

# Detecting small environmental differences: risk-response curves for predator-induced behavior and morphology

Nancy M. Schoeppner · Rick A. Relyea

Received: 15 November 2006 / Accepted: 7 September 2007 / Published online: 6 October 2007  
© Springer-Verlag 2007

**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 traditionally been treated as threshold switches between induced and uninduced states, there is growing evidence that many traits can respond in a continuous fashion. In this experiment we exposed larval anurans (wood frog tadpoles, *Rana sylvatica*) to an increasing gradient of predation risk to determine how organisms respond to small environmental changes. We manipulated predation risk in two ways: by altering the amount of prey consumed by a constant number of predators (*Dytiscus* sp.) and by altering the number of predators that consume a constant amount of prey. We 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 a plateauing response, and whether increasing risk via increasing predator number or via increasing prey consumption induced similar phenotypic

changes. We 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 low levels of risk, suggesting that the chemical cues of predation are effective at extremely low concentrations. In contrast to earlier work, we 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.

**Keywords** Environmental sensitivity · Threshold response · Graded response · Continuous variation

---

Communicated by Steven Kohler.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00442-007-0862-4) contains supplementary material, which is available to authorized users.

---

N. M. Schoeppner · R. A. Relyea  
Department of Biological Sciences,  
University of Pittsburgh, Pittsburgh, PA 15260, USA

N. M. Schoeppner (✉)  
School of Biology, Georgia Institute of Technology,  
Atlanta, GA, USA  
e-mail: nschoeppne3@mail.gatech.edu

## Introduction

Phenotypic plasticity is a common response to changing environments. Organisms that possess adaptive plastic traits 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; DeWitt and Scheiner 2004). The range of phenotypes produced in response to environmental changes depends upon the gradient of the environmental factor that is experienced and the sensitivity of the organism in detecting and responding to environmental change. If sensitivity is low, organisms 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, organisms 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, detection 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 is reflected in the early 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 in nature most organisms experience a wide range of environments that can be arrayed along gradients. As a result, there has been a move to examine how organisms respond to a range of environments (West-Eberhard 2003; Relyea 2004a). When experiments have been conducted at a finer scale than simply high versus 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; Loose and Dawidowicz 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 environments are common, then previous conclusions about how organisms respond to environmental variation based on responses exhibited in two extreme environments may not correctly represent the true ecology and evolution of phenotypic plasticity or the range of phenotypes 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 2004a).

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. Given that some traits require a bigger resource investment, if relatively low-cost traits are effective in less extreme environments and the more costly traits are effective in more extreme environments, the more costly traits should only be induced once more extreme environments are encountered (i.e., have a higher threshold of induction). 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, and whether the function continues to increase or plateaus. 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). As in the threshold response, some detectable limit must first be met and then the magnitude of the response can either increase with increasing cue concentration or increase until some limit is met. 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 appear to display all three types of responses (Loose and Dawidowicz 1994; Anholt et al. 1996; Harvell 1998; Laurila et al. 2004; Van Buskirk and Arioli 2002; Relyea 2004a). Therefore, determining if graded responses are common for traits involved in inducible defenses and whether they plateau is an important step in determining how prey balance the costs and benefits of expressing defended phenotypes.

If plastic traits are sensitive to small changes in the environment, then fine-scale environmental variation (i.e., within a generation; Levins 1968) may drastically affect 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 2003). If individuals can detect and respond to small environmental changes with different phenotypes, prey may continually alter their phenotypes to produce the phenotype that is optimal for the environment. For example, if predator density 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 should affect how defensive traits are expressed over time. Therefore, determining how traits respond to fine-scale changes in the environment is a necessary step in understanding how temporal variability in the environment affects trait expression.

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. If prey are sensitive to both the amount of prey consumed and the number of predators, then we should observe differences in the trait inductions when we compare the same amount of prey consumed by different numbers of predators.

We addressed these issues in an experiment in which we 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, we measured how the tadpoles altered their behavior, growth, and relative morphology. We 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 vs. increased predator number).

## Materials and methods

We used a completely randomized design consisting of 11 treatments replicated 4 times for a total of 44 experimental

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's Aquatic Research Laboratory in northwestern Pennsylvania. The 11 treatments included a no-predator treatment, seven treatments in which we 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 we fed one, two, or six predators a constant amount of prey (200 mg) each day. This design allowed us 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 consuming prey was varied (e.g., one predator consuming 200 mg prey vs. four predators each consuming 50 mg prey). The pair-wise comparisons among the means were done using Fisher's least significant difference (LSD). Such comparisons allowed us to determine whether responses to increased predator number were simply responses to the higher total consumption of prey.

To simulate natural pond conditions, we used 800-l mesocosms (cattle tanks) containing 700 l 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 days 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. 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.

Depending on the treatment, each cage was either empty or contained a single larval beetle (*Dytiscus* sp.) that was fed wood frog tadpoles daily. All predators were fed in the afternoon each day. Almost all of the tadpoles were consumed daily for all of the treatments. When the tadpoles were not completely consumed by the next day, the next day's ration was still added to the cage. In most cases where the tadpoles were not consumed, the beetle larvae had died, and it was replaced.

We 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, we reared the hatchling tadpoles in pools containing aged well

water where they were fed rabbit chow ad libitum. We haphazardly selected groups of 30 tadpoles and added them to each mesocosm on 13 May 2005 (mean mass  $\pm$  SE =  $61 \pm 4$  mg).

Behavioral observations were conducted on 5 different days of the experiment (days 7, 10, 13, 19, and 20) where each tank was observed 12 times over a period of 2 h (three observations by four observers). Using established observation protocols (Relyea and Werner 1999), we counted the number of visible tadpoles in each mesocosm and the number of visible tadpoles that were moving. Thus, our 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 ANOVA to test for an effect of treatment, day, and their interaction. When a significant effect was found, we conducted pairwise comparisons using Fisher's LSD test.

After 24 days, 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, Wash.). We 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 we placed a glass plate under the tadpole's tail to bring both structures into the same plane of focus and ensure that we obtained an undistorted lateral image.

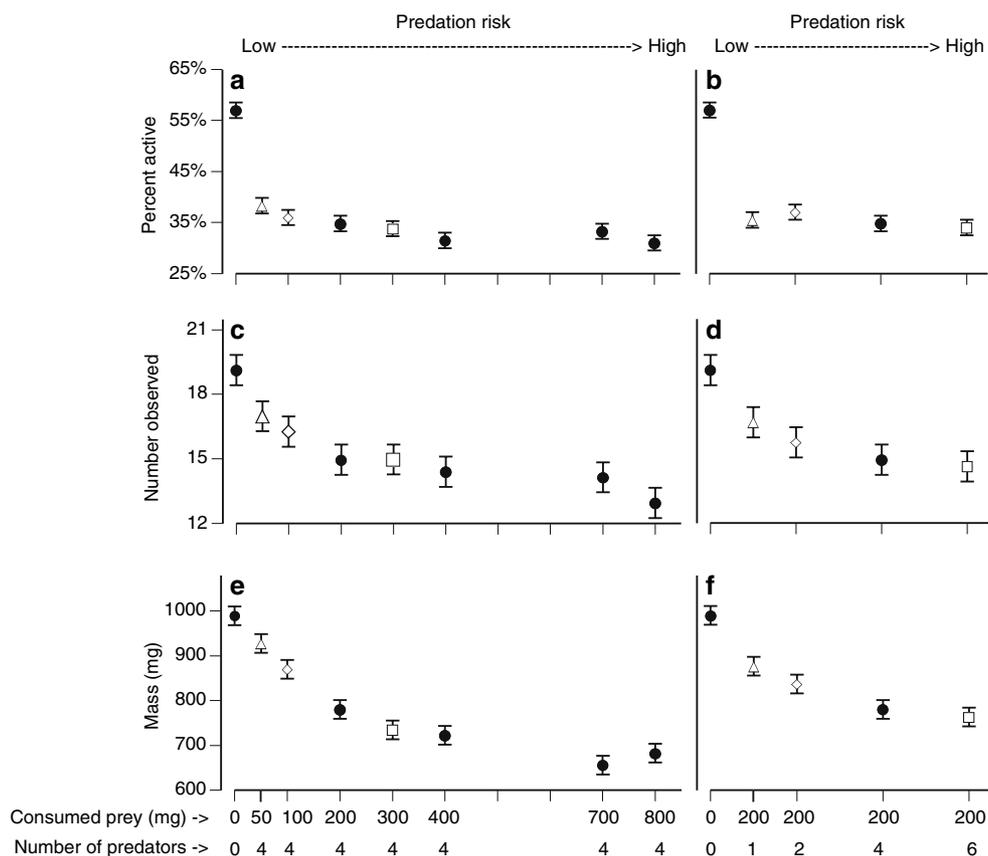
Because we were interested in changes in tadpole shape independent of differences in overall tadpole size (i.e., bigger tadpoles have bigger bodies and tails), we calculated size-adjusted estimates of all the morphological traits. The size-adjusted estimates were obtained using a multivariate analysis of covariance (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. We 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, we 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. We then used a multivariate ANOVA 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 our response variables. For significant univariate effects, we 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. We concluded that the response to increased prey consumption or predator number was not a threshold response when we found significant differences between any of the caged-predator treatments as the amount of risk increased. We summarize our results for each trait and both types of increasing predation risk below. To characterize the differences in sensitivity among the traits with increasing predation risk we also tested for differences in the rate at which each trait accelerated to reach its maximum induction (see "Appendix A").

## Results

We found significant effects of the predator on wood frog tadpole behavior, mass, and morphology. We found that chemical cues released by the predators induced the tadpoles to remain less active and to hide. Using a repeated-measures ANOVA on tadpole activity, we 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 in any of the caged-predator treatments throughout the experiment ( $P \leq 0.001$ ; Fig. 1a,b). Compared to the no-predator treatment, tadpole activity decreased by 33% when exposed to 50 mg consumed prey ( $P < 0.001$ ). In response to increasing numbers of predators, tadpole activity decreased by 38% when exposed to one predator, and increasing the number of predators did not further decrease tadpole activity ( $P \geq 0.494$ ).

In the repeated-measures ANOVA on the number of tadpoles observed (i.e., not hiding), we 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 ( $F_{50,136} = 1.5$ ,  $P = 0.09$ ). The tadpoles in the no-predator treatments hid less than the tadpoles in any of the predator treatments throughout the experiment ( $P \leq 0.001$ ; Fig. 1c,d). The number of tadpoles observed decreased by 11% when exposed to 50 mg consumed prey ( $P = 0.038$ ; Fig. 1c) and an additional 12% when exposed to 200 mg consumed prey ( $P = 0.05$ ). The number observed decreased even further (13%) in response to 800 mg consumed prey ( $P = 0.05$ ). Thus, tadpole hiding did not exhibit a plateauing response to increased prey consumption by predators, although the magnitude of the change per milligram consumed prey grew weaker at higher amounts of consumed prey. In response to increasing numbers of predators, the number of tadpoles observed 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



**Fig. 1** The **a, b** behavior, **c, d** number, and **e, f** mass of wood frog (*Rana sylvatica*) tadpoles when exposed to treatments that varied along an increasing gradient of predation risk (means  $\pm$  1SE). We simulated increasing predation risk by either increasing the amount of prey consumed daily by each of four predators (**a, c, e**) or the number of predators fed a constant daily amount (200 mg) of prey (**b, d, f**). The three open symbols in each panel correspond to the treatments which con-

tained the same total mass of prey consumed but different numbers of predators consuming prey. For example one predator consuming 200 mg prey (*right-hand panels*) versus four predators each consuming 50 mg prey (*left-hand panels*) are indicated by triangles. To aid visual comparisons, two treatments are presented in both left and right columns, zero prey with zero predators and 200 mg prey consumed by four predators

( $P = 0.706$ ). Thus, in response to predator number, tadpole hiding exhibited a graded response (Fig. 1d).

We found a multivariate effect of the treatments on tadpole mass and morphology (Table 1). Univariate analysis (Table 1) 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. 1e,f). Tadpole mass was also affected by increases in prey consumption (Fig. 1e). Tadpoles mass was 6% lower when they were exposed to 50 mg consumed prey ( $P = 0.04$ ). Tadpole mass decreased an additional 16% when exposed to 200 mg consumed prey ( $P < 0.001$ ) and decreased even further (16%) when exposed to 700 mg consumed prey ( $P < 0.001$ ). At 800 mg consumed prey, there was no further decline in mass ( $P = 0.385$ ). Tadpole mass was also affected by increasing numbers of predators

**Table 1** Results of a multivariate ANOVA and subsequent univariate tests that examined the effects of cue concentration and predator number on the mass and seven morphological traits of wood frog tadpoles

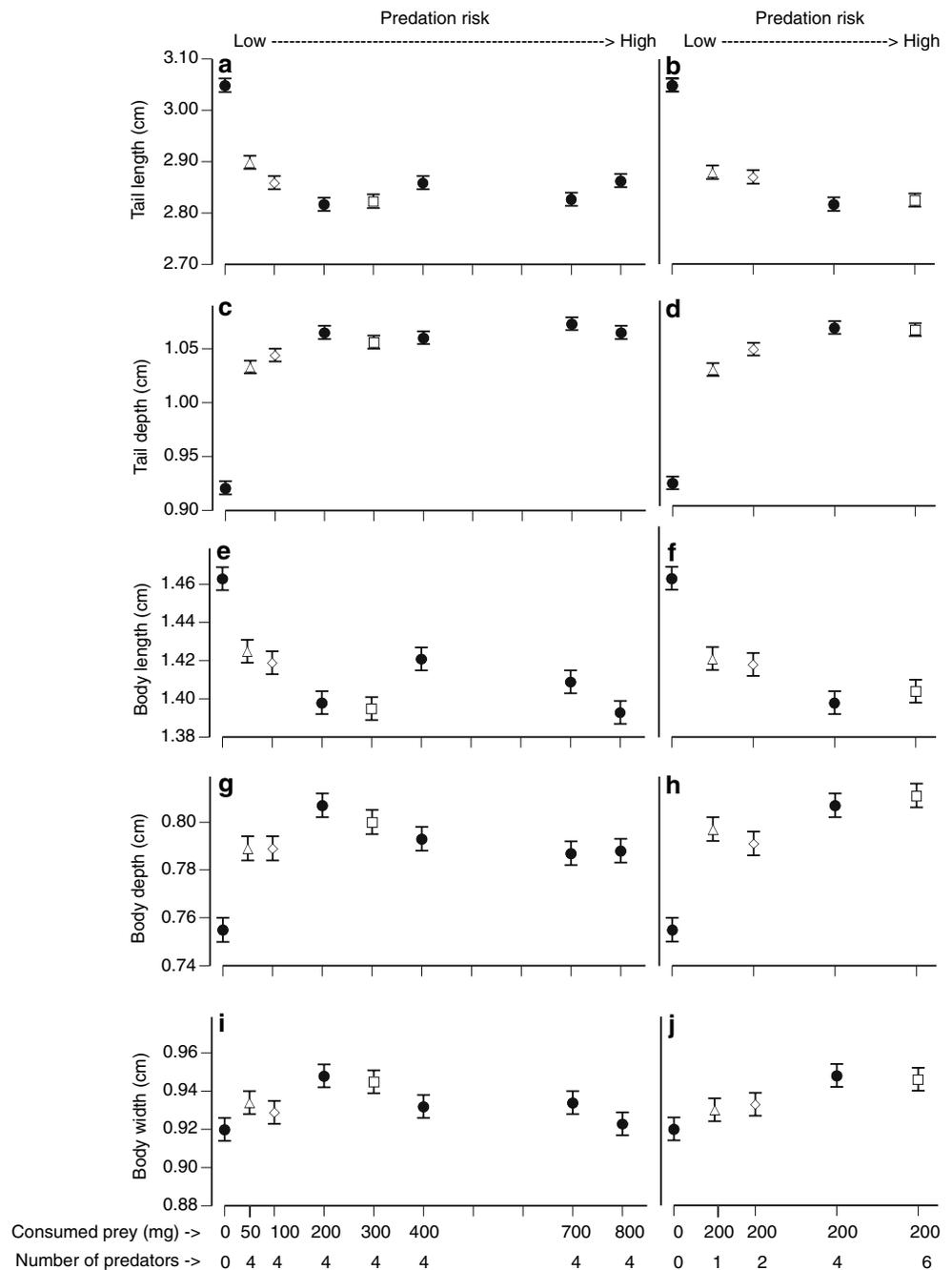
Multivariate test	df	F	P
Treatment	90,180	4.0	<0.001
Univariate test			Predation risk
Mass			<0.001
Body length			<0.001
Body width			0.021
Body depth			<0.001
Tail length			<0.001
Tail depth			<0.001
Mouth width			0.259
Muscle width			0.352
Muscle depth			0.078

(Fig. 1f). Tadpoles mass was 11% lower in response to one predator ( $P = 0.001$ ) and an additional 11% lower in response to four predators ( $P < 0.001$ ).

All of the predator treatments also induced relatively shorter and deeper tails ( $P \leq 0.001$ , Fig. 2a–d) and shorter and deeper bodies ( $P \leq 0.001$ , Fig. 2e–f) than the no-predator treatment. Tadpole tails were 5% shorter when exposed to 50 mg consumed prey ( $P < 0.001$ ; Fig. 2a). Tail length decreased an additional 2% when exposed to 100 mg 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, we did not find clear evidence that the response was plateauing either. Tadpole tails were 6%

**Fig. 2** The relative morphology of wood frog (*R. sylvatica*) tadpoles when exposed to treatments that varied along an increasing gradient of predation risk (means  $\pm$  1SE): **a, b** tail length, **c, d** tail depth, **e, f** body length, **g, h** body depth, **i, j** body width. We simulated increasing predation risk by either increasing the amount of prey consumed daily by each of four predators (**a, c, e, g, i**) or the number of predators fed a constant daily amount (200 mg) of prey (**b, d, f, h, j**). The three open symbols in each panel correspond to the treatments which contained the same total mass of prey consumed, but different numbers of predators consuming prey. For example one predator consuming 200 mg prey (*right-hand panels*) versus four predators each consuming 50 mg prey (*left-hand panels*) are indicated by triangles. To aid visual comparisons, two treatments are presented in both left and right columns, zero prey with zero predators and 200 mg prey consumed by four predators



shorter in response to one predator ( $P < 0.001$ ) and decreased an additional 2% in response to four predators ( $P = 0.002$ ; Fig. 2b). Tadpole tails were 11% deeper when exposed to 50 mg consumed prey ( $P < 0.001$ ; Fig. 2c) and increased an additional 3% in response to 200 mg consumed prey ( $P < 0.001$ ). For increasing predator number, tadpole tails were 10 % deeper when exposed to one predator ( $P < 0.001$ ) and increased an additional 2% when exposed to two predators ( $P = 0.039$ ; Fig. 2d).

The amount of prey consumed by predators also affected the relative size of the tadpole body. Tadpole bodies were 5% shorter when exposed to 50 mg consumed prey ( $P < 0.001$ ; Fig. 2e). The bodies became even shorter in several of the higher consumption treatments (200, 300, 700, and 800 mg;  $P \leq 0.05$ ). However, the body length of tadpoles in the 100- and 400-mg treatments was not different from that in the 50-mg treatment ( $P \geq 0.457$ ). Tadpole bodies were 3% shorter when exposed to one predator ( $P > 0.001$ ; Fig. 2f) and became even shorter (2%) when exposed to four predators ( $P = 0.013$ ). Tadpole bodies were 5% deeper when exposed to 50 mg consumed prey ( $P < 0.001$ ; Fig. 2g). Body depth increased even more in response to 200 mg 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 ( $P = 0.022$ ). Tadpole bodies were 6% deeper when exposed to one predator ( $P < 0.001$ ) and became an additional 2% deeper when exposed to four predators ( $P = 0.049$ ) (Fig. 2h). Tadpoles exposed to 50 mg consumed prey did not change body width compared to the no-predator treatment ( $P = 0.077$ ; Fig. 2i). In fact, it required at least 200 or 300 mg consumed prey to induce an increase in body width ( $P \leq 0.003$ ) and these two treatments were not different from each other ( $P = 0.753$ ). Tadpole body width did not respond to one or two predators ( $P > 0.1$ ) but tadpoles were 3% wider when exposed to four predators ( $P = 0.001$ )

(Fig. 2j). Body width did not increase further when exposed to six predators ( $P = 0.845$ ).

Trait sensitivity

For those traits that exhibited graded responses, we tested for differences in the sensitivity among traits (see “Appendix A”). We found differences among the traits in the rate of change with increasing predation risk ( $k$ ) for increasing amounts of prey consumed ( $F_{3,12} = 71.6, P < 0.001$ ) and for increasing numbers of predators ( $F_{5,17} = 38.6, P < 0.001$ ). When we increased the amount of consumed prey, tail depth was the most sensitive trait (i.e., most rapidly responding) followed by activity, mass, and the number of tadpoles observed (Fig. A1). When we increased the number of predators, tail depth was still the most sensitive trait with a significantly greater slope than all of the other traits (Fig. A2). Tail length, body length, mass, and the number of tadpoles observed all changed at similar rates as the number of predators increased ( $P \geq 0.05$ ), while tadpole body depth increased more slowly than all other traits ( $P < 0.01$ ) except tadpole mass and the number observed ( $P \geq 0.069$ ).

Prey consumed versus predator number

There were two traits (activity and body width) that did not exhibit graded responses to increased predator number but did exhibit graded responses to increased amounts of consumed prey (Table 2). These differences can be explained by the fact that the total amount consumed in the prey-consumption treatments (200–3,200 mg) spanned a much wider range than the predator-number treatments (200–1,200 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.

**Table 2** Summary of the responses observed for the behavioral and morphological traits in response to different amounts of consumed prey or number of predators. The responses were classified as either thresh-

old, graded, or neither. The plateau and  $P$ -values reported indicate the treatment at which no further trait changes were detected

Trait	Amount of prey consumed			Number of predators		
	Classification	Plateau level	$P$ value	Classification	Plateau level	$P$ value
Percent active	Graded	300 mg	$P \geq 0.214$	Threshold	–	–
Number observed	Graded	–	–	Graded	4 Predators	$P = 0.706$
Mass	Graded	700 mg	$P = 0.385$	Graded	4 Predators	$P = 0.563$
Tail length	Neither	–	–	Graded	4 Predators	$P = 0.685$
Tail depth	Graded	200 mg	$P \geq 0.315$	Graded	2 Predators	$P > 0.100$
Body length	Neither	–	–	Graded	4 Predators	$P = 0.521$
Body depth	Neither	–	–	Graded	4 Predators	$P = 0.586$
Body width	Neither	–	–	Threshold	–	–

Because we manipulated both the amount of prey consumed and the number of predators, we could determine whether the response to increased numbers of predators was simply reflecting the greater prey consumption that occurred when there were more predators. We compared treatments that contained different numbers of predators but the same total mass of consumed prey (i.e., four predators each consuming 50 mg prey vs. one predator consuming 200 mg prey, four predators each consuming 100 mg prey vs. two predators each consuming 200 mg prey, and four predators each consuming 300 mg prey vs. six predators each consuming 200 mg prey). We 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.

## 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 anti-predator responses that we 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 2002; 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 2001), but comes 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); while 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 2004b). 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; Turner et al. 1999; Relyea 2001; Vilhunen 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, with prey typically responding more strongly 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 2004a). Fourth, prey 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), we 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 our study shed light onto how tadpole prey use these chemical cues in making their defensive phenotypes. We 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). In that paper we found that the most extreme induction of behavioral defense and any morphological defense require both cue components in combination. In the current study, we 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 or both. By making several comparisons of different numbers of predators consuming the same total amount of prey, we 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. Our results are also consistent with the hypothesis that prey recognize predators through the way the predator modifies the alarm cues during digestion and not by compounds that they emit themselves. However, more studies addressing this question using different model systems are needed before we can arrive at any general conclusions.

The tadpole responses to increasing predation risk were not typically simple “on–off switches” that exhibited threshold responses, but were instead fine-tuned, graded responses to prey consumption, which likely are induced at very low concentrations of predation cues. 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 2004a) although threshold responses were observed for tail length (Van Buskirk and Arioli 2002) and body length (Relyea 2004a). The differences among these studies and the current study may lie in the fact that the current study examined a wider range of prey consumption and predator numbers (200–3,200 mg total consumed prey and from zero to six predators) than the earlier studies (Van Buskirk and Arioli 2002 used 200–800 mg total consumed prey and from zero to three predators; Relyea 2004a used 0–1,200 mg total consumed prey and from zero to four predators). This wider range of treatments would be more likely to detect graded responses where they truly exist. Loose and Dawidowicz (1994) demonstrated that cladocerans exhibit threshold-type responses that then continue to intensify until a plateau is reached. These results clearly show that there is a lower limit to either the organism's ability to detect the cue or profitability to respond to such low levels of cue. However, the question remains as to whether the range of cue concentrations span the actual concentrations encountered in nature in the work to date. This may be a particularly important question if the concentrations of cues detected in nature fall into the range below what was used in this experiment. If predation cues in nature are typically at a concentration far below what was used in our experiment, the traits may already have reached the maximum induction

at the lowest amount of prey consumed. Research which focuses on determining the chemical identity of the predation cues involved in the induction and the ecologically relevant concentrations of the cues is needed to address these questions.

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). Our study did not support this proposition. For increasing prey mass, tadpole tail depth achieved maximum induction more quickly than both behavioral traits (see “Appendix A”). While there were differences in how quickly the trait values increased, 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 prey or one predator consuming 200 mg prey), suggesting that the chemical cues emitted by aquatic predators are effective at very low concentrations (i.e., one predator in 700 l water). In contrast, body width did not exhibit any significant induction until the tadpoles were exposed to four predators consuming 200 mg prey. This indicates that different plastic traits can 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 very 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 relatively 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.

While most of the traits that exhibited plateauing responses had reached the maximum induction in response to 200 or 300 mg consumed prey, two of the traits 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.

#### 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 we 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 day). 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. In a separate study, we use this approach to examine how temporal variation in predation risk impacts the anti-predator traits of wood frog tadpoles when mean risk is held constant (N. M. Schoeppner and R. A. Relyea, unpublished data).

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.

#### Conclusion

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, our 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.

**Acknowledgements** The authors thank Nicole Diecks, Jon Lam, and Dustin Lutomski for their help with the experiment. They also thank Josh Auld, Anthony Bledsoe, Jason Hoverman, and Stephen Tonsor for providing many great comments that improved this manuscript. This work was supported by the NSF, including a Dissertation Improvement Grant (DDIG no. 0508282 to N. M. S.).

#### References

- Andersson S, Shaw RG (1994) Phenotypic plasticity in *Crepis tectorum* (Asteraceae): genetic correlations across light regimes. *Heredity* 72:113–125
- Anholt BR, Skelly DK, Werner EE (1996) Factors modifying antipredator behavior in larval toads. *Herpetologica* 52:301–313
- Anholt BR, Werner E, Skelly DK (2000) Effect of food and predators on the activity of four larval ranid frogs. *Ecology* 81:3509–3521
- Barry MJ, Bayly IAE (1985) Further studies on predator induction of crests in Australian *Daphnia* and the effects of crests on predation. *Aust J Mar Fresh Res* 36:519–535
- Black AR (1993) Predator-induced phenotypic plasticity in *Daphnia pulex*: life history and morphological responses to *Notonecta* and *Chaoborus*. *Limnol Oceanogr* 38:986–996
- Blouin MS (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, Spaak P, De Meester L (1998) Predator-mediated plasticity in morphology, life history, and behavior of *Daphnia*: the uncoupling of responses. *Am Nat* 152:237–248
- Cook SA, Johnston MP (1968) Adaptation to heterogeneous environments. I. Variation in heterophyly in *Ranunculus flammula*. *Evolution* 22:496–516
- DeWitt TJ (1998) Costs and limits of phenotypic plasticity: tests with predator-induced morphology and life history in a freshwater snail. *J Evol Biol* 11:465–480
- DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity: tests with predator-induced morphology and life history in a freshwater snail. *J Evol Biol* 11:465–480
- DeWitt TJ, Scheiner SM (2004) Phenotypic plasticity: functional and conceptual approaches. Oxford University Press, New York
- Dudley SA, Schmitt J (1996) Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *Am Nat* 147:445–465
- Gabriel W (1999) Evolution of reversible plastic responses: inducible defenses and environmental tolerance. In: Tollrian R, Harvell CD (eds) The ecology and evolution of inducible defenses. Princeton University Press, Princeton, N.J., pp 286–305
- Grant JW, Bayly IAE (1981) Predator induction of crests in morphs of the *Daphnia carinata* King complex. *Limnol Oceanogr* 26:201–218
- Greene E (1989) A diet-induced developmental polymorphism in a caterpillar. *Science* 243:643–646
- Gupta AP, Lewontin RC (1982) A study of reaction norms in natural populations of *Drosophila pseudoobscura*. *Evolution* 36:934–948
- Hanazato T, Ooi T (1992) Morphological responses of *Daphnia ambigua* to different concentrations of a chemical extract from *Chaoborus flavicans*. *Freshwater Biol* 27:379–385
- Harvell CD (1998) Genetic variation and polymorphism in the inducible spines of a marine bryozoan. *Evolution* 52:80–86
- Harvell CD (1990) The ecology and evolution of inducible defenses. *Q Rev Biol* 65:323–340
- Havel JE (1985) Predation of common invertebrate predators on long- and short-featured *Daphnia retrocurva*. *Hydrobiologia* 124:141–149
- Hazel W, Smock R, Lively CM (2004) The ecological genetics of conditional strategies. *Am Nat* 163:888–900
- Horat P, Semlitsch RD (1994) Effects of predation risk and hunger on the behavior of two species of tadpoles. *Behav Ecol Sociobiol* 34:393–401
- Houston AI, McNamara JM, Hutchinson JMC (1993) General results concerning the trade-off between gaining energy and avoiding predation. *Philos T Roy Soc B* 341:375–397
- Kats LB, Dill LM (1998) The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* 5:361–394
- Kingsolver JG (1995) Fitness consequences of seasonal polymorphism in western white butterflies. *Evolution* 49:942–954
- Kusch RC, Mirza RS, Chivers DP (2004) Making sense of predator scents: investigating the sophistication of predator assessment abilities of fathead minnows. *Behav Ecol Sociobiol* 55:551–555
- Langerhans RB, DeWitt TJ (2002) Plasticity constrained: over-generalized induction cues cause maladaptive phenotypes. *Evol Ecol Research* 4:857–870
- 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, Kujasalo J, Ranta E (1997) Different antipredator behaviour in two anuran tadpoles: effects of predator diet. *Behav Ecol Sociobiol* 40:329–336
- Laurila A, Jarvi-Laturi M, Pakkasmaa S, Merila J (2004) Temporal variation in predation risk: stage-dependency, graded responses and fitness costs in tadpole antipredator defences. *Oikos* 107:90–99
- Levins R (1968) Evolution in changing environments. Princeton University Press, Princeton, N.J.
- Lima SL, Bednekoff PA (1999) Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *Am Nat* 153:649–659
- Lively CM (1986) Predator-induced shell dimorphism in the acorn barnacle *Chthamalus anisopoma*. *Evolution* 40:232–242
- Lively CM, Hazel WN, Schellenberger MJ, Michelson KS (2000) Predator-induced defense: variation for inducibility in an intertidal barnacle. *Ecology* 81:1240–1247
- Loose CJ, Dawidowicz P (1994) Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. *Ecology* 75:2255–2263
- Marko PB, Palmer AR (1991) Responses of a rocky shore gastropod to the effluents of predatory and non-predatory crabs: avoidance and attraction. *Biol Bull* 181:363–370
- McCollum SA, VanBuskirk J (1996) Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. *Evolution* 50:583–593
- Moczek AP (1998) Horn polymorphism in the beetle *Onthophagus taurus*: larval diet quality and plasticity in parental investment determine adult body size and male horn morphology. *Behav Ecol* 9:636–641
- Morin JP, Moreteau B, Petavy G, Parkash R, David JR (1997) Reaction norms of morphological traits in *Drosophila*: adaptive shape changes in a stenotherm circumtropical species? *Evolution* 51:1140–1148
- Padilla DK, Adolph SC (1996) Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. *Evol Ecol* 10:456–456
- Petranka J (1989) Response of toad tadpoles to conflicting chemical stimuli: predator avoidance versus optimal foraging. *Herpetologica* 1989:283–292
- Pettersson LB, Nilsson PA, Bronmark C (2000) Predator recognition and defence strategies in crucian carp, *Carassius carassius*. *Oikos* 88:200–212
- Pfennig D (1990) The adaptive significance of an environmentally-cued developmental switch in an anuran tadpole. *Oecologia* 85:101–107
- Phillips DW (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). *Am J Bot* 84:887–895
- Pigliucci M, deIorio P, Schlichting CD (1997) Phenotypic plasticity of growth trajectories in two species of *Lobelia* in response to nutrient availability. *J Ecol* 85:265–276
- Relyea RA (2000) Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. *Ecology* 81:2278–2289
- Relyea RA (2001) The relationship between predation risk and anti-predator responses in larval anurans. *Ecology* 82:541–554
- Relyea RA (2002) Costs of phenotypic plasticity. *Am Nat* 159:272–282
- Relyea RA (2003) Predators come and predators go: the reversibility of predator-induced traits. *Ecology* 84:1840–1848
- Relyea RA (2004a) Fined-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology* 85:172–179
- Relyea RA (2004b) Integrating phenotypic plasticity when death is on the line: insights from predator–prey systems. In: Pigliucci M, Preston K (eds) The evolutionary biology of complex phenotypes. Oxford University Press, Oxford, pp 176–194
- Relyea RA (2005) The heritability of inducible defenses in tadpoles. *J Evol Biol* 18:856–866

- Relyea RA, Auld JR (2004) Having the guts to compete: how intestinal plasticity explains costs of inducible defenses. *Ecol Lett* 7:869–875
- Relyea RA, Auld JR (2005) Predator- and competitor-induced plasticity: how changes in foraging morphology affect phenotypic trade-offs. *Ecology* 86:1723–1729
- Relyea RA, Werner EE (1999) Quantifying the relation between predator-induced behavior and growth performance in larval anurans. *Ecology* 80:2117–2124
- Relyea RA, Werner EE (2000) Morphological plasticity in four larval anurans distributed along an environmental gradient. *Copeia*:178–190
- Roff DA (1996) The evolution of threshold traits in animals. *Q Rev Biol* 71:3–35
- Schlichting CD (1989) Phenotypic integration and environmental change. *BioScience* 39:460–464
- Schlichting CD, Pigliucci M (1998) Phenotypic evolution: a reaction norm perspective. Sinauer, Sunderland, Mass.
- Schoepfner NM, Relyea RA (2005) Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. *Ecol Lett* 8:505–512
- Sih A (1992) Prey uncertainty and the balancing of antipredator and feeding needs. *Am Nat* 139:1052–1069
- Skelly DK (1992) Field evidence for a cost of behavioral antipredator response in a larval amphibian. *Ecology* 73:704–708
- Skelly DK (1994) Activity level and the susceptibility of anuran larvae to predation. *Anim Behav* 47:465–468
- Skelly DK, Werner EE (1990) Behavioral and life-historical responses of larval American toads to an odonate predator. *Ecology* 71:2313–2322
- Smith ME, Belk MC (2001) Risk assessment in western mosquitofish (*Gambusia affinis*): do multiple cues have additive effects? *Behav Ecol Sociobiol* 51:101–107
- Spitze K (1992) Predator-mediated plasticity of prey life history and morphology: *Chaoborus americanus* predation on *Daphnia pulex*. *Am Nat* 139:229–247
- Stemberger RS (1988) Reproductive costs and hydrodynamic benefits of chemically induced defenses in *Keratella testudo*. *Limnol Oceanogr* 33:593–606
- Sultan SE, Bazzaz FA (1993) Phenotypic plasticity in *Polygonum persicaria*. I. Diversity and uniformity in genotypic norms of reaction to light. *Evolution* 47
- Tollrian R (1993) Neckteeth formation in *Daphnia pulex* as an example of continuous phenotypic plasticity: morphological effects of *Chaoborus* kairomone concentration and their quantification. *J Plank Res* 15:1309–1318
- Tollrian R, Harvell CD (1999) The ecology and evolution of inducible defenses. Princeton University Press, Princeton, N.J.
- Turner AM, SA Fetterolf SA, Bernot RJ (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 (2002a) A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. *Am Nat* 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, Arioli M (2002) Dosage response of an induced defense: how sensitive are tadpoles to predation risk? *Ecology* 83:1580–1585
- Van Buskirk J, Relyea RA (1998) Selection for phenotypic plasticity in *Rana sylvatica* tadpoles. *Biol J Linn Soc* 65:301–328
- Van Buskirk J, McCollum SA, Werner EE (1997) Natural selection for environmentally induced phenotypes in tadpoles. *Evolution* 51:1983–1992
- van Tienderen PH (1990) Morphological variation in *Plantago lanceolata*: limits of plasticity. *Evol Trend Plants* 4:35–43
- van Tienderen PH (1991) Evolution of generalists and specialists in spatially heterogeneous environments. *Evolution* 45:1317–1331
- Via S, Lande R (1985) Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39:505–522
- Vilhunen S, Hirvonen H (2003) Innate antipredator responses of Arctic charr (*Salvelinus alpinus*) depend on predator species and their diet. *Behav Ecol Sociobiol* 55:1–10
- Walls M, Ketola M (1989) Effects of predator-induced spines on individual fitness in *Daphnia pulex*. *Limnol Oceanogr* 34:390–396
- Werner EE, Anholt BR (1993) Ecological consequences of the trade-off between growth and mortality-rates mediated by foraging activity. *Am Nat* 142:242–272
- Werner EE, Peacor SD (2003) A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84:1083–1100
- West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. *Annu Rev Ecol Syst* 20:249–278
- West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, New York
- Wiackowski K, Staronska A (1999) The effect of predator and prey density on the induced defence of a ciliate. *Funct Ecol* 13:59–65
- Wilson DJ, Lefcort H (1993) The effect of predator diet on the alarm response of red-legged frog, *Rana aurora*, tadpoles. *Anim Behav* 46:1017–1019
- Wolfe LM, Mazer SJ (2005) Patterns of phenotypic plasticity and their fitness consequences in wild radish (*Raphanus sativus*: Brassicaceae). *Int J Plant Sci* 166:631–640