

Dissecting the smell of fear from conspecific and heterospecific prey: investigating the processes that induce anti-predator defenses

Heather M. Shaffery¹ · Rick A. Relyea²

Received: 30 April 2015 / Accepted: 30 August 2015 / Published online: 12 September 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract Prey use chemical cues from predation events to obtain information about predation risk to alter their phenotypes. Though we know how many prey respond to predators, we still have a poor understanding of the processes and chemical cues involved during a predation event. We examined how gray treefrog tadpoles (*Hyla versicolor*) altered their behavior and morphology when raised with cues from different stages of predator attack, predators fed different amounts of prey, and predators consuming different combinations of treefrog tadpoles or snails (*Helisoma trivolvis*). We found that starved predators and predators fed snails induced no defensive responses whereas tadpoles exposed to a predator consuming gray treefrogs induced greater hiding, lower activity, and relatively deeper tails. We also found that the tadpoles did not respond to crushed, chewed, or digested conspecifics, but they did respond to consumed (i.e., chewed plus digested) conspecifics. When we increased the treefrog biomass consumed by predators, tadpoles frequently increased their defenses when only tadpoles were consumed and always increased their defenses when the total diet biomass was held constant via the inclusion of snails. When predators experienced temporal variation in diet composition, including cues from snails to cause additional digestive cues or chemical noise, there was no effect on tadpole phenotypes. Our results suggest

that amphibian prey rely on cues from both chewing and digestion of conspecifics and that the presence of cues from digested heterospecifics play little or no role in adding chemical noise or increased digestive enzymes and by-products that could potentially interfere with induced defenses.

Keywords Chemical cues · Phenotypic plasticity · Dose response · Predation risk · Alarm cues

Introduction

Many organisms have evolved phenotypic plasticity as a strategy to cope with variable environments. For such plastic responses to be adaptive, organisms must discern information about their environment via cues from within a potentially noisy environment (Kats and Dill 1998; Burks and Lodge 2002). From the milieu of multi-sensory cues, organisms need to parse critical information related to numerous activities including mating, foraging, and predator avoidance. When environmental cues come from other individuals in the environment, the information provided is often a by-product of species interactions upon which individuals can eavesdrop, rather than information intentionally conveyed from a sender to a receiver (Dicke and Grostal 2001; Magalhães et al. 2005; Wisenden and Chivers 2006; Dalesman et al. 2007a; Blanchet et al. 2010; Schaefer and Ruxton 2012). As a result, there is a substantial opportunity for prey individuals to modify their phenotypes based on information they can receive via eavesdropping. However, we rarely have a sense of the complexity of information that is conveyed to organisms by these cues (Bourdeau 2010; Donaldson-Matasci et al. 2010), so we need to assess the degree to which organisms detect information that is available in their environment.

Communicated by Joel Trexler.

✉ Rick A. Relyea
relyer@rpi.edu

¹ Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA

² Department of Biological Sciences, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

The study of inducible defenses is one realm of phenotypic plasticity that has provided many insights on the environmental cues that organisms use to match their phenotype to different environments. In aquatic species, prey often rely on water-borne chemical cues that are produced during predation events to assess the risk of predation in their environment and respond accordingly (Nolte et al. 1994; Dicke and Grostal 2001; McCarthy and Fisher 2008). Risk assessment is a complicated process that requires organisms to interpret cues emitted by predators and by prey (both conspecific and heterospecific prey) over time and space and embedded within various chemical backgrounds (Watts 1991; Brown 2003; Stankowich and Blumstein 2005; Fraker 2008; Ferrari et al. 2010). Failure to accurately interpret this information can result in a phenotypic mismatch (DeWitt et al. 1998; Donaldson-Matasci et al. 2010). Since defenses are costly and an accurate assessment of risk is critical to survival, selection should favor prey that can fine-tune their defenses using cues that provide the most reliable information about predation risk (Harvell 1990; Clark and Harvell 1992; Skelly 1992; Kats and Dill 1998; Dicke and Grostal 2001).

Chemical cues emitted during predation events potentially include chemicals emitted by the predator, chemicals emitted by the prey (Wisenden 2000; Ferrero et al. 2011), or prey chemicals that are modified as predators digest the prey (Stabell et al. 2003). During an attack sequence, every step could potentially produce different chemical cues and each cue could provide the prey with information about predation risk [assuming they can detect each cue (Wisenden and Chivers 2006)]. Chemical cues released by prey prior to or during an attack—commonly referred to as alarm cues—are thought to function like the alarm calls of birds and mammals that inform other prey of a predator's presence (Chivers et al. 1996; Chivers and Smith 1998; Summey and Mathis 1998; Wisenden et al. 2004; Jacobsen and Stabell 1999; Mirza and Chivers 2008; Kaliszewicz and Uchmanski 2009). Chemical cues released by predators—commonly referred to as kairomones—allow prey to potentially distinguish predator identity, predator density, and even combinations of predator species (Harvell 1990; Tollrian and Harvell 1999; Turner et al. 1999; DeWitt et al. 2000; Relyea 2003, 2004; Hoverman and Relyea 2007). The process of digestion can release chemical cues that induce different defenses than either predator cues or prey cues (Chivers et al. 1996; Jacobsen and Stabell 1999, 2004). We need to know how cues from different stages in the predation process affect the anti-predator responses of prey and whether this pattern is generalizable across multiple taxa. To date only a few studies have simultaneously examined the impact of the cues released during different stages of a predation event on prey defenses (LaFiandra and

Babbitt 2004; Richardson 2006; Dalesman et al. 2007b; Schoeppner and Relyea 2009a).

When prey assess their environments, they face the challenge of detecting the relevant alarm cues and kairomones in a complex environment containing many other chemical cues that potentially add noise to the chemical environment. For example, many prey defenses track quantitative indicators of predation risk, such as the number of predators present or the biomass of prey that a predator consumes (Helfman 1989; Bouskila and Blumstein 1992). In aquatic species, an increase in prey consumption by a predator commonly induces prey defenses that increase asymptotically (Van Buskirk and Arioli 2002; Mirza and Chivers 2003a; Schoeppner and Relyea 2008). However, such studies have always focused on predators consuming a single species of prey, yet most predators do not consume a single prey species over extended periods of time (Kats and Dill 1998; Bolnick et al. 2007). If prey only track the consumption of conspecifics and they do not respond to predators simultaneously eating heterospecifics, then the induced defenses of prey should be identical with and without the added consumption of heterospecific prey. Alternatively, the inclusion of heterospecific prey could either cause the predator to produce additional digestive cues that have synergistic effects on prey responses to conspecific consumption or simply result in a noisier chemical environment that makes it more difficult for prey to respond appropriately (Chivers and Smith 1998; Burks and Lodge 2002). To our knowledge, no studies have attempted to characterize prey responses specific to increases in conspecific biomass with and without the consumption of heterospecifics.

If the consumption of alternative prey adds noise to a prey's environment in ways that affect the induction of defenses, then we should also find these effects when prey experience temporal variation in predation cues (Shultz et al. 2004; Chivers et al. 2008; Pettorelli et al. 2011). Though the specifics vary among organisms, there is a general consensus that temporal variation in predator presence or attack is an important variable of predation risk that affects defensive decisions (Lima and Bednekoff 1999; Sih et al. 2000). Most investigations of timing seek to identify the specific effects of timing per se on induced defenses (Sih and McCarthy 2002; Laurila et al. 2004; Foam et al. 2005; Mirza et al. 2006; Creel et al. 2008), and do not consider interactions between this timing and variation in other factors such as predator identity, diet, and risk level (but see Ferrari and Chivers 2009). In nature, the timing of predator attacks and which species of prey they are consuming almost certainly vary simultaneously, but we have virtually no idea what effect this has on prey defenses. Given the importance of timing and dietary cues on a prey's ability to induce correct plastic defenses, we need to assess how temporal variation in the consumption of conspecific

and heterospecific prey affects the production of inducible defenses.

Larval anurans (i.e., tadpoles) are an ideal system with which to address these questions. In aquatic environments, tadpoles rely primarily on chemical communication (Kats and Dill 1998; Burks and Lodge 2002; Miner et al. 2005; Houk 2009). They show strong responses to cues from predation events via behavioral and morphological defenses. Behaviorally, tadpoles typically move less and hide more, and this plasticity commonly comes at a cost of slower growth and smaller size at metamorphosis (McCollum and Van Buskirk 1996; Laurila et al. 2004). Morphologically, tadpoles typically induce relatively deep tailfins, short bodies, and other changes which improve their escape chances or make them harder to consume (McCollum and Leimberger 1997; Relyea 2001; Van Buskirk 2000; Relyea and Hoverman 2003; Laurila et al. 2008). In addition, tadpoles can discriminate among different predator diets; they exhibit strong responses to conspecifics being eaten by predators but few or no responses to distantly related prey, such as snails, being eaten by predators (Schoeppner and Relyea 2009a, c). The lack of response to predators consuming snails represents an experimental opportunity because it allows us to manipulate both the total amount of a predator's diet and the portion of the predator's diet that is composed of conspecific tadpoles.

Using the gray treefrog (*Hyla versicolor*), we sought to address the extent to which prey use information from different steps in a predation attack sequence to fine-tune their anti-predator defenses by testing the following hypotheses:

1. Prey defenses are not induced when predators are starved or fed heterospecifics (e.g., snails), but are induced by cues produced when predators consume and digest prey (an established, yet necessary, first step).
2. The magnitudes of prey defenses increase as prey gain more types of information from a predator attack sequence.
- 3a. Prey defenses increase as the mass of consumed conspecific prey increases.
- 3b. Given no response to cues from consumed heterospecifics alone, prey defenses increase with increased biomass of consumed conspecifics even if the total biomass consumed by the predator is held constant by adding heterospecifics.
- 3c. Prey defenses in response to predators consuming a given biomass of conspecific prey are similar to prey defenses in response to predators consuming a given biomass of consumed conspecific prey plus heterospecifics such that total diet biomass is held constant.
4. Given no response to cues from heterospecifics alone, prey will respond to temporal variation in predators

consuming only conspecifics in the same way that they respond to temporal variation in predators consuming conspecifics and heterospecifics.

Materials and methods

To test the above hypotheses, we used a completely randomized design with 13 treatments: a no-predator control (No Predator); a starved-predator control (Starved Predator); 100 mg of crushed tadpoles daily (Crushed 100T); a predator only chewing 100 mg of tadpoles daily (Chewed 100T); a predator only digesting 100 mg of tadpoles daily (Digested 100T); a predator consuming (i.e., chewing and digesting) 100 mg of tadpoles daily (Consumed 100T); a predator consuming 100 mg of snails daily (Consumed 100S); a predator consuming 100 mg of tadpoles or 100 mg of snails on alternate days (Consumed 100T/100S); a predator consuming 100 mg of tadpoles every other day (Consumed 100T/0S); a predator consuming 50 mg of tadpoles and 50 mg of snails daily (Consumed 50T:50S); a predator consuming 25 mg of tadpoles and 75 mg of snails daily (Consumed 25T:75S); a predator consuming 50 mg of tadpoles daily (Consumed 50T); and a predator consuming 25 mg of tadpoles daily (Consumed 25T). The 13 treatments were replicated five times for a total of 65 experimental units. Using subsets of these treatments, we tested our hypotheses with specific predictions (Table 1).

The experimental units were 100-L outdoor mesocosms set up on tables at the University of Pittsburgh's Pymatuning Laboratory of Ecology. On 15 and 16 June 2010, pools were filled with well water and covered in 60 % shade cloth to prevent colonization by insects and other amphibians. The pools were inoculated with 100 g of leaf litter (mostly *Quercus* spp.), 5 g of rabbit chow as an initial nutrient source, and an aliquot of zooplankton and algae that had been collected from three local ponds, screened for invertebrate predators, and pooled. The pools were then allowed to sit for 2 weeks to allow the algae and zooplankton populations to grow.

On 2 July, a predator cage was added to each mesocosm. The predator cages were constructed of black plastic drainpipe with nylon window screen on both ends. Mesocosms assigned a treatment that involved a predator contained a late-instar dragonfly nymph (*Anax junius*) in the cage. All other treatments contained an empty cage. The cages allowed predator cues to diffuse out into the mesocosm but prevented predators from preying on our focal tadpoles.

Fourteen pairs of amplexing pairs of gray treefrogs were collected during 17–24 June and allowed to oviposit their eggs in wading pools that had been filled with well water and covered with a 60 % shade cloth lid to prevent predators from colonizing. Once the eggs hatched, the tadpoles

Table 1 A comparison of hypotheses (*H*) regarding the outcome of cue source and content on prey induced defenses

Hypotheses	Predictions regarding the magnitude of the anti-predator response
H ₁ Prey defenses will increase as prey receive cues from starved predators, predators fed heterospecific prey, and predators fed conspecific prey	No Predator < Starved Predator < Consumed 100S < Consumed 100T
H ₂ Prey defenses will increase as prey are exposed to more cues from a predator attack sequence	No Predator < Crushed 100T < Chewed 100T < Digested 100T < Consumed 100T
H _{3a} Prey defenses will increase as the biomass of consumed conspecific prey increases and produces an increase in the amount of cue produced	No Predator < Consumed 25T < Consumed 50T < Consumed 100T
H _{3b} Given no response to cues from consumed heterospecifics (e.g., snails), prey defenses will increase as the biomass of consumed conspecifics increases while the total biomass consumed is held constant by adding heterospecifics	No Predator < Consumed 25T:75S < Consumed 50T:50S < Consumed 100T
H _{3c} Prey defenses in response to predators consuming a given biomass of conspecific prey are similar to prey defenses in response to predators consuming a given biomass of consumed conspecific prey plus an equal biomass of heterospecifics	Consumed 25T = Consumed 25T:75S and Consumed 50T = Consumed 50T:50S
H ₄ Given no response to cues from heterospecifics (e.g., snails), prey respond similarly to temporal variation in predators consuming conspecifics and temporal variation in predators consuming conspecifics and heterospecifics, both of which will induce weaker defenses than predators consistently consuming conspecifics	No predator < Consumed 100T/100S = Consumed 100T/0S < Consumed 100T

Predictions are rank ordered by strength of induced response. In the predictions, the *number* refers to the mass of the diet
T Tadpoles, *S* snails

were fed rabbit chow ad libitum. On 1 July, tadpoles were selected from a mixture of the 14 clutches and added to the experimental pools. The tadpoles were in early development [Gosner stage 24–25 (Gosner 1960)] with an initial mass of 15 ± 1 mg (mean \pm SE). To assess the possibility of mortality due to handling, 20 tadpoles were placed in a 7-L plastic tub (24-h survival = 100 %).

The next day we applied the predator-cue treatments. Starved predators had not been fed for 5 days prior to addition into the mesocosms, and they were replaced with new starved predators after being in the experiment for 5 days. We applied the Crushed 100T treatment by adding 100 mg of tadpoles (killed via cranial concussion) to 30 mL of filtered well water. The crushed animals were homogenized individually using a mortar and pestle. The Chewed 100T and Digested 100T treatments were carried out by allowing predators in the Chewed 100T pools to consume the entire 100 mg of tadpoles. Once the tadpoles were consumed, we transferred the predators, while still in their cage, to the Digested 100T pools and left them in these pools until the next feeding. An empty cage was placed in the Chewed 100T treatment pools. Predators in any of the snail treatments were fed ramshorn snails (*Helisoma trivolvis*). Dragonflies only consume the soft parts of a snail, so we calculated the total mass of snails for feeding based only on the mass of soft body parts [estimated from shell length-body mass regressions (Turner 2008; E. Cholak, personal communication)]. Across all treatments, the predators

consumed their prey diet within 1 day. Any predators that died during the experiment were replaced with new starved predators or predators that had only been fed gray treefrogs (depending on the treatment).

We took behavioral observations on 3 different days during the experiment. During each observation, we quietly approached each pool and recorded the number of individuals visible (i.e., not hiding beneath the leaf litter) and the number of those visible animals that were moving. We defined tadpole activity as the number of active tadpoles divided by the number of visible tadpoles (Van Buskirk 2001; Schoepner and Relyea 2009a). All observations were conducted before the predators were fed that day. On 10 July (nine observations) and 14 July (ten observations), predators in the Consumed 100T/100S treatment were fed snails the previous day and predators in the Consumed 100T/0S treatment were fed nothing the previous day. On 13 July (eight observations), predators in the Consumed 100T/0S and Consumed 100T/100S treatments were fed tadpoles the previous day. Four to five people collected data on a given day between 0900 and 1100 hours; each person observed all pools. We then calculated the mean activity for each observation day to minimize differences in the number of observations and any individual observer bias.

We ended the experiment on 17 July 2010 when tadpoles across treatments entered Gosner (1960) stage 43, which was 16 days after they were placed in the experiment. At this point in the experiment, the average tadpole had increased its

mass by nearly 60-fold. Continuing the experiment further would have resulted in the tadpoles undergoing metamorphosis, which would have prevented an assessment of their larval morphology.

When we terminated the experiment, we counted and euthanized all tadpoles and then preserved them in 10 % buffered formalin for subsequent morphological analysis. Photos were taken of all preserved tadpoles from a lateral view. Using Image J, we collected landmark data on each individual (Van Buskirk and Schmidt 2000). From the landmark data, we then calculated linear distances for each individual to produce four morphological variables: body length, body depth, tail length, and tail fin depth. These are the most common predator-induced morphological traits in tadpoles (Van Buskirk and Relyea 1998; Van Buskirk 2002; Relyea 2003).

Statistical analyses

The data consisted of mass, behavioral response variables, and morphological response variables. For the behavioral data, we calculated the mean number of individuals observed (i.e., not hiding in the leaf litter) and the mean activity for each pool on each observation day. Because the activity data were proportional, we arcsine transformed these data. We conducted a repeated-measures ANOVA (rm-ANOVA) on the two behavioral responses. Our analysis of the number visible passed the test of sphericity ($W = 0.911$, $p = 0.097$) and equal variances for each time point ($F \geq 0.74$, $p \geq 0.117$). We found deviations from normality in a few of our treatments on different observation days, but ANOVA is typically robust to such violations. The analysis of activity did not pass the test of sphericity ($W = 0.860$, $p = 0.023$), so we used the Greenhouse-Geisser correction for our results. The test of equal variances was met at each time point ($F \geq 1.19$, $p \geq 0.094$).

For the morphological data, linear dimensions of organisms increase as the mass of the organism increases, so we needed to make the morphological dimensions mass independent. The morphological data were first log transformed to improve linearity. To produce mass-independent means, we ran a multivariate analysis of covariance (MANCOVA), with the morphological measurements as dependent variables and log-transformed mass as the covariate. An important assumption of mass-independent data is that the slopes of the relationships do not differ among treatments; we confirmed this by a non-significant treatment-by-mass interaction.

Using the residuals and estimated marginal means saved from the MANCOVA, we calculated size-independent measurements for each individual (Schoeppner and Relyea

2009c). We then calculated the mean morphology and mass of all tadpoles from a given mesocosm. Using these mesocosm means, we examined the effects of the treatments on mass and the four mass-independent morphological dimensions using a MANOVA. We found a few deviations from normality, but ANOVA is typically robust to such violations. We also found one violation of variance homogeneity (body length), so we report Pillai's trace from the MANOVA.

We conducted two types of mean comparisons. Most of our hypotheses involved comparisons between the no-predator treatment versus other treatments that had a directional response that was predicted a priori (i.e., hypotheses 1, 2, 3a, and 3b). For these hypotheses, we used Dunnett's test, which compares a control against all other treatments. Our other two hypotheses (i.e., hypotheses 3c, 4) include comparisons between treatments that are not controls. In these cases, we compared the means using Tukey's test.

Results

To more clearly address each hypothesis, we first report the rm-ANOVA results on the behavioral responses and the MANOVA results on the mass and morphological response variables. For those responses that had significant univariate effects of treatment, we then report the particular mean comparisons on the behavioral and morphological traits to evaluate each of the hypotheses (Table 1).

In the rm-ANOVA on the number of tadpoles observed (i.e., not hiding), there was an effect of treatment ($F_{12,51} = 2.28$, $P = 0.021$) and time ($F_{2,102} = 50.44$, $P < 0.001$), but no interaction ($F_{24,102} = 1.12$, $P = 0.264$). In the rm-ANOVA on the activity of tadpoles, we also found an effect of treatment ($F_{12,51} = 12.05$, $P = 0.038$) and time ($F_{1,8,89} = 1.12$, $P < 0.001$), but no interaction ($F_{21,89} = 1.12$, $P = 0.742$). For both behaviors, the time effect was driven by increases in the number of tadpoles observed and their activity during the experiment.

In the MANOVA on tadpole mass and relative morphology, we found a multivariate effect of the treatment ($F_{60,255} = 1.5$, $P = 0.021$). Subsequent univariate analyses indicated that the multivariate effect was driven by treatment effects on relative tail depth ($P = 0.003$), but not on mass ($P = 0.681$), relative tail length ($P = 0.399$), body depth ($P = 0.357$), or body length ($P = 0.482$). Because we only detected significant effects of the predator-cue treatments on the two tadpole behaviors and relative tail depth, we subsequently tested our hypotheses using these traits.

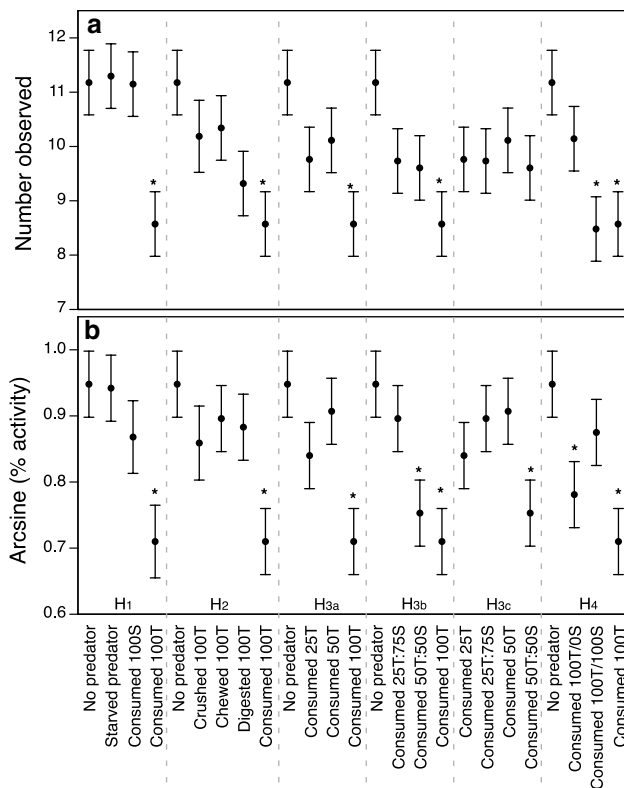


Fig. 1 **a** Number of observed gray treefrog tadpoles and **b** their activity when raised in 13 predator-cue treatments. Data are the least squared mean \pm SE of 27 observations made over 3 days. Treatments are grouped by hypotheses (*H*), so some treatments are repeated within **a** and **b**. Asterisks indicate treatments that differ from the no-predator control based on Dunnett's test ($P \leq 0.05$). Additional Tukey's tests used for H_{3c} and H_4 are not shown with asterisks but are described in the text. For all treatments, $n = 4$ replicates

H₁: do prey induce greater defenses as they receive cues from starved predators, predators consuming heterospecific prey, and predators consuming conspecific prey?

To test the first hypothesis, we compared the No-Predator, Starved Predator, Consumed 100S, and Consumed 100T treatments. We began by examining the two behavioral responses. Compared to the number of tadpoles observed in the No-Predator treatment, there was no effect of Starved Predator ($P = 0.95$) or Consumed 100S ($P = 0.92$), but we observed fewer tadpoles with Consumed 100T, which as a reminder comprises both consumption and digestion ($P = 0.01$; Fig. 1a). There was a 23 % reduction in the number of observable tadpoles between No-Predator and Consumed 100T.

Compared to the activity of the tadpoles in the No-Predator treatment, there was no effect of Starved Predator ($P = 0.91$) or Consumed 100S ($P = 0.51$), but there was an effect of Consumed 100T ($P = 0.01$; Fig. 1b). Activity

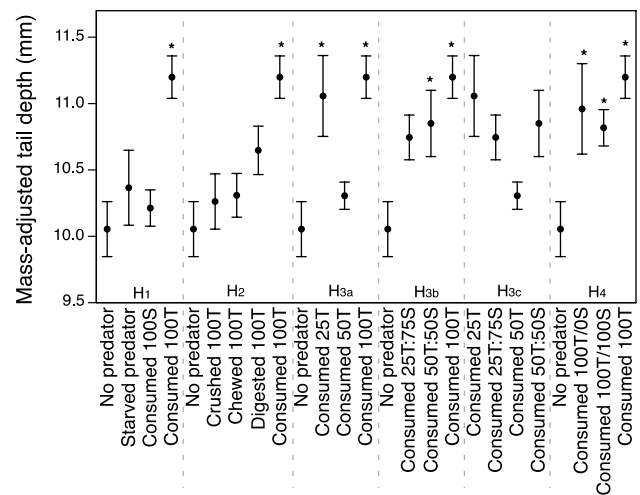


Fig. 2 Mass-independent tail depth for gray treefrog tadpoles when raised in 13 predator-cue treatments. Data are mean \pm SE of animals measured at the end of the experiment on day 16. Treatments are grouped by hypotheses, so some treatments are repeated within panels. Asterisks indicate treatments that differ from the no-predator control based on Dunnett's test ($P \leq 0.05$). Additional Tukey's tests used for H_{3c} and H_4 are not shown with asterisks but are described in the text. For all treatments, $n = 4$ replicates

declined by 18 % from the No-Predator to the Consumed 100T treatment.

We then examined changes in relative tail depth (Fig. 2). Compared to the No-Predator treatment, tail depth was not affected by Starved Predators ($P = 0.54$) or Consumed 100S ($P = 0.76$). However, Consumed 100T induced an 11 % deeper tail fin ($P = 0.001$). Thus, for all three traits, starved predators and predators consuming a distantly related heterospecific did not induce defenses whereas predators consuming conspecific tadpoles did induce defenses.

H₂: do prey defenses increase as prey gain information about a predator attack sequence?

Our second hypothesis was that anti-predator responses would continually increase as tadpoles received cues from crushed conspecifics (Crushed 100T), chewed conspecifics (Chewed 100T), digested conspecifics (Digested 100T), and chewed plus digested conspecifics (Consumed 100T). We began by examining the two behavioral responses. Compared to the number of tadpoles observed in the No-Predator treatment, there was no effect of Crushed 100T ($P = 0.52$), Chewed 100T ($P = 0.58$), or Digested 100T ($P = 0.11$) whereas there was an effect of Consumed 100T (as noted above; Fig. 1a). Compared to the activity of tadpoles in the No-Predator treatment, there was no effect of Crushed 100T ($P = 0.48$), Chewed 100T ($P = 0.70$), or Digested 100T ($P = 0.61$), but there was an effect of Consumed 100T (as noted above; Fig. 1b).

We then examined the tail-depth response (Fig. 2). Across the five treatments, we observed a general trend toward increased tail depth with each subsequent component of the attack sequence. Compared to tail depth in the No-Predator treatment, there were no differences with Crushed 100T ($P = 0.71$), Chewed 100T ($P = 0.62$), or Digested 100T ($P = 0.14$), but there was an effect of Consumed 100T (as noted above). In short, across the three responses, cues from chewed or digested conspecific prey alone did not induce defenses, but cues from prey that were chewed and digested (i.e., Consumer 100T) did induce defenses.

H₃: do prey defenses increase as the biomass of consumed conspecifics increases?

We predicted that prey would induce greater defenses as the biomass of consumed conspecific prey increased. The first way to address this question was to examine increases in predator consumption of tadpoles in the absence of any heterospecific prey (H_{3a}). Comparing the number of tadpoles observed in the No-Predator treatment, there was no effect of Consumed 25T ($P = 0.26$) or Consumed 50T ($P = 0.45$) whereas there was an effect of Consumed 100T (as noted above; Fig. 1a). Comparing the activity of tadpoles in the No-Predator treatment, there was no difference in activity with Consumed 25T ($P = 0.32$) or Consumed 50T ($P = 0.76$), but there was an effect of Consumed 100T (as noted above; Fig. 1b). When we examined relative tail depth, we found that compared to the No-Predator treatment there were deeper tails with Consumed 25T ($P = 0.01$), no effect of Consumed 50T ($P = 0.62$), and deeper tails with Consumed 100T (as noted above; Fig. 2).

In our first corollary to the above hypothesis (H_{3b}), we examined whether prey defenses increase as a function of increased conspecific biomass in the predator's diet irrespective of other heterospecific prey that are consumed while holding constant the total biomass consumed by a predator. We first analyzed the two behavioral responses. Compared to the number of tadpoles observed in the No-Predator treatment, there was no effect of Consumed 25T:75S ($P = 0.51$) or Consumed 50T:50S ($P = 0.20$), but there was an effect of Consumed 100T (as noted above; Fig. 1a). The latter three treatments did not differ ($P > 0.9$). Compared to tadpole activity in the No-Predator treatment, there was no effect of Consumed 25T:75S ($P = 0.70$), but there was lower activity with Consumed 50T:50S ($P = 0.03$) and Consumed 100T (as noted above; Fig. 1b). The latter three treatments did not differ ($P > 0.3$). In regard to tail depth in the No-Predator treatment, we found

that tails became marginally deeper with Consumed 25T:75S ($P = 0.07$), deeper with Consumed 50T:50S ($P = 0.04$), and deeper with Consumed 100T (as noted above; Fig. 2). Once again, the latter three treatments did not differ ($P > 0.9$).

Our second corollary to the above hypothesis (H_{3c}) was that if prey only use consumed conspecific biomass as the cue to produce defenses, we should see no difference in phenotype when prey experience cues from a predator consuming conspecific prey versus predators consuming the same biomass of conspecific prey plus an additional biomass of heterospecifics that, when alone, do not induce any phenotypic change. In terms of the number of tadpoles observed and tadpole activity, we found no significant differences in behavior between Consumed 25T versus Consumed 25T:75S ($P = 1.0$) or between Consumed 50T versus Consumed 50T:50S ($P > 0.6$; Fig. 1a, b). Similarly, there were no differences in tail depth when we compared Consumed 25T versus Consumed 25T:75S ($P = 1.0$) or between Consumed 50T versus Consumed 50T:50S ($P = 0.83$; Fig. 2).

H₄: do prey respond to temporal variation in predator consumption?

Our final hypothesis was that prey would respond to temporal variation in predation cues and produce the same defenses regardless of whether the predator's temporally variable diet consisted of feeding the predator tadpoles or no tadpoles on alternate days versus feeding them tadpoles and snails on alternate days. Moreover, we predicted that the response to alternating diets would be weaker than the response to predators consuming conspecific prey daily.

Compared to the number of tadpoles observed in the No-Predator treatment, there was no effect of Consumed 100T/0S ($P = 0.46$) but there was an effect of Consumed 100T/100S ($P = 0.01$) and Consumed 100T (as noted above; Fig. 1a). There was no difference between Consumed 100T/0S and Consumed 100T/100S ($P = 0.74$).

Compared to tadpole activity in the No-Predator treatment, there was a marginal effect of Consumed 100T/0S ($P = 0.08$) and no effect of 100T/100S ($P = 0.55$), but there was an effect of Consumed 100T (as noted above; Fig. 1b). There was no difference between Consumed 100T/0S and Consumed 100T/100S ($P = 0.98$).

Compared to the No-Predator treatment, tails became deeper with Consumed 100T/0S ($P = 0.02$), Consumed 100T/100S ($P = 0.01$), and Consumed 100T (as noted above; Fig. 2). There was no difference between Consumed 100T/0S and Consumed 100T/100S ($P = 1.00$).

Discussion

Do prey induce greater defenses as they receive cues from starved predators, predators consuming heterospecific prey, and predators consuming conspecific prey (H_1)?

We found that starved predators and predators fed snails induced no changes in gray treefrog behavioral and morphological responses but predators consuming gray treefrogs induced reduced activity, increased hiding behavior, and relatively deeper tails. These defenses are consistent, in both direction and magnitude, with numerous previous studies across a variety of amphibian species (Laurila et al. 1997; Relyea and Werner 2000; Relyea 2001; Van Buskirk 2001). Increased hiding and decreased activity appear to be general defensive strategies that make prey less likely to be detected by predators. In contrast, the induction of deeper tails improves a tadpole's probability of survival once detected and attacked (McCollum and Van Buskirk 1996; Relyea 2001; Hoverman and Relyea 2003; LaFiandra and Babbitt 2004; Richardson 2006). A larger tail could potentially increase swimming speed, but more importantly it can result in a "lure effect" which causes the predator to more likely strike at a large tail rather than the body (Van Buskirk et al. 2003). Whereas dragonfly strikes to a tadpole's body are often fatal, dragonfly strikes to tails can result in tadpoles escaping with torn tails that can be regrown. Given these trait responses, we could then address a number of important questions about the processes and cues that induce these phenotypic changes.

Do prey defenses increase as prey gain information from a predator attack sequence (H_2)?

We predicted that prey would produce stronger defenses as they gained more types of information from the predator attack sequence (i.e., crushed prey, chewed prey, digested prey, and consumed prey). Our results suggest that gray treefrogs hide more, reduce their activity, and increase their tailfin depth as more information about a predation event becomes available. Although there was a pattern of stronger responses as we moved from Crushed 100T to Consumed 100T, only the Consumed 100T induced large enough phenotypic changes in any of the traits to be significantly different from the No-Predator treatment. While few studies have examined all parts of a predation sequence, previous work suggests that cues derived from prey (Chivers and Smith 1998; Summey and Mathis 1998; Stabell et al. 2003; Laforsch et al. 2006) and cues from predator digestion (Jacobsen and Stabell 1999, 2004; LaFiandra and Babbitt 2004; Richardson 2006) can induce morphological and

behavioral defenses. Moreover, organisms tend to increase morphological defenses as predation risk increases (Mirza and Chivers 2003a, b; Bourdeau 2010). However, much of this work also indicates that prey should continuously decrease their activity as more types of information about predation become available (Wisenden et al. 2008; Schoeppner and Relyea 2009a, b). For example, leopard frog tadpoles (*Lithobates pipiens*) do not reduce their activity in response to chewed conspecifics but they do reduce their activity in response to digested or consumed (i.e., chewed plus digested) conspecifics (Schoeppner and Relyea 2009a). In contrast, the gray treefrog tadpoles in the current study did not reduce their activity until they detected consumed conspecifics, which suggests that there can be differences among species in the cues that trigger the threshold of response. For many organisms including amphibians, anti-predator reduction in activity often comes at a cost of decreased growth and mass at metamorphosis (Lima 1988; Skelly 1992; McCollum and Van Buskirk 1996; Van Buskirk 2000; Laurila et al. 2004). Since a smaller mass at metamorphosis can have long-term negative effects on fitness in anurans (Altwegg and Reyer 2003; Ficetola and De Bernardi 2006), gray treefrog tadpoles appear to not respond to the partial cues of predation (i.e., crushed, chewed, or digested conspecifics) but instead hold back from responding until they receive the full suite of predatory cues. We need many more studies to determine whether there are phylogenetic or ecological patterns in how different species respond to the cues emanating from the different stages of predation.

Do prey defenses increase as the biomass of consumed conspecifics increases (H_3)?

We predicted that treefrog tadpoles should increase their defenses as predators consumed greater amounts of treefrog tadpoles, regardless of whether a second distantly related prey was included in the predator's diet. As the predators consumed greater biomasses of conspecific tadpoles, the gray treefrogs spent more time hiding and less time moving. This response is consistent with other studies that have noted an increase in defenses as predator risk increases [i.e., predator abundance (Teplitsky et al. 2004; Fraker 2008, 2009; Schoeppner and Relyea 2009a,b)]. We saw similar responses in behavior when conspecific prey consumption was increased while holding total diet biomass constant by including snail prey, which indicates that for behavioral defenses prey are simply tracking the consumption of conspecifics and are not affected by any additional digestive cues or chemical noise that might be created by consuming distantly related heterospecifics.

The response to increase conspecific biomass was less clear when we examined changes in tailfin depth. Gray treefrogs exposed to the lowest (25 mg) and highest

(100 mg) conspecific biomass induced deeper tails, but those exposed to intermediate biomass (50 mg) did not. In contrast, our manipulations of increasing conspecific prey biomass while holding total diet biomass constant produced a pattern of continuous increases in tail depth. Thus, for reasons that are unclear, increased conspecific consumption produced a monotonic response in morphology when a second, non-inducing prey species was included but not when the second prey species was excluded. To our knowledge, no past studies have held biomass constant while manipulating conspecific biomass. Clearly we need more studies to arrive at any general conclusions.

As a corollary to this hypothesis, we asked whether predator treatments that had the same conspecific biomass but the inclusion or exclusion of a heterospecific in the diet would induce similar defenses (H_{3c}). There was consistent support for this hypothesis in our gray treefrogs, which suggests that the increased amount of digestive cues emitted by the predator when simultaneously consuming heterospecifics, and whatever chemical noise this might create, does not alter the prey's response to the predator eating conspecific prey. There is a significant body of literature about how prey address the complexity of predator identity in their environment, but limited data on how they deal with the complexity of predator dietary cues. Studies focused on variation in predator identity indicate that when faced with conflicting predator information (due to simultaneous predators of different identity) prey produce intermediate defenses (McIntosh and Peckarsky 1999; Eklöv and Werner 2000; Ireland et al. 2007; Lakowitz et al. 2008) or they prioritize defenses to the most dangerous predator (McIntosh and Peckarsky 1999; Teplitsky et al. 2004; Hoverman and Relyea 2007; Bourdeau 2009). Our data suggest that when a predator consumes a mixture of conspecific prey and distantly related prey that by themselves induce no defensive responses, the overall response is largely additive such that the mixed diet induces the same defenses as the conspecific diet.

Do prey respond to temporal variation in predator consumption (H_4)?

Our final hypothesis was that prey responses to temporal variation in predation risk would not be altered by the presence or absence of a heterospecific prey in the predator's diet (which alone does not induce defenses). Our comparisons of the two alternating diets (i.e., Consumed 100T/0S vs. Consumed 100T/100S) found that both treatments induced similar behavioral and morphological phenotypes. Studies looking at temporal variation typically have exposed animals to fluctuating periods of high and low risk (Sih and McCarthy 2002; Laurila et al. 2004; Foam et al. 2005; Mirza et al. 2006; Creel et al. 2008) but we are unaware of studies that have fluctuated predator risk in the presence and

absence of alternative predator diets. Such studies that use a range of relevant heterospecific diets would likely produce new insights into how prey respond to generalist predators that consume a temporally variable diet.

Conclusion

Understanding the complexity of environmental cues is essential to understanding the evolution and ecology of phenotypic plasticity in organisms. Using a model system of gray treefrog tadpoles, we discovered that prey respond to increases in information gained from the different stages in the predator attack sequence and they respond to increased biomass of consumed conspecifics as a primary cue for determining strength of induced defenses. However, the pattern of response to increased biomass can sometimes be altered by the addition of heterospecific cues that alone do not induce prey responses but do hold constant the total amount of biomass consumed by a predator. Direct comparisons of predators consuming tadpole prey with or without the addition of the heterospecific prey caused no change in phenotype at different biomasses of tadpole prey and caused no change in phenotype when prey consumption varied over time. Because heterospecifics in a predator's diet can induce a wide range of defenses to different magnitudes [often related to phylogenetic relatedness (Schoepfner and Relyea 2005)], future work should examine how prey respond to conspecific diets when they are combined with heterospecific diets that range from closely to distantly related species. Future studies should also address the generality of these results in other taxa and across different manipulations of temporal variation.

Acknowledgments We thank Jenise Brown, Emily Cholak, and Sasha Rhode for their assistance in collecting the amplexing treefrogs. We thank Catherine Giancola, Emily Cholak, Jessica Hua, Devin Jones, Dean Matthews, Caitlin Newcamp, Will Brogan, Kate Henderson, and Zach Shelin for observing tadpole behavior and Natalie Roberts for measuring tadpoles. Aaron Stoler, Jessica Hua, Will Brogan, R. J. Bendis, Devin Jones, and Rickey Cothran provided valuable feedback on the manuscript. We also thank the anonymous reviewers and editor for their very helpful comments. All applicable institutional and/or national guidelines for the care and use of animals were followed. The National Science Foundation funded this research.

Author contribution statement H. M. S. and R. A. R. conceived and designed the experiments. H. M. S. performed the experiments. H. M. S. and R. A. R. analyzed the data and wrote the manuscript.

References

- Altwegg R, Reyer HU (2003) Patterns of natural selection on size at metamorphosis in water frogs. *Evolution* 57:872–882
- Blanchet S, Clobert J, Danchin É (2010) The role of public information in ecology and conservation: an emphasis on inadvertent social information. *Ann NY Acad Sci* 1195:149–168

- Bolnick DI, Svanbäck R, Araújo MS, Persson L (2007) Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. *Proc Natl Acad Sci* 104:10075–10079
- Bourdeau PE (2009) Prioritized phenotypic responses to combined predators in a marine snail. *Ecology* 90:1659–1669
- Bourdeau PE (2010) Cue reliability, risk sensitivity and inducible morphological defense in a marine snail. *Oecologia* 162:987–994
- Bouskila A, Blumstein DT (1992) Rules of thumb for predation hazard assessment: predictions from a dynamic model. *Am Nat* 139:161–176
- Brown GE (2003) Learning about danger: chemical alarm cues and local risk assessment in prey fishes. *Fish Fish* 4:227–234
- Burks RL, Lodge DM (2002) Cued in: advances and opportunities in freshwater chemical ecology. *J Chem Ecol* 28:1901–1917
- Chivers DP, Smith RJF (1998) Chemical alarm signalling in aquatic predator–prey systems: a review and prospectus. *Ecoscience* 5:338–352
- Chivers DP, Kiesecker JM, Anderson MT, Wildy EL, Blaustein AR (1996) Avoidance response of a terrestrial salamander (*Ambystoma macrodactylum*) to chemical alarm cues. *J Chem Ecol* 22:1709–1716
- Chivers DP, Zhao X, Brown GE, Marchant TA, Ferrari MC (2008) Predator-induced changes in morphology of a prey fish: the effects of food level and temporal frequency of predation risk. *Evol Ecol* 22:561–574
- Clark CW, Harvell CD (1992) Inducible defenses and the allocation of resources: a minimal model. *Am Nat* 139:521–539
- Creel S, Winnie JA, Christianson D, Liley S (2008) Time and space in general models of antipredator response: tests with wolves and elk. *Anim Behav* 76:1139–1146
- Dalesman S, Rundle SD, Bilton DT, Cotton PA (2007a) Phylogenetic relatedness and ecological interactions determine antipredator behavior. *Ecology* 88:2462–2467
- Dalesman S, Rundle SD, Cotton PA (2007b) Predator regime influences innate anti-predator behaviour in the freshwater gastropod *Lymnaea stagnalis*. *Freshwater Biol* 52:2134–2140
- DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. *Trends Ecol Evol* 13:77–81
- DeWitt TJ, Robinson BW, Wilson DS (2000) Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. *Evol Ecol Res* 2:129–148
- Dicke M, Grostal P (2001) Chemical detection of natural enemies by arthropods: an ecological perspective. *Annu Rev Ecol Syst* 32:1–23
- Donaldson-Matasci MC, Bergstrom CT, Lachmann M (2010) The fitness value of information. *Oikos* 119:219–230
- Eklöv P, Werner EE (2000) Multiple predator effects on size-dependent behavior and mortality of two species of anuran larvae. *Oikos* 88:250–258
- Ferrari MC, Chivers DP (2009) Temporal variability, threat sensitivity and conflicting information about the nature of risk: understanding the dynamics of tadpole antipredator behaviour. *Anim Behav* 78:11–16
- Ferrari MC, Wisenden BD, Chivers DP (2010) Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Can J Zool* 88:698–724
- Ferrero DM, Lemon JK, Fluegge D, Pashkovski SL, Korzan WJ, Datta SR, Spehr M, Fendt M, Liberles SD (2011) Detection and avoidance of a carnivore odor by prey. *Proc Natl Acad Sci* 108:11235–11240
- Ficetola GF, De Bernardi F (2006) Trade-off between larval development rate and post-metamorphic traits in the frog *Rana latastei*. *Evol Ecol* 20:143–158
- Foam PE, Mirza RS, Chivers DP, Brown GE (2005) Juvenile convict cichlids (*Archocentrus nigrofasciatus*) allocate foraging and antipredator behaviour in response to temporal variation in predation risk. *Behaviour* 142:129–144
- Fraker ME (2008) The dynamics of predation risk assessment: responses of anuran larvae to chemical cues of predators. *J Anim Ecol* 77:638–645
- Fraker ME (2009) Predation risk assessment by green frog (*Rana clamitans*) tadpoles through chemical cues produced by multiple prey. *Behav Ecol Sociobiol* 63:1397–1402
- Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190
- Harvell CD (1990) The ecology and evolution of inducible defenses. *Quart Rev Biol* 65:323–340
- Helfman GS (1989) Threat-sensitive predator avoidance in damselfish–trumpetfish interactions. *Behav Ecol Sociobiol* 24:47–58
- Houk LD (2009) Pheromone communication in amphibians and reptiles. *Annu Rev Phys* 71:161–176
- Hoverman JT, Relyea RA (2007) Temporal environmental variation and phenotypic plasticity: a mechanism underlying priority effects. *Oikos* 117:23–32
- Ireland DH, Wirsing AJ, Murray DL (2007) Phenotypically plastic responses of green frog embryos to conflicting predation risk. *Oecologia* 152:162–168
- Jacobsen HP, Stabell OB (1999) Predator-induced alarm responses in the common periwinkle, *Littorina littorea*: dependence on season, light conditions, and chemical labelling of predators. *Mar Biol* 134:551–557
- Jacobsen HP, Stabell OB (2004) Antipredator behaviour mediated by chemical cues: the role of conspecific alarm signalling and predator labelling in the avoidance response of a marine gastropod. *Oikos* 104:43–50
- Kaliszewicz A, Uchmański J (2009) Damage released prey alarm substances or predator odours? Risk assessment by an aquatic oligochaete. *Hydrobiologia* 618:57–64
- Kats LB, Dill LM (1998) The scent of death: chemosensory assessment of predation risk by prey animals. *Ecoscience* 5:361–394
- LaFiandra E, Babbitt K (2004) Predator induced phenotypic plasticity in the pinewoods tree frog, *Hyla femoralis*: necessary cues and the cost of development. *Oecologia* 138:350–359
- Laforsch C, Beccara L, Tollrian R (2006) Inducible defenses: the relevance of chemical alarm cues in *Daphnia*. *Limnol Oceanogr* 51:1466–1472
- Lakowitz T, Brönmark C, Nyström PER (2008) Tuning into multiple predators: conflicting demands for shell morphology in a freshwater snail. *Freshwater Biol* 53:2184–2191
- 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, Järvi-Laturi M, Pakkasmaa S, Merilä J (2004) Temporal variation in predation risk: stage-dependency, graded responses and fitness costs in tadpole antipredator defences. *Oikos* 107:90–99
- Laurila A, Lindgren B, Laugen AT (2008) Antipredator defenses along a latitudinal gradient in *Rana temporaria*. *Ecology* 89:1399–1413
- Lima SL (1988) Initiation and termination of daily feeding in dark-eyed juncos: influences of predation risk and energy reserves. *Oikos* 53:3–11
- Lima SL, Bednekoff PA (1999) Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *Am Nat* 153:649–659
- Magalhães S, Tudorache C, Montserrat M, Van Maanen R, Sabelis MW, Janssen A (2005) Diet of intraguild predators affects antipredator behavior in intraguild prey. *Behav Ecol* 16:364–370
- McCarthy TM, Fisher WA (2008) Multiple predator-avoidance behaviours of the freshwater snail *Physella heterostropha pomila*: responses vary with risk. *Freshwater Biol* 44:387–397

- McCullum SA, Leimberger JD (1997) Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. *Oecologia* 109:615–621
- McCullum SA, Van Buskirk J (1996) Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. *Evolution* 50:583–593
- McIntosh AR, Peckarsky BL (1999) Criteria determining behavioural responses to multiple predators by a stream mayfly. *Oikos* 85:554–564
- Miner BG, Sultan SE, Morgan SG, Padilla DK, Relyea RA (2005) Ecological consequences of phenotypic plasticity. *Trends Ecol Evol* 20:685–692
- Mirza RS, Chivers DP (2003a) Response of juvenile rainbow trout to varying concentrations of chemical alarm cue: response thresholds and survival during encounters with predators. *Can J Zool* 81:88–95
- Mirza RS, Chivers DP (2003b) Predator diet cues and the assessment of predation risk by juvenile brook charr: do diet cues enhance survival? *Can J Zool* 81:126–132
- Mirza RS, Chivers DP (2008) Learned recognition of heterospecific alarm signals: the importance of a mixed predator diet. *Ethology* 107:1007–1018
- Mirza RS, Ferrari MC, Kiesecker JM, Chivers DP (2006) Responses of American toad tadpoles to predation cues: behavioural response thresholds, threat-sensitivity and acquired predation recognition. *Behaviour* 143:877–889
- Nolte DL, Mason JR, Epple G, Aronov E, Campbell DL (1994) Why are predator urines aversive to prey? *J Chem Ecol* 20:1505–1516
- Pettorelli N, Coulson T, Durant SM, Gaillard JM (2011) Predation, individual variability and vertebrate population dynamics. *Oecologia* 167:305–314
- Relyea RA (2001) Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology* 82:523–540
- Relyea RA (2003) How prey respond to combined predators: a review and an empirical test. *Ecology* 84:1827–1839
- Relyea RA (2004) Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology* 85:172–179
- Relyea RA, Hoverman JT (2003) The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs. *Oecologia* 134:596–604
- Relyea RA, Werner EE (2000) Morphological plasticity of four larval anurans distributed along an environmental gradient. *Copeia* 2000:178–190
- Richardson JL (2006) Novel features of an inducible defense system in larval tree frogs (*Hyla chrysoscelis*). *Ecology* 87:780–787
- Schaefer MH, Ruxton GD (2012) By-product information can stabilize the reliability of communication. *J Evol Biol* 25:2412–2421
- Schoeppner NM, Relyea RA (2005) Damage, digestion, and defense: the roles of alarm cues and kairomones for inducing prey defenses. *Ecol Lett* 8:505–512
- Schoeppner NM, Relyea RA (2008) Detecting small environmental differences: risk-response curves for predator-induced behavior and morphology. *Oecologia* 154:743–754
- Schoeppner NM, Relyea RA (2009a) Interpreting the smells of predation: how alarm cues and kairomones induce different prey defenses. *Funct Ecol* 23:1114–1121
- Schoeppner NM, Relyea RA (2009b) Phenotypic plasticity in response to fine-grained environmental variation in predation. *Funct Ecol* 23:587–594
- Schoeppner NM, Relyea RA (2009c) When should prey respond to heterospecific alarm cues? Testing the hypotheses of perceived risk. *Copeia* 2009:190–194
- Shultz S, Noë R, McGraw WS, Dunbar RIM (2004) A community-level evaluation of the impact of prey behavioural and ecological characteristics on predator diet composition. *Proc R Soc Ser B* 271:725–732
- Sih A, McCarthy TM (2002) Prey responses to pulses of risk and safety: testing the risk allocation hypothesis. *Anim Behav* 63:437–443
- Sih A, Ziemba R, Harding KC (2000) New insights on how temporal variation in predation risk shapes prey behavior. *Trends Ecol Evol* 15:3–4
- Skelly DK (1992) Field evidence for a cost of behavioral antipredator response in a larval amphibian. *Ecology* 73:704–708
- Stabell OB, Ogbebo F, Primicerio R (2003) Inducible defences in *Daphnia* depend on latent alarm signals from conspecific prey activated in predators. *Chem Senses* 28:141–153
- Stankowich T, Blumstein DT (2005) Fear in animals: a meta-analysis and review of risk assessment. *Proc R Soc Ser B* 272:2627–2634
- Summey MR, Mathis A (1998) Alarm responses to chemical stimuli from damaged conspecifics by larval anurans: tests of three Neotropical species. *Herpetologica* 54:402–408
- Teplitsky C, Plenet S, Joly P (2004) Hierarchical responses of tadpoles to multiple predators. *Ecology* 85:2888–2894
- Tollrian R, Harvell CD (1999) The ecology and evolution of inducible defenses. Princeton University Press, Princeton
- Turner AM (2008) Predator diet and prey behaviour: freshwater snails discriminate among closely related prey in a predator's diet. *Anim Behav* 76:1211–1217
- Turner AM, 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 (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. *J Evol Biol* 14:482–489
- Van Buskirk J (2002) A comparative test of the adaptive plasticity hypothesis: relationships between habitat and phenotype in anuran larvae. *Am Nat* 160:87–102
- 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) Natural selection for phenotypic plasticity: predator-induced morphological responses in tadpoles. *Biol J Linn Soc* 65:301–328
- Van Buskirk J, Schmidt BR (2000) Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. *Ecology* 81:3009–3028
- Van Buskirk J, Anderwald P, Lüpold S, Reinhardt L, Schuler H (2003) The lure effect, tadpole tail shape, and the target of dragonfly strikes. *J Herpetol* 37:420–424
- Watts BD (1991) Effects of predation risk on distribution within and between habitats in savannah sparrows. *Ecology* 72:1515–1519
- Wisenden BD (2000) Olfactory assessment of predation risk in the aquatic environment. *Phil Trans R Soc Ser B* 355:1205–1208
- Wisenden BD, Chivers DP (2006) The role of public chemical information in antipredator behaviour. *Communication in fishes*. Science Publisher, Enfield, 259–278
- Wisenden BD, Vollbrecht KA, Brown JL (2004) Is there a fish alarm cue? Affirming evidence from a wild study. *Anim Behav* 67:59–67
- Wisenden BD, Karst J, Miller J, Miller S, Fuselier L (2008) Antipredator behaviour in response to conspecific chemical alarm cues in an esociform fish, *Umbra limi* (Kirtland 1840). *Environ Biol Fishes* 82:85–92