change offspring phenotype through two mechanisms, the first by directly providing the offspring with extra resources (a non-informational mechanism) and the second by triggering phenotypes and behaviors that enhance fitness in a food rich environment (an informational mechanism).

Understanding how informational and non-informational mechanisms of transgenerational plasticity interact may help us make better predictions about when it occurs. There has been some success doing this for within-generation plasticity using the marine snail *Nucella* (Bourdeau 2009). Bourdeau (2009) showed that it was possible to induce anti-predatory shell morphology by restricting the snail's feeding rather than exposing it to predation cues. This suggests that the change in shell morphology is driven by a long-lasting effect of reduced foraging rather than being a response to an informational cue from a predator. A manipulation of resource availability could likewise be used to assess how transgenerational plasticity is affected by non-informational mechanisms. When transgenerational plasticity is affected by resource availability, I should observe a difference in the predator induced reaction norms between well fed individuals and food restricted individuals.

Comparing maternal and paternal effects is another strategy that could be used to separate out informational and non-informational parental effects. Informational cues from fathers have been reported in mice (Dias and Ressler 2014). As mothers provide most of the resource investment, as well as the environment in which offspring develop,

non-informational mechanisms of transgenerational plasticity are more likely to appear through maternal effects than paternal effects. Paternal effects in contrast are expected to be driven by informational mechanisms. Thus, by comparing parental effects induced by fathers and mothers, it should be possible to acquire a sense of how much of the parental effect is driven by information dynamics.

The pond snail (*Physa acuta*) presents a good system to test these approaches. Multiple generations of snails can be reared in the lab, and snails have been successfully used in a number of transgenerational studies (Beaty et al. 2016, Laquet and Tariel 2016, Tariel et al. 2020b, Goeppner et al. 2020). The pond snail is also hermaphroditic, and thus the same individual can produce eggs and sperm. This allows the maternal and paternal effect of a treatment to be measured from the same individual (e.g Tariel et al. 2020b). In this experiment, I manipulate resource availability and exposure to cues indicating predation risk to determine how information from predator cues interacts with body condition to affect within and transgenerational plasticity in the pond snail.

Methods

Production and husbandry of F_1 treatment snails

In June of 2019, I collected about 40 wild snails from Sanborn Lake in Stillwater, Oklahoma (Google map coordinates: 36.15498997922014, -97.07793318507633), and placed them in Pyrex bowls to lay eggs. Collected F_0 snails were brought back to a laboratory and placed in a 6L plastic shoebox with about 3L of dechlorinated water. The shoebox was placed in an incubator set to 25C with a 12:12 hour day night cycle. Three days later, I isolated 24 resulting egg masses into individual deli cups, and waited 8 days

for them to hatch. During this time, the eggs were moved from the incubator to an animal room with the same temperature and day:night cycle. Of the 24 egg masses isolated, 22 hatched and 2 failed. From the successful egg masses, I collected 4 haphazardly selected snails and randomly assigned one of each to four treatments: control, full food (CF, n=22); control, food restricted (CS, n=22); predator cue, full food (PF, n=22; 2 died before mating final n=20); and predator cue, food restricted snails (PS, n=22). The ID of the egg mass that snails came from was recorded as their line.

All snails were held individually in 16oz (473mL) deli cups containing about 300 mL of dechlorinated water in a room with a temperature of about 25C and a 12:12 day night light cycle. Predator cue treatments started 10 days after hatching. Snails in the PF and PS treatments received 1 mL of a predator cue consisting of crayfish kairomones mixed with crushed snail water. The predator cues were produced following Beaty et al. (2016) and the methods described in chapter 1. Briefly, 5 crayfish were placed in 600mL of water and fed 0.3g of snails apiece. An additional 1.5g of snails were crushed in a beaker of 200mL of water. 200mL taken evenly from the crayfish bowls and the 200mL of crushed snail water were mixed and then strained through a coffee filter to remove bits, then frozen in 2mL aliquots. Snails in the CF and CS treatments received 1 mL of a control cue consisting of dechlorinated water, frozen and stored in the same manner as the predator cue.

Snails were fed with pieces of Hikari brand algae wafers. To get pieces of the correct mass, I cut chunks of food with a scalpel of approximately the correct size. I then weighed the pieces on an electronic balance, and shaved off the piece until it was within plus or minus 2mg of the desired size. For the first three weeks post hatching, snails

received the same food amounts, 10mg (range 8-12mg) added twice per week. Starting in week 3, snails in the low food treatments (CS and PS) were reduced to ~5mg (range 3-7mg) of food twice per week, while snails in the high food treatments (CF and PF) were increased to 15mg (range 13-17mg) of food twice per week.

Production and husbandry of F_1 mating snails

To produce mating snails with standardized food and no predator experience, I kept the snails from each egg mass that were not are chosen for the treatments described in the previous paragraph group housed in a deli cup and fed them (~30mg) once per week. After 2 weeks, I culled the number of snails from each mass to 10 to avoid stunting their growth due to high density. At 3 weeks of age, I separated these snails into individual deli cups and began feeding them 10mg of food twice per week until they were ready to mate with the treatment snails at 5 weeks of age.

Mating protocol and F_2 husbandry

At five weeks of age I put each snail that had received a treatment with a single line-matched mating snail (painted with red nail polish) together into a cup for 24 hours to allow mating. At the conclusion of the 24-hour mating window, I moved each snail back into their own cup and allowed them to lay eggs. After 72 hours, I removed the adult snails from the cups and left the eggs for 7 days to hatch. Eggs produced by the treatment snails were used to test the maternal effects of food restrictions and predator exposure and are referred to as the "maternal effect snails". Eggs produced by the mating snails were used to test the paternal effects of food restrictions and predator exposure and

are referred to as the "paternal effect snails" (Figure 2.1). When separating the paternal snails into individual deli cups, I observed some egg laying in some of the containers at the point of separating them, suggesting that some of the snails may have prematurely mated. To try to eliminate these snails from the experiment, I monitored egg production for 72 hours post separation and eliminated all snails that laid eggs during this period from the experiment. For the lines in which all snails laid eggs, I used the egg laying snails for mating but did not collect their offspring for the paternal effect portion of the experiment. This affected 4 out of 22 lines. Once hatched, I added 30mg of food to each cup of hatched snails and left them an additional week before culling them into groups of 12. Ten days after culling, I split the groups of 12 into two cups of 6 snails apiece, one designated as a predator cup and one designated as a control cup, and began applying cues (Figure 2.1).

Due to the large number of F_2 cups, I conducted water changes on the snails once per week rather than twice per week. Predator and control cues were still applied twice per week. In order to avoid fouling the water by overfeeding, I started by placing 50mg of food into the cup once per week. After three weeks, once I noted most of the food was being consumed, I increased the food addition to twice per week. I maintained the snails until they reached 7 weeks of age, at which time they were placed into behavioral trials as described below.

Behavioral trials

All F_1 treatment snails participated in behavioral trials (n = 86 snails from 22 lines) and two snails from each F_2 cup were randomly selected to participate in the

behavioral trials (n_{mat} = 286 snails, n_{pat} = 248 snails). I followed the methods in Beaty et al. (2016) to conduct behavioral trials. I started the trials by placing snails in individual deli cups with clean water and no food approximately 19 hours before the start of trials. About 15 minutes before the start of trials, I placed a ~15mg piece of food into each cup which sank to the bottom. I then added control cue to each cup and marked whether the snail was at or above the waterline, or below the waterline. I recorded the position of the snails every 15 minutes for 2.5 hours for a total of 10 observations (control period). Then, I added predator cue to the cups and repeated the procedure (predator period). Crawling to or above the waterline is a common anti-predator behavior in *Physa* snails (Turner et al. 1999). I therefore considered snails at or above the waterline to be engaging in anti-predator behavior. At the conclusion of the behavioral trials, the snails were frozen in a -20C freezer.

Shell shape, shell mass, and soft tissue mass measurements

I conducted shell shape, shell mass, and soft tissue measurements on the same snails that participated in the behavioral trials. To measure shell shape, I photographed the shell, aperture side up using a Canon PowerShot G11 camera attached to an Olympus dissecting microscope (following Beaty et al. (2016) and Goeppner et al. (2020)). I then digitized 28 landmarks on the images using tpsDig2 (Rohlf 2013), 11 fixed landmarks and 17 sliding landmarks (again following Beaty et al. 2016 and Goeppner et al. 2020). I calculated relative warps from these landmarks using a Procrustes analysis in tpsRelw (Rohlf 2010).

To measure shell mass and soft tissue mass, I first measured the combined mass of shell and soft tissue from each intact snail on an electronic balance. I then used forceps and a sharp dissecting probe to carefully pull the snail bodies out of the shells. Four of the paternal effect snails were not possible to separate from their shells, and the soft tissue mass and shell mass of these snails was not measured.

Data analysis F_1 snails for shell mass, soft tissue mass, and behavior

To determine if either the predator cue treatment or the food restriction treatment affected soft tissue mass, I built linear mixed models with lme4 in R with the dependent variable being soft body mass, the potential explanatory variables being predator cue treatment, food treatment, and their interaction, and with line as a random intercept effect variable. I compared the models with AICc. Shell mass was correlated with soft tissue mass ($r^2 = 0.79$). To see how shell mass was affected by predator cue treatment while controlling for soft tissue mass, I created linear mixed models with shell mass as a dependent variable, and predator cue treatment, food treatment, and their interaction as fixed variables and with soft tissue mass as a covariate in every model. Line was included as a random intercept effect.

For behavior, I was interested in the effects of mass, food treatment, and predator cue treatment on the proportion of time snails spent above the waterline in the control and predator cue conditions. I fit generalized linear mixed models including combinations of past exposure to predator cues, mass, food treatment, and predator:mass, predator:food treatment interactions separately for observations during control and predator cue periods. Since food treatment had a large effect on mass, and the goal of my analysis was

to assess how food treatment compared to mass as an explanatory variable, I did not include food treatment and mass in the same models. Line was included as a random intercept effect in all models. I initially fit models assuming a binomial distribution. I observed the residuals of the full and best models in DHARMa (Hartig 2017) and found evidence of overdispersion in the binomial models. To deal with the overdispersion, I refit all models with a beta-binomial distribution. I compared those models using AIC values because Richards (2015) suggests the use of AICc is less reliable for generalized linear models.

For all AIC comparisons, I considered variables unsupported if they had a ΔAIC greater than 7 or if they contained "pretending variables". Pretending variables are variables in a model that has a higher ΔAIC than a simpler nested model without the variable (Richards 2008)

Data analysis F_2 snails for shell mass, soft tissue mass, and behavior

To prevent pseudoreplication from measuring two snails from each cup, I averaged their soft tissue mass and shell mass and combined their measurements of the number of times they exited the water during the control and predator periods of the behavioral trials. This led to one measurement of soft tissue mass, shell mass, and behavior for each cup rather than each individual snail (See figure 1). For both the maternal effect cups and the paternal effect cups, I assessed how parental predator cue treatment, parental food treatment, and individual (within generation) predator cue treatment affected soft tissue mass, shell mass (including soft tissue mass as a covariate) and anti-predator behavior (the probability snails were at or above the waterline during a

control cue period and predator cue period). For soft tissue mass and shell mass, I fit a linear mixed model containing parental predator cue treatment, parental food treatment, and individual predator cue treatment and their two-way interactions. Line was included as a random intercept effect in all models. I compared the model with all simpler nested models using AICc. For anti-predator behavior, I fit the same models as generalized linear models with a binomial distribution. As with the F_1 snails, there was evidence of overdispersion in the residuals of the models, and thus I refit the models with a beta-binomial distribution.

Analysis of shape data for both F_1 and F_2 snails.

For the F_1 snails, I photographed all of the snails. Four snails were excluded because their shells were cracked during photography, leaving 82 snails in the analysis. For the F_2 snails, I randomly selected one snail from each cup to include in the analysis and excluded the cup if shells of both snails were cracked or lacked landmarks (140/143 of the maternal effect cups and 120/124 of the paternal effect cups were included). To analyze the shape data, I conducted a Procrustes analysis in tpsRelw following Beaty et al. (2016) and Goeppner et al. (2020). Relative warps were calculated in tpsRelW and then truncated to include RWs describing up to 95% of the variation in shell shape. I then ran a mixed MANOVA model using ProcMixed in SAS following Goeppner et al. (2020), treating the relative warps as repeated measures for each snail and including a term "var" to indicate the identity of each relative warp. For the F_1 treatment snails, I included food treatment, predator cue treatment, and their interaction as fixed effects. For the F_2 maternal effect snails, I included maternal food treatment, maternal predator cue

treatment, individual predator cue treatment, and the interaction between maternal predator and individual predator cue treatment as fixed effects. For the F_2 paternal effect snails I included paternal food treatment, paternal predator cue treatment, individual predator cue treatment, and the interaction between paternal predator and individual predator cue treatment as fixed effects. All of the models included Line as a random effect and Centroid size as a covariate.

When significant effects were found, I calculated divergence vectors (Langarhans 2009) to visualize the shape change occurring over the significant fixed effect and used tpsReg (Rohlf 2011) to visualize shape change along the divergence vector.

Results

Within generation plasticity for soft tissue mass

To assess the effects of predator cue and food treatment on the soft body mass of the F_1 snails, I fit linear mixed models with predator cue, food treatment, and their interaction and compared them via AICc scores. The model best supported by the data included food and predator cue treatments (Table 2.1, Figure 2.2). Exposure to food restrictions caused a reduction in body mass (β = -22.31, SE = 3.36). Exposure to predator cues caused an increase in body mass, but the effect size was smaller (β = 7.40, SE = 3.36). The ΔAICc of the model that include the interaction of the predator and food treatment was 0.1, suggesting there is weak evidence in favor of including that interaction term. The interaction term in the model suggests the mass increase observed in the predator exposed snails was reduced when the snails were food restricted.

Within generation plasticity for shell mass and shape

To assess the effects of predator cue and food treatments on shell mass, while controlling for soft body mass, I fit linear mixed models with soft body mass, predator cue treatment, food treatment, and the interaction of food and predator cues, and compared them via AICc scores. The best model included predator cue treatment but not food treatment (Table 2.2). As expected, shell mass increased with soft tissue mass (β = 0.63, SE = 0.034) Predator cues led to snails building shells that were lighter than expected for their body mass (β = -3.34, SE = 1.38, Figure 2).

To assess the effects of predator cue and food treatments on shape, I created a MANOVA using the proc Mixed function of SAS, with 11 relative warps accounting for 95% of shape variation as a dependent variable. Significance of the effect of treatment, food, and their interaction on shape were assessed by looking at the p value from the "var" term identifying the relative warps. Neither food nor predator cue treatment affected the shape of snails in the F_1 generation (Table 2.3).

Within generation plasticity for behavior

To assess the effects of food and predator cue treatments and mass on behavior during the control and predator periods, I compared generalized linear models including these variables with AIC scores. The best performing model during the control period included food restriction treatment but not predator cue treatment (Table 2.4a). Snails in the food restricted treatment were less likely to be out of the water during the control period (β = -0.58, SE = 0.23, Figure 2.4a, model predictions: p(anti-predator) fed = 0.257, p(anti-predator) food restricted = 0.162). The models including predator cue treatment

appear to be cases of pretending variables (Richards 2008) and thus the data do not seem to support to including that parameter. The model best supported by the data during the predator period contained predator cue and food treatments (Table 2.4b). Snails that were exposed to either the predator cue treatment (β = -0.49, SE = 0.23) or the food restriction treatment (β = -0.75, SE = 0.23) were less likely to be out of the water during the predator period (model predictions: p(anti-predator) for CF = 0.59, CS = 0.40, PF = 0.47, PS = 0.29) (Figure 2.4b).

Maternal effects on mass, shell shape, and behavior

To assess the effects of maternal predator exposure, maternal food restrictions, and individual predator exposure on the soft tissue mass of the maternal offspring of the treatment snails, I fit linear models including each combination of these variables and their two-way interactions and compared them using AICc. The top model for maternal offspring was the null model (Table 2.5a) suggesting that neither maternal predator cue treatment, maternal food treatment, nor individual predator cue treatment affected soft tissue mass of F2 snails (Figure 2.5a).

To analyze shell mass, while controlling for soft tissue mass, I included soft tissue mass as a covariate in all models. The other independent variables in the models were maternal predator cue treatment, maternal food treatment, individual predator cue treatment, and their two-way interactions. The best performing model included soft tissue mass (β = 0.32 +/- 0.021) maternal food treatment (β = -0.97 +/- 0.68), individual predator cue treatment (-0.89 +/- 0.70), and their interaction (β = 2.46 +/- 0.96) (Table 2.4b, Figure 2.6a).

I analyzed behavior by fitting beta-binomial models with the number of observations that the snail was above the waterline being the dependent variable and parental predator cue treatment, parental food treatment, individual predator cue treatment, and their two-way interactions as the independent variables. For the maternal snails during the control period, the null model performed best, suggesting that maternal predator cue treatment, maternal food treatment, and individual predator cue treatment did not affect the proportion of time the snails spent out of the water when no predator cue was present (Table 2.6a, Figure 2.7a). During the presence of predator cue, the best performing model included maternal food treatment and individual predator cue treatment. Snails from food restricted mothers spent less time at or above the waterline (β = -0.27, SE = 0.14, model predictions: p(antipredator behavior) for maternal food restricted = 0.53, maternal fed = 0.59). Snails exposed to predation cues also spent less time at or above the waterline (β = -0.36, SE = 0.14, model predictions: p(anti-predator) for exposed to predator cues = 0.51, exposed to control cues = 0.60). (Table 2.5b, Figure 2.7b)

I analyzed shell shape using a mixed model MANOVA treating RWs as repeated measures for each snail and including line as a random effect. The relative warps of the maternal effect snails were significantly affected by maternal food treatment and individual predator cue treatment (Table 2.7). Looking at the divergence vector (Figure 2.8), individual predator exposure altered the shape of the aperture making it narrower at the top. Even though it was statistically significant, restricting maternal food appears to have had a smaller effect on shape than individual predator exposure pushing shells towards slightly wider apertures (Figure 2.8).

Paternal effects on mass, shell shape, and behavior

I used the same procedure to assess paternal effects on soft tissue mass as I did to assess maternal effects on soft tissue mass. The null model again performed best (Table 2.8a) suggesting that neither paternal predator cue treatment, paternal food treatment, nor individual predator cue treatment affected soft tissue mass in the paternal offspring of the treatment snails (Figure 2.5b).

I used the same procedure to assess paternal effects on shell mass as I did to assess maternal effects of shell mass. The model containing only soft tissue mass was the best supported model suggesting that neither paternal predator cue treatment, paternal food treatment, nor individual predator cue treatment affected shell mass in the paternal offspring of the treatment snails after controlling for soft tissue mass (Table 2.8, Figure 2.6b).

For the behavior of paternal snails, during the control period, the null model performed best, suggesting that paternal predator cue treatment, paternal food treatment, and individual predator cue treatment did not affect the proportion of time the snails spent out of the water when no predator cue was present (Table 2.9a, Figure 2.9a). During the presence of predator cue, the best supported model included paternal predator cue treatment (Table 2.9b). Snails from a father exposed to predator cues spent less time at or above the waterline during the predator cue period ($\beta = -0.45$, SE = 0.14, Figure 2.9b, model predictions: p(anti-predator behavior) for father predator exposed = 0.58 , father control exposed = 0.47)

I analyzed shell shape using a mixed model MANOVA treating RWs as repeated measures for each snail and including line as a random effect. Paternal predator exposure

alone had a significant effect on shape (Table 2.10). Looking at the divergence vector (Figure 2.10), snails from predator exposed fathers were more elongated than those from control fathers.

Discussion

Overall, I found evidence that both state and information play a role in the phenotypic plasticity of *Physa* snails. In the F_1 snails, as expected, I found that food restrictions reduced snail mass (Figure 2.2, Table 2.1). This is an indication that the food treatments were successful at altering the body conditions of snails. I found evidence that exposure to predator cues caused an increase in the body masses of F_1 snails (Figure 2.2, Table 2.1). This suggests that in spite of the crawl-out behavior snails show when they are exposed to predation cues, they do not actually reduce their total food intake relative to control snails. These results suggest plasticity in response to predation cues in *Physa acuta* is not driven by reduced foraging as was the case in *Nucella* snails (Bourdeau 2009). Increased body size in response to predators has been seen in other studies (Auld 2010, Chivers et al. 2008, Crowl and Covich 1990). It is also consistent with my results in chapter 1 which suggested that the snails in our experimental conditions are not suffering an energetic cost when they respond to predation cues.

The shell mass of the predator exposed snails was slightly lighter than the control snails after controlling for body size (Figure 2.3, Table 2.2). This was in contrast to most studies with mollusks that have found predation cues tend to lead to thicker shells (Appleton and Palmer 1988, Bourdeau 2010) and does not have a clear explanation. One possible explanation is that the predator exposed snails consumed more food than the

control snails and increased their soft tissue mass without increasing their shell mass. Such an explanation would be a reverse version of what Bourdeau (2009) observed in *Nucella* snails with snails having decreased shell mass per unit body mass instead of increased shell mass per unit body mass.

In the F_1 behavioral trials, I compared the model fit of behavioral models containing mass and models containing food treatment in order to assess whether behavior was driven by body condition (mass) or by information about food availability (food treatment). In both the control and predator periods, the models that contained food treatment outperformed the models containing mass, although the difference was more pronounced during the control period (Table 2.4a,b). In both the control and predator periods, snails experiencing food restrictions were less likely to be at or above the waterline, suggesting snails were more willing to spend time at a risky location when they were food restricted. The better performance of the models containing food treatment suggests that snail foraging decisions depend on their perception of food availability in their environment and hunger rather on their body size. This result suggests, at least in snails, perceptions of food availability and hunger may have a larger effect on behavior than asset protection (Clark 1994). This is important because even animals in good body condition may take risks and forage in the presence of predators if they perceive low food in the environment and estimate that a large amount of foraging effort will be needed to obtain food. This result also highlights a difficulty separating phenotypic changes due to state from those caused by informational cues: any stimulus that changes an animal's body condition may also provide information about conditions in the environment. For example, the predictions of the risk allocation hypothesis can be

driven by differences in a prey's perception of the frequency of periods of low predation risk (Lima and Bednekoff 1999) or by changes in their size due to growth (Luttbeg 2017).

In the control period, past exposure to predator cues had no effect on the amount of time snails spent at or above the waterline. However, when the predator cue was present past exposure to predator cues had different effects on F_1 treatment snails, the F_2 maternal offspring of the treatment snails, and the F_2 paternal offspring of the treatment snails. When the predator cue was present past exposure to predator cues caused a reduction in the amount of time that treatment snails and their maternal offspring spent at or above the waterline. This suggests that snails with a history of exposure to predation cues are more tolerant of spending time in a dangerous condition. This result is consistent with our previous finding (Beaty et al. 2016). However, the effect size was smaller in the F_2 maternal offspring of the treatment snails and either absent or greatly reduced in the F_2 paternal offspring of the treatment snails. Overall, the evidence suggests that snails exposed to predator cues reduce the amount of time that they spend in an anti-predator position, but that there is a lot of variation across snails.

Informational models of transgenerational plasticity predict that cues from parents affect their offspring's phenotype when they provide an accurate estimate of the offspring's environment (Leimar et al. 2016, McNamara et al. 2015, Kuijper and Hoyle 2015). Under these models, I expected the maternal and paternal exposure to predators to have similar effects on offspring phenotype, and that parental predator exposure would not interact with parental food treatment. The somatic or state-based hypothesis predicts that phenotypic plasticity, including transgenerational plasticity, depends on environmental stimuli directly changing the phenotype of an animal (Nettle and Bateson

2015). Under this hypothesis, I expected maternal effects to be stronger than paternal effects since mothers are the ones investing resources in eggs. I also expected the effects of parental predator cues to change depending on the food treatment of the parent. The data presented here does not fully support either hypothesis. Contrary to the predictions I made with informational models, the paternal and maternal effects of predation cues were different. For food treatments, there were maternal effects on shell mass, shape, and behavior, but not paternal effects. For predator cue treatments, there were paternal effects on shell shape and behavior, but not maternal effects. As predicted from the somatic hypothesis, maternal food treatment seemed to cause transgenerational plasticity while paternal food treatment did not. The maternal food restriction caused offspring to exhibit less anti-predator behavior, which matches the effect of food restriction in the F_1 snails and suggests food restricted moms invested less in their offspring. However, inconsistent with this hypothesis I did not find evidence that the food restricted mothers produced offspring with less soft tissue mass or shell mass. Paternal predator cue exposure had larger effects on offspring phenotype than maternal exposure to predator cues. It is not clear why this is the case as there is no reason to expect information from paternal cues to be more reliable than those from maternal cues in *Physa acuta*, but perhaps it could due to paternal effects not having a potential somatic effect.

Overall, this experiment found that within generation, predator cues cause an increase in snail mass, a decrease in the proportion of shell mass, and a reduction in anti-predator behavior. Food restrictions decreased total mass and anti-predator behavior but did not affect the reaction norms caused by the predation cues. This suggests that within-generation plasticity is not driven by reduced foraging effort from predator exposed snails

and that it is well explained by the information model of phenotypic plasticity. Trans-generation, this experiment found evidence that maternal food affects behavior and has a small effect on shape, while paternal predator cue exposure reduced anti-predator behavior and generated elongated shell shapes. These results suggest that non-informational mechanisms interact with informational mechanisms to affect transgenerational plasticity, but how is not fully clear.

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Figure 2.1. Experimental design, including sample sizes. For the F_1 snails, C = control cues, P = predator cues, F = Full food, S = Restricted food. Numbers indicate the total number of snails in each treatment. For the F_2 snails, the first two letters of the treatment code indicate their parent's treatments. The third letter indicates the individual treatment of the snails in the group (C = control, P = predator). F_2 sample sizes show the number of individual snails measured with the number of cups they were taken from in parentheses.

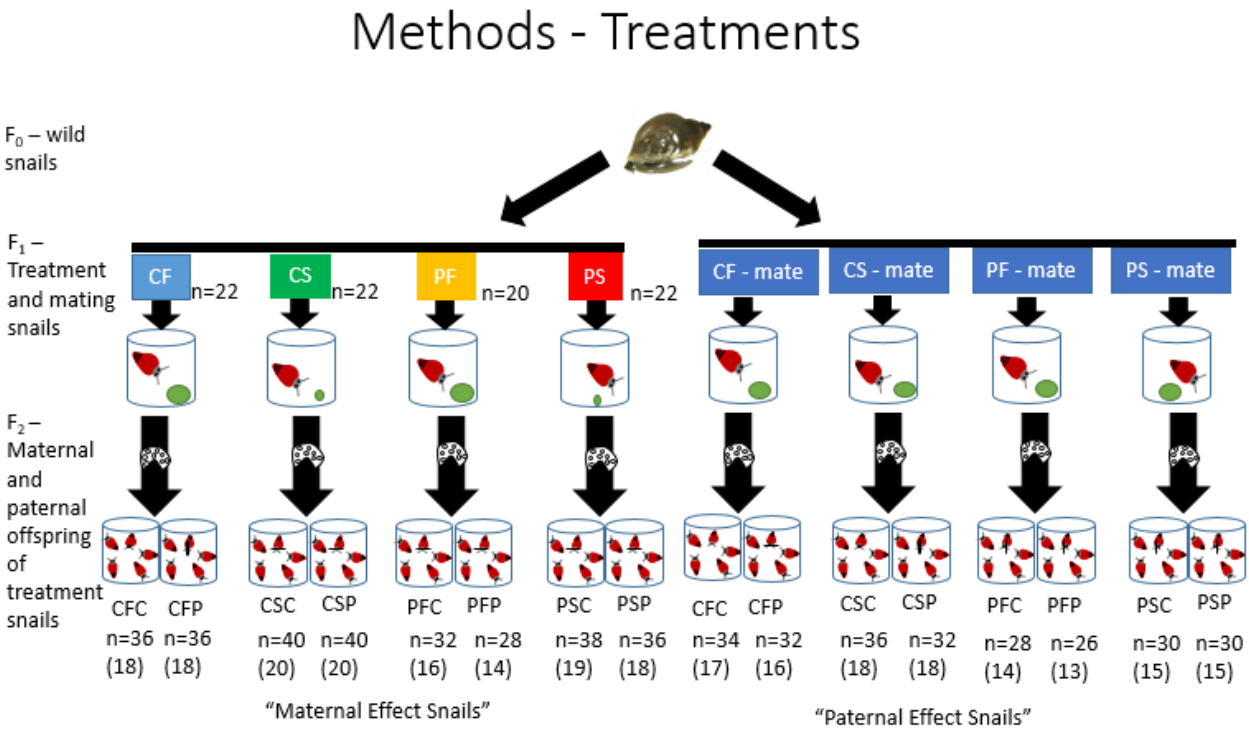


Figure 2.2 Soft Tissue mass of F_1 treatment snails from control full food treatment (CF), control restricted food treatment (CS), predator exposed full food treatment (PF), and predator exposed restricted food treatment (PS). N = 86 snails total from 22 lines, 22 CF, 22 CS, 20PF, and 22 PS. Points are individual snails, the large points are the means, and the error bars are the standard error.

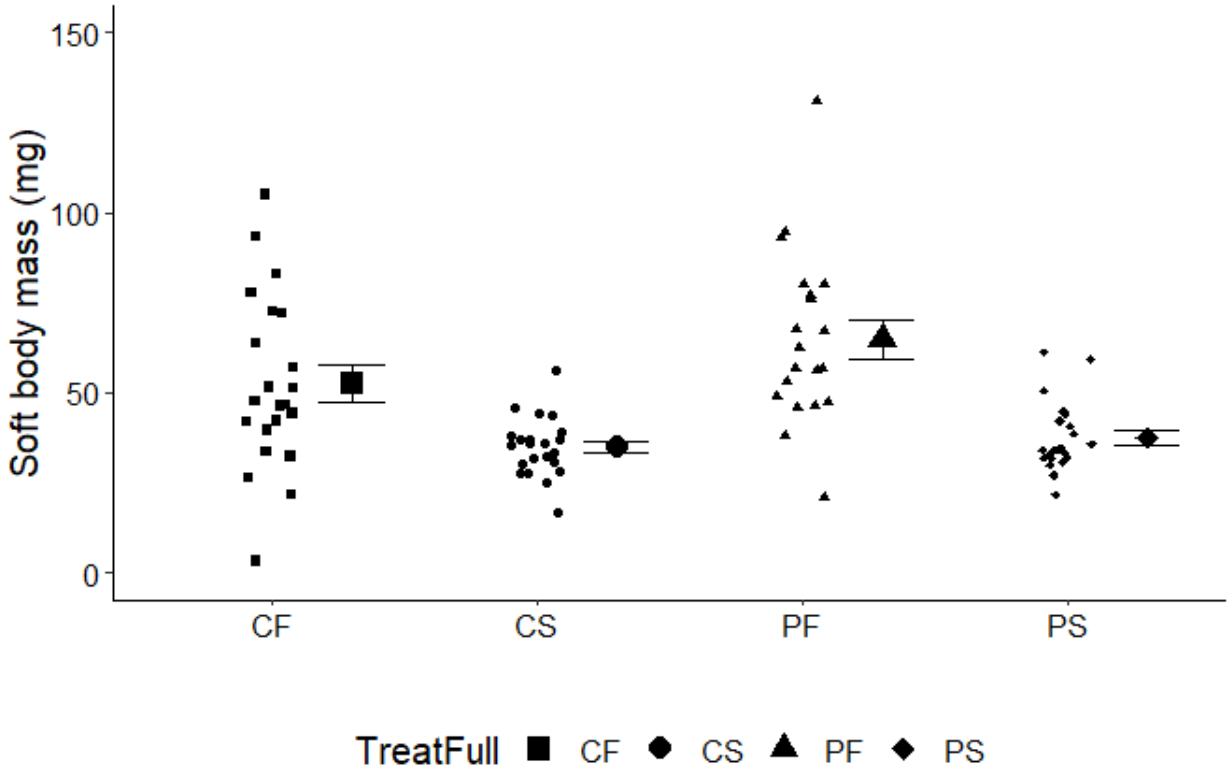
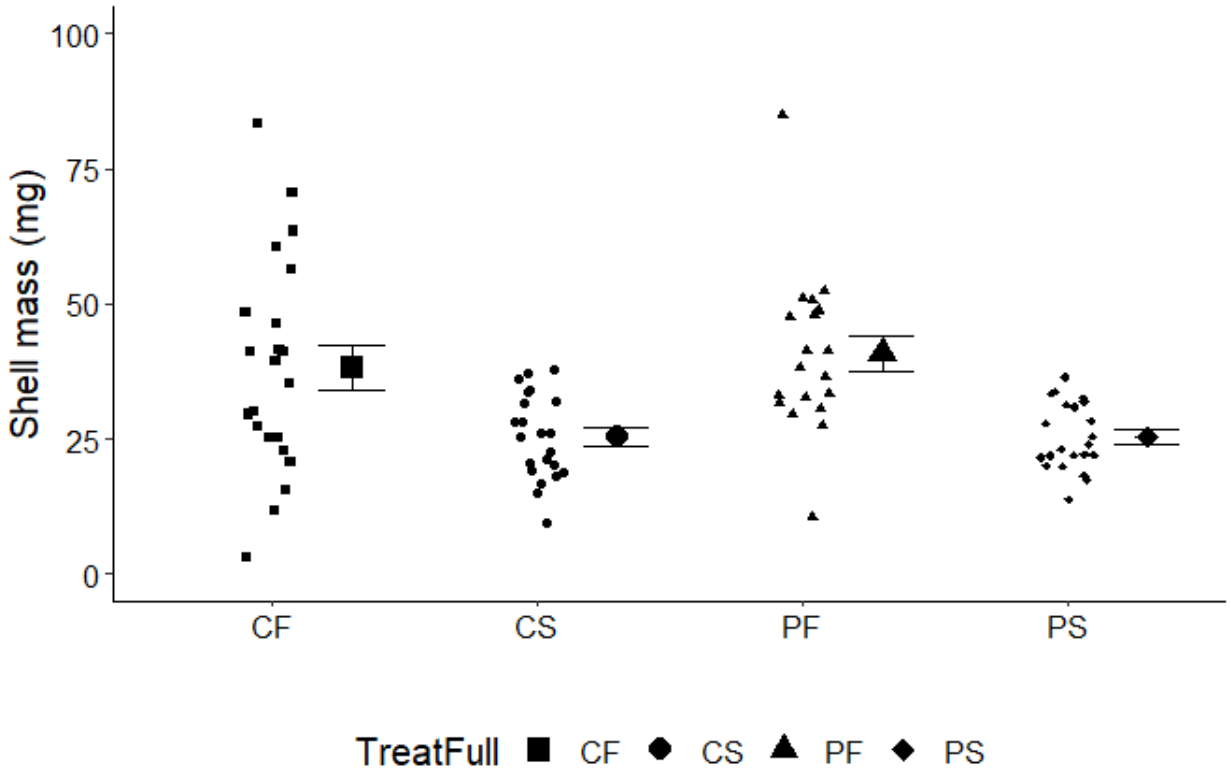


Figure 2.3. A) Shell mass of F_1 treatment snails from control full food treatment (CF), control restricted food treatment (CS), predator exposed full food treatment (PF), and predator exposed restricted food treatment (PS). N = 86 snails total from 22 lines, 22 CF, 22 CS, 20PF, and 22 PS B) Proportion of total mass composed of shell for the same snails. Points are individual snails, the large points are the means, and the error bars are the standard error.

A



B)

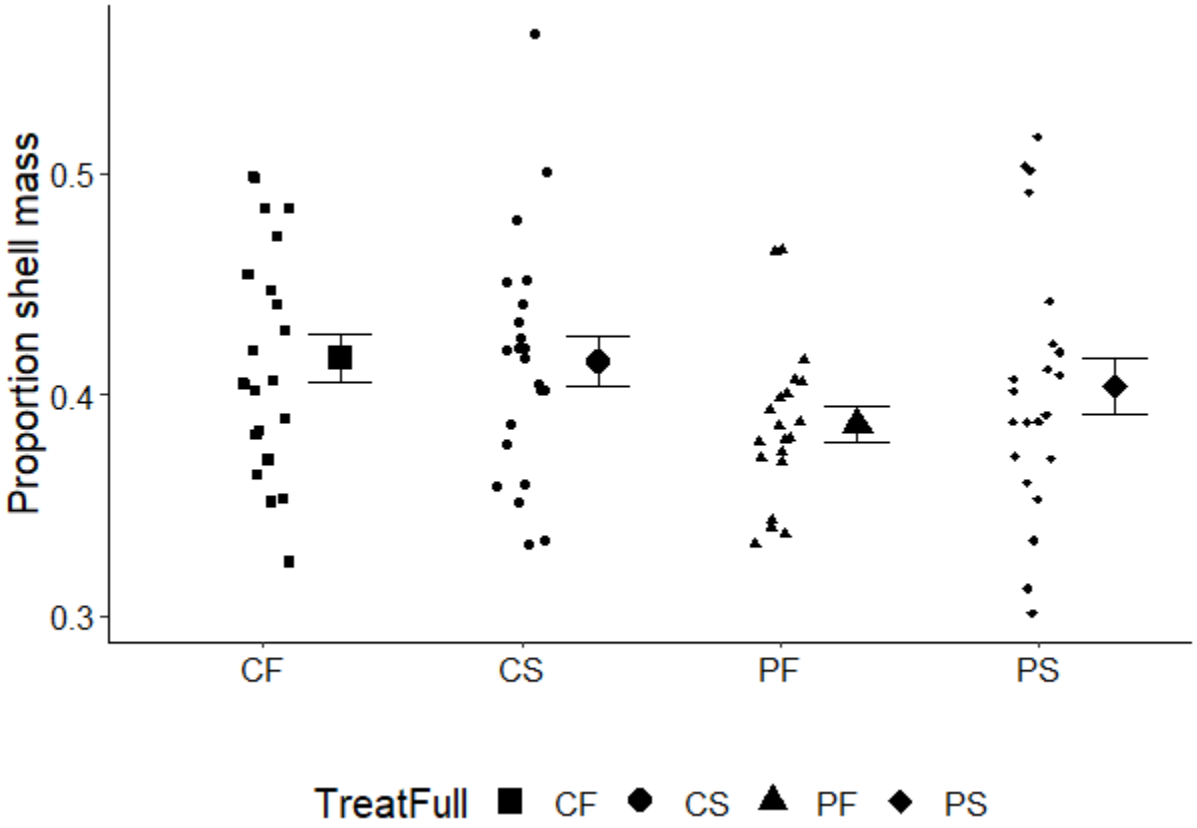
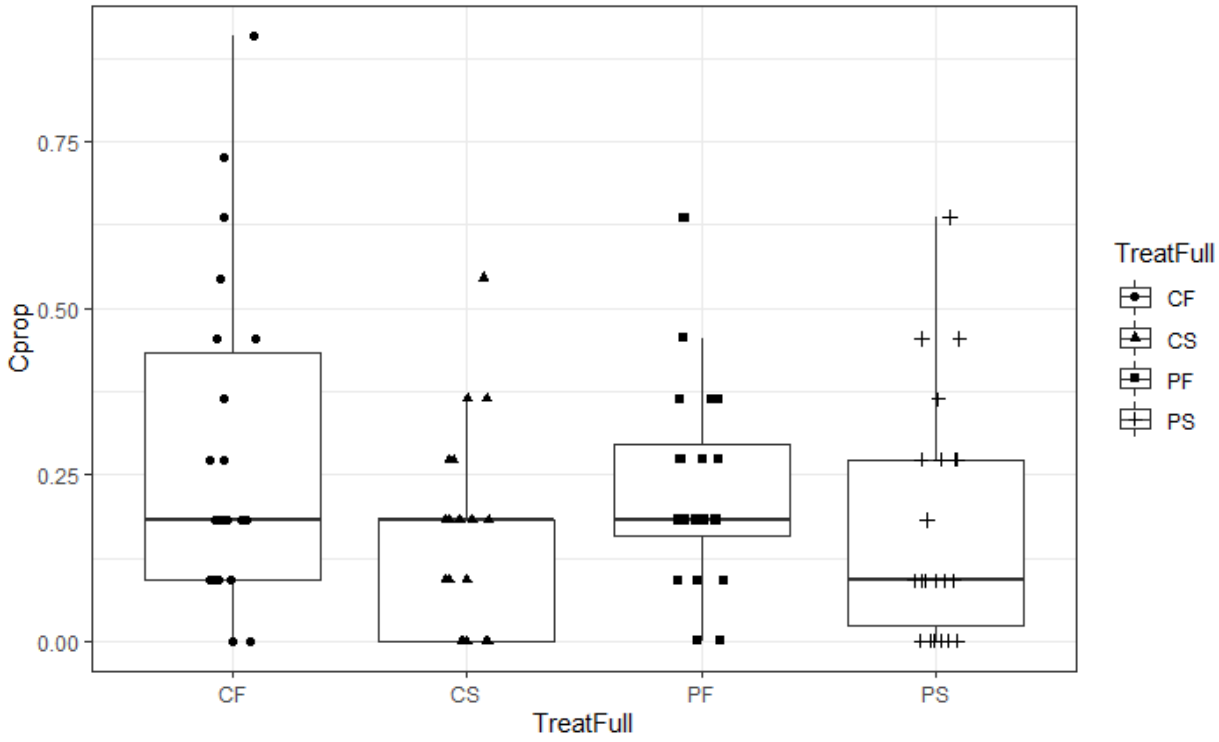

Proportion shell mass
0.5
0.4
0.3
CF
CS
PF
PS
TreatFull
CF
CS
PF
PS

Figure 2.4. A) The proportion of time F_1 snails spent out of the water during the control period. Each dot represents an individual snail. Points are jittered on the x-axis to show the number of snails at each proportion of time out of the water. B) The proportion of time F_1 snails spent out of the water during the predator cue period. Each dot represents an individual snail. Points are jittered on the x-axis to show the number of snails at each proportion of time out of the water.

A)



B)

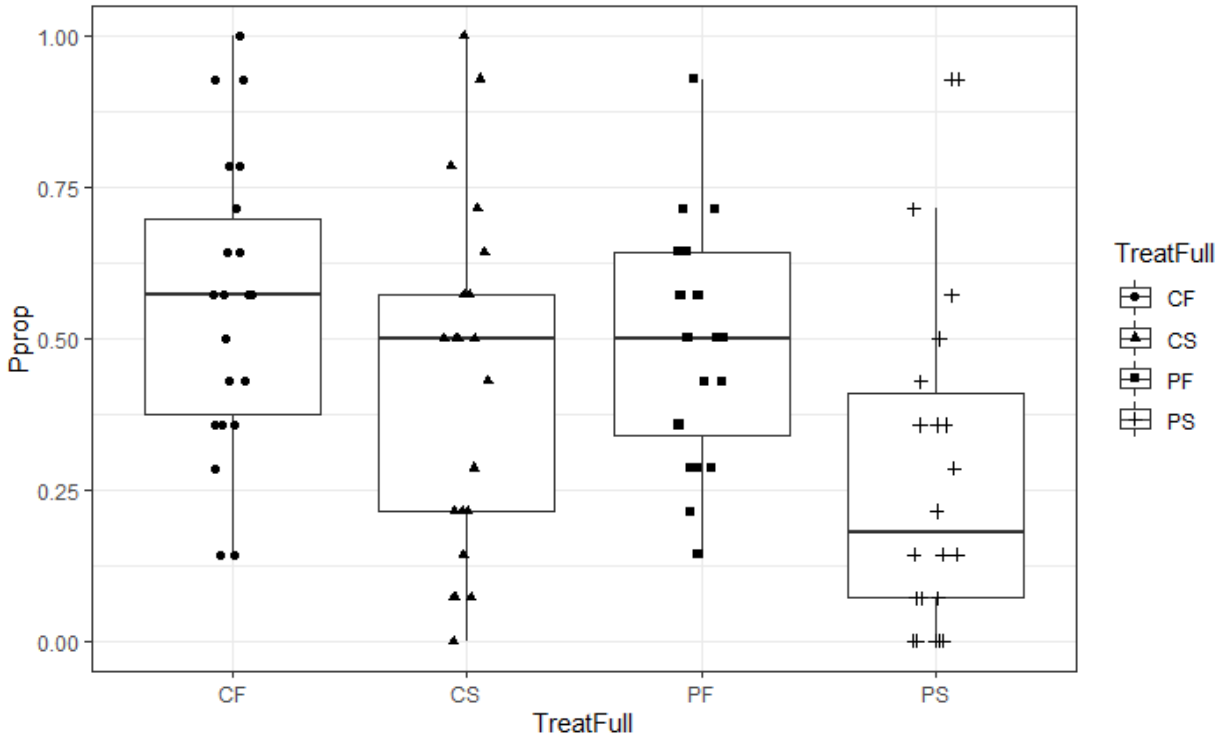
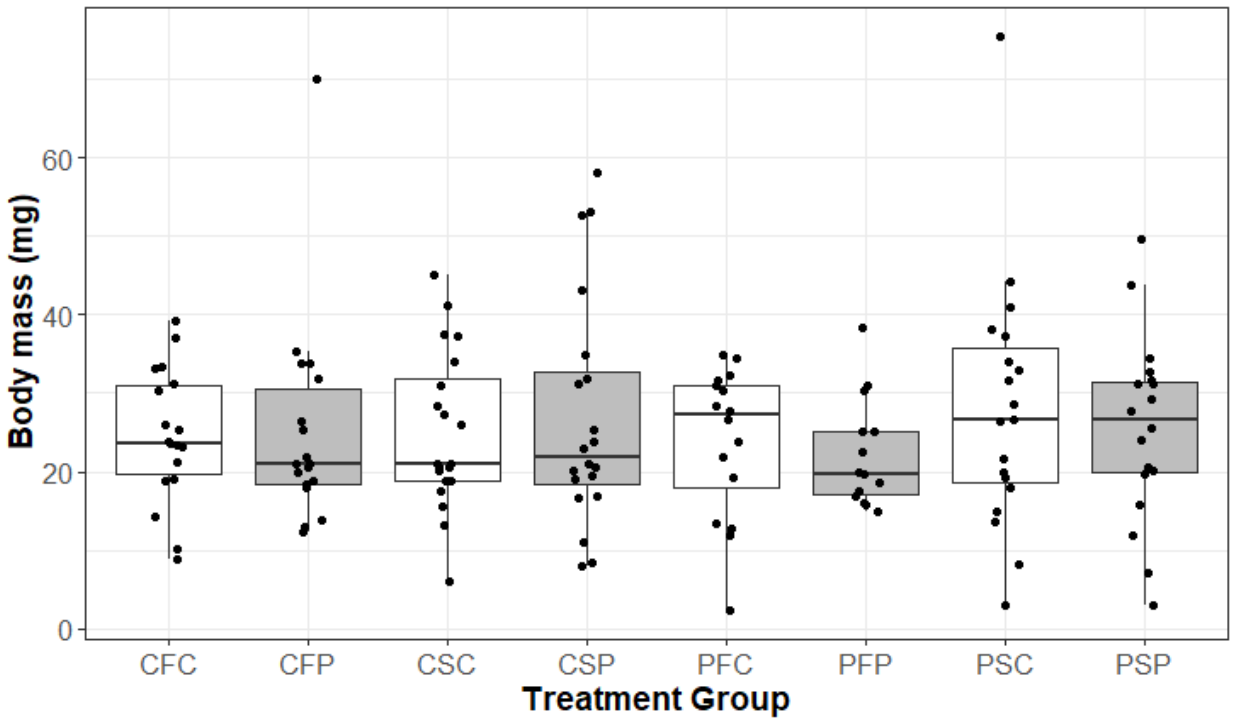

1.00
0.75
0.50
0.25
0.00
Pprop
CF
CS
PF
PS
TreatFull
TreatFull
CF
CS
PF
PS

Figure 2.5. Body mass of the F2 snails who were the maternal offspring of the F_1 treatment snails (A) and the paternal offspring of the F_1 treatment snails (B). Treatments on the x-axis show the parental predator treatment (P = predator, C = Control), the parental food treatment (F = full food, S = food restricted), and the individual treatment (P = predator, C = control). Each point represents the mean mass of offspring from a single cup within the treatment.

A)



B)

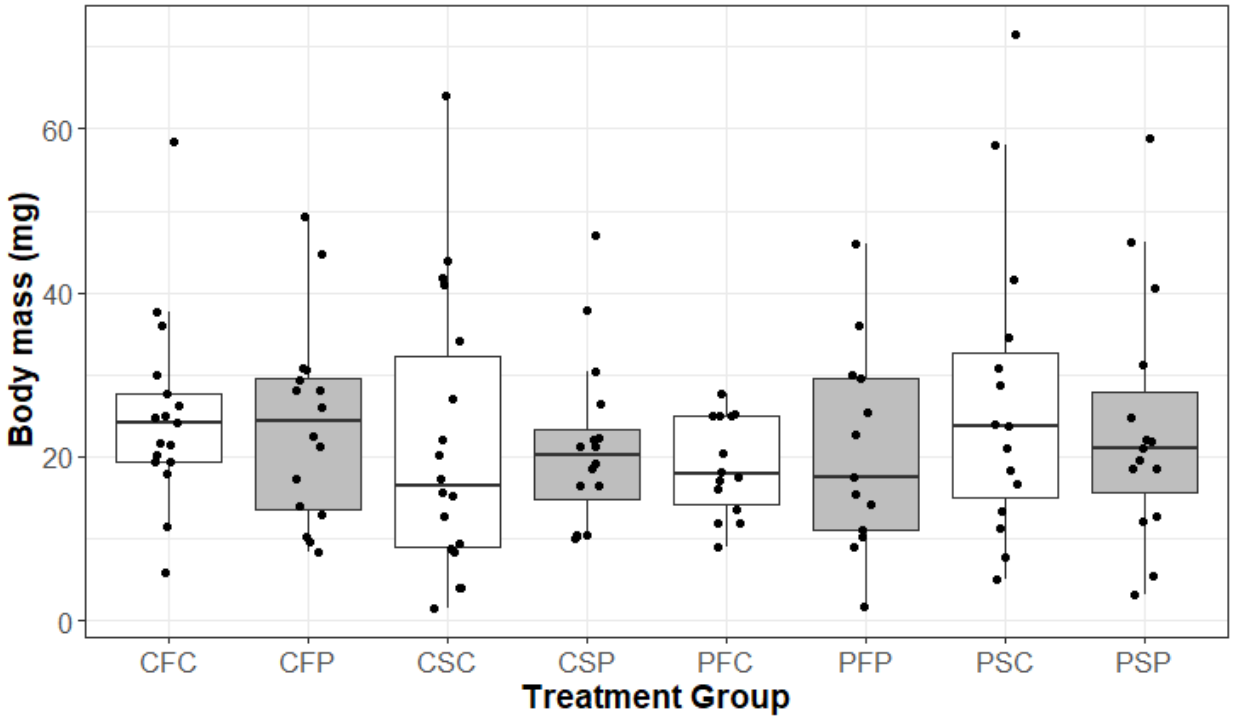
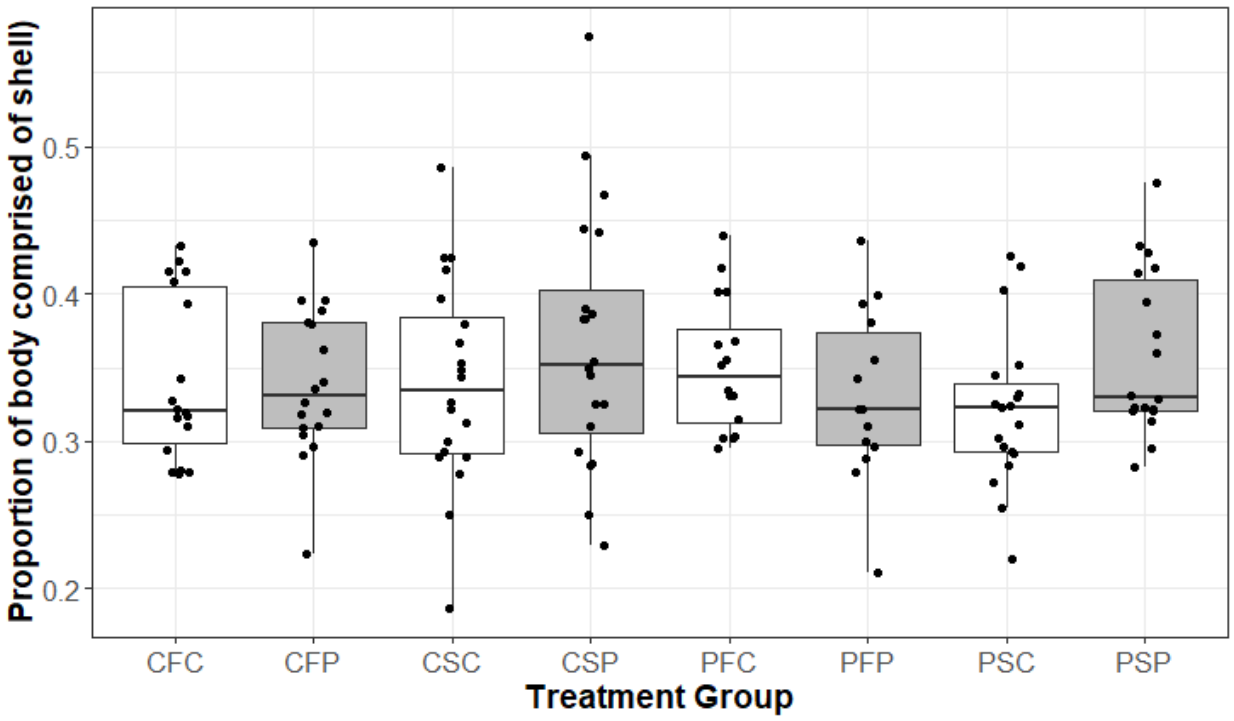

Body mass (mg)
60
40
20
0
CFC
CFP
CSC
CSP
PFC
PFP
PSC
PSP
Treatment Group

Figure 2.6. Proportion of total mass comprised of shell F_2 snails who were the maternal offspring of the F_1 treatment snails (A) and the paternal offspring of the F_1 treatment snails (B). Each point represents the mean proportion shell mass of offspring from a single cup.

A)



B)

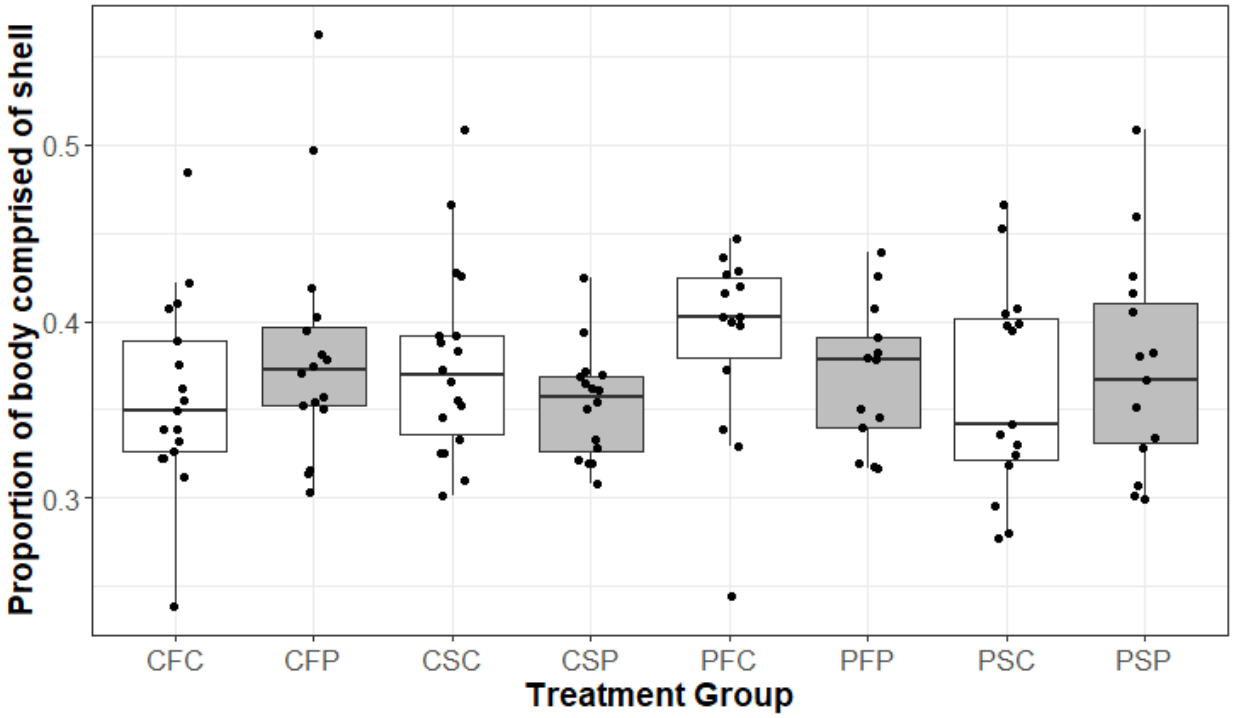
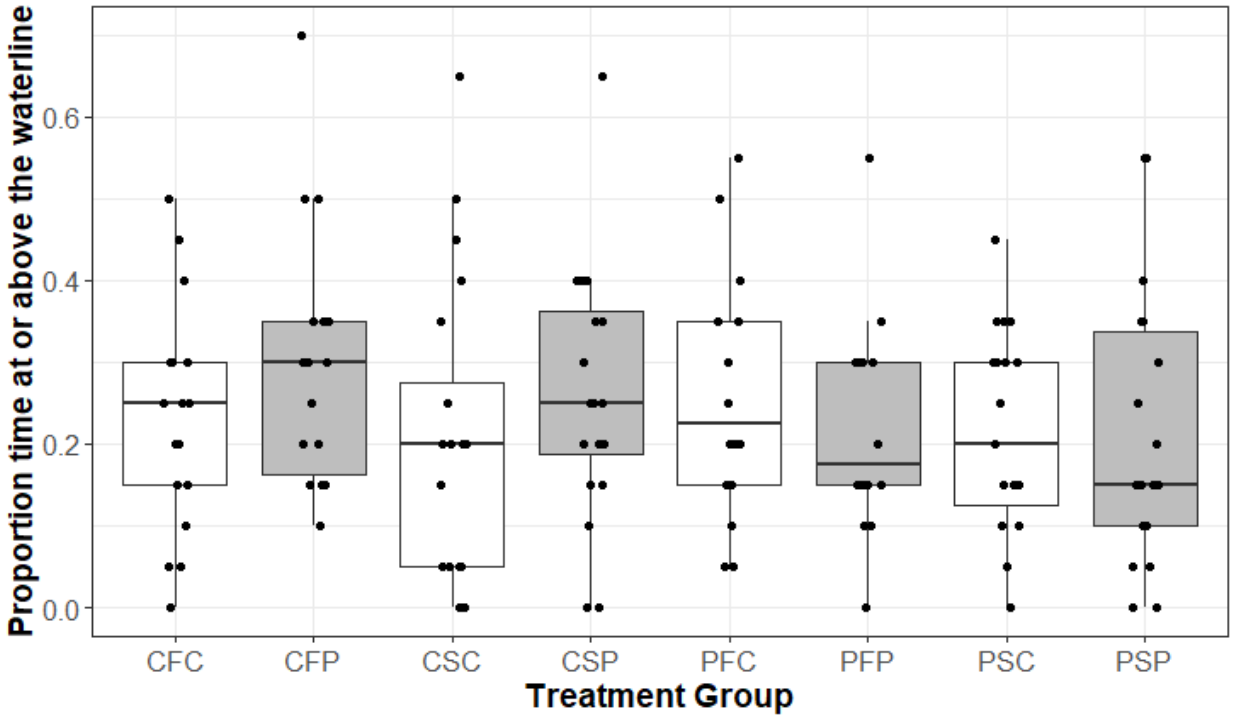

Proportion of body comprised of shell
0.5
0.4
0.3
CFC
CFP
CSC
CSP
PFC
PFP
PSC
PSP
Treatment Group

Figure 2.7. Proportion of time out of the water during the control (A) and predator (B) treatments for the maternal offspring of the F_1 treatment snails. Each point represents the mean proportion of time out of the water for the snails from a single cup.

A)



B)

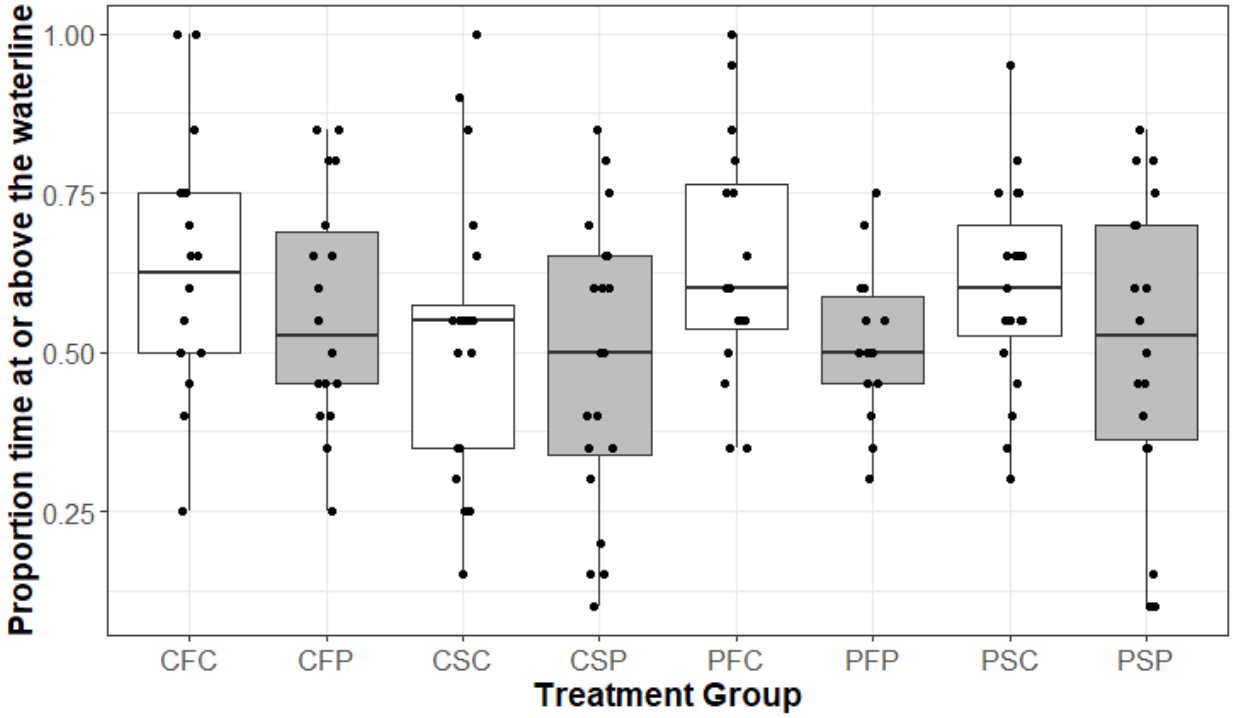

Proportion time at or above the waterline
1.00
0.75
0.50
0.25
CFC
CFP
CSC
CSP
PFC
PFP
PSC
PSP
Treatment Group

Figure 2.8. Shape change across the divergence vector for maternal food and individual predator treatment. Images on the y-axis show thin-plate splines for the 5th percentile divergence vector (DV), the median DV, and the 95th percentile DV. Individual points represent the DV of individual snails. One snail was chosen at random from each maternal effect cup (n = 140).

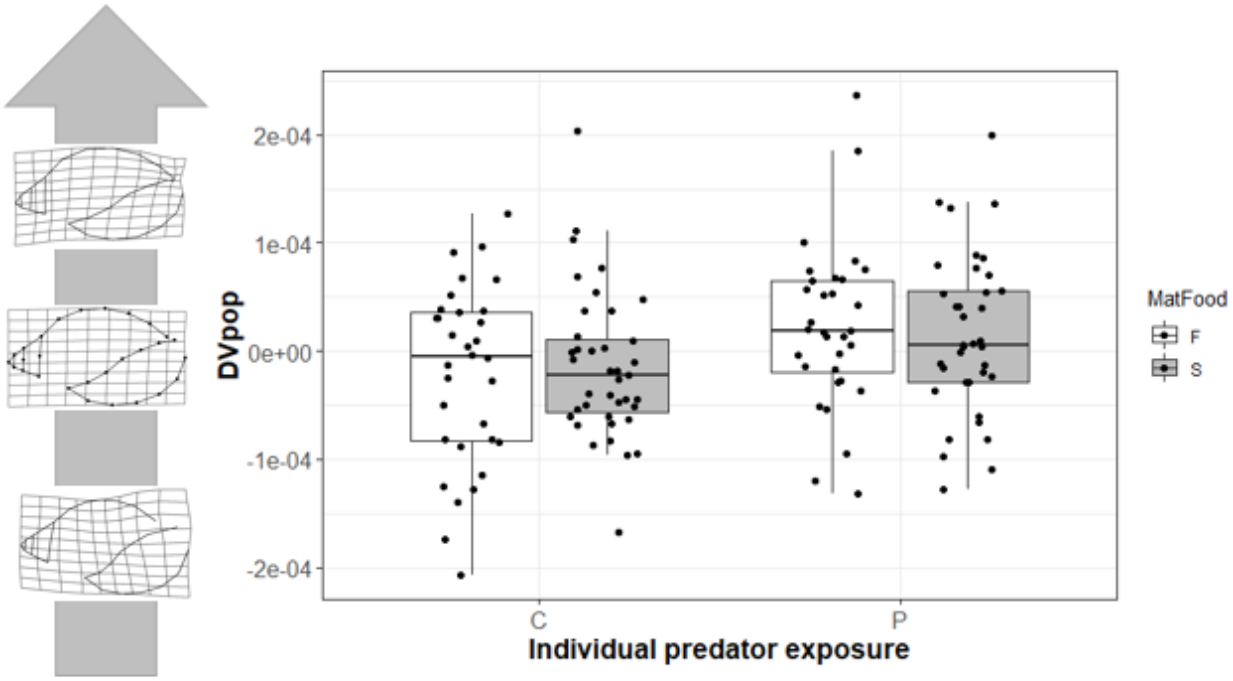
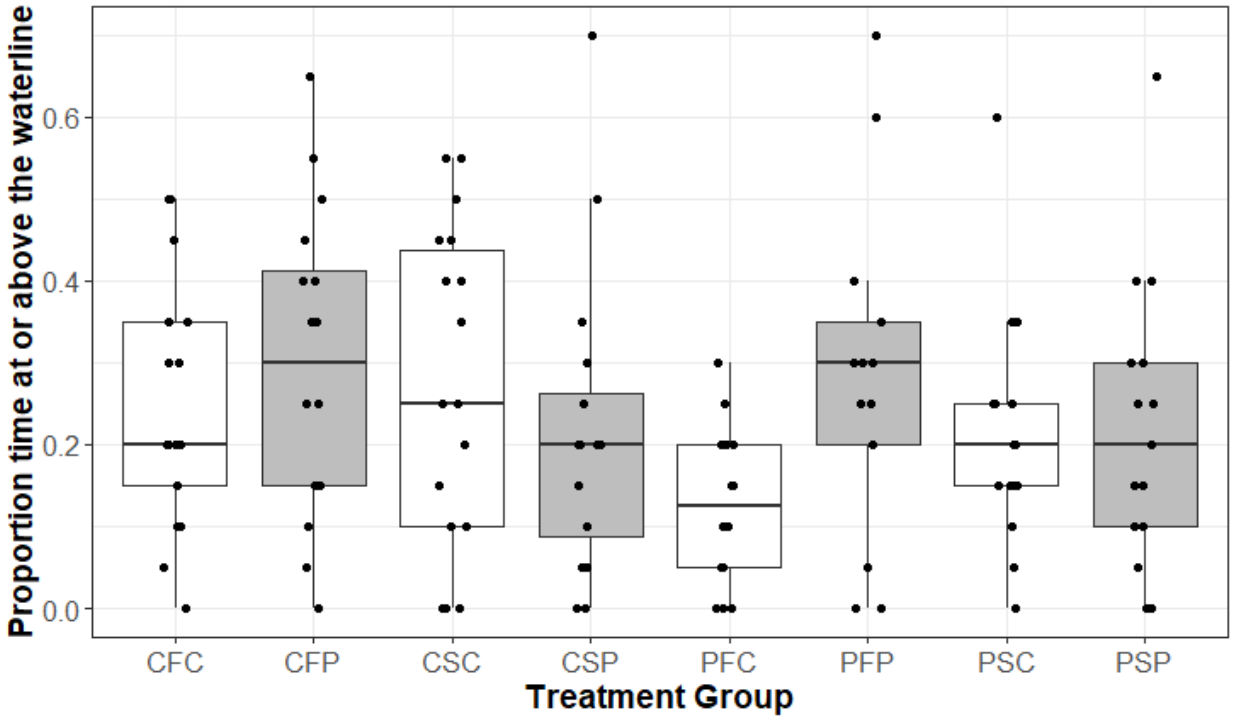


Figure 2.9. Proportion of time out of the water during the control (A) and predator (B) treatments for the paternal offspring of the F_1 treatment snails. Each cup represents the mean proportion of time out of the water for the snails from a single cup.

A)



B)

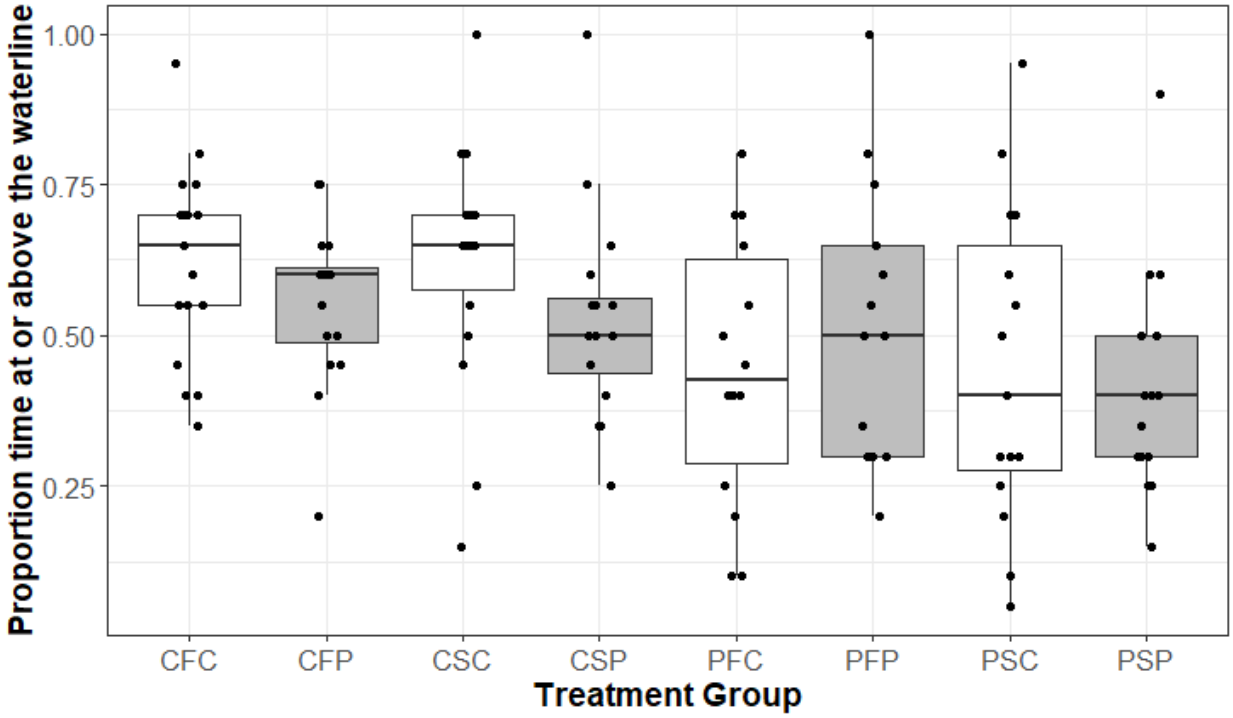


Figure 2.10. Shape change across the divergence vector for paternal predator treatment Images on the y-axis show thin-plate splines for the 5th percentile divergence vector (DV), the median DV, and the 95th percentile DV. Individual points represent the DV of individual snails. One snail was chosen at random from each paternal effect cup (n = 120).

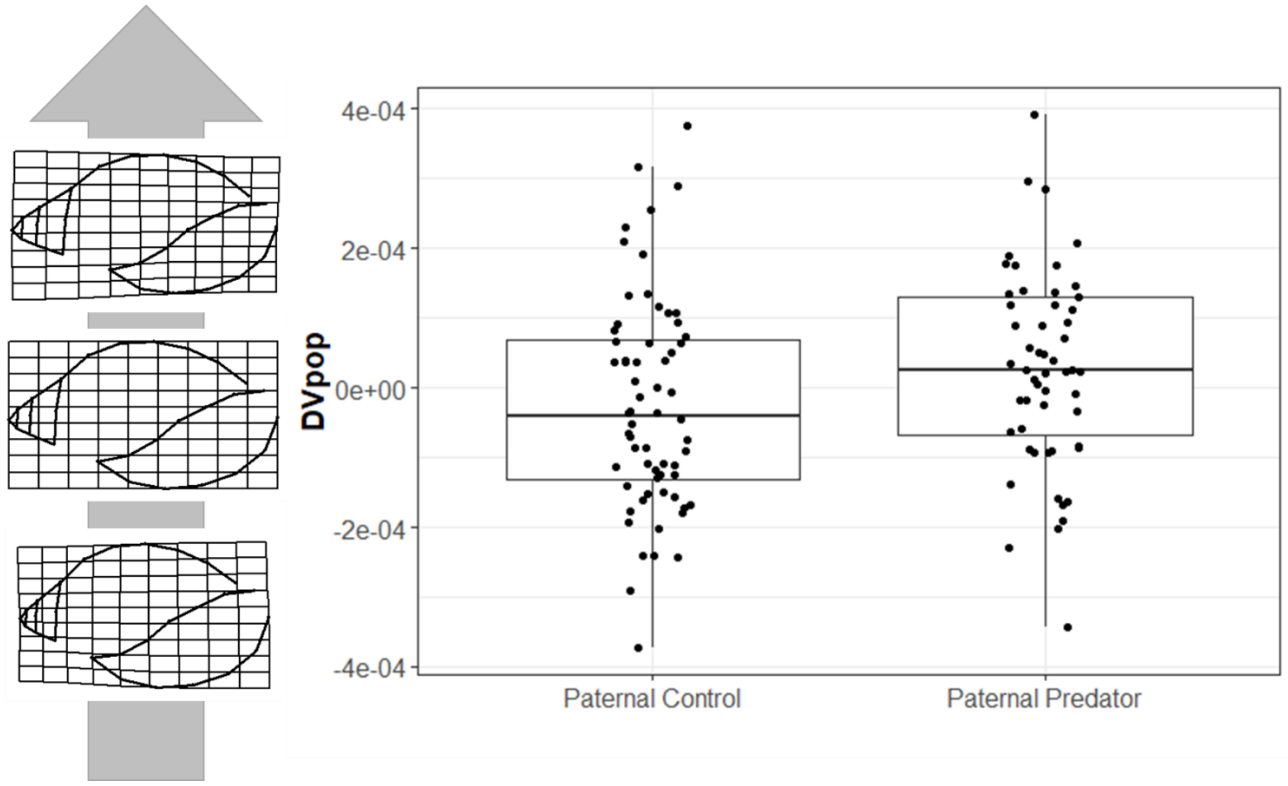


Table 2.1. AICc comparison of models describing the effects of predation treatment, food treatment, and their interaction on the soft tissue mass of F_1 snails. A "*" indicates the interaction between the variables was included in the model.

Model	ΔAICc	DF	Weight
BodyMass ~ Predator + Food + (1|Line)	0	5	0.45
BodyMass ~ Predator * Food + (1|Line)	0.1	6	0.43
BodyMass ~ Food + (1|Line)	2.6	4	0.12
BodyMass ~ Predator + (1|Line)	32.1	4	<0.001
BodyMass ~ 1 + (1|Line)	32.4	3	<0.001

Table 2.2. Models describing the effect of predator treatment, food treatment, and their interaction on the shell mass of the F_1 snails. Body mass was included as a covariate in all of the models to control for it.

Model	ΔAICc	DF
ShellMass ~ Predator + BodyMass	0	5
ShellMass ~ Predator + Food + BodyMass	2.3	6
ShellMass ~ Predator * Food + BodyMass	3.0	7
ShellMass ~ BodyMass	3.5	4
ShellMass ~ Food + BodyMass	5.6	5

Table 2.3. Results of the Mixed MANOVA to test the effects of predator treatment and food treatment on the relative warps (RWs) for F_1 treatment snails. The Index variable is the RW number, and the significance of the predator and food effects was determined by looking at the p-value for the predator/food treatment by Index variable interaction. See Methods for more details.

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
PredTreat	1	244	0.06	0.8030
FoodTreat	1	247	0.16	0.6855
Centroid	1	243	0.05	0.8228
PredTreat*var	10	343	0.43	0.9341
FoodTreat*var	10	343	0.76	0.6703
FoodTre*PredTrea*var	11	340	0.56	0.8598
Centroid*var	10	343	0.73	0.6963

Table 2.4. Models for anti-predator behavior in the F_1 snails. Models are generalized linear mixed models with a beta-binomial distribution

A) During the control period

Model	ΔAIC	DF
C_antipred ~ Food + (1|Line)	0	4
C_antipred ~ Food + Predator + (1|Line)	1.9	5
C_antipred ~ Food * Predator + (1|Line)	3.5	6
C_antipred ~ (1|Line)	4.2	3
C_antipred ~ Mass + (1|Line)	6.0	4
C_antipred ~ Predator + (1|Line)	6.1	4
C_antipred ~ Mass + Predator + (1|Line)	7.9	5
C_antipred ~ Mass * Predator + (1|Line)	9.8	6

B) During the predator period

Model	ΔAIC	DF	Weight
P_antipred ~ Food + Predator + (1|Line)	0	5	0.458
P_antipred ~ Food * Predator + (1|Line)	1.1	6	0.259
P_antipred ~ Food + (1|Line)	2.4	4	0.135
P_antipred ~ Mass + Predator + (1|Line)	3.3	5	0.088
P_antipred ~ Mass * Predator + (1|Line)	5.0	6	0.038
P_antipred ~ Mass + (1|Line)	7.3	4	0.012
P_antipred ~ Predator + (1|Line)	8.1	4	0.008
P_antipred ~ (1|Line)	10.3	3	0.003

Table 2.5. A) Models for soft tissue mass of the maternal F_2 offspring of the treatment snails. Models are linear mixed models with the mean soft tissue mass per cup as the dependent variable. IP is individual predator treatment, MP is maternal predator treatment, and MF is maternal food treatment. B) Models for shell mass of maternal offspring of the treatment snails. Soft tissue mass was included as a covariate in all models.

A)

Model	ΔAICc	DF
BodyMass ~ (1|Line)	0.0	3
BodyMass ~ MF + (1|Line)	0.8	4
BodyMass ~ MP + (1|Line)	2.1	4
BodyMass ~ IP + (1|Line)	2.1	4
BodyMass ~ MF + IP + (1|Line)	2.9	5
BodyMass ~ MF + MP + (1|Line)	2.9	5
BodyMass ~ MP + IP + (1|Line)	4.2	5
BodyMass ~ MP * MF + (1|Line)	4.8	6
BodyMass ~ MP + MF + IP + (1|Line)	5.1	6
BodyMass ~ MF * IP + (1|Line)	5.1	6
BodyMass ~ MP * IP + (1|Line)	5.4	6
BodyMass ~ MF + MP * IP + (1|Line)	6.2	7
BodyMass ~ MF * MP + IP (1|Line)	7.0	7
BodyMass ~ MF * IP + MP (1|Line)	7.3	7
BodyMass ~ MP * IP + MP * MF + (1|Line)	8.2	8
BodyMass ~ MF * IP + MP * IP + (1|Line)	8.4	8
BodyMass ~ MF * IP + MP * MF + (1|Line)	9.3	8
BodyMass ~ MF * IP + MP * IP + MP * MF + (1|Line)	10.5	9

B)

Model	ΔAICc	DF
ShellMass ~ MF * IP + BodyMass + (1|Line)	0	7
ShellMass ~ MF * IP + MP + BodyMass + (1|Line)	0.8	8
ShellMass ~ BodyMass + (1|Line)	0.8	4
ShellMass ~ MP + BodyMass + (1|Line)	1.7	5
ShellMass ~ IP + BodyMass + (1|Line)	2.2	5
ShellMass ~ MF + BodyMass + (1|Line)	2.8	5
ShellMass ~ MF * IP + MP * MF + BodyMass + (1|Line)	3.0	9
ShellMass ~ MF * IP + MP * IP + BodyMass + (1|Line)	3.1	9
ShellMass ~ MP + IP + BodyMass + (1|Line)	3.1	6
ShellMass ~ MF + MP + BodyMass + (1|Line)	3.6	6
ShellMass ~ MF + IP + BodyMass + (1|Line)	4.2	6
ShellMass ~ MF * IP + MP * IP + MP * MF +BodyMass + (1|Line)	5.3	10
ShellMass ~ MP * IP + BodyMass + (1|Line)	5.3	7

ShellMass ~ MP * MF + BodyMass + (1|Line)	5.7	7
ShellMass ~ MF * MP + IP +BodyMass + (1|Line)	7.2	8
ShellMass ~ MF + MP * IP + BodyMass + (1|Line)	7.3	8
ShellMass ~ MP * IP + MP * MF + BodyMass + (1|Line)	9.5	9

Table 2.6. Models for anti-predator behavior of maternal offspring of the treatment snails during the control period (A) and the predator period (B). The dependent variable is the number of checks above and below the waterline for the two snails from each cup.

A)

Model	ΔAICc	DF
C_AP ~ (1|Line)	0	3
C_AP ~ MF + (1|Line)	0.2	4
C_AP ~ MP + (1|Line)	1.2	4
C_AP ~ MF + MP + (1|Line)	1.5	5
C_AP ~ IP + (1|Line)	1.5	4
C_AP ~ MF + IP + (1|Line)	1.7	5
C_AP ~ MP * IP + (1|Line)	1.9	6
C_AP ~ IP * MP + MF + (1|Line)	2.2	7
C_AP ~ MP + IP + (1|Line)	2.7	5
C_AP ~ MF + IP + MP	2.9	6
C_AP ~ MF * MP + (1|Line)	3.5	6
C_AP ~ MF * IP + (1|Line)	3.6	6
C_AP ~ MF * IP + MP * IP + (1|Line)	4.2	8
C_AP ~ MP * MF + MP * IP + (1|Line)	4.2	8
C_AP ~ MF * IP + MP + (1|Line)+ (1|Line)	4.9	7
C_AP ~ MF * MP + IP + (1|Line)	4.9	7
C_AP ~ MF * MP + MF * IP + MP * IP + (1|Line)	6.2	9
C_AP ~ MF * MP + MF * IP + (1|Line)	6.9	8

B)

Model	ΔAICc	DF
P_AP ~ MF + IP + (1|Line)	0	5
P_AP ~ MF * IP + (1|Line)	1.8	6
P_AP ~ IP + (1|Line)	1.8	4
P_AP ~ MF + IP + MP + (1|Line)	1.8	6
P_AP ~ MF * MP + IP + (1|Line)	2.8	7
P_AP ~ MP * IP + MF + (1|Line)	3.4	7
P_AP ~ MF * IP + MP + (1|Line)	3.6	7
P_AP ~ MP + IP + (1|Line)	3.7	5
P_AP ~ MP * MF + MP * IP + (1|Line)	4.4	8
P_AP ~ MF * MP + MF * IP + (1|Line)	4.5	8
P_AP ~ MF + (1|Line)	4.9	4
P_AP ~ MF * IP + MP * IP + (1|Line)	5.2	8
P_AP ~ MP * IP + (1|Line)	5.3	6
P_AP ~ MF * MP + MF * IP + MP * IP + (1|Line)	6.1	9
P_AP ~ (1|Line)	6.6	3
P_AP ~ MF + MP + (1|Line)	6.7	5
P_AP ~ MF * MP + (1|Line)	7.7	6
P_AP ~ MP + (1|Line)	8.4	4

Table 2.7. Results of the Mixed MANOVA to test the effects of predator treatment, maternal predator treatment, and maternal food on the relative warps (RWs) for F_2 maternal effect snails. The Index variable is the RW number, and the significance of the main effects was determined by looking at their interaction with the index variable. See Methods for more details.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
IndvPred	1	575	0.07	0.7937
MatPred	1	575	0.67	0.4127
MatFood	1	576	2.26	0.1329
Centroids	1	575	0.03	0.8732
IndvPred*var	12	688	2.03	0.0196
MatPred*var	12	688	0.76	0.6916
MatFood*var	12	688	1.99	0.0229
Centroids*var	12	688	4.60	<.0001
IndvPred*MatPred*var	13	683	1.19	0.2815

Table 2.8. A)Models for soft tissue mass of paternal offspring of the treatment snails. IP = individual predator treatment, PP = paternal predator treatment, PF = paternal food treatment B) Models for shell mass of paternal offspring of the treatment snails

Model	ΔAICc	DF
BodyMass ~ (1|Line)	0	3
BodyMass ~ IP + (1|Line)	2.0	4
BodyMass ~ PP + (1|Line)	2.1	4
BodyMass ~ PF + (1|Line)	2.1	4
BodyMass ~ PP * PF + (1|Line)	3.1	6
BodyMass ~ PP + IP + (1|Line)	4.2	5
BodyMass ~ PF + IP + (1|Line)	4.2	5
BodyMass ~ PF + PP + (1|Line)	4.3	5
BodyMass ~PF * PP + IP + (1|Line)	5.2	7
BodyMass ~ PF * IP + (1|Line)	6.2	6
BodyMass ~ PP * IP + (1|Line)	6.4	6
BodyMass ~ PF + PP + IP + (1|Line)	6.4	6
BodyMass ~ PF*IP + PP*PF + (1|Line)	7.2	8
BodyMass ~ PP*IP + PP*PF + (1|Line)	7.4	8
BodyMass ~ PF*IP + PP+ (1|Line)	8.4	7
BodyMass ~ PF + PP*IP (1|Line)	8.6	7
BodyMass ~ PF*IP + PP*IP + PP*PF (1|Line)	9.6	9
BodyMass ~ PF*IP + PP*IP (1|Line)	10.7	8

Model	ΔAICc	DF
ShellMass ~ BodyMass + (1|Line)	0	4
ShellMass ~ PF + BodyMass + (1|Line)	1.0	5
ShellMass ~ PP + BodyMass + (1|Line)	1.7	5
ShellMass ~ IP + BodyMass + (1|Line)	2.1	5
ShellMass ~ PF + PP + BodyMass + (1|Line)	2.7	6
ShellMass ~ PF + IP + BodyMass + (1|Line)	3.1	6
ShellMass ~ PP*IP + BodyMass + (1|Line)	3.8	7
ShellMass ~ PP + IP + BodyMass + (1|Line)	3.8	6
ShellMass ~ PF + PP + IP + BodyMass + (1|Line)	4.9	7
ShellMass ~ PP*PF + BodyMass + (1|Line)	4.9	7
ShellMass ~ PF + PP*IP + BodyMass + (1|Line)	4.9	8
ShellMass ~ PF*IP + BodyMass + (1|Line)	5.2	7
ShellMass ~ PF*IP + PP + BodyMass + (1|Line)	7.0	8
ShellMass ~ PF*PP + IP + BodyMass + (1|Line)	7.1	8
ShellMass ~PF * IP + PP*IP + BodyMass + (1|Line)	7.1	9
ShellMass ~ PP*IP + PP*PF + BodyMass + (1|Line)	7.2	9
ShellMass ~ PF * IP + PP*PF + BodyMass + (1|Line)	9.3	9
ShellMass ~ PF*IP + PP*IP + PP*PF + BodyMass + (1|Line)	9.5	10

Table 2.9. Models for anti-predator behavior of paternal offspring of the treatment snails during the control period (A) and the predator period (B)

Model	ΔAICc	DF
C_AP ~ (1|Line)	0	3
C_AP ~ PF * IP + (1|Line)	0.1	6
C_AP ~ IP + (1|Line)	0.1	4
C_AP ~ PP + (1|Line)	0.9	4
C_AP ~ PP + IP + (1|Line)	1.1	5
C_AP ~ PF * IP + (1|Line)	1.2	7
C_AP ~ PF * IP + PP * IP + (1|Line)	1.3	8
C_AP ~ PP * IP + (1|Line)	1.4	6
C_AP ~ PF + (1|Line)	1.7	4
C_AP ~ PF + IP + (1|Line)	1.7	5
C_AP ~ PF * PP + PF * IP + PP * IP + (1|Line)	2.3	9
C_AP ~ PF * PP + PF * IP + (1|Line)	2.3	8
C_AP ~ PF + PP + (1|Line)	2.7	5
C_AP ~ PF + PP + IP + (1|Line)	2.7	6
C_AP ~ IP * PP + PF + (1|Line)	3.0	7
C_AP ~ PF * PP + (1|Line)	3.6	6
C_AP ~ PF * PP + IP + (1|Line)	3.8	7
C_AP ~ PF * PP + PP * IP + (1|Line)	4.2	8

B)

Model	ΔAICc	DF
P_AP ~ PP + (1|Line)	0	4
P_AP ~ PP * IP + (1|Line)	0.9	6
P_AP ~ PP + IP + (1|Line)	1.0	5
P_AP ~ PF + PP + (1|Line)	1.5	5
P_AP ~ IP * PP + PF + (1|Line)	2.2	7
P_AP ~ PF + IP + PP + (1|Line)	2.5	6
P_AP ~ PF * PP + (1|Line)	2.9	6
P_AP ~ PP * PF + PP * IP + (1|Line)	3.6	8
P_AP ~ PF * IP + PP * IP + (1|Line)	3.8	8
P_AP ~ PF * PP + IP + (1|Line)	3.9	7
P_AP ~ PF * IP + PP + (1|Line)	4.2	7
P_AP ~ PF * PP + PF * IP + PP * IP + (1|Line)	5.3	9
P_AP ~ PF * PP + PF * IP + (1|Line)	5.6	8
P_AP ~ (1|Line)	7.4	3
P_AP ~ IP + (1|Line)	8.4	4
P_AP ~ PF + (1|Line)	8.8	4
P_AP ~ PF + IP + (1|Line)	9.8	5
P_AP ~ PF * IP + (1|Line)	11.4	6

Table 2.10. Results of the Mixed MANOVA to test the effects of predator treatment, paternal predator treatment, and paternal food on the relative warps (RWs) for F_2 paternal effect snails. The Index variable is the RW number, and the significance of the main effects was determined by looking at their interaction with the index variable. See Methods for more details.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
IndvPred	1	555	0.33	0.5655
PatPred	1	553	0.78	0.3763
PatFood	1	554	0.61	0.4346
Centroid	1	537	2.35	0.1260
IndvPred*var	14	668	1.44	0.1280
PatPred*var	14	668	1.74	0.0438
PatFood*var	14	668	1.05	0.4025
Centroid*var	14	668	4.76	<.0001
IndvPred*PatPred*var	15	664	0.56	0.9088

CHAPTER III

EFFECTS OF PREDATION RISK ON THE MATING BEHAVIOR OF *PHYSA ACUTA*

Abstract

The presence of predators changes the life history of prey, changes their mating behavior, and induces parental effects in their offspring. Previous research has shown that exposure to predation cues causes delayed reproduction, reduced fecundity, and distinct maternal/paternal effects in *Physa* snails. However, little is known about how exposure to predation cues affects the subsequent mating behavior of snails. A better understanding of this behavior could help determine which parental effects observed in the lab are relevant in the field, and whether reduced mating activity is an explanation for reduced fecundity. To compare the mating behavior of predator exposed and non-exposed snails, I raised snails exposed to either predator cues or control cues and placed them in mating groups of two predator-exposed and two control snails. I recorded the frequency of mountings between the 12 possible male role–female role pairings within each group for ninety minutes, assessed whether the treatments of the two snails in each pair affected the probability the pair mated, and if so for how long they mated. Pairings with a control

snail in the male role and predator-exposed snail in the female role were less likely to occur. Mass did not affect the probability a pair would mount, but pairs with a larger snail in the female role and a smaller snail in the male role remained mounted longer. The mass and treatment of individual snails did not affect the ratio of time steps they spent in the male and female roles, total number of male mountings, or number of shell swings. These results corroborate previous findings that pairings with a smaller snail in the male role are more likely to mate successfully, and suggest that matings of predator and non-predator snails are less likely to occur with implications for parental effect studies.

Introduction

Recent studies across a wide range of taxa have found that when parents are exposed to predators their offspring can experience a range of phenotypic consequences (see review in Tariel and Luquet 2020). This has led to interest in disentangling the effects of exposure to predators through maternal and paternal transgenerational effects (Dias and Ressler 2014, Tariel and Luquet 2020b, Bell et al. 2016, Lehto and Tinghitella 2020). Results from these experiments have shown that parental effects from predation risk vary depending on whether the male, female, or both partners are exposed to predation. Some of these papers (Tariel and Luquet 2020, Chapter 2 of this thesis) take advantage of simultaneous hermaphrodites which an individual can impart a maternal and paternal effect simultaneously. In all of these studies, matings are arranged between individuals of known treatments in order to measure parental effects. However, predation risk affects mating behavior and mate choice (Candolin 1997, DeWitt 1996, Gordon and Briggs 1996), and thus some pairings between predator and non-predator exposed individuals are more likely than others. Thus, fully understanding parental effects

requires understanding how exposure to predation cues affects mate choice when exposed and non-exposed individuals overlap spatially.

Predation risk has widespread effects on mate choice and mating behavior. First, exposure to predators may directly change whether or not individuals engage in mating behavior. For instance, sticklebacks decrease their courtship behavior in the presence of predation risk, a behavior which makes them less attractive to females (Candolin et al. 1997). Animals under predation risk may reduce their activity, which has been shown to lead to a decline in mating in the amphipod *Gamrraus dubeni* (Dunn et al. 2008). Second, predation risk may change which individuals are attractive mates. Female guppies and stickleback fish reduce their preference for brightly colored males in the presence of predators (Godin and Briggs 1996). Likewise, female crickets actually reverse their preference from males with long calls to males with short calls when predators are present (Hedrick and Dill 1993). DeWitt (1996) hypothesized that hermaphroditic freshwater snails exposed to predation risk may be less selective about their mating role because the shell swinging rejection behavior they engage in may attract predators. Predation exposure may also alter reproductive output (Chapter 1, Auld and Houser 2015, Walsh et al. 2015), which could lead to changes in how attractive individuals are as mates.

In addition to deciding who to mate with, simultaneous hermaphrodites face an additional decision about whether to adopt the male or female role (Charnov 1979, Leonard 2005, Anthes 2010. Preference for the male or female role is complex. Preference for the male role may be driven by the lower resource investment required for producing male gametes (Charnov 1979). while preference for the female role may be

driven by the reduced variance in fitness in the female role (Leonard 2005, Leonard and Lukowiak 1991). DeWitt (1996) noted that egg production in pond snails (*P. acuta*) increases with size, and thus the relative fitness of the female role compared to the male role is higher in larger individuals. Thus, he predicted that sex role preference would be size dependent, with smaller individuals having a stronger preference for the male role. Consistent with this, a number of studies have found smaller snails tend to mate in the male role while larger individuals tend to mate in the female role (*Physa*, DeWitt 1996, Wethington and Dillon 1996, Ohbayashi-Hodoki et al. 2004, *Helisoma*: Norton et al. 2008). However, other studies have found no relation between size and sex role preference (Garlick-Ott and Wright 2022, Kone et al. 2007) suggesting other variables besides size influence sex role preference.

The presence of transgenerational plasticity raises the intriguing possibility that mate choice could affect fitness through a non-genetic mechanism. For example, *Daphnia* individuals exposed to fish develop a helmet spire that makes it more difficult for fish to swallow them (Agrawal et al. 1999). Offspring of fish-exposed parents are also more likely to develop the helmet spire. Thus if a Daphnia has detected fish in the environment and developed a helmet spire, it would make sense for them to seek out mates who have also detected fish in the environment and have the helmet spire. In some cases, transgenerational plasticity is maladaptive. For example Goeppner et al. (2020) observed a decrease in crush resistance in sunfish exposed snails. In these cases, it would make sense for individuals to avoid mating with individuals with stress-induced phenotypes.

The pond snail *Physa acuta* is an excellent system to examine the effects of predation exposure on mating behavior. *Physa acuta* readily respond to aquatic predator cues, allowing individuals to be exposed to predation without a risk of their being eaten. They also show within-and transgenerational plasticity in response to predation risk (DeWitt et al. 2000, Luquet and Tariel 2016). There are thus opportunities for within generation plasticity to affect mating behavior and choice, as well as transgenerational plasticity that could affect offspring phenotype. There is also some evidence that certain mating behaviors of *P. acuta* may be affected by predation risk. DeWitt (1996) anecdotally observed shell swinging behavior, in which a snail rejects a potential mating by swinging its shell and dislodging the snail mounting it, are less common in the presence of predators.

Physa fontinalis show aversion to conspecifics outside of mating (Townsend and McCarthy 1980), which suggests they may be trying to avoid detection by predators. Based on this, I expect *P. acuta* raised in an environment with predator cues to show a similar avoidance behavior and mate less frequently than control snails. Predation exposure may have negative effects on reproductive output (Chapter 1) and may reduce hatching success (Auld and Houser 2014). Thus exposure to predation cues may reduce fitness in the female role relative to the male role for predator exposed individuals. Therefore, I predicted that snails exposed to predation cues would mate more frequently in the male role than in the female role. Finally, I predicted that shell swinging would be less common in snails from the predator exposed treatment, based on DeWitt (1996)'s observation this behavior may attract attention from predators.

Methods

Snail collection and husbandry

I raised F_1 snails from F_0 snails using methods similar to those in Chapters 1 and 2. Briefly, about 26 F_0 snails were collected from Sanborn Lake, a freshwater lake in Stillwater OK (Google map coordinates: 36.15498997922014, -97.07793318507633) in June 2020. The snails were housed in individual deli cups and allowed to lay eggs. Seventeen egg masses from these snails were collected, and about 5-10 hatchlings from each egg mass were taken depending on the number of eggs the parent laid. All collected snails were housed in individual 475mL deli cups containing about 300mL of water, and I fed them approximately 0.15mg of Hikari algae wafer twice per week. I exposed one half of the collected hatchlings from each parent to a predator cue, henceforth referred to as the predator treatment. This treatment consisted of a mixture of crushed snails and predator karimones. This predation cue was produced following the same methods in Chapters 1 and 2, and consisted of an equal mixture of water from crayfish feeding on snails and water containing snails crushed by hand. This mixture of cues has been previously shown to induce anti-predator responses in snails (Crowl and Covich 1990). I started cue treatments when the snails were about 2 weeks old and raised the snails to 7 weeks of age. Cues were applied twice per week, and the final cue exposure took place four days before the first mating trials. I kept the snails in isolation prior to mating, so they had no mating experience prior to the start of the mating trials.

Mating trials

I grouped snails into 35 mating groups of 4 snails each, two from the predator treatment and 2 from the control treatment. The groups were created randomly but if I

selected a snail to join the group that matched the line of another member, I reselected to ensure all four snails were unrelated. A timer malfunction affected data from the first 8 groups, and an additional 2 groups had snails die. I removed these groups from the experiment, resulting in 25 groups in the final analysis. I individually marked all four snails in a group with blue, purple, orange, or green nail polish. Colors were randomly assigned relative to snail treatment. I recorded the mass and shell length of each snail.

DeWitt (1991) identified three steps of mating in *P. acuta*. The first, mounting and positioning occurs when the snail in the male role crawls up onto the shell of the snail in the female role. The second, eversion of the penis occurs when the snail in the male role everts its penis and inserts it into the shell of the snail in the female role. The third, intromission, occurs when the snail in the male role has fully inserted its penis and begins sperm transfer to the snail in the female role. A number of rejection behaviors can occur between mounting and intromission, including shell swinging and genital biting (DeWitt 1991, Wethington and Dillon 1996). Because many of the snails mated on the surface of the water or on the side of the cup facing away from me, I was unable to observe whether the penis of the snail in the male role was fully inserted into the mantle of the female snail without disturbing them. I therefore counted mountings rather than intromissions or eversions (Wethington and Dillion 1996). I also counted shell swinging as this was a readily observable behavior.

For each group, I collected data on the amount of time pairs spent mounted and the number of shell swings they engaged in. Pélissié et al. (2014) observed that time spent in intromission was not correlated with paternity. However not all mountings lead to intromission, and DeWitt (1991, 1996) observed that rejection behaviors frequently

lead to dismounts. Thus the amount of time a pair spends in intromission is a good metric for how likely it is intromission occurred. To collect data on mountings and shell swings, I placed the 4 snails in the cup and allowed them 1 minute to acclimate. Every minute for 90 minutes I scanned each cup recording the identities of snails on top of each other. A "mounting" occurred when a snail crawled on top of another snail in a position where it would be able to insert its penis into the other snail. I wanted to avoid counting events where one snail was simply crawling over another snail as a mounting event. Therefore, later when I processed the data, I only counted mounting if the snail in the male role stayed in position for at least 1 full check (60 seconds minimum), thus being on top of the other snail for two consecutive scans. I recorded a number of instances in which two snails attempted to mount a third snail at the same time. When a pair began mounting prior to the arrival of the third snail and continued mounting after the departure of the third snail, I ignored the third snail and counted the number of time steps the pair mounted. When the pair separated prior to the departure of the third snail, I did not count interactions in which two snails were mounted on the third.

The snails were sometimes in chains, with snail A mounting B and B mounting C. I counted these the same as other mounting interactions with one interaction of A in the male role and B in the female role, and one interaction of B in the male role and C in the female role. During each scan, I also counted any observed shell swings, and I recorded whether the swing was being done by the snail in the male role, female role, or unknown if the snail swinging its shell was in the middle of a chain.

Data analysis

I wanted to test how the cue treatment affected the relative amount of time different pairings of snail spent mounted. Each cup contained two control snails (C1 and C2) and two predator snails (P1 and P2). Each snail can potentially mate in both the male and female role. This results in a total of 12 possible mating combinations per cup (C1C2, C2C1, C1P1, P1C1, C1P2, P2C1, C2P1, P1C2, C2P2, P2C2, P1P2, P2P1). For each mating combinations, I recorded the mass difference (female mass – male mass) of the pair and its treatment combination. Treatment combination was the combination of treatments in the pair and was either CC, CP, PC, or PP with the first letter denoting the treatment of the snail in the male role and the second letter denoting the treatment of the snail in the female role. Thus CC indicates a control snail in the male role and a control snail in the female role, CP a control snail in the male role and predator exposed snail in the female role, PC a predator exposed snail in the male role and a control snail in the female role, and PP a predator exposed snail in the male role and a predator exposed snail in the female role.

Next, I calculated the number of checks that I observed a mounting for each of the 12 possible mating combinations in each cup. If the count was 0, this indicated the mating combination never formed. Of the 300 possible mating combinations that could have formed, I observed 156 and the remaining 144 never occurred. I used a two part modelling approach to assess the effects of mass difference and treatment combination on the number of time steps a pair formed. The first part determined if mass treatment or treatment combination affected the probability a pair formed. If the pair formed, the second part determined how many time steps they remained together. This approach, like

a. hurdle model assumes all of the zeroes come from a single source, while a zero-inflated model assumes multiple sources of zeroes (Feng 2021). I considered the two part modelling approach more appropriate than a zero inflated model because the only source of zeros was if the mating combination did not form. Thus there is only a single source of zeros in the data.

For the binomial portion of the model. I used a binomial generalized linear model with a logit link function to estimate how the probability of a mating combination occurring was affected by treatment combination, mass difference, and the interaction between treatment combination and mass difference. Mass difference was standardized in the model by subtracting the mean size difference from each observation and dividing by the standard deviation. I included cup ID as a random intercept to account for non-independence of pairings within the same cup. However, as the variance of the cup ID random effect was close to 0, I dropped the random effect of cup ID and this did not affect parameter estimates for the fixed effects.

For the count portion of the model, I assessed how the number of time steps pairs that came together spent paired was affected by treatments and mass differences. For the 156 observed pairings, I used a zero truncated negative binomial generalized linear model to determine if the sum of the number of time steps combinations of snails were paired was affected by treatment combination, mass difference, or their interaction. Mass difference was again standardized by subtracting the mean from each value and dividing by the standard deviation.

In the next set of analyses, I quantified how the mass and treatment of individual snails affected their mating behavior regardless of who their partner was. I started by

testing whether snail size was affected by treatment. To assess the effect of predator treatment on mass, I fit a linear mixed model with mass as a dependent variable, treatment as an independent variable, and line (the egg mass the snail was from) as a random intercept effect. The resulting model had a singular fit, and since it was not possible to simplify the random effect term further, I fit a linear model without the random intercept and confirmed the coefficients were the same.

Next, I looked at the effects of mass, treatment, and their interaction on the ratio of male mountings to being mounted in the female role, total number of male mountings, and total number of shell swing behaviors. To assess the effects of treatment and mass on the ratio of mountings in the male role to receipts of mountings in the female role, I fit a binomial model. The dependent variable was the number of time steps in the male and female roles for each individual, coded as a "1" for each time step in the male role, and a "0" for each time step in the female role. The independent variables were treatment, mass, and their interaction as independent variables, and line and cup as random intercept effects. After assessment with DHARMa, the resulting model was overdispersed so I refit it as a beta-binomial model.

For the assessment of shell swing behaviors, I fit a Poisson generalized linear mixed model with the count of shell swing behaviors as the dependent variable, and the independent variables being mass, treatment and their interaction, and line and cup as random intercept effects. The model was not overdispersed, but there were deviations between residuals simulated from the model and the observed residuals. Refitting with a negative binomial distribution did not solve the problem, however refitting with a modified version of the negative binomial, "nbinom1" in glmmTMB, solved the problem.

Finally, I analyzed the number of time steps the snail spent mounted on another snail in the male role. I chose to analyze number of time steps mounting in the male role rather than total number of time steps paired because snails solicit matings in the male role (DeWitt 1991). Thus the number of time steps the snail spent mounting in the male role is a good indicator of how frequently they sought out mating interactions. All analyses were performed using the glmmTMB package in R version 4.0.3 I used the package DHARMa (Hartig 2017) to check residuals and ensure they met distributional assumptions.

Results

Effects of size difference and treatment combination on the amount of time a pair spent mating

Pairings containing a snail exposed to predator cues were less likely to occur at least once than pairings of two control snails, but only the CP (control in male role, predator exposed in female role) pairing was significantly less likely than the CC (control in both roles) pairing (Figure 3.1). The PC treatment was borderline significantly less likely to occur than the CC group (Figure 3.1). There were no statistically significant effect of mass or the interaction of mass and treatment on the probability a pairing formed.

Treatment combination did not affect the number of time steps a mounting occurred if the pair mated (Figure 3.2). Size difference was positively correlated with the number of time steps the pair was mounted such that pairs with a larger female than male

were together for more time steps than pairs that had a smaller or negative size difference (Figure 2).

Effects of treatment on mass

Snails that had been exposed to predator cues were heavier than control snails (Figure 3.3, linear model $\beta = 23.04$mg, standard error = 5.48mg, df = 98, $p<0.001$). There was however sufficient overlap in masses between treatments to assess how both mass and treatment affected ratio of mounting as a male to being mounted as a female, number of mountings in the male role, and shell swings.

Effects of individual treatment and mass on mating behavior

Neither snail treatment nor mass affected the proportion of time the snails spent mating in the male vs female role (Figure 3.4), the number of shell swings the snails performed (Figure 3.5), nor the total number of male mountings the snails performed (Figure 3.6).

Discussion

In this experiment, I observed mountings and shell swinging behaviors of predator and control cue exposed snails. I predicted that exposure to predator cues would make the perceived costs of mating (Townsend and McCarthy 1980) and shell swinging behavior (DeWitt et al. 1996) higher. Neither of these predictions were met. I found no difference in the number of time steps pairs spent together based on the treatment combination of the pair (Figure 3.2), nor was there any evidence that predator exposed snails engaged in

fewer mountings or shell swings than control snails (Figure 3.5,3. 6). There was also no evidence that snails in the predator treatment adopted the female role less frequently than the male role (Figure 3.4).

However, there was evidence that control snails may avoid predator exposed snails when seeking out mates. CP pairings occurred significantly less frequently than CC pairings (Figure 3.1) and snails solicit matings by mounting in the male role (DeWitt 1991). Thus even though the total number of mountings did not differ between the control and predator treatment (Figure 3.6) it does appear that control snails preferentially mounted other control snails rather than predator exposed snails (Figure 3.1). Predator exposed snails have a sharper decline in hatching success of their eggs as they age (Auld and Houser 2014), they delay reproduction (Crowl and Covich 1990, Auld et al. 2010, Auld and Relyea 2008, Chapter 1), and they have reduced egg production (Chapter 1). It thus it might be adaptive for control snails to prefer mounting control snails over predator-exposed snails. Less clear is how control snails distinguish predator and non-predator exposed snails without mounting them. Snails pick up information from each other's slime trails (Kirsch in press) and it is possible they are detecting cues in the trails of predator exposed snails, but this would require further testing. There was no evidence that treatment combination affected the amount of time pairs of snails spent together (Figure 3.3), which suggests whatever effect predator cue has may be limited to the pre-mounting stage.

My results somewhat support previous findings that size plays a role in how long snails mate. Consistent with DeWitt (1996), Wethington and Dillon (1996), and Ohbayashi-Hodoki et al. (2004), I found that pairs with a larger female and smaller male

spent more time together than pairs with a smaller female and larger male, although the trend did not reach statistical significance (Figure 3.2). However, there was no evidence that the mass of an individual alone predicted the number of time steps that it spent in the male or female role (Figure 3.4). Thus, my results suggest that it is the relative difference in the masses of snails in a pair rather than individual preferences for the male or female role based on size that predicts how long a pairing will stay together. Interestingly in a similar experiment assessing how temperature affects sex role in *P. acuta*, Garlick-Ott and Wright (2022) failed to find evidence of an effect of size difference within a pair on the duration of copulation. They suggest that their site may have a low density of snails and thus the snails are less selective about mates. Sanborn Lake has a high density of *P. acuta*, and moreover snails appear clumped in areas of the lake with shallow water and fewer fish. Thus, our site resembles that of DeWitt (1996) with a high population of snails more than that of Garlick-Ott and Wright (2022). More work will need to be done to determine how population density affects selectivity of mating in *P. acuta*.

Snail life history traits are affected by both predation cues and mate availability (Auld and Relyea 2008). One of the goals of this experiment was to determine if exposure to predation cues could affect life history traits by reducing the probability that snails mate. My results suggest that this is not the case, as snails exposed to predator cues mated with the same frequency as control snails, and spent the same amount of time in the male and female roles (Figure 3.5, 3.6). Another goal was to determine whether certain combinations of predator and control snails were more or less likely to occur to assist in the interpretation of transgenerational studies (Tariel et al. 2020, Goeppner et al. in revision, chapter 2). My results suggest that CP pairings are less likely to occur than

PC and PP pairings, and thus offspring are more likely to be exposed to predator cues through the father or both parents than the mother alone. Tariel et al. (2020) found that exposing the mother to predator cues a single time led to decreased escape behavior in the offspring, while the same exposure of the father had no effect. The fact that CP matings occur less frequently could suggest that the maternal effect is less common than would be expected by random mating when predator exposed and non-exposed snails overlap. This would lead to fewer offspring suffering the decreased escape speed phenotype in an environment with predators.

Overall, this experiment found evidence that non-exposed snails may be less likely to form pairings with predator exposed snails, and that relative mass differences but not predator treatment combinations affected the duration of mating. Only properties of the pair (treatment combination and size difference) predicted whether pairings mounted and the duration of the mating. Individual properties of the snails, such as treatment and size, failed to predict the duration of mounting other snails, the ratio of time in the male vs female role, and shell swings. The reduction of CP pairings, and of differential pairings of predator and non-predator exposed individuals in general, could help make sense of experiments finding differential maternal and paternal effects of predation.

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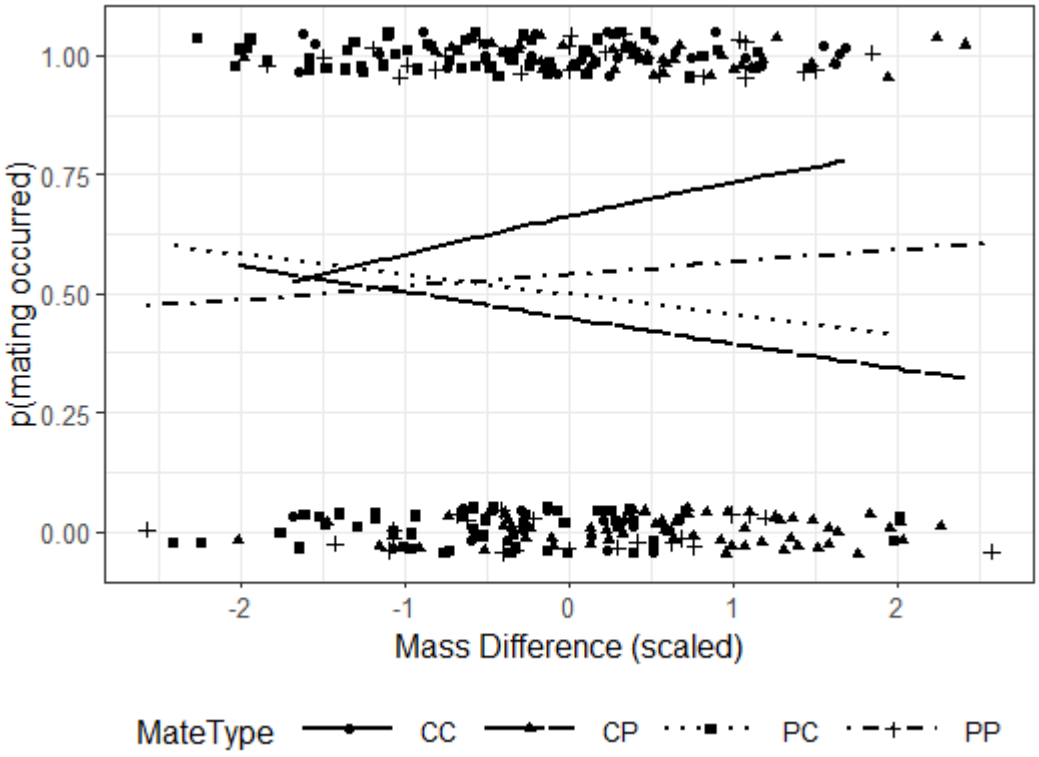
Figure 3.1. Effects of mass difference and treatment combination on the probability of a pair mating at least once during a trial. A) Shows the relation between mass difference (rescaled and standardized) and the probability of mating for the four possible combinations of treatments in a pair. Positive values of mass difference indicate the snail in the female role is larger than the snail in the male role, and negative numbers indicate the snail in the male role is larger. B) A dotplot showing effect sizes in confidence intervals for the fixed effects of the full model. Effect sizes of mate types are shown relative to the CC (control in both male and female role) treatment combination. Mass difference is rescaled and standardized. The dotted line represents an effect size of 0.

A)



B)

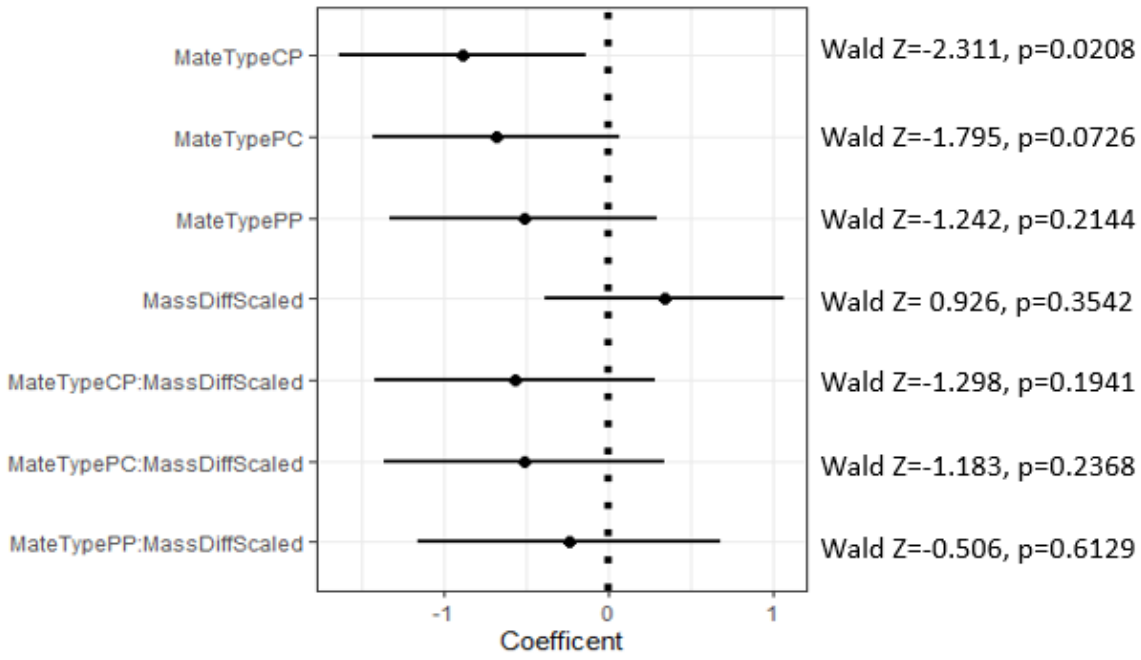
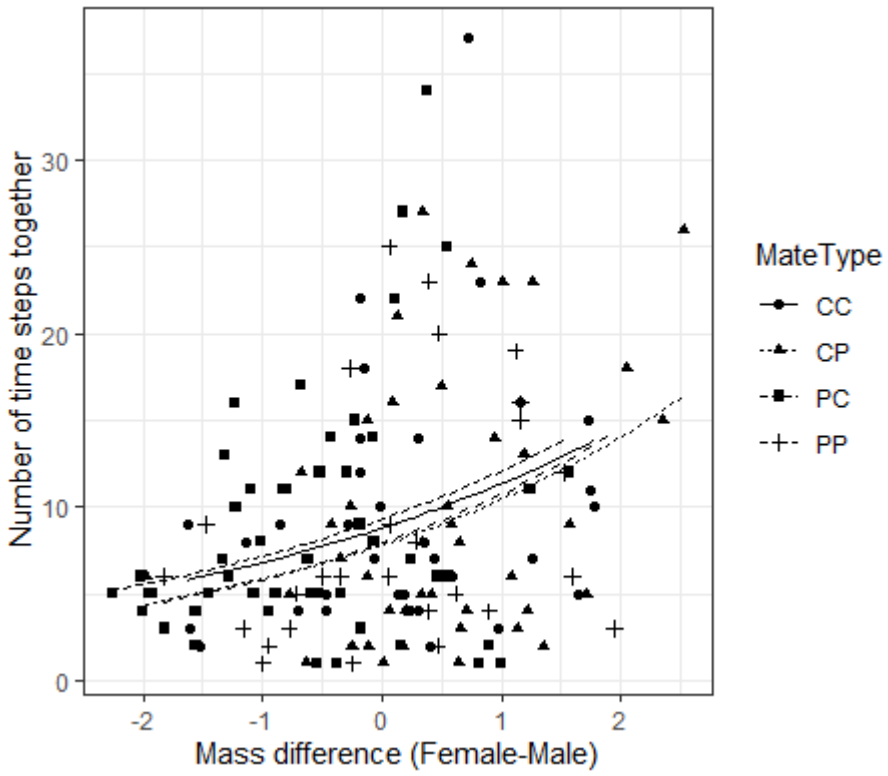

MateTypeCP
MateTypePC
MateTypePP
MassDiffScaled
MateTypeCP:MassDiffScaled
MateTypePC:MassDiffScaled
MateTypePP:MassDiffScaled
Wald Z=-2.311, p=0.0208
Wald Z=-1.795, p=0.0726
Wald Z=-1.242, p=0.2144
Wald Z= 0.926, p=0.3542
Wald Z=-1.298, p=0.1941
Wald Z=-1.183, p=0.2368
Wald Z=-0.506, p=0.6129
-1
0
1
Coefficent

Figure 3.2. Effect of mass and pair treatment on the number of time steps a pair mated if they did mate. A) Shows relation between mass difference in a pair and the number of time steps spent together for pairs in the four treatment combinations. Positive values of mass difference indicate the snail in the female role is heavier than the snail in the male role, 0 is no difference, and negative numbers indicate the snail in the male role is heavier. B) A dotplot showing the effect sizes of treatment combination and mass difference. The effects of treatment are shown in comparison to the CC treatment.

A)



B)

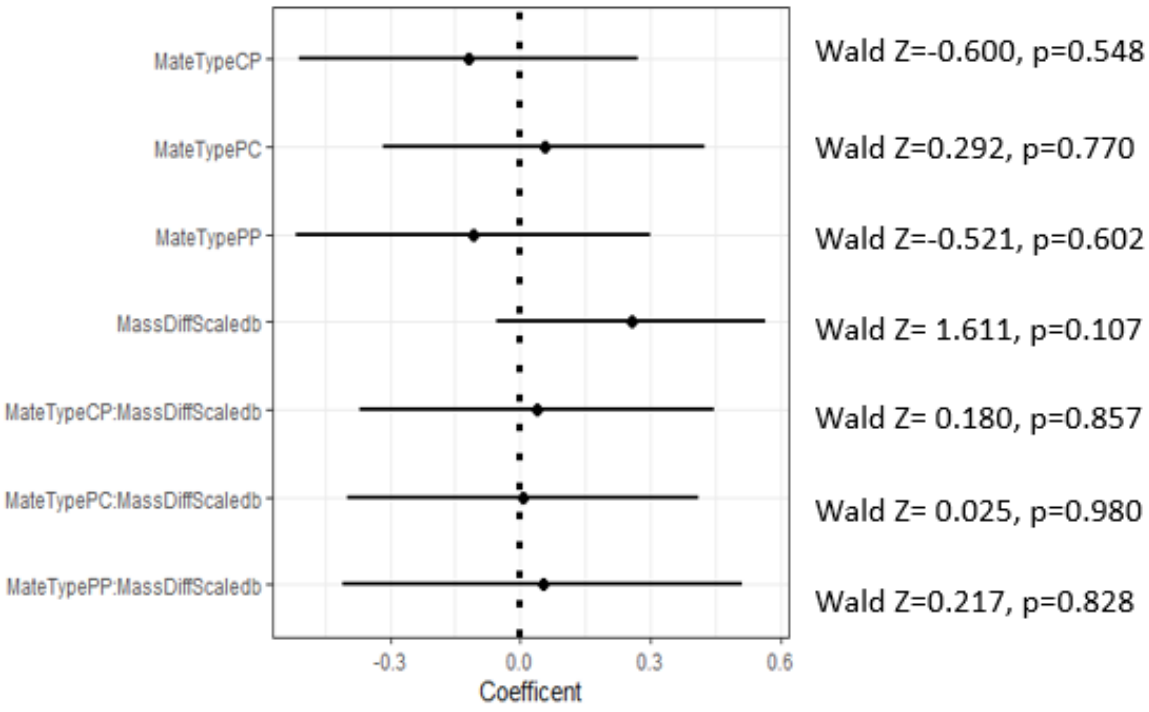

MateTypeCP
MateTypePC
MateTypePP
MassDiffScaledb
MateTypeCP:MassDiffScaledb
MateTypePC:MassDiffScaledb
MateTypePP:MassDiffScaledb
-0.3
0.0
0.3
0.6
Coefficent
Wald Z=-0.600, p=0.548
Wald Z=0.292, p=0.770
Wald Z=-0.521, p=0.602
Wald Z= 1.611, p=0.107
Wald Z= 0.180, p=0.857
Wald Z= 0.025, p=0.980
Wald Z=0.217, p=0.828

Figure 3.3. The effect of treatment on mass (mg).

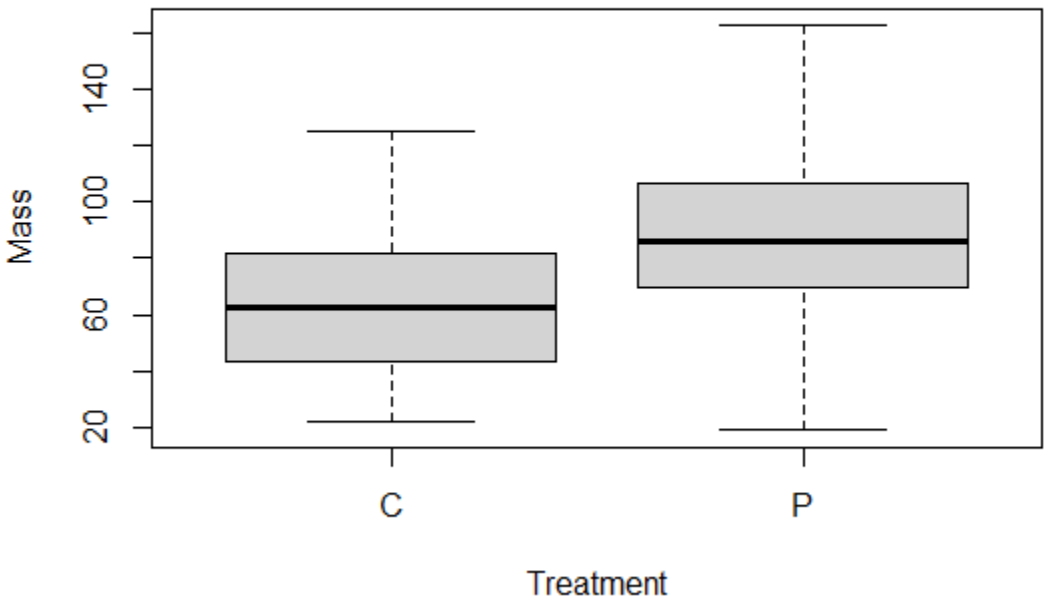
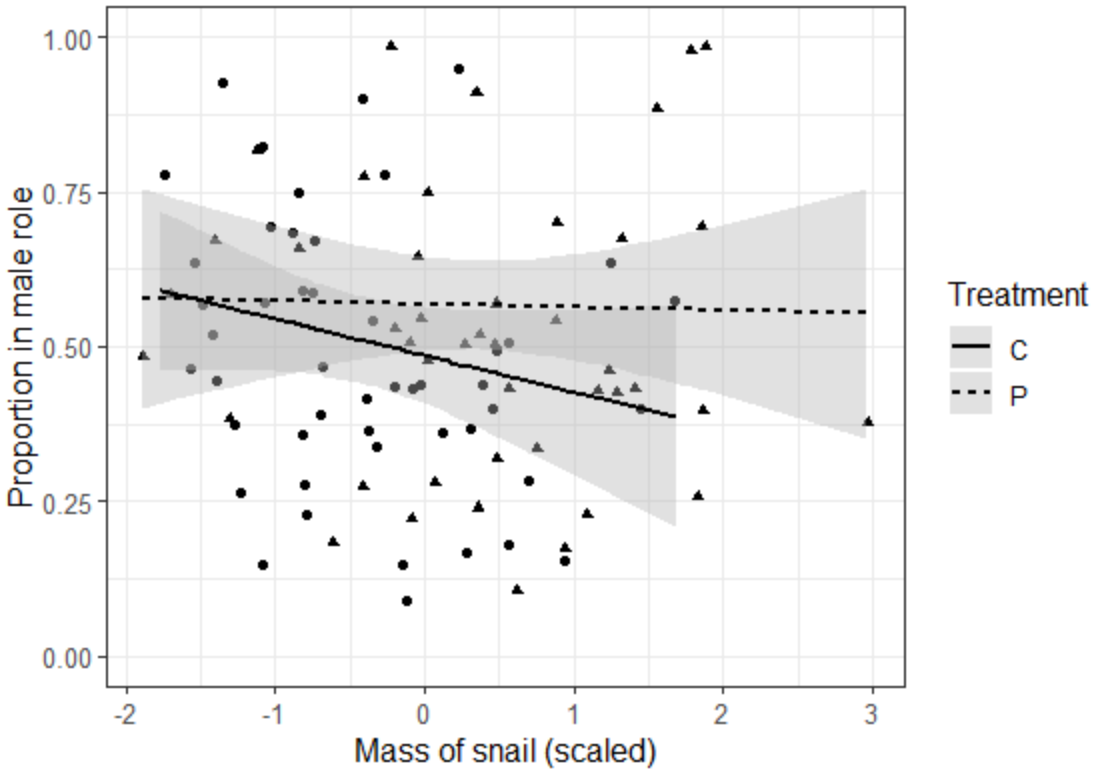


Figure 3.4. The effect of treatment and mass on the ratio of time mounted in the male role to being mounted in the female role. A) The probability of a snail in a mating interaction mounted in the male role rather than the female role. Individual dots represent snails with their scaled mass on the x axis and the proportion of mating time they participated in in the male role on the y axis. Lines are model predictions with 95% confidence intervals shaded. B) A dotplot showing the effects of mass, treatment, and their interaction on the proportion of matings in the male role.

A)



B)

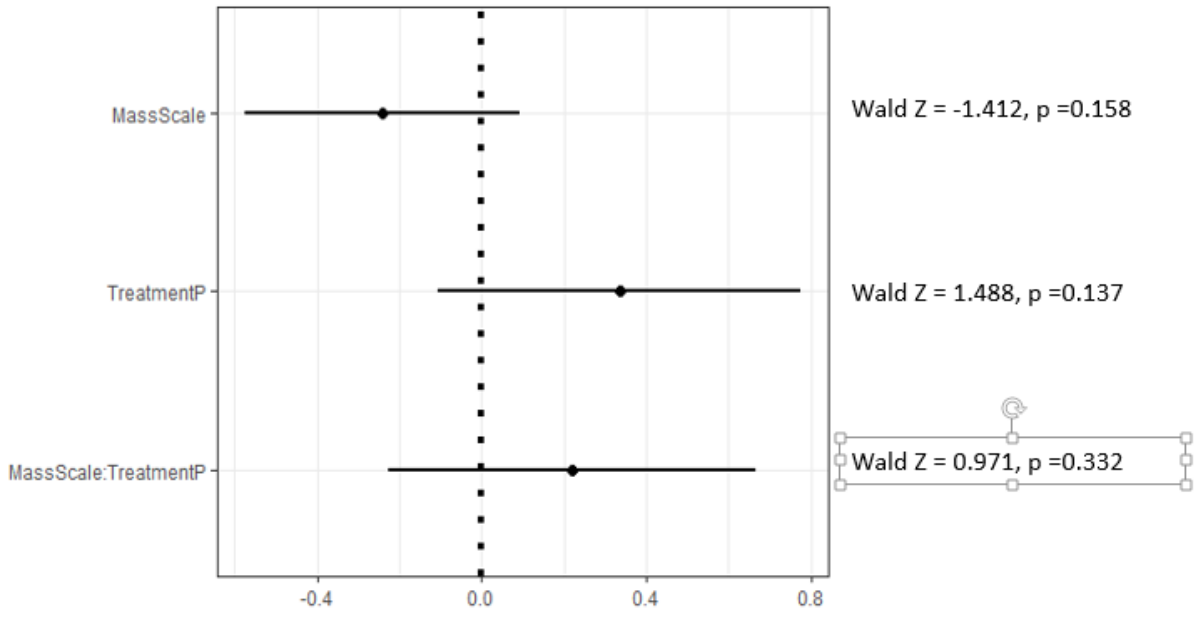
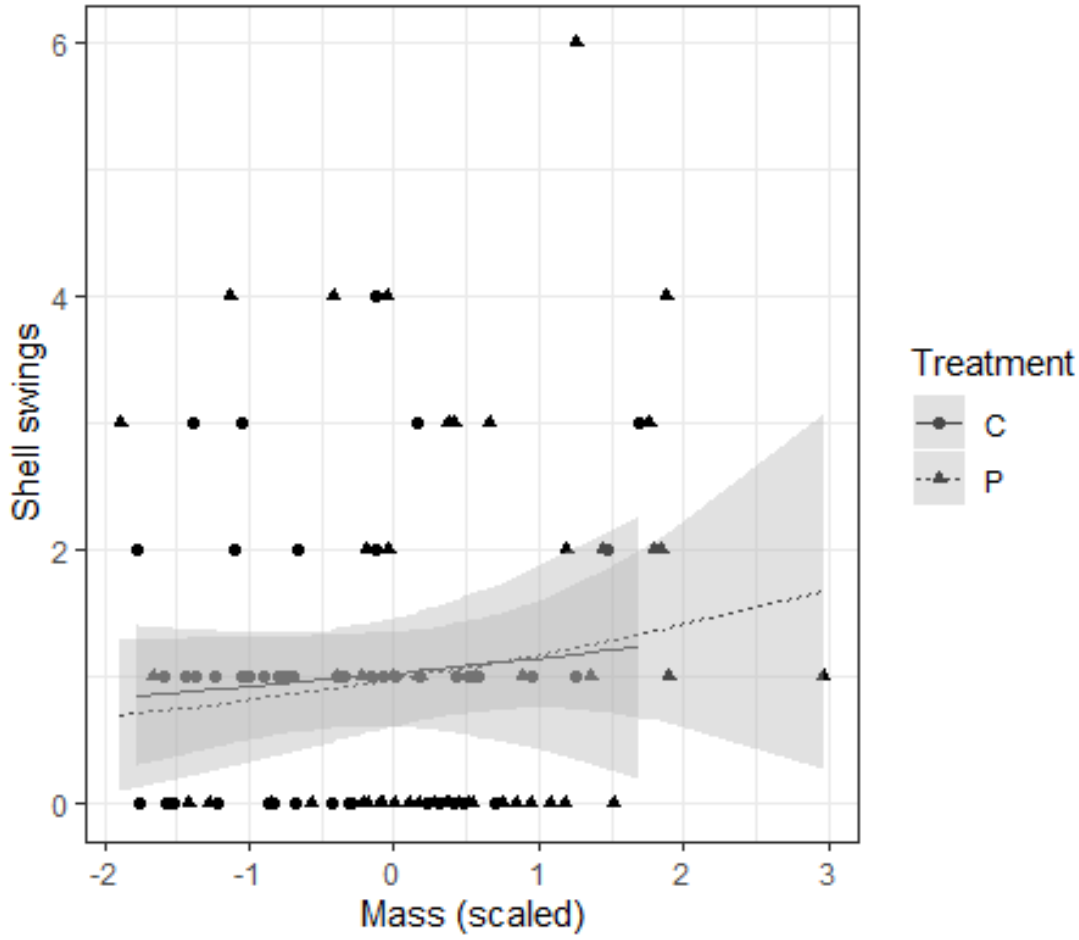

MassScale
TreatmentP
MassScale:TreatmentP
Wald Z = -1.412, p =0.158
Wald Z = 1.488, p =0.137
Wald Z = 0.971, p =0.332
-0.4
0.0
0.4
0.8

Figure 3.5. A) Relationship between number of shell swings (total), snail mass, and treatment. Points represent individual snails with the number of shell swings they engaged in. Lines and shading are the model prediction and confidence intervals. B) Dotplot with the effect sizes and confidence intervals. Effect size of predator treatment is shown in relation to the control treatment.

A)



B)

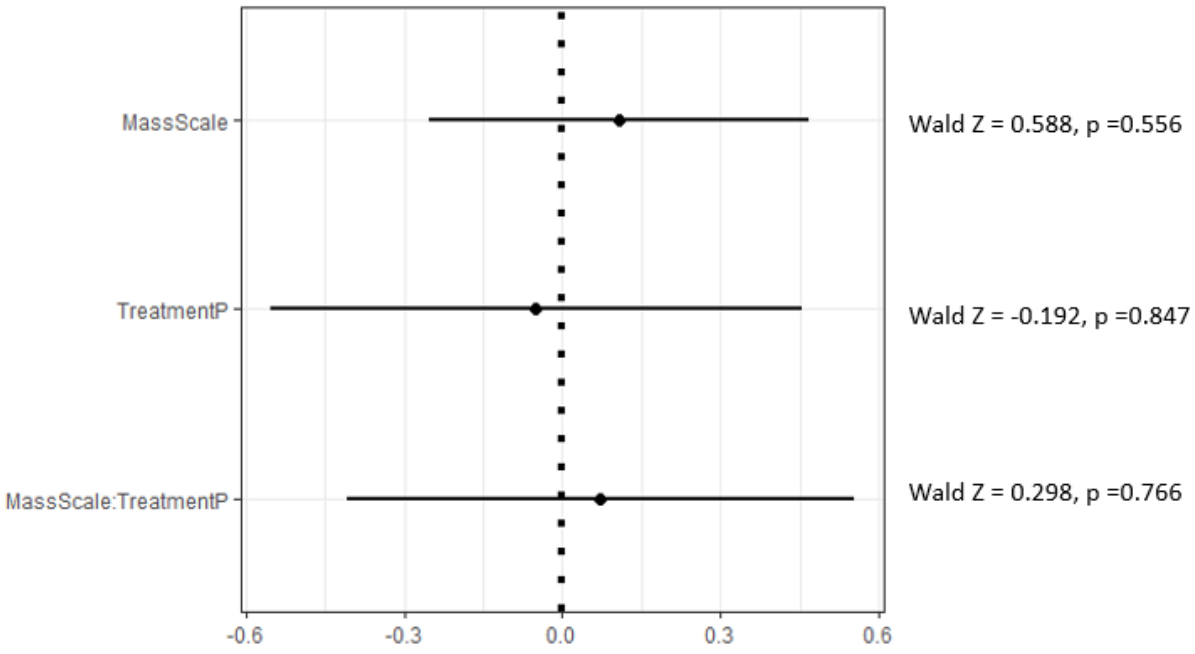
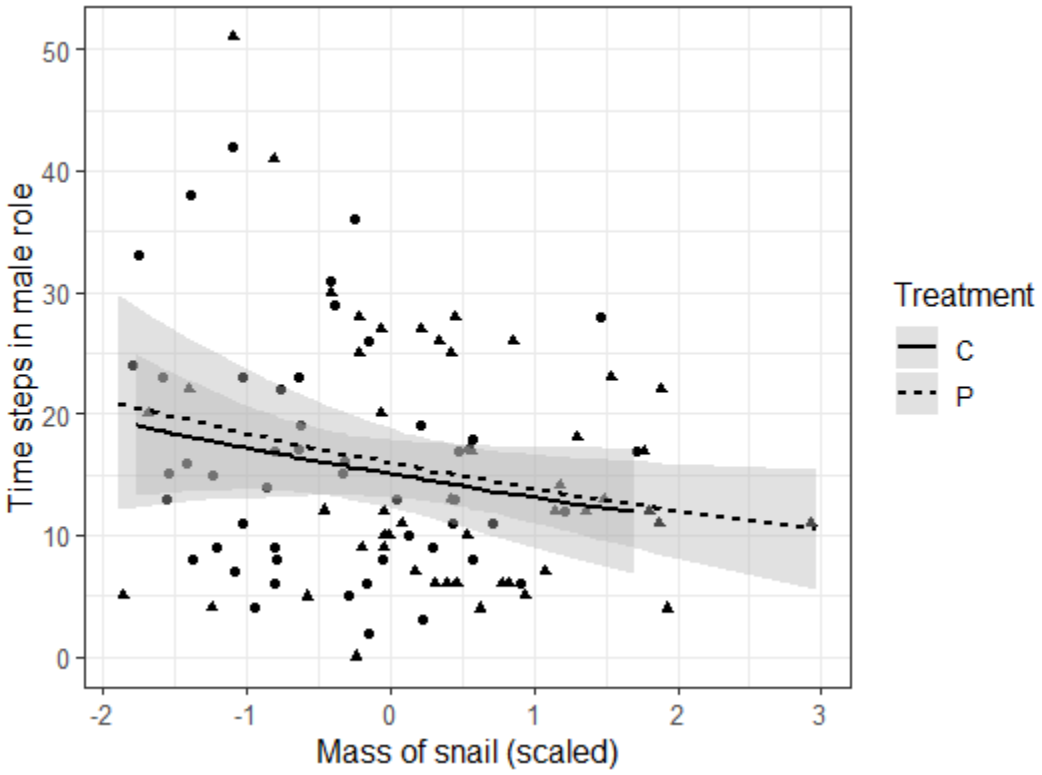
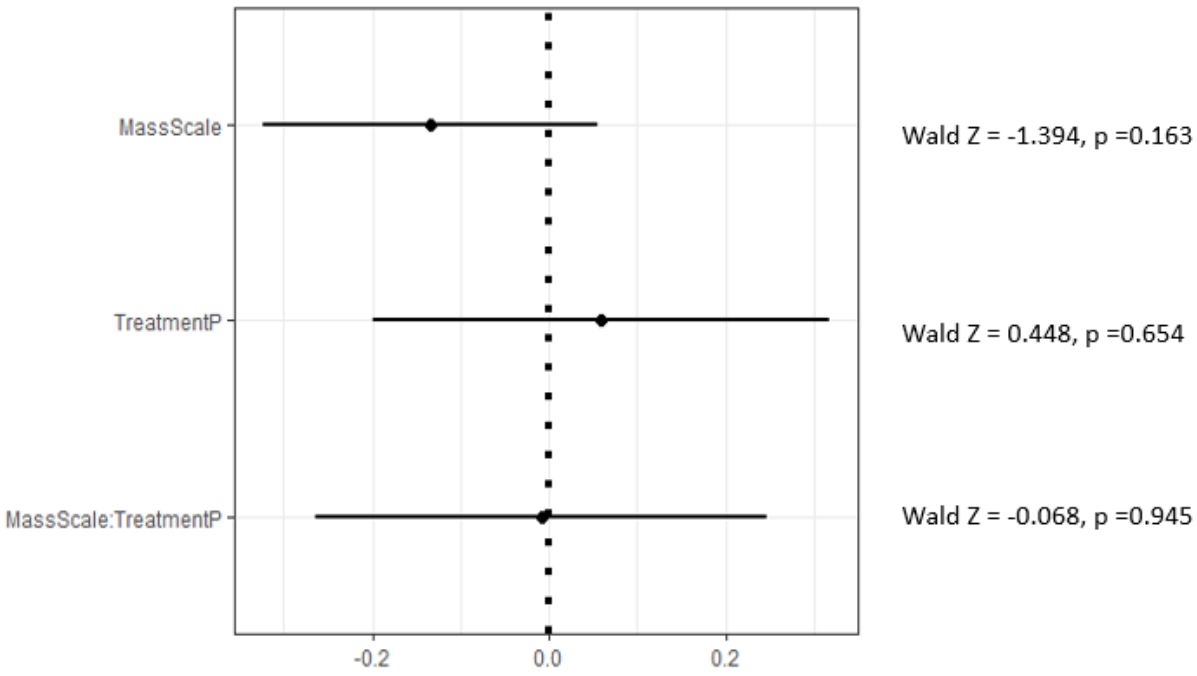

MassScale
TreatmentP
MassScale:TreatmentP
-0.6
-0.3
0.0
0.3
0.6
Wald Z = 0.588, p =0.556
Wald Z = -0.192, p =0.847
Wald Z = 0.298, p =0.766

Figure 3.6. A) Relationship between the number of time steps in the male role, and mass and treatment. . B) Dotplot with the effect sizes and confidence intervals. Effect size of predator treatment is shown in relation to the control treatment

A)



B)


MassScale
TreatmentP
MassScale:TreatmentP
-0.2
0.0
0.2
Wald Z = -1.394, p =0.163
Wald Z = 0.448, p =0.654
Wald Z = -0.068, p =0.945

APPENDICES

VITA

Scott Goeppner

Candidate for the Degree of

Doctor of Philosophy

Thesis: PREDATOR INDUCED PHENOTYPIC PLASITICITY WITHIN AND BETWEEN GENERATIONS IN THE POND SNAIL *PHYSA ACUTA*

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Education:

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Publications

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Professional Memberships:

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Society of Integrative and Comparative Biology