IMPLICATIONS OF SIZE-SELECTIVE PREDATION AND MATE AVAILABILITY FOR MATING-SYSTEM EXPRESSION AND EVOLUTION IN A HERMAPHRODITIC SNAIL (*PHYSA ACUTA*)

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The evolution of environment-specific trait expression (i.e., phenotypic plasticity) represents a seemingly unbeatable evolutionary strategy because a plastic organism may be able to maximize fitness in multiple environments. Traditionally, studies of adaptive plasticity have examined a single type of environment, but organisms in nature may simultaneously adjust their phenotypes to multiple environments. In a series of experiments, I examined whether predation risk and mate availability interact to affect morphology and life history in a hermaphroditic snail (*Physa acuta*). Predation risk was expected to induce an investment in defense at the expense of reproduction. Mate availability was expected to affect the age at first reproduction where isolated snails are expected to delay selfing because this snail is a preferential outcrosser with the potential for self-fertilization at the cost of inbreeding depression. To establish the adaptive benefit of the predator-induced changes, I induced snails by rearing them in the presence and absence of chemical cues from predatory crayfish and exposed both phenotypes to selection by lethal crayfish. Crayfish induced an increase in mass and shell thickness, and snails with these traits experienced higher survival when exposed to a lethal predator. Therefore, predator-induced plasticity was favored by selection. To establish the adaptive benefit of delayed selfing, I quantified inbreeding depression by comparing the fitness of selfed and outcrossed snails reared in predator and no-predator environments. Inbreeding depression occurred in both environments and therefore, delayed selfing is favored by selection. I went on to demonstrate

that inbreeding depression exists for two types of adaptive plasticity (i.e., delayed selfing and an inducible defense). Both types of inbreeding depression in plasticity may act as important constraints on the evolution of self-fertilization. In general, my results highlight the role of enemies in mating-system evolution and the role of mate availability in the evolution of inducible defenses as well as novel forms of constraint on the evolution of plasticity, including the existence of inbreeding depression in adaptive plasticity.

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PREFACE

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1.0 INTRODUCTION

Adaptive phenotypic plasticity can evolve when organisms have the ability to detect environmental variation, phenotypic optima differ among environments and appropriate genetic variation exists (Pigliucci 2001; West-Eberhard 2003; DeWitt and Scheiner 2004). Adaptive plasticity has long been of interest in ecology and evolutionary biology (e.g., Schmalhausen 1949; DeWitt and Scheiner 2004), but our understanding of the constraints on its evolution is still quite limited (Pigliucci 2005; Auld et al., *in review*). Typically, researchers investigate the expression and evolution of plasticity in response to one environmental factor at a time, and studies that investigate the integration of plastic responses to multiple environmental factors can provide insight to how plastic responses are expressed under more natural conditions (e.g., Valladares et al. 2007).

Two variable environmental factors that most organisms experience are predation risk and mate availability. Predation risk is known to induce the expression of defensive phenotypes that can affect behavior, morphology, physiology, life history, and reproduction in a diversity of organisms (e.g., Karban and Baldwin 1997; Tollrian and Harvell 1999). Mate availability can alter the allocation of resources to growth and reproduction (e.g., Puurtinen and Kaitala 2002; Tsitrone et al. 2003*a*; 2003*b*), and can directly affect the mating system (i.e., the pattern of mating among individuals; Jarne and Charlesworth 1993; Ashman et al. 2004). While these two factors are typically considered in isolation, there are good reasons to suspect that they may

interact to affect individual growth and reproduction, amounting to interactive effects on fitness (Steets et al. 2007*a*; Auld and Relyea 2008).

In this dissertation I examine the evolution of phenotypic plasticity in response to predation risk and mate availability using a simultaneously hermaphroditic freshwater snail (*Physa acuta*) as a model system. I focus on the mating system as a central suite of traits that can determine individual reproductive success and well as the larger population genetic structure and possibly speciation (Barrett 1990; Hamrick and Godt 1990; Jarne 1995; Charlesworth 2003; Goodwillie et al. 2005). I evaluate the potential reciprocal implications of predation risk and mate availability by examining the effects that enemies have on mating-system expression and the effects that mating system can have on the expression of inducible defenses.

In Chapter 2, I present some predictions for why predation risk and mate availability may interact to affect individual morphology, life history and fecundity along with an experiment designed to test these predictions. This work was done in collaboration with Dr. Rick Relyea (University of Pittsburgh) and was published in the *Journal of Evolutionary Biology* (Auld and Relyea 2008).

In Chapter 3, I present the results from a selection experiment that was done to assess the adaptive value of predator-induced morphological changes. This was done to evaluate the prediction that the predator-induced changes in shell morphology of *Physa acuta* were adaptive responses. This was also done in collaboration with Rick Relyea and the manuscript is in review at *Evolution* (Auld and Relyea, *in review* A).

Chapter 4 contains the results of an experiment that was conducted for two purposes. First, I assessed the consequences of the mating system in predator and no-predator environments by rearing inbred and outbred individuals with and without access to a mating

partner in the presence and absence of predation risk. With data on individual fitness, I estimated the relative fitness decrement suffered by inbred individuals compared to outbred individuals (i.e., inbreeding depression). This was done to determine whether inbreeding depression differs among environments. Second, I assessed inbreeding depression in adaptive plasticity by examining whether inbred and outbred snails differ in their ability to detect and respond to environmental conditions. This experiment was conducted with Rick Relyea and is being submitted to *Ecology Letters* (Auld and Relyea *in review* B).

Chapter 5 contains my conclusions from this work along with a discussion of the significance and implications of my findings.

2.0 ARE THERE INTERACTIVE EFFECTS OF MATE AVAILABILITY AND PREDATION RISK ON LIFE HISTORY AND DEFENSE IN A SIMULTANEOUS HERMAPHRODITE?

2.1 ABSTRACT

Encountering mates and avoiding predators are ubiquitous challenges faced by many organisms and they can affect the expression of many traits including growth, timing of maturity, and resource allocation to reproduction. However, these two factors are commonly considered in isolation rather than simultaneously. I examined whether predation risk and mate availability interact to affect morphology and life-history traits (including lifetime fecundity) of a hermaphroditic snail (*Physa acuta*). I found that mate availability reduced juvenile growth rate and final size. Predator cues from crayfish induced delayed reproduction, but there were no reduced-fecundity costs associated with predator induction. While there were interactive effects on longevity, lifetime fecundity was determined by the number of reproductive days. Therefore, my results indicate a resource-allocation trade-off among growth, longevity, and reproduction. Future consideration of this interaction will be important for understanding how resource-allocation plasticity affects the integration of defensive, life-history and mating-system traits.

2.2 INTRODUCTION

The life history that an organism employs can be viewed as a strategy for partitioning resources among fitness functions of growth and reproduction. Given some allocation of resources to reproduction, the mating system (i.e., the degree of inbreeding from self-fertilization to outcrossing) can have important fitness consequences by directly affecting the transmission of genetic variation. Therefore, an organism's life history and mating system are intimately connected and both play an important role in determining reproductive success. In addition, both the life history and mating system of an organism may be affected by intra- and interspecific ecological interactions that alter the allocation of resources to growth and reproduction. The avoidance of predators and the search for mates are two such interactions that most organisms face in natural communities. While variation in predation risk and mate availability is ubiquitous and despite many examples of inducible defenses and mating-system plasticity, we are only beginning to consider the ways that these factors may interact.

Inducible defenses have been demonstrated in plants, animals, and protozoans and have served as a fruitful model system for exploring the ecology and evolution of adaptive phenotypic plasticity (Tollrian and Harvell 1999; Relyea 2005*b*). Commonly, predators induce defenses that have fitness costs in prey such as reduced growth rate or fecundity thereby favoring inducible rather than constitutive expression of defensive traits (Tollrian and Harvell 1999). It is important to consider such effects because the induction of a defense can alter allocation of resources between growth and reproduction and costs may not be incurred until late in ontogeny. For example, theory predicts that prey should respond to small-size-selective predators by delaying reproduction in favor of growth to a size refuge (Stearns and Koella 1986). This prediction has been tested and supported by empirical studies (e.g., Crowl and Covich 1990; Hoverman et al.

2005). While many studies of inducible defenses have examined the fitness consequences of expressing a defense, no animal studies to our knowledge have examined the effects of expressing a defense on longevity and lifetime fecundity.

In addition to defense, the mating system employed by an organism can have direct fitness consequences. The benefits of inbreeding include the maintenance of favorable gene complexes (in the context of local adaptation) and the transmission advantage of selfing (Fisher 1941; Jarne and Charlesworth 1993). However, inbreeding increases homozygosity, which can result in inbreeding depression if partially recessive, deleterious alleles are segregating in the population (Jarne and Charlesworth 1993). Conversely, outcrossing can reduce inbreeding depression, but at the cost of decreased gene transfer (Goodwillie et al. 2005). Thus, there is a fitness trade-off between selfing and outcrossing that can favor plasticity in the mating system. Indeed, it has been suggested that in the event of low mate availability, a self-fertile hermaphrodite from a population harboring inbreeding depression should delay selfing for a period of time after achieving reproductive maturity to find a mate and avoid the costs of selfing (Lloyd 1992; Goodwillie et al. 2005). However, at the end of this "waiting time", the organism proceeds with self-fertilization if no mates are present. A recent model predicts that the waiting time should be longer with strong inbreeding depression and efficient resource reallocation to future reproduction (Tsitrone et al. 2003*a*). This model has been tested and supported in hermaphroditic animals (Tsitrone et al. 2003*b*; Schjørring 2004; Escobar et al. 2007; but see Schärer and Wedekind 1999) suggesting that mate availability can influence resource allocation between growth and reproduction in an adaptive fashion.

Clearly, mate availability and predation risk affect many of the same traits. Both mate availability and predation risk can influence individual reproduction (e.g., timing of

reproduction) and may potentially affect the mating system. Interestingly, the manner in which organisms respond to predation risk and mate availability may interact in potentially important ways. For example, if simultaneously hermaphroditic organisms respond to the presence of a small-size-selective predator by delaying reproduction, this delay may affect how long individuals will delay selfing. In other words, the predator-induced delay in reproduction may affect the length of the waiting time in the absence of mates. If the waiting time is altered by predation risk, the mating system may be altered as well. Additionally, when individuals have limited mate availability, resources may be allocated differentially to growth instead of reproduction (Tsitrone et al. 2003*a*, 2003*b*). If this differential allocation results in increased growth, a larger and therefore more defended phenotype can be achieved, thereby providing a benefit in the event of predator colonization. Alternatively, the manner in which organisms respond to variation in predation risk and mate availability may be additive, not interactive, but we currently lack data to evaluate these alternatives. Here, I investigate the potential interaction between predation risk and mate availability for morphology and life-history traits including total lifetime reproduction. Based on previous work, I predict that the availability of mates will lead to early reproduction while the presence of predator cues will induce a delay in reproduction. In addition, I predict a trade-off between growth and reproduction.

2.3 METHODS

2.3.1 Study system, animal collection, and rearing

I examined the effects of predation risk and mate availability on morphology and life history in the freshwater snail *Physa acuta* (Pulmonata, Basommatophora). This snail is a simultaneous hermaphrodite that has been widely used for studying predator-induced plasticity in morphology, behavior, and life history (Crowl and Covich 1990; DeWitt et al. 1999, 2000; Turner et al. 1999; Tsitrone et al. 2003*b*). Specifically, snails display fast growth and narrow shell apertures that appear to increase survival in the presence of small-size-selective, shell-entry predators such as crayfish. *Physa* detects predators via water-borne chemicals (Crowl and Covich 1990; DeWitt et al. 1999), which allows investigators to examine the inductive effects of predators without changes in prey density. *Physa* has also been widely used in studies of mating interactions, mating system expression, and the effects of inbreeding depression (e.g., Jarne et al. 2000; Facon et al. 2006). Because *P. acuta* is easy to culture and has a short generation time (i.e., <3 months), it is an ideal species to use for studies of longevity and lifetime fitness.

Adult *Physa acuta* snails were collected at Geneva pond #3 in northwest Pennsylvania, U.S.A. (41°, 35' N; 80°, 14' W) on 23 January 2006. Snails were transported to the University of Pittsburgh, Pittsburgh, PA within 2 hrs and isolated in 1-liter plastic containers for oviposition. The experimental room was held at 22°C with constant 12-hr light/dark cycles during hatching and the subsequent experiment. Containers were checked daily for eggs and 65 snails were chosen that laid eggs on 29 January (hereafter considered day 0 for determining snail age). *P. acuta* is a preferential outcrosser (Jarne et al. 2000) and can store sperm for long periods of time (e.g., up to 3 months; Dillon et al. 2005) so I assume that all the progeny of these wild-

caught snails were outcrossed. Adults were removed from containers and dissected to assure that the specimens were *P. acuta* (*P. acuta* is superficially similar to other co-occurring *Physa* species (e.g., *P. gyrina*) and positive identification needs to be made based on male genital morphology; Wethington 2004). Hatching began on 10 February (age = 12 d) and all snails were fed ground Spirulina (O.S.I. Marine Lab, Inc., Burlingame, CA) *ad libitum*. From the 65 ovipositing snails, 10 families were randomly selected for use in the experiment. All water used was carbon-filtered and UV-irradiated.

2.3.2 Experimental design

Individual snails were reared in 1-liter plastic containers (filled with 1 liter of water) under a completely randomized design employing a factorial combination of two predator treatments (predator cues present or absent) and two mate-availability treatments (mate available or not [i.e., isolation]). Each treatment was replicated 10 times, yielding 40 experimental units. To equalize genetic differences and potential maternal effects among the treatments, one individual from each of 10 families was used in each treatment (i.e., $n = 10$). Individual snails were added to the containers on 3 March 2006 (age = 33 d; initial mass <1mg), the predator-cue treatment was initiated on 6 March, and the mate-availability treatment began on 10 March (i.e., treatments were applied for approximately two-thirds of the snail's juvenile period). Throughout the experiment, snails were fed three times per week and water was changed weekly. The experiment was conducted for the entire life of the snails (age at death range: 72 – 212 d) to determine longevity and lifetime fecundity.

The predator treatment was implemented by adding water that had been conditioned by a pond-dwelling crayfish (*Procambarus acutus*) that is native to the region and co-occurs with *P.*

acuta. Crayfish (n = 15) were held individually in 10-L plastic tubs containing 3 L of water. Three times per week I collected 1 L of crayfish-conditioned water from each tub, discarded the remaining 2 L, re-filled the tubs with 3 L of fresh water, and fed the crayfish 150 mg of lab-reared *P. acuta* and rabbit chow *ad libitum* (crayfish are omnivores). After pooling the 15 L of predator-cue water, I removed 400 ml of water from each experimental unit assigned the predator treatment and replaced it with 400 ml of predator-cue water. Therefore, the predator-cue concentration in each experimental unit was 20 mg of consumed *Physa* / liter. Snails in the no-predator treatment had 400 ml of water removed three times per week and replaced with 400 ml of fresh water. Predator cues break down, so this static-renewal treatment was implemented to maintain constancy in perceived predation risk.

Mate availability was manipulated without rearing individuals under different densities. Snails in the no-mate-available treatment remained in isolation throughout their entire lives while snails in the mate-available treatment had a marked, sexually mature *P. acuta* added to their container three times per week for 3 hrs at a time (Tsitrone et al. 2003*b*). Mates were selected from lab cultures that were all founded from the same population and represented > 30 families (i.e., isofemale lines). These lines were consistently mixed throughout the experiment and mates were cultured together. Therefore, each time a mate was added, experimental snails potentially had access to a different mate. Mates were marked with fast-drying red nail polish, which is an effective and harmless marking technique (Henry and Jarne 2007). As the majority of oviposition occurs at night (Duncan 1975), it is unlikely that the mates oviposited during these conjugal visits. This duration of mate availability was sufficient to allow copulation of snails and reciprocation of gender roles (Facon et al. 2006; J. R. Auld, *pers. obs*.).

2.3.3 Morphological measurements and analysis

To assess plasticity in shell morphology at the same point in ontogeny, I weighed each snail and took a digital picture using a Canon PowerShot A300 camera on 11 April (age = 72 d). Images were viewed using Optimas (Bothell, WA) and four shell measurements were recorded: shell length, shell width, aperture length, and aperture width (measured at the maximum for each snail). Shell thickness was also measured to the nearest 0.01 mm with digital calipers at the leading edge of the shell. To standardize morphological measurements for differences in overall size, I conducted a MANCOVA with *ln*-transformed mass as a covariate and shell dimensions as response variables (shell thickness was not correlated with mass [$r = 0.074$, $P = 0.653$], so it was not corrected for size). The MANCOVA included predator and mate treatments as fixed effects and the assumptions of the MANCOVA model were verified, including the absence of treatment-by-response variable interactions (i.e., all treatment slopes were parallel). I saved the residuals from the MANCOVA and subsequently used the sum of each individual snail's residual plus the estimated marginal mean (i.e., the mean estimated from the model, including the effects of mass as a covariate) for each treatment as my response variables. This procedure produces estimates of shape variables that are adjusted to remove the effects of overall size and has been successfully used in previous studies of morphological plasticity (e.g., Hoverman et al. 2005). All statistical analyses were performed using SPSS (v.11 for Mac).

To provide a comparison with previous studies (e.g., DeWitt et al. 1999, 2000), I analyzed the aspect ratio of shell and aperture traits (i.e., length divided by width) in addition to analyzing the shape variables independently. I calculated aspect ratios based on size-independent and un-adjusted measures of shell and aperture dimensions and found these two

methodologies to be qualitatively identical. I report test statistics based on the analysis of aspect ratios calculated with un-adjusted shell dimensions.

2.3.4 Life history / reproductive response variables and analyses

The experiment lasted the entire life of the snails to measure a complete set of life-history traits including age/size at first reproduction, growth rate, longevity and lifetime fecundity. Experimental units were checked daily for egg masses and the number of oviposited eggs was counted weekly. During each weekly egg counting, the number of eggs that failed to hatch was also counted to determine egg-hatching success. Individuals were placed in new containers weekly so that I could easily count eggs and evaluate egg hatching. Snails were blotted dry and weighed weekly (to the nearest mg), when they produced their first egg mass (i.e., size at first reproduction), and at death (i.e., size at death). I assessed the effects of my treatments on the allocation of resources between growth and reproduction by comparing growth rate prior to reproduction (i.e., juvenile growth rate) with growth rate during reproduction (i.e., adult growth rate). Juvenile growth rate represents the size at first reproduction divided by the age at first reproduction. Adult growth rate was calculated as the difference between size at death and size at first reproduction divided by the difference between age at death and age at first reproduction. As most snails reproduce up until the day they die, these measures provide a linear estimate of how resource allocation to growth changes when snails initiate reproduction. I also quantified the fraction of total growth that occurs prior to initiating reproduction (i.e., size at first reproduction divided by size at death; SFR/SD) as an additional means of determining how resource allocation between growth and reproduction differs among my treatments.

I used a MANOVA to examine treatment effects on 17 traits: size-independent morphology (i.e., shell length, shell width, aperture length, and aperture width), shell aspect ratio, aperture aspect ratio, shell thickness, age at first reproduction, size (mass) at first reproduction, age at death (i.e., longevity), size at death, the number of reproductive days (age at last reproduction – age at first reproduction), the total number of eggs laid, the proportion of total eggs that hatched, juvenile growth rate, adult growth rate, and the proportion of final size attained prior to reproduction. All life-history / reproduction variables except the three growth variables were *ln*-transformed prior to analysis (except the egg-hatching proportion which was arcsine-square root transformed). When multivariate effects of my treatments were significant I examined univariate effects of the treatments on each variable independently. In an effort to control for multiple testing while balancing the risk of type I and type II errors, I used the methods suggested by Verhoeven et al. (2005) to estimate the false discovery rate. This methodology was initially suggested by Benjamini and Hochberg (1995) as a more powerful means of controlling for multiple testing than the traditional Bonferroni / sequential-Bonferroni tests (Verhoeven et al. 2005). When univariate tests were significant, I conducted mean comparisons using *t*-tests to examine specific comparisons between a pair of treatments (e.g., between mate and no-mate treatments within the no-predator treatment). Two snails were excluded from the final analysis; one of which proved to be a statistical outlier in terms of growth and reproduction while the other never reproduced. Inclusion of the available data from either of these two snails did not qualitatively affect the outcome of the analyses.

2.4 RESULTS

The MANOVA included 17 response variables and revealed significant multivariate effects of predator ($F_{17,18} = 3.023$, $P < 0.05$) and mate ($F_{17,18} = 2.255$, $P < 0.05$) treatments. The predator-by-mate interaction was non-significant ($F_{17,18} = 0.720$, $P > 0.05$). However, univariate tests revealed a significant univariate predator-by-mate interaction for shell thickness, the age at death, and the number of reproductive days (Table 2.1).

2.4.1 Morphology

In my examination of morphology, predator cues did not affect shell width and aperture length, although there was a tendency for snails reared with predator cues to have longer shells and narrower apertures than snails reared without predator cues (Table 2.1). The presence of mates did not affect morphology although there was a tendency for snails with mates to have reduced aperture length than snails reared without mates. Previous studies on predator-induced morphology in freshwater snails have used the ratio of length to width (i.e., aspect ratio; DeWitt et al. 1999, 2000) to describe shell shape. I calculated this statistic for both shell and aperture traits and found no treatment or interaction effects on shell aspect ratio. There were no mate or interaction effects on aperture aspect ratio, but consistent with the tendency for predators to induce relatively narrow apertures, predators tended to increase the aperture aspect ratio.

Predator cues caused an average 13% increase in shell thickness. However, I detected a univariate interaction for shell thickness. This results because snails with mates showed a 25% increase in shell thickness with predator cues (mean $\pm$ S.E.: 0.28 mm $\pm$ 0.01 and 0.35 mm $\pm$ 0.02, no-predator and predator-induced, respectively; $t_{17} = 3.305$, $P = 0.004$) while snails without

mates showed no change in shell thickness with predator cues (0.31 mm $\pm$ 0.01 and 0.32 mm $\pm$ 0.02, no-predator and predator-induced, respectively; $t_{17} = 0.220$, $P = 0.828$).

2.4.2 Life history and reproduction

Predation risk and mate availability affected the allocation of resources to life history traits including growth and the timing of maturity. Predator cues induced larger age and size at first reproduction (Fig. 2.1 A, B; Table 2.1). In addition, mate availability reduced size at first reproduction and size at death (Fig. 2.1 B). There were no significant predator or mate effects on total lifetime fecundity or the egg-hatching proportion.

I observed evidence for a predator-by-mate interaction for age at death and the number of reproductive days. These interactions result because in the no-predator treatment, snails without mates lived 35% longer than snails with mates ($t_{17} = 2.479$, $P = 0.024$), while in the predator treatment there was no mate effect ($t_{17} = 1.344$, $P = 0.197$; Fig. 2.1 A). In the mate treatment, snails lived longer when exposed to predator cues than when not exposed to predator cues ($t_{17} = 3.819$, $P = 0.001$), but snails reared without mates were not affected by predator cues ($t_{17} = 0.664$, $P = 0.515$). A somewhat similar pattern emerges for the predator and mate effects on the number of reproductive days, which is presumably correlated to longevity (Fig. 2.1 C). These patterns of differential longevity and reproductive lifetime produced the pattern of fecundity observed in my treatments.

I explored how predator cues and mate availability altered resource allocation to growth and reproduction by comparing juvenile and adult growth rates. While predator cues did not affect juvenile growth rate, predator-induced snails did experience reduced adult growth rate compared with snails without predator cues (Fig. 2.1 D). Alternately, snails with mates

experienced reduced juvenile growth rate compared to snails without mates, but there was no mate effect on adult growth rate. There was no predator-by-mate interaction for these measures of juvenile and adult growth rate. Additionally, by dividing size at first reproduction by size at death, I found that snails reared without predator cues initiated reproduction when they were approximately 55% of their final mass while snails exposed to predator cues obtained approximately 85% of final mass before reproducing. There was no mate effect or interaction for this measure of growth prior to reproduction.

2.5 DISCUSSION

Although previous studies have reported plasticity in response to predation risk and mate availability, this is apparently the first time they have been considered together. By doing so, I can evaluate an interaction that may occur under natural conditions where both predation risk and mate availability are variable. It is imperative to consider both factors over ontogeny because predation risk and mate availability affect resource allocation between growth and reproduction and the ultimate consequences on lifetime fitness should be evaluated.

Predator cues did not affect overall shell shape, but did affect shell thickness. DeWitt et al. (2000) reported that snails respond to crayfish cues by producing an elongate shell (i.e., increased ratio of length to width). Consistent with these findings, I found a marginally non-significant increase in the aperture aspect ratio with predator cues. Although previous studies have not examined changes in shell thickness, increased shell thickness is likely an important defense; indeed, in additional research with *Physa* from the same population, I have found that crayfish can more easily crush and kill non-induced snails than crayfish-induced snails (Auld and

Relyea, *in review* A [Chapter 2]). Therefore, the predator-induced increase in shell thickness may be an adaptive anti-predator response.

Predator cues caused snails to delay reproduction, which is consistent with theoretical predictions (Stearns and Koella 1986) and previous empirical observations (e.g., Crowl and Covich 1990; Hoverman et al. 2005) that size-selective predation can affect resource allocation between growth and reproduction. Snails reared without predator cues initiated reproduction when they were 55% of their final mass while snails reared with predator cues obtained 85% of final mass before reproducing. As predator cues did not affect size at death, these predator-induced snails had lower growth rates during reproduction (i.e., lower adult growth rates) than snails reared without predator cues. Taken together, predator cues altered the timing of reproduction in ways that cascade to alter the patterns of growth.

While reduced growth and/or fecundity are potential (and commonly mentioned) costs of expressing an inducible defense (Tollrian and Harvell 1999), I found no evidence for such costs in my experiment. However, delayed reproduction can be viewed as a potential cost of expressing a predator-induced phenotype since delayed reproduction leads to a longer generation time. While several studies have examined the effects of predators on reproduction (namely in *Daphnia*; e.g., Black and Dodson 1990; Tollrian 1995; Scheiner and Berrigan 1998), these previous studies have yielded mixed results concerning a fecundity cost; predators often induce delayed reproduction, but fecundity either increases or decreases. In a previous study with a different species of freshwater snail (*Helisoma trivolvis*; Hoverman et al. 2005), crayfish predators induced delayed reproduction and decreased fecundity of snails (all snails were reared with available mates), but this experiment did not last the entire life of the snails. Note that if I had terminated the experiment before the snails died, I would have arrived at similar results. My

study appears to be the first animal study to examine the consequences of an inducible defense over the entire lifetime. However, it is difficult to assess how these results can be extrapolated to field conditions where individuals may not live as long. In general, my approach provides a relatively complete understanding of the potential effects of an inducible defense expressed over the entire lifespan and more studies of this type will greatly contribute to our understanding of the costs and benefits of plastic defenses.

Mate availability had strong effects on the total amount of growth. While isolated and mated snails started reproduction at approximately the same age, isolated snails had larger mass at first reproduction than mated snails. Therefore, isolated snails grew at a faster rate prior to reproduction (i.e., faster juvenile growth rate). One potential explanation for this difference in allocation to growth is that mated snails may have invested more resources in male function than isolated snails. Indeed, theoretical models predict that male allocation should increase with mate availability and that completely selfing individuals should only produce enough sperm to fertilize their own ovules (Charlesworth and Charlesworth 1981; Charnov 1982). Many hermaphroditic organisms increase male allocation with mate availability (e.g., Raimondi and Martin 1991; de Visser et al. 1994; Koene et al. 2006). While this hypothesis is consistent with established theory, it remains speculative and will require further investigation.

Previous studies on simultaneously hermaphroditic animals (including *Physa*) have used reproductive effort and success of isolated and mated individuals to study aspects of the mating system. Past studies, all without predator cues, have found that preferentially outcrossing individuals experience reduced fecundity and progeny survival when isolated (Jarne et al. 1991, 2000; Doums et al. 1996), while preferentially selfing individuals experience increased fecundity when isolated (Wedekind et al. 1998; Gutiérrez et al. 2001*a*, 2001*b*). Interestingly, studies that

have observed reduced fecundity by isolated snails also reported a long waiting time prior to self-fertilization (Jarne et al. 1991, 2000) whereas studies documenting high fecundity of isolated snails report little or no waiting time (Gutiérrez et al. 2001*a*, 2001*b*). In contrast to previous research on different populations of *P. acuta* (Wethington and Dillon 1997; Tsitrone et al. 2003b; Escobar et al. 2007), I did not observe a significant effect of mates on the age at first reproduction, however isolated snails tended to reproduce after mated snails. Importantly, additional research on *P. acuta* has demonstrated substantial among-population variation in waiting time (Escobar et al., *in review*). Therefore, my data are not inconsistent with the model of Tsitrone et al. (2003*a*) predicting a waiting time in outcrossing species.

Research on a diverse array of taxa has demonstrated a general trend that reproductive value gradually declines following the initiation of reproduction (i.e., senescence; Rose 1991). Evolutionary theory of senescence predicts that longevity should be negatively related to growth rate (Metcalfe and Monaghan 2003). In this study, mate availability reduced juvenile growth rate and final size, but the consequences for longevity depended on predation risk. I also found that predation risk induced delayed reproduction, and subsequently, mated snails lived longer under predation risk than mated snails under no predation risk. Without predator cues, where snails initiated reproduction at relatively small size, longevity was reduced due to mating. Comparatively, with predator cues, where snails initiated reproduction at relatively large size, longevity was not affected negatively by mating. This suggests that mating and initiating reproduction at a relatively small size can have detrimental effects on longevity. These findings are in agreement with studies on the effects of mating on longevity in insects (Mishra and Mishra 2005; Maklakov et al. 2007).

2.5.1 Conclusions

My results demonstrate that predation risk and mate availability can affect morphology and life history in *Physa acuta* and while some traits exhibit additive effects of these treatments, I have some evidence for an interaction between predation risk and mate availability. In this study I quantified total fecundity, which represents complete male and female fitness for isolated snails, but only female fitness for mated snails. Individuals reared in isolation should maintain a sperm supply large enough to fertilize their own ovules and engage in mating if a mate shows up, but this sperm storage is most likely never depleted as in the case where individuals encounter mates. Therefore, male allocation is likely to be higher in an individual reared with available mates and increased allocation to male function may result in decreased growth ability. Despite rearing individuals under *ad libitum* food conditions, my results are indicative of a trade-off among growth, longevity, male reproduction and female reproduction. Therefore, these trade-offs are likely to be stronger under more realistic, food-limited conditions. I have shown that mate availability and predation risk act together to influence resource allocation and senescence and future studies should be designed to evaluate these trade-offs over the entire lifespan. Additionally, a number of my insignificant results are indicative of a lack of power. Future experiments with increased sample size will reveal whether the patterns described here are robust. In summary, the mating system (i.e., outcrossing when mated and selfing when isolated) had dramatic consequences for the expression of several life-history traits; most notable were the effects on growth and longevity. Reciprocally, the expression of life-history traits may influence mating-system expression if a trade-off among growth, reproduction and sex allocation occurs under natural circumstances.

Table 2.1. Results of 17 univariate tests showing predator, mate, and interactive effects of the variables included in the MANOVA. Boldface values denote significant tests after controlling for the false discovery rate (see text for details). SFR/SD is size at first reproduction divided by size at death (i.e., the proportion of total mass attained prior to reproduction).

Trait	Predator		Mate		Predator*Mate	
	$F_{1,34}$	P	$F_{1,34}$	P	$F_{1,34}$	P
Shell length	3.724	0.062	1.668	0.205	0.788	0.381
Shell width	0.034	0.855	0.005	0.945	2.066	0.160
Shell aspect ratio	0.769	0.387	0.652	0.425	0.223	0.640
Aperture length	0.001	0.971	3.893	0.057	0.012	0.912
Aperture width	3.787	0.060	0.001	0.975	0.275	0.604
Aperture aspect ratio	3.896	0.057	1.612	0.213	0.503	0.483
Shell thickness	**6.421**	**0.016**	0.006	0.938	**4.968**	**0.033**
Age at first reproduction	**34.836**	**<0.001**	3.164	0.084	0.063	0.803
Size at first reproduction	**17.038**	**<0.001**	**7.948**	**0.008**	1.383	0.248
Age at death	3.011	0.092	1.645	0.208	**7.862**	**0.008**
Size at death	0.341	0.563	**9.014**	**0.005**	0.198	0.659
Reproductive days	2.344	0.135	0.282	0.599	**5.741**	**0.022**
SFR/SD	**20.28**	**<0.001**	0.011	0.917	0.419	0.522
Total eggs laid	3.495	0.070	0.147	0.703	2.833	0.102
Egg-hatching proportion	0.585	0.450	1.975	0.169	0.294	0.591
Juvenile growth rate	2.668	0.096	**5.684**	**0.023**	0.411	0.526
Adult growth rate	**5.968**	**0.020**	0.683	0.414	1.867	0.181

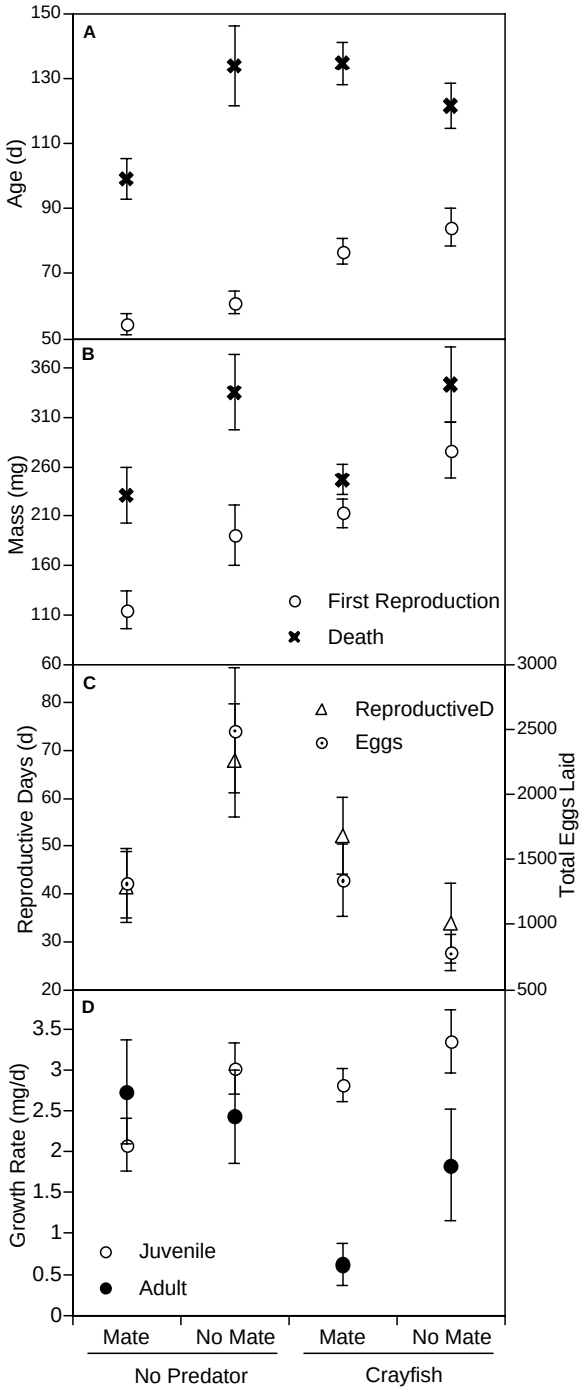

Figure 2.1. The effects of predation risk and mate availability on several life history traits in *Physa acuta*. A) Age and B) Mass at first reproduction and death (symbols are the same in panels A and B). C) The number of reproductive days (age at last reproduction – age at first reproduction) and total lifetime fecundity (total number of eggs laid). D) Juvenile and adult growth rates (see text for details). Data were transformed prior to analysis and symbols represent means $\pm$ 1 S.E.

3.0 PATTERNS OF SELECTION AND MODE OF PREDATION CHANGE BASED ON PREY PHENOTYPE: ADAPTIVE PLASTICITY AND CONSTRAINTS ON INDUCIBLE DEFENSES

3.1 ABSTRACT

Studies of putatively adaptive plasticity, such as inducible defenses, frequently explore the fitness consequences of expressing alternative phenotypes in alternative environments. However, relatively few studies examine *how* and *why* the pattern of selection on a suite of correlated characters changes in relation to the pattern of induction. To address this, I induced freshwater snails in the presence and absence of nonlethal predatory crayfish and exposed both phenotypes (alone and in combination) to selection by lethal crayfish. Crayfish induced an increase in mass and thicker, more compact shells. Crayfish preyed upon uninduced snails rapidly by crushing them, revealing strong selection for increased mass and shell dimensions. Conversely, predation on crayfish-induced snails was less efficient and snails were crushed at a lower rate resulting in a different pattern of selection on induced snails: strong selection for wide apertures and narrow, thick shells. Taken together, I infer that crayfish predation on small, uninduced snails selects for larger shells, while predation on larger, predator-induced snails is more focused on shell architecture. Thus, the pattern of selection changed in response to a

change in the foraging mode of the predator, which itself resulted from the expression of an effective suite of predator-induced defenses.

3.2 INTRODUCTION

Over the past quarter-century, a tremendous number of examples of inducible defenses have accumulated in a great variety of taxa (Karban and Baldwin 1997; Tollrian and Harvell 1999). Such inducible defenses are typically viewed as a form of adaptive phenotypic plasticity where an organism can express a condition-specific phenotype in response to environmental cues (Gotthard and Nylin 1995). Such *inducible* phenotypes represent a potentially optimal way to deal with environmental variation, but the fact that inducible defenses are not expressed in all taxa implies that constraints on the evolution of "perfect" plasticity exist. A series of models have guided our thinking on how such adaptive plasticity has evolved, and one central focus of such models has been the role that phenotypic trade-offs play in favoring inducible expression of certain phenotypes over constitutive expression (Harvell and Tollrian 1999; Berrigan and Scheiner 2004). Across-environmental phenotypic trade-offs in defensive phenotypes emerge when, for example, defended phenotypes experience increased survival in the presence of a predator, but reduced growth, development, and/or fecundity in the absence of a predator (e.g., Ågren and Schemske 1993; Baldwin 1998; Relyea and Auld 2004, 2005; Steiner 2007; Hoverman et al. 2005) or when a defensive phenotype produced in response to one predator increases vulnerability to a different predator (e.g., Smith and Jennings 2000; Relyea 2003; Benard 2006; Hoverman and Relyea 2007*b*). Collectively, variation in predation risk and trade-offs in fitness associated with expression of a defense function to make inducible defenses an

excellent system for studying the evolution of adaptive plasticity in traits that are closely connected to fitness.

However, demonstrating that plasticity in response to some environmental factor (e.g., predation risk) is an adaptive solution to conflicting demands on the phenotype requires evidence of cause and effect underlying the induction and fitness consequences of trait changes, not simply evidence that the trait changes (e.g., induced defenses) are effective (Wade and Kalisz 1990; Gotthard and Nylin 1995; Doughty and Reznick 2004). One method that has been successfully used to establish the adaptive nature of plasticity in a suite of traits is to conduct a selection experiment to demonstrate that the induced changes in the phenotype increase fitness in the inducing environment (e.g., Van Buskirk et al. 1997; Van Buskirk and Relyea 1998). Subsequently, one can examine the pattern of selection on a set of inducible traits and evaluate whether the direction of induction and the direction of selection are congruent for each trait and how correlations among traits influence the pattern of induction and selection. Such correlations among traits may result in an important constraint on the adaptive evolution of the phenotype by restricting what phenotypes are possible (e.g., Raup 1966).

Additionally, the pattern of selection on inducible traits may change across environments, and this alteration can have at least two causes. First, the pattern of selection on induced traits may be altered directly in response to a change in trait values themselves. Second, the pattern of selection on induced traits may change due to trait changes in other, interacting organisms. Such a situation may arise when species interactions result in reciprocal plasticity (e.g., if the predator changes its foraging mode in response to the expression of an inducible defense in its prey). While numerous studies have explored these ideas independently, we lack examples of how the

induction of defensive traits, selection on these traits, and the type of selection (e.g., the mode of predation) are linked and mutually interactive.

Here, I use a common freshwater decapod-gastropod predator-prey interaction to explore the effects of predator induction on the pattern of selection and the importance of understanding the mode of predation for interpreting the pattern of selection. My target organism, the freshwater snail *Physa acuta* (Basommatophora), has been previously used as a model system for studying predator-induced plasticity (e.g., DeWitt 1998; DeWitt et al. 1999, 2000; Turner et al. 1999, 2000; Langerhans and DeWitt 2002; Auld and Relyea 2008). *Physa* detects predators via water-borne chemical cues (Crowl and Covich 1990; Covich et al. 1994), which allows investigators to examine the inductive effects of predators without changing prey density. This previous work has explored how snails adjust shell morphology in the presence of predatory fish and crayfish (e.g., DeWitt 1998; DeWitt et al. 2000). Crayfish-induced snails display elongate shells that increase survival by restricting shell entry by predatory crayfish and rotund, crush-resistant shells in the presence of predatory fish (DeWitt et al. 2000). Based on this previous work, I can predict that the pattern of trait induction will correspond to the pattern of selection (i.e., I predict that the expression of inducible defenses is an adaptive response to the presence of predation risk). Additionally, *Physa* responds to the presence of crayfish by accelerating growth rate at the expense of delayed reproduction (Crowl and Covich 1990; Auld and Relyea 2008). Therefore, while there may be benefits to attaining a size refuge through rapid growth and expressing a predator-induced morphology in the presence of a predator, the cost of such defenses may be incurred in terms of delayed reproduction (as opposed to a cost that involves the same traits). While delayed reproduction may affect fitness in several ways, the effects of predator-induced morphological changes remain less clear.

As described above, documenting adaptive plasticity requires a demonstration of the cause-and-effect relationship that underlies fitness trade-offs for a suite of correlated traits in multiple environments. While previous work in this system has demonstrated the potential for such trade-offs, the relationship between the induction of predator-induced traits and their selective benefits has not been demonstrated. Indeed, studies demonstrating the relationship between induction of and selection on inducible defenses are rare across all systems. Furthermore, variation in the pattern of selection may be related to predator foraging tactics and prey phenotype and these potentially important interactions remain un-explored.

3.3 METHODS

3.3.1 Animal collection and rearing

All snails used in this experiment were descendents of >100 wild-collected snails from Geneva pond #3 in northwest Pennsylvania (41°, 35' N; 80°, 14' W). Ovipositing snails were placed in plastic containers filled with carbon-filtered, UV-irradiated water in the laboratory at the Pymatuning Laboratory of Ecology (PLE; Linesville, PA), and fed ground Spirulina (O.S.I. Marine Lab, Inc., Burlingame, CA) *ad libitum*. The experimental room was held at 22°C with 12-hr light/dark cycles during hatching and the subsequent experiment. Crayfish (*Procambarus acutus*) were collected from the Thompson gravel pit (41°, 40' N; 80°, 30' W) in May 2006, held in 200-liter pools outside, and fed *P. acuta* snails and rabbit chow *ad libitum* until needed.

3.3.2 Trait induction

In order to produce predator-induced and "uninduced" (i.e., no predator exposure) snail phenotypes, I set up 20, 200-liter plastic wading pools outside PLE (hereafter, I refer to snails that were never exposed to predator cues as "uninduced" as a convenient shorthand; I do not mean to assert that they were not *induced* by anything). On 22 May 2006, these pools were filled with well water, supplemented with 5 g rabbit chow as an initial nutrient source and an aliquot of pond water containing zooplankton and phytoplankton from three natural ponds. These pools were covered to prevent colonization by insects and amphibians and aged for two weeks to allow periphyton to grow in the pools as a food source for the snails. Each pool was equipped with a predator cage composed of a 10-cm section of corrugated PVC pipe covered with window screen at both ends. These cages allow chemical cues from predators to diffuse into the pools without allowing the predators to kill any of the focal animals. On 5-6 June, 100 hatchling (i.e., ~2-week old) snails were added to each pool. These snails represent a random sample among all of the offspring of the wild-caught snails (described above) and were not individually marked. Ten of these pools had empty predator cages while the other 10 pools had a crayfish placed into the predator cage. These crayfish were fed ~250 mg of *P. acuta* three times/wk. When feeding the predators, the cages in the predator-free pools were lifted to equalize disturbance. Approximately 5 g of additional rabbit chow was added to these pools once/wk to provide adequate food for the snails. On 9 July all predator cages were removed from the pools. On 10 July the 20 snail pools were drained and all snails were collected. All snails from the 10 predator-free pools were mixed; snails from the predator-induced pools were likewise mixed to randomize any effects of this rearing environment.

3.3.3 Selection experiment

To examine the strength and direction of selection on predator-induced and uninduced traits, one needs to expose predator-induced and uninduced individuals to selection by lethal predators and estimate selection by comparing the phenotypes of the survivors to the phenotypes of the initial samples. To accomplish this, I set up a selection experiment using three combinations of snail phenotypes. All selection trials took place in 10-liter plastic tubs filled with 3 liters of water. To these containers, I added either 10 predator-induced snails, 10 uninduced snails, or 5 predator-induced snails + 5 uninduced snails. To keep track of the predator induction, I marked snails with fast-drying nail polish, which has been shown to be harmless to the snails (Henry and Jarne 2007). To control for any potential effects of marking, I marked one-half of the predator-induced snails and one-half of the uninduced snails.

I had enough snails to set up 143 tubs of 10 snails each. From these, 102 tubs were selected for exposure to a lethal crayfish (34 tubs for each of the three snail-phenotype combinations). All snails in the selection trials were fed and allowed to acclimate for 20 hr. After adding snails to these tubs, I collected 102 crayfish from the outdoor culture pools, isolated the crayfish in 1-liter containers in the lab, and left them overnight. Crayfish were not fed during this period. On 11 July, one randomly selected crayfish (mean carapace length $\pm$ st. dev. = 2.49 cm + 0.29; range = 1.89 – 3.47 cm) was added to each of the 102 tubs and allowed to begin consuming the snails. The total duration of the experiment was 4 d because I expected that predation would be rapid and were most interested in the initial effects of predation (i.e., selection on morphology and not long-term survival). All containers were checked every 1.5 hr for the first 24 hr and every 3 hr for the subsequent 72 hr. A tub was terminated and the surviving snails preserved in 10% formalin when the crayfish had consumed at least 5 snails (i.e.,

50%) or at 96 hr. I recorded the number of snails consumed at each checkpoint to assess predation rate. This protocol facilitates a comparison between the initial phenotypic distribution and the distribution following selection.

Based on previous observations, I knew that crayfish would kill the snails in one of two ways. Crayfish can either crush the shell or reach into the shell and extract the flesh. I quantified how the crayfish killed the snails by recording whether snails were crushed or extracted when I checked survivorship throughout the experiment. Emptied shells were collected and preserved separately from the survivors. Therefore, I could examine how the different phenotypic combinations affected the predation rate (the number of snails killed per hour), the proportion killed (total number of snails killed in a tub divided by 10), and the proportion of killed snails that were crushed. Therefore, for each tub, the proportion crushed, the proportion extracted, and the proportion that survived sum to 1.

The remaining 41 tubs (from my original 143) were placed in the lab in the same manner as the experimental tubs to assess any mortality due to my handling. There were 14 tubs of uninduced snails, 13 tubs of predator-induced snails, and 14 tubs of the uninduced/predator-induced combination. Survival at 24 hours was 100% and these "initial-sample" snails were subsequently preserved in 10% formalin to assess induction and provide a sample of the phenotypes that were exposed to selection. Both marking schemes (uninduced marked and predator-induced marked) were represented equally in these initial samples.

3.3.4 Statistical analysis

I conducted separate analyses to assess the effects of induction by non-lethal crayfish and selection by lethal crayfish. I was specifically interested in examining induction and selection on

overall size and relative shell shape, so the first step in my analysis was to size-adjust all shape variables. I analyzed tub means of uninduced and predator-induced snails that were not exposed to lethal crayfish to assess the effects of predator induction. To understand the relationship among traits, I calculated among-trait correlations using data from individuals. The strength and direction of selection was examined by comparing trait means for snails that were not exposed to a lethal predator with snails that survived predation. Lastly, I explored the consequences of snail defenses by comparing the effect of snail phenotype on predation rate and the mode of predation.

3.3.4.1 Analysis of induction

The first step in my statistical analysis was to determine the relative shape of the snails by making all morphological variables mass-independent. I began by placing the preserved snails in a drying oven for 24 hr to remove any liquid from within the shell. Individual snails were weighed to nearest 0.01 mg and photographed with a digital camera. Images were viewed using Optimas (Bothell, WA) and four shell measurements were recorded: shell length, shell width, aperture length, and aperture width (each dimension was measured at the maximum for each snail; Fig. 3.1). Shell thickness was also measured to the nearest 0.01 mm with digital calipers at the leading edge of the shell. To standardize morphological measurements for size and visualize the effects of induction in my initial sample, I conducted a MANCOVA with log-transformed mass as a covariate and the five log-transformed shell traits as response variables. The MANCOVA included predator induction as a fixed effect and the assumptions of the model were verified, including the absence of treatment-by-response variable interactions (i.e., all treatment slopes were parallel). I saved the residuals from the MANCOVA and subsequently summed each individual's residual value and the estimated marginal mean for each treatment. This procedure produces estimates of shape variables evaluated for individuals of equal size and has

been successfully used in previous studies of morphological plasticity (e.g., Auld and Relyea 2008). Size-independent trait values and mass were then averaged for all individuals within a tub and these tub means served as my final response variables. All statistical analyses were performed using SPSS (v.11 for Mac) and EXCEL.

I examined the effects of predator induction on mass and morphology using tub means from my initial samples (i.e., snails that were not exposed to lethal predators). Only uninduced and predator-induced tubs were included in this analysis (i.e., the combined uninduced / predator-induced treatment was excluded because I was specifically examining the effects of induction and this treatment was expected to be intermediate). I conducted a MANOVA with predator treatment as a fixed effect and used mass and my five size-adjusted shell characters as my response variables. Univariate comparisons were examined when the multivariate effect was significant. To provide a comparison with previous studies (e.g., DeWitt et al. 1999, 2000), I also analyzed the aspect ratio (i.e., length/width) of the entire shell and the shell's aperture. Aspect ratio analyses were conducted using size-adjusted measures of shell and aperture dimensions as well as unadjusted dimensions, but these two methods provided the same answer qualitatively. I calculated bivariate Pearson correlation coefficients among mass and the five shell traits. Correlations were calculated based on individual snail traits and were estimated separately for predator-induced and uninduced snails. I used individual trait values (as opposed to tub means) because I wanted to directly assess the individual phenotypic correlations favored by selection and because all individuals were independently drawn from a mix of all of my induction pools (see above).

3.3.4.2 Analysis of selection

To assess the strength and direction of selection on predator-induced morphology, I needed to size-adjust all the measurements for snails exposed to lethal predators. Because the snails that survived predation represent a phenotypic subset of the initial samples described above, I used the same regression coefficients estimated in the size-adjustment analysis of my initial samples. With my estimate of the slope and intercept for each regression of a shell trait on mass, I calculated the residuals for each individual's traits as

$$e = y - a - bx$$

where e is the residual for a regression of y (log-transformed trait value) on x (log-transformed mass), a is the intercept and b is the regression coefficient (Lynch and Walsh 1998, p. 39). In this way, I size-adjusted the data using a regression based on the phenotypic distribution prior to selection by lethal predators. By adding the estimated residual to the estimated marginal mean for each treatment (calculated from the initial samples), I obtained size-independent measurements of shell dimensions and shell thickness for all snails that survived predation. All size-independent response variables were then averaged within a tub to provide final response variables, as described above. I calculated selection intensity for each phenotype-combination treatment by dividing the difference between each tub exposed to a lethal predator and the mean of all initial samples by the standard deviation of the initial samples. I calculated 95% confidence intervals to assess whether these estimates of selection intensity differed from zero.

I also estimated selection intensity on predator-induced and uninduced snails in the predator-induced/uninduced combination treatment. This was done, as described above, by calculating tub means for predator-induced and uninduced snails (i.e., two estimates from each tub in the combination treatment, one for uninduced snails and one for induced snails), and

dividing the difference between these estimates and their corresponding means calculated from my initial samples by the standard deviation of the initial samples. This was done primarily to explore how selection on predator-induced and uninduced snails changed when they were alone compared with when these phenotypes were combined. Clearly, as I extracted two means from the same tub they are non-independent, but I aim to use to this merely for comparison with selection in the single-phenotype treatments.

Finally, I wanted to assess the consequences of my snail-phenotype-combination treatments on several aspects of predation including predation rate, the proportion of snails that were killed, and the proportion of killed snails that were crushed. Preliminary analyses demonstrated heteroscedasticity-of-error variances and deviations from normality in these variables, so the data were ranked and analyzed using a Kruskal-Wallis test. Predation rate from one tub in the uninduced-phenotype treatment was excluded from this analysis as an outlier (based on Dixon's test; Sokal and Rohlf 1995, p. 406); the predation rate in this tub was more than an order of magnitude greater than the average for this treatment. Additionally, even though crayfish size did not differ among the three snail-phenotype-combination treatments (ANOVA, $F_{2,99} = 1.893$, $P = 0.156$), I examined the effect of crayfish size (i.e., carapace length) on my measures of predation through multiple regressions of carapace length on predation rate, the proportion of snails that were killed, and the proportion of killed snails that were crushed. In short, I found that larger crayfish killed a larger proportion of snails, but crayfish size did not affect predation rate or the proportion of killed snails that were crushed (analyses not presented).

In sum, I ran 13 regressions, 9 Kruskall-Wallis comparisons, and estimated 60 phenotypic correlations. Significance levels for these 82 analyses were adjusted to control for the false-discovery rate (Verhoeven et al. 2005) in making multiple comparisons. The

significance levels for univariate tests that follow a multivariate test do not need to be adjusted as I only evaluated univariate tests when the multivariate test was significant (Zar 1999).

3.4 RESULTS

3.4.1 Trait induction

Predator cues significantly affected the size and shape of *P. acuta* snails (Table 3.1, Fig. 3.1). While the homoscedasticity-of-error-variance assumption of MANOVA was violated for shell length, shell width and aperture width, all other assumptions were upheld. While MANOVAs are generally robust to violating this assumption (Zar 1999), I also utilized a Kruskal-Wallis test and obtained the same qualitative results. Crayfish induced a 78% increase in mass, but a decrease in size-independent shell dimensions (Fig. 3.1 A-C). Predator-induced snails had 2% shorter shells and 38% narrower shells than uninduced snails. Additionally, predator-induced snails had 3% shorter apertures and 5% narrower apertures than uninduced snails (Fig. 3.1D-E). Predator cues also induced a 9% increase in shell thickness (Fig. 3.1 F). Therefore, predator cues led to the production of more compact, dense shells and an increase in overall mass. I found no effect of predator induction on either shell aspect ratio or aperture aspect ratio ($P > 0.1$).

3.4.2 Selection and trait correlations

While cues from predatory crayfish induced significant changes in snail size and shape in a coordinated manner, selection by lethal crayfish had disparate results compared with the pattern of induction (Fig. 3.2). I detected significant selection intensities on each of the six traits I examined (based on the exclusion of zero from 95% confidence intervals), but the magnitude and direction of these estimates varied based on the snail-phenotype treatments (Fig. 3.3 A). In the uninduced-phenotype treatment, I detected positive selection intensities for mass and the four shell dimensions, but no selection on shell thickness. However, in the predator-induced treatment, I observed a different pattern of selection. I did not detect selection on mass, shell length, or aperture length, but did observe selection for increased aperture width, increased shell thickness and decreased shell width. In the predator-induced/uninduced combination treatment, the pattern of selection was intermediate to the pattern of selection in the two single-phenotype treatments. Here, massive snails with wide apertures and thick, narrow shells experienced increased survival (Fig. 3.3 A). Collectively, only one trait (i.e., aperture width) was under consistent selection in all three phenotype-combination treatments, four traits (i.e., mass, shell length, aperture length, and shell thickness) were under selection in some treatments but not others, and one trait (i.e., shell width) was under positive selection in one treatment but negative selection in the other two treatments.

By estimating selection intensities separately for predator-induced and uninduced snails in the predator-induced/uninduced combination treatment, I can explore whether selection changes when predator-induced and uninduced snails are combined compared to when they are separated. Indeed, I see similar, but slightly different patterns of selection on these traits when they are in combination (Fig. 3.3). As when predators selected on only uninduced snails,

selection on uninduced snails in combination with predator-induced snails favored increased mass and all shell dimensions, but also increased shell thickness (*cf*. Fig. 3.3 A, B). Similarly, selection on predator-induced snails in combination with uninduced snails favored increased aperture width, increased shell thickness, and decreased shell width. However, I detected selection for increased mass and decreased aperture length when predator-induced snails were in combination with uninduced snails, but not when predators selected on predator-induced snails alone.

These patterns of differential survival resulted in a change in the phenotypic correlation structure. Prior to selection, shell length, shell width, aperture length, and aperture width were tightly correlated to each other, both in the predator-induced snails and in the uninduced snails (Table 3.2). The correlations among traits were always of greater magnitude for uninduced snails. Following selection, the correlation structure of these six traits changed dramatically such that the magnitude of every correlation among shell and aperture dimensions increased for predator-induced snails and decreased for uninduced snails. Snails that survived predation expressed positive correlations between mass and shell length, aperture length, and aperture width (Table 3.2). Additionally, correlations between shell thickness and mass, shell length, aperture length, and aperture width appeared and were strongly negative. Thus, the disparate patterns of induction and selection are influenced by the combination of snail phenotypes presented to the predator and the underlying pattern of trait correlations.

3.4.3 Mode of predation

Crayfish exhibited different foraging success based on the combination of snails with which they were presented. Analysis with non-parametric Kruskal-Wallis tests revealed

significant differences between the uninduced treatment and the predator-induced treatment for all three variables (i.e., predation rate, proportion killed, and proportion crushed; Fig. 3.4). Predation rate was the highest when predators were presented with uninduced snails and decreased when snails had been induced by predator cues ($X^2 = 28.631$, $P < 0.001$; Fig. 3.4 A). The predation rate on snails in the uninduced/predator-induced combination treatment was intermediate and statistically different from predation rate in the uninduced treatment ($X^2 = 8.645$, $P = 0.003$) and in the predator-induced treatment ($X^2 = 9.447$, $P = 0.002$). This increased predation rate on uninduced snails resulted in an increased proportion of snails that died in the uninduced treatment compared to the predator-induced treatment ($X^2 = 29.259$, $P < 0.001$; Fig. 3.4 B). Similar to predation rate, the proportion killed in the uninduced/predator-induced combination treatment was intermediate and statistically different from the uninduced treatment ($X^2 = 11.392$, $P = 0.001$) and the predator-induced treatment ($X^2 = 9.032$, $P = 0.003$).

Additionally, crayfish used a different foraging mode to kill snails had been exposed to predator cues. While 100% of uninduced snails were crushed, only 70% of the killed predator-induced snails were crushed ($X^2 = 23.210$, $P < 0.001$; Fig. 3.4 C). The remaining 30% were extracted from their shells leaving the shells completely intact. The proportion killed in the uninduced/predator-induced combination treatment was intermediate and statistically different from the predator-induced treatment ($X^2 = 7.596$, $P = 0.006$) and the uninduced treatment ($X^2 = 6.466$, $P = 0.011$). Because predation rate on uninduced snails was quite rapid, it seems unlikely that any induction took place during the selection phase of the experiment (i.e., snails in the uninduced treatment did not have much time to induce a defense).

3.5 DISCUSSION

Predation is a potent agent of selection that is known to affect the distribution of prey phenotypes in nature (e.g., Vermeij and Covich 1978; Vermeij 1979; Osenberg and Mittelbach 1989; Crowl 1990; Reznick et al. 1990; Trussell 1996, 2000a; Trussell and Smith 2000). Many prey organisms have evolved the ability to alter their phenotypes in response to predators in ways that increase survival (Tollrian and Harvell 1999). Such inducible defenses can represent adaptive plasticity if different trait values are favored in the presence and absence of predators. To understand the adaptive nature of inducible defenses, we must assess the relationship between phenotypes expressed in the presence/absence of predation risk and how these phenotypes affect survival in the presence of a predator. Here I have shown that chemical cues from predatory crayfish induce a suite of morphological traits in a common freshwater snail, and while some traits are induced in the direction favored by selection, others respond in the opposite direction. My results also show how the pattern of selection can change based on prey phenotype and highlight a mechanism for this change—flexibility in the predator's foraging mode based on prey defense.

3.5.1 Are predator-induced phenotypic changes adaptive?

I can understand the adaptive value of the observed predator-induced changes in size, shape, and shell architecture by examining whether patterns of induction are consistent with patterns of selection (e.g., Van Buskirk et al. 1997; Van Buskirk and Relyea 1998). If traits are selected in the same direction in which they are induced, the plastic adjustment of a trait in response to predator cues may be favored. Alternatively, when traits are induced and selected in

opposite directions, plastic adjustment of the trait in response to predator cues is not adaptive. In both cases, it is important to examine the correlation structure of the traits because correlations can constrain expression of the optimal phenotype.

Increased mass and shell thickness were induced and selected for by crayfish predators (Fig. 3.2 A, F), but I detected significant selection on mass only in the uninduced and combination treatments and significant selection on shell thickness only in the predator-induced and combination treatments (Fig. 3.3). The pattern of selection in the combination treatment was intermediate to the pattern of selection in the two single-phenotype treatments because selection on induced and uninduced snails in combination was similar to selection on these two phenotypes in isolation (*cf*. Fig. 3.3 A, B). The pattern of selection on mass most likely results because predator-induced snails were 78% larger than uninduced snails and *Procambarus* crayfish are small-size-selective predators that cannot consume prey that have reached a size refuge (J.R. Auld, *pers. obs.*; Juanes 1992). We know from previous studies that this predator-induced increase in size is attained by increasing growth rate and delaying reproduction (Auld and Relyea 2008). This corresponds to results from other studies on *Physa*, other genera of freshwater snails, and other types of organisms (Crowl 1990; Crowl and Covich 1990; Reznick et al. 1990; Riessen 1999; Hoverman et al. 2005) demonstrating a trade-off between early growth and early reproduction.

Given that a large fraction of uninduced snails were killed quickly and that they had relatively thin shells compared to predator-induced snails, the absence of selection on shell thickness in the uninduced treatment may result from the fact that predators could easily crush these smaller uninduced snails and those that survived may have avoided death by some means unrelated to shell thickness. My results for shell thickness are similar to those obtained in a

marine decapod-gastropod predator-prey interaction (Trussell 2000a, b; Trussell and Smith 2000; Rochette et al. 2007). In these studies, predatory crabs (*Carcinus maenus*) induce an increase in shell thickness in marine snails (*Littorina obtusata*) that is an effective defense against crab predation. Additionally, these crab predators utilize multiple foraging modes, crushing the snails if they are small or using a complex, shell-entry tactic termed "winkling" when the snails are large and thick-shelled (Rochette et al. 2007).

In my study, shell thickness and mass also played an apparently strong role in affecting the mode of predation: while 100% of uninduced snails were completely crushed, crayfish switched to shell-entry to kill 30% of their predator-induced snails. Furthermore, I saw the highest predation rate and proportion killed for uninduced snails, compared to predator-induced snails, and the combination-phenotype treatment was intermediate to these two single-phenotype treatments (Fig. 3.4). Therefore, these predator-induced changes in mass and shell thickness represent adaptive forms of phenotypic plasticity.

Conversely, while predator cues induced a decrease in all relative shell dimensions (shell length and width, aperture length and width), selection did not favor relatively small shells overall. Selection in the uninduced-phenotype treatment consistently favored individuals with relatively long, wide shells and long, wide apertures. Therefore, even though these shell dimensions were relative (i.e., independent of mass), having a relatively larger shell provided a survival benefit against a lethal crayfish. Only one of these shell-dimension traits (shell width) was selected in the same direction as it was induced. When crayfish selected upon snails in the predator-induced and combination treatments, snails with relatively narrow shells survived. This is consistent with previous data showing that crayfish predators select for relatively narrow, entry-resistant shells (DeWitt et al. 2000). While the more frequently employed mode of

predation by crayfish in my study was crushing, snails that were killed in the predator-induced treatment were indeed killed by shell entry ~30% of the time. Therefore, snails may respond to chemical cues from crayfish in a consistent manner, even if different species of crayfish employ a variety of foraging modes (see Langerhans and DeWitt [2002] for a similar situation with *Physa* responses to molluscivorous and non-molluscivorous sunfish). One trait in particular, aperture width, is particularly conspicuous. Crayfish cues induced a reduction in relative aperture width, but crayfish selected for increased aperture width in all snail-phenotype treatments (Fig. 3.2 E). Potentially, possessing a wide aperture may confer some crush-resistance (as in DeWitt et al. 2000), and as shell crushing was the most common mode of predation in all snail-phenotype treatments, this provides some explanation for the pattern of selection. However, aperture width was the trait under the most intense selection (Fig. 3.3) and therefore we would expect it to be induced in a direction consistent with selection. Arguably, prey cannot adjust one trait independent of all others and the "maladaptive" response I observed in aperture width may result from a constraint due to other correlated characters.

3.5.2 The importance of trait correlations

The pattern of trait correlations changed before and after selection (Table 3.2). Before selection, all shell dimensions were very tightly correlated for both uninduced and predator-induced snails. Following selection, shell dimensions became less correlated for uninduced snails and more correlated for predator-induced snails. Additionally, all shell dimensions except shell width became positively correlated with mass following selection, signaling that more massive snails with relatively long shells and long, wide apertures survived predation better. Additionally, negative correlations between shell thickness and most shell dimensions arose after

selection meaning that the survivors of predation that had thick shells were relatively less massive and more compact. This is consistent with the view that snails cannot simultaneously produce relatively large and thick shells (*cf.* Trussell 2000b). Interestingly, the only shell thickness-shell dimension correlation that did not appear following selection was between shell thickness and shell width for predator-induced snails. This indicates that snails that produced thick shells and survived predation did not have a consistently wide or narrow shell. Unfortunately, my experimental design does not permit a distinction between direct selection operating on a trait and indirect selection operating on a correlated trait (*sensu* Lande and Arnold 1983), but we can deduce that certain trait combinations increase the chance of survival in an encounter with a lethal predator. This appears to be the first example of how the pattern of correlations among a set of predator-induced defenses changes in response to selection. Importantly, future studies that separate the direct and indirect targets of selection will facilitate a greater understanding of how the suite of traits that are induced by a predator are integrated into a functional response that can be favored by selection (DeWitt and Langerhans 2003; Merilä and Björklund 2004; Relyea 2004). In sum, the pattern of change in trait correlations after selection is consistent with the pattern of selection and the mode of predation on predator-induced snails.

Taken together, we can see some patterns in how phenotypic correlations influence patterns of induction and selection. When crayfish selected on predator-induced snails, narrow shells were favored, presumably because a narrow shell inhibits the shell-entry ability of crayfish (DeWitt et al. 2000). If such a defense is favored when snails are exposed to crayfish, induction of a narrow shell may be favored when individuals detect the presence of a crayfish predator. Interestingly, as mentioned above, this defense was partially effective here, but not as effective as producing a thick shell. Importantly, due to the strong correlation structure among shell

dimension traits (i.e., shell length and width, aperture length and width), the induction of a narrow shell in response to predator cues may also lead to the expression of reduced shell length, aperture length, and aperture width. In this way, traits may be induced in a direction that is counter to that favored by selection. Intense, positive selection on traits like aperture width may essentially be negated by intense, negative selection on other, correlated characters.

In general, phenotypic correlations can result from either underlying genetic correlations or similar environmental factors (Houle 1991), but in practice phenotypic correlations tend to be fairly good approximations of underlying genetic correlations (Roff 1996). Indeed, I found similar results in other work using the same system of crayfish and snails (Auld, *unpubl. data*). This means that the patterns of phenotypic correlation I observed may represent genetic constraints on how traits evolve. Additionally, underlying genetic correlations can create patterns of response to selection that may lead to indirect selection on correlated characters. Given sufficient additive genetic variation, we would expect the traits that are under the most intense selection to respond most directly to selection. As previously stated, I cannot distinguish direct from indirect selection (*sensu* Lande and Arnold 1983), but I can hypothesize that, if phenotypic correlations provide an approximate estimate of genetic correlations, the pattern of induction I observed may be favored by strong selection on at least one of the correlated characters (e.g., shell width).

3.5.3 Toward a mechanistic understanding of the changes in the pattern of selection

Understanding the complex nature of *how* and *why* the pattern of selection on a set of inducible traits changes due to changes in the traits themselves and a change in the mode of predation has made the cause-and-effect relationship underlying the adaptive nature of these

predator-induced defenses more clear. Indeed, experimental manipulation of the environmental factor that both affects the distribution of phenotypes exposed to selection and imposes the covariance between phenotype and fitness that results in selection is critical to evaluate the cause of selection on any phenotype (Wade and Kalisz 1990). In my study, I saw that predators cause selection for large size when their prey are small, but a complicated and somewhat conflicting set of phenotypes when prey are larger and predator-induced. The predator-induced increase in mass is likely to be the best line of defense as vulnerability decreases with increased size. Additionally, increased shell thickness and aperture width are likely to provide some resistance to shell-crushing while the expression of a narrow shell inhibits the shell-entry ability of crayfish (DeWitt et al. 2000; Trussell 2000b). As shell width and aperture width are positively correlated, these traits cannot easily be induced in opposite directions, and therefore the expression of a perfectly defended phenotype (i.e., resistant to shell-crushing and shell-entry) is practically impossible. Therefore, while we can document the constraints involved in producing a defense, the mechanisms underlying the adaptive benefits of maintaining plasticity in these traits is made clearer when we understand the change in the mode of predation that predators demonstrate. Conflicting patterns of induction and selection can arise when the mode of predation is influenced by prey phenotype, and a thorough understanding of the adaptive nature of inducible defenses relies on demonstrating these complexities. Future studies must consider that if predators are capable of adjusting their own traits in response to the expression of a defense in their prey, the coevolution of inducible offenses and inducible defenses may escalate into a sort of predator-prey arms race. Predator-induced offenses have been shown to exist in other species interactions (e.g., Kopp and Tollrian 2003*a*, 2003*b*; Kishida et al. 2006) and the coevolution of plasticity in defense and offense may be a general pattern in numerous systems. One recent

example that highlights the potential generality of such coevolutionary interactions comes from a study demonstrating that a salivary enzyme produced by an herbivorous caterpillar functions to inhibit the anti-herbivore secondary compounds produced by tobacco and tomato plants (Musser et al. 2005). In this system, it is not clear whether the change in the herbivore phenotype will alter the pattern of selection on the plants, but if this does occur (e.g., different physiological or morphological traits are exposed to selection following the induction of an inducible offense) we can hypothesize that an escalatory arms race between enemy and victim may occur. Generally, an increased focus on how all the members of an ecological interaction respond to environmental changes will facilitate a deeper understanding of how and why plasticity evolves (Lima 2002).

3.5.4 Conclusions

I have shown that the predator-induced suite of traits expressed by snails exposed to chemical cues from crayfish increases survival when snails are exposed to lethal crayfish. Subsequent studies using a greater array of predator-densities (i.e., chemical cue concentrations) and/or predator types will further elucidate the complex components that maintain adaptive plasticity in morphology. Selection on predator-induced snails is probably the most natural situation, but as crayfish (and many other predators) can colonize ponds, predation on uninduced snails is feasible in a natural population. Future work using individually marked snails may reveal greater detail on the form of selection (e.g., stabilizing, nonlinear, etc.), but due to the nature of the selective agent (i.e., predation) and the range of phenotypes expressed, I expect that selection is indeed directional in form.

Additionally, I have demonstrated that the pattern of selection on prey traits can change based on changes in prey traits themselves as well as reciprocal changes in the foraging mode

employed by predators. By demonstrating how the pattern of selection and mode of predation change across environments and are mutually interactive, I have made inroads into understanding the complex reasons of *how* and *why* inducible defenses are constructed and maintained. In general, if most inducible defenses are in fact adaptations to variable predation risk, demonstrating the reciprocal changes that occur in predator and prey traits are important but relatively neglected aspects of demonstrating why plasticity in defense has evolved as it has. Therefore, if adaptive plasticity in general affords the opportunity to maintain fitness in multiple environments, we need to consider all of the interacting species that are involved and examine how each player in an interaction adjusts their traits to other species' plastic traits (i.e., is there evidence for the coevolution of adaptive plasticity?).

Table 3.1. Results of a MANOVA on morphological traits with predator-induction treatment as a fixed effect. Shell traits are size-independent and data were transformed prior to analysis (see text for details). Multivariate test results are shown in boldface type. Univariate test results are in plain type.

	df	*F*	*P*
Predator	**6, 20**	**30.44**	**<0.001**
Mass	1, 25	123.65	<0.001
Shell length	1, 25	6.81	0.015
Shell width	1, 25	12.28	0.002
Aperture length	1, 25	17.25	<0.001
Aperture width	1, 25	9.27	0.005
Shell thickness	1, 25	38.93	<0.001

Table 3.2. Phenotypic (Pearson) correlations among mass and five morphological variables before (top value) and after (bottom value) selection by lethal crayfish. Only significant correlations are shown. Correlations for predator-induced snails are given *below* the diagonal; correlations for uninduced snails are *above* the diagonal. Correlations were calculated based on individual traits (Initial samples, N = 201 for predator-induced snails, N = 209 for uninduced snails; Survivors, N = 377 for predator-induced snails, N = 234 for uninduced snails).

	Mass	Shell Length	Shell Width	Aperture Length	Aperture Width	Shell Thickness
Mass		–	–	–	–	–
		0.214	–	0.288	0.346	-0.332
Shell Length	–		0.691	0.848	0.628	–
	0.328		0.556	0.751	0.501	-0.293
Shell Width	–	0.504		0.718	0.580	–
	–	0.603		0.656	0.485	-0.282
Aperture Length	–	0.713	0.605		0.645	–
	0.272	0.753	0.665		0.547	-0.373
Aperture Width	–	0.398	0.355	0.448		–
	0.376	0.521	0.430	0.546		-0.197
Shell Thickness	–	–	–	–	–	
	-0.724	-0.284	–	-0.304	-0.279	

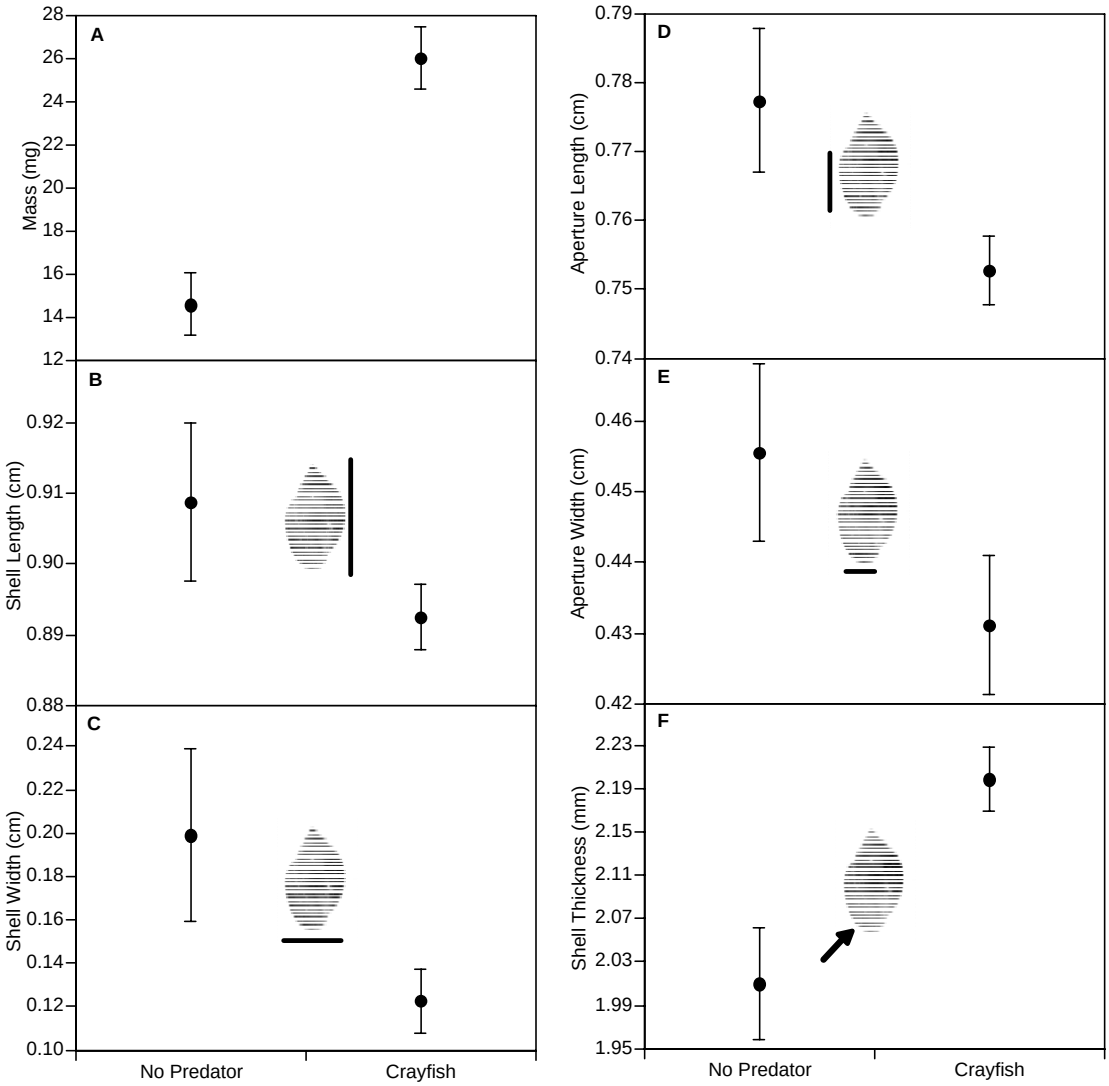

Figure 3.1. Predator-induction effects on mass and five size-independent morphological variables. Morphological variables were log-transformed prior to analysis. Error bars are twice the SE to represent 95% confidence intervals. Inset figures show the shell dimensions that were measured; lines are not drawn to exact scale. The arrow in panel *F* points to the location where shell thickness was measured.

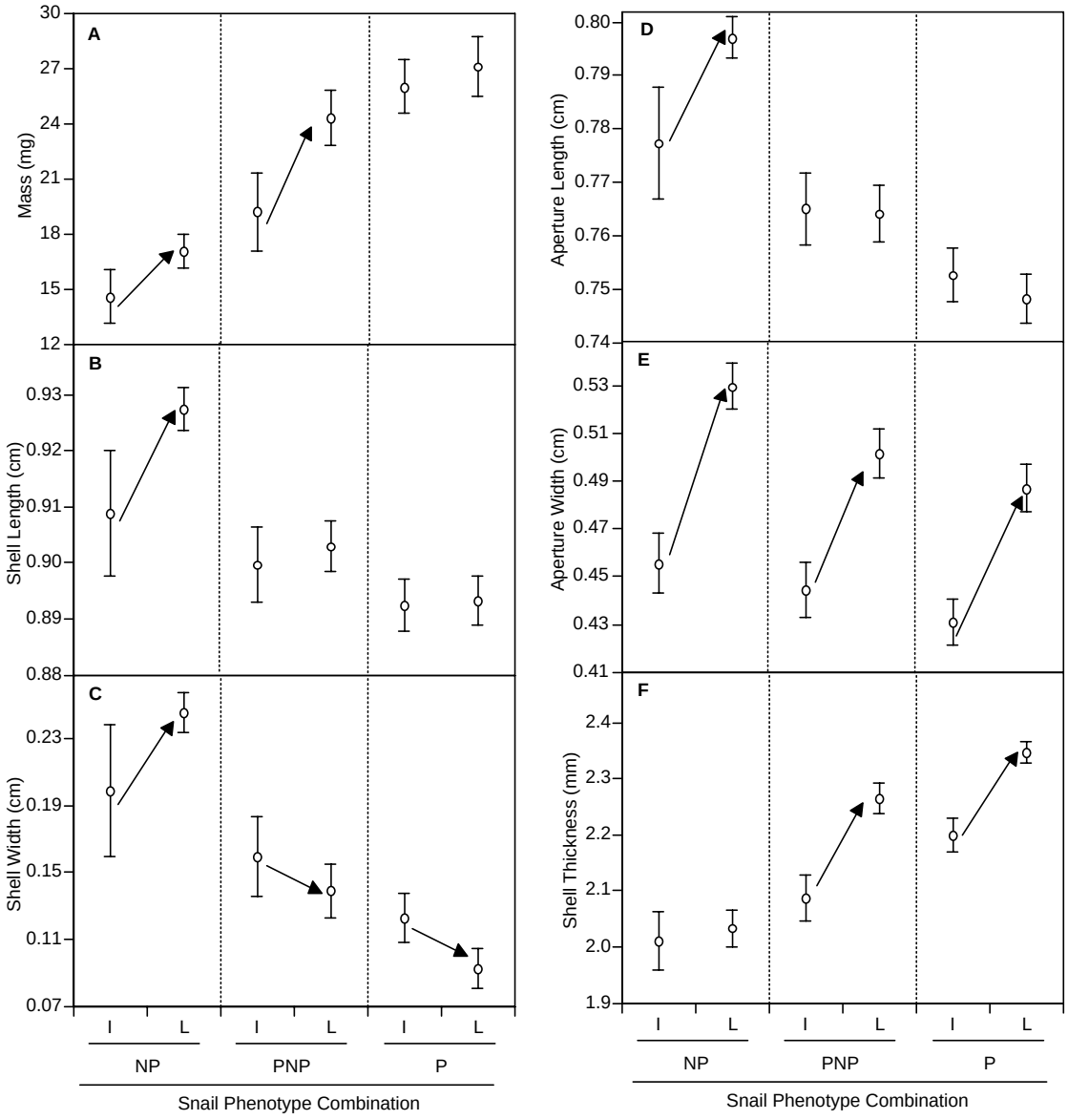

Figure 3.2. Induction and selection by crayfish on mass and five size-independent morphological variables in *Physa acuta*. Along the *x*-axis, "*I*" stands for initial sample meaning induction only. "*L*" stands for lethal, representing the mean of a trait after predation by lethal crayfish. Means for the three different combinations of snail phenotypes are presented separately (predator-induced [P], uninduced [NP], or a mix [PNP]). Error bars are 2*SE. The solid arrows show the direction of change in a trait in response to selection and are provided only for situations where 95% confidence intervals on the selection intensity do not overlap zero.

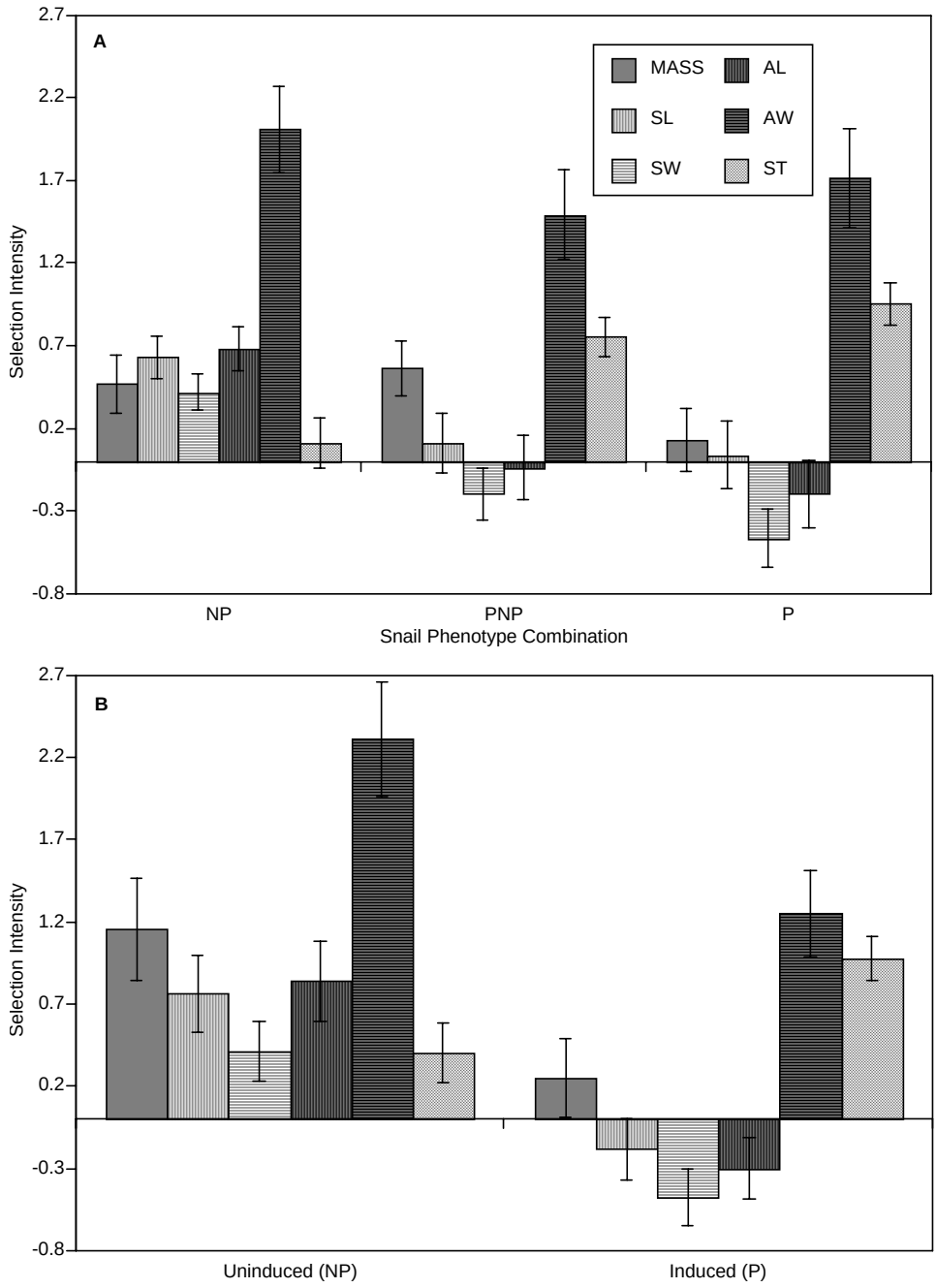

Figure 3.3. Selection intensity of lethal crayfish on mass and five morphological variables. A) Mean selection intensity in each of the three snail-phenotype combinations (abbreviations follow Fig. 3.2). B) Mean selection intensity on the induced and uninduced snails from the combination treatment only (i.e., a decomposition of the middle "PNP" in A). SL, shell length; SW, shell width; AL, aperture width; AW, aperture width; ST, shell thickness. Error bars are 2*SE.

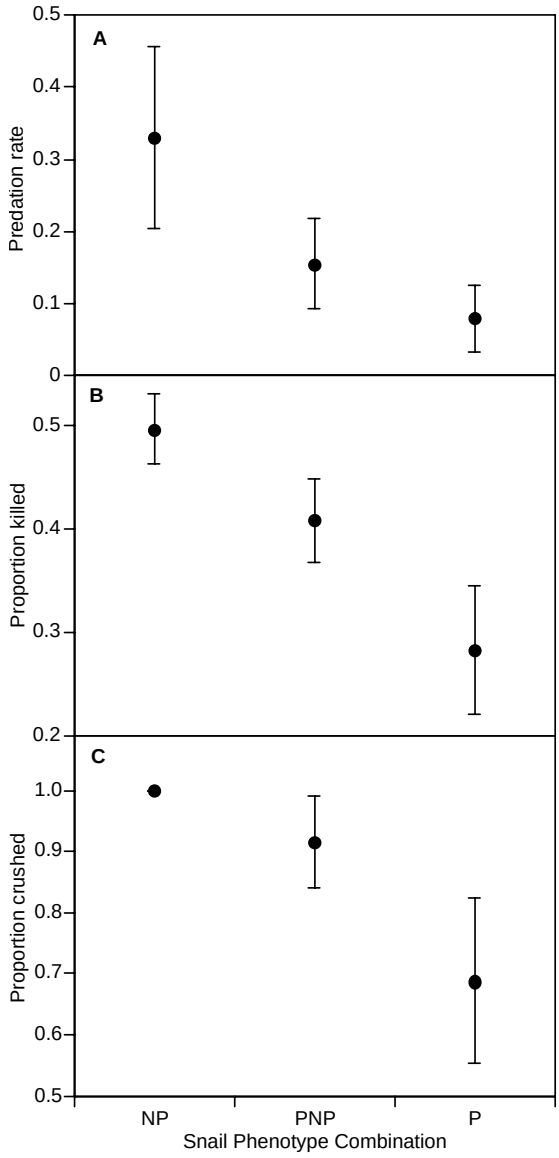

Figure 3.4. Characteristics of predation by crayfish on three combinations of *Physa acuta* phenotypes. Snail-phenotype combination abbreviations follow Figure 3.2. Error bars are 2*SE. A: *predation rate* is the number of snails killed per hour. B: the *proportion killed* is the mean proportion of snails killed in each tub (i.e., by crushing and extraction). C: the *proportion crushed* is the number of snails that were crushed divided by the number of snails killed in each tub. In all tubs 100% of NP snails were crushed (no error bars).

4.0 ENVIRONMENT-DEPENDENT VARIATION IN INBREEDING DEPRESSION IN ADAPTIVE PLASTICITY AND CUMULATIVE FITNESS IN A HERMAPHRODITE

4.1 ABSTRACT

Although a great deal of attention has been paid to the evolution of adaptive plasticity, the potentially important role of inbreeding depression in affecting the expression of such plasticity remains essentially unexplored. I reared selfed and outcrossed freshwater snails (*Physa acuta*) in four environments (predator cues present or absent combined with mate access or no mate access) and quantified changes in snail morphology and life history, inbreeding effects on fitness in different environments, and inbreeding effects on two forms of adaptive plasticity (delayed selfing and an inducible defense). I confirmed previously documented adaptive responses to predator and mate environments. I went on to document that self-fertilization depression occurred in both predator and no-predator environments and that the reduced fitness was due to both inbreeding and isolation. Furthermore, I observed inbreeding depression for both types of adaptive plasticity, demonstrating a novel connection between inbreeding and trait inducibility.

4.2 INTRODUCTION

Adaptive plasticity (i.e., plasticity that has beneficial effects on fitness; Gotthard and Nylin 1995; Dudley and Schmitt 1996) represents a seemingly unbeatable evolutionary strategy in that if an organism can detect and respond appropriately to some environmental cue, fitness can be maximized in multiple environments. Across many systems, we have accumulating evidence that plasticity can be adaptive in that induction of a trait can increase fitness in the inducing environment (e.g., Van Buskirk and Relyea 1998; Tsitrone et al. 2003b; Dechaine et al. 2007; Auld and Relyea, *in review* A [Chapter 2]). However, adaptive plasticity is not ubiquitous and may be constrained by numerous factors (Pigliucci 2001; 2005).

Within natural populations, the mating system (i.e., the pattern of mating among individuals) plays a major role in determining genetic structure and can directly affect fitness (Jarne 1995; Charlesworth 2003). Therefore, the mating system is a major factor influencing genetic variation and may play an important role in the evolution of many traits including adaptive plasticity. The situation is complicated by the fact that the mating system itself is a suite of plastic traits that may be altered under varying environmental conditions. Here, I focus on two main aspects of the relationship between the mating system and adaptive plasticity. 1) How does the relationship between mating system and fitness change among environments (e.g., do the effects of inbreeding change across environments?)? 2) Does the mating system affect how organisms respond to the environment (i.e., does inbreeding affect plasticity?)?

4.2.1 Variation in the effects of inbreeding across environments

In general, the effects of inbreeding on fitness-related traits such as survival and fecundity may differ among environments and this may be due to plasticity in response to environmental conditions (e.g., mate availability) or the actual effects on inbreeding (e.g., inbreeding depression). While many studies on hermaphrodites use enforced self-fertilization as an experimental manipulation to examine the effects of the mating system on fitness, an important distinction should be made between the effects of isolation (i.e., no available mates or pollinators) and inbreeding. Collectively, the combined effects of isolation and inbreeding have been termed self-fertilization depression (Jarne et al. 1991) to reflect the fact that the phenotype may be affected not only by inbreeding but also by mate availability. I examine the consequences of self-fertilization depression by distinguishing the effects of isolation from inbreeding and how these effects change across high-stress and low-stress environments.

It has been hypothesized, with some empirical support, that inbreeding depression may be greater in a stressful environment compared to a more benign environment (Armbruster and Reed 2005). One major natural stressor that has received little if any empirical attention is the effect that predation risk may have on inbreeding depression (Steets et al. 2007a). While previous studies have examined the effects of mating system, mate availability, and predation risk, their potential interactions remain unexplored.

4.2.2 Two types of adaptive plasticity (and how inbreeding may affect them)

It is conceivable that inbreeding could affect many different types of adaptive plasticity. I focus on two well-characterized responses that function to maintain fitness in multiple

environments. First, I examine the effects of inbreeding on plasticity in the age at first reproduction in response to mate availability. Second, I examine the effects of inbreeding on an inducible defense expressed in response to predation risk. Both types of plasticity have been previously demonstrated to be adaptive in my system (freshwater snails; discussed below); however, the effect of inbreeding on these plastic traits is unknown.

4.2.2.1 The waiting time: plasticity in the age at first reproduction

The mating system and the life history of many organisms are closely connected. For example, in a simultaneously hermaphroditic animal with internal fertilization, the age at first reproduction can be adjusted to mate availability (Tsitrone et al. 2003*a*). In preferentially outcrossing organisms, individuals with access to mates often initiate reproduction earlier than individuals without access to mates (Tsitrone et al. 2003*b*; Schjørring 2004; Escobar et al. 2007). Such plasticity is adaptive if it facilitates the avoidance of inbreeding depression (i.e., waiting for a mate to outcross is better than selfing when the relative fitness decrement suffered by inbred offspring is strong). Subsequently, the length of time that individuals delay reproduction in the absence of mates (i.e., the "waiting time") should be under selection corresponding to the magnitude of inbreeding depression (Tsitrone et al. 2003*a*).

Inbreeding has the potential to affect the waiting time in several ways. Inbreeding depression that results in reduced growth may affect the timing of maturity such that the waiting time is altered. Additionally, inbred individuals might express longer waiting times than outbred individuals if there is a compounding negative effect of subsequent inbreeding. When external factors such as small-size-specific predation increase juvenile mortality over adult mortality, the waiting time is predicted to be shorter (Tsitrone et al. 2003*a*). As this is what I have previously observed in this system (i.e., small-size-specific predation; Auld and Relyea, *in review* A

[Chapter 2]), I predict that the waiting time will be reduced in the presence of predation risk. Furthermore, the effects of inbreeding on the waiting time may differ in an environment with enemies if inbreeding depression in growth and survival is different.

4.2.2.2 An inducible defense: plasticity in shell thickness

Many species can induce a defensive phenotype when they detect the presence of an enemy (Tollrian and Harvell 1999). Such inducible defenses are often maintained by allocation trade-offs where the undefended phenotype has higher fitness in a "no-predator" environment or defense against one enemy increases vulnerability to other enemies (e.g., Van Buskirk and Relyea 1998; Relyea 2003). Previous work has shown that snails exposed to predator cues produce thicker shells and that the snails with the thickest shells have the highest survival when exposed to a lethal predator (Auld and Relyea, *in review* A [Chapter 2]). Subsequently, studying how inbreeding can affect the expression of an inducible defense can be informative to how inbreeding alters the perception of environmental cues and the ability to be plastic.

If inbreeding results in a depression in growth or the ability to detect the environment, I predict that the expression of an inducible defense will be impaired in inbred individuals compared to outbred individuals. In this way, inbred individuals may be less defended than outbred individuals, which may be important in natural populations where enemies abound. While it has been previously predicted that inbreeding depression should be stronger in a more stressful environment (e.g., an environment with predators), the possibility for inbreeding depression in an inducible defense has not been previously considered, to the best of my knowledge. If inbreeding not only affects growth and reproduction, but also the ability to produce an environment-specific phenotype, then this represents an important, yet heretofore neglected component of overall inbreeding depression.

4.3 METHODS

To explore the effects of the mating system on fitness and plasticity in morphology and life history, I conducted an experiment using the simultaneously hermaphroditic snail *Physa acuta*. I bred selfed and outcrossed snails and raised them individually under a factorial experimental design. I examined treatment effects on several traits related to fitness, quantified inbreeding depression in multiple environments, and examined the plasticity in several traits to test the hypothesis that inbreeding will not only depress fitness but also disrupt an adaptive plastic response.

All snails used were descendents of wild-caught (G_0; Fig. 4.1) snails from Geneva pond #3 in northwestern Pennsylvania. *Physa acuta* is a preferential outcrosser (outcrossing rates estimated at >90% in numerous populations; Bousset et al. 2004; Henry et al. 2005) that experiences strong inbreeding depression (Jarne et al. 2000; Henry et al. 2003). Additionally, it is known that *Physa acuta* can store sperm (Wethington and Dillon 1991; Dillon et al. 2005) so I assume that the G_1 progeny of these wild-caught snails were outcrossed.

Breeding lines were maintained to produce same-aged selfed and outcrossed G_2 offspring (Fig. 4.1). Siblings from ten G_1 families were split into two groups to be outcrossed or selfed. To outcross the G_1 snails, I placed a new potential mate (marked with non-toxic paint; Henry and Jarne 2007) into each focal snail's container everyday for a two-week period. Selfing snails were left alone until they initiated reproduction. The G_2 offspring from these breeding lines were the basis for the selfed and outcrossed snails utilized in the experiment. All experimental conditions and protocols for these breeding lines and the subsequent experiment were identical to those reported in Auld and Relyea (2008) including snail feeding (*ad libitum* Spirulina three times/week) and water changes (weekly).

Individual G_2 snails were reared in 1 liter of water under a randomized design employing a factorial combination of two previous-mating-system treatments (selfed or outcrossed), two predator treatments (predator cues present or absent), and two mate-availability treatments (mate available or not) yielding eight treatment combinations. Therefore, I simultaneously examined the effects of previous mating history (selfed or outcrossed) and current mating environment (mates present or absent). Using a single individual from each of 10 outcrossed and 10 selfed breeding lines I had a potential total of 80 experimental units. However, two of the selfed lines yielded no offspring when I set up the experiment (n = 8 for selfed treatment combinations) for a total of 72 experimental units. Individual G_2 snails were added to the containers on 30 May 2006 (age = 29 d; initial mass <1 mg); predator-cue and mate-availability treatments began on 31 May and 7 June, respectively, and were implemented as in Auld and Relyea (2008). In short, I produced crayfish-conditioned water by feeding a pond-dwelling crayfish (*Procambarus acutus*) 150 mg of *Physa acuta* three times/week. Prior to each feeding, I collected the water in which each crayfish was held, pooled the water from all crayfish (n = 20), removed 400 ml of water from each experimental unit assigned the predator treatment and replaced it with 400 ml of predator-cue water. Similarly, I removed 400 ml of water three times/week from all no-predator containers and added 400 ml of fresh water. Mate availability was manipulated by adding marked mates to the appropriate containers three times/week for three hours at a time, a duration of time that is sufficient to facilitate copulation (Tsitrone et al. 2003b). Snails in the no-mate treatment remained alone throughout their lives.

4.3.1 Measurement and analysis of snail traits

Following Auld and Relyea (2008), the experiment lasted the entire life of the snails (the last snail died at 267 d old) and I measured a set of life-history traits including age/size at first reproduction, longevity and lifetime fecundity. Experimental units were checked daily, egg masses were marked, and the number of oviposited eggs was counted weekly. During egg counting, the number of eggs that failed to hatch was also counted to quantify egg-hatching proportion. Snails were blotted dry and weighed when they produced their first egg mass and at death (i.e., size at first reproduction and death). Shell thickness, an important defensive trait (Auld and Relyea, *in review* A [Chapter 2]), was measured to the nearest 0.01 mm on 5 July at the leading edge of the shell with digital calipers.

I conducted a MANOVA using R (R Development Core Team 2006) to examine treatment effects on the age and size at first reproduction, age and size at death, the number of reproductive days (age at last reproduction – age at first reproduction), the total number of eggs laid, shell thickness, and the egg-hatching proportion. All variables were *log*-transformed prior to analysis to improve normality (except egg-hatching proportion which was arcsine-square root-transformed). Significant multivariate effects were followed by ANOVAs on specific traits. When appropriate, univariate mean comparisons were conducted. When conducting mean comparisons, I adjusted the significance threshold to control for the false discovery rate (i.e., to balance the risk of Type I and Type II errors; Verhoeven et al. 2005).

4.3.2 Effects of inbreeding on fitness

To provide estimates of inbreeding depression (i.e., the relative fitness decrement suffered by inbred individuals compared to outbred individuals) that were estimated over the lifespan, I used an age-structured model for snails in the eight treatment combinations. These models were constructed with seven stages representing egg, and snails age hatchling – 49 d, 50 d – 99 d, 100 d – 149 d, 150 d – 199 d, 200 d – 249 d, and >250 d (no snails survived to 300 d), where those in the later six stages are capable of reproduction. I used the egg-hatching rate quantified within each treatment as the probability of transitioning from egg to hatchling and estimated the probability of transitioning from hatchling to 50 d old as the probability that an individual within each treatment survived to 50 d. Subsequently, I calculated the probability of surviving from one age class to the next. I therefore estimated age-specific survival probabilities and fecundities.

With these age-specific survival probabilities and fecundities as fitness measures, I estimated fitness depression (δ) using the equation, $\log(\delta) = \log(w_o) - \log(w_s)$, where w is the fitness of outcrossed (w_o) or selfed (w_s) progeny (Johnston and Schoen 1994). Note that these estimates of fitness depression can be back-transformed to the more familiar percent-depression form as $\delta = 1 - 10^{-\log(\delta)}$. I used my fitness estimates to produce five different selfed-outcrossed comparisons (see parallel numbering scheme in Fig. 4.1) in the predator and no-predator treatments:

1) *Outcrossed isolation depression*: the depression in fitness that results from being isolated and forced to self-fertilize for outcrossed individuals. This comparison is made by comparing the fitness of *outcrossed*, *mated* individuals versus *outcrossed*, *isolated* individuals.

2) *Selfed isolation depression*: the depression in fitness that results from being isolated and forced to self-fertilize for selfed individuals. This comparison is made by comparing the fitness of *selfed*, *mated* individuals versus *selfed*, *isolated* individuals.
3) *Inbreeding depression with mates*: the depression in fitness that results from being inbred (i.e., produced through self-fertilization) but still given access to a mating partner and therefore capable of outcrossing. This comparison is made by comparing the fitness of *outcrossed* versus *selfed* snails that are given access to mates.
4) *Inbreeding depression without mates*: the depression in fitness that results from being inbred and forced to self-fertilize compared to individuals that are outcrossed but also forced to self-fertilize. This comparison is made by comparing the fitness of *outcrossed* versus *selfed* snails that are not given access to mates.
5) *Total self-fertilization depression*: the depression in fitness that results from being inbred and forced to self-fertilize compared to individuals that are outcrossed and capable of outcrossing. This comparison is made by comparing the fitness of *outcrossed*, *mated* versus *selfed*, *isolated* individuals.

With these five comparisons, I can make the prediction that total self-fertilization depression ($^{\#}5$) should be greater than all the other depression estimates. Note that $^{\#}5$ should equal the sum of $^{\#}1$ and $^{\#}4$ as well as the sum of $^{\#}2$ and $^{\#}3$ because both of these pairs represent different ways of partitioning total self-fertilization depression. In this way, I can determine whether isolation or inbreeding represents a larger fraction of total self-fertilization depression (i.e., is $^{\#}4 / {}^{\#}5 > {}^{\#}1 / {}^{\#}5$ and is $^{\#}3 / {}^{\#}5 > {}^{\#}2 / {}^{\#}5$?). Following Armbruster and Reed (2005), I also use this data to test the hypothesis that inbreeding depression should be stronger in more stressful environments (i.e., stronger in the predator treatment than in the no-predator treatment).

4.3.3 Effects of inbreeding on adaptive plasticity

I was specifically interested in testing the hypothesis that inbreeding not only depresses fitness (i.e., survival and reproduction), but also that inbreeding impairs the expression of two types of adaptive plasticity: the waiting time prior to selfing and the predator-induced increase in shell thickness. I quantified the waiting time for selfed and outcrossed snails in the presence and absence of predator cues by taking the difference between age at first reproduction in the no-mate and mate treatments (Tsitrone et al. 2003b; Escobar et al. 2007). Similarly, I quantified the plasticity in shell thickness for selfed and outcrossed snails in the presence and absence of mates by taking the difference between shell thickness in predator and no-predator environments. These estimates were obtained by taking the mean of all snails in each treatment combination and calculating the difference between the appropriate pairs (e.g., waiting time for selfed snails in a no-predator environment is obtained by subtracting the mean age at first reproduction for selfed snails in the no-predator environment raised *with mates* from the mean age at first reproduction for selfed snails in the no-predator environment raised *without mates*). To obtain confidence intervals on these estimates, I bootstrap-sampled my data for each treatment combination 1000 times, took the appropriate difference between these randomly aligned bootstrap-sampled means, and calculated the mean difference (i.e., plasticity) and 95% confidence intervals around these plasticities.

4.4 RESULTS

The MANOVA on eight snail traits revealed significant main effects of my three treatments but no significant interaction effects (Table 4.1 A). Previous mating system (i.e., selfed or outcrossed) had a significant effect on age at first reproduction, age at death, number of reproductive days, number of eggs laid, egg-hatching proportion, and shell thickness (Table 4.1 B). In general, selfed snails experienced delayed reproduction and early death (Fig. 4.2 A) compared to outcrossed snails, which resulted in a 44% decrease in the number of reproductive days and a 58% decrease in fecundity (Fig. 4.2 C). Selfed snails had 26% thinner shells than outcrossed snails (Fig. 4.2 D) and experienced a 23% lower egg-hatching proportion (Fig. 4.3). Predator cues had significant effects on size at first reproduction and shell thickness. Predator-induced snails were 25% larger at first reproduction (Fig. 4.2 B) and had 54% thicker shells (Fig. 4.2 D) than snails in the no-predator treatments. Mate availability affected age and size at first reproduction and death. Snails with mates reproduced and died earlier and at a smaller size than snails without mates (Fig. 4.2 A, B). Note that mate availability (i.e., the G_2 mating system, outcrossing with mates and selfing without mates) did not have a significant main effect on the egg-hatching proportion.

Two univariate interaction terms were significant after adjusting significance thresholds to control for the false discovery rate (Verhoeven et al. 2005). I observed a predator-cue-by-mate-availability interaction for size at first reproduction ($F_{1,57} = 5.18$, $P = 0.027$) and a three-way (i.e., mating-system-by-predator-cue-by-mate-availability) interaction for egg-hatching proportion ($F_{1,56} = 5.79$, $P = 0.019$). This predator-by-mate interaction for size at first reproduction (Fig. 4.2 B) emerges because there is a significant effect of mate availability on the size at first reproduction without predator cues ($F_{1,30} = 11.33$, $P = 0.002$), but not with predator

cues ($F_{1,31}$ = 0.30, P = 0.586). The significant three-way interaction for egg-hatching proportion (Fig. 4.2 D) reflects the significant effect of mating system for snails with mates without predator cues ($F_{1,14}$ = 9.93, P = 0.007), but not for snails without mates without predator cues ($F_{1,13}$ = 0.03, P = 0.865). For these snails, inbreeding reduced the egg-hatching proportion, but only for snails that were able to outcross (i.e., with mates). That is, when both inbred and outbred (G_1 mating-system treatment) snails were forced to self-fertilize (i.e., no-mate treatment), there was no effect of previous mating history. The opposite pattern was observed with predator cues in which there was no significant mating-system effect for snails with mates and predator cues ($F_{1,14}$ = 3.61, P = 0.078), but there was a mating-system effect for snails without mates with predator cues ($F_{1,15}$ = 9.13, P = 0.009). Here, the outbred selfing snails (i.e., XS, Fig. 4.1) had higher egg-hatching proportion than inbred selfing snails (i.e., SS). All other interaction effects were not significant and therefore are not reported.

4.4.1 Effects of inbreeding on fitness

The age-structured models revealed substantial self-fertilization depression that stems from both isolation and inbreeding (Table 4.2). Mean survival probabilities and age-specific fecundities are shown in Fig. 4.5. Fitness depression due to both inbreeding and isolation was stronger early in the life cycle than later in the life cycle, where often these estimates of fitness depression were negative later in life. This results from the reduction in longevity that is associated with mating and reproducing by outcrossing. Collectively, these data demonstrate that substantial self-fertilization depression occurs in both predator and no-predator environments and stems from both inbreeding and isolation.

4.4.2 Effects of inbreeding on adaptive plasticity

I also observed substantial effects of inbreeding on the expression of adaptive plasticity. Selfed snails had an approximately 1-d longer waiting time in a no-predator environment and an approximately 7-d longer waiting time in a predator environment (Fig. 4.4 A). Note that the waiting time for outcrossed snails in the predator environment was 6 days shorter than the waiting time in the no-predator environment, which confirms the prediction from the Tsitrone et al. (2003a) model. I also observed a substantial effect of inbreeding on the ability of snails to increase shell thickness in the presence of predator cues (Fig. 4.4 B). This effect occurred both in snails that were given access to mates and those that were not given access to mates, but the effect was more pronounced when snails had access to mates. Collectively, I saw that inbreeding disrupted both types of adaptive plasticity in ways that might compound fitness depression in survival and reproduction.

4.5 DISCUSSION

This study demonstrates that inbreeding not only affects survival and reproduction but also adaptive plasticity. The effects of isolation and predation risk on snail traits were similar to those previously observed (Auld and Relyea 2008). For example, I observed that isolation led to larger size (i.e., improved growth) and longer life, but I expect the effects of inbreeding to counter-balance these fitness benefits. Indeed, inbreeding negatively affected every variable except size at first reproduction and death. Collectively, my results are similar to those observed

in previous studies (e.g., Jarne et al. 1991, 2000; Escobar et al. 2007), and I have unveiled a novel connection between inbreeding depression and adaptive plasticity.

4.5.1 Effects of inbreeding on fitness

I observed substantial evidence for strong self-fertilization depression in fecundity early in life (i.e., 0-49 d and 50-99 d). Another study using the same species estimated total self-fertilization depression (in a no-predator environment) over the entire life cycle at 90% (Jarne et al. 2000). My decomposition of the self-fertilization depression revealed that inbreeding often played a larger role than isolation, which is similar to other studies designed to distinguish these sources of fitness depression (Jarne et al. 1991). Interestingly, in other studies, the effects of isolation on fitness were more negative (e.g., decreased fecundity; Jarne et al. 1991, 2000; Tsitrone et al. 2003b), which may be related to a difference in resource quality. While this and previous studies supplied food *ad libitum*, I used Spirulina while other studies have used boiled lettuce. The former has higher protein and fat content than the latter. Future studies with this and other species are needed to evaluate the effect of additional environmental factors (e.g., resource quality) on the components of self-fertilization depression.

The smaller magnitude of self-fertilization depression with predator cues was surprising and is inconsistent with the prediction that inbreeding depression should be stronger in more stressful environments (Bijlsma et al. 1999; Armbruster and Reed 2005), but is not the first evidence to counter this hypothesis (e.g., Henry et al. 2003; Coutellec and Lagadic 2006; Waller et al. 2008). Future studies with increased sample size and family-level replication are needed to assess how and why inbreeding depression changes among traits, life-history stages and environments (e.g., Escobar et al. 2008).

4.5.2 Effects of inbreeding on adaptive plasticity

In addition to directly depressing fitness, self-fertilization negatively affected both types of adaptive plasticity that I examined. Inbreeding may result in an interruption to an adaptively plastic response for several reasons. For example, inbreeding may lead to the fixation of a mutation at a specific locus involved in either the expression of a trait or the plasticity in that trait. Alternatively, inbreeding may result in an overall fitness depression due to the combined effects of multiple loci and thereby an adaptively plastic response may be precluded due to an overall impairment of the ability to detect and/or respond to the environment. Here, although I have strong evidence that inbreeding does alter the expression of two different adaptively plastic responses, I cannot distinguish among these hypotheses to infer the mechanism underlying this result. Future studies that examine variation among families in the effects of inbreeding on adaptive plasticity may provide insight into the genetic basis of this phenomenon. Regardless, this result points to an important source of constraint on the evolution of adaptive plasticity that has not received much attention heretofore.

Several studies have examined the effects of inbreeding on phenotypic stability in cultivated plants to test hypotheses that heterozygosity and plasticity may be related and that inbreeding may decrease developmental stability (Lerner 1954; Schlichting and Levin 1986, and references therein). As an example, Schlichting and Levin (1986) grew *Phlox* in six environments and found no effect of inbreeding on developmental instability (i.e., plasticity). These results contrast with mine and highlight how little is known about the complex interaction between inbreeding and plasticity.

The waiting time is analogous to delayed selfing observed in several species of plants (e.g., Vogler et al. 1998; Kalisz et al. 1999). Importantly, delayed selfing has been shown to be

positively related to the magnitude of inbreeding depression (Stephenson et al. 2000; Escobar et al. *in review*; but see Escobar et al. 2007). I observed that inbred snails expressed longer waiting times than outbred snails, especially in the presence of predator cues. Such an elongation of the waiting time may reflect an impaired ability for inbred snails to self and may result in increased selection against inbred individuals. Additionally, if this pattern holds in natural populations, this would make conditions that are favorable to the evolution of higher selfing rates (e.g., low mate availability) even less likely to result in a higher selfing rate. Importantly, if inbred individuals delay selfing longer, they are more likely to encounter a partner before reproducing and this may provide an additional negative feedback against the evolution of selfing in populations of *Physa acuta*.

While several recent studies have examined how inbreeding depression may affect tolerance and/or resistance of plants to herbivores and pathogens (e.g., Ouborg et al. 2000; Carr and Eubanks 2002; Carr et al. 2003; Ivey et al. 2004; Stephenson et al. 2004; Ivey and Carr 2005), this appears to be the first study to examine the effects of inbreeding on the *inducibility* of a defensive trait. Indeed, while resistance and tolerance represent two distinct ways in which plants defend themselves against enemies (Strauss and Agrawal 1999; Mauricio 2000; Núñez-Farfán et al. 2007), the effects of inbreeding on the induction of these defensive traits have apparently not been investigated (*cf*. Agrawal et al. 2002; Weinig et al. 2003 as studies that examine herbivore-induced plasticity but not the effects of inbreeding). The current study demonstrates that inbreeding results in impaired expression of a defensive phenotype, which is likely to result in less defended (i.e., more vulnerable) individuals that may be more easily killed.

Taking this into account, inbreeding depression in plasticity is clearly an important component to understanding selection against inbred individuals in a natural population.

Importantly, fitness depression that results from inbreeding may be strong for a particular trait that is only under selection in some environments (e.g., an inducible defense), and thereby the mating system may be under correlated selection through its association with a trait even if that association only exists in certain environments (e.g., with predators). Therefore, depending on the strength and direction of the genetic correlations among traits across environments, the evolution of plasticity may be facilitated or constrained by selection in certain environments (Via and Lande 1985; Gomulkiewicz and Kirkpatrick 1992).

While it has been previously known that inbreeding depression can change across environments, the main result of this study is that inbreeding depression can not only affect traits expressed in one environment, but also the adaptive ability to alter the phenotype across environments (i.e., inbreeding depression in adaptive plasticity). My results point to a novel concern in considering environment-specific inbreeding depression: if inbred organisms not only experience reduced fitness, but also an impaired ability to detect and/or respond to environmental conditions, this may have important implications for understanding the evolution of inbreeding depression and mating systems in natural populations. This points to the importance of considering environmental factors in understanding fitness, particularly as the effects of the mating system on fitness can change across environments. Importantly, inbreeding depression in both forms of adaptive plasticity that I examined is likely to contribute to selection against inbred individuals under natural conditions.

Table 4.1. Results of A) a MANOVA and B) ANOVAs on eight traits (AFR, SFR, age/size at first reproduction; AD, SD, age/size at death; RD, number of reproductive days; EGGS, total number of eggs; EHP, egg-hatching proportion; ST, shell thickness) with treatments as fixed effects. Data were transformed prior to analysis (see text for details). For the univariate analyses, significant interaction terms are discussed in the text. Boldface denotes statistical significance.

A.	*Pillai's Trace*	$F_{8,57}$	*P*
Mating System (MS)	**0.565**	**9.271**	**<0.001**
Predator (PRED)	**0.492**	**6.902**	**<0.001**
Mate Availability (MATE)	**0.443**	**5.673**	**<0.001**
MS*PRED	0.185	1.613	0.141
MS*MATE	0.111	0.892	0.529
PRED*MATE	0.090	0.702	0.688
MS*PRED*MATE	0.113	0.909	0.516

B.	MS		PRED		MATE	
Trait	F_{df}	*P*	*F*	*P*	*F*	*P*
AFR	**$13.561_{1,57}$**	**<0.001**	1.390	0.243	**7.456**	**0.008**
SFR	$0.626_{1,57}$	0.432	**9.240**	**0.004**	**8.778**	**0.004**
AD	**$4.399_{1,59}$**	**0.040**	0.038	0.856	**7.926**	**0.007**
SD	$2.109_{1,54}$	0.152	1.795	0.186	**38.784**	**<0.001**
RD	**$13.682_{1,63}$**	**<0.001**	0.347	0.558	0.099	0.754
EGGS	**$23.097_{1,64}$**	**<0.001**	0.157	0.693	0.309	0.580
EHP	**$18.343_{1,56}$**	**<0.001**	0.037	0.847	2.530	0.117
ST	**$18.852_{1,60}$**	**<0.001**	**40.316**	**<0.001**	0.783	0.380

Table 4.2. Fitness depression that results from isolation, inbreeding and both in survival (A) and fecundity (B; next page) in no predator (NP) and predator (P) treatments. Different types of isolation and inbreeding depression are labeled 1-5 as in Figure 4.1; see text for additional details.

A.		*Survival Probability Depression*						
Type	Predator	Hatching	50d	100d	150d	200d	250d	300d
1	NP	0.06	0	-0.13	-0.44	-0.11	0	0
1	P	-0.01	0	-0.13	-0.4	-0.1	0	0
2	NP	-0.09	0	-0.37	-0.14	-0.14	-0.14	0
2	P	0.12	0.07	-0.14	-0.14	0	0	0
3	NP	0.15	0	0.31	0	0	0	0
3	P	0.06	0	-0.02	0	0	0	0
4	NP	0.01	0	0.17	0.21	-0.03	-0.14	0
4	P	0.18	0.07	-0.03	0.18	0.09	0	0
5	NP	0.08	0	0.06	-0.14	-0.14	-0.14	0
5	P	0.17	0.07	-0.16	-0.14	0	0	0

Table 4.2 (Cont.). Caption on previous page.

B.		*Fecundity Depression*					
Type	Predator	0-49d	50-99d	100-149d	150-199d	200-249d	>250d
1	NP	0.45	0.04	-3.21	-1.96	-0.19	0
1	P	0.45	0.06	-2.68	-1.59	0	0
2	NP	0.25	0.52	-4.98	-4.55	-5.44	-3.27
2	P	0	0.15	-3.60	0	0	0
3	NP	0.73	0.51	0.68	0	0	0
3	P	0.45	0.53	0.72	0	0	0
4	NP	0.63	0.76	0.55	-0.88	-4.42	-3.27
4	P	0	0.58	0.65	0.61	0	0
5	NP	0.80	0.77	-0.90	-4.55	-5.44	-3.27
5	P	0.45	0.60	-0.30	0	0	0

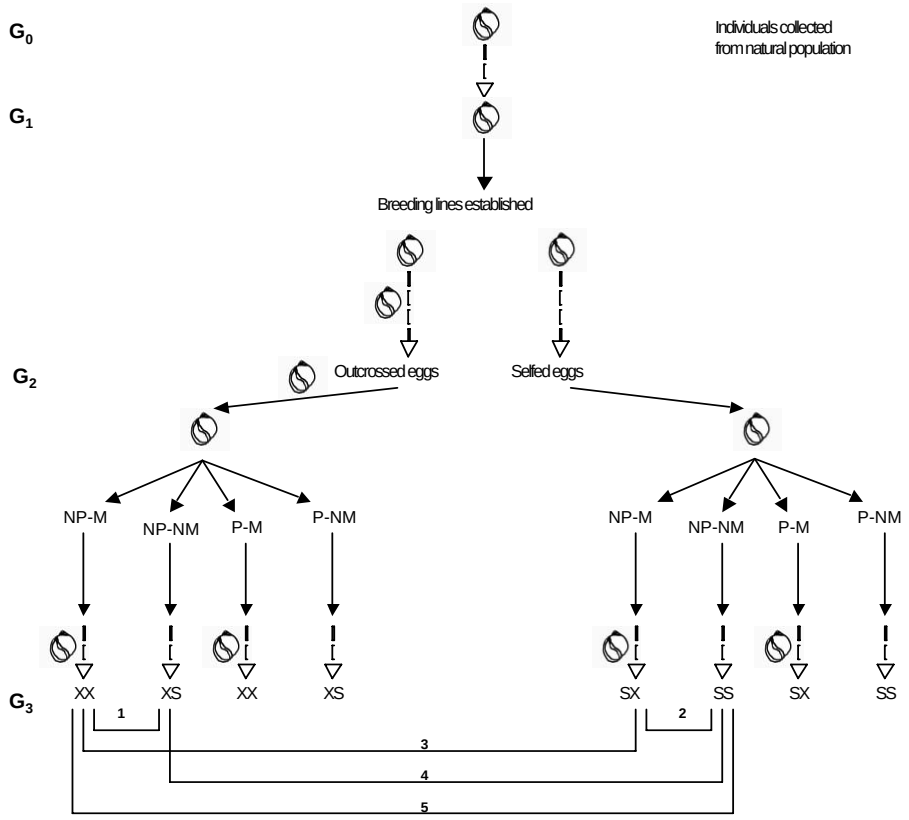

Figure 4.1. Experimental design for one breeding line (i.e., one replicate). Wild (G_0) snails were collected and breeding lines were established using G_1 snails (n = 10). Parallel outcrossing or selfing lines were established using sibs. Selfing was ensured by isolating individuals prior to reproductive maturity while outcrossing was facilitated by providing multiple marked mates in sequence over a two-week period (see text for further details). G_2 (i.e., outcrossed or selfed) snails were used in a factorial experiment where snails were reared in one of four treatments: no predator, mate available (NP-M), no predator, no mate available (NP-NM), predator, mate available (P-M), or predator, no mate available (P-NM). Dashed lines symbolize reproduction, while solid lines facilitate intra-generational connections; the length of these lines is arbitrary. Snails placed along dashed lines are to emphasize outcrossing, while the absence of such snails represents self-fertilization. The letters beneath the dashed arrows for each treatment represent the mating system, where the last letter is the mating system employed by the snails in each experiment and preceding letters symbolize their ancestor's mating system (e.g., SX means that snails produced through self-fertilization were outcrossed). The five possible comparisons used to assess inbreeding and isolation depression are numbered (see text for details). The snail icon comes from Jarne et al. (2000).

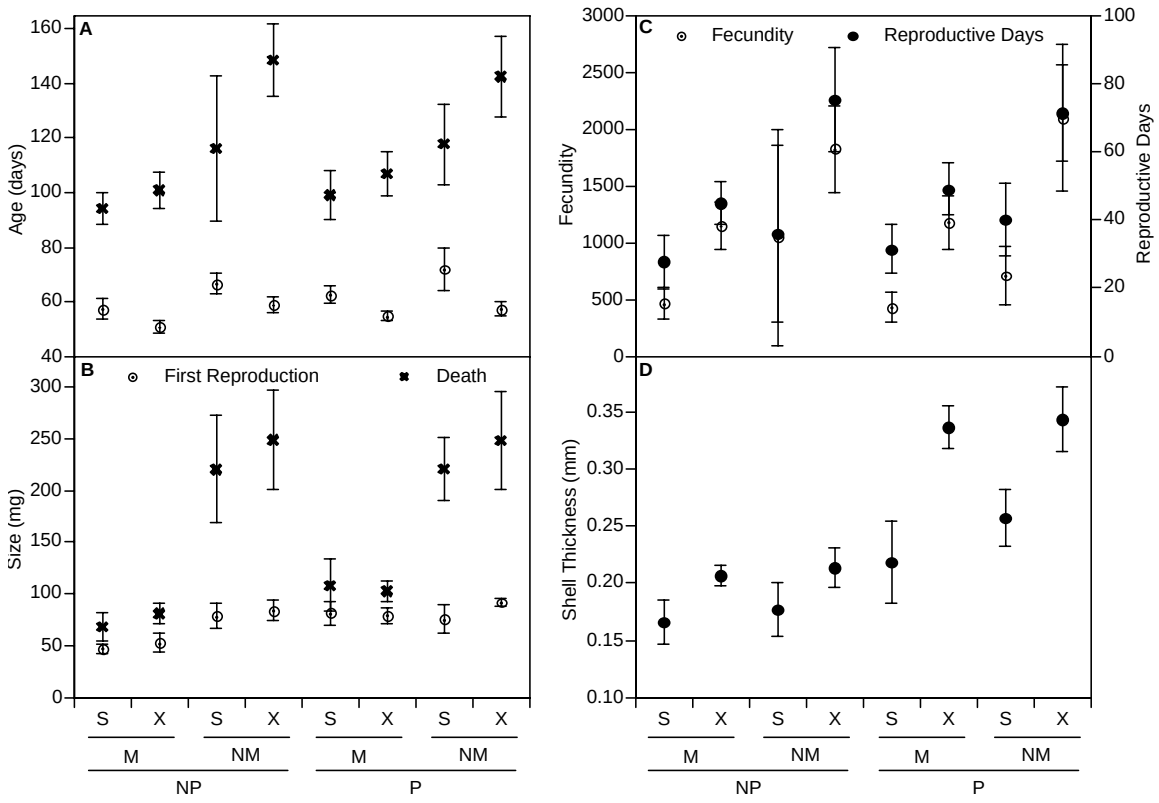

Figure 4.2. The effects of mating system, predation risk and mate availability on age at first reproduction and death (A), size at first reproduction and death (B), total fecundity and the number of reproductive days (C), and shell thickness (D). *S* and *X* along the *x*-axis represent Selfed (*S*) or Outcrossed (*X*) treatments. *M* and *NM* represent mate availability (Mate and No Mate, respectively). *NP* and *P* represent predator treatments (No Predator and Predator, respectively). All data are means $\pm$ 1 S.E.

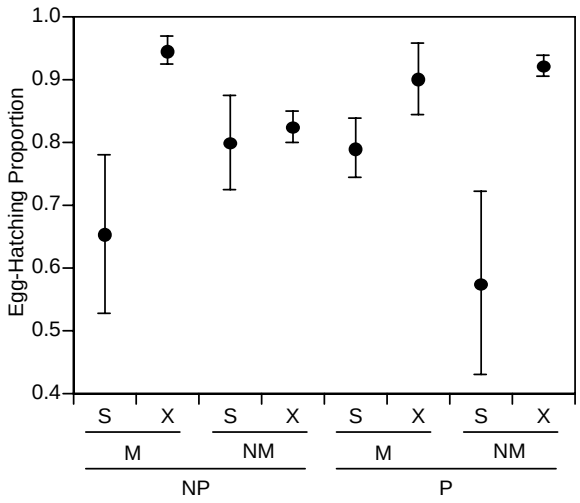

Figure 4.3. Mean egg-hatching proportion (± 1 S.E.) of G_3 *Physa acuta* snails in the presence (*P*) and absence (*NP*) of predator cues. *M* (i.e., mate) and *NM* (i.e., No Mate) represent the mating system of the G_2 snails (i.e., outcrossing and selfing, respectively), while *S* and *X* represent the mating system of G_1 snails, selfed and outcrossed, respectively. See Fig. 4.1 for breeding design.

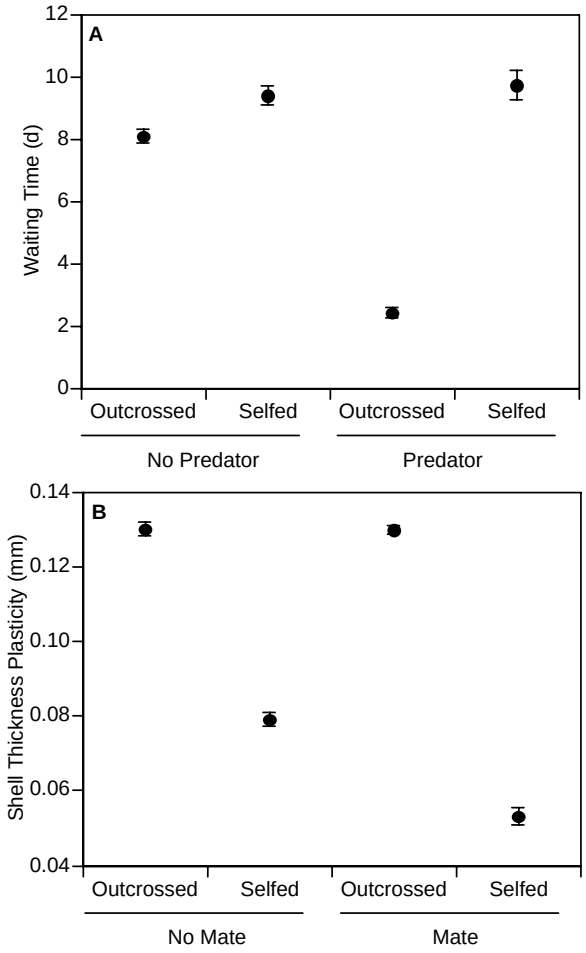

Figure 4.4. Bootstrapped mean plasticities (± 95% confidence intervals) for waiting time (A) and shell thickness (B). Waiting time is the plasticity in age at first reproduction across mate-available/no-mate-available environments (i.e., the delay in selfing in the absence of mates). Shell thickness plasticity is the difference in shell thickness across no-predator and predator environments. See text for further details.

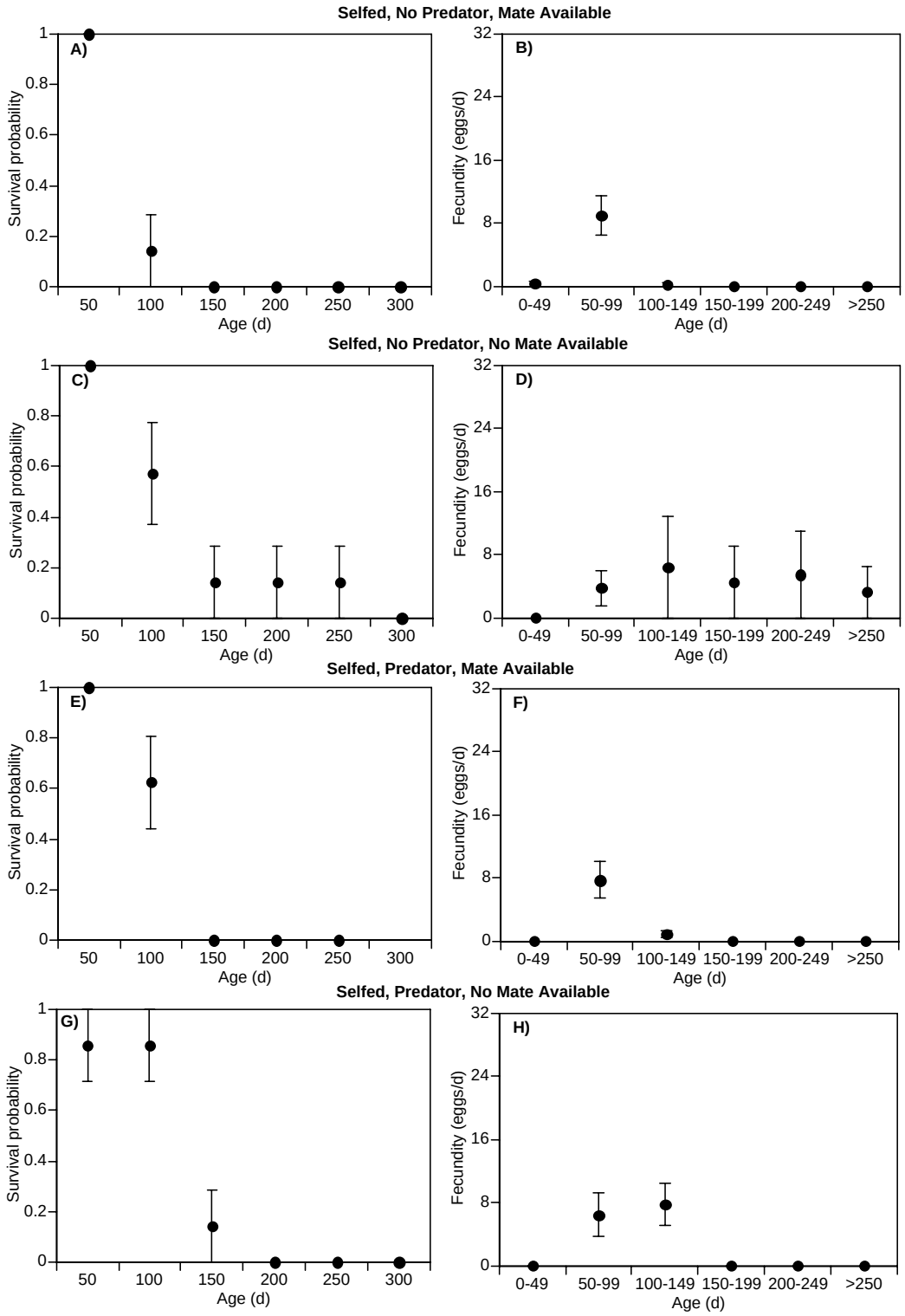

Figure 4.5. Fitness components through development for inbred (A-H) and outbred (I-P; following page) snails reared in four environments (see Fig. 4.1). Left panels show the survival probability (± S.E.); right panels are age-specific fecundities (± S.E.). See text for further description.

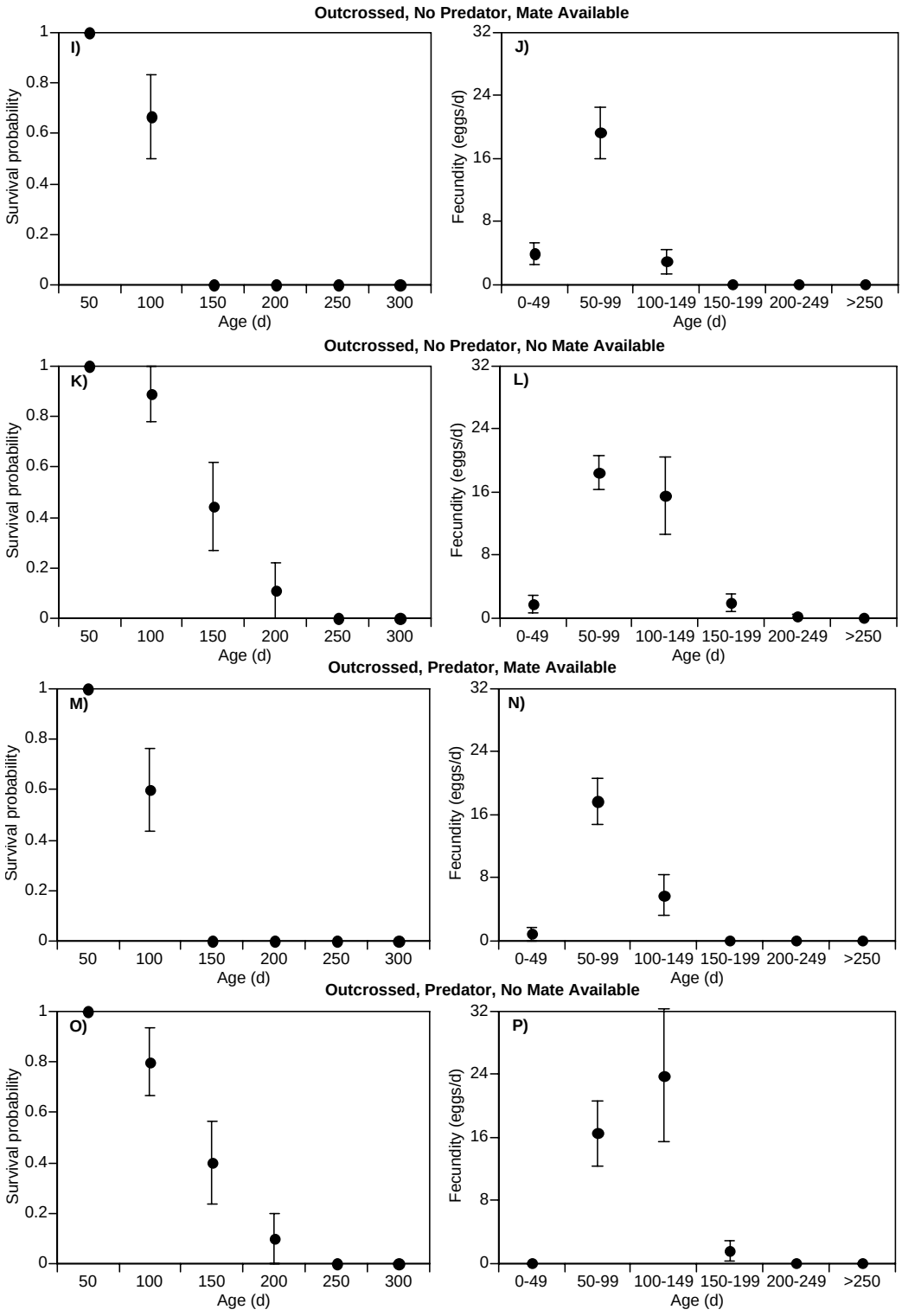

Figure 4.5 I-P. (Caption on previous page).

5.0 CONCLUSIONS

In a broad sense, this dissertation addressed the importance of phenotypic plasticity in response to two different types of environmental variation by exploring factors that facilitate and constrain the evolution of plasticity. In conclusion, I will discuss some key results that warrant future investigation due to their novelty and potential importance.

First, predatory crayfish altered their foraging mode when prey were defended (Chapter 3). Snails were able to detect the presence of crayfish and respond in a way that decreased their risk of being killed, which is in line with numerous studies revealing the ubiquity of adaptive predator-induced defenses in prey (e.g., Tollrian and Harvell 1999). However, we are only beginning to understand how the evolution of reciprocal plasticity occurs and the situations under which it is favored. If prey evolve the ability to defend themselves, there will be an impetus for their enemies to evolve a counter-strategy, providing the potential for a coevolutionary escalation of defense and offense (i.e., an "arms race" between predators and prey). In my results, crayfish switched from crushing 100% of their prey when snails were not predator-induced to crushing 70% of their prey when snails were predator-induced; they extracted the remaining 30% of the snails from their shell. While extracting a snail from its shell is likely a time-consuming process that may require more energy than simply crushing the shell, this provides the predators with an additional means of killing prey. Additionally, this change in the mode of predation alters the pattern of selection on prey such that snails would have to

defend against more than one manner of being killed by the same predator. Future work to unravel the importance of plasticity in each species in this interaction will be important as well as to consider alternate predators and prey and the community-context within which such interactions occur in nature.

Second, I observed inbreeding depression in plasticity, which is a novel result of this dissertation (Chapter 3). Specifically, compared to outcrossed snails, inbred snails showed less shell thickness plasticity to predator cues (i.e., they were not as defended) and more of a waiting time (i.e., they delayed selfing longer). Both of these responses show additional ways by which the evolution of selfing may be inhibited under natural conditions. First, if inbred snails are less able to defend themselves against predators, they may be more easily killed, and if there are alleles that favor selfing, selection by predators may reduce their frequency if they are associated with a more vulnerable shell phenotype. Second, if inbred snails wait longer to find a partner, the chances of them encountering such a partner prior to selfing will increase, which decreases the selfing rate. As inbreeding depression results from an increase in homozygosity, these results have implications for the genetic basis of plasticity, and future studies with family-level replication are needed before further conclusions can be made. In general, the existence of inbreeding depression in plasticity is an important result in its own right and the mechanisms underlying such a phenomenon will need to be worked out in future studies.

I have investigated the effects of one enemy on traits related to reproduction and the pattern of reproduction among individuals. Future studies that examine the effects of different types of enemies are needed to evaluate whether the results that I obtained are unique to crayfish-snail interactions or a general results of size-selective predation.

By exploring a link between the traditionally disparate fields of inducible defenses and mating-system evolution, I have highlighted an interaction that is potentially important under natural conditions. Furthermore, this may be only one example of how disparate factors of an organism's environment (e.g., predation risk and mate availability) can be mutually interactive and points to the exceeding importance of considering the ecological context within which organisms evolve when considering why traits evolve as they do.

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