

**PREDATOR INDUCED DEFENSES IN PREY WITH DIVERSE PREDATORS**

A Thesis

by

MARK ISAAC GARZA

Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2005

Major Subject: Wildlife and Fisheries Sciences

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Approved by:

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Committee Members,	Lee Fitzgerald
	Anthony I. Cognato
Head of Department,	Robert Brown

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## **ABSTRACT**

Predator Induced Defenses in Prey with Diverse Predators. (December 2005)

Mark Isaac Garza, B.S., The University of Texas at San Antonio

Chair of Advisory Committee: Dr. Thomas J. DeWitt

Phenotypic plasticity is an environmentally based change in phenotype and can be adaptive. Often, the change in an organism's phenotype is induced by the presence of a predator and serves as a defense against that predator. Defensive phenotypes are induced in freshwater physid snails in response to both crayfish and molluscivorous fish.

Alternative morphologies are produced depending on which of these two predators snails are raised with, thus protecting them from each of these predators' unique mode of predation. Snails and other mollusks have been shown to produce thicker, differently shaped shells when found with predators relative to those found without predators. This production of thicker, differently shaped shells offers better protection against predators because of increased predator resistance.

The first study in this thesis explores costs and limits to plasticity using the snail-fish-crayfish system. I exposed juvenile physid snails (using a family structure) to either early or late shifts in predation regimes to assess whether developmental flexibility is equally possible early and late in development. Physid snails were observed to produce alternative defensive morphologies when raised in the presence of each of the two predators. All families responded similarly to the environment in which they were raised. Morphology was found to be heritable, but plasticity itself was not heritable. Morphology

was found to become less flexible as snails progressed along their respective developmental pathways.

In the second study, I raised physid snails with and without shell-crushing sunfish and examined the differences in shell thickness, shell mass, shell size and shell microstructural properties between the two treatment groups. Shells of snails raised with predators were found to be larger, thicker and more massive than those raised without predators, but differences in microstructure were found to be insignificant. I conclude that the observed shell thickening is accomplished by the snails' depositing more of the same material into their shells and not by producing a more complex shell composition.

## ACKNOWLEDGEMENTS

I would first like to thank my father for taking me “exploring” outside of the city, in parks and along creeks, and for allowing me to experience nature at an early age. I’d also like to thank my family, especially my uncle Michael, for the many years of taking me along on fishing trips. Without these experiences I may not have developed the interest in nature that took me to this course of study. I also thank my mother for her endless support and encouragement through the years. I would also like to thank Bill Neill for letting me know about the WFSC graduate program; before working with him I really had no idea that I could actually come back to school and spend a lot of time learning so much about so many creatures.

Probably the biggest thanks goes to Thom DeWitt for taking me in, teaching me just about all that went into this thesis, and for being an all around great guy to work with. He has been a tremendous help and I greatly appreciate it.

I would also like to thank my committee members, Lee Fitzgerald and Anthony Cognato, for their valuable comments on this thesis. Not only did they give me great advice throughout graduate school, they are great people to talk to and it is a pleasure just to know them. I would also like to thank Lennie DiMichele for all the help in designing the experiment in Chapter III and arranging for me to use much of the equipment used in the analyses. Thanks to Bill Lackowski of the Materials Characterization Facility for teaching me how to use much of that equipment.

Many thanks to people of the Hispanic Leadership Program in Agriculture and Natural Resources for the many aspects of support they have given me. Not only did they believe in me enough to put me through this graduate program, they were a constant

source of encouragement and knowledge. HLPANR was great program to be a part of and a source many good friends.

Lastly, I thank my wife Rheanna, for her constant support, encouragement and friendship. Financial support for this work was provided by HLPANR fellowship and research support, and CONACYT, National Science Foundation grant 9908528.

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## **CHAPTER I**

### **INTRODUCTION**

Temporal and/or spatial heterogeneity within biotic and abiotic environments can directly affect organisms. Nearly every aspect of the environment can vary. Abiotic variation of the environment includes temperature, day-length, precipitation and chemical concentrations while biotic variation include prey abundance, predator abundance and interspecific competition. These types of changes can profoundly affect the success of an organism, especially if an organism cannot cope with fluctuations in the environment. However, many organisms have flexible phenotypes, which may allow them to optimize fitness according to environmental variation.

Phenotypic plasticity is generally defined as an environmentally based change in phenotype (Bradshaw 1965, Schlichting and Pigliucci 1998, Agrawal 2001). This change in phenotype can stand as a change in an individual's chemistry, physiology, development, morphology, or behavior (DeWitt and Scheiner 2004). Having a flexible phenotype is important to an organism's ability to maintain relatively high fitness in a variable environment (Schlichting 1986, Stearns 1989, West-Eberhard 1989, Scheiner 1993). Identification and response to abiotic or biotic signals are essential to all types of plasticity and it is only through such identification that plasticity can take place. Expression of a particular phenotype to these signals is often adaptive because of improved matching of the phenotype to its surrounding environment (Levins 1968, Moran 1992, Via 1993, Gotthard and Nylin 1995). Many observed cases of adaptive

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This thesis follows the style and format of Ecology.

phenotypic plasticity involve inducible defenses in which the phenotype of prey is induced by the presence of a predator, in turn protecting the prey from that predator (Lively 1986, Dodson 1989, Harvell and Padilla 1990, Van Buskirk and Schmidt 2000, Relyea 2001, DeWitt and Langerhans 2003).

So, the benefits of such adaptive responses are widely known and have been thoroughly documented, but the focus of attention has more recently turned to exploring the existing types of constraints that work on plasticity, meaning, plasticity does not always result in a perfect solution, as illustrated, if only weakly, in recent empirical studies (Weinig and Delph 2001, Langerhans and DeWitt 2002, Relyea 2002).

Constraints on plasticity are generally discussed in terms of costs and limits (DeWitt 1998). *Costs of plasticity* are seen when there is a reduction in fitness by plastic genotypes expressing a given phenotype relative to genotypes that are fixed for that same phenotype (DeWitt et al. 1998). For example, there may be a maintenance cost associated with a plastic genotype, being that there can be sensory and regulatory mechanisms associated with phenotype production which incurs high energetic costs. *Limits of plasticity* are found when a plastic genotype does not produce a trait mean as near the optimum as can fixed development (DeWitt et al. 1998). A type of limit may involve information reliability, for example, when a maladaptive phenotype results because of imperfect cues received in the environment or, correctly responding phenotypically to environmental cues, but then finding that the environment changes (For a complete review of potential costs and limits of phenotypic plasticity, see DeWitt et al. 1998).

In this thesis, I build upon instances of adaptive predator induced morphologies which have been observed in several species of mollusks. The first instance involves the

shell shape produced by the physid snail (*Physella virgata*) when raised with predators that attack snails using contrasting attack modes. When reared with crayfish, physid snails develop a shell that is elongate in shape (DeWitt 1998). Production of an elongate shell results in both an occluded aperture and an elongate spire. Because crayfish and other decapods extract the edible snail body via shell-entry, an occluded aperture hinders a successful attack (Vermeij 1979, Appleton and Palmer 1988, DeWitt et al. 2000, Krist 2002). At the same the elongate spire time may allow for the snail to retract further into the shell (Vermeij 1982). Shell-crushing sunfish (Lauder 1983, Huckins 1997), on the other hand, induce a more rotund shape of the physid snail's overall shell shape (DeWitt 1998), a shape of shell that has been shown to increase its crush resistance (DeWitt et al. 2000).

The second instance of adaptive predator induced plasticity I expand upon in this thesis involves the production of thicker shells by aquatic snails in response to certain molluscivores (Vermeij 1976, Palmer 1979). A thicker shell may obviously benefit the snail because it better protects from attacks by shell-crushing predators (Seeley 1986, Trussell 1996, West and Cohen 1996, Trussell 2000).

The first study in this thesis explores constraints to developmental flexibility of predator induced shell shape. Juvenile physid snail siblings were exposed to early or late shifts in predation regime to assess whether or not developmental flexibility is possible early or late in development, with the trait of focus being overall shell shape.

In the second study, physid snail siblings are raised with or without a shell-crushing species of sunfish and differences in shell shape, mass, thickness and microstructure are examined. Peering into shell microstructure using advanced

nanoindentation techniques is new to the field and what is most unique about this study. What I aim to answer is whether snails produce overall thicker and more massive shells and, if so, whether this thickening is accomplished by layering materials differently within the protein matrix, or, by simply depositing an increased amount of the same material.

## CHAPTER II

# ECOLOGICAL CONSTRAINTS ON THE EVOLUTION OF PHENOTYPIC PLASTICITY

### Introduction

Phenotypic plasticity is the ability of an organism to produce different phenotypes in response to distinct environmental cues (Stearns 1989, Schlichting and Pigliucci 1998). It can be an adaptive strategy in variable or changing environments because of improved phenotype-environment matching (Bradshaw 1965, Levins 1968, Moran 1992). This adaptive matching of phenotype with environment should therefore allow organisms to exploit and tolerate a broader range of environments than would be possible with a fixed phenotype (Schlichting 1986, Scheiner 1993, Schlichting and Pigliucci 1998, Windig et al. 2004). While the benefits of such adaptive plastic responses have been amply documented, constraints on developmental plasticity have been given much less attention.

In general, constraints on plasticity consist of costs or limits (DeWitt 1998). Costs arise when a phenotypically plastic organism exhibits lower fitness while producing the same mean trait value as a developmentally fixed organism. Limits on plasticity are seen when plastic development does not produce a trait mean as near the optimum as can fixed development (DeWitt et al. 1998). Costs and limits have been given much theoretical attention, but only a modest amount of work has tested for costs (Weinig and Delph 2001, Relyea 2002) and few studies have addressed other constraints.

Weinig and Delph (2001) showed that plastic stem elongation responses of the annual weed velvetleaf (*Abutilon theophrasti*) take place at the cost of diminished

plasticity later in life. Because selection favors an increase in stem elongation in certain environments at both early and late life stages (Weinig 2000), a reduction in plasticity later in life may be a significant constraint on the evolution of phenotypic plasticity. Langerhans and DeWitt (2002) demonstrated that physid snails develop rotund shells when reared with either molluscivorous or non-molluscivorous sunfish species. The rotund shape, which increases resistance to shell-crushing fishes, makes snails more vulnerable to shell-entry predators to such invertebrates as insects, leeches, and crayfish. Since the invertebrate predators are ubiquitous in all snail habitats, and perhaps are more common in the absence of fishes, snails that alter morphology to become more rotund in the absence of true molluscivorous fishes make an adaptive error. Such a costly mistake with no obvious benefit may be a major constraint on the evolution of adaptive plasticity.

Although we know that phenotypic plasticity is adaptive for physid snails when expressed to appropriate predators, and have identified several of its potential costs and limits, we still know very little about the limits of developmental flexibility. For example, if the environment changed late in life, would individuals still be able to respond with adaptive developmental shifts? In the present study, I exposed freshwater snails (*Physella virgata*) to either early or late shifts in predation regimes to assess whether developmental flexibility is equally possible early or late in development. Physid snail predation regimes were switched at two points during the developmental period. Subsequent phenotypes were compared for different switches against each other and against controls that are reared in entirely one predation regime. I measured phenotypic responses (overall shell shape) of *Physa* during ontogeny at three exposure intervals. Specifically, I aimed to determine whether recent development is constrained by past



development, a question for which shell morphology is an apt trait as shells are simultaneous records of recent and past development.

### *Study system*

Freshwater snails of the family *Physidae* have a wide distribution and are native to many regions of the world. They exhibit continuous growth, the form of which depends mainly upon the prevailing predation regime. Predation on these snails commonly occurs through two general modes; either by shell-crushing or by way of shell-entry. The redear sunfish (*Lepomis microlophus*) is a prime example of a shell-crushing predator. Having a modified pharyngeal arches with a specialized musculature, this sunfish species is able to maintain a mainly molluscivorous diet (Lauder 1983, Huckins 1997). Crayfish represent a class of shell-entry predators that tend to attack by reaching into to the shell's aperture and extracting the snail body tissue (Alexander and Covich 1991, DeWitt et al. 1999). To reduce success of such predation tactics, physid snails exhibit different morphologies when raised in the presence of these alternative predators, becoming more elongate in shape when raised with crayfish and more rotund when raised with molluscivorous sunfish (DeWitt 1998). Because molluscivorous sunfish are shell crushers, they are deterred by rotund shell shape because shells of this shape are more difficult to crush (DeWitt et al. 1999). In contrast, crayfish are shell-entry predators that are deterred by elongate shell morphology, being that these shells have narrow apertures that are difficult to reach into (Vermeij 1979, Appleton and Palmer 1988, DeWitt et al. 1999). Thus, the induced morphological responses of physid snails to two predator types have significant adaptive benefits.

Since shell morphological responses to the two predator types is opposite, this is an ideal system in which to challenge snails with environmental switches, to assess their ability to respond based on age and history.

## **Methods**

### *Snail rearing*

Approximately 125 *Physella virgata* were collected from a Krenek Tap pond located in Central Park, College Station, Texas, USA. This pond was chosen as my collection site because it is inhabited by both crayfish and redear sunfish. The wild-caught snails were brought to our laboratory, treated with Maracyn ( $0.8 \text{ mg} \cdot \text{l}^{-1}$ ) for two days in group culture and fed *ad libitum* with ground Wardley's brand spirulina flakes. After antibiotic treatment, the 50 largest snails were chosen and placed individually into 300 ml Dixie brand plastic cups containing an RO water/trace elements solution (1ml/15L) (Seachem Fresh Trace). I chose the largest snails as they had the greatest potential for generating egg masses large enough to yield the number of hatchlings required for my experimental design. Water was changed three times weekly by pouring away the unclean water and replacing with fresh water. Replacement water consisted of reverse osmosed water prepared with Seachem Fresh Trace trace elements (1ml/15L). Snails were fed by adding ground spirulina flakes *ad libitum* (approximately 0.1 mg) to each cup after each water change.

Within one week several egg masses were generated. Those egg masses containing at least 24 potential hatchlings were kept in their respective cups. Small egg masses found in the cups were removed using a wooden spatula and discarded. After

hatching, water changes were continued on the same schedule as before, but instead approximately 90% of the water from each rearing cup was removed. This was accomplished by withdrawing water by means of a syringe having a small section of tubing attached to its end. The section of tubing was replaced for each water change of each cup to prevent any possible contamination from one cup to another. Use of a syringe was performed in order to prevent any loss of hatchlings had the water been poured out as had been done prior to hatching. A total of fifteen individuals were determined to have produced at least 24 hatchlings; thus, I had 15 full-sib families. One hatchling from each family was placed into its designated starting treatment using a small paintbrush 10-14 days later as described below.

### *Experimental design*

F<sub>1</sub> snails were raised in using a  $2 \times 3$  factorial design: snails were started in either fish or crayfish environments and experienced either of three switching treatments (no switch, early switch to an alternative predation regime; or late switch). Thus for the six treatments, the sequence of predation treatments for the three successive periods during development was: CCC, CCF, CFF, FFF, FFC, FCC, where CCF indicates a switch from crayfish to fish at the later date (Fig. 1). Twenty-four 57-liter aquaria were established (4 aquaria per treatment) and all tanks were systematically arranged to prevent bias.

Crayfish (*Procambarus clarkii*; 70-85 mm measured from the rostrum dorsally to the telson) were collected from drainage ditches at the Texas A&M University Riverside Campus, College Station, TX; redear sunfish (*Lepomis microlophus*; 70-85 mm TL) were obtained from an area bait supplier. Crayfish and redear sunfish were placed in

Fish block			Crayfish block			Fish block			Crayfish block		
none	late	early	none	late	early	none	late	early	none	late	early
FFF	FFC	FCC	CCC	CCF	CFF	FFF	FFC	FCC	CCC	CCF	CFF
CCC	CCF	CFF	FFF	FFC	FCC	CCC	CCF	CFF	FFF	FFC	FCC
none	late	early	none	late	early	none	late	early	none	late	early
Crayfish block			Fish block			Crayfish block			Fish block		

Fig. 1. Tank arrangement showing the sequence of snail predation treatments for the three successive periods of development. Predation treatment sequences are CCC, CCF, CFF, FFF, FFC, FCC, where CCF indicates a switch from crayfish to fish at the later date.

their appropriate aquaria at least two months prior to adding snail hatchlings. These predators were placed below a plastic grid in their designated tanks and snails were placed individually into cages above the grid (Fig. 2). Each snail-rearing cage was made of 300 ml plastic cups having two (35×38 mm) mesh windows (mesh size = 0.10 mm). These windows allowed water from the tank to flow freely through each rearing cage. This system prohibited contact between snails and predators while still allowing snails to detect chemical and pressure wave cues of the predators.

At six weeks the snails were switched to their next tank which may or may not have contained a different predator than previously, depending on the treatment assigned. At twelve weeks the snails were switched to their final tank/predator regime. Both crayfish and redear sunfish were fed half of a live redworm three times per week. A pinch of ground spirulina was fed to snails on the same day as crayfish and fish were fed. To prevent possible splashing or aerosol transfer of water from a tank of one predator regime to a tank of a different regime, transparent plexiglass walls extending 30 cm above the aquarium rims separated tanks of unlike predator environment. Seachem Trace Elements (1ml/3.8L) was added to each tank weekly. Tank water was filtered and circulated using Whisper II fiber floss filters with no activated carbon. Carbon was not used so that necessary volatiles from the predators needed to induce developmental changes in the snails were not removed from the water. Twenty percent water changes were performed every six weeks in addition to filtration to maintain high water quality.

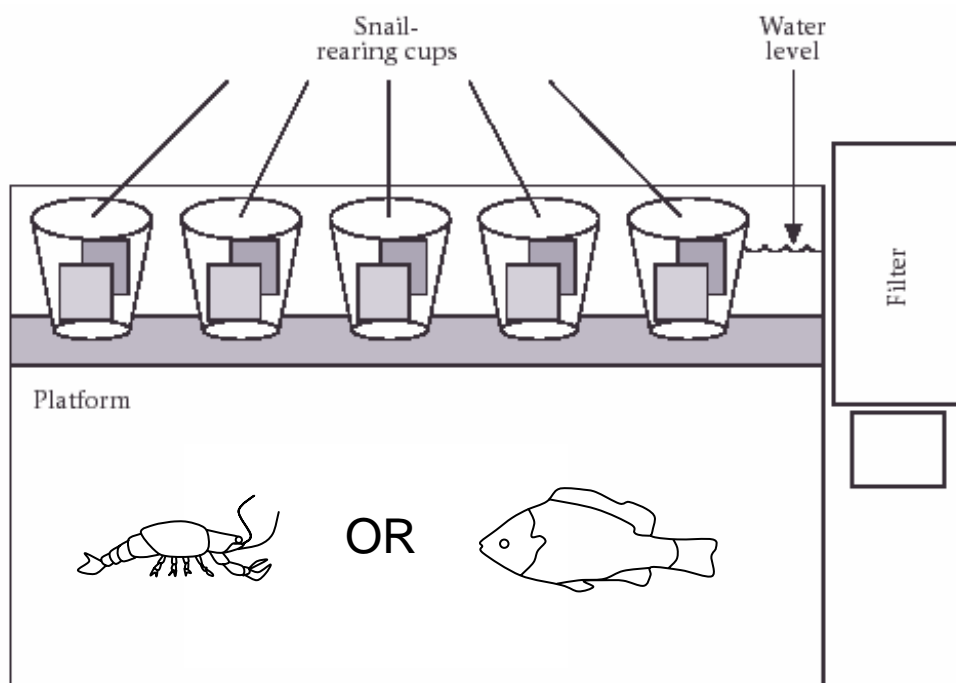


Fig. 2. Diagram of an individual treatment tank. Depending on the type of treatment tank, either a single crayfish or redear sunfish was found below the platform.

### *Morphometrics*

Images of snail shells were captured at the end of six, twelve and eighteen weeks of rearing in their designated predator regimes with a video imaging system and measured using MorphoSys (V. 1.29) morphometric software. Because images of live snails were taken, snails were first encouraged to retreat into their shells by gently pushing at their bodies with a twisted corner of a Kimwipe napkin. Snails were placed aperture down and allowed to rest naturally on a level platform below a video camera. Snail positions could thus be standardized before images were captured.

For each snail, shell outlines and twelve landmarks were digitized (Fig. 3). Landmarks were digitized at the shell apex (LM 3), on sutures connecting the current and previous two whorls (LM 1, 2, 4, and 5), at the farthest point of the shell relative to the coiling axis (LM 6), and at the lower insertion of the aperture (LM 7). The remaining five points found along the apertural region were treated as semilandmarks and were located by projecting at 30° angles from a point existing halfway between landmarks 1 and 7. Each of these five semilandmarks (LM 8 – 12) was confined to “slide” between adjacent points along the apertural curve (Bookstein 1991). Because this curve contains no biologically meaningful points, the sliding landmark method minimizes the bending energies associated with these less informative points. The semilandmarks are slid along the outline until they best conform to the positions of the matching points found along the same curved outline of the reference snail specimen.

Geometric morphometric methods (Rohlf and Marcus 1993) were used to generate detailed information on shell shape. Raw landmark coordinates were aligned by

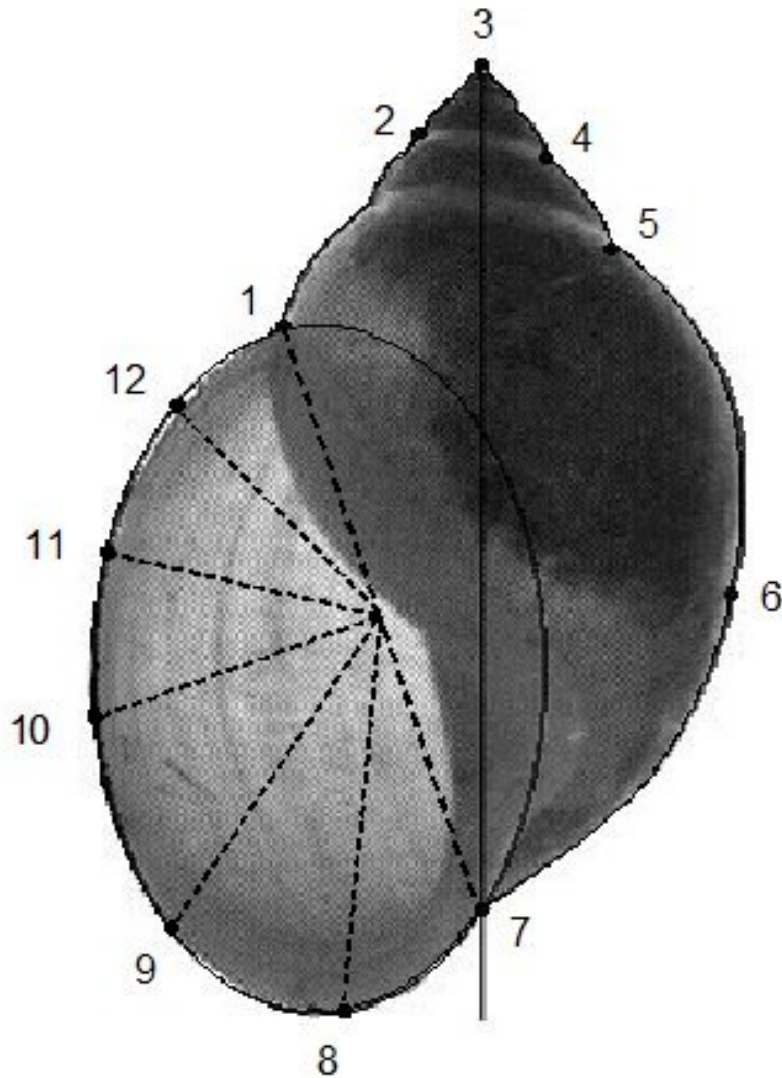


Fig. 3. Landmarks used in geometric morphometric analysis. Landmarks 1 through 7 represent true landmarks. Landmarks 8 through 12 were treated as semilandmarks and were located by projecting 30° angles onto the apertural curve from a point midway between landmarks 1 and 7.



generalized least-squares superimposition from which partial warps and uniform components were calculated using tpsRelw (Rohlf 2004). Such methods are more powerful than traditional methods because information about spatial covariation between landmarks is retained, thus allowing the geometry of shape variation to be conserved throughout the analysis (Rohlf and Marcus 1993).

### *Analysis*

Shape data were analyzed separately for six, twelve and eighteen week time points using multivariate ANCOVA (MANCOVA). Shape parameters (i.e.  $n = 20$  partial warps) from twelve digitized points were tested for variation attributable to treatments, family effects, gene by environment effects, tank effects nested within treatments, and centroid size (covariate). Because interactions between the covariate and main effects were found to be nonsignificant, they were removed from the model.

Heritability estimates of the first three major axes of genetic variation were calculated using a combination of a multiple group ANOVA and the intraclass correlation coefficient. In ANOVA, each canonical axis served as the dependent variable with family as the independent variable. The variance component among families,  $s^2_a$ , is equal to:

$$MS_{\text{among}} - MS_{\text{within}} / n_0.$$

$n_0$  is the harmonic mean number of sibs per family and is equal to:

$$n_0 = (1 / (a - 1)) (\sum n_i - (\sum n_i^2 / \sum n_i))$$

The error variance component ( $s^2_e$ ) is equal to the error mean square ( $MS_{\text{within}}$ ). The intraclass correlation coefficient (t) is equal to:  $s^2_a / s^2_a + s^2_e$ . Division of t by the degree of relatedness within sibling groups (r) gives the heritability estimate,  $h^2 = t / r$  (Falconer

and Mackay 1996). Because I used a full-sibling system,  $r$  is equal to 0.5. All statistical analyses were conducted using JMP software (Version 4.04, SAS Institute Inc., Cary, NC).

## Results

### *Effect at six weeks*

After six weeks only two classes of predator exposure existed for snails in this study—either they were raised with crayfish or fish (C, F). These data were used to establish some background information such as the nature and degree of plasticity, the heritability of shell shape, and the nature of heritability in plasticity (i.e. gene by environment interaction variance). My analysis demonstrated that there were clear differences in overall shell shape ( $F_{20, 280} = 18.37$ ;  $P < 0.0001$ ) between treatments. Snails exposed to crayfish were more elongate in shape and snails exposed to redear were more rotund in shape. The predator regime canonical axis explained 56.7% of phenotypic variation. To visualize the effect of treatment on shape variation after six weeks of exposure, I used TpsRegr (Rohlf 1998) to produce thin-plate spline transformation grids to illustrate shape change along canonical axes of the MANCOVA results (Fig 4). To be sure that canonical axis representation did not distort the true shape variation (DeWitt and Papadopoulos, unpublished data), I additionally conducted the standard MANCOVA using procrustes coordinates (slid and aligned specimens) in place of partial warps. This analysis provides least squares means for the conformations in alternative groups. These alternative conformations were plotted with Morphueus et al. morphometric software (Slice 1998) using thin-plate splines for visualization (Fig. 5).

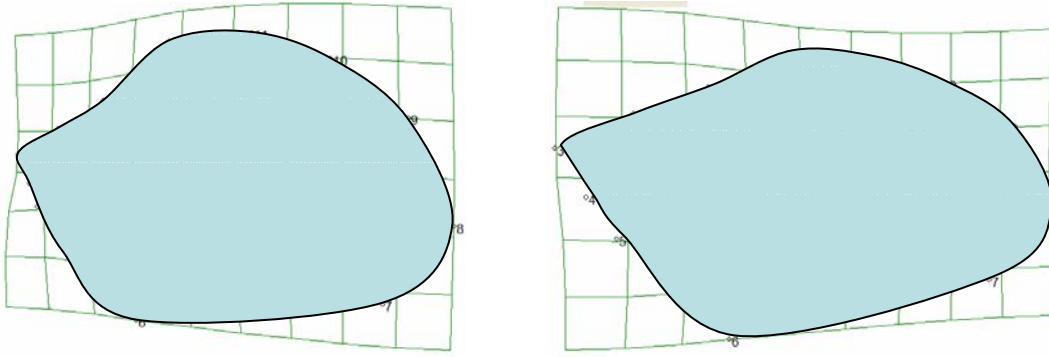


Fig. 4. Thin-plate-spline transformations of redear sunfish (left) and crayfish (right) induced morphologies. Visualizations were produced using tpsRegr (Rohlf 1998) and depict observed range.

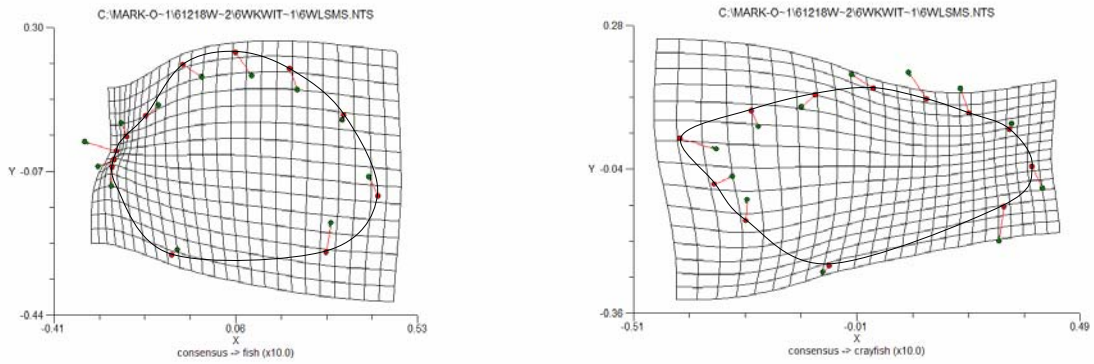


Fig. 5. Transformation between least squares means landmark conformations from a MANCOVA using procrustes coordinates. Image on left pane represents those raised with sunfish, image on right pane represents those raised with crayfish.

I also found a significant effect of family on shape (approx.  $F_{280, 3264.4} = 2.00$ ;  $P < 0.0001$ ). Gene by environment interaction (Family  $\times$  Treatment) was not evident (approx.  $F_{280, 3264.4} = 1.06$ ;  $P = 0.24$ ). In other words, all families responded similarly to the treatment to which they were exposed (i.e., all families were similarly plastic, although family means averaging across treatments differed; DeWitt and Scheiner 2004) (Table 1).

To visualize the reaction norms produced by each family in the analysis, treatment canonical scores were averaged within each environment for each family using the six week data only. These trait means were then graphed to express reaction norms for each family in each environment (Fig. 6). These graphs show the sloped but parallel reaction norms expected when genetic and environmental main effects are significant but the interaction is not (DeWitt and Scheiner 2004). Because a full-sib family system was used, I sought to identify the major axis of genetic variation. That is, I wanted to examine the major manner in which families differed from one another in overall shape. Using the same method I used to visualize the effect of treatment on shape, the first two major canonical axes for family from the MANCOVA were visualized using TpsRegr (Fig. 7 and Fig. 8). I found that the first two major axes of genetic variation looked much like the major axis of variation found for the effect of the predator treatment on shape.

Heritability of the first major axis of genetic variation was estimated to be 0.247. Estimates of heritability of canonical axes two and three were found to be 0.397 and 0.234, respectively. The higher heritability of canonical axis two compared to that of the first axis was unexpected. By definition between group variance is greatest for axis 1, so this result implies there must be lesser within-group variance for canonical axis 2 scores.

Table 1. MANCOVA results for morphological variation at six weeks.

Effects	F	df	P
Treatment	18.37	20, 280	< 0.0001
Family	2.00	280, 3264.4	< 0.0001
Tank (Treatment)	2.04	440, 4225.8	< 0.0001
Family × Treatment	1.06	280, 3264.4	0.25
Centroid size	8.04	20, 280	< 0.0001

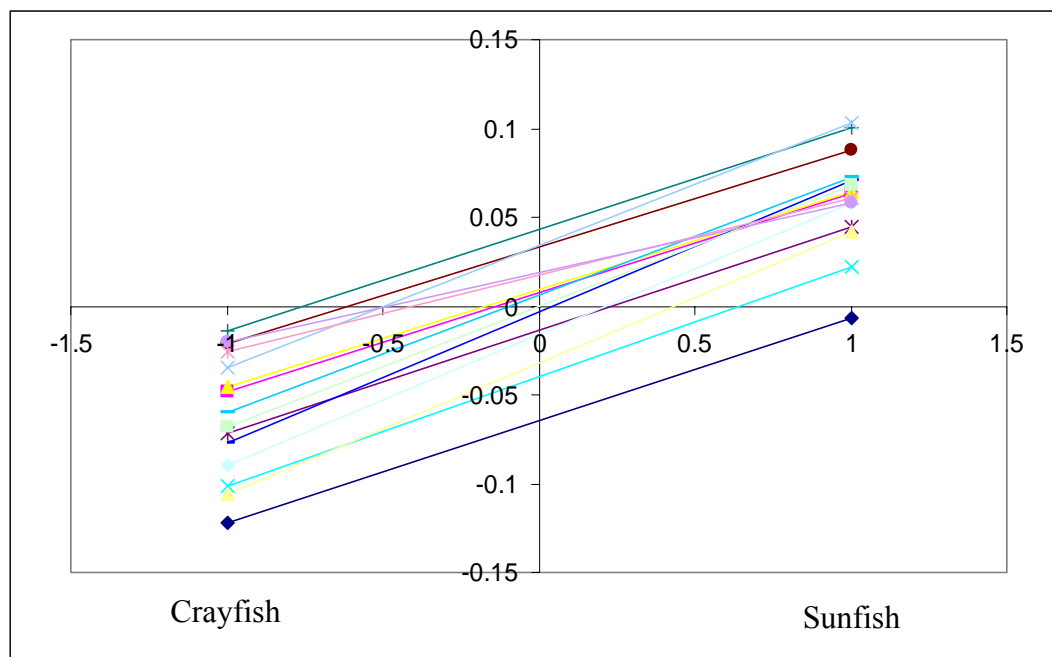


Fig. 6. Reaction norms of genetic variation for the effect of treatment on shape at six weeks. Each family's mean is connected with a line to indicate their particular reaction norm across environments.

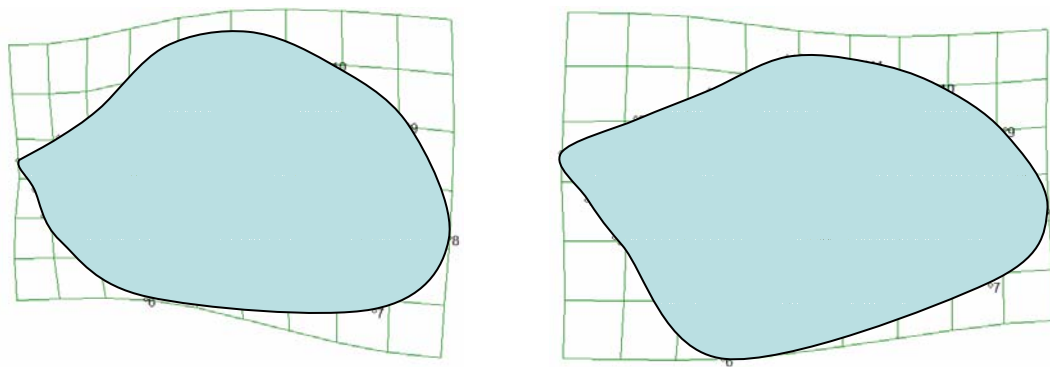


Fig. 7. Visualization of first major (canonical) axis of genetic variation (i.e. variation between families) at six weeks (observed range).



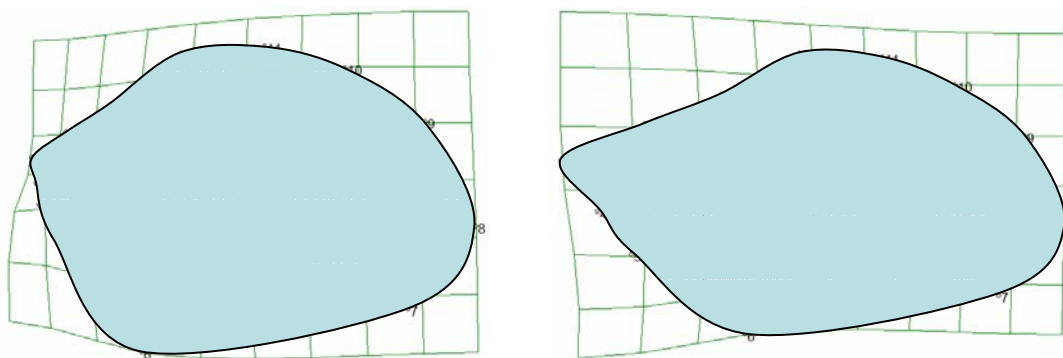


Fig. 8. Visualization of second major (canonical) axis of genetic variation (i.e. variation between families) at six weeks (observed range).

The total number of snails used in the analysis at six weeks was 352 (crayfish = 176, sunfish = 176).

#### *Effect at twelve weeks*

For shape data at twelve weeks, I had snails available from the four treatment groups (CC, CF, FF, FC). I examined canonical axes separating these groups and concluded that canonical axis 1 clearly discriminated between treatments experienced in the first six weeks of exposure, accounting for 50.8% of total phenotypic variation. Examination of canonical axis 2 discriminated between the effect of treatment exposure during the second six weeks, explaining 44% of the residual variation (22% of total variation) (Fig. 9). During this second six week interval snails continued to change shape, but the magnitude of change in shape was not as large as that seen in the first six week interval. The total number of snails used in the analysis at twelve weeks was 330 (CC = 103, CF = 57, FC = 57, FF = 113).

#### *Effect at eighteen weeks*

Snails measured at 18 weeks experienced any of six environmental conditions (CCC, CFF, CCF, FFF, FCC, FFC). From the MANCOVA on shape at eighteen weeks, the first canonical axis discriminated groups by predator experienced in the first six weeks of exposure, the second canonical axis discriminated groups by predator experienced during the second six weeks of exposure, but the third canonical axis did not discriminate between treatments (Fig. 10). Failure of this canonical axis to discriminate between treatments at week eighteen suggests that there was no significant change in

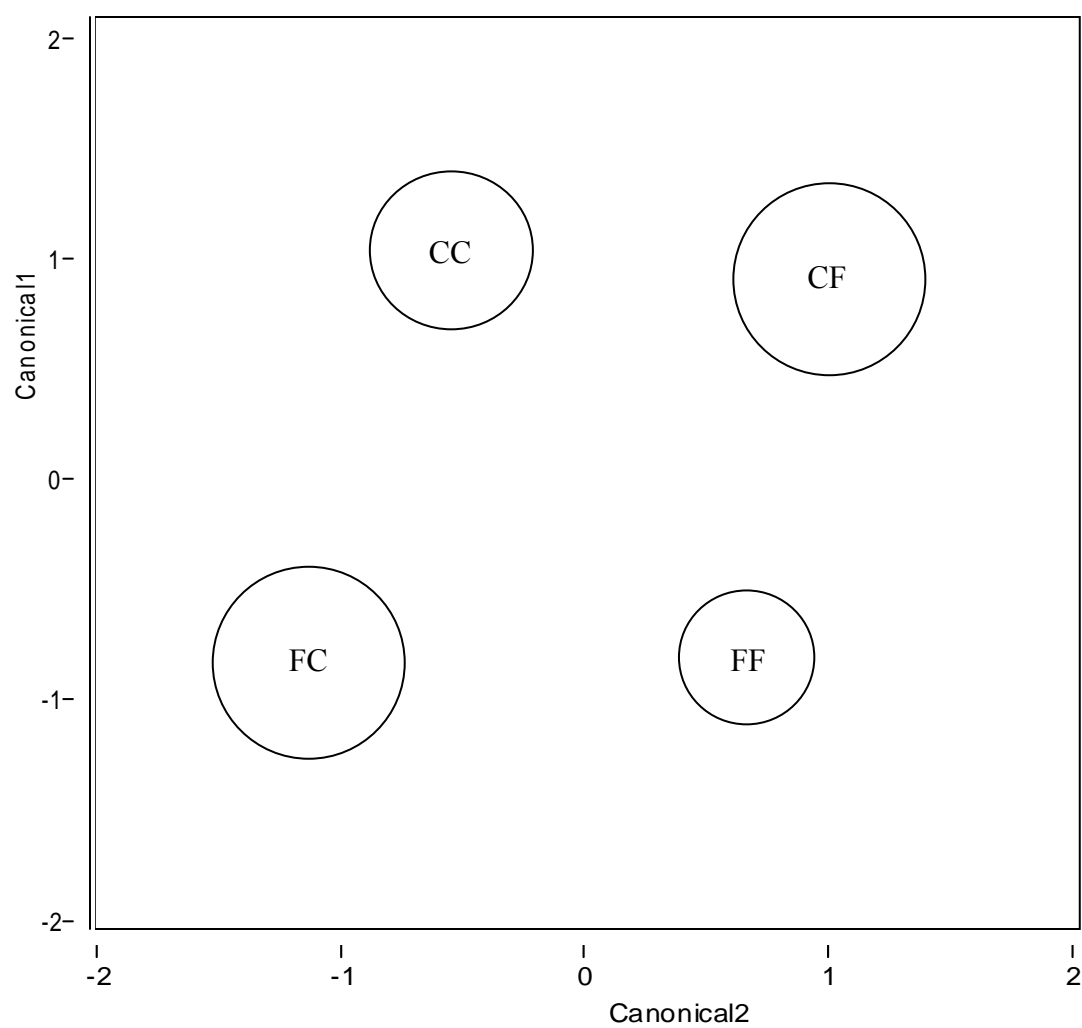


Fig. 9. Group separation by canonical axes after twelve weeks of exposure.

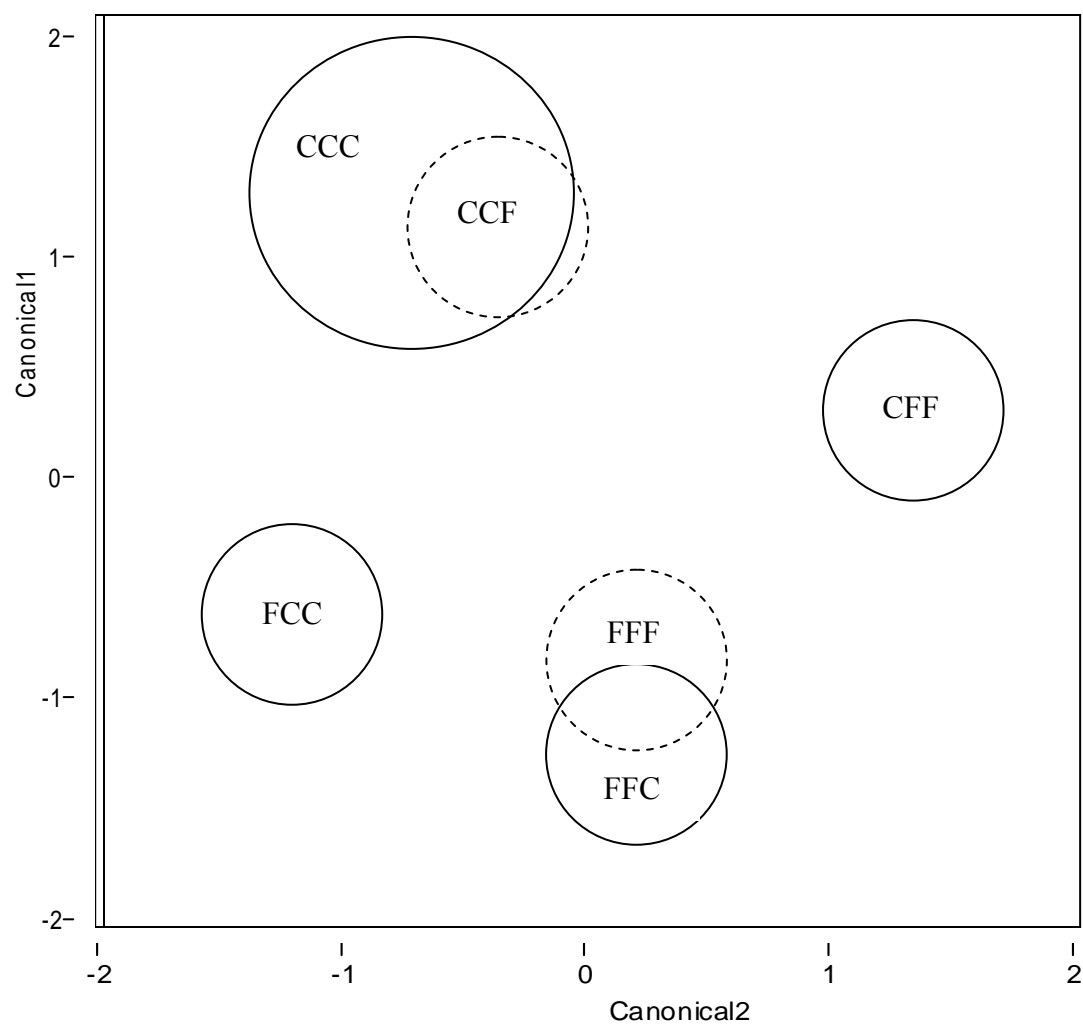


Fig. 10. Group separation by canonical axes after eighteen weeks of exposure.

shape during the final six weeks of predator exposure. The total number of snails used in the analysis at eighteen weeks was 308 (CCC = 42, FFF = 55, FFC = 53, CCF = 52, FCC = 54, CFF = 52).

## Discussion

In this study I found that *P. virgata* produced different morphologies when raised in the presence of alternative predators. Morphology was also found to be heritable, however plasticity itself was not heritable (no genotype  $\times$  environment interaction). Visualization of the first two major axes of genetic variation looked nearly identical to the major axis of variation of treatment on shape. I also found that morphology was less flexible later in life.

Production of an elongate morphology in the presence of crayfish and a rotund morphology in the presence of fish represents adaptive phenotypic plasticity which reduces successful predation by each respective predator (DeWitt et al. 1999). Elongation of the shell not only results in a more occluded aperture, but this shape may provide an area inside the shell into which the snail can further retreat (Vermeij 1982, DeWitt et al. 1999, Krist 2002). This keeps shell-entry predators such as the crayfish from easily accessing the snail body tissue. A rotund shell has been shown to increase crush resistance, thus increasing handling time and rejection rates by durophagous (shell-crushing) predators such as the redear sunfish (DeWitt et al. 1999). For a candid visualization of the effect of treatment on shell morphology, I determined which snails at the study's end had the most extreme predator regime canonical scores and compared their photographs. Figure 11 shows these two individual snails' photographs alongside

one another. The image on the left is of the snail with the most extreme positive canonical score (0.2417) and experienced a CCC treatment throughout ontogeny. The snail image on the right had the most extreme negative score (-0.2902) and experienced a FFF treatment.

Morphology was found to be heritable, but plasticity per se was not heritable. Thus parallel reaction norms describe plasticity in shell shape (i.e., no gene by environment interaction; Fig. 6). This result would be expected where the relative risk of predation by either predator type were variable between generations, or even within generations if the variation were coarse enough that environmental changes could be tracked with developmental responses (DeWitt and Scheiner 2004). If environmental variation were entirely between populations one would expect isolated populations to evolve either fixed or rotund morphology with no developmental flexibility. Yet to the extent that occurs, with a little population mixing one would find that the genetic axis of variation would stretch broadly between the two morphs. This would create the situation where the genetic and plastic axes of variation are parallel in phenotype space. Such was the case in the present data—genetic and plastic axes of variation were highly similar, suggesting that parallelism of variation at multiple levels of the biological hierarchy (genetic, developmental, perhaps species level variation) may be the typical result of divergent natural selection. This is a new topic in evolutionary biology that requires greatly more theoretical and empirical attention (DeWitt, personal communication). For present purposes I just note here the trend toward parallelism.

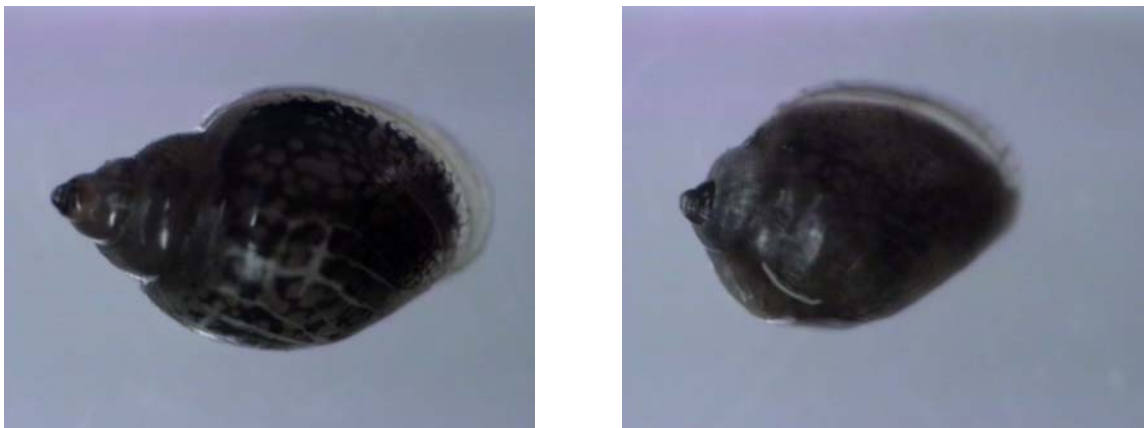


Fig. 11. Photographs of phenotypic extremes induced by predators in this study. The two individuals with the highest and lowest canonical scores for the predator effect axis at six weeks were selected for this visual comparison.

Visualization of each of the first two major axes of genetic variation in TpsRegr showed an effect that was nearly identical to the major axis of variation I found for the effect of the treatment on shape. That the two major axes of genetic variation look much like the effect of predator on shape indicates that this shape axis is important for meting out adaptive solutions for environmental variation occurring on multiple temporal or spatial scales. The second major axis of genetic variation (which is actually more heritable than the first), looks almost identical in all respects (thin-plate spline transformations) to the effect of predator on shape (rotund vs. elongate).

The ability for *P. virgata* to respond to predation with adaptive morphological shifts during ontogeny has previously been shown (DeWitt 1998, DeWitt and Langerhans 2003), but in this experiment I sought to explore constraints on such plasticity. In particular I wanted to know whether the environmental regime in one ontogenetic period affected the response to present shifts in environment. I found that the ability to respond to an environmental shift decays with increasing age, but that response per se is not constrained by the environment in the earlier stages. Since there was so little response to the environment in late ontogeny it should be no surprise that I could not detect an altered nature of response based on earlier environments. After six weeks of predator exposure, snails exhibited extreme change in shape, namely, elongate shell morphology for those exposed to crayfish and a rotund morphology for those exposed to the shell-crushing redear sunfish. During weeks six to twelve, snails continued to change shape but the magnitude of the effect was markedly reduced. During the final six weeks of development (weeks twelve to eighteen), snails exhibited no detectable change in shape despite use of extremely sensitive methods. So, the ability for such change to occur is



reduced as the snails proceed along a developmental trajectory. Such fixing of development after a certain period of exposure (terminating flexibility) represents a major constraint on the evolution of phenotypic plasticity.

The aim of my study was to explore the developmental flexibility of an organism that has become a good model system for plasticity work. In this study physid snails experienced one type of ontogenetic constraint (that due to age) but not the other for which I tested (plasticity early in life constraining potential later in life). These results contrast with that of Weinig and Delph (2001). In annual herbs with modular growth, stem elongation is an adaptive response to foliar shading (detected as a shift in the red:far red wavelength ratio). When shade from competing plants is sensed, they elongate their stem to top their light competitors. In *Abutilon theophrasti*, plasticity expressed early in ontogeny reduced the capacity for response later in life, suggesting a potentially serious constraint on the evolution of phenotypic plasticity. Such constraints limit the value of plasticity relative to fixed development. In the physid system there is still an obvious constraint on the utility of plasticity: developmental shifts that should be adaptive even late in life are apparently not possible (or possible only to a very limited degree). This constraint on adult morphology is likely to be less important in nature than developmental flexibility earlier on, because the risk and fitness consequences of mortality are much greater for young individuals. Old snails do not change much, but there is less imperative to do so.

## CHAPTER III

### STRUCTURAL ASPECTS OF PREDATOR INDUCED SHELLS

#### Introduction

Phenotypically plastic organisms exhibit alternative phenotypes when exposed to different environmental conditions (Bradshaw 1965, Stearns 1989, Schlichting and Pigliucci 1998). Under many circumstances phenotypic plasticity serves an adaptive role, maximizing fitness of individuals in variable environments (Schlichting 1986, Newman 1992, Scheiner 1993, Dudley and Schmitt 1995, Schlichting and Pigliucci 1998). To give an example, several tadpole species alter morphology and produce bright tail colors when raised in the presence of predators such as fish and dragonfly larvae (Van Buskirk and Relyea 1998, Relyea 2001, Teplitsky et al. 2003). A deep and colorful tail serves an adaptive role in that invertebrate predators are more likely to strike at this attractive lure and keep otherwise lethal strikes away from the tadpole's delicate body (Smith and Van Buskirk 1995). Reduced body size and increased tail dimensions can increase burst swimming speed (Dayton et al. 2005) and help avoid strikes by predators (Johnson, Burt and DeWitt, unpublished data). Physid snails live in stochastic environments and use phenotypic plasticity to mitigate natural selection. A classic example of an induced defense serving an adaptive role is seen among many species of gastropods. Shell thickness of aquatic snails (Vermeij 1976, Palmer 1979) has been shown to increase when raised or found in the presence of water-borne molluscivores. One reason why a thicker shell may develop is to offer snails better protection against predators, being that a thicker shell is more difficult to crush with a crab's claw (Seeley 1986, Trussell 1996,

Trussell 2000) or a fish's jaw (Trussell 1996, West and Cohen 1996). Shell shape has also been shown to be influenced by water-borne predation cues. Snails of the family *Physidae* exhibit a shape that is more rotund when reared in the presence of many sunfish species (Langerhans and DeWitt 2002). It is thought that the production of this shape in sunfish environments is adaptive because it increases crush resistance (DeWitt et al. 2000), crushing being the mode which molluscivorous sunfish employ to devour snails (Lauder 1983, Huckins 1997).

My study focuses on predator-induced morphological defenses in a freshwater snail of the family *Physidae*. The aim of my study is three-fold: I first look at differences in shell thickness and shell mass of the freshwater snail (*Physella virgata*) when raised in the presence of the molluscivorous redear sunfish (*Lepomis microlophus*) versus being raised in an environment lacking predators. Second, I examine differences in shell shape between snails raised with and without redear sunfish. Lastly, I explore differences in shell microstructure between snails raised in these two treatments, from the perspective that an increased shell thickness of snails raised with predators can either be due to an increase in crystal layering within the protein matrix of the shell or that, alternately, there are no differences in crystal layering, but that a greater amount of the same material is deposited into their shells.

## **Methods**

### *Snail collecting and rearing*

Freshwater snails (*Physella virgata*) were collected from Krenk Tap pond located in College Station, Texas, USA (30°36'N, 96°17'W). Snails were taken to our

laboratory and treated with Maracyn ( $0.8 \text{ mg} \cdot \text{l}^{-1}$ ) for two days in group culture. During treatment, snails were fed Wardley's brand spirulina flakes *ad libitum*. Spirulina flakes were first ground into powder-form using mortar and pestle. Following treatment with antibiotics, the fifty largest snails of those collected were singly assigned to 300 ml plastic cups containing an RO water/trace elements solution (1ml/15L) (Seachem Fresh Trace). I selected the largest snails as they would be those most likely to produce egg masses large enough to yield an adequate number of hatchlings required for this experiment. Water changes were performed three times per week. Fouled water was poured away and cups were replenished with fresh water containing added trace elements. After each water change, snails were fed with approximately 0.5 mg of ground spirulina flakes.

Several egg masses were noticed within one week. Cups with egg masses containing 12 or more potential hatchlings were kept and parents were removed. In some of these cups I found egg masses having too few potential hatchlings. These smaller egg masses were removed with a wooden spatula and discarded. After hatchlings emerged, water changes were continued as before, but instead approximately 90% of the water from each rearing cup was removed using a syringe. The syringe had a small section of tubing attached to its end and this section of tubing was replaced for each water change of each cup to prevent any possible contamination from one cup to another. Use of a syringe prevented any loss of hatchlings that may have occurred had the spent water been poured from the cups. Eighteen individuals produced clutches of  $\geq 12$  hatchlings. For each of the eighteen families, 12 hatchlings were randomly selected and individually assigned to one of the 12 rearing tanks for which treatments were assigned as described below.

### *Experimental design*

F<sub>1</sub> snail hatchlings were introduced into predator and no predator treatments. Twelve 76-liter aquaria (six containing predators, six containing no predators) were established and systematically arranged to prevent bias. In predator tanks, a single redear sunfish (*Lepomis microlophus*) was placed below a plastic grid in treatment tanks to allow flow-through of fish chemical cues while preventing any physical contact between fish and snails. This species of sunfish was chosen because of it being known as a voracious molluscivore. Tanks containing no predators were set up identically, the only difference being that no predators were introduced into the aquaria. Snails were placed individually into cages above the grid in each tank. Each rearing cage was made of 300 ml plastic cups having two (35×38 mm) mesh windows (mesh size = 0.10 mm) which provided for unrestricted exchange of tank water with the inside of each cage (Fig. 12); this system ensured that cues from the surrounding environment were being received by the snails.

Twelve hatchlings from each of the eighteen families were split into the treatments such that one individual from each family was represented in each tank. Redear sunfish (*Lepomis microlophus*) were purchased from a local supplier. The sunfish were placed in their appropriate aquaria at least two months prior to adding snail hatchlings.

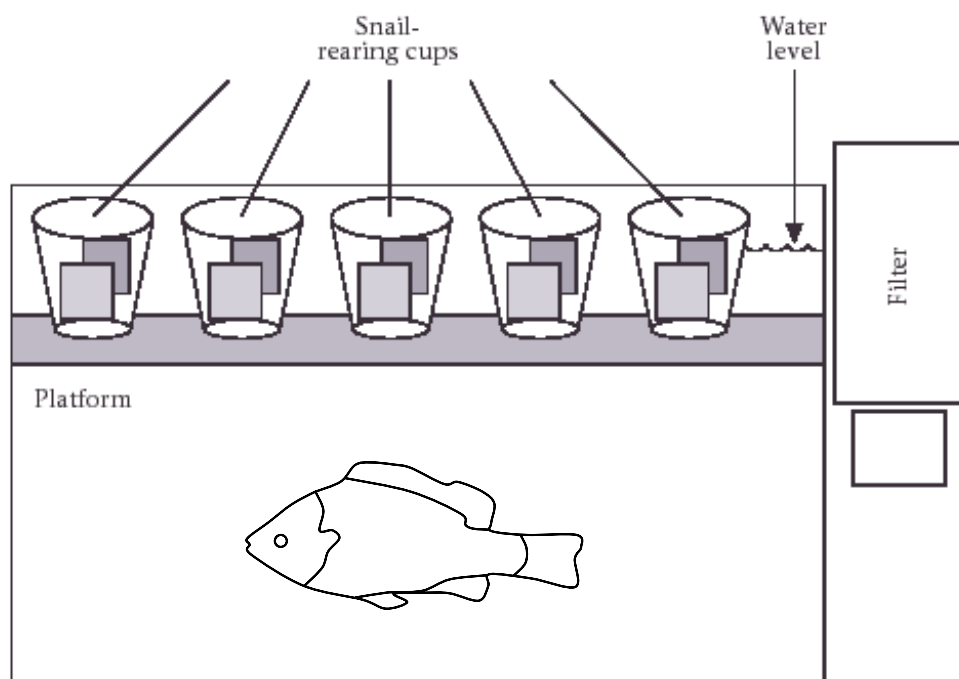


Fig. 12. Diagram of an individual treatment tank (with predator). Tanks without predators were identical except that nothing was placed below the platform on which snail rearing cages rest.

The sunfish were fed commercial pellets three times per week. Snails were fed a pinch of ground spirulina flakes on the same day the sunfish were fed. Extreme care was taken to prevent water in aquaria containing predators from mixing with aquaria containing sunfish and vice versa during feeding or when topping off aquaria as necessary due to water evaporation. Epsom salt was added to each aquarium ( $0.087\text{g} \cdot \text{L}^{-1}$ ) to ensure that the snails had an adequate calcium source during development. Twenty percent water changes were performed twice during the experiment (every 4 weeks) in order to maintain high water quality.

#### *Shell thickness and mass*

Snails were removed after 67 days (approximately three months) of rearing in their respective treatments. The live snails were transferred to Falcon brand 24-well tissue culture plates, as this method was found to be ideal for easy labeling, tracking and containment of individual snails. The snail-containing plates were then placed in a freezer for 24 h and then set out at room temperature and defrosted until ice crystals were no longer visible (as in Vaughn et al. 1993). Snails were individually blotted with a Kimwipe brand tissue, returned to culture plates, then placed into a drying oven and dried at  $60^{\circ}\text{C}$  for 24 h (as in Vaughn et al. 1993). The dried snail body tissue was found to be negligible in total snail mass; therefore all snail bodies were removed from their shells using forceps. Shell weights were taken using a digital laboratory balance accurate to 0.1 g. The total number of shells which weights were measured was 177 (89 predator, 88 no predator). After weighing, shell images were then captured for morphometric analysis as described in the next section.

In measuring shell thickness, I made sure to be consistent in the area of the shell that was to be measured. I chose to measure thickness at the top portion of the shell's final whorl, adjacent to the snail's aperture. I first removed the shell's spire with a razor blade and then picked away the apertural lip using tweezers in order to expose the shell's final whorl. The portion of the shell chosen for measurement was then affixed with epoxy to a zinc-plated steel plumbing washer. The washers (with snail fragments fixed in epoxy) were placed atop modeling clay in order to position the fragment such that a direct view of each shell's cross-section could be achieved. Snail thickness measurements were determined at  $600\times$  magnification using a Hirox 3-D Microscope equipped with measurement software. Because of the shells' brittle nature, several were destroyed during the epoxy-fixing process. Thus the final number of shells on which thickness was measured was 160 (86 predator, 74 no predator).

### *Morphometrics*

After weighing, shell images were captured using a video imaging system. Shells were placed aperture down on a stage below a video camera such that perimeter of the aperture was flush with the stage. The total number of shells' images captured was 164 (84 predator, 80 no predator). Morphometric software (MorphoSys Version 1.29) was used to digitize twelve landmarks along each shell's contour. For each snail, shell outlines and twelve landmarks were digitized (Fig 13). Landmarks were digitized at the shell apex (LM 3), on sutures connecting the current and previous two whorls (LM 1, 2, 4, and 5), at the farthest point of the shell relative to the coiling axis (LM 6), and at the lower insertion of the aperture (LM 7). The remaining five points found along the



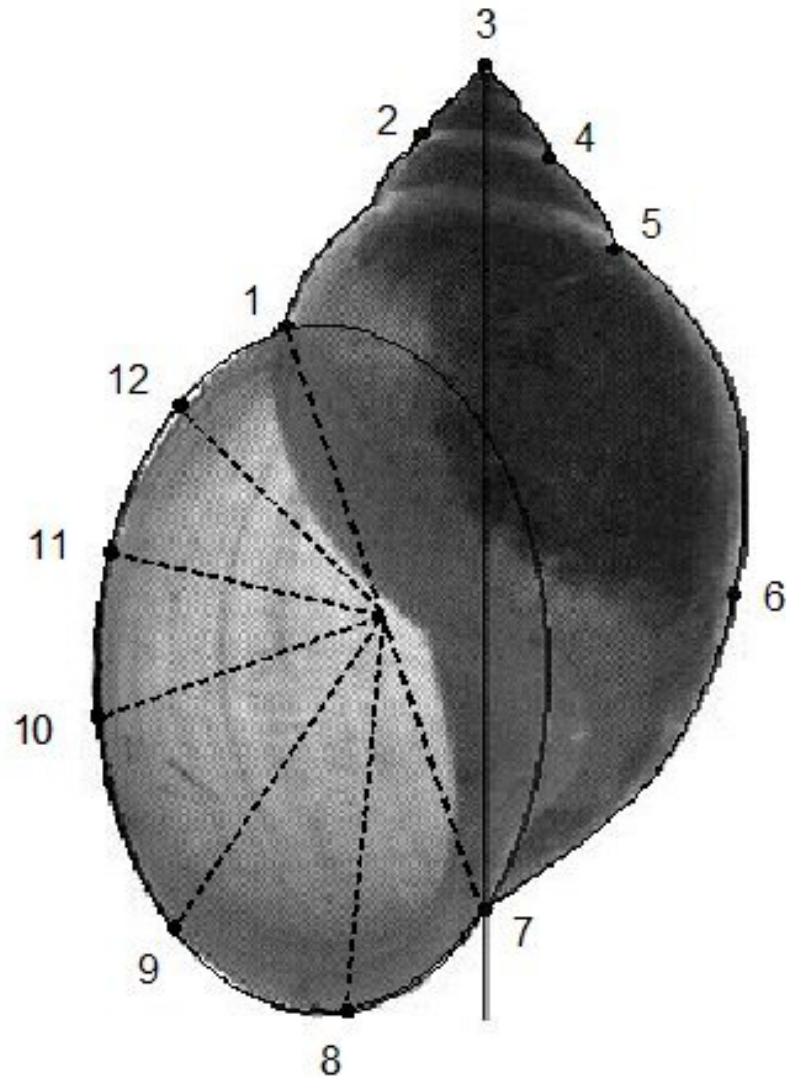


Fig. 13. Landmarks used in the shape analysis. Landmarks 1-7 were digitized manually. Landmarks 8-12 were digitized by projection from the midpoint of chord 1-7 every 30 degrees to the shell margin. These latter points were treated as sliding semilandmarks following Bookstein 1991.

apertural region were treated as semilandmarks and were located by projecting at 30° angles from a point existing halfway between landmarks 1 and 7. Because these semilandmarks (LM 8-12) occur along a curve lacking any truly significant points, they were allowed to “slide” between adjacent points (Bookstein 1991). This method acknowledges the difference between true landmarks and semilandmarks and by design minimizes the bending energy associated with semilandmarks while conserving information about the shape of the curve.

For all specimens, raw landmark coordinates were aligned by generalized least-squares superimposition and used to calculate uniform and partial warps using tpsRelw (Rohlf 2004). In contrast to traditional linear-distance methods, such geometric morphometric methods (Rohlf and Marcus 1993) are much more powerful because spatial covariation between landmarks is retained. Differences in shape are thus conserved throughout the analysis and can be reconstructed using thin-plate splines for visualization (Rohlf and Marcus 1993).

### *Nanomechanical properties*

I examined shell microstructure of snails raised in both treatments using a Hysitron Nanoindenter (Triboscope, Hysitron, Inc.). Nanoindentation techniques have been used extensively to measure the nanomechanical properties of hard thin films. Such techniques are used to gather information about the hardness and elastic modulus of thin films and coatings. For biological materials, nanoindentation experiments have been performed to examine the behavior of materials such as bone and tooth enamel (Kinney et al. 1996, Zysset et al. 1999). For the purposes of this experiment, I used the same

technique to explore shell microstructural differences between snails raised between treatments (i.e., sunfish predators versus no predators). On the same epoxy imbedded shell fragments I used to measure shell thickness, the Nanoindenter was used to gather high-sensitivity force and displacement measurements. Because the shell fragments were imbedded into the epoxy with their outer layer (periostracum) down, indentations were made on the inside portion of the shell that is in contact with the snail body tissue. Due to budgetary and time constraints, I chose to analyze only a subset of the total number of shells available.

Twelve shells from each predator and no predator treatments were analyzed (all from different tanks and different families). Shells were examined using a nanoindentater tip with a 90° cube-corner diamond tip of nominal radius of curvature of 30 nm. Four indentations per shell were performed using a trapezoidal loading curve. Indentations were performed for a maximum load of 9000  $\mu\text{N}$  at a constant loading/unloading rate of 450  $\mu\text{N/s}$ . Data for only ten shells of each treatment were available for analysis due to instrument error.

### *Analysis*

To compare shell size between treatments, shell mass (log transformed) and centroid size were treated jointly using a MANOVA and were tested for effects due to treatment, tank nested in treatment, family, and the effect of gene by environment. Because I had two measures of size (log weight and centroid size) these were combined using principal components analysis. Principal component 1 was used as my measure for size and was used as the controlling factor in comparing shell thickness between

treatments. This comparison was accomplished using an ANOVA, testing for effects due to treatment, tank nested in treatment, family and the interaction of genotype by environment. Statistical analyses of mass, thickness and centroid size data were conducted using JMP software (Version 4.04, SAS Institute Inc., Cary, NC).

For shape analysis, I performed multivariate ANCOVA (MANCOVA) on geometric morphometric data. Shape parameters (i.e.  $n = 20$  partial warps) from twelve digitized points were tested for variation attributable to treatments, family effects, gene by environment effects, tank effects nested within treatments, and centroid size as a covariate. Interactions between the covariate and main effects were tested, found to be nonsignificant and were removed from the model. Shape data for snails from one predator tank and from one no predator tank were left out of the analysis (twelve snails total) due to high mortality, stunted growth and overall poor health of snails found in these two tanks. Statistical analyses of shape data were conducted using JMP software (Version 4.04, SAS Institute Inc., Cary, NC).

To analyze shell nanomechanical properties, I first obtained the average loading slope by linear regression of the nanoindentation load-displacement curves. I took the average slope to be the best estimate of hardness which I term  $H_{\text{best}}$ . However, often the curves displayed a tendency to increase in a stepwise fashion, indicating inhomogeneous layers of shell material. To characterize the magnitude of this stepping, I performed a quadratic regression and measured the deviation between this function and the actual curve (Fig. 14). A high step naturally creates a large deviation from this function and this degree of mismatch is captured readily as  $1 - R^2$  from the regression. I termed this value  $I$ , for inhomogeneity of the loading curve. Measures of material stiffness and

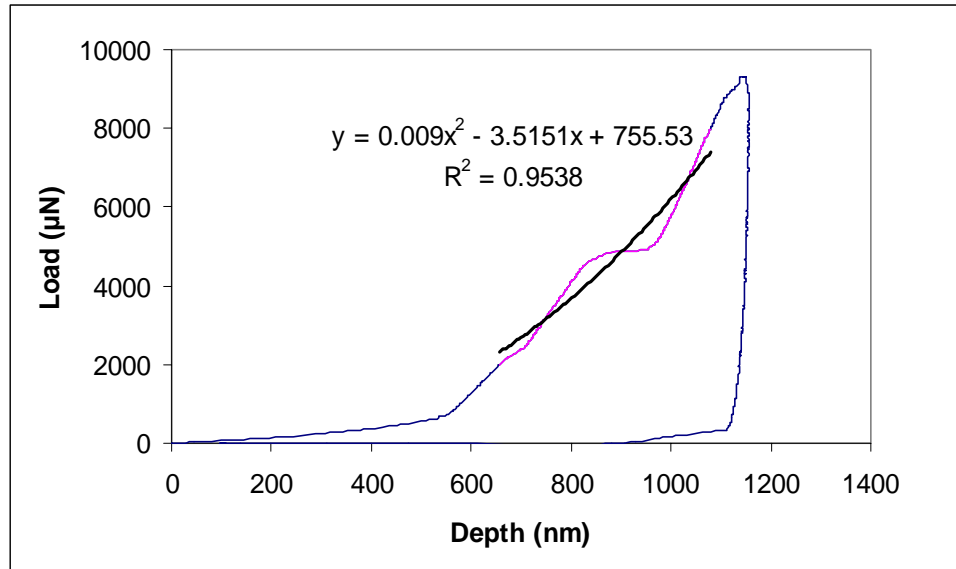


Fig. 14. Example load profile for a shell during nanoindentation. Load increases as the indenter tip penetrates the shell material. The relevant part of the curve for calculating XYZ is given in fuscia above. Shown in blue overlay upon the fuscia line is the linear regression of these points used to calculate X. The difference in R2 between the (blue) linear regression and the (fuscia) quadratic regression was taken as  $I$ , a measure of inhomogeneity of the shell material over the depth of the indentation.

effective hardness of the material were also obtained by the test instrument. Stiffness ( $S$ ) is the slope of the initial unloading portion of the load-displacement curve (i.e. load / displacement) and is a measure of the overall material stiffness. Effective hardness ( $H_{\text{eff}}$ ) is the value of the maximum displacement used for the data analysis. Values of  $H_{\text{best}}$ ,  $I$ ,  $S$  and  $H_{\text{eff}}$  for each of four indentations were averaged for each individual; between-treatment mean differences for these values were tested using a two-tailed Mann-Whitney U-test and was conducted using SPSS Version 13.0 (SPSS Inc., Chicago, IL).

## Results

Snails raised in the presence of predators had both thicker (LSM no predator =  $21.3\mu$ , SE = 0.85, LSM predator =  $28.4\mu$ , SE = 0.75) and more massive (LSM no predator = 9.1 mg, SE = 0.45, LSM predator = 14.2 mg, SE = 0.68) shells than those raised without predators (Fig. 15, Fig. 16). This represents a 33 percent greater thickness and a 56 percent greater mass of those snails raised in a molluscivorous sunfish environment compared to those raised in a predator-free environment. Additionally, centroid size of snails was found to be greater when raised with predators (LSM no predator = 8.99, SE = 0.11, LSM predator = 9.99 mg, SE = 0.10) representing an 11 percent increase in overall shell size compared to those raised without predators (Fig. 17).

Shell shape was influenced by both environmental and genetic effects ( $F_{20, 88} = 4.38$ ;  $P < 0.0001$ ; Table 2). Predator treatment was the strongest determinant of shell shape. Those snails reared with sunfish exhibited a shape that was more rotund than snails reared without predators. The treatment canonical axis explained 49.7% of phenotypic variation. I used TpsRegr (Rohlf 1998) to produce thin-plate spline

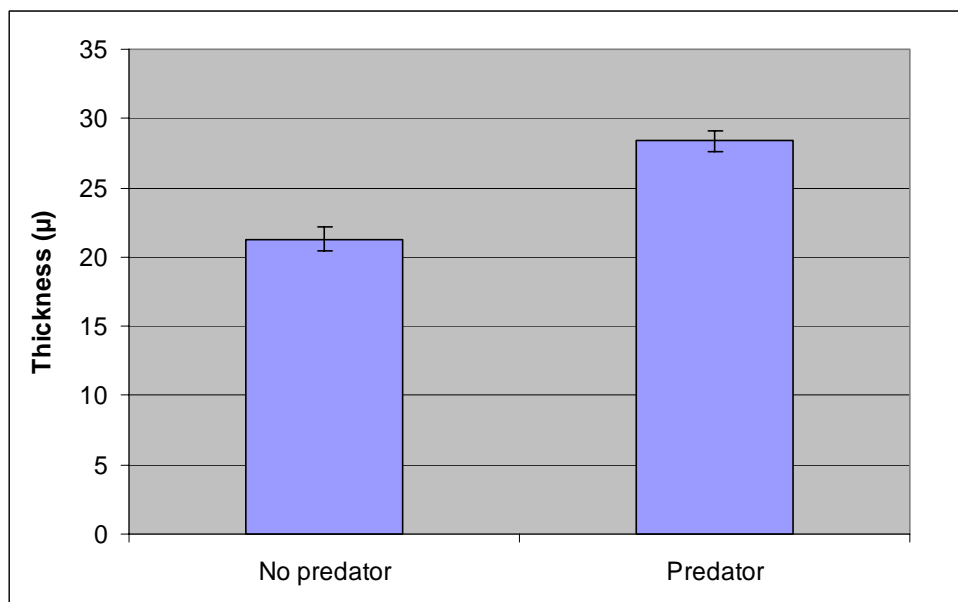


Fig. 15. Predator treatment effect on shell thickness.

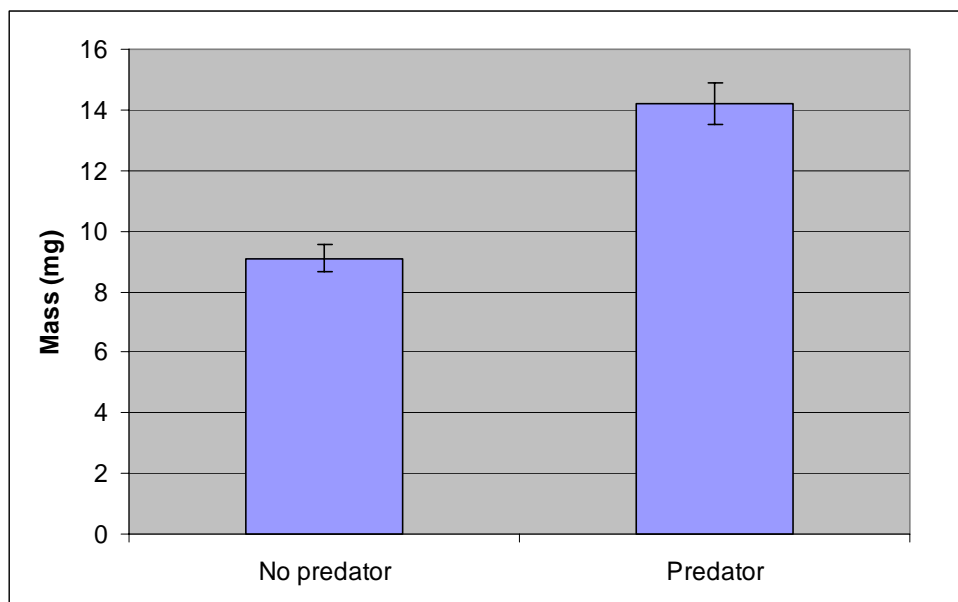


Fig. 16. Predator treatment effect on shell mass.



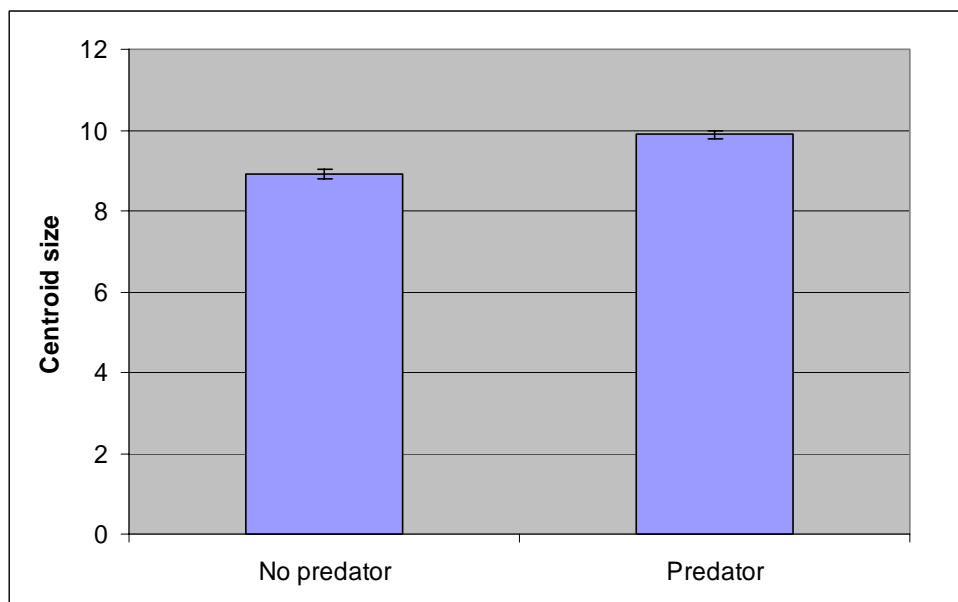


Fig. 17. Predator treatment effect on shell size.

Table 2. MANCOVA results for shell shape variation.

Effects	F	df	P
Treatment	4.35	20, 88	< 0.0001
Family	1.41	340, 1196	< 0.0001
Tank (Treatment)	1.58	160, 671.5	< 0.0001
Family × Treatment	0.95	340, 1196	0.73
Centroid size	1.56	20, 88	0.08

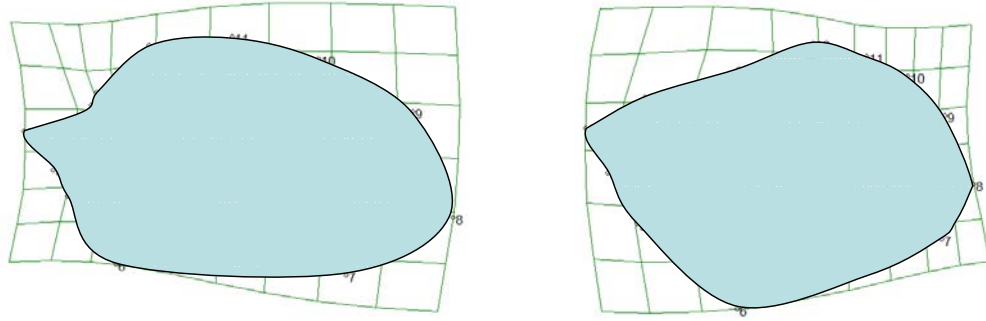


Fig. 18. Predator induced shell shape visualized by TpsRegr visualization using canonical scores from the predator effect in the MANCOVA on partial warps. Image on left pane represents those raised without sunfish, image on right pane represents those raised with sunfish.

transformation grids to illustrate shape change along canonical axes of the MANCOVA results (Fig. 18). I also performed the standard MANCOVA using slid and aligned specimens (procrustes coordinates) in place of partial warps in order to be certain that my original canonical axis representation did not distort true shape variation. Canonical spaces can become distorted if patterned variation exists in the data error matrix (DeWitt and Papadopoulos, unpublished data). The procrustes analysis provides least squares means for the conformations in alternative groups. These alternative conformations were plotted with Morpheus et al. (Slice 1998) morphometric software using thin-plate splines for visualization (Fig. 19).

Shell shape variation was also attributable to a genetic component (i.e. the family effect; approx.  $F_{340, 1196} = 1.41$ ;  $P < 0.0001$ ), but there was no evidence for gene by environment interaction (Family  $\times$  Treatment; approx.  $F_{340, 1196} = 0.95$ ;  $P = 0.73$ ). In other words, all families responded similarly to the treatment to which they were exposed (exhibited similar plasticity) (Table 2).

Nanomechanical analysis of shells showed no significant microstructural differences between shells of snails raised in no-predator and predator environments (Table 3).

## **Discussion**

Physid snails responded to predation cues of a shell-crushing sunfish by altering their shape, overall size, shell thickness and mass. Contrary to what I had expected, I found no between-treatment differences in shell microstructure.

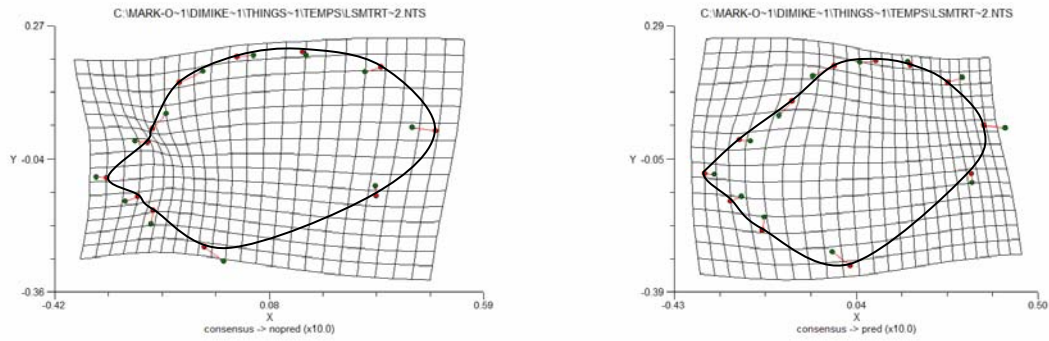


Fig. 19. Predator induced shell shape visualized by transformation between least squares means landmark conformations from a MANCOVA using procrustes coordinates. Image on left pane represents those raised without sunfish, image on right pane represents those raised with sunfish.

Table 3. Mann-Whitney U results for comparison of nanoindentation data between predator and no-predator shells.

	Z-value	P-value
Inhomogeneity (1 - Rsquare)	-0.093	0.354
Stiffness (S)	-1.457	0.145
Effective hardness (Heff)	-0.751	0.453
Hardness best estimate (Hbest)	-0.044	0.965

The phenotypically plastic responses I observed are likely to offer at least three adaptive benefits. First, production of a more rotund shape can increase the likelihood of rejection (DeWitt et al. 2000). Secondly, increasing shell thickness has been shown to increase the force required by a shell-crushing predator to ‘crack’ a shell (Vermeij 1993). Lastly, the additional increase in overall size further may offer an advantage by deterring gape-limited sunfish. These developmental responses likely work together to give the snail greater protection from successful predation by shell-crushing sunfish, thus increasing their chance of survival.

In my examination of shell microstructure, I was interested in identifying any differences in between-treatment crystal layering. The outer covering of shells in freshwater snails is known as the periostracum and protects the shell from chemical dissolution and physical erosion. The underlying shell layers are laid into an organic matrix by the snail’s mantle as calcium carbonate crystals (aragonite). I chose to measure the portion of the shell that is in contact with the mantle to avoid any interference that the periostracum may have given had I chosen to measure top-down, so to speak. Although shell thickness was greater overall for those snails raised with sunfish, this thickness was increased by depositing more of the same material into the shell and not by altering the manner in which aragonite was layered.

The nanoindentation approach I used in this research to my knowledge has not been applied before in ecological studies. Yet the potential for uncovering microstructural differences in the material properties of organismal phenotypes is potentially great. In the present case I found no shell microstructure differences when snails were reared with shell-crushing redear sunfish versus rearing in the absence of predators. The differences I

found all pointed to developing shells that were larger, thicker and more difficult to crush when predators were present. Although I had expected that shells may layer crystals differently in their shells, I found none. The only innovations I uncovered regarding shell defenses against predation involved the amount of material (shell thickness and size) and the gross conformation of the material (shell shape).



## CHAPTER IV

### SUMMARY

Many organisms respond to their environment with adaptive developmental shifts. In the case of the physid snail, morphology and structure can be profoundly influenced by the presence of particular predators. These phenotypes are adaptive in that in each case fitness is enhanced by improved phenotype-environment matching. In this thesis, I illustrate morphological responses of physid snails to the presence of different predation regimes (sunfish, crayfish and no predator).

The experiment discussed in Chapter II illustrates how physid snails produce different morphologies in the presence of different predators (redear sunfish vs. crayfish). Because I used a family structure, after six weeks of exposure I was able to illustrate reaction norms for each family across the two environments, determine heritability estimates of shell shape, show that morphology was heritable, and show major axes of genetic variation. Monitoring shape change throughout development in changing predation regimes allowed me to discover that the ability for snails to change shape is reduced as development progresses.

In Chapter III, I explored not only differences in shape between snails raised with and without predators, but I also looked at differences in shell thickness, mass, overall size and microstructure. Snails raised with predators developed a defensive shell shape and also produced thicker, larger, more massive shells. These morphological responses likely work together to reduce mortality by shell-crushing predators. Although shell thickness, mass and size differed between treatments, I found no differences in shell

microstructure. This is taken to mean that snails raised with predators increase shell thickness by adding more of the same material to the shell.

This body of work, taken with previous studies, suggests a remarkable degree of developmental flexibility on the part of prey exposed to diverse predators in nature. Physid snail responses to predators are many and varied, though I did find limits to how developmentally flexible snails can be. The complexity and breadth of induced responses in physid snails suggests that equally broad and complex adaptations may be common for other organisms. Only by including such complexity in our analysis of adaptations are we likely to fully understand the nature of adaptation.

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