INFLUENCE OF MULTIPLE ENVIRONMENTS ON TADPOLE PHENOTYPES:
PLASTICITY, ADAPTATION, AND CONSERVATION IMPLICATIONS

A Dissertation

Submitted to the Graduate School
of the University of Notre Dame
in Partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy

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July 2009
INFLUENCE OF MULTIPLE ENVIRONMENTS ON TADPOLE PHENOTYPES:
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Abstract

by

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Phenotypic plasticity is the ability of an organism to alter its phenotype in response to changing environmental conditions. The recognition that phenotypic changes often improve the fitness of individuals (i.e., are adaptive) has expanded interest in plasticity as an important driver of many ecological and evolutionary processes.

Predation risk and competition density are important environmental factors that induce adaptive phenotypic changes in many species. However, the specific impact of these factors on an individual’s phenotype depends on the foraging behavior employed by the predator, the type of competition experienced (interference vs. exploitative), and other environmental conditions.

For anuran larvae (i.e., tadpoles), the presence of vertical habitat structure may affect phenotypic responses by altering: 1) capture success and foraging behavior of predators and 2) the type and level of competition experienced. Using large-scale mesocosm experiments, I found that, compared to simple environments, tadpoles were
less likely to express adaptive behavioral and morphological phenotypes in habitats containing greater environmental complexity. For example, tadpole phenotypic responses to changes in habitat structure and competition density were adaptive, but only in the absence of predation risk. However, in a survey of natural ponds, predation risk and competition density, but not habitat structure, explained substantial portions of tadpole phenotypic variance. Even though tadpoles experience changes in multiple environmental variables, predation and competition seem to exert the greatest influence in shaping tadpole phenotypes.

Predation risk and competition intensity also interacted to modify the development of a maladaptive tadpole malformity linked to ultraviolet-B radiation overexposure. Behavioral changes of tadpoles induced by predation and competition, such as activity levels, likely mediated this effect.

Through this research, I have demonstrated that increasing environmental complexity constrains the ability of organisms to express adaptive phenotypes. Patterns of phenotypic plasticity observed in laboratory conditions do translate to natural environments, but are limited to phenotypic responses to environments that exert strong inductive and selective pressures. Furthermore, phenotypic plasticity can modify the susceptibility of organisms to the effects of environmental stressors.
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ACKNOWLEDGMENTS

I would like to first thank my advisor, Gary Belovsky, for accepting me into his lab and granting me the independence to decide on my own research project. He has helped in every aspect of this thesis, from the design of experiments to the interpretation of the results. Many more thanks are extended to the entire Belovsky lab including Angela Laws, whose sarcastic wit cannot be topped; Dave Choate, who, more than anyone, has helped improve my scientific writing (although he would cringe at the split infinitive within the first sentence); David Hoekman, who helped me keep atop the newest ecology literature; Chelse Prather, who kept me sane during my final year; and the newest members, Chris Patrick and David Flagel. Thanks are also extended to Gary’s non-graduate student team, including Kelly Stanton, Heidi Mahon, and Brad Wright.

I thank Arthur J. Schmitt for his generous contributions to the University of Notre Dame and establishment of the Schmitt graduate fellowships.

I would also like to thank my committee members. Jessica Hellmann provided great encouragement and advice on early experimental designs. Hope Hollocher unselfishly helped untangle messy results, and our discussions together were tremendous learning opportunities for me. Rick Relyea provided great expertise and was always available for quick chats, even though he was about 400 miles away.

Other faculty at the University of Notre Dame were very helpful, including the UNDERC assistant directors, Michael Cramer and Gretchen Gerrish. Jason McLachlan was extremely helpful and encouraging during a difficult statistical modeling period. Jeff
Feder was a constant source of knowledge. David Lodge was extremely helpful in experimental design issues.

I should save one paragraph to thank Kerry Yurewicz who was the major reason how I got started working with tadpoles. Discussions with Kerry greatly improved my experiments, and she was very helpful in guiding me around the various ponds at UNDERC.

The graduate student community at Notre Dame also deserves a thank you, especially to Andrew and Tori Forbes who remain great friends and have great scientific minds. Dave Costello, Konrad Kulacki, Tom Powell, Brett & Jody Peters, Matt Barnes, Ashley Baldridge, Natalie Griffiths, and others all helped make graduate school an enjoyable experience. Many others helped out with specific experiments, and they are thanked at the end of all data chapters.

Finally, I would like to thank my family, especially my amazingly supportive parents for the many selfless sacrifices they have made to make this entire dissertation possible. My brothers, James and Andrew, and my sister, Anna, have been encouraging and supportive.
1.1 Overview

When confronted by environmental change, organisms may alter the expression of their morphological, behavioral, or developmental phenotypes, an ability termed phenotypic plasticity (Pigliucci 2001, West-Eberhard 2003, DeWitt and Scheiner 2004). Historically considered as developmental noise and an “embarrassment” to experimental studies (Bradshaw 1965, p. 148, as cited by West-Eberhard 1989, p. 249), phenotypic plasticity has recently become an increasing area of research for the ecology and evolution of species communities (Pigliucci 2001, 2005, West-Eberhard 2003, Scheiner and DeWitt 2004, Miner et al. 2005). This increased interest has been prompted largely by the recognition that many plastic phenotypic changes improve the fitness of the individual inhabiting the new environment (i.e., are adaptive). Consequently, the ability of an organism to express adaptive plastic traits often becomes selectively favored within populations (Bradshaw 1965).

Within a food web, phenotypic changes of one species can affect other species, termed a trait-mediated interaction (Werner and Peacor 2003). For example, many prey species react to the presence of predators via reductions in foraging activity (Lima and Dill 1990, Lima 1998), which can subsequently promote the growth of the prey’s food resource (Agrawal 2001, Werner and Peacor 2003, Schmitz et al. 2004). Phenotypic
plasticity can also affect nutrient cycling (Miner et al. 2005), population dynamics (e.g., Verschoor et al. 2003) and the susceptibility of organisms to environmental stressors such as pesticides (e.g., Relyea and Mills 2001, Relyea 2003) or parasites (e.g., Decaestecker et al. 2002).

For species evolution, phenotypic plasticity allows organisms to rapidly adapt to changing environments by facilitating the expression of different phenotypes without the need for corresponding changes to the genotype. Among natural systems, plasticity is thought to have contributed to the adaptive radiation of several taxa groups including Caribbean Anolis lizards (Losos et al. 2000), African cichlids (Galis and Metz 1998), and fishes of northern postglacial lakes (Robinson and Parsons 2002). Plasticity has also been suggested as a significant promoter of speciation through the process of genetic accommodation, in which the expression of a once-plastic phenotype becomes developmentally canalized due to constant environmental conditions (Waddington 1975, West-Eberhard 2003, Schlichting 2004). Canalization occurs because maintaining the molecular machinery required for phenotypic plasticity is thought to be costly (West-Eberhard 2003).

Phenotypic plasticity is clearly an important component of many ecological and evolutionary research areas. However, despite a recent intensification in plasticity research, there remain several unanswered questions and issues, particularly regarding phenotypic responses in complex environments. How do phenotypes change in response to multiple environmental variables? Most studies of phenotypic plasticity only examine the trait responses of individuals to changes in one type of environment. Do plastic phenotypes that are adaptive in simple environments retain their fitness advantages
within more complex environments? Also, are patterns of phenotypic plasticity observed within laboratory conditions consistent with patterns observed in natural environments? Due to the inherent complexity of natural environments, answering these types of questions is critical to understanding the causes and implications of environmentally-induced phenotypes within natural environments. The objective of this dissertation was to address these and other related questions through a variety of laboratory experiments and field surveys.

1.2 Study System

The tadpole stage of frogs is an ideal study system to address these questions. Tadpole phenotypic responses to several environmental variables have already been documented in a number of laboratory studies, providing important a priori knowledge that can greatly inform hypotheses that address the effects of multiple environmental factors on tadpole phenotypes. Additionally, tadpole laboratory experiments can be easily replicated using artificial ponds (Wilbur 1997). Replication is especially crucial for complex experimental designs in which several treatment levels of multiple environmental factors need to be manipulated. Finally, natural tadpole habitats provide well-defined units (i.e., ponds) as well as subunits (i.e., microhabitats within ponds) for which phenotype-environment correlations at multiple spatial scales can be inferred.

Predation risk and competition are two important environmental variables that induce a variety of tadpole phenotypic changes (Smith and Van Buskirk 1995, Relyea 2002, Benard 2004, Miner et al. 2005), and only recently have the interactions between predation risk and competition on tadpole phenotypes been investigated (e.g., Relyea
The magnitude of predator- and competitor-induced traits expressed by tadpoles, though, is dependent on specific environmental conditions. For example, the abundance of vertical structure can affect phenotypic responses by altering a tadpole’s perception of predation risk and competitive intensity, although this hypothesis has never been empirically tested. Therefore, in order to more accurately predict predator- and competitor-induced tadpole phenotypes in natural environments, it is necessary to examine how structural complexity affects tadpole phenotypes independently as well as synergistically with predation risk and competition.

1.3 Summary of Chapters

In chapter 2, I determine how the presence of structural complexity affects the foraging behavior of two common predators of tadpoles. Structure can either interfere with the ability of the predator to capture prey (reviewed in Horinouchi 2007) or induce the predator to shift from an active to a sit-and-pursue (SAP) foraging mode (e.g., James and Heck 1994, Laurel and Brown 2006). Because tadpoles exhibit different suites of behavioral and morphological defenses in response to active and SAP predators (Teplitsky et al. 2005, Touchon and Warkentin 2008), a shift in predator foraging mode induced by structure could subsequently modify predator-induced responses of tadpoles inhabiting structured habitats.

In chapter 3, I examine how the presence of predators, competitors, and structural complexity affect tadpole growth rates through their effects on tadpole foraging behavior and the abundance of food resources. Empirical and theoretical studies on food web
dynamics generally assume that activity levels of prey provide a direct quantification of foraging effort (e.g., Anholt and Werner 1995, Eklov and Werner 2000, Peacor 2002). Consequently, changes to overall activity levels are thought to affect individual growth rates as well as the abundance of the individual’s food resource (Peacor 2002). This assumption is especially prevalent in predator-prey theory and has led to the concept that the presence of predators can cause a trophic cascade mediated by behavioral changes of the prey (e.g., Schmitz et al. 2004). However, it is rarely recognized that foraging effort of many prey species is a composite of several different behaviors, such as searching for food and actual feeding time (but see McCollum and Van Buskirk 1996, Van Buskirk and Yurewicz 1998). Additionally, many studies that examine environmental impacts on prey growth rates attribute changes in growth to the effects of the environment on either prey foraging behavior or resource abundance (but see Werner and Peacor 2006). This chapter addresses how environmental factors affect both these components of growth rates.

In chapter 4, I examine how the presence of predators, competitors, and structural complexity affects tadpole morphological traits. The adaptive plasticity hypothesis posits that phenotypic changes of an individual in response to certain environmental variables improve the fitness of the individual inhabiting that new environment (Dudley and Schmitt 1996). However, most tests of the adaptive plasticity hypothesis consider trait responses to changes in one environmental factor (e.g., Van Buskirk and Relyea 1998). Recent research suggests that many organisms can fine-tune their phenotypic responses to multiple environments (e.g., Relyea 2004), but we lack explicit tests of the adaptiveness of these responses. In this chapter, I provide such a test by comparing the
predator-induced traits of tadpoles with the traits that are selected for by predators foraging within habitats containing different structure densities.

In chapter 5, I examine how phenotypes of tadpoles collected from natural ponds correlate with multiple environmental variables. Environmental heterogeneity favors the development of phenotypic plasticity (Via and Lande 1985, Scheiner 1998, Ernande and Dieckmann 2004, Leimar et al. 2006) and local polymorphisms (e.g., Juenger and Bergelson 2002, Svensson and Sinervo 2004). However, the scale at which environmental variables exhibit heterogeneity can differ. Therefore, the strength of phenotype-environment associations of organisms in natural environments should likewise be scale-dependent. In this chapter, I use hierarchical linear models and measures of spatial autocorrelation to test this relationship for tadpoles inhabiting temporary ponds.

In chapter 6, I examine how predators and competitors can modify the harmful effects of ultraviolet-B radiation (UVBR) on tadpoles through their changes to tadpole behavior. Amphibian populations worldwide are currently experiencing drastic declines (Rohr et al. 2008), and several hypotheses have been proposed, including the increase of UVBR caused by the depletion of stratospheric ozone (e.g., Collins and Storfer 2003, Blaustein and Kiesecker 2002). Indeed, amphibian eggs and larvae exposed to high UVBR levels do experience increased malformations (e.g., Blaustein et al. 1997) and mortality (Bancroft et al. 2008). Ecological interactions, such as predation or competition, often affect the impacts of several environmental stressors, including pesticides (Relyea and Mills 2001, Relyea 2003) and parasites (Thiemann and Wassersug 2000, Decaestecker et al. 2002, Johnson et al. 2006), on various aquatic organisms.
However, the effects of predators and competitors on susceptibility of organisms to UVBR are unknown. This chapter provides the first empirical test of how behavioral plasticity can potentially modify harmful effects of UVBR on aquatic organisms.

In chapter 7, I present a synthesis of these previous five chapters and suggestions for future research directions.

1.4 References


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CHAPTER 2: 
DIFFERENTIAL EFFECTS OF STRUCTURAL COMPLEXITY ON PREDATOR 
FORAGING BEHAVIOR

2.1 Abstract

The choice of predator foraging mode has important consequences for ecological communities. Foraging mode designations are often made on the basis of predator activity, yet activity can be affected by various environmental stimuli independent of changes in foraging mode. Structural complexity can reduce predator activity by either 1) interfering with predator vision and mobility or 2) as part of a foraging mode shift. To distinguish between these two possible outcomes, I examined the effects of simulated aquatic vegetation on multiple behaviors of two aquatic insect predators feeding on anuran larvae (i.e., tadpoles). Larvae of the diving water beetle (*Dytiscus* spp.) shifted from an active predator in treatments without structure to a sit-and-pursue (SAP) predator in treatments containing structure, as indicated by a decrease in activity and prey encounter rates and an increase in probability of capture. This tradeoff between encounter rates and probability of capture resulted in an equal number of prey captures among the treatments. Dragonfly nymphs (*Anax juniqus*) remained SAP predators in both treatments, although interference from the simulated vegetation significantly reduced activity. Structure also slightly decreased the number of aeshnid prey captures. Physiological attributes of the predators, such as mode of respiration and method of prey
detection, seemed to influence foraging behavior. This study emphasizes the benefits of measuring multiple predator behaviors when classifying predators to particular foraging modes.

2.2 Introduction

The foraging behavior that a predator adopts within a particular environmental context has multiple effects on ecological communities, including the strength of predator consumptive effects (Coen et al. 1981, Huey and Pianka 1981) and nonconsumptive effects (Preisser et al. 2007), the types of inducible defenses exhibited by prey (Teplitsky et al. 2005), the effects of multiple predators on prey mortality (Schmitz 2005, 2007), the occurrence of trophic cascades (Post et al. 1999) and changes in ecosystem function (Schmitz 2008).

Traditionally, predator foraging modes are classified as one of three alternatives (McLaughlin 1989, Schmitz 2005, 2007): (1) active, wherein a predator moves through its environment to find and capture prey, (2) sit-and-pursue (SAP), in which a predator waits for prey to approach before striking and (3) sit-and-wait (SAW), wherein a predator remains at a fixed location for a prolonged period of time (i.e., days or weeks). Measurements of predator activity are often used as the sole basis to differentiate between these three modes (e.g., Savino and Stein 1989, Perry 1999), yet other metrics, such as encounter rates (Werner and Anholt 1993, Scharf et al. 2006) are known to differ among modes.

Predator behavior is quite dynamic in response to changing environmental conditions (Lima 2002). Indeed, several studies have documented shifts in predator
foraging mode in response to changes in prey density (Huey and Pianka 1981, Formanowicz 1982, Hirvonen 1999), prey species (Savino and Stein 1989), the presence of structural complexity (James and Heck 1994), and even reproductive condition (Crowl and Alexander 1989). However, not all predator species exhibit these shifts (e.g., Savino and Stein 1982, Ostrand et al. 2004, Laurel and Brown 2006), therefore, it is important to determine which predator species show foraging mode plasticity in response to particular environmental stimuli.

I tested for the presence of a shift in foraging mode induced by structural complexity for two aquatic insect predators. For lentic, aquatic systems, structure is typically vertical in nature and is comprised of submerged or emergent vegetation that rises from the bottom of the water body (Heck and Crowder 1991). Many studies have demonstrated that structured microhabitats increase prey survival from predation (reviewed in Heck and Crowder 1991, Denno et al. 2005, Horinouchi 2007), while a few studies have shown decreased prey survival (e.g., Flynn and Ritz 1999).

Whether structure inhibits or facilitates predators depends on the behavior and physical hunting characteristics of the predator. Structure hinders predators that rely on highly developed vision to detect prey as well as mobility to pursue and capture prey (Savino and Stein 1982, Folsom and Collins 1984, Manatunge et al. 2000), yet can aid other predators by providing perches to ambush prey (James and Heck 1994) or by concealing predators that are cryptic (Laurel and Brown 2006, Manderson et al. 2000). Some predators exhibit foraging mode plasticity in response to structure. For example, in non-structured habitats, seahorses (*Hippocampus erectus*) are active foragers, but shift to an SAP tactic when placed in artificial seagrass beds (James and Heck 1994). Foraging
mode shifts hold important implications for prey populations because predators that shift foraging strategies often do not experience a reduction in the total number of prey captures (e.g., Formanowicz 1982, Laurel and Brown 2006), while predators that lack this plasticity—and are instead impeded by structure—usually capture less prey (e.g., Babbitt and Tanner 1998, Manatunge et al. 2000).

The two different predator species used in this study, larvae of the diving water beetle (*Dytiscus* spp.: Dytiscidae) and nymphs of the common green darner (*Anax junius*: Aeshnidae, Anisoptera), differ in their hunting behavior and physical characteristics. Dytiscid larvae have poor vision, and rely on chemical and tactile cues to detect prey (Formanowicz 1987), while aeshnids detect prey using highly developed compound eyes (Sherk 1977). Dytiscids are slow swimmers but highly maneuverable, whereas aeshnids can propel themselves rapidly through the water using a jet-propulsion system (Nachtigall 1985). They also differ in respiration mode: dytiscids breathe atmospheric air through a spiracle located in the terminal abdominal segment, whereas aeshnids are gill-respirators. Due to the physiological differences between these two predators, I examined if structural complexity will differentially affect their foraging behavior. Previous studies have established that, in habitats without structure, dytiscid larvae are active foragers (Formanowicz 1982) and aeshnid nymphs are SAP foragers (Pritchard 1965). I hypothesize that 1) structure will induce dytiscids to shift from an active to an SAP foraging mode by providing perches for them to hunt and breathe simultaneously, and 2) structure will induce aeshnids to become more active predators, in order to offset any structural interference of vision or mobility. Because predator activity reductions in response to structure may be due to either a foraging mode shift or structural interference,
I measured multiple predator behaviors in addition to activity, such as encounter rates, probability of capture, and capture success, to better characterize predator foraging modes. For example if structural complexity reduces predator activity and capture success, then interference is inferred; if structural complexity reduces predator activity, but not capture success, a shift in predator foraging mode is inferred.

2.3 Methods

2.3.1 Predator and prey

The prey species used in the predation trials were tadpoles of the eastern gray treefrog (*Hyla versicolor*). At the study site, *H. versicolor* tadpoles are important food sources for both dytiscids and aeshnids, however, the two predator species show limited temporal overlap, as dytiscid larvae typically begin pupation when dragonflies begin depositing eggs. Field surveys and laboratory experiments indicate that both predator and prey species inhabit open and vegetated microhabitats within ponds (Formanowicz and Bobka 1989, Chapter 5, Michel unpresented data).

In early June 2006, a total of eight amplexing *H. versicolor* pairs from three open-canopy ponds located at the University of Notre Dame Environmental Research Center (Gogebic County, MI) were collected in 5-gallon buckets and allowed to breed overnight. All eggs were then transferred to a 1 m diameter plastic wading pool filled with 55 L of well water and inoculated with 500 ml of pond water. After hatching, tadpoles were fed pulverized rabbit food ad libitum until they reached a minimum size appropriate for predation trials (about 200 mg).
Dytiscus and Anax larvae were collected with minnow traps placed in three open-canopy ponds. Individuals were also opportunistically dipnetted from these same ponds. Trapping for Dytiscus larvae was conducted between May and mid-June, while Anax larvae were trapped between late July and August. Predators were kept indoors in 500-mL polypropylene jars and fed one H. versicolor tadpole daily. Each predator was starved for 24 h before being used in experimental trials.

2.3.2 Experimental Arena

Experiments were conducted in two 48 cm × 24 cm glass aquaria. To simulate vertical structure, 112 strands of 0.64 cm diameter × 7 cm green polypropylene rope were tied to a black plastic mesh cut to fit the bottom of the aquarium, yielding a density of 972 stems·m⁻² (equivalent to 40% cover, Savino and Stein 1982). This density falls within the range observed in natural ponds located within the study site (0 – 1,234 stems·m⁻², Michel unpresented data). The mats were placed into the aquarium and covered completely with approximately 1 cm of washed gravel to anchor the mats and allow the rope strands to float. For the treatment without structure (hereafter, open treatment), the aquarium contained only the black mesh and the washed gravel, but no rope. Both aquaria were filled with day-old well water kept outside to mimic temperature regimes in open-canopy ponds. Previous observations had indicated that cold, fresh well water markedly reduced tadpole activity.

2.3.3 Experimental Trials

Before observations began, one predator was placed in each tank and allowed to acclimate for one hour. Predators of similar lengths and developmental stage (3rd instar)
were used for each particular trial. Twelve randomly selected tadpoles (corresponding to a density of 104 tadpoles·m⁻², consistent with tadpole densities in natural ponds) were weighed and introduced into each tank. Tadpole body size was similar among treatments (independent two-sample t-tests: *Dytiscus – Hyla*, *t*₁₈ = 0.583, *p* = 0.567; *Anax – Hyla*, *t*₁₈ = 0.037, *p* = 0.971). One observer simultaneously watched both tanks for one hour and recorded the following predator behaviors with the assistance of a digital voice recorder: % activity (calculated as the number of seconds the predator moved divided by the total number of seconds observed), number of encounters with prey (whenever the predator and a prey individual came within approximately 1 cm of each other), number of strikes, and number of prey captures. The use of a digital voice recorder allowed for the simultaneous observation of both tanks.

Predator and tadpole individuals were used only once and water was changed between every trial to minimize the effects of accumulated chemical alarm cues and predator kairomones. Due to different phenologies, *H. versicolor* tadpoles and *Dytiscus* were observed in July, and *H. versicolor* tadpoles and *Anax* were observed in August. Ten replicates of each species pair treatment combination were conducted; however, percent activity for one trial of the *Anax - H. versicolor* predator-prey pair was lost due to human error.

A potential confounding factor of this experiment is that behavioral responses of tadpoles to the structure may mediate changes in predator behavior more than the direct effect of the structure. However, this possibility seems unlikely because previous experiments have found that similar structure treatments had no significant effect on *H.*
*versicolor* tadpole activity in either the presence or absence of predators (Michel unpresented data, see Chapter 3 for data on wood frog tadpoles).

### 2.3.4 Statistical Analysis

Because phylogeny can potentially confound comparisons of foraging modes between distantly related predator species (Perry 1999), I did not explicitly make comparisons of hunting behaviors between the two predator species. Only percent activity, after an arcsine, square root transformation, met the assumptions for parametric statistics and was analyzed using an independent two-sample *t*-test. The three remaining response variables (# encounters, # strikes, # captures) were analyzed using a Kruskal-Wallis test, with a Bonferroni alpha adjustment of \( \alpha = 0.0167 \). Analyses were performed using Systat 10.

Initially, the effect of structure on predator probability of capture (defined as # captures/# strikes) and probability of attack (# strikes/# encounters) was tested using each predator trial as a separate replicate. However, some predators had no strikes or captures; therefore, biasing the estimate. For example, a capture success proportion of 0/17 is identical to a proportion of 0/3, yet, given that the energy expenditure of a predator striking 17 times is greater than a predator striking 3 times, these two values should be regarded as different. Therefore, for each treatment, I obtained an aggregate capture and attack probability by dividing the sum of all 10 captures (or strikes) by the sum of all 10 strikes (or encounters). Then I calculated the difference between the capture or attack probabilities of the two treatments. To test the probability of obtaining a larger difference in capture or attack probabilities, I used a bootstrap approach to randomly generate a distribution of these differences. From the original dataset of 20 experimental
trials (i.e., 10 replicates from the structure treatment and 10 replicates from the open treatment), I randomly selected without replacement two sets of 10 data pairs (i.e., captures and strikes for one predator), obtained an aggregate capture or attack probability for each set, and then calculated the difference between these two sets. I repeated this process 1000 times. A two-tailed probability value was obtained by dividing the number of generated differences greater than the ± original difference by the 1000 replications. Bootstrap procedures were performed using R 2.7.0.

2.4 Results

2.4.1 Dytiscus - H. versicolor

Structural complexity significantly reduced the number of dytiscid strikes by 69%, the number of encounters by 67% (Table 2.1A), and dytiscid activity by 57% ($t_{18} = 7.245, p < 0.001$, Figure 2.1A). The number of captures did not differ by treatment. Aggregate capture probability of dytiscids in the structure treatment was 0.500 compared to 0.244 for the predators in the open treatment (Table 2.1B; structure: $0.583 \pm 0.148$, open: $0.302 \pm 0.084$, Figure 2.1B). The difference between the two treatments ranked 27th out of 1000 runs giving a significant $p$-value of 0.027. Dytiscid attack probability in the structure treatments (0.500) did not differ significantly from predators in the open treatments (0.523, $p = 0.742$).
2.4.2 Anax - H. versicolor

Aeshnids in the structure treatment significantly reduced activity by 46% \((t_{16} = 2.497, p = 0.024, \text{Figure 2.1A})\). The number of strikes, captures, and encounters was not affected by structure (Table 2.1A). The differences in aeshnid capture and attack probabilities between the structure and open treatments were also not significantly different \((p = 0.872, \text{and } p = 0.982, \text{respectively, Table 2.1B})\).
TABLE 2.1

PREDATOR RESPONSE VARIABLES FOR THE TWO PREDATOR-PREY COMBINATIONS.

\[ \text{Dytiscus – Hyla} \quad \text{Anax - Hyla} \]

(a) Kruskal-Wallis \( p \)-values

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SE</th>
<th>( P )</th>
<th>Mean</th>
<th>SE</th>
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<tr>
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</tr>
<tr>
<td>Structure</td>
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<td>&lt;0.001</td>
<td>6.60</td>
<td>1.19</td>
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<tr>
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</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Structure</td>
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</table>

(b) Bootstrap \( p \)-values

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<td><strong>Probability of attack</strong></td>
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<tr>
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<tr>
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</table>
Figure 2.1 A) Percent activity and B) probability of capture for the two predator-prey combinations. Data are mean ± 1 SE.
2.5 Discussion

Structure had multiple effects on the foraging behavior of dytiscid larvae but affected only the activity level of aeshnid nymphs. As hypothesized, dytiscid larvae shifted from an active forager in the open treatments to an SAP forager in the structure treatments. Changes in activity, encounter rates, attack rates, and capture probability were indicative of this shift. Contrary to my hypothesis, aeshnids actually decreased activity when foraging in structured environments. However, because no other aeshnid behavior changed in response to structure, I believe this activity reduction is a result of interference from structure rather than a shift to a more sedentary foraging mode. These results highlight the necessity of examining multiple predator behaviors when classifying predators to a specific foraging mode. Because recent papers have stressed the impact that predator foraging mode has on the interactions within ecological communities (Schmitz 2005, 2007, 2008, Teplitsky et al. 2005, Preisser et al. 2007), it is important that predator mode designations are accurate.

A reduction in predator activity in response to the presence of structural complexity is common among aquatic systems (reviewed in Denno et al. 2005, Horinouchi 2007), and arises from one of two mechanisms. First, structure may hinder the swimming speed of predators, especially if the predator is much larger than both the prey and the unit of structure (Savino and Stein 1989, Priyadarshana et al. 2001, Horinouchi 2007) and may also obstruct predator vision and thus encumber the active pursuit of prey (Manatunge et al. 2000; Denno et al. 2005). Second, structure may induce a predator to shift between foraging modes, in which a reduction in activity comprises one part of the shift (e.g., Savino and Stein 1982).
Physiological attributes and the body plan of the predator species seem to have determined the outcome of structural complexity on foraging behavior. Dytiscid larvae are unlikely to be hindered by structure because they are highly maneuverable yet slow swimmers (approximately 6 cm·s\(^{-1}\); Nachtigall 1985) and rely on chemical and tactile cues rather than vision to detect prey (Formanowicz 1987). I also observed that, in the presence of structure, dytiscids preferred to perch on rope stems near the water surface to ambush prey, which simultaneously allows for the respiration of atmospheric air. Aeshnid nymphs, conversely, are highly visual predators (Sherk 1977) and use swim bursts, with a maximum velocity of 30 cm·s\(^{-1}\) (Nachtigall 1985), to capture prey. Structure may have delayed the initial visual detection of tadpoles, as indicated by an ad hoc comparison demonstrating that structure significantly increased the time for an aeshnid to capture its first individual (mean ± 1 SE; open = 431.4 s ± 174.2 s, structure = 1058.8 ± 224.5; Mann-Whitney \(U = 15.0, p = 0.024\)). However, whether these predator attributes can be used to predict the foraging mode of predators has come under debate (Perry 1999).

Foraging mode shifts in response to structural complexity are not uncommon among aquatic systems. James and Heck (1994) found that seahorses became ambush foragers in simulated seagrass environments by perching onto grass blades. Other explanations for shifting foraging modes in response to vertical structure include concealment (i.e., sculpins, Laurel and Brown 2006; summer flounder, Manderson et al. 2000) and energy minimization (i.e., largemouth bass, Savino and Stein 1982). In all these studies, predators switched from an active foraging mode in the treatments lacking
structure to an SAP mode in the treatments containing structure, which may indicate a general trend.

As SAP foragers, dytiscids had significantly fewer encounters with prey but higher capture probabilities than as active foragers, which reflects a tradeoff between each foraging strategy (Werner and Anholt 1993, Scharf et al. 2006). Because attack rates did not differ between treatments, this tradeoff resulted in similar capture rates across treatments. These tradeoffs may generally result from foraging mode shifts as Formanowicz (1982) found that a shift in foraging behavior in response to increasing prey densities did not result in a difference in the total number of prey captures.

Structure did not decrease the number of aeshnid prey captures, contrary to the results of previous studies (Babbitt and Jordan 1996, Babbitt and Tanner 1997, Tarr and Babbitt 2002). Prey survival from predators that do not shift foraging modes in response to structure may function on a structural complexity gradient. As stem density increases, impediments to vision and movement would become more pronounced, resulting in reduced prey captures.

Hirvonen (1999) found that larval damselflies (Lestes sponsa) gradually shifted from SAP to active foragers as prey densities increased and then back to SAP foragers as prey densities increased even more. These shifts were mediated by the relative energetic profitability of each foraging mode, believed to be determined mostly by prey encounter rates. Such a nonlinear, gradual foraging mode shift in response to increasing prey densities does not seem likely for predators responding to structural complexity. For dytiscids, the reason for the foraging mode shift in structured habitats seems to be the
availability of perch sites. Therefore, even at very low stem densities, dytiscids would be expected to show a distinct shift in foraging mode.

It is important to understand how environmental factors alter predator foraging behavior, because the distinction between, for example, structure causing a shift in predator foraging mode or structure merely interfering with predator foraging activity can have wide-ranging ecological and evolutionary impacts. Predation has two different effects on prey populations: consumptive effects (also termed “density-mediated interactions”), which include the death of prey individuals due to predation, and nonconsumptive effects (also termed “trait-mediated interactions”), such as the phenotypic changes that some prey species undergo in response to cues indicating the presence of a predator (Werner and Peacor 2003, Abrams 2007). This experiment provides a test for the former and concludes that, due to a shift in foraging mode, dytiscid capture rate does not differ among open and structured environments. In contrast, aeshnid capture rate may decline as the density of structurally complexity increases due to interference of aeshnid vision and movement (Babbitt and Jordan 1996, Tarr and Babbitt 2002).

While this experiment did not test for predator nonconsumptive effects on prey, several conjectures can be made. First, if the magnitude of antipredator responses is related to predation risk (Lima and Dill 1990), then prey inhabiting densely vegetated environments should exhibit fewer or reduced inducible defenses to predators obstructed by structure, but not to predators that exhibit behavioral plasticity in foraging mode. Second, Teplitsky et al. (2005) observed that an active predator induced a different suite of prey morphological and behavioral traits than those induced by an SAP predator.
Consequently, depending on the structural complexity of a microhabitat, prey exposed to predators that shift foraging modes may exhibit different suites of inducible traits, even though the predator cues remain the same. Third, in a meta-analysis, Pressier et al. (2007) found that SAP foragers induced stronger prey responses than both active and SAW predators. Therefore, the magnitude of antipredator traits in response to predators may depend on the structural complexity of the environment. Given the abundance of studies on the effects of predator foraging mode and structural complexity on predator consumptive effects, future studies should focus on the effects of predator behavior and structural complexity on the phenotypic plasticity of prey organisms.

2.6 Acknowledgments

I would like to thank Melinda Adams, whose dedication to this experiment made it possible and publishable. L. Huebner and C. Spears assisted in the field. I thank G. Belovsky, T. Crowl, D. Choate, A. Laws, R. Relyea and two anonymous reviewers for their helpful comments on previous versions of this manuscript. I also thank N. Preston for assistance with data analysis. This research was conducted with approval from the Institutional Animal Care and Use Committee of the University of Notre Dame (Protocol No.: 07-085).

2.7 References


CHAPTER 3:
ENVIRONMENTAL CHANGE ALTERS PREY GROWTH RATES BY MODIFYING FOOD ABUNDANCE BUT NOT PREY ACTIVITY

3.1 Abstract

Environmental factors can influence growth rates by altering multiple mechanisms of growth, such as the foraging activity of an individual and the food abundance in a given habitat. Many studies, however, examine how environmental factors alter growth rates through their modifications to a single growth mechanism. Additionally, foraging behavior is often quantified only by an estimation of overall activity levels, even though foraging is actually a composite of several different behaviors, such as searching for food and actual feeding time. In this study, I examine how intraspecific competitor density, predation risk, and density of structural complexity impact the growth rates of wood frog tadpoles (*Rana sylvatica*) through their effects on tadpole food abundance (i.e., periphyton) and various components of tadpole foraging behavior (overall activity, swimming and feeding times). Structure had only weak effects on tadpole growth rates and did not interact with competition or predation risk. Competition decreased growth rates through a reduction in per-capita food abundance while the presence of predators increased tadpole growth rates by promoting the growth of periphyton. Competition and predation risk did alter overall tadpole activity levels, but results from focal observations demonstrate that tadpoles adjusted swimming time
and not feeding time in response to these treatments. Therefore, the increase in periphyton abundance among predator treatments was more likely a result of added nutrients from the wastes of the predator rather than an indirect effect from reduced tadpole activity. This study concludes that tadpole growth was more affected by changes in food abundance, rather than prey activity levels, caused by the three environmental variables.

3.2 Introduction

The rate at which an individual grows and develops is an important fitness component of many prey organisms with complex life histories (Wilbur and Collins 1973, Werner 1986). In aquatic systems, faster growth allows individuals to metamorphose rapidly and escape potential hazards such as predator colonization (Benard 2004, Relyea 2007) or the threat of desiccation (e.g., Petranka and Sih 1987). Large body size can increase individual competitive ability (Brooks and Dodson 1965), fecundity (Tilley 1968) and survival (Smith 1987), decrease susceptibility to predation through a size refuge (Kishida and Nishimura 2004), and provide greater access to potential mates (Berven 1981). Performance traits such as overall speed (Van Buskirk and McCollum 2000) and predator escape speed (Richardson 2002, Wilson et al. 2005) increase with body size, however, excessively rapid growth may negatively affect burst speed (Arendt 2003).

Two major determinants of growth rates are the amount of time an individual spends foraging and the abundance of available food resources (Werner and Anholt 1993, Peacor and Werner 2000). Environmental factors can alter these two components,
resulting in changes in prey growth rates. However, many studies examine the effects of environmental factors on growth rates by quantifying their effects on only one of several components of growth, such as foraging behavior (e.g., Relyea 2002a) or resource abundance (e.g., Burley et al. 2006). Because environmental factors often have multiple, complex effects on these growth rate determinants, it is difficult to ascribe with confidence the mechanisms by which changes in the environment affect growth rates. Additionally, most empirical and theoretical studies that examine the effects of foraging activity on growth rates consolidate various foraging behaviors into a singular metric of overall activity level (e.g., Anholt and Werner 1995, Eklov and Werner 2000, Peacor 2002, but see McCollum and Van Buskirk 1996, Van Buskirk and Yurewicz 1998). Parsing out the various components of overall activity, such as search time, vigilance time, and feeding time will provide more information on how prey adjust these specific behaviors in response to changes in the environment (Lind and Cresswell 2005), and, subsequently, how these changes affect growth rates.

In this study, I examine how growth rates of a focal prey species are affected by the combinations of three different environmental factors—predation risk, intraspecific competition and density of structural complexity—as mediated by their effects on prey foraging behavior and prey food resource abundance. Predators often induce a reduction in prey foraging activity, which can lead to a decrease in growth rates (Werner and Anholt 1996, Peacor and Werner 1997, Brown et al. 1999, Relyea and Werner 1999, Relyea 2000, Turner 2004, Werner and Peacor 2006). Conversely, by consuming prey and inducing foraging activity reductions, predators can lessen the pressure on food resources, subsequently causing an increase in growth rates of surviving individuals.
Additionally, predators can increase resource abundance by the introduction of nutrients from organic wastes and prey carcasses (McCollum et al. 1998, Vanni 2002, McIntyre et al. 2004). Competition reduces prey growth rates through interference (i.e., competitors directly interact with each other) and exploitative (i.e., competitors share a limiting resource) mechanisms. Competition induces behavioral changes in some species, such as increased foraging activity, but these changes are often not adequate to overcome the depletion of a limiting resource (e.g., Relyea 2002).

Structural complexity is defined as the density of physical structure (e.g., vegetation, woody debris) in the habitat. Structure can either discourage (e.g., Crowder and Cooper 1982) or promote (e.g., see Results section, this chapter) the abundance of an organism’s food resources. Additionally, by providing a temporary refuge from predation (e.g. Babbitt and Tanner 1998), structural complexity may allow prey to relax antipredator behavior (Woodley and Peterson 2003), which may then permit increased foraging time. However, few studies have examined the possible interactions between predation risk, competition, and structural complexity on prey growth rates.

I conducted this study using tadpoles of the wood frog (*Rana sylvatica*) and a common predator, the larvae of the diving water beetle (*Dytiscus* spp.). *R. sylvatica* are mostly benthic algal scrapers and feed primarily on periphyton (Sciesari et al. 2009). *R. sylvatica* tadpoles decrease overall activity in response to chemical cues from dytiscid larvae, resulting in reduced growth rates (Relyea 2003, Schoeppner and Relyea 2008, but see Relyea 2001), but no studies have examined how tadpoles alter components of foraging in response to predation risk from dytiscid predators. Intraspecific competition reduces *R. sylvatica* growth rates (Van Buskirk and Yurewicz 1998, Relyea 2002b, 2004,
even though the presence of competitors induces an increase in *R. sylvatica* activity (Van Buskirk and Yurewicz 1998, Relyea 2002b, 2004). Predation risk and competition can also interact to affect *R. sylvatica* activity levels and, subsequently, growth rates: the degree to which tadpoles reduce activity in response to predation risk increases as competitor density decreases (Relyea 2004). In pond habitats, structural complexity is present mostly in the form of aquatic vegetation. Periphyton grows readily on aquatic vegetation; thus, the presence of structure will likely increase tadpole food resources. I hypothesize that: 1) predation risk will decrease prey growth rates, caused by predator-induced changes in prey activity, 2) competition will decrease growth rates, caused by changes in per capita resource abundance and 3) structural complexity will increase growth rates, caused by increases in prey food abundance provided by the structure. I further hypothesize the following interactions between these three factors: 1) the magnitude of the predator-induced decrease in growth rates will be greater at low competition densities (Peacor and Werner 2000), and 2) the negative effect of predation risk and competition on growth rates will decrease as the level of structure increases due to the effects of structure on prey activity and prey food resource levels.

3.3 Methods

3.3.1 Collection of organisms

In early May 2006, *R. sylvatica* tadpoles were collected, using dipnets, from three temporary ponds located within the University of Notre Dame Environmental Research Center (UNDERC, Gogebic Co., MI) and placed in outdoor wading pools (~ 1 m
diameter, 15 cm water depth) before utilization in experiments. Tadpoles were approximately Gosner developmental stage 27 (Gosner 1960) when collected. From May to June 2006, larvae of the diving water beetle (*Dytiscus* spp.) were collected from six temporary ponds using minnow traps and dipnets. Dytiscids were kept individually in 500-ml polypropylene jars and fed tadpoles once daily.

3.3.2 Experimental mesocosms

The experiment was replicated using 60 60.5-l circular tubs (48 cm diameter, 24 cm water depth) covered with fiberglass window screening. Tubs were filled with well-water and inoculated with 1 g crushed rabbit food and 500 ml of pond-water two weeks before the experiment to stimulate algal growth for tadpole consumption. Each tub was then randomly assigned one level from each of three factors: 1) predators – no predator or one caged predator, 2) competitors – 0 or 14 additional conspecific tadpoles, and 3) structure – 0, 28 or 84 rope strands—to simulate the physical effects of submerged aquatic vegetation (Savino and Stein 1982). All factors were fully crossed for a total of 12 treatments and each treatment was replicated 5 times. Predators were housed in 10 cm × 10 cm PVC pipe covered with window screening on both ends; no-predator tubs received an empty cage. Predators were fed 500 mg of *R. sylvatica* tadpoles every other day throughout the duration of the experiment. Green polypropylene rope strands (23 cm length, 0.635 cm diameter) were tied to a black plastic mesh cut to fit the bottom of the tub. One 7 cm × 7 cm clay tile (to obtain estimates of periphyton abundance, see below) and three scrubbed rocks anchored the mesh to the tub bottom.

In order to estimate periphyton growth on the rope strands and in the experimental mesocosms in the absence of tadpoles, nine additional tubs—three of each structure
treatment—were assembled as described above with the exception that no tadpoles were added. These tubs will be referred to as ‘no-tadpole’ tubs.

In early May, 7 or 21 *R. sylvatica* tadpoles (depending on the competitor treatment) were weighed (initial mass ± 1 SE; 73.23 ± 0.94 mg) and then added to each tub. After a 24h acclimation time, one empty cage or one cage containing a dytiscid larva was then added. Twenty-two days later, when the first tadpoles reached Gosner stage 42 (emergence of the forelimbs), the experiment was terminated and all surviving tadpoles were counted, weighed and then preserved in 10% formalin for later morphological measurements (see Chapter 4). Tadpoles were not replaced during the experiment (survival was relatively high at 96.2% and did not differ significantly among treatments).

3.3.3 Response variables

Three sets of response variables were measured: 1) tadpole behavior, 2) tadpole growth and development, and 3) periphyton abundance. Behavior was measured using both scan and focal sampling. For scan samples, I recorded the proportion of moving tadpoles out of the number of visible tadpoles (e.g., Relyea 2001). For focal samples, I watched one individual tadpole in each tub for one minute and recorded the number of seconds it spent resting, swimming, or feeding (McCollum et al. 1997, Van Buskirk and Yurewicz 1998), as well as the substrate on which it was feeding. Feeding was characterized by the movement of the mouth to scrape algae combined with the distinctive tail flick tadpoles use to generate force during feeding. *R. sylvatica* tadpoles are facultative filter feeders (Seale and Wassersug 1979) but I did not observe any filter feeding during this experiment. Focal and scan sampling were performed on five
separate occasions during similar time periods. Behavioral observations began five days into the experiment and ended two days before the experiment was concluded.

Average growth rates per tadpole (mg/tadpole/day) were estimated by subtracting the total initial mass from the total final mass and dividing by the number of tadpole in each tank and the duration of the experiment in days. Developmental stage was obtained using a Gosner developmental stage table.

The day after tadpoles were removed, I collected the clay tile and two rope strands (i.e., when available) near the clay tile for all tubs including the no-tadpole tubs. Estimates of periphyton abundance were conducted separately for both tile and rope. Periphyton was removed and filtered onto two pre-weighed ash-dried and two fresh Whatman GF/F 25 μm filters. The ash-dried filters were then weighed, ashed, and then weighed again to obtain ash-free dry mass (AFDM). The two fresh filters were soaked overnight in 10 ml of methanol. This extract was then analyzed with a fluorometer to estimate the concentration of chlorophyll α.

3.3.4 Statistical analyses

Measures of tadpole behaviors from scan and focal sampling were analyzed with a multivariate analysis of variance (MANOVA), using only two of the three focal behaviors because of interdependence issues (see Van Buskirk and Yurewicz 1998). Average growth rates (mg/tadpole/day) and Gosner stages showed significant deviance from a multivariate normal distribution (Henze-Zirkler $T = 4.46, p < 0.001$) and were therefore analyzed separately with univariate analysis of variances (ANOVAs). Clay tile chlorophyll α concentration (μg/cm²) and AFDM (mg/cm²) were analyzed with a
MANOVA. However, these two variables demonstrated a modest departure from a multivariate normal distribution (Henze-Zirkler $T = 3.01, p = 0.0026$) due to two positive outliers in the AFDM dataset (both for the high competition, no predator, 0 rope treatment). Reviewing the data revealed no justifiable reason for removing these two points; therefore they were included in the statistical analyses, and Pillai’s trace was examined as the MANOVA test statistic since it is robust to modest violations of the multivariate normality assumption (Gotelli and Ellison 2004). Rope strand chlorophyll $\alpha$ concentration and AFDM was analyzed with a three-way factorial MANOVA. Zero stem density treatments were not included in this analysis, because they lacked rope stems.

For the no-tadpole tubs, tile periphyton data was analyzed using a one-way MANOVA with structure (3 levels: none, low, high) as the factor, and rope strand periphyton was analyzed using a one-way MANOVA with structure (2 levels: low, high) as the factor. Behaviors were arcsine square root transformed and the remaining variables were log-transformed before statistical analyses. Univariate ANOVAs were used whenever significance was detected with the MANOVAs. All analyses were performed using the GLM procedure in SAS (SAS Institute 2000 – 2004).

3.4 Results

3.4.1 Behavior

Competition, predation risk, and their interaction significantly affected *R. sylvatica* behavior (Table 3.1). Predators induced a decrease in overall tadpole activity regardless of the competition treatment, while competition induced an increase in tadpole
activity only among the predator tubs (Figure 3.1B). For the focal sample results, competitors caused an increase in swimming time, a decrease in resting time, and no change in feeding time, while predators caused a decrease in swimming time, an increase in resting time, and no change in feeding time (Figure 3.1A). Structure affected none of the tadpole behaviors and interacted with none of the main effects. Focal and scan sampling methods showed evidence of congruence, as percent activity obtained from the scan samples was negatively correlated with percent inactivity obtained from the focal samples ($r = -0.509, P < 0.001$). During feeding bouts, tadpoles fed from the tub walls approximately 50% of the time, regardless of structure treatment (Figure 3.2).
### TABLE 3.1

A) RESULTS OF A MULTIVARIATE ANALYSIS OF VARIANCE TESTING THE EFFECTS OF COMPETITION, PREDATION RISK, AND STRUCTURAL COMPLEXITY ON TADPOLE BEHAVIOR AND B) *P*-VALUES FROM SUBSEQUENT UNIVARIATE ANOVAS.

#### A) Multivariate statistics

<table>
<thead>
<tr>
<th>Source</th>
<th>Wilk’s $\lambda$</th>
<th>df</th>
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<th>$P$</th>
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<tbody>
<tr>
<td>Competition</td>
<td>0.660</td>
<td>3, 46</td>
<td>7.887</td>
<td>&lt;0.001</td>
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<tr>
<td>Predation Risk</td>
<td>0.520</td>
<td>3, 46</td>
<td>14.111</td>
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<tr>
<td>Structure</td>
<td>0.879</td>
<td>6, 92</td>
<td>1.021</td>
<td>0.417</td>
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<td>Comp. * Pred.</td>
<td>0.825</td>
<td>3, 46</td>
<td>3.243</td>
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<tr>
<td>Comp. * Struct.</td>
<td>0.919</td>
<td>6, 92</td>
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<td>0.683</td>
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<tr>
<td>Pred. * Struct.</td>
<td>0.958</td>
<td>6, 92</td>
<td>0.337</td>
<td>0.916</td>
</tr>
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<td>C * P * S</td>
<td>0.989</td>
<td>6, 92</td>
<td>0.086</td>
<td>0.998</td>
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#### B) Univariate ANOVAs

<table>
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<tr>
<th>Variable</th>
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<th>Predation Risk</th>
<th>Structure</th>
<th>Comp. * Pred.</th>
</tr>
</thead>
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<tr>
<td>% active</td>
<td><strong>0.009</strong></td>
<td>&lt;<strong>0.001</strong></td>
<td>0.818</td>
<td><strong>0.003</strong></td>
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<tr>
<td>% resting</td>
<td>&lt;<strong>0.001</strong></td>
<td>&lt;<strong>0.001</strong></td>
<td>0.298</td>
<td>0.400</td>
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<tr>
<td>% feeding</td>
<td>0.675</td>
<td>0.603</td>
<td>0.739</td>
<td>0.662</td>
</tr>
<tr>
<td>% swimming</td>
<td>&lt;<strong>0.001</strong></td>
<td>&lt;<strong>0.001</strong></td>
<td>0.082</td>
<td>0.423</td>
</tr>
</tbody>
</table>

Note: Bold indicates significance at $\alpha = 0.05$
Figure 3.1 Behavioral responses (mean ± 1 SE) of R. sylvatica to competition and predation risk. A) Percentage of time tadpoles spent resting, feeding or swimming. B) Overall activity levels obtained from scan sampling.
3.4.2 Growth and development

Average growth rate and Gosner developmental stage decreased with the addition of competitors and increased with the presence of predators (Table 3.2, Figure 3.3A). Predation risk induced an increase in mean Gosner developmental stage only among the high competition tubs (Figure 3.3B). Structure had a significant negative effect on growth rates, although this effect was only evident for the low competition, no predator treatment.
TABLE 3.2
RESULTS OF AN ANALYSIS OF VARIANCE TESTING THE EFFECTS OF COMPETITION, PREDATION RISK, AND STRUCTURE ON A) AVERAGE GROWTH RATES (MG/TADPOLE/DAY) AND B) GOSNER DEVELOPMENTAL STAGES.

A) Average growth rate (mg/t/d)

<table>
<thead>
<tr>
<th>Source</th>
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<tr>
<td>Competition</td>
<td>1</td>
<td>1077.170</td>
<td>&lt;0.001</td>
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<tr>
<td>Predation Risk</td>
<td>1</td>
<td>24.545</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Structure</td>
<td>2</td>
<td>4.028</td>
<td>0.024</td>
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<tr>
<td>Comp. * Pred.</td>
<td>1</td>
<td>1.142</td>
<td>0.290</td>
</tr>
<tr>
<td>Comp. * Struct.</td>
<td>2</td>
<td>0.296</td>
<td>0.745</td>
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<tr>
<td>Pred. * Struct.</td>
<td>2</td>
<td>0.043</td>
<td>0.958</td>
</tr>
<tr>
<td>C * P * S</td>
<td>2</td>
<td>2.066</td>
<td>0.138</td>
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</tbody>
</table>

B) Gosner developmental stage

<table>
<thead>
<tr>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>Competition</td>
<td>1</td>
<td>1025.670</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Predation Risk</td>
<td>1</td>
<td>8.091</td>
<td>0.007</td>
</tr>
<tr>
<td>Structure</td>
<td>2</td>
<td>1.632</td>
<td>0.206</td>
</tr>
<tr>
<td>Comp. * Pred.</td>
<td>1</td>
<td>4.210</td>
<td>0.046</td>
</tr>
<tr>
<td>Comp. * Struct.</td>
<td>2</td>
<td>1.156</td>
<td>0.323</td>
</tr>
<tr>
<td>Pred. * Struct.</td>
<td>2</td>
<td>0.130</td>
<td>0.879</td>
</tr>
<tr>
<td>C * P * S</td>
<td>2</td>
<td>0.129</td>
<td>0.880</td>
</tr>
</tbody>
</table>

Note: Bold indicates significance at $\alpha = 0.05$
Figure 3.3 A) Per capita growth rates (mean ± 1 SE) of *R. sylvatica* all three treatments. Circles designate low competition treatments and triangles designate high competition treatments. Open symbols are no-predator treatments and closed symbols are predator treatments.  B) Gosner developmental stage (mean ± 1 SE) of *R. sylvatica* in response to competitors and predators.

3.4.3 Resources

The presence of predators increased chlorophyll α concentration on the clay tile and increased both chlorophyll α concentration and AFDM on the rope strands (Table 3.3, Figure 3.4). There were significantly lower amounts of AFDM on the rope strands in the high structure treatment compared to the low structure treatment. This trend was similar for periphyton growth on the tiles, but was only marginally significant. Competition had no significant effect on total periphyton abundance; therefore per-capita resource abundance was lower in the high competitor treatments. For the no-tadpole tubes, structure significantly affected periphyton growth on the rope strands (Wilk’s λ = 0.119, $F_{4,10} = 4.747, P = 0.021$) but not on the clay tile (Wilk’s λ = 0.262, $F_{4,10} = 2.382, P = 0.121$). The high structure treatment had lower concentrations of chlorophyll α ($P =$
0.003) and densities of AFDM ($P = 0.004$) on the rope strands than the low structure treatment (Figure 3.4).

### TABLE 3.3

**A) MANOVA RESULTS FOR PERIPHYTON GROWING ON THE TILES AND**

**SUBSEQUENT $P$-VALUES FROM THE UNIVARIATE ANOVAS.**

**B) MANOVA RESULTS FOR PERIPHYTON GROWING ON THE ROPE STRANDS AND**

**SUBSEQUENT $P$-VALUES FROM THE UNIVARIATE ANOVAS.**

#### A) Multivariate statistics for periphyton abundance on the clay tiles

<table>
<thead>
<tr>
<th>Source</th>
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<th>df</th>
<th>$F$</th>
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</thead>
<tbody>
<tr>
<td>Competition</td>
<td>0.059</td>
<td>2, 47</td>
<td>1.480</td>
<td>0.238</td>
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<td>Predation Risk</td>
<td>0.365</td>
<td>2, 47</td>
<td>13.291</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Structure</td>
<td>0.171</td>
<td>4, 96</td>
<td>2.244</td>
<td>0.070</td>
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</table>

#### B) Multivariate statistics for periphyton abundance on the rope strands

<table>
<thead>
<tr>
<th>Source</th>
<th>Wilk’s $\lambda$</th>
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<th>$P$</th>
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<tbody>
<tr>
<td>Competition</td>
<td>0.891</td>
<td>2, 31</td>
<td>1.899</td>
<td>0.167</td>
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<tr>
<td>Predation Risk</td>
<td>0.451</td>
<td>2, 31</td>
<td>18.903</td>
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<tr>
<td>Structure</td>
<td>0.531</td>
<td>2, 31</td>
<td>13.699</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

#### Variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Competition</th>
<th>Predation Risk</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll $\alpha$</td>
<td>0.263</td>
<td>&lt;0.001</td>
<td>0.017</td>
</tr>
<tr>
<td>AFDM</td>
<td>0.090</td>
<td>0.637</td>
<td>0.050</td>
</tr>
<tr>
<td>Chlorophyll $\alpha$</td>
<td>0.483</td>
<td>&lt;0.001</td>
<td>0.377</td>
</tr>
<tr>
<td>AFDM</td>
<td>0.559</td>
<td><strong>0.005</strong></td>
<td><strong>0.016</strong></td>
</tr>
</tbody>
</table>

*Note: All treatment interactions were non-significant and subsequently excluded from this table for clarity. Bold indicates significance at $\alpha = 0.05$.***
Figure 3.4 A) Chlorophyll $\alpha$ concentration and B) ash-free dry mass on tile and rope in the experimental tubs. Circles designate tile substrate and squares designate rope substrate. Open symbols are no-predator treatments, closed symbols are predator treatments, and gray-shaded symbols are from the no-tadpole tubs. Data are mean ± 1 SE.
3.5 Discussion

The three environmental factors influenced prey growth rates predominantly through their effects on periphyton resources and not through changes in prey behavior. The strongest effect on tadpole growth was competition, which caused an approximate fourfold decrease in average growth rates. Competition had no significant effect on tadpole feeding time, suggesting that differences in per capita resource abundance produced these large growth rate differences. The presence of predators increased tadpole growth rates—despite inducing a decrease in overall activity levels—and also stimulated the growth of periphyton. Because tadpole feeding rates were similar between predator treatments, the increase in resource abundance was likely attributable to a nutrient enrichment via the mineralization of organic wastes from the predator. However, it should be noted that unmeasured tadpole traits (i.e., food injection rate, digestion assimilation) might have also contributed to changes to growth rate. Structural complexity decreased growth rates slightly, possibly by reducing the abundance of periphyton on substrates preferred by tadpoles, but had no effect on any aspects of tadpole activity.

Predation risk affected tadpole growth rates by inducing an increase in periphyton abundance. Because predators did not affect the amount of time tadpoles spent feeding, it is likely that periphyton growth was promoted by subsidies from the organic wastes of the caged predator, although this effect was not quantified directly in this study. Fertilization from predator wastes seems to be a common side effect among studies of prey growth rates that use experimental mesocosms (e.g., McCollum et al. 1998, Anholt
et al. 2000, Relyea and Werner 2000, Babbitt 2001, Barnett and Richardson 2002, McIntyre et al. 2004, Stav et al. 2007). However, several studies have not experienced this subsidy effect (Peacor 2002, Werner and Peacor 2006). This discrepancy may arise from differences in experimental venue size and nutrient loads between the experiments. All seven of the studies cited above that implicate predator subsidies as contributing to the growth of tadpoles were conducted in small mesocosms (mean volume = 31.2 l per predator added), while Peacor (2002) and Werner and Peacor (2006) used 1300 l tanks (325 l per predator added) and frequently added nitrogen and phosphorus fertilizer. Therefore, predator-mediated nutrient subsidies may have greater implications for small, nutrient-poor habitats [e.g., closed-canopy ponds (Skelly et al. 2002, Schiesari 2006)], where wastes generated by predators can have a more significant effect on nutrient levels.

As expected, overall tadpole activity decreased in the presence of predators and increased with the addition of competitors, but these activity differences were mostly due to adjustments of swimming time rather than feeding time. In regards to foraging, swimming time reflects search effort for new food patches. As food resources become more patchily distributed, changes in the amount of time an individual spends searching for food becomes an important determinant in the amount of food acquired (Lima and Dill 1990, Chase et al. 2001). Within experimental mesocosms, however, periphyton is more uniformly distributed (that is, periphyton grows within the entire mesocosm, and there are no patches lacking periphyton). Consequently, because periphyton was present throughout all areas of the mesocosm, changes in swimming time in response to competitors and predators had little effect on growth rates. These results suggest that the adaptiveness of behavioral changes in response to different environmental factors may
depend on the environmental context of a given habitat. Specifically for tadpoles, adjustments to swimming time will become more critical in natural temporary ponds, where food resources show clumped distributions (Michel unpresented data).

For prey that reduce overall activity levels in response to predators, the decision on which behavioral component to alter (i.e., swimming or feeding) may depend on the hunting behavior of the predator. In this study, dytiscid larvae induced a decrease in tadpole swimming time, while other studies have found that the presence of dragonfly nymphs (*Anax junius*) induce a decrease in both swimming and feeding time (McCollum and Van Buskirk 1996, Van Buskirk and Yurewicz 1998). Activity reduction in response to predators is thought to decrease the probability of being detected by a predator (Lima and Dill 1990, Skelly 1994, Lima 1998), yet predator species use different cues to detect prey. In response to highly visual predators like dragonfly nymphs (Pritchard 1965), which can detect tadpoles by the characteristic tail flutter tadpoles use when foraging, tadpoles may reduce both swimming and feeding time to avoid detection. For predators like dytiscid larva that require tactile and chemical cues to detect prey (Formanowicz 1987), tadpole feeding activity may elicit a weaker detection response from dytiscids than swimming. Considering that prey organisms show predator-specific behavioral and morphological inducible defenses in both aquatic (Relyea 2001, Teplitsky et al. 2005) and terrestrial systems (Schmitz 2008), the possibility of predator-specific foraging costs is not surprising.

Foraging models predict that activity is greater at low food abundance (Abrams 1991, Werner and Anholt 1993); therefore reductions in tadpole activity in predator tanks may be attributed to the increase in periphyton abundance caused by the organic wastes
of the caged predators. While it is unfeasible to isolate the effects of food abundance and predators on tadpole activity for this experiment, this argument cannot explain why tadpole activity levels were similar among the competition treatments for the no-predator tubs, despite greater per capita resource abundance for the low competition treatment.

The highest rope density treatment decreased tadpole growth by approximately 10%, despite providing more surface area for the growth of periphyton. However, the presence of rope strands also decreased periphyton abundance on the clay tiles presumably by shading light and, by promoting the growth of algae on the rope, increasing algal competition for nutrients. Because tadpoles preferred to feed from the tub wall, which provides a harder surface for algae scraping than rope strands, the structure treatments probably reduced the densities of easily accessible and available food resources. A weak effect of structural complexity on tadpole growth rates has been demonstrated in previous studies (Sredl and Collins 1992, Babbitt and Tanner 1998).

Structure did not affect the magnitude of tadpole behavioral responses to increased conspecific densities or the presence of a predator. I hypothesized that structure would decrease tadpole foraging activity through an increase in food resources. However, structure actually discouraged the growth of periphyton on the substrate preferred by tadpoles. I also hypothesized that structural complexity would reduce the degree of tadpole antipredator behavior, because the protective benefits from predation provided by structure would allow tadpoles to increase activity (Woodley and Peterson 2003). However, tadpoles did not modify antipredator behavior in the presence of structure. Structure may not actually protect tadpoles from dytiscid predation. In Chapter 2, I found that dytiscid larvae are able to switch from an active to a sit-and-
pursue predator when hunting in structured habitats, and consequently do not suffer a reduction in number of prey captures (Michel and Adams 2009, Chapter 2). Because sit-and-pursue predators tend to capture more active prey (Huey and Pianka 1981, Scharf et al. 2006), it is perhaps not surprising that tadpoles from predator treatments maintained low activity levels independent of the density of structural complexity. It would be interesting to examine how structure modifies prey antipredator behavior in response to predators that are negatively affected by structure.

Growth rates have significant implications for the fitness of prey individuals. This study demonstrates that individual prey growth rates are strongly dependent on the per capita abundance of food resources available given certain environmental conditions. The effects of these environmental conditions on the abundance and availability of food resources have a strong influence on the growth response of prey individuals. Environmental conditions can also alter prey activity; however, the context of particular environmental variables (e.g., spatial distribution of food resources), and the behavioral components that prey species modify in response to different environments may affect the importance of behavioral changes on growth rates. This context-dependency suggests that future studies on indirect food web interactions should move away from isolating the effects of individual factors and employ a more comprehensive approach, by testing how multiple environmental factors affect various attributes of all trophic levels (sensu Werner and Peacor 2006).
3.6 Acknowledgments

I thank D. Costello, L. Kinsman, and C. Patrick for help with analysis of periphyton. I thank M. Alldred and E. Zervoudakis for their help in weighing tadpoles. G. E. Belovsky and D. Choate provided helpful comments on earlier manuscripts. This research was conducted with approval from the Institutional Animal Care and Use Committee of the University of Notre Dame (Protocol No.: 07-085).

3.7 References


CHAPTER 4:
ADAPTIVE AND NON-ADAPTIVE MORPHOLOGICAL PLASTICITY IN
RESPONSE TO MULTIPLE ENVIRONMENTAL FACTORS

4.1 Abstract

Research on phenotypic plasticity has been mostly limited to studies that examine trait responses to changes in one or two environmental variables. However, because multiple biotic and abiotic factors in natural environments can vary and the expression of plastic traits is often context-dependent, studies should focus on measuring trait responses to simultaneous changes in multiple environmental factors. In this study, I examine how structural complexity affects predator- and competitor-induced morphological traits of wood frog (*Rana sylvatica*) tadpoles. I also provide a measure of the adaptiveness of predator-induced morphological responses by comparing the traits induced by various combinations of predators and structure with the traits selected for by predators among different structure densities. The presence of structure decreased the magnitude of several known competitor-induced responses of tadpoles including gut length, body depth and tail length. However, this pattern was only observed in the absence of predators. Presumably, structure provided more space for the growth of periphyton (a tadpole food source) and interfered with the visual assessment of competitor density by tadpoles, although the exact mechanism is unknown. Structure had complex effects on tadpole predator-induced morphology. Only two traits, body length and tail length, were induced
and selected for by predators in a similar magnitude and direction among the different structure densities. Additionally, the predator [larvae of the diving water beetle (*Dytiscus* spp.)], preferentially killed tadpoles with deep tailfins, contradicting the previously established notion that a deep tailfin is a ubiquitous morphological defense against all tadpole predators. This study demonstrates that plastic responses of individuals to changes in multiple environments are complex and are not always adaptive within the experienced environmental conditions.

4.2 Introduction

Phenotypic plasticity, the ability of a genotype to alter its phenotype in response to changing environmental conditions, is a characteristic shared by many species responding to a wide variety of environmental factors (Tollrian and Harvell 1999, Pigliucci 2001, West-Eberhard 2003, DeWitt and Scheiner 2004). Such phenotypic changes are believed to improve the fitness of the individual inhabiting the subsequent environment (i.e., the adaptive plasticity hypothesis, Dudley and Schmitt 1996). Support for the adaptive plasticity hypothesis is obtained when the traits induced by a particular environment match the traits that are selected for within that same environment (Dudley and Schmitt 1996, Van Buskirk et al. 1997, Van Buskirk and Relyea 1998, Teplitsky et al. 2005). Failure to demonstrate such a relationship suggests that the induced trait value may be nonadaptive—a consequence of physiological (Gotthard and Nylin 1995, Nussey et al. 2007) or genetic constraints (DeWitt et al. 1998, Donohue et al. 2001).

The majority of studies on phenotypic plasticity measure phenotypic responses of an organism to changes in one environmental factor, while controlling for other
potentially important variables. However, relating the results of these studies to complex natural systems is problematic because the expression of phenotypic plasticity in response to one environmental factor can be affected by the presence of other factors (e.g., Peacor 2002, Turner 2004). This context-dependence has significant implications for natural ecosystems, due to the many ecological and evolutionary consequences of phenotypic plasticity (reviewed in Miner et al. 2005, Pigliucci 2005, 2007, Fordyce 2006). Likewise, if we wish to make inferences on the adaptiveness of plastic traits in natural environments, tests of the adaptive plasticity hypothesis should also be conducted within more complex environments. However, few studies have examined trait responses of individuals to changes in multiple environments or the adaptiveness of these responses.

In this chapter, I examine how three environmental factors—predation risk, competition intensity, and structural complexity—interact to affect the expression of phenotypes. Predation and competition are two important environmental factors that induce phenotypic change in a wide variety of species (Miner et al. 2005). Adaptive predator-induced traits decrease the vulnerability of the individual to predation (Tollrian and Harvell 1999), whereas adaptive competitor-induced traits improve the ability of the individual to acquire a limiting resource, usually related to growth (Relyea 2002a). Additionally, the magnitude of these trait changes can be modified by the presence of other environmental factors, such as the abundance of food resources (e.g., Relyea 2002a, Turner 2004). Less is known regarding the influence of structural complexity on the phenotypic plasticity of organisms. Structural complexity is a component of many natural ecosystems (McCoy and Bell 1991), and can potentially affect both predator- and
competitor-induced traits. For example, structure can interfere with predator foraging success (reviewed in Denno et al. 2005, Horinouchi 2007), thus reducing the need for costly predator-induced defenses (e.g., Woodley and Peterson 2003). Additionally, some predators shift from an active to a sit-and-pursue (SAP) foraging mode in response to structure (e.g., Savino and Stein 1982, James and Heck 1994, Laurel and Brown 2006, Michel and Adams 2009). Consequently, because defensive traits exhibited by prey often depend on the hunting strategy of the predator (e.g., Teplitsky et al. 2005, Touchon and Warkentin 2008), structure may cause a corresponding shift in predator-induced traits that provide protection against that specific predator foraging mode. For competitor-induced traits, structure may affect the abundance of food resources, interfere with the ability of an individual to visually assess the density of competitors in a given area, or both.

To address the influence of predators, competitors, and structure on the phenotypes of individuals, I used tadpoles of the wood frog (*Rana sylvatica*). In response to predators, *R. sylvatica* tadpoles usually decrease the size of their body and increase the depth of their tailfin (Van Buskirk and Relyea 1998, Relyea 2001, Relyea 2002b, Relyea 2003a, Schoeppner and Relyea 2008). These responses are considered adaptive because predators preferentially feed on tadpoles with large bodies and shallow tailfins (Van Buskirk and Relyea 1998). In response to conspecific competitors, *R. sylvatica* tadpoles develop large bodies and shallow tailfins (Relyea 2002b) as well as longer intestines (Relyea and Auld 2004) and larger mouthparts (Relyea and Auld 2005). Tadpoles exhibiting these competitor-induced morphological changes experience faster growth rates than noninduced tadpoles (Relyea 2002b). Predation and competition can also
interact to affect tadpole morphology, such that the degree of predator or competitor-
induced plasticity is reduced with the addition of the other environmental factor (Relyea
2004, Relyea and Auld 2004, 2005). However, no study has examined how structure can
modify predator- and competitor-induced traits or how structure affects the adaptiveness
of predator-induced traits.

In this chapter, I first assess the morphological changes of *R. sylvatica* tadpoles to
combinations of predators, intraspecific competitors, and structural complexity. Then, to
test the adaptiveness of predator-induced phenotypic responses of tadpoles in habitats
with structural complexity, I conduct predator selection trials in which the morphological
traits of surviving individuals are quantified. Adaptive phenotypic plasticity is inferred
when the direction and magnitude of a morphological trait matches the direction and
magnitude of traits selected for by predators.

4.3 Methods

4.3.1 Experiment I – Trait induction

Wood frog tadpoles of approximately Gosner developmental stage 26 (Gosner
1960) were collected from three temporary ponds at the University of Notre Dame
Environmental Research Center (Gogebic Co., MI) and transferred to 1 m diameter
wading pools filled with well water. Each pool contained tadpoles originating from the
same pond population.

Experimental mesocosms were constructed using 60.5-l plastic tubs (48 cm
diameter) filled to 24 cm with well water, inoculated with 500-ml of filtered pond water
and 1 g of crushed rabbit food to stimulate algal growth, and then covered with fiberglass window screening to prevent invasion of aquatic insects. Each tub also received periodic introductions of zooplankton to prevent bacterial blooms. Tubs were randomly assigned one level from each of three main factors: predator (presence or absence), competition (7 or 21 conspecific tadpoles), and structural complexity (0, 28 or 84 strands of green polypropylene rope). These factors were fully crossed and replicated 5 times for a total of 60 tubs. Predator presence was manipulated by placing one large (approximately 5 cm) diving water beetle larvae (*Dytiscus* spp.) within a 10 cm by 10 cm PVC pipe enclosed on both ends with fiberglass window screening. Dytiscid larvae are common tadpole predators and inhabit the three temporary ponds from which the experimental tadpoles were collected (see chapter 5 of this thesis). Because dytiscid larvae breathe atmospheric air, one 2.5-cm Styrofoam cube was placed in each cage to maintain buoyancy. During the experiment, predators were fed 500 mg of *R. sylvatica* tadpoles every other day. For the predator absence treatment, an empty cage was placed in the tub. Structural complexity was simulated using 23 cm long strands of green polypropylene rope attached to a black plastic hardware net cut to fit the bottom of the tub. The hardware net was anchored to the tub bottom using three scrubbed rocks and one clay tile (see Chapter 3). The zero structural complexity treatment contained the net, but no rope.

In May 2006, 7 or 21 *R. sylvatica* tadpoles (depending on the assigned level of competition) were weighed (mean mass ± 1 SE: 73.23 ± 0.94 mg) and then added to each tub. After 24 h, predators were added to cages. The experiment was terminated after 22 days, at which point some tadpoles began metamorphosis (pre-eruption of forelimbs,
approximately Gosner stage 42). *R. sylvatica* exhibit morphological responses to caged predators within 7 days of constant exposure (Relyea 2003b). All surviving tadpoles were collected, euthanized, and preserved in 10% formalin. Tadpoles were removed from the preservative after three months, weighed, and photographed in the lateral and dorsal view. Then, to obtain a measure of mouthpart size, five tadpoles from each tub were selected, inverted in a petri dish, and photographed with a Nikon digital camera attached to a dissecting scope. Afterwards, these same five tadpoles were dissected and the total length of the gut (Gut) was recorded (Relyea and Auld 2004). Malformed tadpoles (see Chapter 6) were not used in any morphological measurements.

Seven morphological variables were measured from the body photographs using the program ImageJ (Rasband 1997-2008): body length (BL), depth (BD) and width (BW), tail muscle depth (TMD) and width (TMW), tail length (TL) and the depth of the top half of the tailfin (HTFD). Most tadpole morphology studies measure the depth of the entire tailfin, however an obstruction in the photographs taken for experiment II (see below) restricted measurements to the top half only. To maintain consistent phenotypes among induction and predator selection experiments, HTFD was measured for both. HTFD and full tailfin depth were highly correlated for experiment I (raw measurements: Pearson’s *r* = 0.930; size-adjusted measurements: Pearson’s *r* = 0.758). For the mouthpart photographs, the length of the upper jaw sheath as well as the first three upper and lower labial toothrows were measured using ImageJ. To obtain an overall measure of mouthpart size, these 7 mouthpart variables were condensed using a principal components analysis (PCA) using a correlation matrix. All variables loaded positively into the first component, which explained 66.7% of the total variance (eigenvalue =
4.67). The scores from this first component were saved and regarded as an overall measure of mouthpart size (Mouth).

Because morphology scales with body size, each morphological trait was standardized to a common body mass using an analysis of covariance (ANCOVA) with treatment combination as the factor and tadpole mass as the covariate. Slope values were homogenous for all morphological measurements ($P > 0.10$ for all traits). The resulting residuals for each individual were then added to the estimated marginal mean for each treatment combination and means for each tub were obtained. Mass and all nine morphological variables, except the PC factor scores for mouthpart size, were log-transformed before this standardization. A 3-way multivariate analysis of variance (MANOVA) was performed using all 9 standardized morphological traits as the response variables with predator, competition, and structural complexity and their interactions as the factors. In the event of a significant multivariate statistic (at $\alpha = 0.05$) for a factor, a univariate analysis of variance (ANOVA) was conducted to determine which response variables were significantly affected by the factor. Statistical analyses were performed using Systat 10.0.

4.3.2 Experiment II – Trait selection

The objective of this experiment was to measure the morphological traits of tadpoles that are preferred by predators foraging among three levels of structural complexity. Hence, the desired outcome is a measure of selection intensity based on differential mortality of tadpoles.

In mid-April 2008, a total of nine *R. sylvatica* egg masses (i.e., sibships) were collected from eight different ponds known to contain dytiscid larvae (see Chapter 5).
Egg masses were kept in separate 1 m diameter wading pools filled with well water. After hatching, tadpoles were fed ad libitum so that tadpole body sizes remained relatively consistent among the nine different sibships. Healthy tadpoles (i.e., normal swimming, no malformations) within an approximate size range of 150 – 400 mg were used in predator selection trials.

Selection trials were conducted using similar experimental mesocosms described in experiment I. Tubs were randomly assigned one of three structural complexity treatments (0, 28, or 84 strands of polypropylene rope), constructed as previously described. Ten predator selection trials were conducted for each structure treatment for a total of 30 tubs. At the beginning of each predator trial, two tadpoles were randomly chosen from each of the nine sibships, for a total of 18 tadpoles. A high number of tadpoles were chosen in order to maximize the variation in trait values available for predator selection. Each individual was anaesthetized with a low concentration of MS-222 (Anholt et al. 1998), weighed, and then photographed simultaneously from the dorsal and lateral view using a specially constructed Plexiglass chamber (Van Buskirk and Schmidt 2000). Tadpoles were checked for healthy recovery from the anesthesia (i.e., resumption of normal swimming behavior within 30 minutes) and then introduced to the experimental mesocosm. After 24 h acclimation, one large, starved dytiscid larva (approximately 5 cm) was then introduced to the mesocosm and allowed to forage. After another 24 h, the predator was removed and all remaining tadpoles were preserved in 70% ethanol. I conducted 3 – 6 such trials per day for seven successive days, and I used each predator for only one trial.
Approximately 2 weeks after the experiment, tadpoles were removed from the preservative, weighed and photographed in the dorsal and ventral view. For each individual, the same seven body and tail morphological traits used for experiment I were measured with the exception of body depth (an obstruction in the majority of photographs prevented reliable measurements).

For each tub, an ANCOVA was conducted for each morphological trait with time (pre-predator vs. post-predator) as a factor and tadpole mass as a covariate. The pre-predator estimated mean was subtracted from the post-predator estimated mean to obtain a measure of selection differential. This selection differential was then divided by the pre-predator standard deviation of the particular trait to obtain the selection intensity (Arnold and Wade 1984, Van Buskirk and Relyea 1998). A MANOVA was performed with the selection intensities of each morphological trait as the response variables and structure density as the main factor. A constant term was included in the statistical model to test whether any selection intensities differed significantly from zero. Univariate ANOVAs were conducted when the multivariate statistic was significant (at $\alpha = 0.05$).

4.3.3 Comparing induced traits and selected traits

First, the mean morphological traits from the induction experiment (Experiment I) were transformed into ‘induction differentials’ to attain consistency with the selection intensities obtained in Experiment II. Induction differentials were calculated by subtracting the mean trait value of all high-competition, predator-free tubs of the $i$th structure treatment from the mean trait value of each high-competition, predator tub of the $i$th structure treatment. This calculation resulted in 5 induction differential measurements for each structure treatment, from which a mean and standard error were
calculated. Only high-competition tubs were used because: 1) that tadpole density (21 per tub) was most similar to the density used in the selection experiments (18 per tub), and 2) the traits of the tadpoles used in the selection experiment (i.e., Experiment II) most closely matched the traits of the tadpoles raised in the high-competition treatments from the induction experiment (i.e., Experiment I). Selection intensities were then plotted against induction differentials for each structure treatment. An adaptive response to predation risk (from the perception of a tadpole) was defined as a trait that was induced and selected in similar directions (i.e., quadrants I and III of a Cartesian coordinate system).

4.4 Results

4.4.1 Experiment I – Trait Induction

At the multivariate level, all three main factors and their interactions had significant effects on tadpole morphology (Table 4.1). The results from univariate ANOVAs demonstrated that every morphological trait was affected by at least one factor or interaction (Table 4.1). To facilitate interpretation, two figures were created: Figure 4.1 compares the mean value of a trait in a no-predator environment to the mean value of the same trait in a predator environment, and Figure 4.2 compares the mean value of a trait in a low competition environments to the mean value of the same trait in a high competition environment.
### TABLE 4.1

A) RESULTS FROM A MULTIVARIATE ANALYSIS OF VARIANCE EXAMINING THE EFFECT OF COMPETITION, PREDATION RISK, STRUCTURAL COMPLEXITY ON NINE TADPOLE PHENOTYPES.  B) P-VALUES FROM UNIVARIATE ANALYSES OF VARIANCES FOR EACH PHENOTYPE.

<table>
<thead>
<tr>
<th>Source</th>
<th>Wilk’s Lambda</th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Competition</td>
<td>0.206</td>
<td>9, 40</td>
<td>33.640</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Predation Risk</td>
<td>0.508</td>
<td>9, 40</td>
<td>5.245</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Structure</td>
<td>0.378</td>
<td>18, 20</td>
<td>2.862</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Comp.*Pred.</td>
<td>0.580</td>
<td>9, 40</td>
<td>3.931</td>
<td>0.005</td>
</tr>
<tr>
<td>Comp.*Structure</td>
<td>0.273</td>
<td>18, 80</td>
<td>2.195</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pred.*Structure</td>
<td>0.301</td>
<td>18, 80</td>
<td>3.657</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Comp.*Pred.*Str.</td>
<td>0.369</td>
<td>18, 80</td>
<td>2.874</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

B) P-values from univariate ANOVAs

<table>
<thead>
<tr>
<th>Trait</th>
<th>Comp.</th>
<th>Pred.</th>
<th>Struct.</th>
<th>C.*P.</th>
<th>C.*S.</th>
<th>P.*S.</th>
<th>C.*P.*S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>ns</td>
<td>0.014</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>BD</td>
<td>ns</td>
<td>0.013</td>
<td>ns</td>
<td>ns</td>
<td>0.008</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>BW</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.002</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>HTFD</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>ns</td>
<td>0.037</td>
<td>ns</td>
<td>0.003</td>
<td>ns</td>
</tr>
<tr>
<td>TL</td>
<td>&lt;0.001</td>
<td>0.012</td>
<td>ns</td>
<td>ns</td>
<td>0.036</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TMW</td>
<td>0.0190</td>
<td>ns</td>
<td>0.004</td>
<td>ns</td>
<td>&lt;0.001</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>TMD</td>
<td>ns</td>
<td>0.021</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Gut</td>
<td>0.020</td>
<td>ns</td>
<td>0.002</td>
<td>ns</td>
<td>0.038</td>
<td>ns</td>
<td></td>
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<tr>
<td>Mouth</td>
<td>ns</td>
<td>0.007</td>
<td>ns</td>
<td>ns</td>
<td>&lt;0.001</td>
<td>0.004</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Note: Non-significant P-values (at α = 0.05) designated as ‘ns’.
Figure 4.1 Mean (± 1 SE) values of nine phenotypes in the absence and presence of predators. The dashed line indicates equal phenotypes between the two environments; symbols above the dashed line indicate the predator induced larger trait values, symbols below the dashed line indicate the predator induced smaller trait values. Open symbols indicate low competition environments; filled symbols indicate high competition environments. Circles designate zero structure, triangles indicate low structure, and squares indicate high structure.
Figure 4.2 Mean (± 1 SE) values of nine phenotypes in the low competition and high competition environments. Symbol designation is the same as in Figure 4.1, except open symbols indicate no predator environments and filled symbols indicate predator environments.
**Tail muscle depth** – Tail muscle depth was significantly affected only by predation risk. Averaged across treatments, predators induced a 1.8% increase in muscle depth (Figure 4.1A, 4.2A).

**Mouthpart size** – Mouthpart size was affected by predation risk, and interactions between structure and predation risk, structure and competition, and a three-way interaction among these factors. Overall, predation risk induced smaller mouthparts, except among the low competition, low structure tubs (Figure 4.1B). In the absence of structure, competition induced smaller mouthparts, but with structure, competition induced larger mouthparts (Figure 4.2B).

**Body length** – Overall, predation risk induced shorter bodies while high competitor density induced longer bodies. Predators induced a 2.8% reduction in body length at low competition, but only a 0.7% reduction in body length at high competition (Figure 4.1C, 4.2C).

**Body depth** – Predation risk significantly induced deeper bodies, but this effect interacted with structure. At high stem density, predation risk induced a 3.4% increase in body depth (Figure 4.1D, 4.2D). At zero and low stem density, predation risk did not induce any significant changes to body depth; although, at zero stem density, there was a slight trend for predators to induce deeper bodies at low competition but shallower bodies at high competition.

**Tail muscle width** – Overall, competition induced a decrease in tail muscle width, but this effect was only evident at high stem density, where tail muscles were 6.4% smaller (Figure 4.1E, 4.2E). At high competition, structure had no effect on muscle width, but at low competition, low stem density induced 4.3% and 6.9% narrower tail
muscles than zero and high stem density, respectively (i.e., compare the open triangle with the open circle and open rectangle in Figure 4.1E).

*Gut length* – Gut length was significantly affected by competition, structure, and an interaction between structure and predation risk. Overall, competitors induced a 3.2% increase in gut length (Figure 4.1F, 4.2F). In the absence of predation risk, high and low stem density induced 6% and 4.7% longer guts, respectively, than zero stem density. However, predation risk induced a 6.4% reduction in gut length at low stem density, but no change at zero and high stem density.

*Top half tailfin depth* – Overall, competition induced shallow tails and predation risk induced deeper tailfins, however these two factors significantly interacted with each other, and predation risk interacted with stem density. Predators induced a 2% increase in top half tailfin depth at low competition, but only a 0.4% increase at high competition (Figure 4.1G, 4.2G). In the presence of predation risk, stem density did not modify tadpole top half tailfin depth, yet in the absence of predation risk, low and high stem density induced a 2.2% and 2.3% reduction in top half tailfin depth, respectively, compared to tadpoles at zero stem density.

*Body width* – Predation risk significantly interacted with stem density, and there was also a significant three-way interaction between all factors. At high competition, there were no significant effects of predation risk or stem density (Figure 4.1H, 4.2H). At low competition, predators induced 2.2% and 2.9% wider bodies at zero and high stem density, respectively, but induced 2.5% narrower bodies at low stem density.

*Tail length* – Overall, both competition and predation risk induced shorter tails, but both of these factors interacted significantly with structure, and there was also a
significant three-way interaction. In the absence of predation risk, high competition induced shorter tails at every stem density (Figure 4.1I, 4.2I). Among the predator treatments, high competition induced shorter tails only at low stem density. At zero stem density, predation risk induced shorter tails at low competition, but longer tails at high competition. At low stem density, predation risk induced longer tails at low competition, but did not modify tail length at high competition. And at high stem density, predation risk induced shorter tails regardless of competition.

4.4.2 Experiment II – Trait selection

The number of surviving tadpoles did not differ among the structure treatments (Kruskal-Wallis test: \( K = 3.78, \text{df} = 2, P = 0.151 \)). The results from the MANOVA yielded a significant constant term (Wilk’s \( \lambda = 0.028, F_{7,21} = 1052, P < 0.001 \)). A univariate ANOVA demonstrated that the selection intensities for tadpole mass \( (P < 0.001) \), tail muscle depth \( (P < 0.001) \), tail muscle width \( (P < 0.001) \), top half tailfin depth \( (P < 0.001) \), and body width \( (P < 0.001) \) all differed significantly from zero. Body mass, top half tailfin depth, and body width all had negative selection differentials (i.e., the predator killed tadpoles with larger values of these traits), and tail muscle depth and width had positive selection differentials (Figure 4.3). Stem density did not significantly affect the selection intensities of any trait (Wilk’s \( \lambda = 0.428, F_{14,42} = 1.588, P = 0.123 \)).
Figure 4.3 Mean (± 1 SE) selection intensities for tadpole mass and six phenotypes responding to predation by *Dytiscus* larvae. Values greater than zero indicate that predators selected for larger trait values (i.e., killed individuals with small values of the trait), and values less than zero indicate that predators selected for smaller trait values (i.e., killed individual with large values of the trait). Black bars indicate zero structure treatment, light-gray bars indicate low structure treatment, and dark-gray bars indicate high structure treatment.

4.4.3 Comparison of induced and selected traits

Three traits (body length, tail length and tail muscle depth) shared similar induction and selection directions at all three stem densities (Figure 4.4). Two traits (tail muscle width and top half tailfin depth) were induced and selected in similar directions at only zero stem density, although the magnitude of selection for tail muscle width did not closely match the magnitude of induction. At low and high stem density, top half tailfin depth was maladaptive; that is, tadpoles in these environments grew relatively deep tailfins in response to predators, but predators selectively killed tadpoles with deep
tailfins during selection trials. Tail muscle width was also similarly maladaptive at low and high stem density: predators induced narrow muscles but selectively killed tadpoles with narrow muscles. At low and high stem density, the direction of body width induction matched that of selection, but not at zero stem density.

Figure 4.4 Comparison of traits selected and induced by a tadpole predator, *Dytiscus* larvae. Selection intensities are identical to those in Figure 3. Induced trait values show the direction and magnitude of the response of the trait to cues from *Dytiscus* larvae when raised in a high competition environment. Bars are + or – standard errors, depending on position of data point in relation to the origin. Italicized text refers to the specific phenotype, see text for abbreviations. Circles, triangles, and squares indicate zero, low, and high structure treatments, respectively.
4.5 Discussion

Tadpoles exhibited complex morphological responses to the twelve different combinations of predator, competitor, and structurally complex environments. In isolation from the other environmental factors, structure had only small effects on tadpole morphology. However, structure did significantly interact with both competitors and predators. In the absence of predation risk, structure decreased the magnitude of several competitor-induced traits, including gut length and body depth. For predator-induced traits, three traits shared similar patterns of induction and selection among the three structure treatments. For several other traits, observable effects of structure on the direction, but not magnitude, of predator-induced traits did concur with the effect of structure on the direction of predator selection intensities. However, in general, few patterns of predator-induced trait changes matched patterns of predator trait selection—especially with the addition of structure—suggesting that the ability to express adaptive phenotypic changes is compromised in response to changes in multiple environments.

4.5.1 Competitor-induced plasticity

In habitats lacking structure tadpoles grew deeper bodies, shorter tails, and longer guts in response to competition. These changes agree with previous studies on \textit{R. sylvatica} morphological changes with competitors and are thought to increase competitive ability (Relyea 2002a, Relyea and Auld 2004). With the addition of structure, however, competitors only induced small changes in tail and gut length, and did not alter body depth at all. This result suggests that the presence of structure modified these specific competitor-induced traits by decreasing the competitive intensity perceived by tadpole individuals.
Competition arises through either exploitative (i.e., changes in food abundance) or interference (i.e., direct interactions between individuals) mechanisms. Structure can potentially affect the degree to which individuals experience these methods of competition by altering food resource abundance (exploitative) or the visual assessment of other individuals (interference). Results from Chapter 3 concluded that structure actually causes a slight decrease in available tadpole food resources. Therefore, structure likely modifies competitor-induced responses by interfering with a tadpole’s visual assessment of competitors. Vision appears to be an important cue predicting competitor density, as several studies have found that mirrors and clay models of tadpoles placed within experimental mesocosms also induce competitor-induced tadpole traits (Rot-Nikcevic et al. 2005, 2006).

Among predator environments structure did not reduce the magnitude by which tadpoles altered gut length, body depth and tail length in response to competitors. For example, with predators, tadpoles did not alter gut lengths and actually decreased body depth in response to competition. These patterns may reflect a tradeoff between adaptive phenotypic responses to competition and predation risk: phenotypes that improve the competitive ability of an individual are also thought to increase susceptibility to predators (Relyea 2002a). For example, tadpoles with large bodies grow faster than small-bodied tadpoles (Van Buskirk et al. 1997, Van Buskirk and Relyea 1998, Relyea 2002a), but are selectively killed by predators (Van Buskirk et al. 1997, Van Buskirk and Relyea 1998, Teplitsky et al. 2005, Johnson et al. 2007). Unfortunately, this study could not assess whether dytiscid larvae selectively kill deep-bodied tadpoles.
4.5.2 Predator-induced plasticity

Body and tail length were the only two traits of tadpoles for which the pattern of induction matched the pattern of predator selection among the different structure densities. Predators induced and selected for long tails and normal bodies among structure-less habitats and short tails and bodies among structured habitats. These trait changes may reflect adaptive responses to different predator foraging modes. Active predators induce and select for long tails (e.g., Teplitsky et al. 2005), while sit-and-pursue predators induce and select for short bodies (e.g., Van Buskirk and Relyea 1998). In response to structure, dytiscid larvae shift from an active to a sit-and-pursue foraging mode (Michel and Adams 2009, Chapter 2). Therefore, even though tadpoles experience identical chemical cues from predators, it seems that they can specifically modify body and tail length in response to structural complexity that is adaptive to the foraging mode of the predator. However, patterns of tail length plasticity in response to predators is highly variable among amphibian species (Van Buskirk 2002), especially for wood frog tadpoles (Relyea 2001, Relyea 2003a, Schoeppner and Relyea 2008).

For several other traits, trait induction matched predator selection intensity in direction but not magnitude (Figure 4). This result may be a consequence of using only chemical cues from predators and consumed conspecifics to elicit morphological responses, as experiments using only cues from predators often underestimate the true magnitude of trait changes (Abrams 2008).

A deep tailfin is widely regarded as an important predator-induced tadpole defense for the larvae of many amphibian species because of the ubiquity of its expression in response to several different species of predators (e.g., Lardner 2000,
Relyea 2001, Van Buskirk 2002, Teplitsky et al. 2005). My results are somewhat contradictory: in habitats without structure, predators induced no change in upper tailfin depth among low competition treatments and actually induced a significant decrease in upper tailfin depth among high competition treatments. This response appears to be adaptive, because dytiscid larvae preferentially captured tadpoles with deep tailfins regardless of structure density. Conversely, for the tubs with structure, tadpoles developed deeper tailfins in response to predators, a response that the predator selection experiments suggest is disadvantageous. This maladaptive response may reflect conflicting pressures from structural complexity and predation risk on the induction of upper tailfin depth. Tailfin depth may be under such strong selection pressure from predators (e.g., Teplitsky et al. 2005, Johnson et al. 2007) that tadpoles are constrained to produce a specific depth of the tailfin whenever chemical cues from predators are present. Because the presence of structure alone induced shallow tailfins, a deep tailfin becomes disadvantageous when the individual inhabits a habitat with structure.

Considering that other predators selectively kill shallow-tailed individuals (e.g., Van Buskirk and Relyea 1998, Teplitsky et al. 2005, Johnson et al. 2007) why did dytiscid larvae selectively capture tadpoles with deep tailfins? A deep tailfin is thought to lure predators to strike the tail instead of the more vulnerable body region of a tadpole (Van Buskirk et al. 2003, 2004, Johnson et al. 2007). Consequently, this defense would only be effective against highly visual predators, yet dytiscid larvae have poor eyesight (Gilbert 1994). While this explanation addresses why deep-tailed tadpoles did not gain a survival advantage, it does not explain why they suffered a survival disadvantage. Instead of visual cues, dytiscid larvae detect prey predominantly using chemical and
tactile cues (Formanowicz 1987). The movement of a deep tailfin would displace more water, possibly facilitating detection by dytiscid larvae. Indeed, given the poor vision of dytiscid larvae, tadpoles with relatively large values of any morphological trait may be more susceptible. This hypothesis is supported by the preferential predation of dytiscid larvae on larger massed and wider bodied tadpoles.

Dytiscid larvae also selectively killed tadpoles with small tail muscles, corresponding with previous selection experiments with dragonfly nymphs (Van Buskirk et al. 1997, Van Buskirk and Relyea 1998) and sticklebacks (Teplitsky et al. 2005). Tail muscle size often correlates positively with burst swimming speed (Dayton et al. 2005), suggesting that tadpoles with large tail muscles are able to effectively escape predator attacks. Selection pressure for a large tail muscle may be stronger in the presence of predators that actively pursue prey as opposed to ambush predators (Teplitsky et al. 2005). This hypothesis is supported by the current study: selection intensities for both tail muscle depth and width were greater (although not significantly so) among the zero structure treatments, where dytiscids are active predators (Michel and Adams 2009, Chapter 2). It should be noted that some results from the predator selection experiment contradict known phenotypic correlations of wood frogs. For example, predators selectively killed tadpoles with deep tailfins and narrow tail muscles, yet tail muscle width and tailfin depth are highly correlated (Relyea 2005). This discrepancy may have arisen due to differences in the quantification of tailfin depth (i.e., full tailfin vs. upper half of tailfin), or effects of the ethanol preservative on the tadpole traits. Experiments are currently underway to address the latter.
Several trait responses to the three environmental factors were nonadaptive, which could have been a consequence of genetic or physiological constraints (Gotthard and Nylin 1995, DeWitt et al. 1998). For example, predator selection intensities for both top half tailfin depth and body width were negative. However, because tailfin depth and body shape are negatively correlated (Relyea 2005), the ability of tadpoles to develop shallow tailfins and narrow bodies may be genetically constrained. Physiological constraints may also affect phenotypic responses (Gotthard and Nylin 1995). Dytiscid larvae strongly selected for narrow-bodied tadpoles, but predator-induced plasticity of this trait was weak. A small body size negatively impacts tadpole competitive ability (Relyea 2002a), and thus body width plasticity may be constrained by selective pressures to grow and metamorphose.

4.5.3 Conclusions

The prevalence of nonadaptive trait responses suggests that individuals may not be able to accurately respond to a large number of cues from different types of environmental variables. Therefore, the complexity of natural environments may constrain the ability of individuals to exhibit adaptive phenotypes. However, within natural habitats, individuals can employ one of many strategies when confronted with complex environmental changes. For example, individuals lacking the ability to express strong predator-induced morphological traits may select habitats that confer greater protection from predation. However, we have limited knowledge on how an individual’s capacity to express plastic phenotypes interacts with habitat selection behavior, as many laboratory experiments constrain habitat choice of the individual. The link between morphology and habitat selection behavior has been established in terrestrial systems.
For example, lizards with long limbs, which confer faster sprinting speeds, occupy open habitats that are distant to protective rock cover (Goodman 2009). Therefore, it is recommended that future studies examine correlations between habitat selection behavior and morphology, as well as the capacity of an individual to morphologically respond to environmental change.

The effects of multiple environmental factors on the phenotypes of organisms are rarely studied despite repeated requests (e.g., Chapin et al. 1987, Sultan et al. 1998, West-Eberhard 2003, Miner et al. 2005, Stillwell et al. 2007). Although these experiments typically require complicated experimental manipulations and difficult interpretations (Stillwell et al. 2007), they are critical towards our understanding of how organisms phenotypically respond to a complex environment. The results of the current study demonstrate that it is possible to accurately predict the response of some traits to combinations of three different environmental variables. However, not all traits responded in predictable patterns, suggesting that additional mechanisms, such as genetic correlations, developmental constraints, or habitat selection behavior, contribute significantly to the development of plasticity in natural environments.

4.6 Acknowledgments

I thank M. Alldred and E. Zervoudakis for assistance with tadpole photography. J. Van Buskirk and B. Peters aided in construction of the tadpole photobox. Many thanks to H. Hollocher for helpful discussions. G. E. Belovsky and A. A. Forbes made helpful comments on previous versions of this manuscript.
4.7 References


CHAPTER 5:
PHENOTYPE-ENVIRONMENT ASSOCIATIONS OF TADPOLES IN NATURAL PONDS: THE IMPORTANCE OF SPATIAL SCALE

5.1 Abstract

Environmental heterogeneity can favor the development of local polymorphisms and phenotypic plasticity; however, the spatial scale at which environmental variables exhibit such heterogeneity can differ. Therefore, examinations of phenotype-environment associations of organisms within natural environments should employ a multiscale approach. Using hierarchical linear models, I determine how multiple environmental variables at two different spatial scales affect the morphological phenotypes of wood frog (*Rana sylvatica*) tadpoles collected from natural ponds. Additionally, morphology of wild-caught tadpoles was compared with morphology of tadpoles raised in a common garden experiment to assess genetic effects on phenotypes. Among ponds, mean tadpole phenotypes were strongly correlated with mean predation risk and tadpole density, two environmental variables known from laboratory experiments to exert strong selective and inductive pressure on tadpole morphology. However, within specific ponds, environmental variables such as water depth and abundance of leaf litter were more important than predation risk or competition for the majority of tadpole traits. Water depth and leaf litter abundance exhibited significantly clustered spatial distributions within most ponds, suggesting that the spatial properties of
environmental variables influence the spatial scale at which they affect phenotypes. Strong genetic effects were only found for tadpole tailfin depth, which corresponds to the claim that tailfin depth is under strong selective and inductive pressure. This study highlights the importance of examining phenotype-environment associations across multiple spatial scales.

5.2 Introduction

Environments can influence phenotypes by inducing phenotypic change (i.e., phenotypic plasticity) or by selecting for traits conferring the highest fitness within that environment (Langerhans et al. 2007). Consequently, phenotypes of organisms collected in natural habitats are often associated with the environmental characteristics of the habitat that exert strong inductive or selective pressure (e.g., Losos 1990, Keeley et al. 2005, Kerfoot, Jr. and Schaefer 2006, Langerhans et al. 2007).

Within natural populations, the spatial variance (i.e., heterogeneity) of an environmental variable is a strong promoter of phenotypic variance. Theoretical models predict that spatially heterogeneous environments favor the development of adaptively plastic traits (e.g., Via and Lande 1985, Moran 1992, Scheiner 1998, Sultan and Spencer 2002, Ernande and Dieckmann 2004, Leimar et al. 2006) and the divergence of phenotypes into local polymorphisms (Sultan and Spencer 2002, Scheiner 1998). Additionally, several empirical studies confirm these predictions (e.g., Juenger and Bergelson 2002, Svensson and Sinervo 2004, Lind and Johansson 2007). However, environmental variables exhibit heterogeneity at different spatial scales (Wiens 1989, Conover et al. 2006). Therefore, phenotype-environment associations should likewise
differ among multiple spatial scales, and should be strongest at the scale in which the environmental variable is heterogeneous.

Examinations of phenotype-environment associations of natural populations should also consider how the genotype contributes to the development of the phenotype (e.g., Keeley et al. 2007). Usually, this is accomplished through common garden experiments, in which individuals from different populations are collected and raised under identical conditions (Conover et al. 2006). Expressed phenotypes under these identical conditions provide an estimate of a population-level genetic component to phenotypic development.

In this study, I examine the influence of five cross-scale environmental variables and phenotypic measurements of common garden individuals on the morphology of wild-caught wood frog (\textit{Rana sylvatica}) tadpoles. \textit{R. sylvatica} tadpoles inhabit temporary ponds that can span several environmental gradients, such as hydroperiod and predator and competitor composition (Wellborn et al. 1996, Van Buskirk 2005). Additionally, environmental factors can demonstrate spatial variability within ponds, creating local microhabitats (Alford 1986, see results of current study).

Several environmental variables impose strong inductive and selective pressure on tadpole morphological phenotypes. Laboratory experiments demonstrate that predators induce and select for deep tailfins and short bodies (Van Buskirk and Relyea 1998). Increased intraspecific competition induces tadpoles to develop long bodies, shallow tailfins (Relyea 2002a), longer intestines (Relyea and Auld 2004), and larger mouthparts (Relyea and Auld 2005), all of which increase the competitive ability of tadpoles (Relyea 2002a).
The objective of this study was to identify environmental variables that are strongly correlated with tadpole phenotypes at a local, microhabitat scale (within ponds) and at a broader scale (among ponds). I predict that environmental variables will have the strongest association with tadpole phenotypes as the same scale in which they demonstrate heterogeneity. For example, if predators and competitors exhibit strong heterogeneity within ponds (i.e., ‘hotspots’, or ‘coldspots’; Brodie, Jr. et al. 2002), then I expect tadpole phenotypes to be strongly correlated with estimates of predation risk and competitive intensity within ponds. To analyze such a multiscale dataset, I used hierarchical linear models (Bryk and Raudenbush 1992, Gelman and Hill 2007, McMahon and Diez 2007), which permitted the partitioning of phenotypic variance, as well as its relationship with environmental factors, into within-pond and among-pond scales.

5.3 Methods

5.3.1 Field sampling

I selected five open and five closed canopy ponds (Table 5.1) known to be wood frog (Rana sylvatica) breeding sites at the University of Notre Dame Environmental Research Center (Gogebic county, MI). Within each pond, I established a 2 m by 2 m sampling grid, which served as sampling locations (hereafter termed “sites”, or “sampling sites”) for tadpoles and data on five environmental variables: tadpole predation risk, tadpole abundance, water depth, macrophyte abundance and amount of leaf litter. A
systematic sampling scheme allowed for subsequent spatial analysis of all five environmental variables (Fortin and Dale 2005).

**TABLE 5.1**
INFORMATION FOR THE 10 SAMPLED PONDS

<table>
<thead>
<tr>
<th>Pond</th>
<th>Canopy Designation</th>
<th>Area (m²)</th>
<th># Sampling sites</th>
<th># Sites with tadpoles</th>
<th>Sampling Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBB</td>
<td>Open</td>
<td>191.0</td>
<td>49</td>
<td>24</td>
<td>June 2</td>
</tr>
<tr>
<td>NFL</td>
<td>Open</td>
<td>26.8</td>
<td>8</td>
<td>2</td>
<td>June 4</td>
</tr>
<tr>
<td>SML</td>
<td>Open</td>
<td>168.9</td>
<td>44</td>
<td>30</td>
<td>June 5</td>
</tr>
<tr>
<td>VP12</td>
<td>Closed</td>
<td>86.7</td>
<td>20</td>
<td>3</td>
<td>June 11</td>
</tr>
<tr>
<td>VP27</td>
<td>Open</td>
<td>110.9</td>
<td>35</td>
<td>18</td>
<td>June 9</td>
</tr>
<tr>
<td>VP9B</td>
<td>Open</td>
<td>143.2</td>
<td>48</td>
<td>26</td>
<td>June 8</td>
</tr>
<tr>
<td>VPEFGH</td>
<td>Closed</td>
<td>33.9</td>
<td>11</td>
<td>0</td>
<td>June 11</td>
</tr>
<tr>
<td>VPGD</td>
<td>Closed</td>
<td>56.7</td>
<td>13</td>
<td>11</td>
<td>June 3</td>
</tr>
<tr>
<td>VPJ</td>
<td>Closed</td>
<td>59.2</td>
<td>31</td>
<td>7</td>
<td>June 10</td>
</tr>
<tr>
<td>VPN</td>
<td>Closed</td>
<td>47.1</td>
<td>18</td>
<td>10</td>
<td>June 11</td>
</tr>
</tbody>
</table>

Tadpole predators were sampled using one 42 cm by 23 cm unbaited Gee minnow trap composed of 6.4 mm galvanized wire mesh and two 22 mm diameter entrance holes. Six pieces of Styrofoam were mounted inside each trap to prevent sinking and potentially drowning air-breathing organisms. For approximately three weeks before tadpole sampling, traps were checked once daily and all potential tadpole predators were counted and released. Predators included large-sized diving water beetle larvae (Dytiscidae, > 4 cm length, 19.4% of all captures), medium-sized dytiscid larvae (> 1.5 cm and < 4 cm
length, 15.5%), large-sized dytiscid adults (> 4 cm length, 35.6%), medium-sized dytiscid adults (> 1.5 cm and < 4 cm, 18.0%), adult giant water bugs (Belostomatidae, 7.1%), adult newts [(Notophthalmus viridescens), 2.0%], scavenger beetle larvae (Hydrophilidae, 1.4%), adult water scorpions [(Ranatra spp.) 0.6%], and dragonfly larvae (Aeshnidae, 0.3%). Because different predator species pose varying mortality risks for tadpoles, I multiplied a predation risk weight factor, based on Van Buskirk and Arioli (2005) and personal observations, to the site-specific abundance totals for each predator. Large dytiscid larvae, belostomatids, and aeshnids received a weight of 3, medium dytiscid larvae received a weight of 2, and all other predators received weights of 1. Larger weights denoted a more dangerous tadpole predator. I then standardized by the number of nights each site was trapped to obtain a predation risk per trap-night measurement.

*R. sylvatica* tadpole abundance was quantified using two methods. Tadpoles freely entered minnow traps, and a count was recorded only on the last day of trapping. Two days after minnow traps were removed from a pond, each site was then sampled for tadpoles using a 30 cm diameter PVC pipe that was thrust vertically through the water column to the pond substrate. This sampling method does not disturb tadpole captures at sites less than 1 m away (Mullins et al. 2004). The number of tadpoles dipnetted within the pipe provided the second measure of tadpole abundance. Both methods were performed only once per pond. I assumed that these two methods provided reasonably equal capture probabilities, and obtained an average tadpole count for each site. *Rana sylvatica* was the only anuran species present among the closed canopy ponds. Spring peeper (*Pseudacris crucifer*) tadpoles were present in most open canopy ponds, but had
recently hatched and were rarely caught in dipnets. All tadpoles that were captured using pipe sampling were taken back to the laboratory, euthanized, and preserved in 10% formalin for later morphological measurements.

Water depth and macrophyte abundance were recorded at the time of tadpole sampling. Before I dipnetted for tadpoles within each pipe, all macrophyte stems including submerged and emergent plants were counted and removed. The majority of aquatic plants were sedges and bulrushes (Cyperaceae), pondweeds (Potamogeton spp.) and buttercups (Ranunculus spp.). Woody debris consisted mostly of logs on the bottom of the ponds, and, consequently did not contribute to vertical structure. Leaf litter abundance was recorded at the end of June after all 10 ponds had dried. I placed a 30 cm diameter PVC ring over each site and counted the number leaf litter pieces greater than 30 mm diameter.

5.3.2 Common-garden tadpoles

In mid-April, portions of three wood frog egg masses from each pond were collected and placed in separate 1 m diameter wading pools kept outdoors and covered with 60% shade cloth. Due to low egg mass abundances, three portions from the same egg mass were sampled from ponds VP12 and VPEFGH. After hatching, 40 tadpoles from each pool were randomly selected and transferred to one of 30 previously established outdoor aquatic mesocosms, so that one pool contained tadpoles from one sibship collected from one pond (10 ponds × 3 sibships = 30 pools). These mesocosms were composed in 1 m diameter wading pools, filled with well water, and inoculated with 1 L of filtered pond water, an aliquot of zooplankton and 5 g of crushed rabbit food to encourage algal growth. When tadpoles reached a similar size as those collected from the
natural ponds [Gosner developmental stage 31 – 37 (Gosner 1960), approximately 40 days], I removed all individuals with a dipnet and preserved them in 10% formalin for later morphological measurements. These phenotypic values provide baseline morphological traits for each pond population.

5.3.3 Morphological measurements

I was interested in four tadpole morphological traits: maximum depth of the tailfin (TFD), body length (BL), gut length (Gut), and size of the labial toothrows (Mouth). For each tadpole, I first obtained a mass and then photographed the lateral view after propping the tail on two microscope slides to present a more natural representation (Relyea and Werner 2000). Each photograph was viewed using the program ImageJ, scaled to size using a millimeter gradient present in the photograph, and measured for BL (tip of snout to body terminus) and maximum TFD. After the body photograph, the mouth of each tadpole was photographed using a 3.34 megapixel Nikon Coolpix 990 digital camera affixed to a dissecting scope (Relyea and Auld 2005). Using ImageJ, these photographs were scaled to size and the length of each labial toothrow and the perimeter of the upper jaw sheath were measured. Wood frog tadpoles typically have a 3/4 toothrow formula, and only lengths of toothrows that had fully-formed teeth were measured. After mouth photography, each tadpole was then dissected and the length of the intestine from the midgut to rectum was measured using a ruler (Relyea and Auld 2004). Procedures for all four morphological measurements were identical for both wild-caught and laboratory-raised tadpoles.

Before statistical analyses were conducted on morphological traits, they were standardized by tadpole body size. For TFD, BL, and Gut, I log-transformed each
variable and then conducted a univariate analysis of covariance (ANCOVA) with pond-of-origin as the factor and log-transformed mass as the covariate. After confirming slope homogeneity, I saved the residuals and added them to the marginal means estimated from the ANCOVA to obtain size-adjusted morphology. For mouthpart morphology, I first conducted a principal components analysis (PCA) using the length of each toothrow and the upper jaw sheath as the variables. I combined both wild-caught and lab-raised tadpoles in this analysis in order to better perceive mouthpart size differences between the two groups. All variables loaded positively into the first factor score, which explained 62.3% of the variation (eigenvalue = 4.99). I identified this factor score as a representation of total mouthpart size and saved the score for each individual. I then removed variation due to body size in the same manner described above. Using the standardized traits, I obtained a mean value of each morphological trait for each site (i.e., for the wild-caught tadpoles) or for each population (i.e., for the lab-raised tadpoles). For all 4 traits, I performed separate ANCOVAs for wild-caught and lab-raised tadpoles because inclusion of all tadpoles resulted in heterogeneous slopes.

5.3.4 Spatial analyses

Each environmental variable and tadpole morphological variable was analyzed for evidence of spatial autocorrelation at a distance class of 2 meters using the global Moran’s $I$ statistic tool in ArcGIS 9.2. Directional patterns were not anticipated for any of the variables, thus all measures of autocorrelation were omnidirectional. A distance class of 2 meters was chosen as a compromise between selecting a large value to eliminate effects of tadpole movement within ponds and an adequate sample size within the smaller ponds. Values of Moran’s $I$ close to 1 represent a clustered spatial pattern,
values close to –1 represent a dispersed pattern and values close to zero represent a random pattern (Fortin and Dale 2005). The spatial autocorrelation tool in ArcGIS 9.2 provides a test of the null hypothesis of no spatial pattern (i.e., \( I = 0 \)) using a permutation test.

5.3.5 Model building

The objective of this analysis was to determine how environmental variables at the sampling site scale (level 1) and environmental and genetic factors at the pond scale (level 2) affect morphology of wild-caught tadpoles (see Table 5.2 for a description of the hierarchical framework). Because hierarchical models can quickly become complicated with the inclusion of explanatory variables and their interactions at any level, I initially conducted an exploratory data analysis to determine which independent variables explained the most variation in tadpole morphology at both scales. First, I excluded ponds NFL, VP12, and VPEF GH from analyses due to a low number of sites in which tadpoles were captured. Then, for within-pond phenotypic variation, I ran a multiple linear regression using measurements of all five site-specific environmental variables and included a spatial error term to account for any spatial autocorrelation (Wang 2006). I then identified which environmental variables were significant (\( \alpha = 0.05 \)) for each particular morphological variable (for inclusion in subsequent hierarchical linear modeling) and repeated this process for every pond. Within-pond regressions were conducted using GeoDa 0.9.5 (Anselin 2003). To assess any potential multicollinearity between environmental variables, I ran correlations of all within-pond variables for each pond. For pond SML, water depth was negatively correlated with leaf litter (Pearson’s \( r = -0.466 \), Bonferroni adjusted \( P = 0.026 \)). For pond VP9B, water depth was negatively
correlated with stem density (Pearson’s $r = -0.567$, adjusted $P = 0.002$), and positively correlated with tadpole abundance (Pearson’s $r = 0.487$, adjusted $P = 0.019$). All other possible correlations were not statistically significant.
TABLE 5.2
DESCRIPTION OF HIERARCHICAL POND FRAMEWORK WITH PREDICTORS AT LEVEL 1 (WITHIN-POND) SCALE AND LEVEL 2 (AMONG-POND) SCALE.

<table>
<thead>
<tr>
<th>Response Variables</th>
<th>Level 1 (within-pond)</th>
<th>Level 2 (among-pond)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphological traits of wild-caught tadpoles:</td>
<td>Site-specific values or means:</td>
<td>Mean values for each pond:</td>
</tr>
<tr>
<td>• Tailfin depth (TFD)</td>
<td>• Water depth (S.Depth)</td>
<td>• Water depth (M.Depth)</td>
</tr>
<tr>
<td>• Body length (BL)</td>
<td>• # macrophyte stems (S.Stems)</td>
<td>• # macrophyte stems (M.Stems)</td>
</tr>
<tr>
<td>• Gut length (Gut)</td>
<td>• Leaf litter abundance (S.Leaf)</td>
<td>• Leaf litter abundance (M.Leaf)</td>
</tr>
<tr>
<td>• Mouthpart size (Mouth)</td>
<td>• Tadpole abundance (S.Tads)</td>
<td>• Tadpole abundance (M.Tads)</td>
</tr>
<tr>
<td></td>
<td>• Predation risk intensity (S.Risk)</td>
<td>• Predation risk intensity (M.Risk)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Mean value of morphological trait of common-garden tadpoles (CgTFD, CgBL, CgGut, or CgMouth)</td>
</tr>
</tbody>
</table>

Note: Abbreviations are given in parentheses.
For among-pond phenotypic variance, I used a backwards stepwise multiple linear regression for each morphological variable; however, because of small sample size (n = 7 ponds), I excluded independent variables that, from analyzing scatterplots, did not seem to be correlated with morphological variables. All remaining environmental variables in the final regression model (at $\alpha = 0.05$) were included in subsequent hierarchical linear modeling. Among-pond multiple linear regressions were conducted using R 2.7.0. Additionally, I tested for correlations between pond-mean environmental variables, as well as between morphology of common garden tadpoles and the pond-mean environmental variables. No correlations were significant (Table 5.3, 5.4).

**TABLE 5.3**

VALUES OF PEARSON’S $R$ (ABOVE DIAGONAL) AND CORRESPONDING UNCORRECTED $P$-VALUES (BELOW DIAGONAL) FOR POND-MEAN ENVIRONMENTAL VARIABLES.

<table>
<thead>
<tr>
<th></th>
<th>Depth</th>
<th>Stems</th>
<th>Leaf Litter</th>
<th>Tad Index</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td></td>
<td>0.531</td>
<td>-0.238</td>
<td>0.084</td>
<td>0.510</td>
</tr>
<tr>
<td>Stems</td>
<td>0.220</td>
<td></td>
<td>-0.527</td>
<td>-0.248</td>
<td>0.501</td>
</tr>
<tr>
<td>Leaf Litter</td>
<td>0.608</td>
<td>0.225</td>
<td></td>
<td>0.371</td>
<td>-0.615</td>
</tr>
<tr>
<td>Tad Index</td>
<td>0.858</td>
<td>0.591</td>
<td>0.412</td>
<td></td>
<td>-0.319</td>
</tr>
<tr>
<td>Risk</td>
<td>0.243</td>
<td>0.252</td>
<td>0.141</td>
<td>0.485</td>
<td></td>
</tr>
</tbody>
</table>

Note: $P$-values evaluated at a Bonferroni adjusted $\alpha = 0.005$. 
TABLE 5.4
VALUES OF PEARSON’S R FOR MORPHOLOGY OF COMMON GARDEN TADPOLES AND POND-MEAN ENVIRONMENTAL VARIABLES.

<table>
<thead>
<tr>
<th></th>
<th>Tailfin Depth</th>
<th>Body Length</th>
<th>Gut Length</th>
<th>Mouthpart Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td>-0.179</td>
<td>0.344</td>
<td>-0.493</td>
<td>-0.195</td>
</tr>
<tr>
<td>Stems</td>
<td>0.229</td>
<td>0.190</td>
<td>-0.088</td>
<td>-0.429</td>
</tr>
<tr>
<td>Leaf Litter</td>
<td>-0.385</td>
<td>0.047</td>
<td>0.592</td>
<td>0.378</td>
</tr>
<tr>
<td>Tad Index</td>
<td>-0.165</td>
<td>0.673</td>
<td>0.365</td>
<td>0.289</td>
</tr>
<tr>
<td>Risk</td>
<td>0.440</td>
<td>0.393</td>
<td>-0.321</td>
<td>0.175</td>
</tr>
</tbody>
</table>

Note: All P-values > 0.10.

After obtaining potentially important within-pond and among-pond predictors (Table 5.3), I began building hierarchical linear models separately for each morphological variable, following the procedures outlined in Gelman and Hill (2007) and Bryk and Raudenbush (1992). I started with an unconditional model that included an intercept that was allowed to vary by pond:

\[ Y_{ij} = \beta_{0j} + r_{ij}, \]  \hspace{1cm} (5.1)

\[ \beta_{0j} = \gamma_{00} + u_{0j}, \]  \hspace{1cm} (5.2)

where \( Y \) is the morphological variable of interest for the \( i \)th sampling site of the \( j \)th pond, \( r \) is residual error assumed to have a normal distribution of \((0, \sigma^2)\) and describes phenotypic variance within ponds, \( \gamma \) is the level 2 intercept (i.e., the grand mean of the
morphological variable), and $u$ is a random variable with a normal distribution of $(0, \tau_{00})$ and describes phenotypic variance among ponds. I then calculated the intraclass correlation, $\rho$, which is defined as $\tau_{00}/(\tau_{00} + \sigma^2)$ and estimates the proportion of variance in $Y$ that is among ponds (Bryk and Raudenbush 1992). Subtracting this value from one gives the proportion of phenotypic variance within ponds.

### TABLE 5.5

SIGNIFICANT WITHIN-POND AND AMONG-POND PREDICTORS OBTAINED FROM EXPLORATORY DATA ANALYSIS FOR EACH TADPOLE MORPHOLOGICAL TRAIT.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Within-Pond Predictors</th>
<th>Among-Pond Predictors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tailfin depth</td>
<td>S.Depth</td>
<td>M.Stems</td>
</tr>
<tr>
<td></td>
<td>S.Risk</td>
<td>M.Risk</td>
</tr>
<tr>
<td>Body length</td>
<td>None</td>
<td>M.Tads</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M.Risk</td>
</tr>
<tr>
<td>Gut length</td>
<td>S.Depth</td>
<td>M.Tads</td>
</tr>
<tr>
<td></td>
<td>S.Leaf</td>
<td>M.Risk</td>
</tr>
<tr>
<td>Mouthpart size</td>
<td>S.Leaf</td>
<td>M.Tads</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M.Risk</td>
</tr>
</tbody>
</table>

I then subsequently added among-pond predictors to the $\beta_{0j}$ intercept term while keeping equation 5.1 constant:

$$\beta_{0j} = \gamma_{00} + \gamma_{01}(X)_j + u_{0j}, \quad (5.3)$$
where $X_j$ is an among-pond predictor. I compared the $\tau_{00}$ estimates of these models with the unconditional model (i.e., equation 5.1) to estimate the proportion of among-pond phenotypic variance explained by each predictor. For each morphological variable, all among-pond predictors identified from exploratory data analysis, as well as any interactions, were tested. I then removed all among-pond predictors and subsequently added within-pond predictors:

$$Y_{ij} = \beta_{0j} + \beta_{1j}(Z_{ij} - \bar{Z}_j) + r_{ij},$$  \hspace{1cm} (5.4)

$$\beta_{0j} = \gamma_{00} + u_{0j},$$  \hspace{1cm} (5.5)

$$\beta_{1j} = \gamma_{10} + u_{1j},$$  \hspace{1cm} (5.6)

where $Z$ is the level 1 predictor that is centered around the grand mean. Centering aids in interpretation of the intercept and reduces any correlations between intercept and slope estimates (Bryk and Raudenbush 1992). The random intercept ($u_{0j}$) and slope error ($u_{1j}$) are now assumed to have a multivariate normal distribution of:

$$\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} \tau_{00} & \tau_{01} \\ \tau_{10} & \tau_{11} \end{pmatrix}$$

where $\tau_{01}$ is the covariance of the two random error terms (Gelman and Hill 2007). To estimate the proportion of within-pond phenotypic variance explained by the predictor, I compared the $\sigma^2$ estimates with those from the unconditional model.

I then combined within-pond and among-pond predictors to determine their effects on the within-pond and among-pond variance estimates. From each model, I also obtained estimates of Akaike’s information criterion (AIC) and deviance information criterion (DIC) to evaluate the fit of each model. Smaller numbers of these information
criterions in terms of absolute values indicate a better model fit. However, model selection was based on the proportion of total phenotypic variance explained by the predictors, and AIC and DIC measurements were only used to ensure that each model provided a better fit than the unconditional model (equations 5.1, 5.2). Models were fit using the linear mixed effects (lmer) function in R.2.7.0 (Gelman and Hill 2007).

5.4 Results

5.4.1 Spatial autocorrelation estimates

For all ponds, there was no evidence of significant spatial patterning for any tadpole morphological variable. Values of Moran’s $I$ for TFD ranged from -0.38 to 0.10; for BL, -0.59 to -0.01; for Gut, -0.15 to 0.12; and for Mouth, -0.10 to 0.04. For the environmental variables, water depth, stem abundance and leaf litter abundance showed significantly clustered spatial patterns in most ponds, while tadpole abundance and predation risk intensity showed mostly random spatial patterns (Table 5.6).
### TABLE 5.6

VALUES OF MORAN’S I AND SUBSEQUENT SPATIAL PATTERN (C = CLUSTERED, R = RANDOM) AS DETERMINED BY A PERMUTATION TEST OF THE NULL HYPOTHESIS I = 0.

<table>
<thead>
<tr>
<th>Pond</th>
<th>Water Depth</th>
<th>Stems</th>
<th>Leaf Litter</th>
<th>Tadpole Abundance</th>
<th>Predation Risk Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>Pattern</td>
<td>I</td>
<td>Pattern</td>
<td>I</td>
</tr>
<tr>
<td>NBB</td>
<td>0.440</td>
<td>C</td>
<td>0.230</td>
<td>C</td>
<td>0.180</td>
</tr>
<tr>
<td>SML</td>
<td>0.470</td>
<td>C</td>
<td>-0.020</td>
<td>R</td>
<td>0.190</td>
</tr>
<tr>
<td>VP27</td>
<td>0.650</td>
<td>C</td>
<td>0.190</td>
<td>C</td>
<td>0.140</td>
</tr>
<tr>
<td>VP9B</td>
<td>0.700</td>
<td>C</td>
<td>0.300</td>
<td>C</td>
<td>0.280</td>
</tr>
<tr>
<td>VPGD</td>
<td>-0.004</td>
<td>R</td>
<td>--</td>
<td>--</td>
<td>0.110</td>
</tr>
<tr>
<td>VPJ</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>VPN</td>
<td>0.480</td>
<td>C</td>
<td>--</td>
<td>--</td>
<td>-0.090</td>
</tr>
</tbody>
</table>

Note: Dashes indicate no value of Moran’s I was calculated due to a low sample size.
5.4.2 Tailfin depth model results

The unconditional model provided a within-pond variance estimate of $4.4 \times 10^{-4}$ and an among-pond estimate of $1.39 \times 10^{-11}$, suggesting that more than 99.9% of the variation in TFD was within a pond (Table 5.7). The inclusion of pond-level risk intensity decreased among-pond variance to $2.26 \times 10^{-13}$, a 98.4% reduction. The inclusion of mean tailfin depth of the common garden tadpoles decreased among-pond variance to $2.34 \times 10^{-13}$, a 98.3% reduction. Both these variables were positively correlated with pond-mean TFD (Figure 5.1B, 5.1C). Pond-level stem density did not explain any among-pond variation. Site-specific water depth and risk intensity reduced within-pond variation by 6.8% and 2.3% respectively, and, combined, reduced within-pond variation by 10.5%. The full model (combining all within and among-pond predictors, as well as the interactions between them) reduced among-pond variation by 99% and within-pond variation by 9.3%. Removal of the interactions between level 1 and level 2 led to a model in which pond-mean risk intensity and site-specific water depth were the only two significant positive predictors, as judged by the standard error estimate (Figure 5.1A). The full model had the lowest AIC and DIC values.
TABLE 5.7
RESULTS OF VARIOUS HIERARCHICAL MODELS FOR TAILFIN DEPTH (TFD).

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<td>-602</td>
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Figure 5.1 A) Relationship between site-specific standardized tadpole tailfin depth and water depth grouped by ponds. For clarity, only ponds with significant positive slopes are shown. B) Relationship between pond-mean standardized tadpole tailfin depth and predation risk intensity, and C) standardized mean tadpole tailfin depth from common garden tadpoles.

5.4.3 Body length model results

The unconditional model suggested that 25.3% of BL variation existed among ponds while 74.7% was within a pond (Table 5.8). The inclusion of pond-mean tadpole abundance reduced among-pond variance to \(2.66 \times 10^{-5}\), a 59.1% reduction, while the inclusion of pond-mean risk intensity reduced among-pond variance to \(2.70 \times 10^{-5}\), a 58.5% reduction. A combined model reduced among-pond variance by 91.3%. This model also produced the lowest AIC and DIC estimates. Inclusion of an interaction term did not substantially reduce among-pond variance. Pond-mean tadpole abundance was positively correlated to mean BL, while pond mean risk intensity was negatively correlated (Figure 5.2). Regarding pond-mean tadpole abundance, one pond (VPGD) had
several orders of magnitude more tadpoles than the other ponds, and may be an outlier.

Exploratory data analysis suggested that there were no important site-specific predictors, which was confirmed by the models in which not one predictor was able to explain more than 1.5% of within-pond variation.

### TABLE 5.8

RESULTS OF VARIOUS HIERARCHICAL MODELS FOR BODY LENGTH (BL).

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<th>Parameter</th>
<th>Estimate</th>
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Figure 5.2 Relationship between standardized body length and A) pond-mean predation risk intensity and B) pond-mean tadpole abundance.

5.4.4 Gut length model results

The unconditional model estimated that 26.7% of the variation in gut length existed among ponds while 73.3% of the variation existed within a pond (Table 5.9). The inclusion of pond-mean tadpole abundance and pond-mean risk intensity reduced among-pond variance by 3.3% and 17.8% respectively. A model combining these two predictors reduced among-pond variation by 52.8% and the inclusion of an interaction term reduced this variation by 69.2%. Within-pond variables failed to explain a substantial proportion
of the within-pond variance. However, a model combining pond-mean predation risk intensity and tadpole abundance, as well as site-specific water depth reduced within-pond variation by 1.6% and among-pond variation by 93%. There was a strong negative interaction term for pond-mean predation risk and tadpole abundance. Generally, gut length decreased as predation risk intensity increased, except for the pond with the highest tadpole abundance, at which tadpoles had short gut lengths (Figure 5.3). Within ponds, tadpole gut length tended to decline with increasing water depth and leaf litter.
### TABLE 5.9

**RESULTS OF VARIOUS HIERARCHICAL MODELS FOR GUT LENGTH (GUT)**

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<tr>
<th>Parameter</th>
<th>Estimate</th>
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Figure 5.3 Interactive effects of pond-mean predation risk intensity and tadpole abundance on standardized tadpole gut length. Size of symbol corresponds to the logarithm of tadpole abundance per sampling site of a pond.

5.4.5 Mouthpart size model results

The unconditional model estimated that 44.2% of the variation in mouthpart size was among ponds, while 55.8% existed within a pond (Table 5.10). The inclusion of pond-mean tadpole abundance and pond-mean risk intensity each separately reduced among-pond variation by 20%, but a combined model was only able to reduce among-pond variation by an additional 1%. Site-specific leaf litter abundance reduced within-pond variation by 4.5%. A model combining both among-pond predictors and site-specific leaf litter abundance, but no interactions among them, reduced among-pond variation by 33.1% and within-pond variation by 6.3%. However, this model had larger...
values of AIC and DIC than the unconditional model. Mouthpart size was positively correlated with pond-mean tadpole abundance (although this relationships was strongly driven by the outlier pond VPGD), and negatively correlated with pond-mean predation risk intensity (Figure 5.4B). Mouthpart size also tended to decrease with site-specific leaf litter abundance (Figure 5.4A).
### TABLE 5.10

RESULTS OF VARIOUS HIERARCHICAL MODELS FOR MOUTHPART SIZE

(MOUTH)

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<th>$\tau_{00}$</th>
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Figure 5.4 A) Relationship between site-specific standardized tadpole mouthpart size and leaf litter abundance. For clarity, only ponds with significant negative slopes are shown. B) Relationship between pond-mean standardized tadpole mouthpart size and predation risk intensity and C) tadpole abundance.

5.5 Discussion

Tadpole phenotype-environment associations showed strong evidence of scale-dependence. As predicted, environmental variables that demonstrated heterogeneity at a within-pond scale explained the most phenotypic variance at that scale. Within ponds, water depth and leaf litter were the strongest predictors of three of the four morphological phenotypes (Table 5.5). These environmental variables also showed clustered spatial distributions within most ponds (Table 5.6). Conversely, predation risk and tadpole abundance exhibited random spatial distributions in the majority of ponds. Thus, at the within-pond scale, the absence of ‘hotspots’ or ‘coldspots’ precluded their importance in explaining tadpole phenotypic variance. Among ponds, predation risk and tadpole
abundance were the most important predictors of tadpole phenotypic variance, suggesting that tadpoles adjust their phenotypes in response to pond-wide levels of these two variables. Tailfin depth was the only trait that demonstrated the possibility of a strong genetic component, and likely represents strong natural selection from predators.

Spatiotemporal properties of the environmental variables influenced the scale at which these variables associated with tadpole phenotypes. Important within-pond predictors (i.e., water depth and leaf litter) are relatively discrete and less temporally dynamic than the important among-pond predictors (i.e., predation risk intensity and tadpole abundance), which are mobile, temporally heterogeneous, and emit chemical cues that disperse throughout a pond. This distinction is supported by the spatial analyses of each environmental variable: water depth and leaf litter showed significant clustering in most ponds, while predation risk and tadpole abundance were mostly randomly distributed. Consequently, tadpole perceptions of water depth and leaf litter microhabitat differences may be more accurate than perceptions of local predator and tadpole density. This conclusion agrees with theoretical models that show, for a given scale, the ability of an organism to accurately predict environmental change is a significant prerequisite for plastic trait responses (e.g., Moran 1992, Scheiner 1998, Leimar et al. 2006). Therefore, tadpoles were able to adjust their phenotypes in response to predictable differences in water depth and leaf litter abundance. This phenotype-environment correlation could have arisen through either nonrandom habitat selection behavior or inducible changes in morphology, although this study could not distinguish between these two mechanisms.

The presence of discrete environmental heterogeneity at a microhabitat scale are believed to have impelled the divergence of several natural populations, including
sticklebacks (*Gasterosteus* spp.) responding to limnetic and benthic habitats (Day et al. 1994), various fish species of northern postglacial lakes responding to littoral and pelagic habitats (reviewed in Robinson and Parsons 2002), and anoles (*Anolis* spp.) responding to ground, bush, trunk and crown habitats (Losos 1990, Losos et al. 2000). However, because water depth and leaf litter presumably exert weak inductive or selective pressure, it does not appear that microhabitats within ponds play an important role in the divergence of amphibian populations. This conclusion is supported by the observation that none of the four tadpole traits exhibited strong spatial clustering within ponds.

Pond-mean predation risk intensity and tadpole abundance explained much of the variation in pond-mean tadpole traits. However, results from hierarchical linear models indicate that most phenotypic variation occurred within rather than among ponds. This difference is especially evident for tailfin depth, for which within-pond variation accounted for 99% of the total variation. Phenotypic variation can be reduced by stabilizing selection. Therefore, such cross-scale differences in phenotypic variation suggest that stabilizing selection is stronger at a pond-wide level than among microhabitats within ponds. Furthermore, much of the among-pond variation was explained by predation risk intensity and tadpole abundance, which are two environmental variables known to impose strong selection on tadpole traits (Van Buskirk et al. 1997, Van Buskirk and Relyea 1998, Relyea 2002a, Teplitsky et al. 2005). Conversely, within-pond environmental gradients could only explain approximately 2 – 10% of trait variation within ponds and are not thought to impose strong selective pressure on tadpole phenotypes. These results suggest that the induction and selection
pressures for tadpole morphological traits are stronger at a pond-wide scale, than among microhabitats within ponds.

Why could only a small portion of within-pond trait variation be explained? First, environmental variables that I did not quantify, such as the level of tadpole food resources, could have been significant within-pond predictors. Second, tadpoles simply may not phenotypically respond to local-scale environmental variables. The environmental variables known to impose strong selective and inductive pressure on tadpole phenotypes—predation risk and tadpole abundance—were mostly randomly distributed within a pond. Thus, the unpredictability of these environments may have precluded strong phenotypic responses (Scheiner 1998, Leimar et al. 2006). Finally, substantial phenotypic variation may have been explained by variation in genotypes and genotype-by-environment interactions. Keeley et al. (2007) estimated that heritable differences explained 52.7% of variation in rainbow trout (Oncorhynchus mykiss) morphology, as opposed to environmental differences, which explained 7.3%. Proulx and Magnan (2004) also found that genetic factors explained approximately 2% more variation in brook charr (Salvelinus fontinalis) morphology. I was only able to obtain an estimate of population-level genetic variation, so future studies should examine individual differences in genotypes and genotype by environment interactions.

Tailfin depth was the only trait in which the pond-mean value matched the mean value of individuals raised under common garden conditions, suggesting that the tailfin depth of each population reacted to natural environments in a similar manner (i.e., parallel reaction norms, Figure 5.5). Laboratory studies demonstrate that tadpoles from different populations show similar magnitudes of tailfin depth plasticity in response to
predation risk (Lardner 1998, Relyea 2002b, Van Buskirk and Arioli 2005). I also found evidence for this relationship, as tailfin depth of common garden tadpoles was positively correlated with the predation risk intensity of the source pond (see Table 5.4). Furthermore, in the present study, tadpoles of natural populations were mostly responding to predation risk, as it was the only pond-mean environmental variable that was significantly correlated with tailfin depth. Therefore, in natural populations, predators likely exert strong selective pressures on tailfin depth of tadpoles (Van Buskirk and McCollum 1998, Van Buskirk and Arioli 2005).

Figure 5.5 Mean tadpole tailfin depth among the common garden tadpoles and wild-caught tadpoles from natural ponds, grouped by pond. Filled symbols are open-canopy ponds, open symbols are closed-canopy ponds. Tailfin depth was back-transformed to obtain similar units among environments.
The results of this field survey also suggest that tadpole phenotypic responses to predation risk and competitor density observed in laboratory experiments are accurate predictors of tadpole phenotypic change among natural populations. Many tadpole predators both select for (Van Buskirk et al. 1997, Van Buskirk and Relyea 1998, Teplitsky et al. 2005) and induce deep tailfins and long bodies (Lardner 1998, Relyea 2003, McIntyre et al. 2004, Schoeppner and Relyea 2008). One notable exception to this pattern is given in chapter four, where I found that dytiscid larvae select for shallow tailfins among open and structured habitats, and induce shallow tailfins among open habitats (see chapter four). Thus, the presence of other predator species within the natural ponds and chemical alarm cues from killed conspecifics likely contributed to the relationship between pond-mean predation risk and tailfin depth. Decreases in gut length and mouthpart size are thought to be costs incurred by tadpoles in order to produce deep tailfins and short bodies (Relyea and Auld 2004, 2005). Ponds with high tadpole densities produced tadpoles with longer bodies and larger mouthparts, which is also likely due to a combination of selection and plasticity. Laboratory experiments demonstrate competitors induce longer bodies (Relyea 2002a, 2004) and larger mouthparts (Relyea and Auld 2005) in *R. sylvatica* tadpoles, and that these changes enhance growth and developmental rates in highly competitive environments (Relyea 2002a). Competitors also induce longer gut lengths and shallower tailfins (Relyea 2002a, Relyea and Auld 2004), but these patterns were not observed. These results have important implications for phenotypic plasticity research, as they demonstrate that patterns of plasticity observed among laboratory studies are very similar to patterns observed among natural populations.
In this chapter, I have demonstrated that phenotype-environment associations differ among multiple spatial scales and that the importance of environmental factors on phenotypic development depends on their spatiotemporal properties. Whether tadpoles actively chose habitats based on their morphology or were induced by environments to exhibit morphological changes is unknown. Therefore, a fruitful avenue of research for a wide variety of species could be an integration of habitat selection behavior with morphology and ability to express morphological plasticity. Hierarchical linear models and measures of spatial autocorrelation facilitated the recognition of these patterns and they have the potential to be valuable statistical tools to investigate these types of patterns in other systems (e.g., McMahon and Diez 2007).

5.6 Acknowledgments

T. Frauendorf and L. Kinsman aided in predator trapping. K. Balzer, M. Barnes, K. Davilia, J. Dreyer, B. Peters, and V. Velez helped with collection of tadpoles and environmental data. L. Whiting helped with tadpole photography and trait measurement. J. Van Buskirk and B. Peters helped with the construction of the tadpole photobox. Many thanks to J. MacLachlan for helpful discussions on hierarchical linear modeling.

5.7 References


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6.1 Abstract

Ultraviolet-B radiation (UVBR) is an important stressor of many aquatic organisms, but actual concentrations of UVBR reaching aquatic microhabitats differ due to absorption by surrounding vegetation and attenuation with depth. Because many aquatic species alter habitat selection in response to various environmental factors, exposure levels of organisms to UVBR may be directly modified by the presence of these factors. I tested whether behavioral changes of amphibian larvae in response to conspecific competitors and predation risk affected the development of an amphibian malformity linked with UVBR overexposure. Predation risk decreased malformation frequency, and behavioral observations suggest that predator-induced reduction in amphibian activity levels decreased their susceptibility to harmful UVBR effects. Malformed individuals were also more vulnerable to predators, suggesting that sublethal UVBR effects may play an important role in the global decline of amphibian populations. However, because only 9% of individuals developed the malformity, losses of this magnitude may be compensatory.
6.2 Introduction

Aquatic organisms are negatively impacted by a variety of abiotic and biotic stressors acting alone or synergistically (Cech Jr. et al. 1998 Bancroft et al. 2008). Ecological interactions, such as competition and predation, often amplify the negative impacts of these stressors by adding physiological stress to the individual (Relyea and Mills 2001, Relyea 2003) or inducing behavioral changes that render the organism more susceptible to the effects of the stressor (Thiemann and Wassersug 2000, Decaestecker et al. 2002, Johnson et al. 2006). Ultraviolet-B radiation (UVBR, 280 – 320 nm wavelength) is a particularly harmful environmental stressor to many aquatic species (Bancroft et al. 2007, Häder et al. 2007), and satellite data demonstrate that levels of UVBR reaching the surface of the earth have increased over the last several decades due to depletion of stratospheric ozone (Kerr and McElroy 1993, Corn and Muths 2002). These levels are projected to remain elevated for the next three decades (Madronich et al. 1998). However, the actual amount of UVBR reaching aquatic microhabitats varies spatially and is negatively affected by water depth, concentration of dissolved organic carbon (DOC), water turbidity, and density of aquatic vegetation (Peterson et al. 2002).

Amphibians that inhabit aquatic habitats for some part of their life history (e.g., as eggs or larvae) are vulnerable to increases in UVBR concentrations because, as ectotherms, they must be exposed to sunlight to regulate body temperature. Amphibian larvae are especially susceptible because they lack specialized epidermal structures, such as scales or feathers that can provide protection from UVBR (Blaustein and Belden 2003). Exposure to high levels of UVBR can reduce hatching success of amphibian eggs (Blaustein et al. 2001), increase mortality rates of eggs and larvae (Bancroft et al. 2008)
and cause various sublethal effects including skin lesions (Flamarique et al. 2000), retinal damage (Fite et al. 1998, Flamarique et al. 2000) and malformations (Blaustein et al. 1997) depending on the dose of UVBR received (Ankley et al. 2000, 2002).

Consequently, UVBR has been identified as a major environmental factor contributing to the global amphibian decline (Blaustein and Kiesecker 2002, Collins and Storfer 2003, but see Licht 2003).

During the larval developmental stage, many amphibian species exhibit behavioral plasticity in response to environmental factors. For example, chemical cues from predators induce reductions in larval amphibian activity (Richardson 2001, Van Buskirk 2002), spatial avoidance of predators (Van Buskirk and Schmidt 2000) and increased refuge use (Van Buskirk 2001) whereas the presence of competitors induces increases in overall activity (Relyea 2002). Because UVBR levels vary spatially within aquatic habitats and harmful effects of UVBR are dose-dependent, such behavioral plasticity of amphibian larvae may modify negative effects of UVBR exposure (Peterson et al. 2002, Licht 2003). By promoting a sedentary, benthic lifestyle where exposure to UVBR is diminished, predators may decrease overall harmful UVBR effects. Likewise, by promoting an active lifestyle, which may increase the cumulative amount of time spent in areas of high UVBR concentration, competitive environments may increase these harmful effects.

In previous years, I had noticed that a small proportion of wood frog (*Rana sylvatica*) tadpoles raised in outdoor mesocosms had developed scoliosis, a lateral curvature of the tail muscle (Hisaoka & List 1957; Figure 6.1). Scoliosis afflicts a diverse range of amphibian species and its expression is linked to overexposure to UVBR
(e.g., Worrest and Kimeldorf 1976, Pahkala et al. 2001, Weyrauch and Grubb, Jr. 2006, Van Uitregt et al. 2007, Croteau et al. 2008). Using the frequency of scoliosis as an indicator of harmful UVBR effects, I tested the hypothesis that competition, predation risk and aquatic vegetation will modify the development of tadpole scoliosis and, hence, UVBR exposure. Additionally, I quantified tadpole activity levels to assess if the effects of predators and competitors on scoliosis development were mediated by tadpole behavioral responses to these manipulated environments.

Figure 6.1 One normal tadpole (far right) and two tadpoles with varying levels of scoliosis.
Additionally, I was also interested in quantifying any negative effects of scoliosis on natural tadpole populations. Because tadpole swimming speed depends on tail morphology, and swimming speed subsequently determines escape rates from predators (Dayton et al. 2005), I tested if scoliotic tadpoles suffer from reduced swimming ability, and, consequently experience greater mortality from predation than normal tadpoles. If scoliosis does render tadpoles more vulnerable to predators, then such sublethal impacts of UVBR should be considered when evaluating the contributions of UVBR to the global decline of amphibian populations. This paper provides the first evidence that ecological interactions can modify harmful effects of UVBR overexposure, and that such harmful effects can incur substantial fitness costs for amphibian larvae.

6.3 Materials and Methods

6.3.1 Experiment I: Effects of environmental variables on scoliosis frequency

I present data on scoliosis development that was collected as part of a larger experiment testing the effects of competitors, predation risk and aquatic vegetation on tadpole behavior and morphology. For further details on experimental methods see Chapter 3. I collected *R. sylvatica* tadpoles of approximately Gosner developmental stage 25 (Gosner 1960) from three temporary ponds within the University of Notre Dame Environmental Research Center (Gogebic Co., MI) and placed them into 1 m diameter plastic wading pools kept outside. After approximately one week, I placed tadpoles into 60.5-l experimental mesocosms in which one treatment level of each of the following environmental factors were manipulated: competitors (7 or 21 total *R. sylvatica* tadpoles),
predation risk (no predator or one caged predator), and aquatic vegetation (simulated using 0, 28, or 84 strands of green polypropylene rope). One diving water beetle larva (*Dytiscus* spp.) was used as the predator species and fed 500 mg of *R. sylvatica* tadpoles every other day. I caged predators so that tadpole densities established by the competitor treatments were maintained. Because *Dytiscus* larvae breathe atmospheric air, I kept cages near the water surface using Styrofoam cubes. I fully crossed each factor (2 × 2 × 3) and replicated each treatment five times for a total of 60 experimental mesocosms. I kept all mesocosms outside where they experienced only ambient UVBR levels, and also covered each mesocosm with fiberglass windowscreening to prevent invasion from aquatic insects. After 30 days, I removed all surviving tadpoles from the mesocosms and preserved them in 10% formalin. I later examined all individuals for scoliosis and a proportion was calculated for each tub.

I used scoliosis:normal phenotype ratios as a binomial response variable. Analysis of data consisted of a modeling approach, using binomial errors, with competition, predation risk, simulated aquatic vegetation and their interactions as categorical independent variables. I started with all factors and began removing non-significant interactions and then main effects. Because there was evidence of overdispersion when all factors were included, I employed quasibinomial errors (Crawley 2005). At each step, I tested whether the removal of the term improved the fit of the model by comparing estimates of deviance with the previous model using an analysis of deviance (ANODEV). I saved the most parsimonious model and tested for significance of included effects using an ANODEV. Model building and significance testing was conducted using R.2.7.0.
To test the effect of tadpole activity on scoliosis development, I used a logistic regression with binomial errors (Crawley 2005) with scoliosis:normal phenotype ratios per mesocosm as the dependent variable and mean overall activity per mesocosm (after an arcsine, square root transformation) as the independent variable. Because an analysis of the residuals demonstrated violations of the assumptions of parametric statistics, I used a Monte Carlo approach to test the hypothesis that activity level has no effect on scoliosis development. I randomly selected with replacement scoliosis:normal phenotype ratios from each tub and paired these with measures of activity levels. Using a generalized linear model (GLM), I obtained an estimate of the slope and Akaike’s Information Criterion (AIC; used to evaluate model fit). This process was repeated 5,000 times. A one-tailed $P$-value for the slope estimate was obtained by dividing the number of randomly generated slope estimates that were greater than the slope estimate of the actual data by the 5,000 simulations. I followed a similar procedure to obtain a one-tailed $P$-value for AIC except that we used the number of randomly generated AIC estimates less than the AIC estimate of the actual data. Monte Carlo simulations were performed with a code written in R 2.7.0.

6.3.2 Experiment II: Effects of scoliosis on survival from predators

In late April 2008, I collected portions of *R. sylvatica* egg masses from five ponds and placed them in five outdoor wading pools filled with well water. In early May after eggs hatched, I randomly selected 50 tadpoles from each pool and placed them into one of two 1-m diameter wading pools kept in an open field that receives full sunlight, with the intent that exposure to sunlight would induce some of the tadpoles to develop scoliosis. I covered each pool with a lid composed of fiberglass windowscreening to
discourage colonization from other aquatic organisms. When tadpoles were
approximately at Gosner developmental stage 31, I randomly selected 50 scoliotic and 50
non-scoliotic tadpoles from the two pools to be used in swimming and survival trials.

To test burst swimming speed of tadpoles, I constructed a Plexiglas chamber (35
cm × 7.5 cm) filled with approximately 3 cm of ambient temperature well water. At the
bottom of the chamber we delineated a 1 cm × 1 cm grid, and then placed a Sony digital
camcorder (29.97 frames·s⁻¹) 40 cm above the water surface to obtain a top-down view.
For each of the 100 tadpoles, we first obtained a mass (average mass ± 1 SE: scoliotic
0.338g ± 0.011; normal 0.306g ± 0.012) and then placed the individual in the chamber.
After a one-minute acclimation time, I simulated a predator attack by flicking the tadpole
at the base of the tail muscle with a dissecting probe (Dayton et al. 2005). A trial
consisted of three successive probes. Swimming trials were conducted over two days.
During digital playback of the video, I calculated burst speeds by measuring the distance
traveled by the tadpole within the first 0.3 – 0.6 s following the simulated attack. Of the
three burst speed measurements, I used the fastest speed for statistical analyses.

After each tadpole was tested for swimming burst speed, it was placed into one of
two 60.5-l tubs, according to phenotype, for a 24-h recovery period. I then randomly
selected five scoliotic and five normal individuals and placed them together into one
60.5-l tub containing 24 strands of green polypropylene rope representing habitat
structure. I allowed 24-hour acclimation and then introduced one large dytiscid beetle
larva (average mass ± 1 SE: 0.964 g ± 0.068) to each tub. I checked tubs at 9 h, 15 h, and
18 h post-introduction of the predator to ensure that the predator did not consume all
tadpoles. When I observed that approximately five tadpoles were remaining in a tub, I
collected the predator and sorted the survivors by phenotype. All mortality was assumed to be due to predators, since no tadpole carcasses were found. Overall, I performed 10 predation trials using a different predator individual in each.

To analyze the effects of scoliosis on burst swimming speeds, I log-transformed burst speed and body mass and then conducted an analysis of covariance (ANCOVA) with phenotype as the factor and body mass as the covariate. The interaction between phenotype and body size was not significant ($P = 0.993$), indicating homogeneity of slopes in estimates of burst swimming speed. This analysis was conducted using Systat 10.0. For the predation trials, I used a chi-square test to determine if survival with predators was independent of phenotype. Data from all 10 replicates was used, and an expected distribution was generated internally from the observed data using a contingency table.

6.4 Results

6.4.1 Experiment I: Effect of environmental variables on scoliosis frequency

Tadpole survival was high (96.2%) and did not differ between treatments. Overall, 8.7% of tadpoles exhibited some degree of scoliosis. The most parsimonious model for explaining the proportion of scoliosis phenotypes included terms for competition, predation risk, and their interaction. The results from the ANODEV indicated that predation risk and the interaction with competition had significant effects on the proportion of tadpoles afflicted with scoliosis (Table 6.1). The presence of predators induced lower frequencies of scoliosis across all treatments, while the presence
of competitors induced higher frequencies only among predator treatments (Figure 6.2). Due to large variance, simulated aquatic vegetation had no significant effect on scoliosis frequency, although the proportion of scoliosis in the high stem density treatment was 50.5% and 50.8% lower than those of the zero and low stem density treatments, respectively.

**TABLE 6.1**

RESULTS FROM AN ANALYSIS OF DEVIANCE (ANODEV) TESTING THE SIGNIFICANCE OF THE DEVIANCE OF THREE MODELS FROM THE NULL MODEL (NO FACTORS).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Deviance</th>
<th>Residual df</th>
<th>Residual Deviance</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null</td>
<td>59</td>
<td>83.649</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Competition</td>
<td>1</td>
<td>2.942</td>
<td>58</td>
<td>80.708</td>
<td>2.246</td>
<td>0.123</td>
</tr>
<tr>
<td>Predation risk</td>
<td>1</td>
<td>5.726</td>
<td>57</td>
<td>74.982</td>
<td>4.781</td>
<td>0.033</td>
</tr>
<tr>
<td>Comp * P. risk</td>
<td>1</td>
<td>6.290</td>
<td>56</td>
<td>68.691</td>
<td>5.253</td>
<td>0.026</td>
</tr>
</tbody>
</table>
Tadpole activity levels were positively correlated with the proportion of scoliotic tadpoles (slope ± 1 SE; 2.50 ± 1.97, Figure 6.3). This slope value ranked 330th out of 5,000 Monte Carlo simulations, yielding a *P*-value of 0.066. The AIC estimate for the actual data was 164.22, and this estimate ranked 2,523rd out of 5,000 simulations, yielding a *P*-value of 0.5046.
6.4.2 Experiment II: Effects of scoliosis on survival from predators

Anecdotal observations of tadpole swimming motion suggest that scoliosis restricts tail movement to the portion of the tail that is distal to the site of the tail muscle curvature (Video 6.1 and 6.2). After controlling for variation in body size (Figure 6.4), average burst swimming speed of normal tadpoles was approximately 14% greater than that of tadpoles afflicted with scoliosis ($F_{1,97} = 14.59$, $P < 0.001$, Figure 6.5). The predator, larval dytiscid beetles ($Dytiscus$ spp.) consumed significantly more scoliotic...
than normal tadpoles ($\chi^2 = 12.98$, df = 1, $P < 0.001$). On average, 66% of the normal phenotypes survived compared to 30% of the scoliosis phenotype (Figure 6.5).

Figure 6.4 Burst swimming speeds of normal and scoliotic tadpole phenotypes as a function of tadpole body mass (grams). Normal phenotypes are represented by closed symbols and solid trendline, scoliotic phenotypes are represented by open symbols and dotted trendline.
Figure 6.5 Proportion of tadpoles surviving predator trials and average burst swimming speed (mm/s) adjusted for body size for normal (top) and scoliotic (below) phenotypes. Error bars are one standard error.

6.5 Discussion

These results indicate that the impact of UVBR on aquatic organisms depends on the environmental characteristics of a particular habitat. Competition and predation risk had significant interactive effects on the frequency of scoliosis. The proposed mechanism for this result is that behavioral changes of the tadpoles induced by competition and predation risk subjected tadpoles to varying levels of UVBR. Similar to their effects on scoliosis frequency, competition and predation risk also have non-additive effects on tadpole activity levels such that the magnitude of predator-induced activity reduction is greater at low competition densities (Relyea 2004, Teplitsky and Laurila 2007). Indeed, I found that experimental mesocosms with more active tadpoles produced a higher proportion of scoliotic tadpoles (Figure 6.3). However, because the strength of
this relationship was weak and could not significantly explain model deviance, changes to other tadpole behaviors may be equally important. For example, tadpoles also spatially avoid predators (Van Buskirk and Schmidt 2000) and increase the use of benthic refugia, such as leaf litter, in the presence of predators (Van Buskirk 2001). Therefore, because predator cages were kept near the water surface, most tadpoles reacted to predators by remaining near the bottom of mesocosms, where UVBR levels were diminished. Interestingly, a negative effect of predation risk on the susceptibility of organisms to environmental stressors seems to be rare and has been documented in only a few cases (e.g., Parris et al. 2006).

The presence of dense structural complexity (i.e., 84 stem density treatment) reduced the proportion of scoliotic tadpoles by approximately 50%. This reduction is consistent with in situ measurements of UVBR in densely vegetated microhabitats, where emergent vegetation reduces UVBR concentrations by 40 – 50% at a depth of 5 cm (Peterson et al. 2002). Aquatic vegetation is present in many aquatic habitats, and likely buffers many aquatic organisms from harmful UVBR effects.

Scoliotic tadpoles were approximately 50% more susceptible to predators, and my results suggest that reduced swimming ability was a significant cause of this increased vulnerability. Burst swimming speed is important for determining prey escape from a predator attack (Lima & Dill 1990), especially for tadpoles (Dayton et al. 2005). Therefore, reductions in the swimming ability of individuals should negatively impact avoidance of predators. However, this study cannot exclude other mechanisms, such as any possible effects of scoliosis on tadpole behavior.
The contribution of increasing UVBR levels to the current global amphibian
decline is a highly debatable issue (Licht and Grant 1997, Blaustein and Kats 2003, Licht
2003, Blaustein and Bancroft 2007) and the results of this study provide conflicting
evidence. For instance, tadpole predators are present in the vast majority of open-canopy
ponds (Van Buskirk and Arioli 2005, Chapter 5) and their presence would presumably
reduce harmful effects of UVBR to tadpoles through induced behavioral plasticity.
However, any malformations that are induced by UVBR are likely to be lethal because of
predator selection. As for the effects of competitors, tadpole densities in natural ponds
can be up to 2.5 times greater than the densities used in the high competition treatments,
which could further exacerbate harmful UVBR effects through increased tadpole activity.
Regardless, the overall frequency of scoliosis in my experiment was relatively low (<
10%), and models demonstrate that such small decreases in larval amphibian abundance
are mostly compensatory and have little effect on amphibian population dynamics (Biek
et al. 2002, Vonesh and De la Cruz 2002). Thus, even though sublethal effects of UVBR
exposure become lethal when considered in natural environments, it seems unlikely that
such losses to predation are responsible for drastic declines in amphibian populations.

Many aquatic species are negatively affected by UVBR (Bancroft et al., Häder et
al. 2007) and some of these same species exhibit behavioral plasticity to competitors,
predators, and other environmental factors (Tollrian and Harvell 1999). For example,
harmful UVBR effects may be minimized in zooplankton species that undergo diel
vertical migration, the movement into deeper areas during daylight to avoid visual
predators (Stich and Lampert 1981). Conversely, benthic predators can induce
zooplankton to undergo reverse vertical migration and become exposed to higher levels
of UVBR (Boeing et al. 2004). Therefore, the patterns observed in this study have the potential to be applicable to a wide variety of aquatic ecosystems.

6.6 Acknowledgments

I would like to thank Stephanie Burke for her help in all aspects of Experiment II. G. E. Belovsky, G. Gerrish and J. J. Hellmann provided helpful comments on previous versions of the manuscript.

6.7 References


CHAPTER 7:
CONCLUSIONS AND FUTURE DIRECTIONS

7.1 Overview

Two major challenges for phenotypic plasticity research have been the identification of trait responses to simultaneous changes in multiple environments (Sultan et al. 1998, West-Eberhard 2003, Miner et al. 2005, Stillwell et al. 2007) and the assessment of the adaptiveness of these responses (Via et al. 1995, Gotthard and Nylin 1995). Because natural systems inherently experience fluctuations in multiple environmental conditions, this research is critical for accurately predicting adaptive patterns of plasticity within field populations. More accurate predictions will subsequently lead to an enhanced understanding of the many ecological and evolutionary implications of phenotypic plasticity in natural environments (Miner et al. 2005, Pigliucci 2005, 2007, Fordyce 2006). The goal of this dissertation was to utilize a combination of laboratory experiments and field surveys to analyze how multiple environments interact to shape tadpole phenotypes. The conclusions from these experiments have not only answered some critical questions, but also suggest some new and exciting directions for future research. Below I highlight the major insights provided by this dissertation and outlines for future experiments.
7.2 Conclusions and Future Directions

An important conclusion from my dissertation was that the inclusion of changes in multiple environments constrains the ability of an organism to adaptively match its phenotype to the experienced environment. Tadpole phenotypic responses to a combination of structure and competition environments were only adaptive in the absence of predators. Additionally, only one trait exhibited strong adaptive plasticity in response to structure and predation risk. These nonadaptive results are particularly disappointing because they limit our ability to accurately predict patterns of phenotypic change among individuals within natural populations. However, in chapter 5, I showed that only two environmental factors—predation risk and competition density—were important predictors of tadpole phenotypic variation between different ponds. Because predation risk and competition density also exert strong selective and inductive pressure on tadpole phenotypes (Van Buskirk and Relyea 1998, Relyea 2002), these two variables may be the most important environmental determinants of tadpole phenotypes. Therefore, predictions of tadpole phenotypes based on an assessment of pond-mean predation risk and competition density would be reasonably accurate.

When confronted with environmental variability, organisms may adjust their phenotypes to better fit the environment, or actively select habitats that are best suited to their phenotype (Donohue 2003). In Chapter 5, I demonstrated that predation risk and competition density were randomly distributed within ponds, suggesting that tadpoles cannot accurately choose microhabitats based on densities of predators or competitors. Conversely, tadpoles could potentially discriminate microhabitats based on other environmental variables that exhibit clustered distributions within ponds and are
relatively temporally invariable, such as habitat structure (i.e., stem density). Therefore, differences in the spatiotemporal properties of environmental variables may determine if an organism responds through phenotypic plasticity or nonrandom habitat selection (although in chapter 5 I was unable to distinguish between these two mechanisms). This hypothesis is supported by the conclusions of chapters 3 and 4 where, under laboratory conditions, structure did not have strong affects on most tadpole phenotypes. Most laboratory experiments that analyze plastic responses of organisms constrain the habitat choice of the organism. However, I believe a fruitful avenue of research will be experiments that integrate habitat choice with the expression of plastic phenotypes. For example, would individuals that lack the ability to express predator-induced plastic responses select microhabitats that offer protection from predators?

In chapter 4, I demonstrate that the magnitude of competitor-induced traits decreases with the addition of habitat structure. Previous studies also suggest that the addition of additional environmental factors seems to decrease the magnitude of phenotypic responses. For example, Relyea (2004) demonstrated that the magnitude of predator-induced behavioral and morphological changes decreases as the number of conspecific competitors within the habitat increases. This effect of multiple environments on phenotypic changes likely results from trade-offs organisms face when experiencing cues from different environments. For example, individuals that are better defended against predators are often poor competitors (e.g., Van Buskirk and Relyea 1998). If the addition of multiple environmental variables does modify phenotypic plasticity, then the magnitude of the expression of plastic traits should be minimal among natural conditions. Consequently, phenotypic plasticity may only be a lab artifact that is
only expressed in response to changes in simple environments. Because a growing number of researchers are beginning to examine phenotypic plasticity in response to changes in multiple environments, I suggest that a meta-analysis should be conducted that examines if the magnitude of phenotypic responses to simple environmental change decreases with the addition of other environmental variables.

The results from chapter 5 demonstrated that predation risk and competition density are important predictors of tadpole phenotypes among natural environments on a pond-wide scale. Importantly, this result is concordant with laboratory experiments that suggest predation risk and competition exert significant selective and inductive pressure on tadpole phenotypes. However, the results from hierarchical linear models suggest that substantial tadpole phenotypic variation exists within-ponds. Individual-level differences in genotypes and genotype by environment interactions could potentially explain this phenotypic variance. Therefore, future studies should incorporate genetic factors at a within-pond scale. The logistics of such a study would be daunting (i.e., quantifying plastic responses of the majority of sibships within a pond, obtaining genetic markers that could be matched with wild-caught tadpoles, etc.), but would help identify the scale at which genotypes, environments, and their interaction affect phenotypic development of organisms in natural populations.

Chapter 6 provided compelling evidence that ecological interactions, such as predation and competition, modify the harmful effects of ultraviolet-B radiation on tadpoles by inducing changes in tadpole behavior. The results from this chapter further emphasize the importance of assessing the impacts of stressors within more environmentally realistic conditions (e.g., Relyea 2003). Because many aquatic
organisms are affected negatively by ultraviolet-B radiation (Bancroft et al. 2007), and also exhibit behavioral plasticity to predation risk and competition (Tollrian and Harvell 1999), I believe similar types of experiments should be conducted for a wide variety of species that are negatively affected by ultraviolet-B radiation.

In chapter 3, I hypothesized that the costs of antipredator behavior depend on the predator species to which the prey are responding, and likely reflect the cues that predators use to detect prey. Given that prey organisms also exhibit predator-dependent behavioral and morphological inducible defenses (Relyea 2001, Van Buskirk 2001, Teplitsky et al. 2005, Schmitz 2008), the possibility of predator-dependent costs of plasticity is likely. One prediction from chapter 3 is that a reduction in swimming time, as opposed to feeding time, in response to predators will be costly when food resources are patchily distributed. Such an experiment would provide an elegant test of the hypothesis of predator-dependent costs of plasticity.

Phenotypic plasticity is an exciting research topic with many implications for the ecology and evolution of species communities. The results of this thesis have addressed: 1) if organisms can adaptively respond to multiple environments, 2) the important environmental variables that shape phenotypes of organisms in natural conditions, and 3) the effects of plasticity on the susceptibility of organisms to the effects of environmental stressors. Studies examining the effects of >2 environmental variables on the phenotypes of organisms are rare; thus, it is recommended that future studies address this question using a wide range of study systems. This dissertation provides a framework for such research as well as testable predictions.
7.3 References


