EAVESDROPPING ON THE ENEMY: THE IMPORTANCE OF CHEMICAL CUES FOR INDUCIBLE DEFENSES

by

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Many species rely on phenotypically plastic traits to defend themselves against predators and the induction of these phenotypes require reliable environmental cues. In aquatic systems, defensive phenotypes are induced by chemical cues emitted during predation events. Using larval amphibians as a model system, my dissertation focuses on how prey use the different types of chemical information available from predators (kairomones) and prey (alarm cues) and how prey integrate their defensive decisions in response to chemical cue variation over space and time.

Predation cues contain information on the identity of the predator (kairomones) and the identity of the attacked prey (alarm cues). I have shown that different alarm cues (from different predator diets) induce different magnitudes of prey defense and discovered that the magnitude of the response depends on the evolutionary divergence time between the diet and the responding prey. Because chemical cues from consumed prey induce different suites of traits than cues from starved predators or damaged prey, I have also performed experiments to determine the role the predators themselves play in producing the cue (i.e. releasing a kairomone or digesting alarm cues). I found that digestion of the prey is essential to induce the complete suite of defensive traits.

Because induced defenses have associated costs, prey should balance these costs and benefits by fine-tuning their responses to their environment over space and time. To do this, prey must be able to detect and respond to changes in risk when they move into new environments (spatially) or when predators come and go (temporally). I have found that tadpoles can detect small differences in risk, but that experiencing pulses of risk, when compared to a constant risk, largely does not alter their defensive decisions. Collectively, this work demonstrates the important role of environmental cues in understanding the ecology and evolution of inducible defenses.

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PREFACE

The research that I present in this dissertation would not have been possible without the help of my colleagues and friends. Foremost among them is my advisor, Rick Relyea, who has provided me with the support, guidance, and friendship that helped me to succeed as a graduate student. All of the encouragement, camaraderie, and ridiculous conversations will never be forgotten. I would also like to thank Rick for finally realizing the superiority of spelling gray with an "e" instead of an "a". I thank my labmates, Jason Hoverman and Josh Auld for making these past six years some of the best times of my life. I especially thank Christine Relyea for being the best friend I could have asked for. Thank you for allowing me to be a part of your life and a part of your family. I hope I can always live up to my honorary status as "crazy aunt" for Isabelle and all of the little Relyeas to come.

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The support of my family has been unfaltering throughout my time at the University of Pittsburgh. For that I am unendingly grateful. Their unwavering confidence has helped me to accomplish things that I wasn't sure that I was going to be able to do. I have also been incredibly lucky to have enjoyed some very dear friendships while in Pittsburgh. I'm particularly grateful to Tony Baumert, Nicole Diecks, Angela Boule and April Randle for their unconditional friendship and support. Finally, I thank Brian Regan, for providing the lab with so many jokes and for teaching us about imbicilin and boxen, and president George W. Bush, for providing us all with inspirational quotes like "If we don't succeed we run the risk of failure".

1.0 INTRODUCTION

Phenotypic plasticity, the ability of a single genotype to produce multiple phenotypes, is an important aspect of how organisms respond to environmental variation (Pigliucci 2001). While not all phenotypic plasticity is adaptive, a plethora of research has shown that organisms modify their phenotypes in response to environmental change, and that those changes result in the induced organism having higher fitness than non-induced organisms (West-Eberhard 2003). To produce a phenotype that is well-suited for its environment, organisms must have reliable cues that indicate either the current (if the phenotype can be changed quickly) or future (if the phenotype requires more time to change) state of the environment (Moran 1992).

In aquatic systems, many organisms possess plastic defenses that are induced by chemical cues emitted during predation events (Larsson and Dodson 1993, Chivers and Smith 1998). Chemical cues induce phenotypic changes that not only affect prey survival and performance (Smith and Van Buskirk 1995, Pijanowska 1997, Van Buskirk et al. 1997, Van Buskirk and Relyea 1998, Wisenden et al. 1999, McIntyre et al. 2004), but also affect interspecific interactions in ecological communities (i.e. trait-mediated effects, Lima 1998, Turner et al. 2000, Werner and Peacor 2003). While chemical cues are important to both individuals and communities, we actually know little about how prey detect and interpret chemical cues when making their phenotypic decisions (Pigliucci 2001, Iyengar and Harvell 2002). In this dissertation, I present the results of five studies designed to determine how prey use different types of chemical information, available from predators (kairomones) and prey (alarm cues), and how prey integrate their defensive decisions in response to chemical cue variation over space and time.

Phenotypic variation in nature is determined by both genotypes and the extent of environmental variation that organisms encounter. In nature, predation risk varies in both time and space, and the extent that this environmental variation translates into phenotypic variation

will depend on how sensitive an organism is to differences in predation risk (i.e. differences in the amounts of chemical predation cues). Given that predator-induced defenses come at a cost of reduced growth and development, selection should select for the ability to detect small differences in predation risk (Moran 1992, Werner and Anholt 1993). I address this question in chapter 2 by determining how wood frog tadpoles (*Rana sylvatica*) respond to an increasing gradient of predation risk produced by either feeding a constant number of predators increasing amounts of prey or feeding increasing numbers of predators a constant mass of prey. This work was done in collaboration with Rick Relyea and is being prepared for submission to Ecology.

A major tenet for the evolution of inducible defenses is that organisms must experience variation in predation risk over space or time. While most attention has focused on course-grained (i.e. intergenerational) variation in risk, fine-grained (i.e. intragenerational) differences might also be important (Lima and Bednekoff 1999, Miner and Vonesh 2004, Ruehl and DeWitt 2005). While the majority of previous work has focused on the effects of chronic exposure to a single environment, spatial and temporal variation within an organism's lifetime may have substantial impacts on phenotypic expression. To date, several studies have examined the impact of temporal variation on prey behavior by comparing prey responses in constant environments to prey responses when switched between predator and no-predator environments (Hamilton and Heithaus 2001, Sih and McCarthy 2002, Van Buskirk et al. 2002, Pecor and Hazlett 2003, Laurila et al. 2004, Foam et al. 2005). However, these manipulations alter both mean risk and the variation in risk, so the effects of temporal variation *per se* cannot be determined. Therefore, we need to ask whether prey can alter their phenotypic decisions in response to variation in risk independent of the mean level of risk experienced. I address this question in chapter 3 by exposing wood frog tadpoles (*Rana sylvatica*) to different types of variation in predation risk while holding mean predation risk constant (mg of prey/caged predator/day). This work was done in collaboration with Rick Relyea and is being prepared for submission to Ecology.

Predation cues are complex mixtures that contain information about both the predator (kairomones) and species of prey being consumed (Chivers and Smith 1998). However, the role that kairomones and alarm cues play in shaping prey phenotypes has been largely restricted to prey behavior and has focused on either kairomones or alarm cues. In nature, inducible defenses are often predator-specific and involve several behavioral and morphological traits. Given that kairomones and alarm cues provide different information about predation risk, the prey may not

commit to a specific defense when only one cue type is present. In chapter 4, I address this question with an experiment in which I quantified behavioral and morphological responses of grey tree frog tadpoles (*Hyla versicolor*) exposed to either no predators, starved predators (a source of kairomones), nine crushed diets (sources of alarm cues), or nine consumed diets (sources of kairomones *plus* alarm cues). This work was done in collaboration with Rick Relyea and has been published in Ecology Letters.

When prey discriminate among predator diets, cues from consumed conspecifics typically induce strong responses but cues from consumed heterospecifics can induce either weak or strong responses (Wilson and Lefcort 1993, Chivers and Mirza 2001). Two mechanisms have been proposed to explain the variable responses to heterospecific diets: 1) prey respond strongly to coexisting heterospecifics and weakly to non-coexisting heterospecifics (the ecological mechanism) or 2) prey respond strongly to heterospecifics that are closely-related and weakly to heterospecifics that are distantly-related (the phylogenetic mechanism). However, our ability to discriminate between these mechanisms has been limited because the phenotypic responses to the diets needed to discriminate between these mechanisms have not been compared within a single experiment. In chapter 5, I present data from an experiment in which I exposed grey tree frog tadpoles (*Hyla versicolor*) to a variety of predator diets including conspecifics, heterospecifics that coexist with grey tree frogs (e.g., spring peepers, wood frogs, salamanders, insects and snails), and a closely-related heterospecific that does not overlap with grey tree frogs (the Pacific tree frog, *Hyla regilla*) to determine the mechanism underlying the response to heterospecific alarm cues. This work was done in collaboration with Rick Relyea and is being prepared for submission to Behavioral Ecology and Sociobiology.

The work presented in chapter 4 shows that either starved predators or alarm cues alone do not induce the full suite or magnitude of traits induced by predators consuming prey. This observation suggests several possible roles for both the predator and the prey in generating kairomones and alarm cues. First, alarm cues and kairomones from starved predators alone may not provide enough information to induce a complete anti-predator phenotype and the prey may simply need to encounter both cues simultaneously. Alternatively, the weak responses to each component may be because the cues are only produced or are modified during prey digestion. To determine the roles of both the predator and the consumed prey in inducing defended phenotypes we need to determine if phenotypes differ when: 1) alarm cues from the prey and

kairomones from starved predators are encountered simultaneously; 2) the predator digests any diet or 3) the predator digests conspecific prey. In chapter 6, I present the results from an experiment using leopard frog tadpoles (*Rana pipiens*) that addresses this question. This work was done in collaboration with Rick Relyea and is being prepared for submission to Ecology Letters.

Finally, in Chapter 7, I discuss the implications of my work for understanding the ecology and evolution of inducible defenses and predator-prey interactions. I also discuss directions for future research into how prey integrate and respond to the information provided by chemical cues.

2.0 DETECTING SMALL ENVIRONMENTAL DIFFERENCES: RISK-RESPONSE CURVES FOR PREDATOR –INDUCED BEHAVIOR AND MORPHOLOGY

2.1 ABSTRACT

Most organisms possess traits that are sensitive to changes in the environment (i.e. plastic traits) which results in the expression of environmentally-induced polymorphisms. While most phenotypically plastic traits have been traditionally treated as threshold switches between induced and uninduced states, there is growing evidence that many traits can respond in a continuous fashion. Using larval anurans (wood frog tadpoles, *Rana sylvatica*), I manipulated predation risk in two ways: 1) by altering the amount of prey consumed by a constant number of predators (*Dytiscus* sp.) and 2) by altering the number of predators that consume a constant amount of prey. I then quantified the expression of predator-induced behavior, morphology, and mass to determine the level of risk that induced each trait, the level of risk that induced the maximal phenotypic response for each trait, whether the different traits exhibited graded or threshold responses, and whether increasing risk by increasing the numbers of predators or by increasing the consumption of prey induced similar phenotypic changes. I found that all of the traits exhibited fine-tuned, graded responses and most of them exhibited a plateauing response with increased predation risk, suggesting either a limit to plasticity or the reflection of high costs of the defensive phenotype. For many traits, a large proportion of the maximum induction occurred at the very lowest level of risk, suggesting that the chemical cues of predation are effective at extremely low concentrations. Interestingly, the maximum response in all traits was not induced at the same level of predation risk and not all traits plateaued at the same level of predation risk. In contrast to earlier work, I found that behavioral and morphological responses to increased predator number were simply a response to increased total prey consumption. These results have important implications for models of plasticity evolution, models of optimal

phenotypic design, expectations for how organisms respond to fine-grained changes (i.e. within generation) in their environment, and impacts on ecological communities via trait-mediated indirect effects.

2.2 INTRODUCTION

Phenotypic plasticity is a common response to changing environments. Organisms that possess trait plasticity have the ability to alter their traits in response to environment cues to produce phenotypes that perform better under the new environmental conditions (Schlichting and Pigliucci 1998, West-Eberhard 2003). The range of phenotypes produced in response to environmental changes depends both upon the gradient of the environmental factor that can be experienced and the sensitivity of the organism in detecting and responding to environmental change. If sensitivity is low, the organism may only detect a difference once a threshold level is encountered, resulting in a discrete polymorphism (Moczek 1998, Lively et al. 2000). If there is genetic variability among individuals in the point of this threshold induction, then different points along the environmental gradient will produce different proportions of induced and uninduced individuals (Roff 1996, Lively et al. 2000, Hazel et al. 2004). However, if sensitivity is high, the organism may have the ability to detect and respond to an environmental gradient with graded phenotypic responses, where increased cue intensity increases the magnitude of the induction and not just the proportion of induced individuals (Harvell 1990, 1998). Hence, environmental sensitivity will determine the range of phenotypic variation that can be produced and how closely the organism can "match" its phenotype to the environment.

The classical perception is that adaptive plastic phenotypes are switches between induced and uninduced states **(**Cook and Johnston 1968, Grant and Bayly 1981, Havel 1985, Stemberger 1988, Greene 1989, Pfennig 1990**)**. This perception arises from the earliest models of plasticity evolution which examined two-environment scenarios for mathematical simplicity (Via and Lande 1985, van Tienderen 1991) and from the early empirical investigations of plasticity which typically examined how organisms altered their traits in two environments (i.e. long vs. short photoperiod, hot vs. cold temperature, high vs. low competition, light vs. shade, predator vs. no-predator; Lively 1986, Schlichting 1989, Van Tienderen 1990, Blouin 1992, Spitze 1992,

Andersson and Shaw 1994, Kingsolver 1995, Dudley and Schmitt 1996, Pigliucci et al. 1997). However, there has been an increasing appreciation that most organisms in nature experience a wide range of environments that can be arrayed along gradients and, therefore, there has been a move to examine how organisms respond to a range of environments (West-Eberhard 2003, Relyea 2004). When experiments have been conducted at a finer-scale than simply high vs. low environmental state, researchers have often discovered that many traits display graded responses to continuous environmental changes (Gupta and Lewontin 1982, Barry and Bayly 1985, Walls and Ketola 1989, Hanazato and Ooi 1992, Tollrian 1993, Sultan and Bazzaz 1993, Horat and Semlitsch 1994, Morin et al. 1997, Pigliucci 1997, Harvell 1998, Wiackowski and Staronska 1999, Kusch et al. 2004, Wolfe and Mazer 2005). If graded responses to continuous environmental variation are common, then previous conclusions about how organisms respond to environmental variation based on responses exhibited in two environmental extremes may not correctly represent the true ecology and evolution of phenotypic plasticity or the range of phenotypes that will be available for selection to act upon in nature (Schlichting 1989). Thus, to determine the extent that organisms can detect and respond to small differences among environments , we need to examine how multiple traits are expressed along multiple points of an environmental gradient (Horat and Semlitsch 1994, Van Buskirk and Arioli 2002, Relyea 2004).

It is becoming clear that organisms alter suites of traits in dealing with environmental change and that the sensitivity to the environment can differ among traits (Schlichting and Pigliucci 1998, Boersma et al. 1998). One way that sensitivity can differ among traits is the point along an environmental gradient at which a trait is induced. If relatively low-cost traits are effective in less extreme environments, but only high-cost traits are effective in more extreme environments, the high-cost trait should only be induced once more extreme environments are encountered. For example, Harvell (1998) showed that low levels of predation risk induced bryozoans to form small corner spines but higher levels of predation risk were required to induce larger membranous spines. A second way that sensitivity can differ among traits is whether traits are induced in a threshold or graded fashion. Theory predicts that threshold response should evolve when the fitness function underlying the traits is discontinuous such that, after the initial induction, increases in the trait provide no additional fitness benefit. In contrast, graded responses should evolve when there is a continuous fitness function associated with the trait such that increases in the expression of the trait are associated with increases in fitness (Lively 1986,

Roff 1996). For inducible defenses, theory often implicitly assumes that prey can respond to variation in predation risk with a graded phenotypic response. However, traits that change in response to predation cues display both types of responses (Anholt et al. 1996, Harvell 1998, Laurila et al. 2004, Van Buskirk and Arioli 2002, Relyea 2004). Therefore, determining if graded responses are common for traits involved in inducible defenses is an important step in determining how prey balance the costs and benefits of inducible defenses.

If plastic traits are sensitive to small changes in the environment, then fine-scale (i.e. within generation; Levins 1968) environmental variation may drastically effect phenotypic expression. Previous work has shown that plastic traits can be reversible when the environment switches from one extreme to the other in an environmental gradient. For example, predator-induced defenses (behavioral and morphological) converge on the no-predator phenotype when predators leave the environment (Van Buskirk 2002b, Relyea 2003b). If individuals can detect and respond to small environmental changes with different phenotypes, then prey may continually alter their phenotypes to produce the phenotype that is optimal for the environment. For example, if predator number or foraging efficiency varies during the prey's ontogeny, and the prey detect these changes and interpret them as changes in predation risk, then fine-grained variation in risk may affect how defensive traits are expressed over time. Determining how traits respond to fine-scale differences in the environment is a necessary step in understanding how temporal variability in the environment may affect phenotypic expression of these traits.

When examining how organisms respond to an environmental gradient, the conclusions we make about environmental sensitivity may depend on what cues we manipulate. Many organisms use more than one cue to predict future environments. For example, most aquatic prey use both alarm cues (from damaged prey) and kairomones (from predators) for inducing plastic defensive traits. Increases in the amount of prey consumed by a constant number of predators would increase the alarm cue:kairomone ratio while increases in the number of predators that consume a given amount of prey would decrease the alarm cue:kairomone ratio. Therefore, prey may use differences in the ratios of these components to determine if the cues they encounter are being produced by a single predator consuming a large amount of prey or several predators that are consuming fewer prey per predator. Being able to detect these differences may be important if different traits are effective against different size classes of predators. Therefore, if multiple cues are used to assess the environment, the observed

sensitivity may be dependent on the ratio at which those cues are encountered.

I addressed these issues in an experiment in which I exposed larval anurans to a gradient of predation risk via either increasing the prey consumption by a fixed number of predators or increasing the number of predators that are fed a fixed amount of prey. In response to these gradients, I measured how the tadpoles altered their behavior, growth, and relative morphology. I tested the following predictions: 1) All traits are induced at the same level of predation risk 2) All traits respond to increasing predation risk with graded responses, and 3) The magnitude of the response to predation risk will differ depending upon the way that the risk is experienced (i.e. increased prey consumption versus increased predator number).

2.3 METHODS

I used a completely randomized design consisting of 11 treatments replicated four times for a total of 44 experiment units to quantify the magnitude of tadpole defensive responses across a range of predation risk environments. The experiment was conducted in pond mesocosms (cattle watering tanks) located at the Pymatuning Laboratory of Ecology Aquatic Research Laboratory in northwestern Pennsylvania. The 11 treatments included a no-predator treatment, seven treatments in which I fed four caged predators a constant mass of prey each day (50, 100, 200, 300, 400, 700, or 800 mg), and three additional treatments in which I fed 1, 2, or 6 predators a constant amount of prey (200 mg) each day. This design allowed me to manipulate predation risk in two different ways (via increased prey consumption and via increased predator number) and make several treatment comparisons in which the total amount of prey consumed was the same but the number of predators doing the consuming was varied (e.g., one predator consuming 200 mg of prey versus four predators each consuming 50 mg of prey). Such comparisons allowed me to determine whether responses to increased predator number were simply responses to the higher total consumption of prey.

To simulate natural pond conditions, I used 800-L mesocosms (cattle tanks) containing 700 L of aged well water, 200 g leaf litter, 15 g rabbit chow (as an initial nutrient source), and an aliquot of pond water containing algae and zooplankton. All components were added to the mesocosms 10 d prior to the start of the experiment to allow algal growth. Six predator cages,

constructed of 10 cm black plastic drainpipe and covered on both ends with fiberglass mesh screens, were placed into each tank. Depending on the treatment, each cage was either empty or contained a single larval beetle (*Dytiscus* sp.) that was fed wood frog tadpoles daily. The cages allowed chemical cues, which are released when the predators consume prey, to diffuse throughout the tank while preventing the predator from consuming the tadpoles in the experiment. All tanks were covered with 67% shade cloth lids to prevent colonization by any predatory insect or amphibian larvae during the experiment.

I used wood frog tadpoles (*Rana sylvatica*) that were collected as hatchlings from a nearby pond (Shrub Pond; Crawford County, PA) on 24 March 2005. The tadpoles were newly hatched from a group of more than 50 egg masses and had not yet left the egg masses. To prevent exposure to predation cues prior to the experiment, I reared the hatchling tadpoles in pools containing aged well water where they were fed rabbit chow *ad libitum*. I haphazardly selected groups of 30 tadpoles and added them to each mesocosm on 13 May 2005 (mean mass ± SE = 61 ± 4 mg).

Behavioral observations were conducted on five different days of the experiment (days 7, 10, 13, 19, and 20) where each tank was observed 12 times over a period of 2 hrs (three observations taken by four observers). Using established observation protocols (Relyea and Werner 1999), I counted the number of visible tadpoles in each mesocosm and the number of visible tadpoles that were moving. Thus, my behavioral response variables were the mean number of tadpoles observed (the inverse of tadpole hiding) and the mean proportion of active tadpoles in each tank. The data were analyzed with a repeated-measures analysis of variance (rmANOVA) to test for an effect of treatment, day, and their interaction. When a significant effect was found, I conducted pairwise comparisons using Fisher's LSD test.

After 24 d, all tadpoles were removed from the mesocosms, euthanized, and preserved in 10% formalin for subsequent morphological measurements (mean survival = 93 $\pm$ 0.73%). Tadpole morphology was measured using an image analysis system (Optimas Bioscan; Bothell, Washington, USA). I weighed each tadpole and then measured eight morphological dimensions: body depth, length, and width; tail length and depth; tail muscle depth and width, and mouth width (see Fig.1 in Relyea 2000). Because the tadpole's body is round I placed a glass plate under the tadpole's tail to bring both structures into the same plane of focus and ensure that I obtained an undistorted lateral image.

Because I was interested in changes in tadpole shape independent of differences in overall tadpole size (i.e. bigger tadpoles have bigger bodies and tails), I calculated size-adjusted estimates of all the morphological traits. The size-adjusted estimates were obtained using a multivariate analysis of covariance (MANCOVA, SPSS version 11.0.2 for Mac OS X) with mass as the covariate. To improve the linearity of the mass-trait relationship before the analysis, tadpole mass was log-transformed when necessary. I found no mass-by-treatment interactions for any of the traits, indicating that the regression lines among treatments were parallel for each trait (a requirement for making the size-adjustment). To produce the size-adjusted measurements of each morphological trait, I added the residuals from the within-group regression to the estimated marginal mean for the appropriate treatment and averaged the measurements for all tadpoles in each tank for each of the eight traits. I then used a multivariate analysis of variance (MANOVA) to examine the effect of prey consumption and predator number on wood frog mass and the eight mass-independent morphological traits using tank means as my response variables. For significant univariate effects, I compared treatment means using Fisher's LSD. These pairwise comparisons were used to assess the evidence for either a threshold or graded responses to predator cues. I concluded that the responses to increased prey consumption or predator number was not a threshold response when I found significant differences among any of the treatments containing caged predators.

2.4 RESULTS

2.4.1 General response to predators

I found significant effects of the predator treatments on wood frog tadpole behavior, mass, and morphology. In the repeated-measures ANOVA on tadpole activity, I found an effect of treatment ($F_{10,33} = 21.0$, $P < 0.001$) and time ($F_{5,29} = 25.5$, $P < 0.001$) but no treatment-by-time interaction ($F_{50,136} = 1.3$, $P = 0.125$). The tadpoles in the no-predator treatment were more active than any of the caged-predator treatments ($P \leq 0.001$; Fig. 2.1). Similarly, I found an effect of predator treatment ($F_{10,33} = 5.7$, $P \leq 0.001$), time ($F_{5,29} = 139.1$, $P < 0.001$), but no treatment-by-time interaction ($F_{50,136} = 1.5$, $P = 0.09$) on the number of tadpoles observed (i.e. not hiding).

The tadpoles in the no-predator treatments hid less than the tadpoles in any of the predator treatments ($P \leq 0.001$; Fig 2.1). In short, predators induced tadpoles to hide and remain less active.

I found a multivariate effect of the treatments on tadpole mass and morphology (Table 2.1A). Univariate analysis (Table 2.1B) indicated differences in final mass, body dimensions (length, depth, and width), and tail dimensions (length and depth). Tadpoles exposed to any of the predator treatments had lower mass than the no-predator control ($P \leq 0.043$, Fig. 2.1). All of the predator treatments also induced relatively shorter and deeper tails ($P \leq 0.001$, Fig. 2.2) and shorter and deeper bodies ($P \leq 0.001$, Fig. 2.3) than the no-predator treatment. The one trait that did not exhibit consistent induction by the 10 treatments containing caged predators was body width. Only tadpoles in the 200-mg, 300-mg, and 6-predator treatments had narrower bodies than the tadpoles in the no-predator treatment ($P \leq 0.003$). The remaining three traits (muscle width, muscle depth, and mouth width) were not affected by the treatments.

2.4.2 Increasing consumption of prey: Graded or threshold response?

All of the traits showed a graded response to increases in the amount of prey consumed by the predator, but not all of the traits showed responses that plateaued at the highest risk levels. For each trait, I begin by comparing the responses between the no-predator control and the lowest amount of consumed prey (50 mg). I then compare how the trait changed as prey consumption increased above 50 mg.

Compared to the no-predator treatment, tadpole activity decreased by 33% when exposed to 50 mg of consumed prey ($P < 0.001$; Fig. 2.1) and decreased an additional 11% when exposed to 300 mg of consumed prey ($P \leq 0.046$). Activity did not decrease further at higher amounts of consumed prey ($P \geq 0.214$). Thus, tadpole activity exhibited a plateauing response to increased prey consumption by predators.

The number of tadpoles observed decreased by 11% when exposed to 50 mg of consumed prey ($P = 0.038$; Fig. 2.1) and decreased an additional 12% when exposed to 200 mg of consumed prey ($P = 0.05$). The number observed decreased even further (14%) in response to 800 mg of consumed prey ($P = 0.05$; Fig. 2.1). Thus, tadpole hiding did not exhibit a plateauing response to increased prey consumption by predators, although the magnitude of the change per

mg consumed prey grew weaker at higher amounts of consumed prey.

Tadpoles mass was also affected by increases in prey consumption (Fig. 2.1). Tadpoles were 6% less massive when exposed to 50 mg of consumed prey ($P = 0.04$). Tadpole mass decreased an additional 16% when exposed to 200 mg of consumed prey ($P < 0.001$) and decreased even further (16%) when exposed to 700 mg of consumed prey ($P < 0.001$). At 800 mg of consumed prey, there was no further decline in mass ($P = 0.385$), confirming a plateauing response.

I next examined relative tail size. Tadpoles tails were 5% shorter when exposed to 50 mg of consumed prey ($P < 0.001$; Fig. 2.2). Tail length decreased an additional 2% when exposed to 100 mg of consumed prey ($P = 0.038$), and decreased even more (2%) when exposed to 200 mg consumed prey ($P = 0.033$). In environments with greater than 200 mg consumed prey, tail length exhibited small increases compared to 200 mg consumed prey (e.g. 400 or 800 mg consumed prey), but tadpole tails in these environments were always shorter than the tails observed for 50 mg consumed prey ($P < 0.05$). Therefore, tail length was not consistent with a simple threshold response across an increasing gradient of predation risk however I did not find clear evidence that the response was plateauing. Tadpole tails were 11% deeper when exposed to 50 mg of consumed prey ($P < 0.001$; Fig. 2.2). Tail depth increased an additional 3% in response to 200 mg of consumed prey ($P < 0.001$) but did not increase any more at higher amounts of consumed prey ($P \geq 0.315$); showing a platueating response.

The amount of prey consumed by predators also affected the relative size of the tadpole body. Tadpole bodies were 5% shorter bodies when exposed to 50 mg consumed prey ($P < 0.001$; Fig. 2.3). The bodies became even shorter in several of the higher consumption treatments (200, 300, 700, and 800 mg; $P \leq 0.05$). However, body length in the 100- and 400-mg treatments was not different than 50-mg treatment ($P \geq 0.457$), which does not support a plateauing response. Tadpoles bodies were 5% deeper when exposed to 50 mg of consumed prey ($P < 0.001$; Fig. 2.3). Body depth increased even more in response to 200 mg of consumed prey ($P = 0.031$). At greater amounts of prey consumption, body depth exhibited a small gradual decline until the 800-mg treatment induced shallower bodies than the 200-mg treatment ($P = 0.022$). Tadpoles exposed to 50 mg of consumed prey did not change body width compared to the no-predator treatment ($P = 0.077$; Fig. 2.3). In fact, it required at least 200 or 300 mg of consumed prey to induce an increase in body width ($P \leq 0.003$) and these two treatments were

not different from each other (P = 0.753). No other treatments caused a change in body width. In summary, the body dimensions exhibited graded responses and two of the responses were generally suggestive of plateaued responses. However, several treatments induced body changes that diverged from a clear plateauing response.

2.4.3 Increasing predator number: Graded or threshold response?

I found evidence of a continuous response to increasing predator number for six out of the eight tadpole traits. For all of the traits, I begin by comparing the response between the no-predator control and the one-predator treatment. I then indicate how the trait changed as the number of predators increased above one predator.

I first examined the behavioral traits. Tadpole activity (Fig. 2.1) decreased by 38% when exposed to one predator, but increasing the number of predators did not further decrease tadpole activity ($P \geq 0.494$). In contrast, the number of tadpoles observed (Fig. 2.1) decreased by 13% in response to one predator (P = 0.008) and decreased an additional 10% in response to four predators (P = 0.04). However, there was no difference between four and six predators (P = 0.706). Thus, in response to predator number, activity exhibited a threshold response while hiding exhibited a graded response.

Tadpole mass also was affected by increasing numbers of predators (Fig. 2.1). Tadpoles were 11% less massive in response to one predator (P = 0.001) and an additional 11% less massive in response to four predators (P < 0.001). However, mass did not decrease any further from four to six predators (P = 0.563).

Next I examined relative tail morphology. Tadpole tails were 6% shorter in response to one predator (P < 0.001) and decreased an additional 2% in response to four predators (P = 0.002; Fig. 2.2). There was no further decrease when exposed to six predators (P = 0.685). Tadpoles tails were 10 % deeper when exposed to one predator (P < 0.001) and increased an additional 2% when exposed to two predators (P = 0.039). Tail depth did not decrease further when exposed to four or six predators (P > 0.1). Thus, both tail dimensions exhibited graded and plateauing responses to increased numbers of predators.

Finally, I examined the relative body dimensions. Tadpoles bodies were 3% shorter when exposed to one predator (P > 0.001; Fig. 2.3) and became even shorter (2%) when exposed

to four predators (P = 0.013). However, bodies did not become any shorter when exposed to six predators (P = 0.521). Tadpole bodies were 6% deeper when exposed to one predator (P < 0.001) and became an additional 1% deeper when exposed to four predators (P = 0.049). Body depth did not increase further when tadpoles were exposed to six predators (P = 0.586). Tadpole body width did not respond to one or two predators (P > 0.1) but was 3% wider when exposed to four predators (P = 0.001). Body width did not increase further when exposed to six predators (P = 0.845). Thus, two of the three body dimensions exhibited graded and plateauing responses when exposed to increased numbers of predators.

As noted above, there were two traits (activity and body width) that exhibited apparent threshold responses to increased predator number but graded responses to increased amounts of consumed prey. These differences can be explained by the fact that the total amount of consumed in the prey-consumption treatments (200 to 3200 mg) spanned a much wider range than the predator-number treatments (200 to 1200 mg). This wider range of chemical cue allowed the induction of more extreme phenotypic changes that could fully demonstrate the trait's ability to exhibit a graded response.

2.4.4 Prey consumed versus predator number

Because I manipulated both the amount of prey consumed and the number of predators, I could determine whether the response to increased numbers of predators was simply reflecting the greater prey consumption that occurred when there were more predators. I compared treatments that contained different numbers of predators but the same total mass of consumed prey (i.e. four predators each consuming 50 mg of prey versus one predator consuming 200 mg of prey, four predators each consuming 100 mg of prey versus two predators each consuming 200 mg of prey, and four predators each consuming 300 mg of prey versus six predators each consuming 200 mg of prey). I found no differences in the magnitude of the defensive trait for any of the comparisons made for the behavioral traits ($P \geq 0.214$), mass ($P \geq 0.090$) and the morphological traits ($P \geq 0.170$). This suggests that the tadpole response to increased predator number was simply reflecting the increased amount of prey being consumed.

2.5 DISCUSSION

The results of this study demonstrate that tadpoles alter a suite of traits in response to predation risk and that both the initiation and magnitude of the defensive responses depend upon the level of predation risk and the trait in question. The antipredator responses that I observed were consistent with past studies and are thought to be adaptive (McCollum and Van Buskirk 1996, Van Buskirk et al. 1997, Van Buskirk and Relyea 1998, Anholt et al. 2000, Laurila 2000, Relyea and Werner 2000, Relyea 2002b, Van Buskirk 2002a). The reduction in activity and increase in hiding makes prey less noticeable to predators and this can translate into a reduction in predation (Sih 1992, Skelly 1994, Relyea 2001b). However, these behavioral responses come at a cost of reduced foraging and, therefore, reduced growth (Skelly and Werner 1990, Skelly 1992, DeWitt 1998, Relyea 2002a). The morphological responses also appear to be adaptive because tadpoles with deeper tails and shorter bodies escape predation better than tadpoles with the opposite morphology (Van Buskirk et al. 1997, Van Buskirk and Relyea 1998). Larger tails are thought to serve as sacrificial targets for predatory strikes that can tear away and be regrown (Blair and Wassersug 2000, Van Buskirk et al. 2002, 2003). However, tadpoles with large tails and short bodies experience slower growth because they have relatively smaller mouthparts for scraping periphyton and relatively shorter (and likely less efficient) intestines (Relyea and Auld 2004, 2005). Moreover, in wood frog tadpoles, we know that these traits and, in some cases, the plasticity of these traits contain substantial additive genetic variation which allows them to be subject to selection in predator and no-predator environments (Relyea 2005).

While most studies of predator-induced defenses have taken a two-environment approach (e.g. predators present and absent; Tollrian and Harvell 1999), it is clear that this is rarely the reality that most prey species face in nature (reviewed in Relyea 2004). Indeed, prey can experience and respond to a wide range of predation risk that can manifest itself in several ways. First, a number of studies have found that prey can respond to different species of predators that vary in riskiness (Phillips 1976, Marko and Palmer 1991, Black 1993, Relyea 2001b, Vilhuren and Hirvonen 2003; but see Langerhans and DeWitt 2002). Second, prey appear to assess differences in predation risk when a given predator consumes conspecific versus heterospecific prey. Typically, prey respond stronger to the consumption of conspecifics (Wilson and Lefcort 1993, Laurila et al. 1997, Pettersson et al. 2000, Smith and Belk 2001, Schoeppner and Relyea

2005). Third, a limited number of studies (including the current study) have examined prey responses to different predator densities and found that prey are able to detect and respond appropriately to increased predator numbers (Barry and Bayly 1985, Harvell 1998, Van Buskirk and Arioli 2002, Relyea 2004). Fourth, prey might also be able to detect and respond to differences in risk when a predator consumes more prey (Barry and Bayly 1985, Walls and Ketola 1989, Anholt et al. 1996, Van Buskirk and Arioli 2002, see also Petranka 1989 for similar response to increased amounts of crushed prey). Consistent with previous work using tadpoles (Van Buskirk and Arioli 2002), I found that wood frog tadpoles were quite sensitive to differences in prey consumption and they were capable of exhibiting more extreme defenses when predators consumed more prey. Thus, the collective evidence is that aquatic prey are generally capable of assessing different levels of predation risk including detecting different species of predators, different predator diets, different densities of predators, and different amounts of prey consumption. The fact that all of this occurs via water-borne chemical cues suggests that aquatic prey are attuned to an impressive diversity of cues and cue concentrations.

The results of my study shed light onto how tadpole prey use these chemical cues in making their defensive phenotypes. I recently demonstrated that the kairomones from starved predators alone or the alarm cues from damaged prey alone fail to induce the full suite and magnitude of anti-predator defenses (Schoeppner and Relyea 2005). Inducing the complete magnitude of behavioral defense and any morphological defense requires both cue components in combination. In the current study, I found that increased prey consumption by a fixed number of predators and increased predator number (consuming a fixed per-capita prey ration) both induced more extreme defenses. This suggests that the increased prey defenses could be either due to greater concentrations of kairomones or greater concentrations of alarm cues. By making several comparisons of different numbers of predators consuming the same total amount of prey, I found that the response to increased predator density was apparently not due to predator number *per se*, but rather to the greater consumption of prey that was occurring with more predators. This conclusion is in agreement with Van Buskirk and Arioli's (2002) conclusions on tadpole behavioral defenses but is in contrast with their conclusions concerning tadpole morphological defenses. Van Buskirk and Arioli (2002) found that morphological traits were more sensitive to the number of predators that were present because starved predators induced morphological defenses. We need many more studies addressing this question before we can

arrive at any general conclusions.

The tadpole responses to increasing predation risk were never simple "on-off switches" that exhibited threshold responses, but were instead fine-tuned, graded responses to prey consumption. This is precisely what one would predict when organisms experience a continuous range of spatial environmental heterogeneity and when more extreme responses are associated with greater costs (Houston et al. 1993, Werner and Anholt 1993). Moreover, the result is in general agreement with most anti-predator traits examined in previous studies (Van Buskirk and Arioli 2002, Laurila et al. 2004, Relyea 2004) although threshold responses were observed for tail length by Van Buskirk and Arioli (2002) and for body length by Relyea (2004). The differences between these two previous studies and the current study may lie in the fact that the current study examined a wider range of prey consumption and predator numbers (200 to 3200 mg of total consumed prey and 0 to 6 predators) than the earlier studies (Van Buskirk and Arioli 2002, 200 to 800 mg of total consumed prey and 0 to 3 predators; Relyea 2004, 0 to 1200 mg of total consumed prey and 0 to 4 predators). This wider range of treatments would be more likely to detect graded responses where they truly exist.

Because behavioral traits are easily altered and do not require morphological remodeling, it has been proposed that behavioral traits should be more sensitive to changes in predation risk (West-Eberhard 1989, Padilla and Adolph 1996, Gabriel 1999, VanBuskirk 2002b). My study did not support this proposition; behavioral and morphological traits showed strikingly similar sensitivities to increased predation risk and exhibited graded responses. Although all of the traits exhibited graded responses, the point at which a particular trait was induced was not identical. For example, most of the traits exhibited a large amount of induction when exposed to the lowest level of predation risk (four predators each consuming 50 mg of prey or one predator consuming 200 mg of prey), suggesting that the chemical cues emitted by aquatic predators are effective at very low concentrations (i.e. one predator in 700 L of water). In contrast, body width did not exhibit any significant induction until the tadpoles were exposed to four predators consuming 200 mg of prey. This indicates that different plastic traits might have unique sensitivities to an environmental gradient. To better assess this situation, we need to more intensively explore the sensitivity of prey at even lower levels of predation risk than we have explored in the current experiment. Only by examining extremely low levels of predation risk could we determine if the other behavioral and morphological traits are induced at different levels of the predation risk

gradient.

The graded responses mostly exhibited plateaus at high levels of predation risk. Such a relationship is common in studies of plasticity (Schlichting and Pigliucci 1998, West-Eberhard 2003) and is thought to be due to either physical or physiological limits of plasticity or due to the continually increased costs that typically accompany more extreme phenotypes (Werner and Anholt 1993, DeWitt et al. 1998, Kats and Dill 1998). Interestingly, there were a few traits that did not respond in this way. For example, body width showed generally weak responses to the predator environments by exhibiting an initial increase and then a decrease with greater prey consumption. While this pattern of response confirms the continuous nature of the response, it does not suggest a plateauing response. The reasons underlying such response patterns remain unclear, but one possible explanation is that only the traits that are under the strongest direct selection (e.g., activity, tail depth) show a clear plateauing pattern of increased induction.

For those responses that did plateau, the environmental state that induced maximal trait expression was similar for most of the traits including activity, tail length and depth, and body length and depth (i.e. 200 to 300 mg of prey consumption). There were two other traits that exhibited distinctively different responses. In the first case, tadpole hiding continued to increase across the entire range of predation risk, although it did exhibit a pattern of change that suggested a plateau would exist somewhere just beyond the maximum level of predation risk. In the second case, tadpole mass continued to decline until the second-highest level of predation risk (700 mg). As noted earlier, reduced mass is commonly associated with predator-induced behavior and morphology. However, all of the morphological variables and one of the two behavioral traits plateaued at a much lower level of predation risk. This suggests either that the one non-plateauing response (tadpole hiding) was responsible for the continual mass loss with increased predation risk or that there were additional (i.e. unmeasured) traits that were also changing at the higher levels of predation risk that caused a loss of mass.

2.5.1 Implications of understanding how prey respond to gradients in predation risk

The ability of prey to sense small differences in predation risk has a number of interesting implications for the ecology of predator-prey interactions and for the larger ecological community. Sensitivity to differences in predator number and predator consumption means that

prey can attempt to balance the costs and benefits of their defenses and potentially arrive at an optimal solution. While I have shown the phenomenon using a single predator species (beetle larvae), the phenomenon likely exists with many other species of predators as well. This ability means that prey can detect small changes in predation risk even at the microhabitat level providing that predators and their cues are not well mixed throughout the aquatic habitat and that the cues do not persist for long periods (i.e. < 1 d). Under these conditions, prey could tailor an appropriate defensive phenotype to the riskiness of their particular microhabitat.

Possessing the ability to detect and respond to small differences among different constant predation risk environments also means that prey should be able to detect temporal changes in predation risk within a given environment. If prey experience pulses of risk instead of a chronic level of risk and can reverse the induction of their defensive traits, prey may be able to exploit periods of low risk by adjusting their phenotype (Lima and Bednekoff 1999). By quantifying how prey alter their traits at each level of predation risk, we can then make quantitative predictions about how prey should respond to temporal variation in predation risk using a variety of potential decision rules. I use this approach in a companion study in which I examine how temporal variation in predation risk impacts the anti-predator traits of wood frog tadpoles when mean risk is held constant (see Chapter 3).

Graded responses across a range of predation risk also have potential effects on the larger ecological community. For example, ecologists are growing to appreciate the importance of trait-mediated indirect effects in aquatic systems in which there is a change in interaction strength between two species because the traits of one species are altered (without altering its density; reviewed in Werner and Peacor 2003). Given that prey can adjust their traits in a very fine-tuned fashion with changes in predator number and the amount of consumed prey, this suggests that the strength of these trait-mediated indirect effects should also vary with predator number and the amount of consumed prey. This prediction appears to have not yet been tested, but it should be a profitable topic of future investigations.

2.5.2 Conclusions

The results of this study indicate that prey can be highly sensitive to the number of predators in their environment and the amount of prey being consumed. The most sensitive range appears to

be within a very narrow window of low predation risk, consistent with the expectation that aquatic prey detect the chemical cues of their predators at very low concentrations. While both kairomones and alarm cues are important for inducing prey defenses, my results suggest that more extreme behavioral and morphological defenses are a function of the total amount of prey consumed and not a function of predator number *per se*. Future studies should examine how this sensitivity affects prey at the microhabitat scale, how risk-response curves can be used to predict responses to temporal variation in predation risk, and how different magnitudes of risk translate into different magnitude of trait-mediated effects in the community.

Table 2.1 Results of a multivariate analysis of variance (A) and subsequent univariate tests (B) that examined the effects of cue concentration and predator number on the mass and seven morphological traits of wood frog tadpoles. PVE = percent of variance explained by treatment effects

A. Multivariate test	df	F	P
Treatment	90,180	4.0	<0.001

B. Univariate test	Predation risk (P)	PVE (%)
Mass	<0.001	85
Body length	<0.001	69
Body width	0.021	23
Body depth	<0.001	58
Tail length	<0.001	84
Tail depth	<0.001	91
Mouth width	0.259	7
Muscle width	0.352	3
Muscle depth	0.078	17

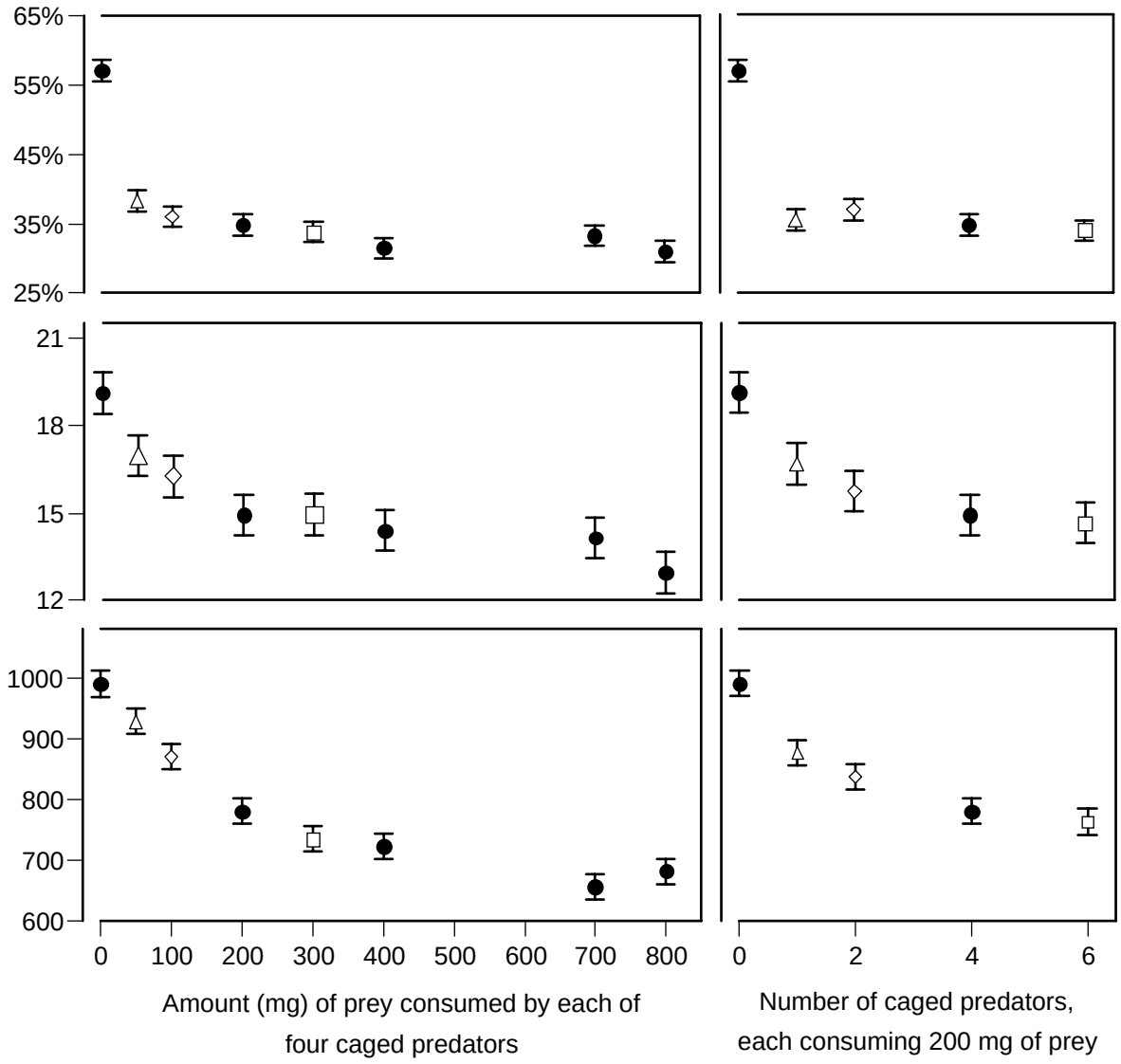

Figure 2.1 The behavior and mass of wood frog tadpoles when exposed to treatments that varied in the amount of prey consumed by each of four predators (left panels) or the number of predators fed a constant (200 mg) amount of prey (right panels). The three open symbols in the left and right panels indicate the three treatments which contained the same total mass of prey consumed, but different numbers of predators doing the consuming. To visually assist the reader in making the comparisons, one of the treatments is presented twice (left panel, 200 mg of prey consumed; right panel, 4 predators). Data are means $\pm$ 1 SE.

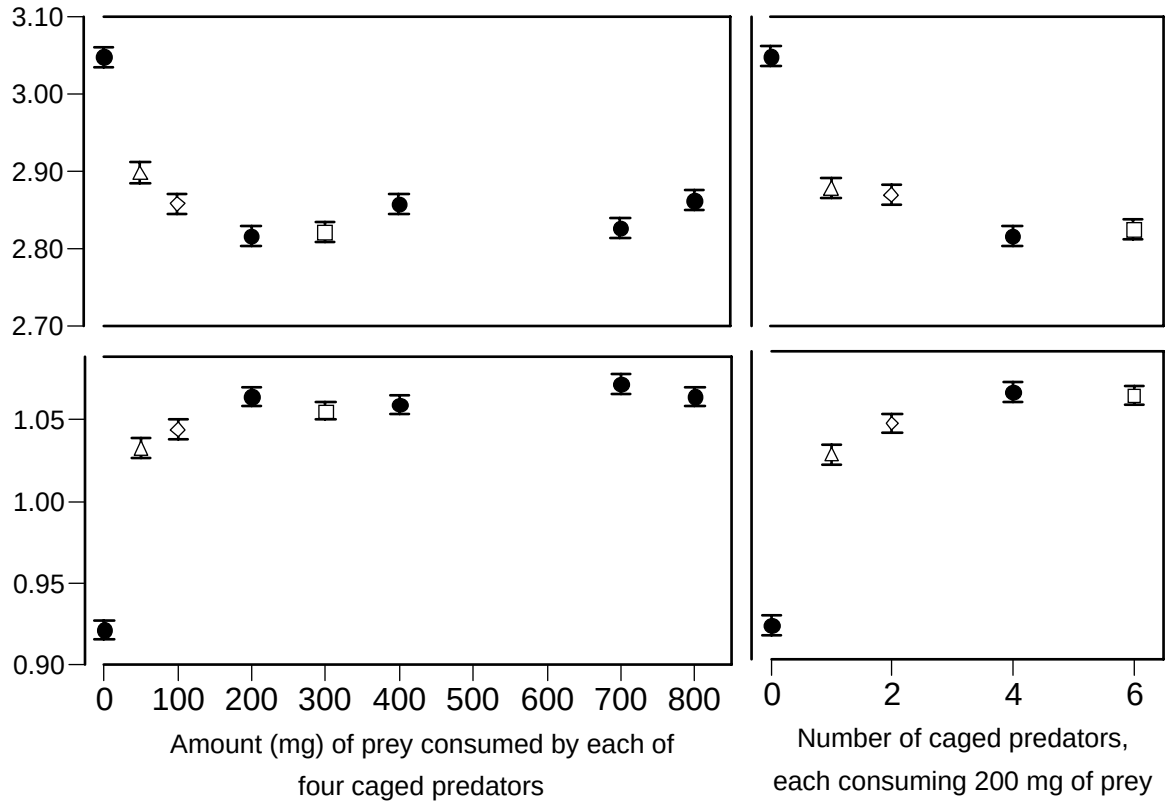

Figure 2.2 The relative tail morphology of wood frog tadpoles when exposed to treatments that varied in the amount of prey consumed by each of four predators (left panels) or the number of predators fed a constant (200 mg) amount of prey (right panels). The three open symbols in the left and right panels indicate the three treatments which contained the same total mass of prey consumed, but different numbers of predators doing the consuming. To visually assist the reader in making the comparisons, one of the treatments is presented twice (left panel, 200 mg of prey consumed; right panel, 4 predators). Data are means $\pm$ 1 SE.

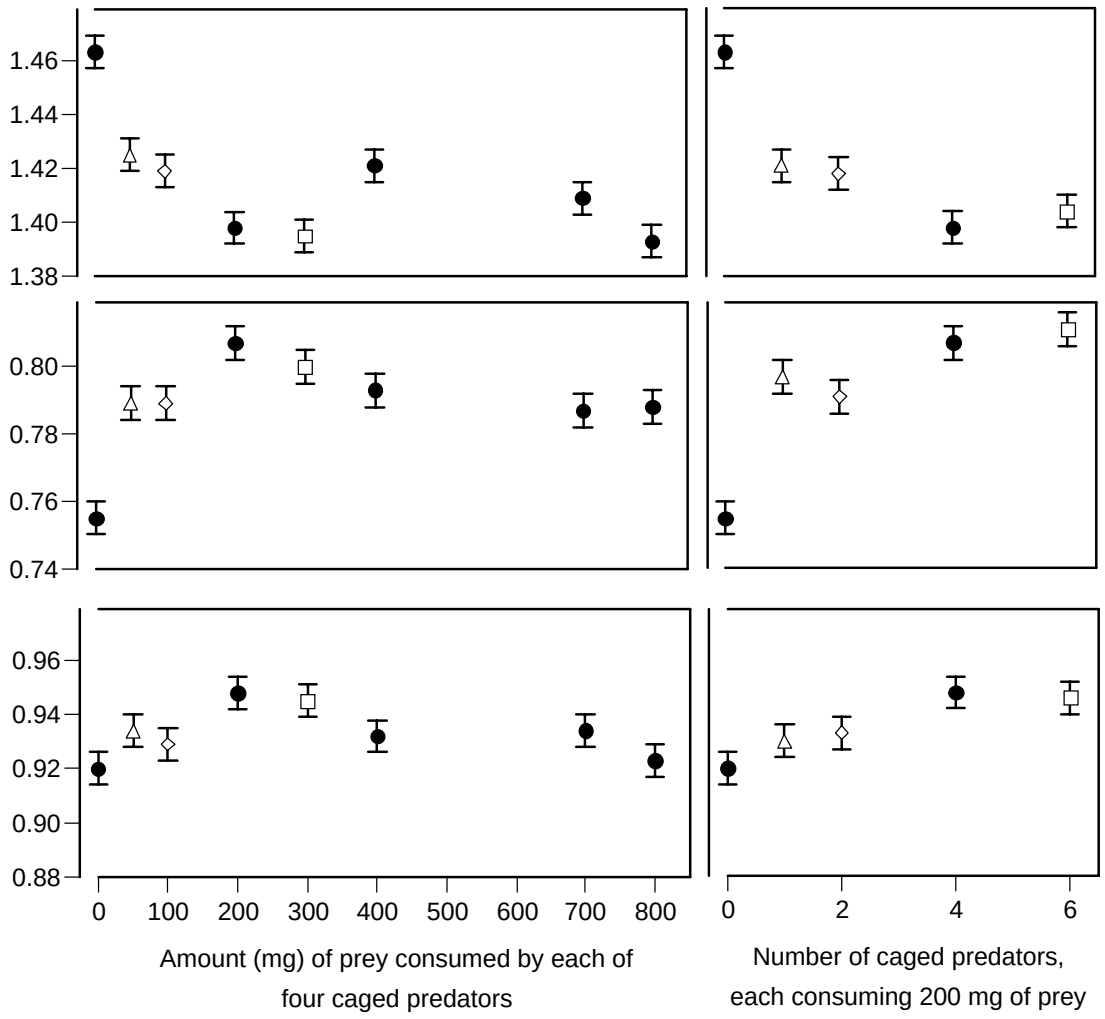

Figure 2.3 The relative body morphology of wood frog tadpoles when exposed to treatments that varied in the amount of prey consumed by each of four predators (left panels) or the number of predators fed a constant (200 mg) amount of prey (right panels). The three open symbols in the left and right panels indicate the three treatments which contained the same total mass of prey consumed, but different numbers of predators doing the consuming. To visually assist the reader in making the comparisons, one of the treatments is presented twice (left panel, 200 mg of prey consumed; right panel, 4 predators). Data are means $\pm$ 1 SE.

3.0 PHENOTYPIC PLASTICITY AND FINE-GRAINED ENVIRONMENTAL VARIATION: PREY RESPONSES TO TEMPORAL VARIATION IN PREDATION RISK

3.1 ABSTRACT

In nature, organisms experience environmental variability at both coarse-grained (inter-generational) and fine-grained (intra-generational) scales, and a common response to environmental variation is phenotypic plasticity. The emphasis of most empirical work to date has been on examining coarse-grained variation with the goal of understanding the costs and benefits of plastic responses. However, fine-grained variation can also have fitness consequences. Few studies have examined the importance of fine-grained variation for phenotypically plastic responses and none have made quantitative predictions as to how organisms should alter their phenotype. In this study, I investigated the effects of fine-grained variation in predation risk on the inducible defenses of larval wood frogs (*Rana sylvatica*). I produced temporal variation in risk by altering the density and feeding schedule of caged predators (*Dytiscus spp.*) while holding the average risk constant. Using dose-response relationships for plastic defensive traits from a companion study, I was able to make quantitative predictions about how the tadpoles should respond to temporal variation in risk and then test these predictions against the observed responses. I found that temporal variation in risk did not affect behavioral traits but it did affect at least one of the morphological traits. Therefore, both the average environment experienced and the variation around that average are important in determining the induction of plastic traits. Therefore, fine-grained variation must be considered when interpreting phenotypes observed in nature.

3.2 INTRODUCTION

Few organisms live in static environments but instead experience fluctuations in both biotic and abiotic factors. These fluctuations can affect their fitness and limit their distribution. When there are reliable environmental cues, many organisms exhibit phenotypic plasticity in response to changes in their environment and improve their performance (Pigliucci 2001, DeWitt and Scheiner 2004, West-Eberhard 2003). Most empirical work on phenotypic plasticity has focused on how organisms respond to a constant exposure to different environments. This approach has produced a wealth of knowledge about the costs and benefits of phenotypic plasticity when organisms experience variable environments across generations (i.e. coarse-grained environmental variation; Schlichting and Levin 1984, Lively 1986, van Tienderen 1990, Sultan and Bazzaz 1993, Tollrian 1993, Dudley and Schmitt 1996, Pigliucci et al. 1997, Relyea 2004). In nature, however, organisms often encounter substantial environmental variation within their lifetimes over both space and time (i.e. fine-grained environmental variation). If fine-grained variation encompasses the same environmental range as coarse-grained variation, then organisms that express the "wrong" phenotype will suffer the same range of fitness costs that favor plasticity in coarse-grained environments, but for shorter periods of time (i.e. some fraction of the lifetime). Therefore, fine-grained variation may have substantial effects on individual fitness. As a result, selection should favor individuals that can adjust their phenotypes in response to fine-grained environmental variation. If organisms can detect and respond to fine-grained variation, then understanding these effects will be particularly important for extrapolating experimental results to the interpretation of phenotypic patterns observed in nature.

Surprisingly few studies of phenotypic plasticity have directly manipulated fine-grained variation. The majority of empirical work has addressed the effects of temporal variation in resource levels (Kacelnik and Bateson 1996, Wayne and Bazzaz 1993, Winn 1996, Siems and Sikes 1998, Ali and Wootton 1999, Novoplansky and Goldberg 2001, Englemann and Schlichting 2005, Miner and Vonesh 2004, Ruehl and DeWitt 2005) or predation risk (Hamilton and Heithaus 2001, Sih and McCarthy 2002, Van Buskirk et al. 2002, Pecor and Hazlett 2003, Foam et al. 2005). However, because most of the above studies were not designed to examine temporal variation *per se*, most studies that have manipulated temporal variation in the environment have simultaneously altered the average environment that the organism experiences

(but see Wayne and Bazzaz 1993, Siems and Sikes 1998, Novoplansky and Goldberg 2001, Englemann and Schlichting 2005, Miner and Vonesh 2004). For example, several tests of the risk allocation hypothesis (Lima and Bednekoff 1999) have been conducted to determine how prey behavioral defenses are affected by fluctuating periods of high and low predation risk compared to a constant high-risk predator environment (Hamilton and Heithaus 2001, Sih and McCarthy 2002, Van Buskirk et al. 2002, Pecor and Hazlett 2003, Laurila et al. 2004, Foam et al. 2005). Thus, individuals in the fluctuating-risk treatment not only experience greater fine-grained variation, but also a lower average risk compared to individuals in the constant high-risk environment. To address the effects of temporal variation *per se*, we need to manipulate fine-grained variation while holding the average experience constant among treatments (e.g., Wayne and Bazazz 1993, Siems and Sieks 1998, Novoplansky and Goldberg 2001, Englemann and Schlichting 2005, Miner and Vonesh 2004).

The effects of fine-grained variation on traits will depend on whether the induced responses are reversible or irreversible. If induced traits are irreversible, then organisms with induced traits obviously cannot respond to future environmental changes. When traits are reversible, the pattern of response to temporal variation will depend on whether responses are threshold or graded. For threshold responses, organisms would be limited to switching between alternative trait states and the observed response to temporal variation will depend on the last environment encountered. For graded responses, organisms can produce a wide range of possible phenotypes, and the final phenotype exhibited will depend on the frequency of the variation, the intensity of the variation, how individuals average variation over time, and how frequently the environment changes (i.e. whether it is longer than the time required to change the trait, Padilla and Adolph 1996). Thus, behavioral traits, which can be rapidly induced, should be able to be modified quickly and track fine-grained variation to produce a phenotype that is continuously suited to the environment. In contrast, morphological traits, which require longer times for induction and reversal (Van Buskirk 2001, Relyea 2003b), may favor a strategy that integrates fine-grained variation over time according to some decision rule.

When organisms cannot rapidly track fine-grained variation, there are a number of potential decision rules. First, when there are high costs of incorrectly assessing a particular environmental state or the environment changes frequently, organisms might respond to just one of the environmental extremes experienced. Alternatively, when organisms try to achieve an

optimal response in the face of trade-offs (i.e. balancing resource acquisition and defense allocation) a decision rule that averages the risk may be the best solution. When considering how an averaging of fine-grained variation would effect the phenotype, it is important to recognize that most environmentally induced responses are curvilinear responses that exhibit saturating effects as the environment gradient becomes more extreme (either because organisms reach a biological limit or because the costs of a more extreme response outweigh the benefits; Dewitt et al. 1998). If the individual 1) cannot detect the fine-grained changes in risk (i.e. they do not sample environmental conditions often enough) or 2) cannot respond because of the costs of changing their phenotype, the best strategy may be to respond to the average environment and ignore the fine-grained variation. Alternatively, an individual could average fine-grained variation over time by producing an average of the phenotypes induced by each environmental state. In this scenario, individuals experiencing fine-grained variation would exhibit a less extreme phenotype than individuals experiencing a constant environment (because the average of each environment's induced phenotype is not equivalent to the phenotype in the average environment; i.e. Jensen's inequality, Ruel and Ayres 1999, Miner and Vonesh 2004). While one can easily test these predictions in a qualitative fashion, one would need to first quantify how species alter their traits across an environmental gradient (i.e. risk-response curves) to test these predictions in a quantitative fashion. A few studies have assessed the qualitative effect of fine-grained variation on plastic phenotypes (Wayne and Bazzaz 1993, Novoplansky and Goldberg 2001, Englemann and Schlichting 2005, Miner and Vonesh 2004); none have made *a priori*, quantitative predictions about how strongly fine-grained temporal variation should affect phenotypic induction.

In this study, I examined the effect of fine-grained variation on larval anurans (i.e. tadpoles), a system that has become well documented for its plasticity in response to predators (Smith and Van Buskirk 1995, Relyea 2001a, 2002a,b; Laurila and Kujasalo 1999, Lardner 2000, Van Buskirk 2002a,b; Laurila et al. 2002). Inducible defenses are well-studied and often show continuous responses to increases in predation risk (Van Buskirk and Arioli 2002, Relyea 2004). Predator-induced defenses in tadpoles are continuous and reversible, making tadpoles a prime candidate for studying the effects of temporal variation on plasticity (Van Buskirk 2002b, Relyea 2003b). By observing how behavior, morphology, and mass were affected by temporal variation in predation risk (while holding average risk constant) and comparing these responses to

predictions generated from a companion study in which I quantified dose-response relationships for predation risk (see Chapter 2), I asked whether tadpole phenotypes were affected by fine-grained variation in risk and, if so, if the responses were consistent with the mechanisms of highest risk, the lowest risk, average risk, or average phenotype.

3.3 METHODS

I conducted the experiment at the Pymatuning Laboratory of Ecology's Aquatic Research Facility located in northwestern Pennsylvania in the spring of 2003. I used a completely randomized design with eight treatments replicated five times for a total of 40 experimental units. My goal was to expose tadpoles to predation risk (i.e. chemical cues from caged predators) that varied in intensity over time but had the same average level of risk overall. Therefore, my eight treatments included a no-predator control, a constant predation risk treatment in which four predators were each fed 100 mg prey/predator/tank/d, and six treatments in which I created fine-grained variation in predation risk while keeping the average amount consumed (100 mg prey/predator/tank/d). I produced temporal variation in predation risk in three different ways. First, I varied the frequency that four predators were fed: 1) 200 mg every 2 d, 2) 400 mg every 4 d, or 3) 800 mg every 8 d. Second, I varied the amount that four predators were fed on a set time schedule. Using a 2-d feeding schedule, I rotated four predators through cycles of 100 mg, 200 mg, and 300 mg of prey (100-200-300 2 d); using a 4-d feeding schedule, I rotated four predators through cycles of 100 mg, 400 mg, and 700 mg (100-400-700 4 d). Third, I varied the number of predators. For this treatment, I rotated through cycles of two, four, and six caged predators (feeding the predators 200 mg of prey every 2 d). Collectively, these treatments allowed us to manipulate fine-grained variation in predation risk in a variety of ways (see Table 3.1 for a summary of the treatments and the feeding schedule).

The experimental units were 800-L pond mesocosms (cattle watering tanks) designed to simulate the types of ponds where these amphibians are typically found. Each mesocosm contained 700 L of aged well water, 200 g leaf litter, 15 g rabbit chow (as an initial food source), and an aliquot of pond water containing algae and zooplankton. Because up to six predators were added to some tanks, I placed six predator cages into each tank. The cages were

constructed of 10-cm black plastic drainpipe covered on both ends with a fiberglass mesh screen which allowed the predator cues to diffuse into the tank. Depending on the treatment, each cage was either empty or contained a single larval beetle (*Dytiscus* sp.). All mesocosms were covered with shade cloth lids to prevent colonization by other organisms. The wood frog tadpoles were collected from two populations (Shrub pond and Staub pond; 10 egg masses/population) as newly laid egg masses on 28 March 2003. The eggs were hatched and the tadpoles were reared in pools containing aged well water. The wood frogs were fed rabbit chow *ad libitum* prior to the experiment. Using a mixture of tadpoles from the two populations, I added 30 tadpoles to each mesocosm on 9 May 2003 (initial mean mass ± SE = 139 ± 5 mg).

Behavioral observations were conducted using established observation protocols (Relyea and Werner 1999). I counted the number of visible tadpoles in each mesocosm and the number of visible tadpoles that were moving. From these data, I calculated the proportion of active tadpoles. I began observations the day after all of the predators were fed (i.e. day 9) and continued to conduct observations until the day before all the predators were scheduled to be fed again (i.e. day 15). The number of observations per tank were as follows: seven observations on day 9, seven observations on day 11, six observations on day 12, and six observations on day 15. On each day, I used the mean number observed and the mean proportion of active tadpoles from each tank as my response variables. The data for each behavioral trait were analyzed with a repeated-measures analysis of variance (rmANOVA). When a significant effect was found, I conducted pair-wise comparisons using Fisher's LSD test.

After 24 d, all tadpoles were removed from the mesocosms and preserved in 10% formalin for subsequent morphological measurements (mean survival = 93 $\pm$ 0.2%). Tadpole morphology was measured using an image analysis system (Optimas Bioscan; Bothell, Washington, USA). I weighed each tadpole and then measured five morphological dimensions: body depth, length, and width; and tail length and depth (see Fig.1 in Relyea 2000). Because the tadpole's body is round I placed a glass plate under the tadpole's tail to bring both structures into the same plane of focus and ensure that I obtained an undistorted lateral image.

Because I was interested in the effects of the treatments on tadpole shape independent of tadpole mass, I first performed a multivariate analysis of covariance (MANCOVA) and saved the residuals. Prior to performing the MANCOVA I transformed the data when necessary to improve the linearity of the relationship between each trait and mass. I found no mass-by-

treatment interactions for any of the traits, indicating that the regression lines among treatments were parallel for each trait (a requirement for making the size-adjustment). To produced mass-independent estimates of each tadpole trait for every tadpole measured, I added the residuals saved from the MANCOVA to the estimated marginal mean for each treatment. For each trait, I averaged the size-adjusted data for all of the tadpoles in each tank, and then used these tank means, along with mean tadpole mass, as my response variables in a multivariate analysis of variance (MANOVA) to examine the effects of the treatments. When significant multivariate effects were found, I then conducted univariate tests and used Fisher's LSD to make pair-wise comparisons among the treatment means. I excluded one tank from the analysis (treatment = 100-400-700 4 d) because the tank contained a large amount of mold, the water was cloudy, and the tadpoles had hardly grown over the course of the experiment.

3.3.1 A few assumptions

While manipulating predator number is a direct manipulation of predation risk, manipulating predator feeding schedules is a more indirect manipulation of predation risk that relies on a number of important assumptions. First, it assumes that caged predators consume their prey shortly after prey are added to the cage. Based on the risk-response experiment (see Chapter 2), this assumption is well-supported; *Dytiscus* larvae consistently consumed up to 800 mg of tadpoles within 1d. Second, it assumes that predators produce chemical cues in a relatively short pulse after consuming the prey and then stop producing the cues. While there are no data available for wood frog tadpoles and *Dytiscus* predators, there is some support for this assumption for other species of tadpoles and predators. While the time required for a cessation of cue production is currently unknown, larval dragonfly nymphs (*Anax junius*) that have not fed for more than 4 or 5 d induce no defensive responses in larval tree frogs (*Hyla versicolor*; Schoeppner and Relyea 2005) and only weak morphological defenses in larval pool frogs (*Rana temporaria*; Van Buskirk and Arioli 2002). Finally, it assumes that chemical cues break down rapidly. There is good support for this assumption; chemical cues from dragonfly nymphs induce no behavioral responses in larval leopard frogs (*R. pipiens*) after being aged for 24 h (R. A. Relyea, *unpublished data*). Additionally, chemical cues from sunfish induce weak behavioral defenses in snails (*Physa acuta*) after being aged for 24 h and have no effect after being aged for

41 h (Turner and Montgomery 2003). In short, the assumptions I made are moderately to strongly supported by existing data.

3.3.2 Making quantitative predictions

For those traits that were affected by temporal variation in predation risk, I made *a priori*, quantitative predictions about how the tadpoles should respond using data from a companion study in which I quantified how each level of predation risk affected the traits of wood frog tadpoles (termed "the risk-response experiment;" see Chapter 2). In the risk-response experiment, I raised tadpoles under a wide range of predator densities (from zero to six predators) and predator rations (from 0 to 800 mg of tadpole prey per day) and quantified how tadpoles altered their behavior, morphology, and mass. Importantly, the companion study and the current study were conducted using the same mesocosm set-up, the same duration of time, and the same wood frog populations. Because the experiments were conducted in different years, there were small differences in tadpole mass and the magnitude of trait plasticity between the two experiments. For example, at the end of the current experiment, tadpoles were 12% larger than in the risk-response experiment. These size differences can be attributed to differences in initial tadpole mass (139 mg for the current experiment vs. 61 mg for the response-curve experiment) and ambient temperature between the two years. Additionally, the tadpoles in the current experiment responded a bit more strongly to the predator cue than the tadpoles in the response-curve experiment. The small differences in plasticity between years was not unexpected and likely stems from small differences in resource availability and the specific genotypes of the tadpoles present in the experiment. To permit the risk-response data to predict how tadpoles should respond to fine-grained variation in predation risk, I made scalar adjustments such that the two experiments were equivalent in the no-predator environment and had similar magnitudes of plasticity. I corrected for differences in tadpole size and trait plasticity by making adjustments to all of the tank means in the risk-response experiment using the two treatments that both experiments had in common, the no predator and the 100 mg prey/predator/d treatments. First, to correct for the difference in overall tadpole size, I subtracted the mean no-predator trait value of the current experiment from the mean no-predator trait value observed in risk-response experiment. I then added that difference to each tank mean in the risk-response

experiment for each of the morphological traits and mass. This size adjustment had to be performed for all the morphological traits and not just for mass because even though the morphological traits were size-adjusted measurements within an experiment the size-adjusted estimates were calculated for a larger mass in the current experiment; therefore, the traits are not size-independent between experiments. Next, to correct for differences in trait plasticity, I calculated a correction factor that scaled the magnitude of trait plasticity in the risk-response experiment to the magnitude of plasticity observed between the 100 mg prey/predator/d treatment and no-predator treatment in the current experiment. I corrected for plasticity differences for the morphological traits and mass by first subtracting the tank mean from the no-predator treatment mean. I then multiplied that difference by the proportional difference in plasticity between the two experiments (i.e. the no-predator treatment – the 100 mg prey treatment for the current experiment/the no-predator treatment – the 100 mg prey treatment in the risk-response experiment) and then I subtracted this adjusted plasticity from the tank mean.

Once the data adjustments were completed, I used the response-curve data to make predictions about how tadpoles should respond to temporal variation in predation risk. I first used a nonlinear regression to quantify the curvilinear relationship in the response-curve experiment between: 1) predator number and tadpole phenotypes; and 2) predator consumption of prey and tadpole phenotypes. If the response-curve relationship was negative and plateauing, I used an one-phase exponential decay regression equation:

$$Y = (min - max) * exp((-k * X) + min)$$

If the response-curve relationship was positive and plateauing, I used a one-phase exponential association equation (GraphPad Prism 4).

$$Y = min + (max- min) * (1 - exp(-k*X)))$$

Using these equations, I calculated predictions for the three hypotheses posed earlier concerning how prey should respond to temporal variation in predation risk: 1) exhibit the phenotype that is used at the highest level of predation risk experienced; 2) exhibit the phenotype that is used at the lowest level of predation risk experienced; or 3) exhibit the average of the high and low risk phenotypes, weighted by the frequency of each environment's occurrence. I did not calculate predictions for exhibiting the phenotype appropriate for the average environment because support for that decision rule was evaluated by comparing the response in the variable-risk treatments to the constant-risk treatment.

For clarity, the following example illustrates how I developed the three quantitative predictions for tadpoles exposed to a temporal-variation treatment in which predators were fed 400 mg of prey once every 4 d. For the highest risk prediction, I determined how tadpoles responded to predators consuming 400 mg of prey every day in the response-curve experiment. For the lowest risk prediction, I determined how tadpoles responded to a no-predator environment in the response-curve experiment. For the average phenotype prediction, I used data from the response-curve experiment to average the phenotype exhibited when prey were exposed to predators consuming 400 mg of prey and the phenotype exhibited when prey were exposed to a no-predator treatment (the latter was weighted three times the former). I also examined calculations using geometric averages, but I do not present those predictions because they did not differ substantially from the predictions made by using arithmetic averages. Using the predicted values from the response-curve experiment, I determined whether the predicted values fell outside of the 95% C.I. of the data observed in current experiment. If so, I concluded that the predictions were significantly different from the observations.

3.4 RESULTS

I found significant effects of the predator treatments on tadpole behavior. In the repeated-measures ANOVA on tadpole activity, I found a significant effect of predator treatment ($F_{7,31}$ = 54.7, $P < 0.001$) and time ($F_{3,29}$ = 39.2, $P < 0.001$) but no treatment-by-time interaction ($F_{21,84}$ = 1.2, $P = 0.272$). Mean comparisons indicated that tadpoles in the no-predator treatment were more active than the tadpoles in all treatments containing predators (for all observation days, $P < 0.001$; Fig. 3.1). However, compared to the constant-risk treatment (100 mg prey/predator/tank/d), variation in predation risk had no effect on tadpole activity ($P \geq 0.348$).

In the repeated-measures ANOVA on the number of tadpoles observed (i.e. the number not hiding), I found a significant effect of predator treatment ($F_{7,31}$ = 5.0, $P = 0.001$), time ($F_{3,29}$ = 41.6, $P < 0.001$), and a treatment-by-time interaction ($F_{21,84}$ = 2.4, $P = 0.002$). The interaction occurred because the tadpoles hid more early in the experiment compared to later in the experiment. Comparisons among the first three observation days showed no differences in tadpole hiding over time ($P \geq 0.149$) and, within each day, tadpoles in all of the predator

treatments hid more than the tadpoles in the no-predator treatment ($P \leq 0.05$; Fig. 3.2). Among the predator treatments, I found that the amount of hiding exhibited in the constant-risk treatment (100 mg/predator /tank/d) was never different from the variable-risk treatments ($P \geq 0.129$). On the fourth observation day, there was no treatment effect on hiding ($P = 0.402$).

There was a significant multivariate effect of the treatments on wood frog mass and morphology ($F_{42,125} = 4.6$, $P < 0.001$). The multivariate effect was caused by univariate effects of mass, body length, tail length, tail depth ($P < 0.001$ for all tests); there were no univariate effects of body width ($P = 0.111$) or body depth ($P = 0.756$).

Based on mean comparisons of tadpole mass, tadpoles were smaller in all treatments containing predators compared to the no-predator treatment ($P \leq 0.001$; Fig. 3.3A). However, compared to the constant-risk treatment (100 mg prey/predator/tank/d), variation in predation risk had no effect on tadpole mass ($P \geq 0.171$). When the data were compared to the three predictions, none of the three predictions consistently fell within the C.I. of the observed data. Rather, the observed predictions typically fell between the highest-risk and average-phenotype predictions.

Based on mean comparisons of tail length, tadpoles had relatively shorter tails in all treatments containing predators compared to the no-predator treatment ($P \leq 0.001$; Fig. 3.3B). However, compared to the constant-risk treatment (100 mg prey/predator/tank/d), variation in predation risk generally had no effect on tail length ($P \geq 0.315$) except that tadpoles exposed to predators consuming 400 mg every 4d and 100-400-700 mg every 4d induced tails that were nearly significantly longer ($P = 0.057$ and 0.078, respectively). When the data were compared to the three predictions, the observed data overlapped the CI of the highest-risk prediction while others were intermediate to the highest-risk and average-phenotype predictions.

Based on mean comparisons of tail depth, tadpoles had relatively deeper tails in all treatments containing predators compared to the no-predator treatment ($P \leq 0.001$; Fig. 3.3C). Compared to the constant-risk treatment (100 mg prey/predator/tank/d), tadpoles in the 200 2d were not different (100 1d; $P = 0.909$) but tadpoles in the 400 4d and 800 8d treatments had shallower tails ($P \leq 0.012$). Hence, tail depth responded to temporal variation in predation risk. Tadpole tail depth was not affected by variation in the amount fed to predators or variation in predator number ($P \geq 0.389$). When the data were compared to the three predictions, many of

the observed data were similar to the highest-risk prediction while others were intermediate to the highest-risk and average-phenotype predictions.

Based on mean comparisons of body length, tadpoles had relatively shorter bodies in all treatments containing predators compared to the no-predator treatment ($P \leq 0.001$; Fig. 3.3D). However, compared to the constant-risk treatment (100 mg prey/predator/tank/d), variation in predation risk had no effect on body length ($P \geq 0.129$). When the data were compared to the three predictions, the most common observation was intermediate to the highest-risk and average-phenotype predictions.

3.5 DISCUSSION

Tadpoles responded to the presence of chemical cues from predators with changes in behavior and morphology. In some cases, fine-grained variation in predation risk affected the traits. The predator-induced phenotypic changes were consistent with past experiments and are thought to be adaptive in tadpoles. A combination of reduced activity and the development of deeper tails and shorter bodies lowers the risk of predation but at the cost of slower growth due to reduced time spent foraging, the induction of relatively smaller mouthparts, and the induction of relatively shorter, less efficient intestines (Skelly 1992, 1994, Relyea 2001a, 2002c,d; Van Buskirk 2002b, Relyea and Auld 2004, 2005). Moreover, these traits are under selection (Van Buskirk et al. 1997, Van Buskirk and Relyea 1998, Relyea 2002a) and, in wood frogs, have a heritable basis (Relyea 2005).

I predicted that tadpole behavior would track fine-grained variation in risk such that periods of high predation risk would induce low activity and increased refuge use, while periods of low predation risk would induce high activity and decreased refuge use. I observed tadpole behavior at several periods during the course of the experiment and found no differences among the treatments on any of the observation days regardless of the type or magnitude of temporal variation in predation risk. It was particularly striking that even when predators were only fed once every 8 d, tadpole activity did not increase 7 d after the feeding (i.e. day 15; Fig. 3.1D). This result contradicts the predictions made by the risk allocation hypothesis (Lima and Bednekoff 1999) which predicts that prey experiencing variable risk should forage more during

periods of low risk and less during periods of high risk compared to prey reared in constant low or high risk environments, respectively. In my experiment, I found no differences in tadpole behavior among the treatments that differed in the proportion of time they spent in high risk environments. In another test of the risk allocation hypothesis using tadpoles, Van Buskirk et al. (2002) found that tadpoles also did not respond to increased proportion of time at risk. They proposed that the lack of response was due to the tadpole's ability to maintain high growth rates under high predation risk, and that the risk allocation hypothesis may only apply in situations where some minimum growth requirement cannot be met in the high-risk environment. It is also possible that one would not observe responses consistent with the risk allocation hypothesis when prey are already maximizing their foraging in the constant-low-risk environment. While variation in predation risk did not affect behavior, the tadpoles did change their behavioral decisions over ontogeny such that the tadpoles used refuges less later in the experiment (Fig. 2). This is consistent with previous work showing that defensive behavior decreases as the tadpoles grow and develop morphological defenses (Anholt and Werner 1998, Van Buskirk 2002b, Relyea 2003b).

I predicted that morphological defenses would be affected by fine-grained variation when the periods between changes in the risk were longer than the time needed to alter the morphology. Given that uninduced individuals require 4d to develop morphological defenses that are equivalent to an individual that has experienced continuous predation risk (Van Buskirk 2001, Relyea 2003), I predicted that the tadpoles exposed to longer periods between predator feedings would respond by producing a phenotype that was consistent with either 1) the highest-risk environment, 2) the lowest-risk environment, 3) the average-risk environment, or 4) the average phenotype. For mass and body length, I not only found support for the hypothesis that the tadpoles were responding to the average-risk environment, but I could also reject the hypotheses that the tadpoles were responding to the highest-risk environment, the lowest-risk environment, or that they were responding by exhibiting the average phenotype.

The support for the decision rule hypotheses differed for the two tail dimensions. Tail length did respond to one of the variation treatments (400 mg 4d) with a marginally non-significant response ($P = 0.059$), suggesting that this trait does not necessarily follow the average-risk environment. However, the other three hypotheses were also frequently rejected; the pattern of tail length responses was typically intermediate to the average-risk/highest-risk

prediction and the average-phenotype prediction (one cannot discriminate between the highest-risk and the average-risk predictions for tail length because the maximum change in tail length is induced at < 100 mg/prey/predator, making the two predictions nearly identical; see Chapter 2). Tail depth was the one trait measured that did respond to temporal variability in predation risk. When I compared the magnitude of the change to my quantitative prediction, I found that the observed response was intermediate to the highest-risk and average-phenotype predictions, suggesting that tail depth follows an as yet unidentified decision rule. Interestingly, while tadpole tail depth was affected by variation in predator feeding frequency, it was not affected by variation in predator consumption or predator number. This suggests that tadpoles can discriminate among different types of temporal variation and respond to each type in unique ways. Overall, these results indicate that tadpoles can detect and respond to temporal variation in predation risk but that different traits appear to have different decision rules.

Because I did not switch prey between predator and no-predator environments, but instead produced variation in predation risk by feeding the predators at different times, I had to make some assumptions about the production and breakdown of the chemical predation cues in my mesocosms. The identities of the chemicals used to detect predators are not known; therefore, I could not directly track the changes in cue concentration over time and the possibility exists that some of my assumptions were incorrect. As noted above (see Methods) the assumption that predators consumed their prey within 1 d was met and the evidence of rapid breakdown times of chemical cues is well supported. However, the duration that predators continue to produce chemical cues is still an open question. In deriving my predictions, I assumed that predators release a pulse of chemicals (alarm cues and kairomones) for 1 d. If predators actually release chemical cues over a longer period of time, then prey in the shorter periods of variability (e.g., predators feeding every 2 d) may not have experienced fluctuations in cues that were different from the constant-risk treatment. Data concerning the amount of time that chemical cues are produced following prey consumption are sparse and equivocal. Starved predators (dragonfly naiads) sometimes induce behavioral and morphological responses in tadpoles, but whether a response is observed appears to depend on the length of time that the predator has been starved and the size of the experimental venue (Anholt et al. 1996, Anholt and Werner 1998, Van Buskirk and Arioli 2002, Schoeppner and Relyea 2005). I am unaware of any studies that document the amount of time needed for prey to stop responding to a predator once it

has been fed. Further experiments are needed to determine if the lack of response observed for morphological traits is due to the scale of the fine-grained variation or due to the cue release dynamics.

Prey also may not respond to very short-term changes in predator cues because such short-term changes are a poor indicator of actual predation risk. If predators produce chemical cues for a short period while they digest their prey, and then do not produce cues while they are hunting, tadpoles that immediately increase activity when they detect decreases in cue concentrations would be more likely to encounter the predator when it resumes hunting. If this were the case, tadpoles that increase their activity immediately following a decrease in cue concentration would have lower survival than tadpoles that behaved more cautiously. Therefore, the lack of a behavioral response to fine-grained temporal variation may be adaptive, because over evolutionary time the prey that have ignored short-term fluctuations in risk would have survived better. However, this is less likely to be the case when fluctuation in risk occurs over longer time scales (4 to 8 d) because the lost growth opportunities of being overly cautious would be more substantial. Indeed, the tail depth response to 4- and 8-d temporal variation suggests that prey do not ignore temporal variation on these longer time scales.

The effects of fine-grained variation in predation risk on prey morphology has important implications for interpreting the phenotypic patterns observed in nature. In this experiment, I found that tadpoles exposed to some types of fine-grained variation in risk produced shallower tails than tadpoles in the constant-risk environment. In nature, tadpoles likely experience fine-grained variation in the chemical cues that indicate risk. Therefore, the magnitude of the defenses observed in constant-risk experiments likely over-estimate what is ever achieved in nature. Given that phenotypes are often viewed as the product of balancing conflicting demands (i.e. growth and defense) less intense defenses are often interpreted as indicative of increased competition. This experiment has shown that the magnitude of the defense can also be decreased by fine-grained variation in predation risk and care must be taken when interpreting phenotypic differences observed in nature.

This study supports the findings of previous work examining the effects of temporal variation in resources on an individual's phenotype. In all studies to date that have manipulated fine-scaled variation while holding the average environment constant, at least one trait was affected by environmental variability. In animals, sea urchin larvae had longer feeding arms and

fathead minnows had longer guts when food availability varied (Miner and Vonesh 2004, Siems and Sieks 1998). In plants, both Wayne and Bazzaz (1993) and Novoplansky and Goldberg (2001) found that total biomass was lower when resources were more variable (light and water respectively). In addition, Novoplansky and Goldberg (2001) found that variable water conditions altered competitive hierarchies among species particularly at low overall resource levels. Additionally, Englemann and Schlichting (2005) found that fine-grained variation in water availability affected bolting date, plant height, and survival; however, the effects of variability were only observed when overall water availability was low. Overall, these results indicate that fine-grained environmental variability is important to the expression of phenotypically plastic traits.

3.5.1 Conclusions

Previous work has shown that when prey experience temporal variability in predation risk they often respond by altering their defensive decisions (Hamilton and Heithaus 2001, Sih and McCarthy 2002, Van Buskirk et al. 2002, Pecor and Hazlett 2003, Laurila et al. 2004, Foam et al. 2005). However, these studies have simultaneously varied both variation in risk and average risk. In this experiment, I demonstrated that prey behavior is not affected by the fine-grained variation in predation risk. Conversely, the temporal pattern of risk variation is important to the expression of one of the most prevalent morphological defenses in tadpoles. These results contradict the conventional wisdom that traits which can be altered quickly should track temporal environmental change while traits which cannot be altered quickly should not be affected by fine-grained temporal variation. The results also highlight the need to understand how prey integrate temporal variation in the chemical cues that they use to estimate risk, and also determine the extent of temporal variation in risk that the prey actually encounter in nature. In addition, my study further supports the results of work on fine-scaled variation in resource availability which has shown that fine-scaled temporal variation decreases the magnitude of the induction of plastic traits.

Table 3.1 Feeding schedule where the mean level of predation risk was held constant across treatments while amount fed, frequency fed, or the number of predators were varied over 24 d. The treatment labels at the top of the table give the number of predators (P) in the first row (4 or switching among 2,4, and 6 predators), the amount fed to each predator in grams of prey in the second row, and the time between feedings in the third row.

	4P	4P	4P	4P	4P	4P	2,4,6P	2,4,6P
	100	200	400	800	100,200,300	100,400,700	200	400
Day	1d	2d	4d	8d	2d	2d	2d	4d
1	400	800	1600	3200	400	400	400	800
2	400	0	0	0	0	0	0	0
3	400	800	0	0	800	0	800	0
4	400	0	0	0	0	0	0	0
5	400	800	1600	0	1200	1600	1200	1600
6	400	0	0	0	0	0	0	0
7	400	800	0	0	400	0	400	0
8	400	0	0	0	0	0	0	0
9	400	800	1600	3200	800	2800	800	2400
10	400	0	0	0	0	0	0	0
11	400	800	0	0	1200	0	1200	0
12	400	0	0	0	0	0	0	0
13	400	800	1600	0	400	400	400	800
14	400	0	0	0	0	0	0	0
15	400	800	0	0	800	0	800	0
16	400	0	0	0	0	0	0	0
17	400	800	1600	3200	1200	1600	1200	1600
18	400	0	0	0	0	0	0	0
19	400	800	0	0	400	0	400	0
20	400	0	0	0	0	0	0	0
21	400	800	1600	0	800	2800	800	2400
22	400	0	0	0	0	0	0	0
23	400	800	0	0	1200	0	1200	0
24	400	0	0	0	0	0	0	0
Total (mg)	9600	9600	9600	9600	9600	9600	9600	9600

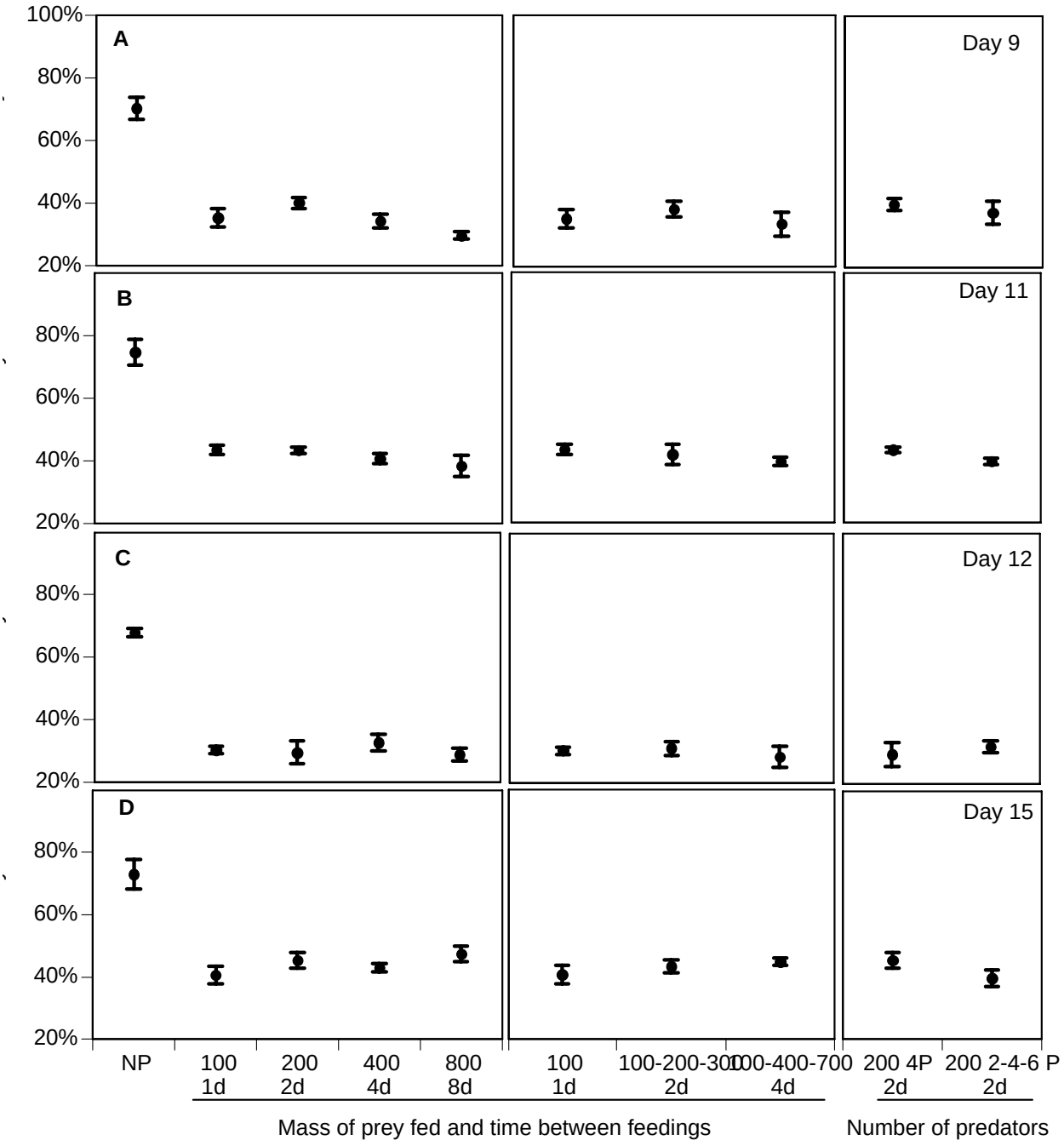

Figure 3.1 Wood frog tadpole activity in response to variable predation risk on four different days. In all predator treatments, tadpoles experienced an average risk of 100 mg prey/predator/tank/d but varied in the amount and frequency that predators were fed (right panel). Data are means $\pm$ 1 S.E. For ease of comparisons, the middle and left panel repeat the data from the 100 1d and 200 2d treatments respectively.

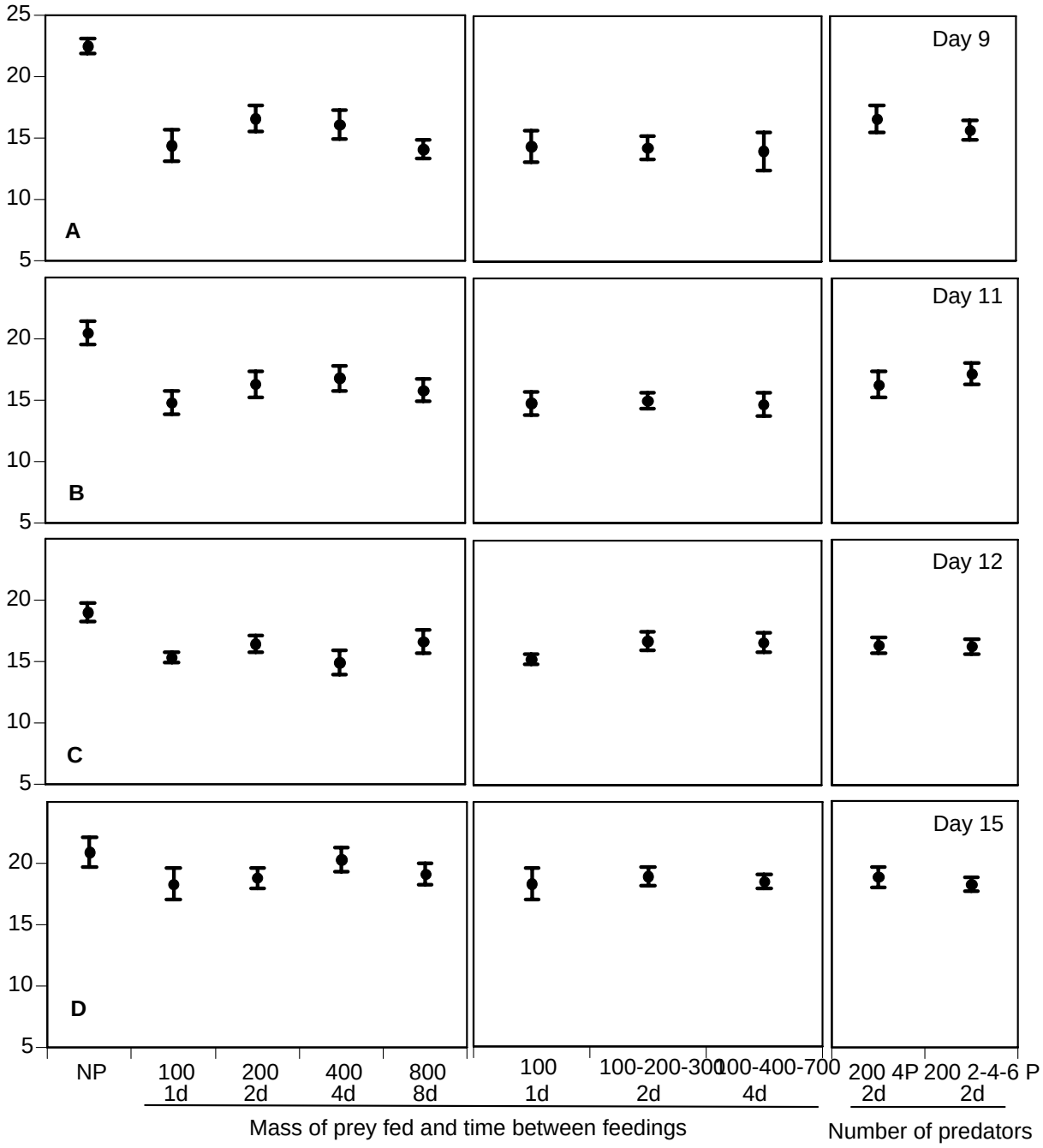

Figure 3.2 Wood frog tadpole refuge use in response to variable predation risk on four different days. In all predator treatments, tadpoles experienced an average risk of 100 mg prey/predator/tank/d but varied in the amount and frequency that predators were fed (left and middle panel) or the number of predators that were fed (right panel). Data are means $\pm$ 1 S.E. For ease of comparisons, the middle and left panel repeat the data from the 100 1d and 200 2d treatments respectively.

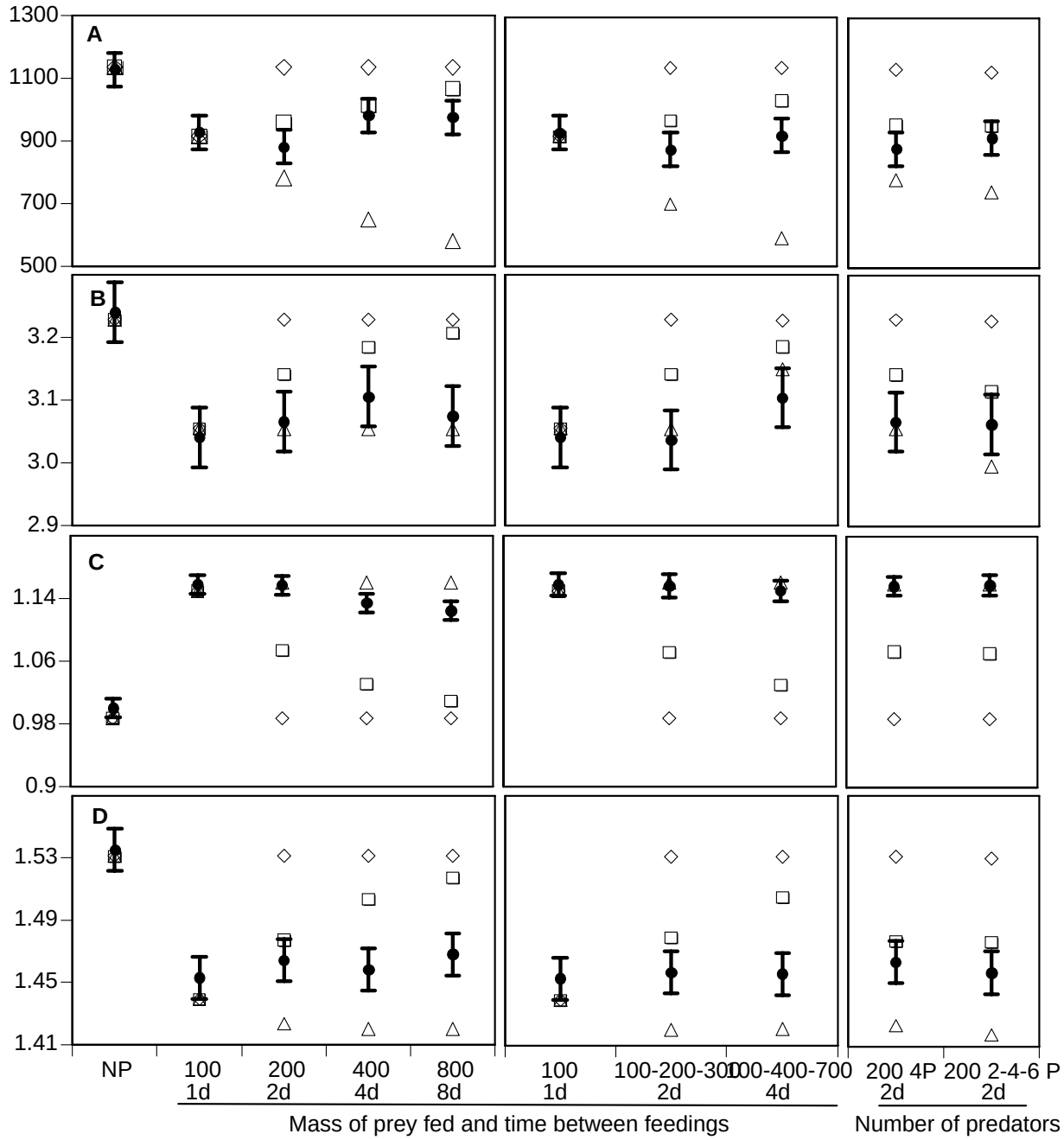

Figure 3.3 Wood frog tadpole mass and relative morphology in response to variable predation risk at the end of the experiment. In all predator treatments, tadpoles experienced an average risk of 100 mg prey/predator/tank/d but varied in the amount and frequency that predators were fed (left and middle panel) or the number of predators that were fed (right panel). The observed data are means with 95% C.I. and are represented by the closed circles. For ease of comparisons, the middle and left panel repeat the data from the 100 1d and 200 2d treatments respectively. Open symbols are the three decision rule predictions: highest risk = triangle, lowest risk = diamond, and average phenotype = square

4.0 DAMAGE, DIGESTION, AND DEFENSE: THE ROLES OF ALARM CUES AND KAIROMONES FOR INDUCING PREY DEFENSES

4.1 ABSTRACT

Inducible defenses are widely used for studying phenotypic plasticity, yet frequently we know little about the cues that induce these defenses. For aquatic prey, defenses are induced by chemical cues from predators (kairomones) and injured prey (alarm cues). Rarely has anyone determined the separate and combined effects of these cues, particularly across phylogenetically diverse prey types. I examined how tadpoles (*Hyla versicolor*) altered their defenses when ten different prey were either crushed by hand or consumed by predators. Across all prey types, crushing induced only a subset of the defenses induced by consumption. Consuming versus crushing produced additive responses for behavior but synergistic responses for morphology and growth. Moreover, I discovered the first extensive evidence that prey responses to different alarm cues depends on prey phylogeny. These results suggest that the amount of information available to the prey affects both the quantitative and qualitative nature of the defended phenotype.

4.2 INTRODUCTION

From simple single-celled organisms to plants and animals, most individuals can alter their phenotype in response to changes in biotic and abiotic factors (i.e. phenotypic plasticity; Pigliucci 2001). Many phenotypic changes appear to be adaptive, resulting in higher fitness in the inducing environment than alternative phenotypes (e.g., Dudley and Schmitt 1996, Van Buskirk and Relyea 1998). However, for organisms to properly adjust their phenotype, there

must be reliable environmental cues that indicate the current or future environmental conditions (Moran 1992). In many systems, identifying the source and function of these cues poses a tremendous challenge (Burks and Lodge 2002).

Numerous plants and animals exhibit plastic defenses against herbivores and predators (Karban and Baldwin 1997, Tollrian and Harvell 1999) and in many animals the defensive traits are induced by chemical cues that are produced during predation events (Petranka et al. 1987, Chivers and Smith 1998). These chemicals contain components from predators (termed "kairomones") and components from injured prey (termed "alarm cues"). As a result, the environmental information available to prey is potentially quite complex, including information about the species and density of predator present and the species of prey being consumed (Larsson and Dodson 1993). A major question in the field of inducible defenses asks how prey interpret this information when making their phenotypic decisions (Chivers and Smith 1998, Kats and Dill 1998, Chivers and Mirza 2001).

Because the chemical cues produced during predation contain both kairomones and alarm cues, prey may require both types of information when making their defensive decisions (the identity of the predator and the identity of the killed prey). Alarm cues (from damaged or crushed prey) have frequently been used as surrogates of predation, with the implicit assumption that the cues from damaged prey induce the complete suite of predator-induced defenses. However, prey that do respond to predation cues often do not respond to damaged conspecifics alone (Alexander and Covich 1991, Brönmark and Pettersson 1994, Summey and Mathis 1998, Slusarczk 1999; but see Stabell and Lwin 1997**,** Pijanowska 1997). The lack of consistent responses to alarm cues may occur because prey responses to alarm cues alone are small (and thus difficult to detect) or because some prey only alter their traits when they obtain information from both alarm cues and kairomones. To discriminate between these two possibilities and determine how prey use alarm cues, we must directly compare prey responses to damaged versus consumed prey.

Prey should use the information contained in alarm cues to estimate their predation risk and develop their defenses. Previous investigators have hypothesized that prey responses to alarm cues from heterospecifics should be related to either the frequency of coexistence between species that share a common predator (i.e. alarm cues from prey that frequently coexist should induce stronger responses than non-coexisting prey) or the phylogenetic relatedness between the

responding prey and the prey that released the alarm cues (i.e. closely related prey should produce similar alarm cues and, thus, induce stronger responses than distantly related prey; Chivers and Smith 1998). While a number of behavioral experiments have examined the impacts of different alarm cues, support for either hypothesis has been equivocal because the majority of these studies have not been specifically designed to distinguish between the hypotheses. Given that these studies have primarily used only two diets or three diets, the results often support both hypotheses. More definitive tests require a large number of prey types that span across a wide range of prey phylogeny while controlling for coexistence.

When testing the impact of alarm cues and kairomones on prey defenses, we also need to take an integrated approach that recognizes the full suite of defenses that prey employ because damaged and consumed prey may not induce all traits in the same way (i.e. behavior vs. morphology; Van Buskirk and Arioli 2002). To date, the focus has been on behavioral traits, yet biologists are becoming increasingly aware that many prey also defend themselves with inducible morphology and life history (Crowl and Covich 1990, Brönmark and Pettersson 1994, Relyea 2001a, Laurila et al. 2002). To understand how alarm cues and kairomones affect prey defenses, we need to simultaneously examine behavior, morphology, and life history.

I addressed these challenges using larval anurans (tadpoles), which are well known for their ability to alter their behavior, morphology, and life history in response to predators (Van Buskirk 2002a,b; Relyea 2001a, 2002b). I exposed grey tree frog tadpoles (*Hyla versicolor*) to a wide range of coexisting prey types that were either crushed by hand or consumed by a caged dragonfly predator (*Anax junius*) and then observed how the tadpoles altered their behavior, morphology, and growth. I used prey types that all commonly coexist so that any differences among prey types could not be explained by the coexistence hypothesis. Further, by using predator-naïve tadpoles, I prevented any potentially confounding affects of learning. I tested the following hypotheses: 1) different alarm cues should induce different phenotypes; 2) crushed and consumed prey induce different suites and magnitudes of defenses; and 3) alarm cues from closely related prey should induce stronger defenses than alarm cues from distantly related prey.

4.3 METHODS

I exposed grey tree frog tadpoles to chemical cues emitted from a factorial combination of 10 prey types experiencing two modes of prey death (crushed by hand or consumed by *Anax*) in a randomized block design. The 20 treatments were replicated five times (five spatial blocks) for a total of 100 experimental units. The 10 prey types spanned a wide range of phylogeny: no prey, grey tree frog tadpoles, spring peeper tadpoles (*Pseudacris crucifer*), wood frog tadpoles (*Rana sylvatica*), leopard frog tadpoles (*R. pipiens*), spotted salamander larvae (*Ambystoma maculatum*), damselfly nymphs (*Lestes* spp.), dragonfly nymphs (*Sympetrum* spp.; a small dragonfly species that is quite small and induces few changes as a predator (Relyea 2003a)), and two snail species (*Physa acuta* and *Stagnicola elodes*). Crossing these 10 prey types with the two modes of prey death (crushed or consumed) produced two types of controls. The first control was an empty predator cage to quantify tadpole phenotypes when no predation cues were present. The second control was a starved dragonfly nymph to quantify tadpole phenotypes when only predator kairomones were present. Although this experiment did not include a treatment of starved predators plus crushed conspecifics, subsequent experiments have confirmed that this treatment induces changes similar to starved predators alone (Schoeppner and Relyea, unpublished data).

I conducted the experiment in outdoor pond mesocosms (wading pools). Each mesocosm contained 80 L of well water, 100g of leaf litter (*Quercus* spp.), 5g of rabbit chow, and an aliquot of pond water containing algae and zooplankton. These mesocosms have been used in previous studies with great success (Relyea 2001a, 2002c). Each pool contained one predator cage (a 500 ml plastic cup covered with 1 x 2 mm mesh screen that prevented predators and prey types from escaping) that was either empty or held a single larval dragonfly. All pools were covered with 60% shade cloth lids to prevent colonization by amphibians and invertebrates during the experiment. On 30 June 2002, I added 20 predator-naïve hatchlings to each pool (haphazardly selected from a mixture of hatchlings from 32 clutches of eggs). These 32 clutches of eggs were laid in the lab by amplecting pairs of tree frogs that were collected on 16 May 2002, and then reared as tadpole in wading pools prior to the experiment. In short, the tadpoles had not been exposed to predator cues as either eggs or hatchling tadpoles.

I added the crushed or consumed prey to the pools three times per week. Equal masses of each prey type (350 mg) were either crushed by hand or fed to the larval dragonflies. Because the diets differed in individual size, the number of prey could not be held constant, but differences in prey number do not affect anti-predator responses (Schoeppner and Relyea, unpublished data). At each feeding, the consumed prey were added to the predator cages and I checked that each predator had consumed its diet. If the predator had not eaten, the uneaten prey were left in the cage and the predator was replaced. At the end of the experiment, only a few of the treatments had any uneaten prey. Because this was a small fraction of the total amount of prey fed to the predator during the experiment, these pools were not excluded. The prey used for the crushed cue treatments were first euthanized and then macerated in a blender for 30 sec. The crushed prey were then distributed evenly to the appropriate pools. To equalize disturbance during feeding, I lifted all empty cages and then returned them to the pools.

After 17d, I observed tadpole behavior (24 hrs after cue addition). For each pool, the number of tadpoles visible and the number of visible tadpoles that were active (moving) was recorded, permitting us to quantify the proportion of tadpoles observed (i.e. not hiding) and the proportion of tadpoles active. Each pool was observed ten times and I used the mean behaviors of each pool as my behavioral response variables.

After 20d, all tadpoles were removed and preserved in 10% formalin for subsequent morphological measurement. Survival was excellent across all treatments (98.23 ± 0.03%) and there was no pattern among the treatments. Tadpole morphology was measured using an image analysis system (Optimas Bioscan; Bothell, Washington, USA). I weighed each tadpole and then measured seven morphological dimensions: tail length and depth; tail muscle depth and width; and body depth, length, and width (see Fig. 1 in Relyea 2000). Because the tadpole's body is round, I placed a glass plate under the tadpole's tail in the lateral view. For simplicity, I only report on the two tadpole dimensions that most consistently respond to predators (tail depth and body length).

Because I was interested in differences in tadpole shape, I had to first correct for differences in overall size. To make the morphological dimensions size-independent, I regressed the two morphological measurements (log-transformed to improve the linearity of the relationship) against the log-transformed mass of each individual and then saved the residuals. I calculated the mean residuals from each pool and used these mean residuals as my

morphological response variables. This approach has been widely applied in past studies of morphological plasticity (Relyea 2000, 2001a, 2002c).

I analyzed all of the data in a single multivariate analysis of variance (MANOVA) that examined the effects of block, cue type (crushed or consumed), prey type, and their interactions on grey tree frog behavior, mass, and the two size-independent morphological dimensions. Block interactions were never significant; thus, I pooled the block interaction degrees of freedom with the error term. For significant univariate effects, I conducted mean comparisons using Fisher's LSD test.

To test the relationship between the grey tree frog's phenotypic responses and the phylogenetic relatedness of the different crushed and consumed prey, I used phylogenetic divergence times. For example, invertebrates diverged from chordates 990 million years ago (mya) and salamanders diverged from anurans 250 mya (Feller and Hedges 1998, Kumar and Hedges 1998). Within the anurans, ranids (wood frogs and leopard frogs) and hylids (grey tree frogs and spring peepers) diverged 100 mya (Wallace et al. 1971). Within the hylids, *Pseudacris* and *Hyla* diverged approximately 50 mya (Hedges 1986). Because some of the taxa are not phylogenetically independent (e.g., the four invertebrates, the two ranids), I averaged the values for each taxonomic group (within a block) to represent invertebrates and ranids, respectively. In short, the nine taxa were reduced to five independent taxa: grey tree frogs, peepers, ranids, salamanders, and invertebrates. Using these dates, I conducted a multivariate analysis of covariance (MANCOVA) using blocks, cue type (crushed versus consumed), and divergence date as a covariate (using log (divergence date + 10 mya)) and the tadpole activity, hiding, mass, and mean residuals for the two morphological traits as the response variables.

4.4 RESULTS

There were significant multivariate effects of block (Wilks' $F_{20,236} = 5.3$, $P < 0.001$), prey type (Wilks' $F_{45,321} = 2.9$, $P < 0.001$), cue type (Wilks' $F_{5,71} = 65.1$, $P < 0.001$), and the prey type-by-cue type interaction (Wilks' $F_{45,321} = 1.8$, $P = 0.003$). Block effects occurred for all traits (univariate tests, $P < 0.02$), likely due to block position in the field. Blocks closer to the forest edge experienced more shade, likely producing differences in periphyton which can affect the

magnitude of predator-induced phenotypes (Relyea 2002c). Importantly, the lack of a prey type-by-cue type interaction confirms that the pattern of response to the different treatments was consistent across all blocks.

The percentage of tadpoles observed in the pools was affected by prey species ($F_{9,75}$ = 5.2, $P < 0.001$) and cue type ($F_{1,75}$ = 48.4, $P < 0.001$) but not their interaction ($F_{9,75}$ = 0.9, P = 0.527; Fig. 4.1). Across all prey treatments, consumed prey caused 8% more hiding than crushed prey. Across both cue types, there was strong hiding when the treatments used grey tree frogs or spring peepers ($P < 0.001$), moderate hiding with the other amphibian species ($P < 0.01$), and little hiding with the invertebrate prey ($0.15 > P > 0.01$). Compared to grey tree frogs reared with no cues, there was a 12% increase in hiding with crushed conspecifics (P = 0.001), a 12% increase in hiding with starved predators (P = 0.004), and a 21% increase in hiding with consumed conspecifics ($P < 0.001$),

Tadpole activity was affected by prey species ($F_{9,75}$ = 3.1, P = 0.003) and cue type ($F_{1,75}$ = 26.8, $P < 0.001$) but not their interaction ($F_{9,75}$ = 0.9, P = 0.495; Fig. 4.1). Across all prey species, consumed prey induced 17% lower activity than crushed prey. Compared to the control treatment, consumed amphibians induced the largest activity reductions ($P < 0.008$) while invertebrate prey induced the smallest activity reductions ($P > 0.03$). Compared to grey tree frogs reared with no cues, I found a 10% reduction in activity with crushed conspecifics (P = 0.027), a nonsignificant 3% reduction in activity with starved predators (P = 0.460), and a 20% reduction in activity when conspecifics were fed to predators ($P < 0.001$).

Tail depth was affected by prey species ($F_{9,75}$ = 11.2, $P < 0.001$), cue type ($F_{1,75}$ = 328.3, $P < 0.001$) and their interaction ($F_{9,75}$ = 6.0, $P < 0.001$; Fig. 4.1). The interaction occurred because there were no differences among the crushed prey (univariate P = 0.109), but there were substantial differences among the consumed prey (univariate $P < 0.0001$). Compared to dragonflies consuming no prey, increases in tail depth were large when dragonflies consumed grey tree frogs and peepers ($P < 0.001$), moderate when dragonflies consumed wood frogs, leopard frogs, and salamanders ($P < 0.001$), and small when dragonflies consumed invertebrates (damselfly larvae, P = 0.002; dragonfly larvae, P = 0.022; *Stagnicola* snails, P = 0.034; *Physa* snails, P = 0.153). Compared to grey tree frogs reared with no cues, crushed conspecifics and starved predators each caused small effects on tail depth (P = 0.055 and P = 0.022, respectively)

while predators consuming conspecifics caused a five-fold larger increase in tail depth ($P < 0.001$).

Body length was affected by prey species ($F_{9,75} = 4.3$, $P < 0.001$), cue type ($F_{1,75} = 83.6$, $P < 0.001$) and their interaction ($F_{9,75} = 3.1$, $P = 0.003$; Fig. 4.1). The interaction occurred because crushed prey had no effect on body length (univariate $P = 0.789$) while consumed prey had significant effects (univariate $P < 0.001$). Compared to starved dragonflies, all consumed prey induced relatively shorter bodies ($P < 0.05$) except the invertebrate prey ($P \geq 0.05$). Compared to grey tree frogs reared with no cues, I found no effect of crushed conspecifics ($P = 0.867$) or starved predators ($P = 0.481$) but a large decrease in body length when predators consumed conspecifics ($P < 0.001$).

Tadpole mass was affected by prey species ($F_{9,75} = 4.5$, $P < 0.001$) and cue type ($F_{1,75} = 2.5$, $P = 0.116$) with a nearly significant interaction ($F_{9,75} = 1.8$, $P = 0.080$; Fig. 4.1). The marginal interaction occurred because the crushed prey had no impact on tadpole mass (univariate $P = 0.086$) whereas consumed prey had a significant impact (univariate $P = 0.002$). Compared to starved dragonflies, consumed grey tree frogs and peepers caused reductions in mass ($P < 0.04$) while the remaining consumed prey had no effect ($P > 0.2$). Compared to grey tree frogs reared with no cues, I found no effect of crushed conspecifics or starved predators ($P > 0.35$), but predators consuming conspecifics caused a 15% reduction in mass ($P < 0.001$).

When I examined the relationships between the phylogenetic distance of each prey and the grey tree frog's response, I found significant multivariate effects of block (Wilks' $F_{20,127} = 3.6$, $P < 0.001$), cue type (Wilks' $F_{5,38} = 13.9$, $P < 0.001$), divergence date (Wilks' $F_{5,38} = 9.1$, $P < 0.001$), and the cue type-by-divergence date interaction (Wilks' $F_{5,38} = 3.8$, $P = 0.007$). For the two behavioral traits (percent observed and percent activity; Fig. 4.2), the traits were affected by cue type ($P \leq 0.014$) and divergence time ($P \leq 0.01$), but not by their interaction ($P > 0.22$). For mass and the two morphological traits (tail depth and body length; Fig. 4.3), the traits were affected by cue type ($P \leq 0.003$), divergence time ($P < 0.02$), and their interaction ($P < 0.01$). For these latter three traits, I found significant effects of divergence date when the prey were consumed ($P \leq 0.002$) but not when they were crushed ($P > 0.3$).

4.5 DISCUSSION

The results of this study indicate that prey make use of the diverse information available from alarm cues and kairomones when making their defensive decisions. The phenotypic changes induced by the caged dragonfly larvae are likely adaptive. For example, increased hiding and decreased activity in response to predators are consistent with a plethora of previous studies (Kats and Dill 1998). In general, less apparent prey have increased survival due to decreased detection by predators (Skelly 1994), but this behavior comes at the cost of slower growth in predator-free environments (Harvell 1992, Skelly 1992). The increase in tail depth and decrease in body length is consistent with past studies of morphological defenses in tadpoles (Relyea 2003a, Van Buskirk 2002b). Tadpoles with relatively deeper tails and smaller bodies survive better in the presence of predators (Van Buskirk and Relyea 1998), but this phenotype experiences slower growth (Van Buskirk 2000). I observed reduced growth in my experiment, with the largest growth reductions occurring in the treatments that induced the strongest defenses. For amphibians, reduced growth is important to fitness because it results in delayed metamorphosis (which can be deadly in a drying pond), decreased size at maturity, and decreased future egg production (Berven and Gill 1983, Semlitsch et al. 1988).

Cues from crushed prey alone did not induce the same suite of defenses as cues from consumed prey. Crushed and consumed prey both induced increased hiding and decreased activity, but only the consumed prey consistently induced deep tails and short bodies. This result supports the hypothesis that the additional information provided by the simultaneous exposure to both kairomones and alarm cues allow prey to mount more complete and effective anti-predator defenses. This difference may exist because behavioral defenses are typically more easily reversed than morphological defenses (see Relyea 2003b). Thus, if alarm cues provide incomplete information about predation risk, perhaps prey use easily reversible behavioral defenses so that their defensive decision can be quickly reversed if the information turns out to be incorrect. Similarly, prey may require more complete information (alarm cues plus kairomones) before investing in defenses that are more difficult (or impossible) to reverse.

Within the subset of traits induced by both crushed and consumed prey (the two behavioral traits), crushed prey induced weaker defenses. There has been equivocal support for the importance of alarm cues alone for inducing behavioral defenses. For example, across 20

species of larval anuran, nearly half of the species did not respond behaviorally to crushed conspecifics (Wilson and Lefcort 1993, Summey and Mathis 1998). The species that did respond were distributed across three families, suggesting that the lack of response is not limited to the loss of alarm cues in one family. Moreover, the equivocal impact of crushed prey on prey behavior is also found in other taxa including *Daphnia* (Stirling 1995, Pijanowska 1997), snails (Alexander and Covich 1991, Turner 1996), and sea urchins (Parker and Shulman 1986, Hagen et al. 2002). Collectively, these data suggest that while crushed prey can induce some phenotypic changes, the changes are often restricted to behavioral traits and the magnitude of the change is frequently small compared to the magnitude induced by consumed prey.

If prey simply detect and respond to kairomones and alarm cues, the response to the consumed cues should be equivalent to the additive combination of the responses to the crushed cues alone and the predator kairomones alone. My data indicated that responses to consumed conspecifics are more than additive for morphology and growth. From these data, one cannot determine if the synergism is simply the result of encountering both cues simultaneously, or if there is something about consuming the prey in and of itself that causes the synergy. For example, the latter scenario could occur if actual predation produces compounds that are not produced by starved predators (i.e. digestive enzymes or digested prey tissues; Stabell et al. 2003). Further studies are needed to identify the mechanism responsible for the synergistic responses.

The fundamental difference between cues from crushed and consumed prey also can be found in my analysis of alarm cue phylogeny. The phylogenetic-relatedness hypothesis predicts that an organism's defensive responses will be strong when closely related prey are killed but weak when distantly related prey are killed (Chivers and Smith 1998, Chivers and Mirza 2001). The decrease in the magnitude of response with phylogenetic relatedness could arise from one of two mechanisms: 1) more distantly related prey do not release the same chemicals; or 2) predation on more distantly related prey communicates a decreased risk of predation (due to predator search images; Persons et al. 2001). This hypothesis appears to have never been tested across a wide range of prey relatedness. For the two behavioral traits, I found support for the hypothesis when the prey were either crushed or consumed. While a number of behavioral experiments have examined the impacts of different alarm cues, past experiments have not used both closely related (within the same order) and distantly related prey. In my study, all

consumed amphibians induced strong responses while the insect and snail prey induced weak (or no) response. For mass and the two morphological traits, I also found support for the phylogenetic relatedness hypothesis, but only when prey were consumed (crushed prey never induced any morphological changes). There have been very few studies of predator diet on morphology and mass (Brönmark and Pettersson 1994, Stabell et al. 2003) and no previous tests of the phylogenetic hypothesis. My results provide the first extensive evidence that prey responses to different alarm cues (from a group of coexisting prey) can follow a strong phylogenetic pattern. More studies are needed to determine the generality of this pattern in other species. While several authors have stated that fish respond more strongly to alarm cues from closely related fish than from distantly related fish (Smith 1982, Mathis and Smith 1993, Stabell and Lwin 1997), no study to date has tested the hypothesis using a large number of coexisting diets that span a range of phylogenetic relatedness.

4.5.1 Conclusions

The use of environmental cues is critical for organisms to exhibit adaptive plasticity, yet for organisms with predator-induced defenses we know relatively little about the complexity of the chemical cues that are used. My results suggest that the chemical cues associated with predation are complex, but not without pattern. Despite the fact that many researchers use crushed prey as surrogates of predation (reviewed in Chivers and Smith 1998), it appears that the cues emitted by damaged or crushed prey can be fundamentally different from the cues emitted by consumed prey; crushed prey frequently do not induce the full suite or magnitude of traits that are induced by consumed prey. In such cases, prey have apparently evolved a reliance on both alarm cues and kairomones. However, this is not to say that alarm cues are unimportant. When alarm cues are combined with the kairomones, they can have large impacts on the induced defense. This reliance may have evolved because alarm cues alone provide no information about which predator is present and kairomones alone (i.e. from starved predators) provide no information about which prey species are being killed by the predator (which may be critical information when predators preference changes over time). This research underscores the importance of simultaneously examining the impacts of crushed and consumed prey across a wide range of phylogeny and a diversity of traits. With this approach, we can better arrive at generalizable

patterns as to how prey obtain information from their environment and make their phenotypically plastic decisions.

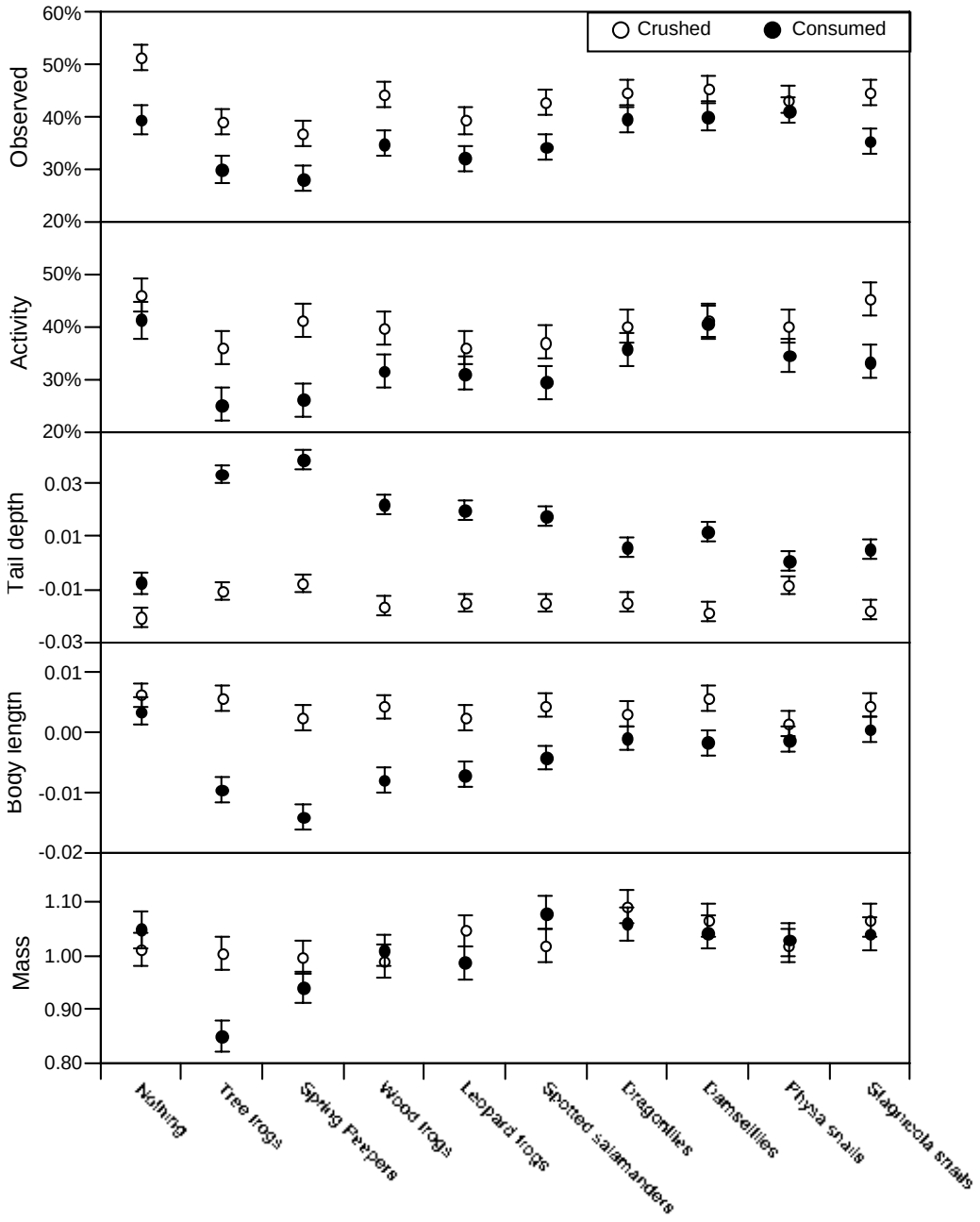

Figure 4.1 Behavior, relative morphology, and mass of larval grey tree frogs (mean residuals + 1 SE) exposed to chemical cues from crushed (open symbols) or consumed (filled symbols) diets from a wide range of phylogeny. For crushed diets, the treatment termed "nothing" indicates a cue-free environment. For consumed diets, the treatment termed "nothing" indicates a starved-predator environment. Relative morphology was calculated by regressing the log-transformed dimensions of all individuals against their log-transformed mass and then saving the mean residuals from each pool.

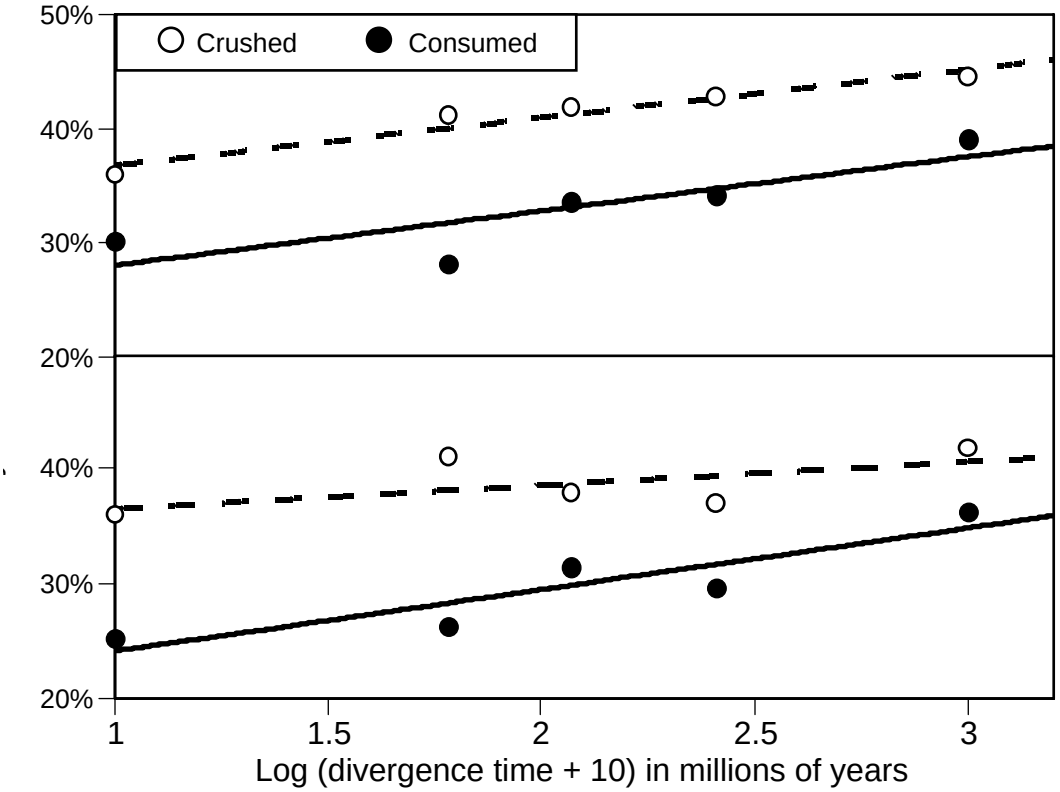

Figure 4.2 The relationship between phenotypic responses of grey tree frog tadpoles and the phylogenetic distance of either crushed prey (open symbols, dashed lines) or consumed prey (closed symbols, solid lines) for the behavioral traits. The analysis was based upon 50 experimental units but only the 10 treatment means are plotted to provide graphical clarity.

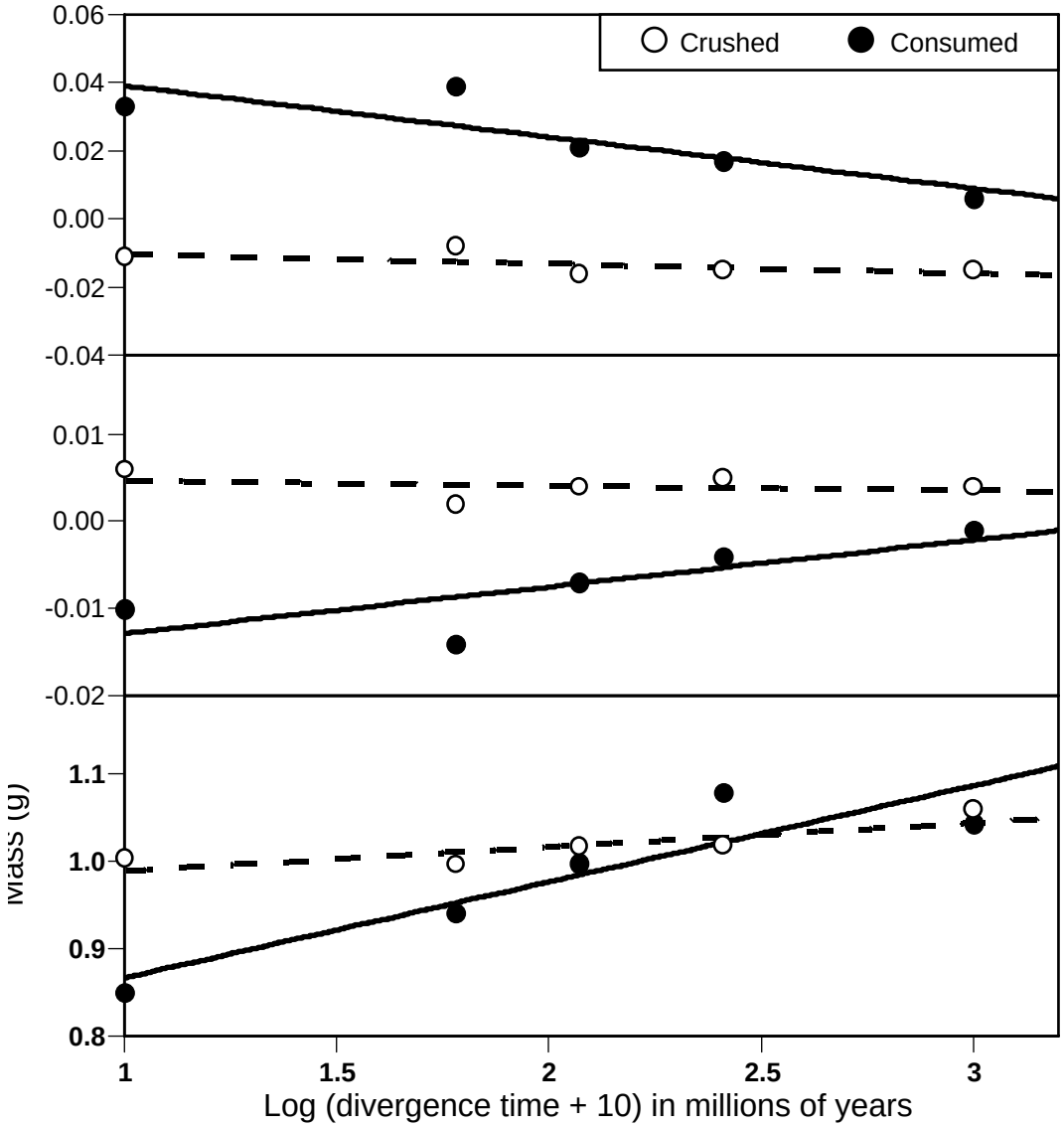

Figure 4.3 The relationship between phenotypic responses of grey tree frog tadpoles and the phylogenetic distance of either crushed prey (open symbols, dashed lines) or consumed prey (closed symbols, solid lines). The analysis was based upon 50 experimental units but only the 10 treatment means are plotted to provide graphical clarity.

5.0 WHEN SHOULD PREY RESPOND TO HETEROSPECIFIC ALARM CUES? TESTING THE MECHANISMS OF PERCEIVED RISK

5.1 ABSTRACT

Inducible defenses have been studied from a diverse array of perspectives with a major focus on how prey use environmental cues in making their phenotypic decisions. In aquatic systems a long-standing question is why chemical cues from different diets consumed by the same type of predator induce strong responses while others induce weak responses or no response at all. In a previous study I showed that the magnitude of the response was related to the phylogenetic relatedness of the predator's diet to the unconsumed prey; where cues from closely related species induced strong responses and distantly related species induced weaker responses. In this study I performed a behavioral assay to determine if the strong responses to the closely related diets was due to 1) similarity of alarm cues among closely related species or 2) shared risk among coexisting organisms that share a common predator. I compared the behavioral defenses of grey tree frog tadpoles (*Hyla versicolor*) to cues from a dragonfly nymph (*Anax junius*) that consumed one of seven diets that span a wide range of phylogenetic relatedness and coexist with grey tree frogs to one diet that is closely related to grey tree frogs but has an allopatric range. Consistent with previous results, I found that the tadpoles could discriminate among the predator diets and that the magnitude of behavioral response was strongly related to phylogenetic relatedness but not to coexistence. In addition, differences in the responses to different predator diets were not due to differences in prey size when diet mass was held constant. Collectively, these data suggest that prey are quite proficient at discriminating among predator diets and that different predator diets induce different magnitudes of defense not due to differences in prey size or prey coexistence, but due to the changes in the chemical composition of prey alarm cues as the diets increase in phylogenetic distance from the target.

5.2 INTRODUCTION

Prey use a wide range of behaviors to decrease their encounter rates with predators and hence increase their survival (Lima and Dill 1990). Because the use of anti-predator behavior often comes at the cost of reduced growth and reproduction compared to individuals that do not display the behavior (Sih 1980, Anholt and Werner 1995), theory predicts that organisms should exhibit responses that are commensurate with the magnitude of predation risk faced (Helfmann 1989). To accomplish this, prey must be able to perceive their current predation risk and balance the cost of defense with other requirements (e.g., foraging or finding a mate). While many organisms use environmental cues (chemical, mechanical, visual) to detect their predators and make decisions about allocation to defensive behaviors (Tollrian and Harvell 1999, Venzon et al. 2000), we still lack an understanding of what aspects of the cues are important in communicating information about predation risk.

In aquatic systems, prey generally assess predation risk via chemical cues released by both the predator and the consumed prey (Larson and Dodson 1993). Predators produce chemicals, termed kairomones that the prey use to determine the species of predator against which they must defend themselves. The specificity of kairomones has been confirmed by showing that different predator species induce different behavioral responses, either in the magnitude or type of traits induced (Turner et al. 1999, Dewitt et al. 2000, Relyea 2001a,b). Prey also respond to chemicals from other injured prey, termed alarm cues, which are released when predators capture and consume prey. Prey typically exhibit the strongest behavioral defenses when the predator consumes a diet of conspecific prey (Wilson and Lefcort 1993). However, when predators consume heterospecific prey, researchers have observed that the prey's behavioral defenses vary in strength (Smith 1992, Chivers and Mirza 2001). To explain the wide range of responses to different predator diets, two hypotheses have been proposed: ecological coexistence and phylogenetic relatedness.

The ecological coexistence hypothesis posits that prey should respond strongly to alarm cues from coexisting heterospecifics and weakly to alarm cues from non-coexisting heterospecifics (Chivers and Mirza 2001). This hypothesis assumes that responses to heterospecific cues are a result of natural selection favoring the ability to detect and respond to alarm cues that communicate information about a shared predator (i.e. if you are being eaten, I

am in danger too). Under this scenario, the predator consumes the two prey species with similar probabilities and, therefore, alarm cues from the consumed heterospecifics are interpreted as representing a level of risk that is similar to cues from consumed conspecifics. A number of studies have supported this hypothesis by demonstrating that coexisting prey (that are relatively closely related) induce similarly strong defenses (Chivers and Smith 1998, Mirza and Chivers 2003, Mirza et al. 2003). However, few studies have examined whether closely related, non-coexisting prey also induce strong defenses. Such a result would allow us to reject the ecological-coexistence hypothesis.

The phylogenetic-relatedness hypothesis posits that prey should respond strongly to alarm cues from closely related heterospecifics while alarm cues from distantly related heterospecifics should induce weaker responses regardless of coexistence (Parker and Shulman 1986, Mathis and Smith 1993, Sullivan et al. 2003, Schoeppner and Relyea 2005). This hypothesis is based upon the premise that closely related prey produce similar chemical alarm cues and, therefore, induce similar behavioral defenses. Therefore, alarm cues from two species that are allopatric but of similar phylogenetic-relatedness to a target species should induce similar behavioral defenses in the target. In one of the most extensive tests of the phylogenetic-relatedness hypothesis to date, I exposed grey tree frog tadpoles (*Hyla versicolor*) to chemical cues from a wide range of predator diets including conspecifics, several anuran diets, one caudate, two insect nymphs, and two snails. I found that as predicted by the phylogenetic-relatedness hypothesis, chemical cues from consumed conspecifics induced the strongest responses in tadpole behavior and morphology, and that the magnitude of the response decreased as phylogenetic-relatedness of the diet to the tree frog tadpoles decreased (Schoeppner and Relyea 2005). However, because all of the diets used in that study commonly coexist with the grey tree frog, cues from the closely related diets (i.e. amphibians) may be inducing strong responses either because 1) closely-related heterospecifics emit similar alarm cues or 2) heterospecifics emit different alarm cues but the prey have evolved the ability to detect those cues because organisms that present a similar search image to a common predator share a similar level of risk. Therefore, to definitively distinguish among the ecological coexistence hypothesis and the phylogenetic relatedness hypothesis we need to expose prey to diets that both do and do not coexist with the responding species.

When we examine different predator diets, it is critical that we feed the same mass of prey to the predators because the amount of chemical cues being released likely depends on the mass of prey consumed (Van Buskirk and Arioli 2002). However, even when prey mass is controlled, there can be differences in the number of individuals that compose each diet due to differences in the size of different prey species (e.g., Smith and Belk 2001). If alarm cues are contained in the skin as in many fish species (Hara 1993, Smith 1992) and toads (Hews 1988), then differences in prey number could be important because a larger number of small individuals would contain a greater surface:mass ratio, producing more chemical cue per unit mass and a stronger anti-predator response. The impact of prey number (while holding diet mass constant) appears to have never been tested. Without knowing if differences in the number of prey consumed affects the magnitude of the observed behavioral responses we cannot rule out the possibility that any difference among diets is due to differences in the amount of cue detected. Therefore, we need to know how differences in the number of items consumed affect prey behavior.

I addressed these issues in a series of laboratory experiments in which I exposed grey tree frog tadpoles (*Hyla versicolor*) to a variety of treatments where I fed larval dragonflies several different diets. I quantified grey tree frog activity in each environment and tested the following hypotheses: 1) prey can discriminate among a wide range of predator diets; 2) behavioral defenses will be strong when predators consume closely related prey and weak when predators consume distantly related prey; 3) behavioral defenses will be strong when predators consume coexisting prey and weak when predators consume non-coexisting prey; and 4) prey responses to predator diets are affected by the number of items in the diet (while controlling for total diet mass).

5.3 METHODS

I performed two experiments that addressed how prey respond to different predator diets. The first experiment tested how grey tree frog tadpoles responded to predator diets that varied in phylogeny and coexistence (the "mechanism experiment"). The second experiment tested how

grey tree frog tadpoles were affected by the number of prey items that the predator consumed (the "prey-number experiment").

5.3.1 The mechanism experiment

I exposed grey tree frog tadpoles to chemical cues from caged dragonfly naiads that consumed different species of prey. I employed a randomized block design in which I exposed tadpoles to nine different treatments replicated 10 times for a total of 90 experimental units across two spatial blocks (5 reps/ experimental shelf in the laboratory). The nine treatments consisted of 1) no predator, 2) a caged starved predator, 3) caged dragonflies fed one of six diets that coexist with grey tree frog larvae (conspecifics; spring peeper larvae, *Pseudacris crucifer*; wood frog larvae, *Rana sylvatica*; spotted salamander larvae, *Ambystoma maculatum*; libellulid dragonfly naiads, *Sympetrum internum*; and freshwater snails, *Physa acuta*), and 4) caged dragonflies fed a diet that does not coexist with grey tree frog larvae (Pacific tree frog larvae; *Pseudacris regilla*). The starved predator treatment was included to control for the effect of the predator alone on tree frog behavior. Pacific tree frogs were chosen as a diet in this experiment because they are closely related (confamilial) to grey tree frogs but the two species have allopatric ranges. Pacific tree frogs are restricted to the west coast from British Columbia south through California extending east into Montana, Idaho, and Nevada while grey tree frogs are found on the east coast from south Ontario through north Florida and extending west into Manitoba, Oklahoma, and central Texas (Behler and King 1991). Based on these current distributions, it is unlikely that these species have coexisted during the last 20,000 years (i.e. since the beginning of the last ice age).

I conducted the experiment in the laboratory using 90 10-L plastic tubs filled with 7 L of filtered well water. I obtained grey tree frog tadpoles by collecting 18 pairs of amplected frogs from Mallard Pond (Crawford County, PA) on 11 May 2004 and allowing them to oviposit in laboratory tubs containing aged well water. To ensure that the hatchling tadpoles were kept predator-naïve, I reared them outdoors in covered wading pools and fed them rabbit chow *ad libitum*. From these hatchlings, I haphazardly selected 10 tadpoles for each tub (initial mean mass ± 1 SE = 105 $\pm$ 6 mg). Each tub also contained a 250-mL opaque plastic cup covered with a mesh screen, which served as the predator cage. For tubs assigned a predator treatment, I

added a single late-instar dragonfly naiad (*Anax junius*) and fed each predator 300 ± 10 mg of the assigned diet; for the no-predator control treatment, I added an empty cup. To protect against problems of predators emitting chemical cues from different diets consumed prior to the experiment, I fed the assigned diets to the dragonflies for at least two weeks prior to the start of the experiment. Dragonflies assigned the "starved" treatment were not fed for one week prior to the experiment.

I added the tadpoles and caged predators to the tubs on 11 July 2004. I observed tadpole activity the next morning between 1000 and 1200 hrs. I used scan sampling where I counted the number of tadpoles moving in each tub to determine tadpole activity (Altmann 1974). I performed 10 observations on each tub and used the mean proportion of active tadpoles as my response variable. I analyzed tadpole activity using an analysis of variance (ANOVA) in which I looked for the effect of predator treatment and block on tadpole activity. Differences among treatment means were compared using Fisher's LSD test.

I performed two linear regressions using the treatment means for each experimental unit to test the hypothesis that the mean activity induced by each diet was positively correlated with the time since divergence of each diet species with grey tree frogs. I included all of the coexisting diet species in the first regression but replaced the spring peeper diet with the Pacific tree frog diet (both in the genus *Pseudacris*) in the second regression to determine if diet coexistence affected the phylogenetic correlation. My data would support the phylogenetic relatedness hypothesis if replacing the coexisting diet with a non-coexisting diet of similar phylogenetic relatedness produced a similar correlation. The divergence dates were taken from the literature: invertebrates diverged from chordates 990 million years ago (mya, Kumar and Hedges 1998) and salamanders diverged from anurans 250 mya (Feller and Hedges 1998). Among the anuran diets, I assumed that hylids diverged from ranids 100 mya (Wallace et al. 1971) and that *Pseudacris* and *Hyla* diverged 50 mya (Hedges 1986). Prior to the analysis, I log-transformed the divergence dates (log (divergence date + 10 mya)) to improve the linearity of the relationship between time since divergence and tree frog tadpole activity.

5.3.2 The prey-number experiment

The prey-number experiment tested how a target species (grey tree frog tadpoles) responded when a predator consumed a diet of small, medium, or large prey of a given taxa (while controlling for total diet mass). These experiments followed a protocol similar to the mechanism experiment. Predators were fed similar masses (within each diet species) of either 1 large, 3 medium, or, 5 small tadpoles of three species: wood frogs (total diet mass ± 1 SE; 656 ± 5, 703 ± 7, and 694 ± 30 mg, respectively), leopard frogs (975 ± 7, 917 ± 7, and 955 ± 38 mg, respectively), and grey tree frogs (677 ± 5, 687 ± 9, and 708 ± 25, respectively). The initial mean mass ($\pm$ 1 SE) of the target tadpoles was 94 $\pm$ 11 mg. There were a total of 10 treatments (nine diets plus a no-predator control) replicated eight times for a total of 80 experimental units. Behavioral observations were taken between 1000 and 1200 hrs. on 25 July 2001. I analyzed tadpole activity using an analysis of variance (ANOVA) in which I looked for the effect of predator treatment and block on tadpole activity. Differences among treatment means were compared using Fisher's LSD test.

5.4 RESULTS

5.4.1 The mechanism experiment

The ANOVA found a significant effect of the treatments ($F_{8, 71} = 19.8$, $P < 0.0001$) but no significant block effect ($F_{1, 71} = 1.1$, $P = 0.269$) or block-by-treatment interaction ($F_{8, 71} = 1.4$. $P = 0.227$), therefore the block and block-by-treatment degrees of freedom were pooled into the error term. The strongest reduction in activity occurred when predators consumed any of the amphibian diets (Fig. 5.1). There were no differences among these amphibian diet treatments ($P > 0.125$), but the treatments had significantly lower activity than the no-predator treatment, the starved-predator treatment, and both the insect and snail diets ($P \leq 0.006$). The libellulid diet induced an intermediate tadpole activity that was weaker than the response to the amphibian diets ($P \leq 0.006$) but stronger than the response to the snail or starved dragonfly diets ($P \leq$

0.031). Both the freshwater snail diet and starved-predator treatment were not different from the no-predator control ($P \geq 0.368$).

Both regressions showed a significant positive correlation between time since divergence and tadpole activity (Fig. 5.2). The two analyses, using either the Pacific tree frog diet (which does not coexist; $P < 0.001$, $r^2 = 0.39$, $N = 60$) and the spring peeper diet (which does coexist; $P < 0.001$, $r^2 = 0.39$, $N = 60$), both showed that the tadpoles responded with weaker behavioral changes as phylogenetic relatedness increased regardless of congeneric species coexistence.

5.4.2 The prey-number experiment

In the prey-number experiment, there were significant block ($F_{9, 58} = 7.0$, $P < 0.001$; due to temperature differences among shelf heights) and treatment effects ($F_{9, 58} = 19.7$, $P < 0.001$) but no significant block-by-treatment interaction ($P = 0.254$). All predator diets induced lower activity than the no-predator control ($P < 0.0001$; Fig. 5.3). However, there were no differences among predators consuming one large, three medium, or five small size tadpoles either between or within any of the species treatments ($P > 0.05$).

5.5 DISCUSSION

My results provide evidence that the magnitude of a prey's response to alarm cues can be predicted by the phylogenetic relatedness of the prey to the responding species regardless of coexistence. In this experiment, chemical cues from consumed Pacific tree frog tadpoles (which are allopatric to grey tree frogs) induced activity reductions that were equivalent to those induced by the alarm cues from all of the coexisting amphibian species. These results are consistent with a previous experiment that supported the predictions of the phylogenetic-relatedness hypothesis but only included diets that coexisted with the target tadpole species (Schoeppner and Relyea 2005). In both studies, the experiments were performed on predator-naïve targets, indicating that the tadpole responses are innate. Because the Pacific tree frog diet induced strong defenses that were similar to the congeneric spring peeper diet, my results suggest that the cues detected by the

tadpoles were similar among all amphibians and therefore induced similar responses (a prediction of the phylogenetic-relatedness hypothesis) while the cues from the insect and snail diets must have been different from the amphibian diets and therefore induced weaker responses. Because I was able to include only one diet that did not coexist with the target species, further studies that include multiple diets that do not coexist should be conducted to ensure that this result is a general phenomenon and not unique to the taxa I used.

While I found strong support for the phylogenetic-relatedness hypothesis, using tadpoles as my model system, other studies have found results that do not support this hypothesis. For example, Parker and Shulman (1986) found that the induction of hiding behavior in seven species of sea urchins in response to damage-released alarm cues was not consistent with either the phylogenetic-relatedness or ecological-coexistence hypotheses. Cues from more closely related sea urchins and sea urchins that share similar habitats did not always induce hiding. Similarly, in an experiment using fish, damage-released cues from closely related heterospecifics did not induce behavioral responses while damage-released cues from more distantly related species did induce behavioral responses (Commens and Mathis 1999, Chivers et al. 2000). While these results could indicate that phylogenetic relatedness is not a consistent predictor of prey responses to heterospecific cues, there are several methodological differences between my experiment and previous work addressing the role of phylogenetic relatedness that may account for the differences among the findings (including diet identity, cue type, target experience, and degree of relatedness among the diets).

The equivocal nature of the support for either hypothesis may be explained in part by the identity of the diets chosen. Some alarm cues may provide "mixed information" if the diet used is also a predator or potential prey of the target (Petranka 1989, Wildy et al.1999, Mirza et al 2003, Sullivan et al 2003). If the diet is a predator of the focal species, the focal species may detect and respond to both alarm cues and kairomones from the diet and, as a result, respond strongly regardless of phylogenetic relatedness (Mirza et al 2003, Sullivan et al. 2003). If the diet is a potential prey item for the focal species, the focal species may respond to the cues by increasing its foraging rather than exhibiting anti-predator behavior (Petranka 1989, Wildy et al. 1999). In the current study, one of the distantly related diets, the *Sympetrum* dragonfly naiad, is a small predator that can only consume small tadpoles and induce very weak tadpole defenses (Relyea 2003a). I found that tadpoles responded to the *Sympetrum* diet with an activity level that

was lower than the no-predator, starved-predator, and snail treatments but higher than any of the amphibian diets. Because this dragonfly naiad is also sometimes a tadpole predator, I cannot rule out that a small part of the activity reduction could have been caused by kairomones from *Sympetrum* in addition to the alarm cues from *Sympetrum*. This illustrates the importance of only using diets that do not also emit cues that can serve as kairomones or food cues to the target prey when we evaluate the evidence supporting either the phylogenetic-relatedness or ecological-coexistence hypothesis.

When considering the evidence for the phylogenetic-relatedness hypothesis, we must consider whether experiments were performed using damaged prey or consumed prey. For example, the phylogenetic-relatedness hypothesis is well-supported in tadpole studies (Wilson and Lefcort 1993, Laurila et al 1997, Schoeppner and Relyea 2005) but not well-supported in fish studies. However, most amphibian studies use cues from consumed diets whereas most fish studies use cues from crushed fish skin (Chivers and Mirza 2001). This difference in protocol may be critically important because crushed prey can induce much weaker anti-predator behaviors, making responses to crushed prey much more difficult to detect (Schoeppner and Relyea 2005). To determine whether the lack of consistency across taxa is due to taxonomic group or experimental protocol, we need to conduct experiments in other taxonomic groups, which examine the effects of cues from crushed and consumed diets concurrently.

The tadpoles used in my study were predator naïve; however, many experiments that document prey defensive behaviors in response to chemical cues have used wild-caught target species. Numerous studies have shown that prey can learn to respond strongly to heterospecific cues once the target has simultaneously encountered the heterospecific and conspecific alarm cues (Chivers and Smith 1994, Chivers et al. 1996,Wisenden and Millard 2001, Mirza and Chivers 2001a, Chivers et al. 2002). When animals can learn to associate alarm cues from heterospecifics with predation risk, the behaviors of experienced prey no longer reflect previous selection for responses to cues that reliably predict risk. It is likely that many organisms respond innately to some cues (i.e. cues from conspecifics and heterospecifics that have cues that are structurally similar) while they learn to respond to other cues (cues from heterospecifics that are also consumed by a common predator), and both types of responses are important in producing effective anti-predator defenses. Therefore, in evaluating the phylogenetic-relatedness or the ecological coexistence hypotheses we must recognize that these two types of responses (innate

and learned) generate different predictions about the expected patterns of responses. The phylogenetic-relatedness hypothesis predicts that prey should respond innately to cues from heterospecifics and any cue that is structurally similar to the conspecific alarm cue. If prey learn that other cues communicate information about predation risk, then the innate pattern of responses may be obscured when experienced organisms are tested. Another concern when using experienced targets is that prey that have been in contact with predation cues prior to the experiment may have formed morphological defenses and because they are already defended no longer use behavioral defenses (Relyea 2003b). Therefore, it is crucial that we understand how experience affects prey responses to chemical cues and consider the effect of such experience on the predicted behavioral responses.

When testing the phylogenetic-relatedness hypothesis, we must also consider our subjective definitions of "closely related" versus "distantly related." For example, in studies of fish responses to predators eating a variety of heterospecifics (different species of fish), diets that diverged approximately 250 mya relative to the focal species have been classified as distantly related (Commens and Mathis 1999, Mirza and Chivers 2001b, Mirza et al. 2001). However, in studies of amphibian responses to predators eating a variety of heterospecifics (from amphibians to invertebrates), diets that diverged 250 mya relative to the focal species have been classified as closely related (i.e. the amphibian diets) whereas diets that diverged 900 mya have been classified as distantly related (i.e. the invertebrate diets; Schoeppner and Relyea 2005). A strong response to the heterospecific diet would be interpreted as rejecting the phylogenetic-relatedness hypothesis under the first scenario, but supporting the phylogenetic-relatedness hypothesis under the second scenario. This highlights the fact that if a study focuses on a narrow range of relatedness it can miss the point at which the alarm cues become dissimilar and the prey responses weaken. For example, if I had only considered the amphibian diets in this study I would have concluded that predator diet had no effect on prey behavior. Therefore, a rigorous evaluation of the phylogenetic-relatedness hypothesis requires that diets span a wide range of relatedness based on a standardized measure (such as divergence time) making comparisons among studies more meaningful.

Finally, when we conduct experiments in which we examine prey responses to different predator diets, we make an implicit assumption about the amount of cue produced by each diet. For example, in this study, I assumed that providing the predator with an approximately

equivalent mass of each diet resulted in approximately the same amount of alarm cue being released in each treatment. The chemical nature of alarm cues is known or has been proposed for only a few species (Smith 1992, Pfeiffer et al. 1985, Brown et al. 2000, Brown et al. 2001) and it is often thought that the alarm cues are located in prey skin (at least in fishes; Hara 1993, Mirza et al. 2001). If this were generally true in other species, one would predict that a diet of smaller individuals would produce more cues than a diet of larger individuals of the same species (due to a higher surface area:mass ratio). I found no evidence that the number of individuals in a diet matters (when total diet mass is controlled). My results suggests that either the alarm cues of tadpoles are not contained in the skin, that the smaller tadpoles produce less cue per unit surface area compared to larger tadpoles (thus equalizing the total amount of cue produced), or that the tubs were saturated with cues in all treatments (thus, preventing any behavioral differences from being expressed). While additional tests are needed to verify that my results are not due to cue saturation, they do support the hypothesis that the differences observed in the mechanism experiments are due to differences in the cues released by different diets and are not confounded by differences in the number of prey fed to the predator.

5.5.1 Conclusions

While many studies have shown that chemical cues from heterospecific prey can alter prey behavior and morphology, we still do not understand why some heterospecific cues induce strong behavioral defenses while others do not. This ambiguity likely results from both differences in methodology among experiments and in differences among species in their perception and response to chemical cues. Future experiments that strive to determine the role of phylogenetic relatedness and ecological coexistence in prey responses to heterospecific alarm cues should be conducted using predator-naïve animals and cues from consumed prey. Ultimately, we need to understand more about the chemical nature of alarm cues and predator kairomones before we can understand how chemical cues communicate information about predation risk to prey.

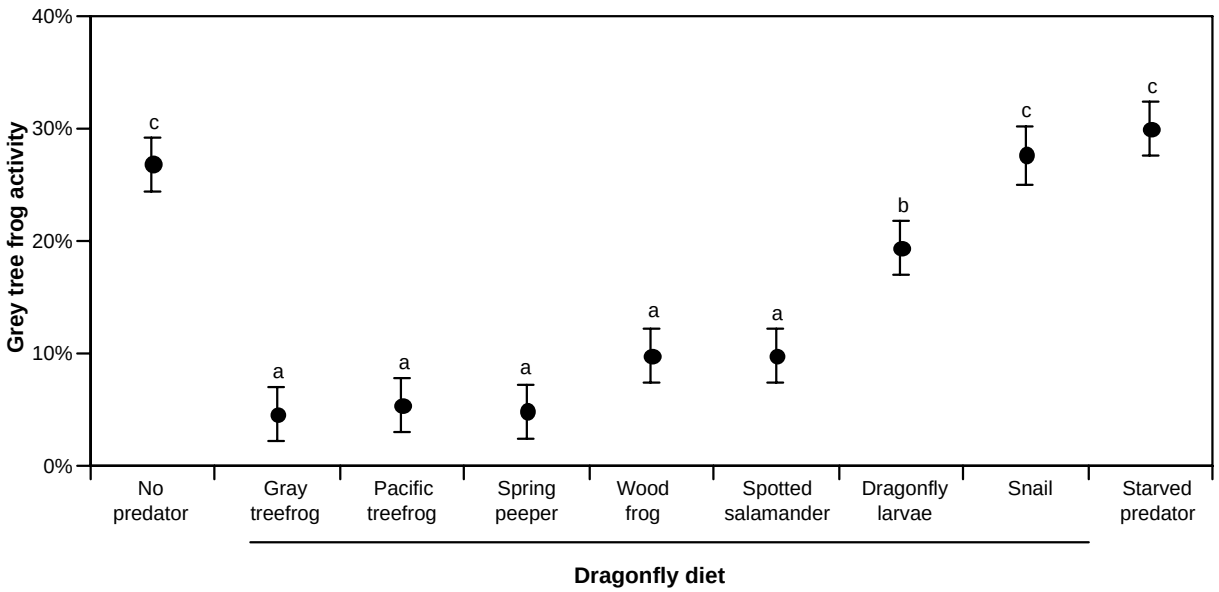

Figure 5.1 Activity of grey tree frog tadpoles in response to no predator cues, cues from a starved predator, or cues from caged dragonflies that had consumed one of seven different diets. Data are means + 1 SE and different letters indicate significantly different means based on Fisher's LSD.

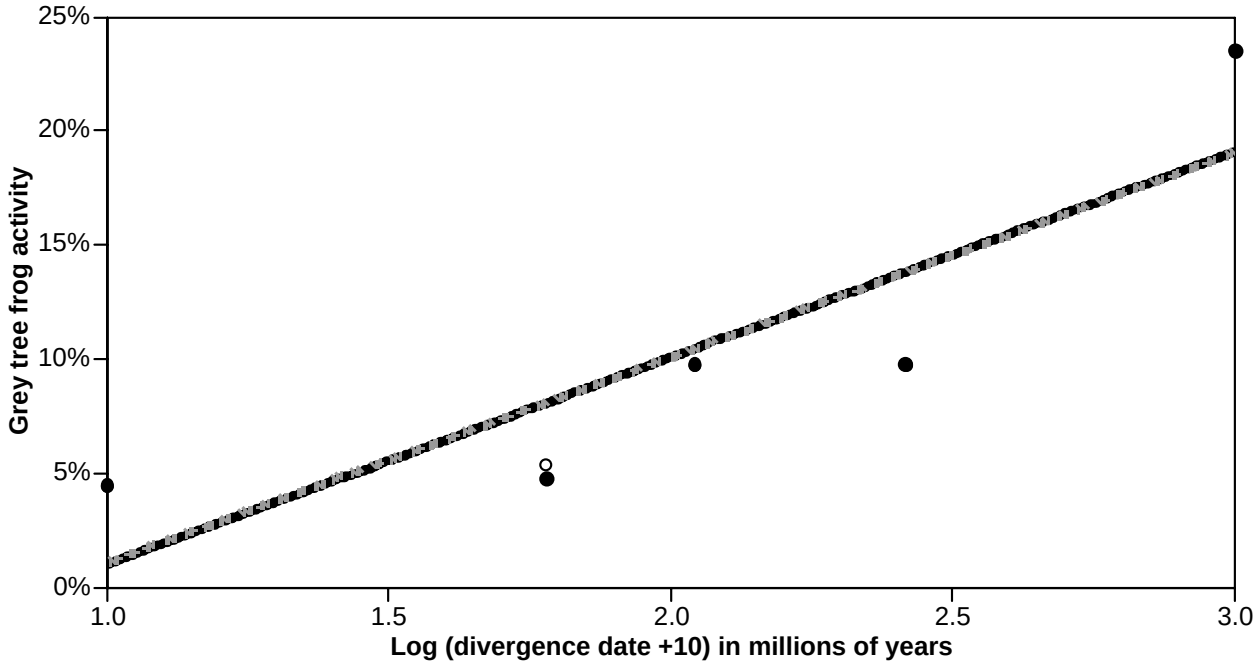

Figure 5.2 A regression of divergence time of the different predator diets and the corresponding activity of grey tree frog tadpoles. Divergence dates of the prey type from grey tree frogs were taken from the literature. The solid line shows the results of a regression including only the coexisting species whereas the dashed line shows the regression when the spring peeper (*Pseudacris crucifer*, closed circle) is replaced with a non-coexisting diet of similar phylogenetic divergence (Pacific tree frogs, *Pseudacris regilla*, open circle).

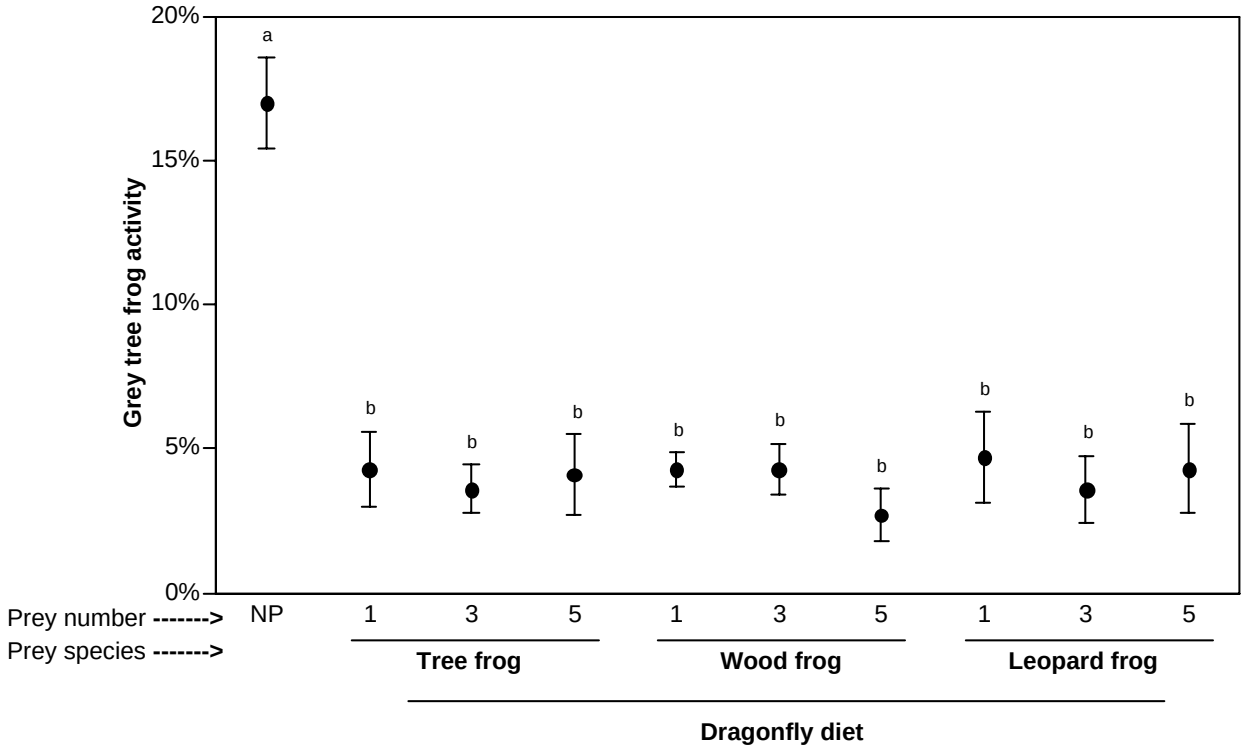

Figure 5.3 Activity of grey tree frog tadpoles exposed to larval dragonflies fed different numbers of each of three diets (trees, woods, leopards) while holding total mass of the diet constant within each species. Different letters indicate significantly different means based on Fisher's LSD. Data are means $\pm$ 1 SE.

6.0 DISSECTING THE CHEMICAL CUES OF PREDATION

6.1 ABSTRACT

Phenotypic plasticity is thought to evolve when organisms have reliable cues about changing environments. In aquatic systems, prey often use chemical cues that are produced when predators consume prey. The cues that the prey use to detect predators could potentially be coming from two sources 1) the predator could be emitting a chemical that the prey can detect (i.e. a kairomone) and 2) the prey could be releasing chemicals when tissue is damaged (i.e. alarm cues). Previous work has shown that alarm cues and kairomones from starved predators alone often induce weaker responses in fewer traits when compared to the phenotypes expressed in response to cues from consumed prey. However, a mechanistic understanding of why the cues have synergistic effects is lacking. For example, alarm cues from damaged prey and kairomones from starved predators may induce weaker defenses because the prey simply lacks the information provided by the missing cue when either is encountered alone. Alternatively, the increased response to cues from consumed prey may be due to some aspect of predator digestion. To address this question I exposed leopard frog tadpoles (*Rana pipiens*) to nine treatments consisting of either alarm cues from crushed prey, kairomones from starved predators, kairomones from predators digesting conspecific or heterospecific diets, and combinations of these cues. My results indicate that the cues that prey use to detect their predators are specific to the digestion of the prey and that kairomones are not constitutively released by the predator. Additionally, I found that cues released during prey consumption and digestion both contribute to the induction of the defended phenotypes that are exhibited in response to cues from consumed prey. Future work should focus on determining how the dynamics of predator digestion and predator behavioral decisions affect the combinations of cues that the prey encounter and the defensive phenotypes that are produced.

6.2 INTRODUCTION

A well-established paradigm in ecology and evolution is that the combination of traits that an organism possesses will determine the outcomes of interspecific interactions (Werner 1992, Wootton 1994, Abrams 1995). However, few organisms experience a single environment throughout their lifetime, which means that individuals that display a single fixed phenotype will be "mismatched" to their environment during part of their life. A common response to environmental variation is phenotypic plasticity, where an organism can alter its phenotype in response to environmental cues and thereby improve its performance in the prevailing environment (West-Eberhard 2003). Whether organisms employ fixed or plastic phenotypes is thought to depend upon the balance of the costs, benefits, and limits of plasticity and the proportion of time that the organism spends in a particular environment (Scheiner 1993, DeWitt et al. 1998, Pigluicci 2001). For individuals that do employ plastic strategies, the magnitude and specificity of the response depends upon how quickly traits can change relative to the speed of the environmental change (Padilla and Adolph 1996), resource availability (Relyea 2004), and the amount of information that the organism can collect about the environment (Sih 1992, Moran 1992, Burks and Lodge 2002). When the environment doesn't change too frequently and resources are not limiting, the magnitude of the induction and the specificity of the plastic response (i.e. the number and combination of traits induced) should increase with amount of information available about the environment.

Inducible defenses are a well-studied area of phenotypic plasticity, where environments containing predators induce changes in the behavior, morphology, and life-history traits of the prey. In aquatic systems, chemical cues have been identified as an important source of information about predation risk for a wide range of taxa including algae (Hessen and Van Donk 1993, Lampert et al 1994), ciliates (Kuhlmann and Hackmann 1985, Kusch 1993), rotifers (Gilbert and Stemberger 1984, Stemberger and Gilbert 1987), bryozoans (Harvell 1986), cladocerans (Krueger and Dodson 1981, Dodson 1989, Tollrian 1993), gastropods (Crowl and Covich 1990), amphibians (Petranka et al. 1987, Hews 1988,), and fish (Keefe 1992, Brönmark and Petterson 1994). When predators capture and consume prey, a miasma of chemicals are released that have the potential to provide detailed information about predation risk. Previous work has demonstrated that prey can distinguish among different species of predators and

produce predator-specific defenses that are often linked to the riskiness of the predator and the predator's hunting strategy (Barry and Bayly 1985, Turner et al. 1999, McCarthy and Fisher 2000, Relyea 2001a, Iyengar and Harvell 2002). This suggests that different predator species release unique cues (i.e. kairomones; Turner et al. 1999, Grostal and Dicke 2000, McCarthy and Fisher 2000, Relyea 2001a, 2004, Iyengar and Harvell 2002). In addition, prey can distinguish among different predator diets and produce diet-specific defenses (Wilson and Lefcort 1993, Chivers et al. 1996, Laurila et al. 1997, Pettersson et al 2000, Schoeppner and Relyea 2005). The magnitudes of diet-specific defenses can be correlated to the phylogenetic relatedness of the predator's diet, with closely-related diets inducing stronger responses than distantly-related diets (Smith 1982, Mathis and Smith 1993, Mirza and Chivers 2001b, Schoeppner and Relyea 2005). This suggests that different prey species release unique cues (i.e. alarm cues). Collectively, these studies indicate that kairomones and alarm cues both provide essential information for prey when inducing their anti-predator defenses.

While alarm cues from different predator diets are important to prey defensive decisions, it is interesting that alarm cues by themselves are often insufficient for inducing prey defenses. For example, alarm cues alone can induce behavioral responses in a range of taxa, but the response is not consistent among species within a taxonomic group or even among populations within a species (Walls and Ketola 1989, Summey and Mathis 1998, Hazlett 1994, Turner 1996, 1997, Pijanowska 1997, Petranka and Hayes 1998, Huryn and Chivers 1999, Stabell et al. 2003, Jacobsen and Stabell 2004). Moreover, when researchers have compared the traits induced by predators eating prey to the traits induced by alarm cues alone, they find that alarm cues alone do not induce the full suite and magnitude of traits (Turner et al. 1999, Hagen et al. 2002, Schoeppner and Relyea 2005). This suggests that alarm cues are necessary, but not sufficient, to induce anti-predator defenses in most prey. To induce the full suite and magnitude of prey defenses, alarm cues must either be combined with kairomones from the predator or be modified during digestion by the predator.

A multitude of studies have demonstrated the existence of kairomones, but the source and composition of kairomones have not been well-characterized. One possibility is that predators always produce kairomones (because the kairomones are tied to an ongoing metabolic function of the predator) and therefore prey would always be aware of the predator's presence. A second possibility is that kairomones are chemicals related to a predator's consumption and digestion of

prey (e.g., digestive enzymes or by-products) and prey have evolved the ability to "eavesdrop" on these chemicals in assessing their risk of predation (Crowl and Covich 1990, Covich et al. 1994, Pettersson et al. 2000). Supporting this scenario is the fact that starved predators often do not induce prey defenses (Crowl and Covich 1990, Stirling 1995, McCollum and Leimberger 1997, Slusarczk 1999, Schoeppner and Relyea 2005). If kairomones are digestive enzymes, one would predict that the digestion of any diet would induce prey defenses (although the digestive enzymes might have to be combined with prey-specific alarm cues to induce the full suite and magnitude of defenses). In contrast, if kairomones are digestive by-products, one would predict that the complete anti-predator response would only be observed when the predator consumed the prey and not when undigested cues are encountered in any combination. Such cues could either be modified prey tissues or the chemicals emitted by the bacterial flora of the predator's digestive system when digesting a particular species of prey (termed "predator labeling"; Crowl and Covich 1990, Mathis and Smith 1993, Petterson et al. 2000, Jacobsen and Stabell 1999, Stabell et al. 2003, Jacobsen and Stabell 2004). To understand the source and role chemical cues play in inducing prey defenses, we need to evaluate all of these alternative scenarios. While a number of studies have examined pieces of this question, there have been few complete tests.

The goal of my study was to determine the source and effectiveness of alarm cues and kairomones for inducing behavioral and morphological defenses in prey. Using larval anurans, a model system well known for its plasticity (Relyea 2001a, Van Buskirk 2002a,b, Miner et al. 2005), I examined the separate and combined effects of alarm cues from crushed conspecifics and kairomones from either starved predators, predators fed heterospecific prey, predators fed conspecific prey, predators that chew but do not digest conspecific prey, and predators that digest but do no chew conspecific prey. Using these treatments, I tested the following predictions: 1) If alarm cues are sufficient to induce prey defenses, then crushed prey should induce the same traits as predators fed conspecific prey; 2) If kairomones are always produced by the predator, then starved predators should induce the same traits as predators fed conspecific prey; 3) If kairomones are always produced but must be detected in combination with alarm cues, then starved predators plus crushed prey should induce the same traits as predators fed conspecific prey; 4) If kairomones are only produced once prey are eaten and the kairomones are digestive enzymes, then predators fed heterospecific prey should induce the same traits as predators fed

conspecific prey; 5) If kairomones are digestive enzymes, but must be detected in combination with alarm cues, then predators fed heterospecific prey plus crushed prey should induce the same traits as predators fed conspecific prey; 6) If kairomones are not digestive enzymes but are digestive by-products, then predators fed heterospecific prey plus crushed prey should induce weaker defenses than predators fed conspecific prey; and 7) if kairomones are digestive by-products, then predators that only chew conspecific prey should induce weaker defenses than predators that only digest conspecific prey.

6.3 METHODS

I used a completely randomized design consisting of nine treatments replicated five times for a total of 45 experimental units. The nine treatments were as follows: 1) a no-predator control; 2) crushed tadpoles (i.e. alarm cues alone); 3) a caged predator that was starved; 4) crushed tadpoles plus a caged predator that was starved; 5) a caged predator that consumed and digested snails (*Physa integra*); 6) crushed tadpoles plus a caged predator that consumed and digested snails; 7) a caged predator that only consumed tadpoles; 8) a caged predator only digested tadpoles; and 9) a caged predator that consumed and digested tadpoles. Collectively, these nine treatments allowed us to identify the sources of the chemical cues that induce anti-predator defenses in tadpoles.

I performed the experiment in 100-L wading pool mesocosms that contained well water, 5 g rabbit chow, 100 g leaf litter (primarily *Quercus* spp.), and zooplankton and algae collected from three nearby ponds. These mesocosms were set up in an old field at the Aquatic Research Laboratory of the Pymatuning Laboratory of Ecology in northwestern Pennsylvania on an array of benches that raised the pools 50 cm off the ground. The wading pools were filled with well water on 27 and 28 April 2004 and covered with 60% shade cloth lids to prevent colonization by insects and other amphibians during the experiment. I added one predator cage to each pool that was either empty or contained a single late-instar dragonfly nymph (*Anax junius*) as dictated by the treatment. I used 450-ml plastic cups covered with fiberglass mesh screen as my predator cages, which allowed the cues from the predator and consumed prey to diffuse through the pools

while preventing the predator from preying on the tadpoles in the experiment (Petranka et al. 1987, Relyea and Werner 2000, Relyea 2000, 2001a,2003a; Schoeppner and Relyea 2005).

I used leopard frog tadpoles (*Rana pipiens*) that were collected as newly laid egg masses on 17 April 2004 and hatched and reared in wading pool mesocosms to prevent their exposure to predator cues prior to the experiment. The tadpoles were fed rabbit chow *ad libitum* prior to the experiment. On 10 May 2004, I added 20 tadpoles to each pool. The tadpoles were selected haphazardly from a mixture of tadpoles from ten egg masses. The initial mass of the tadpoles was 25.1 $\pm$ 1.4 mg (mean $\pm$ S.E.). Twenty tadpoles were placed in a 7-L plastic tub to assess mortality caused by handling (24-hr survival was 100%).

I added the chemical cue treatment to the pools three times per week. The first cue addition took place on 12 May, 2 d after the tadpoles were added to the mesocosms. All of the treatments employing prey consumption by predators received 300 mg of prey (snails or tadpoles). All of the treatments employing prey crushing received 300 mg of tadpoles (in 100 ml of water) that had been euthanized and then macerated in a blender for 1 min. The starved predators were not fed for five days prior to being used in the experiment and were kept in the pools for no more than 5 d before being replaced with a new starved predator. To create the treatments that employed only predator consumption or only predator digestion, predators from the digestion-only pools were removed, placed into the consumption-only pools, and fed 300 mg of tadpoles. Once the predators had consumed the tadpoles, they were returned to digestion-only pools. To equalize disturbance among pools, all empty predator cages were lifted each time the chemical cue treatments were applied and 100 ml of water was added to all treatments that did not receive crushed tadpoles. Any predators that died were replaced with either a starved predator or a predator that had previously been fed leopard frog tadpoles in the lab (depending upon treatment). All of the prey fed to the predators were consumed by the end of the experiment.

I observed tadpole behavior on six different days over the course of the experiment. I recorded the number of tadpoles visible (i.e. not hiding in the leaf litter) and the number of observed tadpoles that were moving. By dividing the latter by the former, I could quantify the proportion of tadpoles that were active (Peacor and Werner 1997, Relyea 2000, 2001a, 2003a; Schoeppner 2005) On three of the observation days, the predators had been fed the previous day: 13 May (6 observations 2-4 pm); 27 May (6 observations); 1 June (10 observations). On the

other three observation days, the predators had been fed earlier in the day: 17 May (12 observations); 19 May (9 observations); 21 May (10 observations). The observations were taken by multiple observers and all observations were completed within a 2-hr period. I ended the experiment on 2 June 2004, 24 d after tadpoles were added to the mesocosms. All tadpoles in the experiment were counted, euthanized, and preserved in 10% formalin for subsequent morphological analysis. Preserved tadpoles were measured using an image analysis system (BioScan Optimas; Bothell, WA) in which I measured tail depth and length, tail muscle depth and width, mouth width, and body depth, length, and width. All tadpoles were positioned with a glass plate under their tail during measuring to provide an undistorted lateral image.

Because observations were taken on multiple days, the behavioral data were analyzed using a repeated-measures analysis of variance to test for the effect of the chemical cue treatments, observation day, and their interaction. The data analyzed were pool means averaged across observations on each observation day. When I found a significant treatment affect, I performed subsequent pairwise comparisons using Fisher's LSD.

To determine how the different chemical cue treatments affected tadpole shape independent of differences in size, I first conducted a multivariate analysis of covariance (MANCOVA) using tadpole mass as the covariate and the eight morphological traits measured for all of the tadpole as the response variables. Prior to the analysis, tadpole mass was cube-root transformed to improve the linearity of the relationship between mass and each of the morphological traits. Within-group regression lines were parallel. I saved the residuals from this analysis and then added the residuals for each tadpole to the estimated marginal means for the appropriate treatment to produce size-independent estimates of the traits for all of the tadpoles. Using tank means for all traits as the response variables, I then used a multivariate analysis of variance to determine how the chemical cue treatments affected mass and the size-adjusted morphological traits. I used Fisher's LSD tests to make pairwise comparisons for all traits that showed a significant univariate effect.

6.4 RESULTS

The repeated-measures analyses indicated that tadpole activity (Fig. 6.1) was affected by treatment ($F_{8,36}$ = 5.0, P < 0.001) and time ($F_{5,32}$ = 16.6, P < 0.001) but showed no time-by-treatment interaction ($F_{40,142}$ = 1.4, P = 0.074). Chemical cues from starved predators, crushed tadpoles, consumed and digested snails, or any combinations of these cues produced activity levels that were not different from the no-predator treatment (P $\geq$ 0.096). Tadpoles exposed to cues from consumed and digested tadpoles were less active than all other treatments (P $\leq$ 0.014) except for the digested-(but not chewed) treatment (P = 0.237). Compared to tadpoles in the no-predator treatment, tadpoles exposed to chewed-(but not digested) conspecifics exhibited 7% lower activity (P = 0.030), tadpoles exposed to digested-(but not chewed) conspecifics exhibited 11% lower activity (P = 0.001), and tadpoles exposed to chewed-and-digested conspecifics exhibited 15% lower activity (P < 0.001). Tadpoles in the digested-(but not chewed) conspecifics exhibited activity that was intermediate to that observed in the chewed-(but not digested) conspecifics treatment (P = 0.173) and the chewed-and-digested conspecifics treatment (P = 0.237).

I found a multivariate effect of the treatments on tadpole morphology (Wilks' $F_{72,128}$ = 2.4, P = < 0.001). Univariate tests indicated significant effects on tail depth and body length (univariate tests, P < 0.001) but no effects on mass (univariate test, P = 0.114) or the other six morphological dimensions (univariate tests, P > 0.28). When I examined tail depth (Fig. 6.2A), I found that tadpoles exposed to starved predators, crushed tadpoles, chewed-and-digested snails, chewed-and-digested snails plus crushed tadpoles, and chewed-(but not digested) tadpoles had no effect compared to the no-predator control (P $\geq$ 0.178). However, compared to the no-predator control, tadpoles exposed to digested-(but not chewed) tadpoles had 4% deeper tails and tadpoles exposed to digested and chewed tadpoles had 6% deeper tails (P $\leq$ 0.001). Interestingly, tails were deeper when tadpoles were digested and chewed than when tadpoles were only digested (P = 0.021).

The treatments also affected body length (Fig. 6.2B). Tadpoles exposed to cues from starved predators, crushed tadpoles, starved predators plus crushed tadpoles, and chewed-and-digested snails plus crushed tadpoles had relatively longer bodies than tadpoles in the no-predator treatment (P < 0.02). Tadpoles exposed to the chewed-and-digested snails had bodies

that did not differ from the no-predator treatment ($P = 0.101$). Compared to the no-predator treatment, tadpole body length was not affected by the treatments in which tadpoles were chewed-(but not digested), digested-(but not chewed), or chewed and digested ($P \geq 0.101$).

6.5 DISCUSSION

The defended phenotype expressed by the leopard frog tadpoles depended on the combination of cues that the tadpoles encountered. I found that tadpoles responded to chemical cues from consumed and digested tadpoles by becoming less active and forming deeper tails. Previous work on inducible defenses in tadpoles has consistently shown that chemical cues from consumed conspecifics induce reductions in activity and that reduced activity increases prey survival by making the prey less conspicuous to the predator (Skelly 1994, Anholt et al. 1996). Cues from consumed prey also induce tadpoles to form relatively deeper tail fins and shorter bodies when compared to tadpoles not exposed to predator cues (McCollum and Van Buskirk 1996, Lardner 2000, Relyea 2000, 2001a, 2002b, Laurila et al. 2004). These morphological responses allow tadpoles to survive predation better than non-induced tadpoles (Van Buskirk et al. 1997, Van Buskirk and Relyea 1998) but predator-induced tadpoles grow more slowly than non-induced tadpoles (Skelly 1992, Van Buskirk 2000). In this study, I did not find a significant effect of exposure to predation cues on leopard frog mass but this may be because tadpole mass was measured late in ontogeny. Previous studies have shown that mass differences often only occur early in ontogeny (Relyea and Werner 2000, Van Buskirk 2001).

In contrast to past work using larval leopard frogs and other tadpole species, the tadpoles in this study did not develop shorter bodies when predators ate conspecific tadpoles. Because my analysis used mass-adjusted morphology, when the relative size of one morphological trait increases we expect to see a concurrent decrease in another morphological trait. Given that I found no significant effect of consumed cues on any other morphological traits, I must conclude that the leopard frog tadpole from the population used in this study were altering their body dimensions in a way that was not captured by the measurements that I made in this study (i.e. making small changes in several body dimensions). Further, I found that tadpole body length increased when the tadpoles were exposed to cues from crushed prey or starved predators. While

this response is in the opposite direction of the expected response to cues from consumed conspecifics, there are very few comparative data to evaluate this result. In what appears to be the only other tadpole study examining the effects of alarm cues and starved predators on body length, Schoeppner and Relyea (2005) found that these treatments had no effect on the body length of grey tree frog tadpoles (*Hyla versicolor*). Hence, I cannot say whether this response is a generalized response to only alarm cues or starved predators. Because a change in body length is not known to be an adaptive response to predators, for the remainder of the discussion I focus on the two significantly affected traits (activity and tail depth) that did exhibit typical responses to predators and are known to serve as effective anti-predator defenses.

I found that cues from crushed leopard frog tadpoles were ineffective at inducing changes in both tadpole activity and tail depth. A multitude of studies spanning a wide range of aquatic species has shown that alarm cues from crushed conspecifics can induce behavioral responses (flat worms, Wisenden and Millard 2001, amphipods, Wisenden et al. 2001, echinoderms, Parker and Schulman 1986, crustaceans, Pijanowska 1997, snails, Turner 1996, McCarthy and Fisher 2000, insects, Chivers et al. 1996, Huryn and Chivers 1999, tadpoles, Hews 1988, Petranka 1989, Summey and Mathis 1998, fish, Smith 1982, Mathis and Smith 1993; for a review see Chivers and Smith 1998). However, many studies have shown that alarm cues alone do not induce behavioral responses (Crowl and Covich 1990, Alexander and Covich 1991, Wilson and Lefcort 1993, Stirling 1995, Magurran et al 1996, Turner 1997, Lefcort 1998, Summey and Mathis 1998); that alarm cues induce weaker responses than those induced when predators consume prey (Hazlett and Schoolmaster 1998, McCarthy and Fisher 2000, Hagen et al. 2002); or that alarm cues induce responses in fewer traits than those induced when predators consume prey (Hazlett and Schoolmaster 1998, Turner et al. 1999, McCarthy and Fisher 2000, Hagen et al. 2002). For morphological defenses, the majority of previous work has shown that alarm cues alone do not induce morphological changes in prey (Walls and Ketola 1989, Brönmark and Pettersson 1994, Schoeppner and Relyea 2005; but see Stabell and Lwin 1997, Stabell et al. 2003). Thus, while alarm cues alone can induce behavioral changes in some taxa, alarm cues are frequently insufficient for inducing the full suite and magnitude of behavioral and morphological defenses. This indicates either that the alarm cues must be encountered simultaneously with the kairomones or that the alarm cues become modified during digestion.

I found that cues from starved predators alone were not sufficient to induce changes in leopard frog activity or tail depth. This indicates that dragonfly naiads do not produce a constitutive kairomone. Several previous experiments using starved predators have reported no induction of defenses in a wide range of species (Crowl and Covich 1990, Stirling 1995, McCollum and Leimberger 1997). However, a few studies have reported that starved predators can induce defensive behavior (Hazlett and Schoolmaster 1998, McCarthy and Fisher 2000, Pettersson et al. 2000) and morphology (Walls and Ketola 1989, Iyengar and Harvell 2002). In those cases in which investigators compared induction by starved predators and predators fed conspecific prey, the responses induced by starved predators were relatively weak (Walls and Ketola 1989, Vilhunen and Hirvonen 2003; but see Pettersson et al. 2000). Previous studies in tadpoles have reported that behavioral and morphological responses to starved predators are either weak or non-existent (Anholt et al. 1996, McCollum and Leimberger 1997, Van Buskirk and Arioli 2002). A common thread in all of these studies is that starved predators induce little or no defensive response in their prey. This indicates that the starved predators emit little or no kairomone.

A possible explanation for the observation that cues from crushed prey alone and cues from starved predators alone are not sufficient to induce a complete anti-predator response is that the prey may simply need to encounter the two cues simultaneously. Because not all defenses are effective against all types of predators, not all species of predators pose the same degree of threat, and some predators only pose a threat at specific times in ontogeny (Turner et al. 1999, Puttlitz et al. 1999, Lardner 2000, Relyea 2001a,b; Mirza and Chivers 2001c, Van Buskirk 2001, Mirza et al. 2003), prey may require information about both predator species and predator diet before committing to a complete anti-predator response. In this experiment, I found that cues from starved predators plus cues from crushed conspecifics did not induce changes in either tadpole activity or tail depth. Few studies have examined this combination of cues and no study has examined this cue combination in tadpoles. In a study using predatory crabs (*Callinectes bellicosus*), Jacobsen and Stabell (2004) found that crabs that had consumed marine snails (*Tegula funebralis*) induced a crawl out response in the snails while cues from a starved crab combined with cues from crushed snails did not induce a response. These two studies indicate that the lack of response to alarm cues alone or starved predators alone is not simply because the two cues have to be detected simultaneously. Furthermore, I can conclude, at least for my

system, and probably for many other systems, that the kairomones detected by prey are only released when the predator is consuming and digesting prey.

If predators have to be eating prey to produce the cues that induce the full suite and magnitude of anti-predator defenses in prey, then one might hypothesize that the kairomone could be either a digestive enzyme (or mixture of enzymes) or that the kairomone is a digestive by-product including potentially modified alarm cues (Crowl and Covich 1990, Hagen et al. 2002, Stabell et al. 2003). In the current study I showed that, in agreement with previous work (Schoeppner and Relyea 2005), cues from a predator consuming snails did not induce changes in tadpole activity or tail depth. These results indicate that the chemicals that induce defensive responses cannot be simply digestive enzymes (or at least not enzymes that are used to digest a variety of prey species). In addition, numerous studies have reported diet-specific responses in a range of species (Wilson and Lefcort 1993, Chivers et al. 1996, Laurila et al. 1997, Pettersson et al. 2000, Schoeppner and Relyea 2005); such responses cannot be explained by the production of digestive enzymes alone. However, such responses might be inducible by the combination of digestive enzymes plus alarm cues that would be emitted during the attack on the prey. I addressed this possibility by combining the cues of a predator consuming snails plus cues from crushed tadpoles and found no change in tadpole activity or tail depth. Therefore, from my experiment I can conclude that the cues that induce the complete anti-predator response in tadpoles are either 1) prey-specific alarm cues that are uniquely modified by each predator during digestion (i.e. a digestive by-product) or 2) a combination of prey-specific alarm cues and predator-specific digestive enzymes. Distinguishing among these possibilities will require future studies into the signature of enzymes that are produced for specific predator diets or identification of the chemical components of the alarm cue to determine how the alarm cues are modified during digestion.

When prey are consumed, the cues that induce anti-predator defenses may be released when the predator chews the prey, digests the prey, or both. For activity, I found that the cues from chewed-(but not digested) tadpoles induced a small activity reduction, while cues from digested-(but not chewed) tadpoles induced a moderate activity reduction, and cues from chewed-and-digested tadpoles induced a large activity reduction. For tail depth, I also found that the cues from chewed-(but not digested) tadpoles induced no increase in tail depth, cues from digested-(but not chewed) tadpoles induced a moderate increase in tail depth, and cues from

chewed-and-digested tadpoles induced a large increase in tail depth. These results suggest that while the cues emitted by chewing alone induce little or no response on their own, when combined with the cues of digestion, the cues of chewing cause a stronger induction of defensive traits. This result suggests either that some alarm cues are lost during consumption (making the cues emitted by digestion more dilute) or that the predator may produce some cues while chewing (i.e. salivary enzymes or compounds that immobilize the prey) that can induce weak activity reductions. Two previous studies have also addressed the effects of metabolites alone (digested but not chewed) on tadpole morphology. Richardson (2006) found that cues from digested prey induced a weaker change in tail shape when compared to cues from consumed prey using tree frog tadpoles (*Hyla chrysoscelis*), which supports my conclusions that the cues from digestion do not induce the complete anti-predator response. However, LaFiandra and Babbitt (2004) found no difference in the induction of tadpole tail depth between cues from prey that were digested-(but not chewed) and cues from prey that were chewed and digested; but they did find that tadpole tail color was differentially effected by the digested-(but not chewed) and chewed-and-digested treatments. Therefore, while more studies are needed to arrive at generalities about the relative importance of cues released when prey are chewed-(but not digested) and digested-(but not chewed), it is clear that both types of cues are involved in inducing the complete antipredator phenotype. Nevertheless, my results indicate that prey can discriminate between predators chewing conspecific prey and predators digesting conspecific prey. Therefore, if predators only consume but do not digest prey where they hunt then the non-consumed prey would only encounter a fraction of the chemical cues released. This has important implications regarding the optimal behavior of a foraging predator that might try to emit digestive cues in an area away from where it attacks prey to limit the information available for the induction of defenses in other potential prey.

6.5.1 Conclusions

I have shown that the kairomone used by anuran larvae to identify dragonfly naiads is not a chemical that is constitutively produced by the predator and that both the act of prey consumption and digestion play a role in the induction. This result is consistent with previous assertions that kairomones should not be constitutively produced because selection should act to

eliminate the production of any constitutive chemical that allowed prey to detect their predators. However, while kairomones are not constitutive, these results also highlight the fact that the prey still can detect their predators and that the predators themselves play a role in producing the cues that induce the most intense anti-predator responses. This implies that the predator can be "chemically invisible" to the prey when they consume diets that do not contain alarm cues that the prey recognize (Stabell et al. 2003). These results also emphasize that predator behavior may play an important role in determining what defensive phenotype the prey exhibits (Lima 2002). If the predator does not consume and digest the prey in the same area or if the predator switches diets often, the prey may not encounter the chemical environment that induces the most extreme defenses. Understanding the dynamics of inducible defenses in nature will require studies to determine 1) how long the predators remain chemically "visible" due to prey digestion and 2) how predator behavior alters the cues that prey are able to detect.

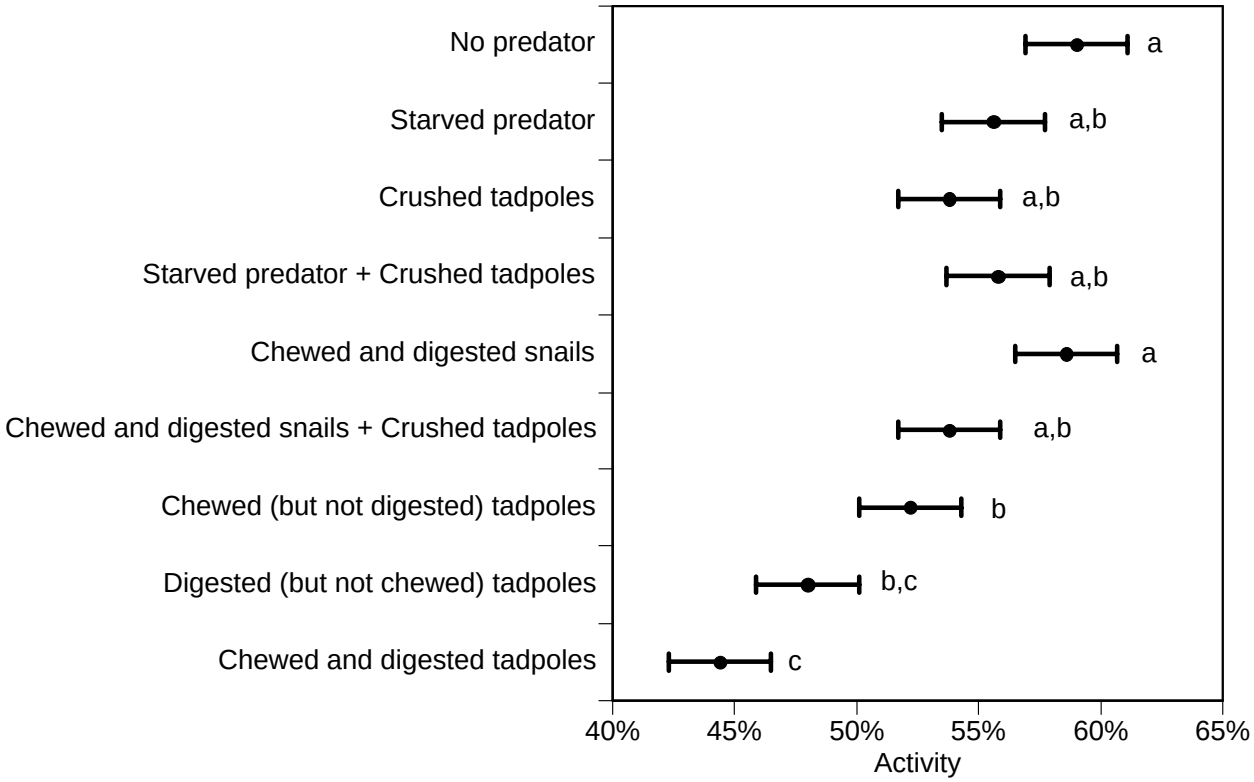

Figure 6.1 The effects of different combinations of chemical cues on tadpole activity. Activity data was taken on six separate days. However, because I found no treatment-by-time interaction, the data are presented as means $\pm$ 1 SE for all observations.

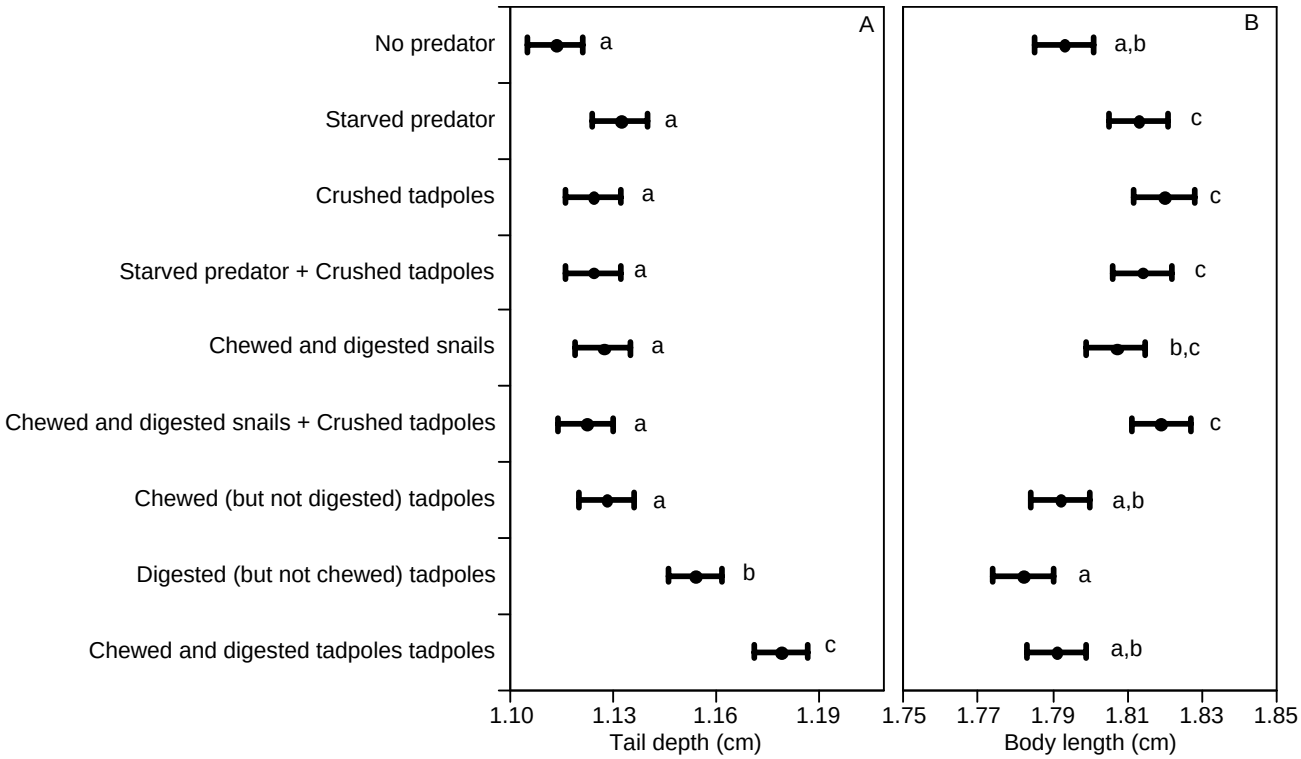

Figure 6.2 The effects of different combinations of chemical cues on the relative tail depth and body length of tadpoles. The data are treatment means + 1 S.E.

7.0 CONCLUSIONS AND FUTURE DIRECTIONS

This dissertation highlights the fact that inducible defenses are not a simple phenotypic dimorphism, but that prey have an amazing ability to modify their phenotypes in response to differences in the intensity of predation risk, fine-scale variation in risk, and the combinations of kairomones and alarm cues that they encounter. Plasticity theory predicts that selection should favor the detection of environmental cues that provide increasingly specific and accurate information about the environment (Moran 1992). When one considers the vast number of combinations of environmental conditions that organisms may encounter through their lifetime, and the conflicting demands of allocating resources to growth, reproduction, and defenses, it is not surprising that organisms can detect small changes in the environment and modify their phenotypes in directions consistent with decisions that maximize growth while minimizing mortality (Sih 1980, Horat and Semlitsch 1994, Relyea 2004). Further research is needed to understand how closely organisms can track environmental changes with phenotypic changes and to quantify the associated fitness benefits of these changes.

The risk-response curves found for wood frog tadpole behavior, morphology, and mass highlight some important points about how prey respond to risk that will be valuable in predicting and evaluating prey defended phenotypes. I found that a large fraction of the changes in inducible traits were induced at very low levels of predation and that the traits were changing at different rates (i.e. some increased and plateaued more quickly than others). However, determining if different traits display different induction thresholds will require a characterization of the risk-response curve for lower levels of predation risk. These data also highlight the need for future studies that look at the effects of predation risk on prey physiology. While all of the morphological and behavioral traits had plateaued or were clearly pleatueaing, tadpole mass continued to decline with increasing levels of risk. While the decreases in mass could be the product of the accumulated effects of several small trait changes at the highest risk,

it is also possible that the decrease in mass is due to underlying physiological responses to predation risk (McPeek et al. 2001). Determining the mechanism underlying the continued decreases in mass at the highest levels of predation risk will be an important advance in our understanding of the costs involved in responding to predation risk and possibly to stressful environments in general.

For amphibians, I also showed that alarm cues from damaged conspecifics induce weak behavioral responses and no changes in morphology. Studies using other species also have shown that alarm cues often induce weaker phenotypic responses when compared to the phenotypes induced by cues from consumed prey (Petranka and Hayes 1998, Jacobsen and Stabell 1999, Hagen et al. 2002). I have also showed that the magnitude of the prey's response to predation cues is concentration dependant. Therefore, depending upon the shape of the dose-response curve for alarm cues alone and prey defensive traits, alarm cues at very high concentrations may induce a similar magnitude of response as cues from consumed prey. This could be important for the interpretation of data from lab experiments. Many lab experiments are performed in small venues containing a few liters of water. When cues from a caged predator or damaged prey are added to these venues, the final amount of consumed prey/ volume of water is often much higher than any concentration used in larger mesocosm experiments. While these results would appear to show that alarm cues are equivalent to cues from consumed prey, the result would not be meaningful to ecological communities because they are outside the scope of what is ever encountered in nature. Determining the extent of this problem will require that dose-response relationships are determined in response to increasing concentrations of alarm cues alone and that these dose-response curves be compare to the responses to cues from consumed prey. To date, I am aware of only one study that compares the dose-response relationships for cues from damaged prey and consumed prey but the range of concentrations tested was limited (Hagen et al. 2002). We will also need to determine the chemical identity of the compounds involved in inducing the defenses to be able to determine the ecologically realistic concentrations of the compounds, so that future experiments are not performed under ecologically irrelevant conditions.

I demonstrated that defended phenotypes are induced by chemical cues from closely-related species. While it is clear that amphibians detect chemical cues from conspecifics and other species that emit similar chemicals, work using other species has shown different patterns.

Several studies have found that some species can respond strongly to alarm cues from distantly-related heterospecifics (Chivers et al. 1996, Wisenden et al 1999,Chivers and Mirza 2001). However, such responses are often found when the responding prey have been taken from the wild and, therefore, are not predator-naïve. This may be important because learning through a process of template updating can modify how prey use the information they obtain from chemical sues. If prey commonly learn to associate cues from heterospecifics (that do not induce responses in predator naïve individuals) with predation risk over time, then the pattern of responses observed in predator naïve individuals will only be predictive of prey responses during a short window in prey ontogeny. In preliminary work on chemical cue learning in amphibians, I have shown that the response to chemical cues is innate and that pairing heterospecific cues with alarm cues and kairomones does not alter their response. However, these experiments have all been short-term behavioral observations in the laboratory. Therefore, future work needs to address the importance of the duration and frequency of pairing heterospecific and conspecific cues association learning.

An important goal of future work should be to incorporate more ecological reality into our experiments and determine if the predictions from the lab and mesocosm experiments are predictive of phenotypic variation in more complex environments (Irving and Magurran 1997, Petranka and Hayes 1998). Communication theory predicts that the prey will respond to less informative cues if there is a potentially high cost not responding to the cues (i.e. not responding when there is a true risk of predation; Greenfield 2002). For example, if prey live in an exposed habitat without refuges (where they are much more vulnerable to predation), they should be more likely to respond to less informative cues. Interestingly, while prey refuges are common in nature, most studies of alarm cues have been conducted under lab conditions that lack refuges. These studies have found that alarm cues often induce strong behavioral defenses, but it is precisely under these conditions that we might expect to observe strong responses to alarm cues. In one of the few case studies of this phenomenon, Magurran et al. (1996) found that fathead minnows (*Pimephales promelas*), a species that commonly exhibits strong behavioral responses to alarm cues in the laboratory, showed no response to cues under natural conditions. If responses to predation cues are exaggerated under laboratory conditions, then we may be over-estimating the strength of responses to predatory cues. To understand how environmental context affects our understanding of predator-induced defenses, we need to quantify responses to

alarm cues and kairomones across a range of refuge availability. When we do this, we also need to expand beyond our focus on behavioral defenses by also incorporating morphological defenses to determine if both types of defenses decrease as refuge availability increases.

The chemical complexity of the environment is another important aspect that should be considered in future work. To detect an accurate signal of predation risk, an organism must be able to distinguish the informative cue from uninformative background noise. Aquatic prey live in complex environments that contain chemicals that are both intentionally released (e.g., sex pheromones), and chemicals that are simple by-products of metabolism. Most of these chemicals are not informative about predation risk and, in fact, may interfere with the prey's ability to detect predation cues. Communication theory terms this the signal:noise ratio (Greenfield 2002). As the amount of noise increases, the ability of the prey to distinguish the information about predation risk from the mélange of other chemicals is expected to diminish. However, when we evaluate the effects of chemical cues on prey phenotype in experiments, it is typically done in a simple chemical landscape consisting of cues from only the predator and the prey. Under these conditions, there is little potential for chemical noise. Indeed, many studies have found that extremely low concentrations of alarm cues are sufficient to induce behavioral responses in prey (Pettersson et al. 2000, Brown et al. 2001, Mirza and Chivers 2003). However, these studies were performed in the absence of natural chemical background noise. Without including background noise, we will likely overestimate the prey's ability to detect and respond to predation cues under natural conditions. To understand the effects of "chemical noise," we need to conduct experiments that compare the prey's behavioral and morphological defenses across a range of simple to complex chemical landscapes.

Empirical studies of prey responses to chemical cues have provided us with an incredible amount of information about the nature and source of predation cues. The experiments presented in this dissertation along with studies employing a diversity of organisms have shown that the cues that prey use to detect predation are specific to the digestion of prey. A crucial but very difficult step in understanding the role of chemical cues in predator prey interactions will be in determining the chemical identity of the compounds that different species of prey detect. Once we know what chemicals a given species uses we can look for differences in sensitivities to those chemicals among populations, and among species to address questions about how differences in the detection and response to predation cues may be related to local differences in predation risk

and if differences in the relative importance of alarm cues and kairomones can be associated with large taxonomic groups (e.g. amphibians detect products of digestion but fish rely more on alarm cues alone). While it is no doubt a daunting challenge, a critical step in understanding the ecology of predation cues will be to determine the chemical identity of the cues.

As more and more studies document the importance of trait-mediated effects on the outcomes of species interactions and community structure, it is clear that predicting trait expression for organisms that employ inducible defenses will require a good deal of information about the predation regime to which they are exposed (Beckerman et al. 1997, Lima 1998, Turner et al. 2000, Werner and Peacor 2003). Trait changes induced by the addition of a predator can alter the outcome of competition. Given that predator diet also determines the induction of defenses, predator diet may be able to change the nature of species interactions. Being able to form generalities about the degree of induction and the types of traits that organisms should use in environments containing predators is very valuable to studies concerned with trait-mediated effects, but we need to understand more about how prey integrate information from their cues, how the decisions may vary among populations, and how the different decisions may vary depending on the types of traits that are being considered.

In summary, while we have an excellent understanding of predator-induced plasticity, investigating the chemical identity of the cues and incorporating more ecological complexity into our experiments are the next critical steps toward understanding the ecology and evolution of predator-induced plasticity (Dodson et al. 1994, Chivers and Smith 1998, Kats and Dill 1998, Burks and Lodge 2002). Hundreds of studies have used chemical cues from caged predators to study the impacts of predation cues on prey behavior, morphology, and life history. While we have long known that these cues induce phenotypic changes, we are just beginning to understand the extent of the information conveyed by the cues and the specificity of the responses (Burks and Lodge 2002). By understanding how predator diet and environmental context affect prey traits, we will be better able to correctly predict if and how these trait changes should affect the community as a whole.

BIBLIOGRAPHY

Abrams, P. A. 1995. Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. American Naturalist **146**:112-134.

Alexander, J. E., and A. P. Covich. 1991. Predator avoidance by the freshwater snail *Physella virgata* in response to the crayfish *Procambarus simulans*. Oecologia **87**:435-442

Ali, M., and R. T. Wootton. 1999. Coping with resource variation: Effect of constant and variable intervals between feeding on reproductive performance at first spawning of female three-spined sticklebacks. Journal of Fish Biology **55**:211-220.

Altmann, J. 1974. Observational study of behavior: sampling methods. Behavior **49**: 227-267.

Andersson, S., and R. G. Shaw. 1994. Phenotypic plasticity in *Crepis tectorum* (Asteraceae): genetic correlations across light regimes. Heredity **72**:113-125.

Anholt, B. R. and E. E. Werner. 1995. Interaction between food availability and predation mortality mediated by adaptive behavior. Ecology **76**: 2230-2234.

Anholt, B. R., and E. E. Werner. 1998. Predictable changes in predation mortality as a consequence of changes in food availability and predation risk. Evolutionary Ecology **12**:729-738.

Anholt, B. R., D. K. Skelly, and E. E. Werner. 1996. Factors modifying antipredator behavior in larval toads. Herpetologica **52**:301-313.

Anholt, B. R., D. K. Skelly, and E. E. Werner. 2000. Effect of food and predators on the activity of four larval ranid frogs. Ecology **81**:3509-3521.

Barry, M. J., and I. A. E. Bayly. 1985. Further studies on predator induction of crests in Australian *Daphnia* and effects of crests on predation. Australian Journal of Marine and Freshwater Research **36**:519-535.

Beckerman, A. P., M. Uriarte, and O. J. Schmitz. 1997. Experimental evidence for a behavior-mediated trophic cascade in a terrestrial food chain. Proceedings of the National Academy of the Sciences of the United States of America **94**:10735-10738.

Behler, J. L., and F. W. King. 1991. The Audubon Society Field Guide to North American Reptiles & Amphibians. Alfred A. Knopf Inc., New York.

Berven, K. A., and D. E. Gill. 1983. Interpreting geographical variation in life history traits. American Zoologist **28**:85-97.

Black, A. R. 1993. Predator-induced phenotypic plasticity in *Daphnia pulex*: life history and morphological responses to *Notonecta* and *Chaoborus*. Limnology and Oceanography **38**:986-996.

Blair, J., and R. J. Wassersug. 2000. Variation in the pattern of predator-induced damage to tadpole tails. Copeia **200**:390-401.

Blouin, M. S. 1992. Comparing bivariate reaction norms among species: time and size at metamorphosis in three species of *Hyla* (Anura: Hylidae). Oecologia **90**:288-293.

Boersma, M., L. De Meester, and P. Spaak. 1998. Predator-mediated plasticity in morphology, life history, and behavior of *Daphnia*: The uncoupling of responses. American Naturalist **152**:237-248.

Brönmark, C., and L. B. Pettersson. 1994. Chemical cues from piscivores induce a change in morphology in crucian carp. Oikos **70**:396-402.

Brown, G. E., J. C. Adrian, and M. L. Shih. 2001. Behavioral responses of fathead minnows to hypoxanthine-3-N-oxide at varying concentrations. Journal of Fish Biology **58**: 1465-1470.

Brown, G. E., J. C. Adrian, E. Smyth, H. Leet and S. Brennan. 2000. Ostariophysan alarm pheromones: Laboratory and field tests of the functional significance of nitrogen oxides. Journal of Chemical Ecology **26**: 139-154.

Burks, R. L., and D. M. Lodge. 2002. Cued in: Advances and opportunities in freshwater chemical ecology. Journal of Chemical Ecology **28**:1901-1917.

Chivers, D. P. and R. S. Mirza. 2001. Predator diet cues and the assessment of predation risk by aquatic vertebrates: A review and prospectus. In: Chemical signals in vertebrates 9 (Ed. by Marchlewska-Koj, A., Lepri, J. J. & Mèuller-Schwarze, D.), pp. 277-284. New York: Kluwer Academic/Plenum Publishers.

Chivers, D. P. and R. J. F. Smith. 1994. The role of experience and chemical alarm signaling in predator recognition by fathead minnows, *Pimephales promelas*. Journal of Fish Biology **44**: 273-285.

Chivers, D. P., and R. J. F. Smith. 1998. Chemical alarm signaling in aquatic predator-prey systems: A review and prospectus. Ecoscience **5**:338-352.

Chivers, D. P., B. D. Wisenden, and R. J. F. Smith. 1996. Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. Animal Behaviour **52**:315-320.

Chivers, D. P., R. S. Mirza, and J. G. Johnston. 2002. Learned recognition of heterospecific alarm cues enhances survival during encounters with predators. Behavior **139**: 929-938.

Chivers, D. P., M. H. Puttlitz and A. R. Blaustein. 2000. Chemical alarm signaling by reticulate sculpins, *Cottus perplexus*. Environmental Biology of Fishes **57**: 347-352.

Commens, A. M. and A. Mathis. 1999. Alarm pheromones of rainbow darters: Responses to skin extracts of conspecifics and congeners. Journal of Fish Biology **55**: 1359-1362.

Cook, S. A., and M. P. Johnston. 1968. Adaptation to heterogeneous environments. I. Variation in heterophylly in *Ranunculus flammula*. Evolution **22**:496-516.

Covich, A. P., T. A. Crowl, J. E. Alexander, and C. C. Vaughn. 1994. Predator-avoidance responses in freshwater decapod-gastropod interactions mediated by chemical stimuli. Journal of the North American Benthological Society **13**:283-290.

Crowl, T. A., and A. P. Covich. 1990. Predator-induced life-history shifts in a freshwater snail. Science **247**:949-951.

DeWitt, T. J. 1998. Costs and limits of phenotypic plasticity: Tests with predator-induced morphology and life history in a freshwater snail. Journal of Evolutionary Biology **11**:465-480.

DeWitt, T. J., A. Sih, and D. S. Wilson. 1998. Costs and Limits of phenotypic plasticity. Trends in Ecology and Evolution **13**:77-81.

DeWitt, T. J., and S. M. Scheiner. 2004. Phenotypic Plasticity: Functional and Conceptual Approaches. Oxford Univ. Press, New York.

DeWitt, T. J., B. W. Robinson and D. S. Wilson. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. Evolutionary Ecology Research **2**: 129-148.

Dodson, S. I. 1989. The ecological role of chemical stimuli for the zooplankton: predator-induced morphology in *Daphnia*. Oecologia **78**:361-367.

Dodson, S. I., T. A. Crowl, B. L. Peckarsky, L. B. Kats, A. P. Covich, and J. M. Culp. 1994. Non-visual communication in freshwater benthos: An overview. Journal of the North American Benthological Society **13**:268-282.

Dudley, S. A., and J. Schmitt. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. American Naturalist **147**:445-465.

Engelmann, K. E., and C. D. Schlichting. 2005. Coarse- versus fine-grained water stress in *Arabidopsis thaliana* (Brassicaceae). American Journal of Botany **92**:101-106.

Feller, A. E. and S. B. Hedges. 1998. Molecular evidence for the early history of living amphibians. Molecular Phylogenetics and Evolution **9**: 509-516.

Foam, P. E., R. S. Mirza, D. P. Chivers, and G. E. Brown. 2005. Juvenile convict cichlids (*Archocentrus nigrofasciatus*) allocate foraging and antipredator behaviour in response to temporal variation in predation risk. Behaviour **142**:129-144.

Gabriel, W. 1999. Evolution of reversible plastic responses: Inducible defenses and environmental tolerance. Pages 286-305 *in* R. Tollrian and C. D. Harvell, editors. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey.

Gilbert, J. J., and R. S. Stemberger. 1984. *Asplanchna*-induced polymorphism in the rotifer *Keratella slacki*. Limnology and Oceanography **29**:1309-1316.

Grant, J. W. G., and I. A. E. Bayly. 1981. Predator induction of crests in morphs of the *Daphnia carinata* King complex. Limnology and Oceanography **26**:201-218.

Greene, E. 1989. A diet-induced developmental polymorphism in a caterpillar. Science **243**:643-646.

Greenfield, M. D. 2002. Signalers and Receiver: Mechanisms and Evolution of Arthropod Communication. Oxford University Press, New York.

Grostal, P., and M. Dicke. 2000. Recognising one's enemies: a functional approach to risk assessment by prey. Behavioral Ecology and Sociobiology **47**:258-264.

Gupta, A. P., and R. C. Lewontin. 1982. A study of reaction norms in natural populations of *Drosophila pseudoobscura*. Evolution **36**:934-948.

Hagen, N. T., A. Andersen, and O. B. Stabell. 2002. Alarm responses of the green sea urchin, *Strongylocentrotus droebachiensis*, induced by chemically labeled durophagous predators and simulated acts of predation. Marine Biology **140**:365-374.

Hamilton, I. M., and M. R. Heithaus. 2001. The effects of temporal variation in predation risk on anti-predator behaviour: An empirical test using marine snails. Proceedings of the Royal Society of London Series B-Biological Sciences **268**:2585-2588.

Hanazato, T., and T. Ooi. 1992. Morphological responses of *Daphnia ambigua* to different concentrations of a chemical extract from *Chaoborus flavicans*. Freshwater Biology **27**:379-385.

Hara, T. J. 1993. Role of olfaction in fish behaviour. Pages 171-195 *in* T. J. Pitcher, editor. Behaviour of teleost fishes. Chapman & Hall, London.

Harvell C.D. 1992. Inducible defenses and allocation shifts in a marine bryozoan. Ecology **73**: 1567-1576.

Harvell, C. D. 1986. The ecology and evolution of inducible defenses in a marine bryozoan: Cues, costs, and consequences. American Naturalist **128**:810-823.

Harvell, C. D. 1990. The ecology and evolution of inducible defenses. Quarterly Review of Biology **65**:323-340.

Harvell, C. D. 1992. Inducible defenses and allocation shifts in a marine bryozoan. Ecology **73**:1567-1576.

Harvell, C. D. 1998. Genetic variation and polymorphism in the inducible spines of a marine bryozoan. Evolution **52**:80-86.

Havel, J. E. 1985. Predation of common invertebrate predators on long- and short-featured *Daphnia retrocurva*. Hydrobiologia **124**:141-149.

Hazel, W., R. Smock, and C. M. Lively. 2004. The ecological genetics of conditional strategies. American Naturalist **163**:888-900.

Hazlett, B. A. 1994. Alarm responses in the crayfish *Orconectes virilis* and *Orconectes propinquus*. Journal of Chemical Ecology **20**:1525-1535.

Hazlett, B. A., and D. R. Schoolmaster. 1998. Responses of cambarid crayfish to predator odor. Journal of Chemical Ecology **24**:1757-1770.

Hedges, S. B. 1986. An electrophoretic analysis of holarctic hylid frog evolution. Systematic Zoology **35**:1-21.

Helfmann, G. S. 1989. Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. Behavioral Ecology and Sociobiology **24**: 47-58.

Hessen, D. O., and E. van Donk. 1993. Morphological changes in *Scenedesmus* induced by substances released from *Daphnia*. Archiv Fur Hydrobiologie **127**:129-140.

Hews, D. K. 1988. Alarm responses in larval western toads, *Bufo boreas*: Release of larval chemical by a natural predator and its effects on predator capture efficiency. Animal Behaviour **36**:125-133.

Horat, P., and R. D. Semlitsch. 1994. Effects of predation risk and hunger on the behavior of two species of tadpoles. Behavioral Ecology and Sociobiology **34**:393-401.

Huryn, A. D., and D. P. Chivers. 1999. Contrasting behavioral responses by detritivorous and predatory mayflies to chemicals released by injured conspecifics and their predators. Journal of Chemical Ecology **25**:2729-2740.

Houston, A. I., J. M. McNamara, and J. M. C. Hutchinson. 1993. General results concerning the trade-off between gaining energy and avoiding predation. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences **341**:375-397.

Irving, P. W., and A. E. Magurran. 1997. Contest-dependent fright reactions in captive European minnows: The importance of naturalness in laboratory experiments. Animal Behaviour **53**:1193-1201.

Iyengar, E. V., and C. D. Harvell. 2002. Specificity of cues inducing defensive spines in the bryozoan *Membranipora membranacea*. Marine Ecology-Progress Series **225**:205-218.

Jacobsen, H. P., and O. B. Stabell. 1999. Predator-induced alarm responses in the common periwinkle, *Littorina littorea*: dependence on season, light conditions, and chemical labeling of predators. Marine Biology **134**:551-557.

Jacobsen, H. P., and O. B. Stabell. 2004. Antipredator behaviour mediated by chemical cues: The role of conspecific alarm signaling and predator labeling in the avoidance response of a marine gastropod. Oikos **104**:43-50.

Kacelnik, A., and M. Bateson. 1996. Risky theories- The effects of variance on foraging decisions. American Zoologist **36**:402-434.

Karban, R., and I. T. Baldwin. 1997. Induced Responses to Herbivory. University of Chicago Press, Chicago, IL.

Kats, L. B., and L. M. Dill. 1998. The scent of death: Chemosensory assessment of predation risk by prey animals. Ecoscience **5**:361-394.

Keefe, M. 1992. Chemically mediated avoidance behavior in wild brook trout, *Salvelinus fontinalis:* The response to familiar and unfamiliar predaceous fishes and the influence of fish diet. Canadian Journal of Zoology-Revue Canadienne De Zoologie **70**:288-292.

Kingsolver, J. G. 1995. Fitness consequences of seasonal polymorphism in western white butterflies. Evolution **49**:942-954.

Krueger, D. A., and S. I. Dodson. 1981. Embryological induction and predation ecology in *Daphnia pulex*. Limnology and Oceanography **26**:219-223.

Kuhlmann, H.-W., and K. Heckmann. 1985. Interspecific morphogens regulating prey-predator relationships in protozoa. Science **227**:1347-1349.

Kumar, S. and S. B. Hedges. 1998. A molecular timescale for vertebrate evolution. Nature **392**: 917-920.

Kusch, J. 1993. Behavioral and morphological changes in ciliates induced by the predator *Amoeba proteus*. Oecologia **96**:354-359.

Kusch, R. C., R. S. Mirza, and D. P. Chivers. 2004. Making sense of predator scents: investigating the sophistication of predator assessment abilities of fathead minnows. Behavioral Ecology and Sociobiology **55**:551-555.

LaFiandra, E. M., and K. J. Babbitt. 2004. Predator induced phenotypic plasticity in the pinewoods tree frog, *Hyla femoralis*: Necessary cues and the cost of development. Oecologia **138**:350-359.

Lampert, W., K. O. Rothhaupt, and E. von Elert. 1994. Chemical induction of colony formation in a green alga (*Scenedesmus acutus*) by grazers (*Daphnia*). Limnology and Oceanography **39**:1543-1550.

Langerhans, R. B., and T. J. DeWitt. 2002. Plasticity constrained: over-generalized induction cues cause maladaptive phenotypes. Evolutionary Ecology Research **4**:857-870.

Lardner, B. 2000. Morphological and life history responses to predators in larvae of seven anurans. Oikos **88**:169-180.

Larsson, P. and S. I. Dodson. 1993. Chemical communication in planktonic animals. Archiv Fur Hydrobiologie **129:**129-155.

Laurila, A. 2000. Behavioural responses to predator chemical cues and local variation in antipredator performance in *Rana temporaria* tadpoles. Oikos **88**:159-168.

Laurila, A., and J. Kujasalo. 1999. Habitat duration, predation risk and phenotypic plasticity in common frog (Rana temporaria) tadpoles. Journal of Animal Ecology **68**:1123-1132.

Laurila A., S. Karttunen and J. Merila. 2002. Adaptive phenotypic plasticity and genetics of larval life histories in two *Rana temporaria* populations. Evolution **56**: 617-627.

Laurila, A., J. Kujasalo, and E. Ranta. 1997. Different antipredator behaviour in two anuran tadpoles: effects of predator diet. Behavioral Ecology and Sociobiology **40**:329-336.

Laurila, A., M. Jarvi-Laturi, S. Pakkasmaa, and J. Merila. 2004. Temporal variation in predation risk: Stage-dependency, graded responses and fitness costs in tadpole antipredator defenses. Oikos **107**:90-99.

Lefcort, H. 1998. Chemically mediated fright response in southern toad (*Bufo terrestris*) tadpoles. Copeia:445-450.

Levins, R. 1968. Evolution in Changing Environments. Princeton University Press, Princeton, NJ.

Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions: what are the ecological effects of anti-predator decision-making? Bioscience **48**:25-34.

Lima, S. L. 2002. Putting predators back into behavioral predator-prey interactions. Trends in Ecology and Evolution **17**:70-75.

Lima, S. L., and P. A. Bednekoff. 1999. Temporal variation in danger drives antipredator behavior: The predation risk allocation hypothesis. American Naturalist **153**:649-659.

Lima, A. P., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: A review and prospectus. Canadian Journal of Zoology **68**:619-640.

Lively, C. M. 1986. Predator-induced shell dimorphism in the acorn barnacle *Chthamalus anisopoma*. Evolution **40**:232-242.

Lively, C. M., W. N. Hazel, M. J. Schellenberger, and K. S. Michelson. 2000. Predator-induced defense: Variation for inducibility in an intertidal barnacle. Ecology **81**:1240-1247.

Magurran, A. E., P. W. Irving, and H. P.A. 1996. Is there a fish alarm pheromone? A wild study and critique. Proceedings of the Royal Society of London Series B-Biological Sciences **263**:1551-1556.

Mathis, A., and R. J. F. Smith. 1993. Intraspecific and cross-superorder responses to chemical alarm signals by brook stickleback. Ecology **74**:2395-2404.

Marko, P. B., and A. R. Palmer. 1991. Responses of a rocky shore gastropod to the effluents of predatory and non-predatory crabs: avoidance and attraction. Biological Bulletin **181**:363-370.

McCarthy, T. M., and W. A. Fisher. 2000. Multiple predator-avoidance behaviours of the freshwater snail *Physella heterostropha pomila*: responses vary with risk. Freshwater Biology **44**:387-397.

McCollum, S. A., and J. D. Leimberger. 1997. Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. Oecologia **109**:615-621.

McCollum, S. A., and J. Van Buskirk. 1996. Costs and benefits of a predator-induced polyphenism in the gray tree frog *Hyla chrysoscelis*. Evolution **50**:583-593.

McIntyre, P. B., S. Baldwin, and A.S. Flecker. 2004. Effects of behavioral and morphological plasticity on risk of predation in a Neotropical tadpole. Oecologia **141**:130-138.

McPeek, M. A., M. Grace, and J. M. L. Richardson. 2001. Physiological and behavioral responses to predators shape the growth/predation risk trade-off in damselflies. Ecology **82**:1535-1545.

Miner, B. G., S. E. Sultan, S. G. Morgan, D. K. Padilla, and R. A. Relyea. 2005. Ecological consequences of phenotypic plasticity. Trends in Ecology and Evolution **20**:685-692.

Miner, B. G., and J. R. Vonesh. 2004. Effects of fine grain environmental variability on morphological plasticity. Ecology Letters **7**:794-801.

Mirza, R. S. and Chivers, D. P. 2001a. Learned recognition of heterospecific alarm signals: The importance of a mixed predator diet. Ethology **107**:1007-1018.

Mirza, R. S., and D. P. Chivers. 2001b. Are chemical alarm cues conserved within salmonid fishes? Journal of Chemical Ecology **27**:1641-1655.

Mirza, R. S., and D. P. Chivers. 2001c. Do juvenile yellow perch use diet cues to assess the level of threat posed by intraspecific predators? Behaviour **138**:1249-1258.

Mirza, R. S. and Chivers, D. P. 2003. Fathead minnows learn to recognize heterospecific alarm cues they detect in the diet of a known predator. Behavior **140**:1359-1369.

Mirza, R. S., D. P. Chivers and J. J. Scott. 2001. Differential responses of male and female red swordtails to chemical alarm cues. Journal of Fish Biology **59**:716-728.

Mirza, R. S., S. A. Fisher, and D. P. Chivers. 2003. Assessment of predation risk by juvenile yellow perch, *Perca flavescens*: Responses to alarm cues from conspecifics and prey guild members. Environmental Biology of Fishes **66**:321-327.

Moczek, A. P. 1998. Horn polymorphism in the beetle *Onthophagus taurus*: Larval diet quality and plasticity in parental investment determine adult body size and male horn morphology. Behavioral Ecology **9**:636-641.

Moran, N. A. 1992. The evolutionary maintenance of alternative phenotypes. American Naturalist **139**:971-989.

Morin, J. P., B. Moreteau, G. Petavy, R. Parkash, and J. R. David. 1997. Reaction norms of morphological traits in *Drosophila*: adaptive shape changes in a stenotherm circumtropical species? Evolution **51**:1140-1148.

Novoplansky, A., and D. E. Goldberg. 2001. Effects of water pulsing on individual performance and competitive hierarchies in plants. Journal of Vegetation Science **12**:199-208.

Padilla, D. K., and S. C. Adolph. 1996. Plastic inducible morphologies are not always adaptive: The importance of time delays in a stochastic environment. Evolutionary Ecology **10**:456-456.

Parker, D. A., and M. J. Shulman. 1986. Avoiding predation: Alarm responses of Caribbean sea urchins to simulated predation on conspecific and heterospecific sea urchins. Marine Biology **93**:201-208.

Peacor, S. D., and E. E. Werner. 1997. Trait-mediated indirect interactions in a simple aquatic food web. Ecology **78**:1146-1156.

Pecor, K. W., and B. A. Hazlett. 2003. Frequency of encounter with risk and the tradeoff between pursuit and antipredator behaviors in crayfish: A test of the risk allocation hypothesis. Ethology **109**:97-106.

Persons M.H., S. E. Walker, A. L. Rypstra, and S. D. Marshall. 2001. Wolf spider predator avoidance tactics and survival in the presence of diet-associated predator cues (Araneae : Lycosidae). Animal Behavior **61**:43-51.

Petranka, J. W. 1989. Response of toad tadpoles to conflicting chemical stimuli: Predator avoidance versus "optimal" foraging. Herpetologica **45**:283-292.

Petranka, J., and L. Hayes. 1998. Chemically mediated avoidance of a predatory odonate (*Anax junius*) by American toad (*Bufo americanus*) and wood frog (*Rana sylvatic*a) tadpoles. Behavioral Ecology and Sociobiology **42**:263-271.

Petranka, J. W., L. B. Kats, and A. Sih. 1987. Predator-prey interactions among fish and larval amphibians: Use of chemical cues to detect predatory fish. Animal Behaviour **35**:420-425.

Pettersson, L. B., P. A. Nilsson, and C. Brönmark. 2000. Predator recognition and defense strategies in crucian carp, *Carassius carassius*. Oikos **88**:200-212.

Pfeiffer, W., G. Riegelbauer, G. Meier, and B. Schiebler. 1985. Effects of hypoxanthine-3-N-oxide and hypoxanthine-1-N-oxide on central nervous excitation of the black tetra, *Gymnocorymbus ternetzi* (Characidae, Ostariophysi, Pisces) indicated by dorsal light response. Journal of Chemical Ecology **11**:507-523.

Pfennig, D. 1990. The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. Oecologia **85**:101-107.

Phillips, D. W. 1976. The effect of a species-specific avoidance response to predatory starfish on the intertidal distribution of two gastropods. Oecologia **23**:83-94.

Pigliucci, M. 1997. Ontogenetic phenotypic plasticity during the reproductive phase in *Arabidopsis thaliana* (Brassicaceae). American Journal of Botany **84**:887-895.

Pigliucci, M. 2001. Phenotypic plasticity: Beyond nature and nurture. Johns Hopkins University Press, Baltimore, MD.

Pigliucci, M., P. deIorio, and C. D. Schlichting. 1997. Phenotypic plasticity of growth trajectories in two species of *Lobelia* in response to nutrient availability. Journal of Ecology **85**:265-276.

Pijanowska, J. 1997. Alarm signals in *Daphnia*? Oecologia **112**:12-16.

Puttlitz, M. H., D. P. Chivers, J. M. Kiesecker, and A. R. Blaustein. 1999. Threat-sensitive predator avoidance by larval Pacific tree frogs (Amphibia, Hylidae). Ethology **105**:449-456.

Relyea R.A. 2000. Trait-mediated indirect effects in larval anurans: Reversing competition with the threat of predation. Ecology **81**:2278-2289.

Relyea, R. A. 2001a. Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology **82**:523-540.

Relyea, R. A. 2001b. The relationship between predation risk and antipredator responses in larval anurans. Ecology **82**:541-554.

Relyea, R. A. 2002a. Costs of phenotypic plasticity. American Naturalist **159**:272-282.

Relyea, R. A. 2002b. Local population differences in phenotypic plasticity: Predator-induced changes in wood frog tadpoles. Ecological Monographs **72**:77-93.

Relyea R.A. 2002c. Competitor-induced plasticity in tadpoles: Consequences, cues, and connections to predator-induced plasticity. Ecological Monographs **72**:523-540.

Relyea, R. A. 2002d. The many faces of predation: How induction, selection, and thinning combine to alter prey phenotypes. Ecology **83**:1953-1964.

Relyea, R. A. 2003a. How prey respond to combined predators: A review and an empirical test. Ecology **84**:1827-1839.

Relyea, R. A. 2003b. Predators come and predators go: The reversibility of predator-induced traits. Ecology **84**:1840-1848.

Relyea, R. A. 2004. Fined-tuned phenotypes: Tadpole plasticity under 16 combinations of predators and competitors. Ecology **85**:172-179.

Relyea, R. A. 2005. The heritability of inducible defenses in tadpoles. Journal of Evolutionary Biology **18**:856-866.

Relyea, R. A., and J. R. Auld. 2004. Having the guts to compete: How intestinal plasticity explains costs of inducible defenses. Ecology Letters **7**:869-875.

Relyea, R. A., and J. R. Auld. 2005. Predator- and competitor-induced plasticity: How changes

in foraging morphology affect phenotypic trade-offs. Ecology **86**:1723-1729.

Relyea, R. A., and E. E. Werner. 1999. Quantifying the relation between predator-induced behavior and growth performance in larval anurans. Ecology **80**:2117-2124.

Relyea, R. A., and E. E. Werner. 2000. Morphological plasticity of four larval anurans distributed along an environmental gradient. Copeia 2000:178-190.

Richardson, J. L. 2006. Novel features of an inducible defense system in larval tree frogs (*Hyla chrysoscelis*). Ecology **87**:780-787.

Roff, D. A. 1996. The evolution of threshold traits in animals. The Quarterly Review of Biology **71**:3-35.

Ruehl, C. B., and T. J. DeWitt. 2005. Trophic plasticity and fine-grained resource variation in populations of western mosquitofish, *Gambusia affinis*. Evolutionary Ecology Research **7**:801-819.

Ruel, J. J., and M. P. Ayres. 1999. Jensen's inequality predicts effects of environmental variation. Trends in Ecology and Evolution **14**:361-366.

Scheiner, S. M. 1993. Genetics and evolution of phenotypic plasticity. Annual Review of Ecology and Systematics **24**:35-68.

Schlichting, C. D. 1989. Phenotypic integration and environmental change. Bioscience **39**:460-464.

Schlichting, C. D., and D. A. Levin. 1984. Phenotypic plasticity of annual *Phlox*: tests of some hypotheses. American Journal of Botany **71**:252-260.

Schlichting, C. D., and M. Pigliucci. 1998. Phenotypic Evolution: A Reaction Norm Perspective. Sinauer, Sunderland, Massachusettes, U.S.A.

Schoeppner, N. M., and R. A. Relyea. 2005. Damage, digestion, and defense: The roles of alarm cues and kairomones for inducing prey defenses. Ecology Letters **8**:505-512.

Semlitsch R.D., D. C. Scott and J. H. K. Pechmann. 1988. Time and size at metamorphosis related to adult fitness in *Ambystoma talpoideum*. Ecology **69**:184-192.

Siems, D. P., and R. S. Sikes. 1998. Tradeoffs between growth and reproduction in response to temporal variation in food supply. Environmental Biology of Fishes **53**:319-329.

Sih, A. 1980. Optimal behavior: Can foragers balance two conflicting demands? Science **210**:1041-1043.

Sih, A. 1992. Prey uncertainty and the balancing of antipredator and feeding needs. American Naturalist **139**:1052-1069.Werner, E. E. 1992. Individual behavior and higher-order species Interactions. American Naturalist **140**:S5-S32.

Sih, A., and T. M. McCarthy. 2002. Prey responses to pulses of risk and safety: Testing the risk allocation hypothesis. Animal Behaviour **63**:437-443.

Skelly, D. K. 1992. Field evidence for a cost of behavioral antipredator response in a larval amphibian. Ecology **73**:704-708.

Skelly, D. K. 1994. Activity level and susceptibility of anuran larvae to predation. Animal Behaviour **47**:465-468.

Skelly, D. K., and E. E. Werner. 1990. Behavioral and life-historical responses of larval American toads to an odonate predator. Ecology **71**:2313-2322.

Slusarczk, M. 1999. Predator-induced diapause in *Daphnia magna* may require two chemical cues. Oecologia **119**:159-165.

Smith, D. C., and J. Van Buskirk. 1995. Phenotypic design, plasticity, and ecological performance in two tadpole species. American Naturalist **145**:211-233.

Smith, M. E. and M. C. Belk. 2001. Risk assessment in western mosquitofish (*Gambusia affinis*): Do multiple cues have additive effects? Behavioral Ecology and Sociobiology **51**:101-107.

Smith, R. J. F. 1982. Reaction of *Percina nigrofasciata*, *Ammocrypta beani*, and *Etheostoma swaini* (Percidae, Pisces) to conspecific and intergeneric skin extracts. Canadian Journal of Zoology **60**:1067-1072.

Smith, R. J. F. 1992. Alarm signals in fishes. Review of Fish Biology and Fisheries **2**:33-63.

Spitze, K. 1992. Predator-mediated plasticity of prey life history and morphology: *Chaoborus americanus* predation on *Daphnia pulex*. American Naturalist **139**:229-247.

Stabell, O. B., and M. S. Lwin. 1997. Predator-induced phenotypic changes in crucian carp are caused by chemical signals from conspecifics. Environmental Biology of Fishes **49**:145-149.

Stabell, O. B., F. Ogbebo, and R. Primicerio. 2003. Inducible defenses in *Daphnia* depend on latent alarm signals from conspecific prey activated in predators. Chemical Senses **28**:141-153.

Stemberger, R. S. 1988. Reproductive costs and hydrodynamic benefits of chemically induced defenses in *Keratella testudo*. Limnology and Oceanography **33**:593-606.

Stemberger, R. S., and J. J. Gilbert. 1987. Multiple species induction of morphological defenses in the rotifer *Keratella testudo*. Ecology **68**:370-378.

Stirling, G. 1995. *Daphnia* behavior as a bioassay of fish presence or predation. Functional Ecology **9**:778-784.

Sullivan, A. M., D. M. Madison, and J. R. Rohr. 2003. Behavioral responses by red-backed salamanders to conspecific and heterospecific cues. Behavior **140**:553-564.

Sultan, S. E., and F. A. Bazzaz. 1993. Phenotypic plasticity in *Polygonum persicaria*. I. Diversity and uniformity in genotypic norms of reaction to light. Evolution **47**.

Summey, M. R., and A. Mathis. 1998. Alarm responses to chemical stimuli from damaged conspecifics by larval anurans: Tests of three neotropical species. Herpetologica **54**:402-408.

Tollrian, R. 1993. Neckteeth formation in *Daphnia pulex* as an example of continuous phenotypic plasticity: Morphological effects of *Chaoborus* kairomone concentration and their quantification. Journal of Plankton Research **15**:1309-1318.

Tollrian, R., and D. Harvell. 1999. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, New Jersey, U.S.A.

Turner, A. M. 1996. Freshwater snails alter habitat use in response to predation. Animal Behaviour **51**:747-756.

Turner, A. M. 1997. Contrasting short-term and long-term effects of predation risk on consumer habitat use and resources. Behavioral Ecology **8**:120-125.

Turner, A. M., R. J. Bernot, and C. M. Boes. 2000. Chemical cues modify species interactions: the ecological consequences of predator avoidance by freshwater snails. Oikos **88**:148-158.

Turner, A. M., S. A. Fetterolf, and R. J. Bernot. 1999. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. Oecologia **118**:242-247.

Turner, A. M., and S. L. Montgomery. 2003. Spatial and temporal scales of predator avoidance: experiments with fish and snails. Ecology **84**:616-622.

Turner, A. M., S. A. Fetterolf, and R. J. Bernot. 1999. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. Oecologia **118**:242-247.

Van Buskirk, J. 2000. The costs of an inducible defense in anuran larvae. Ecology **81**:2813-2821.

Van Buskirk, J. 2001. Specific induced responses to different predator species in anuran larvae. Journal of Evolutionary Biology **14**:482-489.

Van Buskirk, J. 2002a. A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. American Naturalist **160**:87-102.

Van Buskirk, J. 2002b. Phenotypic lability and the evolution of predator-induced plasticity in tadpoles. Evolution **56**:361-370.

Van Buskirk, J., and R. A. Relyea. 1998. Natural selection for phenotypic plasticity: predator-induced morphological responses in tadpoles. Biological Journal of the Linnean Society **65**:301-328.

Van Buskirk, J., and M. Arioli. 2002. Dosage response of an induced defense: how sensitive are tadpoles to predation risk? Ecology **83**:1580-1585.

Van Buskirk, J., S. A. McCollum, and E. E. Werner. 1997. Natural selection for environmentally induced phenotypes in tadpoles. Evolution **51**:1983-1992.

Van Buskirk, J., C. Muller, A. Portmann, and M. Surbeck. 2002. A test of the risk allocation hypothesis: tadpole responses to temporal change in predation risk. Behavioral Ecology **13**:526-530.

Van Buskirk, J., P. Anderwald, S. Lupold, L. Reinhardt, and H. Schuler. 2003. The lure effect, tadpole tail shape, and the target of dragonfly strikes. Journal of Herpetology **37**:420-424.

van Tienderen, P. H. 1990. Morphological variation in *Plantago lanceolata*: limits of plasticity. Evolutionary Trends in Plants **44**:35-43.

van Tienderen, P. H. 1991. Evolution of generalists and specialists in spatially heterogeneous environments. Evolution **45**:1317-1331.

Venzon, M., A. Janssen, A. Pallini, and M.W. Sabelis. 2000. Diet of a polyphagous arthropod predator affects refuge seeking of its thrips prey. Animal Behavior **30**:369-375.

Via, S., and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution **39**:505-522.

Vilhunen, S., and H. Hirvonen. 2003. Innate antipredator responses of Arctic charr (*Salvelinus alpinus*) depend on predator species and their diet. Behavioral Ecology and Sociobiology **55**:1-10.

Wallace, D. G., L. R. Maxson and A. D. Wilson. 1971. Albumin evolution in frogs: A test of the evolutionary clock hypothesis. Proceedings of the National Academy of the Sciences of the United States of America **68**:3127-3129

Walls, M., and M. Ketola. 1989. Effects of predator-induced spines on individual fitness in *Daphnia pulex*. Limnology and Oceanography **34**:390-396.

Wayne, P. M., and F. A. Bazzaz. 1993. Birch seedling responses to daily time courses of light in experimental forest gaps and shadehouses. Ecology **74**:1500-1515.

Werner, E. E. 1992. Individual behavior and higher-order species interactions. American Naturalist **140**:S5-S32.

Werner, E. E., and B. R. Anholt. 1993. Ecological consequences of trade-off between growth and mortality rates mediated by foraging activity. American Naturalist **142**:242-272.

Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology **84**:1083-1100.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics **20**:249-278.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press, Oxford; New York., U.S.A.

Wiackowski, K., and A. Staronska. 1999. The effect of predator and prey density on the induced defense of a ciliate. Functional Ecology **13**:59-65.

Wildy, E. L.,D. P. Chivers and A. R. Blaustein. 1999. Shifts in life-history traits as a response to cannibalism in larval long-toed salamanders (*Ambystoma macrodactylum*). Journal of Chemical Ecology **25**:2337-2346

Wilson, D. J., and H. Lefcort. 1993. The effect of predator diet on the alarm response of red-legged frog, *Rana aurora*, tadpoles. Animal Behaviour **46**:1017-1019.

Winn, A. A. 1996. Adaptation to fine-grained environmental variation: An analysis of within-individual leaf variation in an annual plant. Evolution **50**:1111-1118.

Wisenden, B. D., A. Cline, and T. C. Sparkes. 1999. Survival benefit to antipredator behavior in the amphipod *Gammarus minus* (Crustacea : Amphipoda) in response to injury-released chemical cues from conspecifics and heterospecifics. Ethology **105**:407-414.

Wisenden, B. D., S. G. Pohlman, and E. E. Watkin. 2001. Avoidance of conspecific injury-released chemical cues by free-ranging *Gammarus lacustris* (Crustacea: Amphipoda). Journal of Chemical Ecology **27**:1249-1258.

Wisenden, B. D., and M. C. Millard. 2001. Aquatic flatworms use chemical cues from injured conspecifics to assess predation risk and to associate risk with novel cues. Animal Behaviour **62**:761-766.

Wolfe, L. M., and S. J. Mazer. 2005. Patterns of phenotypic plasticity and their fitness consequences in wild radish (*Raphanus sativus*: Brassicaceae) **166**:631-640.

Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics **25**:443-466.