Prey responses to fine-scale variation in predation risk from combined predators

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While it is well documented that organisms can express phenotypic plasticity in response to single gradients of environmental variation, our understanding of how organisms integrate information along multiple environmental gradients is limited in many systems. Using the freshwater snail *Helisoma trivolvis* and two common predators (water bugs *Belostoma flumineum* and crayfish *Orconectes rusticus*), we explored how prey integrate information along multiple predation risk gradients (i.e. caged predators fed increasing amounts of prey biomass) that induce opposing phenotypes. When exposed to single predators fed increasing amounts of prey biomass, we detected threshold responses; intermediate amounts of consumed biomass induced phenotypic responses, but higher amounts induced little additional induction. This suggests that additional increases in predator-induced traits with greater predator risk offer minimal increases in fitness or that a limit in the response magnitude was reached. Additionally, the response thresholds were contingent on the predator and focal trait. For shell width, responses were generally detected at a lower amount of consumed biomass by water bugs compared to crayfish. Within the crayfish treatments, we found that the shell thickness response threshold was lower than the shell width response threshold. When we combined gradients of consumed biomass from both predators, we found that the magnitude of response to one predator was often reduced when the other predator was present. Interestingly, these effects were often detected at consumed biomass levels that were lower than the threshold concentration necessary to elicit a response in the single-predator treatments. Moreover, our combined predator treatments revealed that snails shifted from discrete responses to more continuous (i.e. graded) responses. Together, our results reveal that organisms experiencing multiple environmental gradients can integrate this information to make phenotypic decisions and demonstrate the novel result that an exposure to multiple species of predators can lower the response threshold of prey.

A core concept in phenotypic plasticity is the reaction norm, which is the function that describes the phenotype produced by a genotype in response to a series of environments (Schlichting and Pigliucci 1998, Pigliucci 2001, West-Eberhard 2003). Because many organisms encounter continuous environmental variation, quantifying the shape of reaction norms is a critical step in exploring the ecology and evolution of phenotypic plasticity. For instance, theory predicts that selection should favor continuous phenotypic responses when the underlying fitness function is continuous (i.e. increasing fitness associated with expressing more extreme phenotypes; Lively 1986, Roff 1996). In this scenario, organisms should exhibit more extreme phenotypes as the magnitude of environmental change becomes more extreme. However, organisms are not infinitely plastic; phenotypic constraints or increasing fitness costs can limit the magnitude of responses along an environmental gradient (Houston et al. 1993, Werner and Anholt 1993, Loose and Dawidowicz 1994, DeWitt et al. 1998, West-Eberhard 2003). As a result, reaction norms can plateau or even crash at environmental extremes. For example, thermal reaction norms in ectotherms increase as temperatures increase but precipitously decline as organisms reach their critical thermal maximum (Huey and Kingsolver 1993, Kingsolver and Gotulskiewicz 2003). Moreover, the level of sensitivity to environmental change can vary by species and trait (e.g. behavior, morphology), and be shaped by the fitness tradeoffs associated with phenotypic decisions and the type of cues available to the organism (reviewed by Schlichting and Pigliucci 1998, Pigliucci 2001, West-Eberhard 2003).

Given the complexity of natural systems, there has also been growing interest in understanding how complex environmental gradients influence reaction norms (Stamp and Bowers 1990, Sultan et al. 1998, Kingsolver et al. 2006, Gianoli et al. 2007, Maestre et al. 2007, Stillwell et al. 2007, Golubski and Abrams 2011, Kasumovic 2013). Natural systems are characterized by a broad array of abiotic (e.g. pH, precipitation, nutrients, temperature) and biotic (e.g. competition, predation, parasitism) factors that can influence the expression of phenotypes (Schlichting and Pigliucci 1998, Pigliucci 2001, West-Eberhard 2003). Importantly, organisms often simultaneously encounter different environmental factors and must adjust their phenotypes based on the fitness costs and benefits associated with phenotypic
responses to each environment (Gurevitch et al. 2000, Miner et al. 2005, Valladares et al. 2007). The situation becomes more complex when continuous variation in each environment is considered; organisms must integrate information on the magnitude of environmental change and the underlying fitness functions for each environment to fine-tune their phenotypes (Castellanos and Barbosa 2006, Kingsolver et al. 2006, Stillwell et al. 2007). Because multiple environments are likely to interact to influence the reaction norm, research that integrates environmental combinations with naturally observed gradients in each factor will help to develop a more comprehensive understanding of plasticity within complex environments (Sultan et al. 1998, Stillwell et al. 2007, Valladares et al. 2007).

The inducible defenses of prey have been a model system for addressing phenotypic plasticity (Tollrian and Harvell 1999). Recently, a greater focus has been placed on understanding the expression of inducible defenses within complex environments. In particular, research has increasingly addressed how prey respond to combinations of predators (Sih et al. 1998, Persons and Rypstra 2001, Bourdeau 2009, Beckerman et al. 2010, Golubski and Abrams 2011, Herzog and Laforsch 2013, Walzer and Schausberger 2013). Because predators can differ in how they consume prey and the level of risk they pose to prey, prey must make decisions on how to modulate their phenotypic expression. While our knowledge of how prey respond to combined predators has progressed, research has typically utilized relatively simple predator treatments (e.g. predator A, predator B, and A + B; reviewed by Relyea 2003). However, growing evidence suggests that many inducible defenses are graded in response to the level of risk posed by predators; predator density as well as the amount of prey consumed by the predator influence phenotypic expression (Harvell 1998, Kusch et al. 2004, Schoeppner and Relyea 2008, Beckerman et al. 2010). When prey encounter combinations of predators that differ in prey consumption rates, phenotypic expression will depend on several factors including predator cue detection thresholds of the prey, the riskiness of each predator, the effectiveness of the defenses against each predator, and the costs associated with expressing the defense. However, our understanding of how prey respond to combinations of predator gradients remains limited. Only by examining phenotypic responses to combinations of predators along predation risk gradients can we begin to address the complexities of predator-prey interactions within natural communities.

Our model system consisted of the freshwater snail *Helisoma trivolvis* and two common snail predators (water bugs *Belostoma flumineum* and crayfish *Orconectes rusticus*). While *H. trivolvis* expresses inducible defenses against both predators, the responses to each predator are in opposite directions (Hoverman et al. 2005, Hoverman and Relyea 2007b, 2008, 2009, 2012). In the presence of water bugs, snails form wider shells that reduce the water bug’s ability to contact the snail’s body when retracted within the shell. In contrast, snails form thicker shells in the presence of crayfish that increase the shell’s ability to withstand shell crushing and chipping by crayfish. Because the resource required for building shells (i.e. calcium carbonate) is limited in freshwater snails, they cannot simultaneously invest in shell width and shell thickness. Thus, the wider shells produced in the presence of water bugs tend to be thinner while the thicker shell produced in the presence of crayfish tend to be narrower. As a result, snails face a survival tradeoff associated with response to each predator (Hoverman and Relyea 2009). Given that these two predators induce traits in opposing directions, how do snails respond when both are encountered simultaneously? In this scenario, we have shown that *H. trivolvis* tends to bias phenotypic expression towards water bugs (i.e. wider and thinner shells) rather than balancing their phenotypic responses (Hoverman and Relyea 2007b). In that study, however, prey responses were only examined at a single level of risk for each predator. It remains unknown how these snails respond to gradients of predation risk from multiple predators that induce traits in opposing directions.

Here, we examined the responses of *H. trivolvis* to water bugs and crayfish fed increasing amounts of prey biomass (i.e. increasing risk). Our first objective was to quantify reaction norms along a predation risk gradient for each predator alone. More specifically, we assessed the response threshold (i.e. the amount of consumed biomass necessary to elicit a response) for multiple traits for each predator and whether increased consumed biomass caused continual increases in prey defenses or whether prey defenses exhibited a response that reaches a plateau. Our second objective was to determine how snails integrate information on the amount of predation risk from combined predators to form inducible defenses. Because *H. trivolvis* faces a phenotypic tradeoff between investment in shell thickness and shell width in response to each of the predators, we expected that the magnitude of phenotypic responses to one predator would decrease as the amount of predation risk associated with the other predator increased.

**Methods**

We conducted the experiment at the University of Pittsburgh’s Pymatuning Laboratory of Ecology. On 30 March 2006, we collected 180 adult Helisoma trivolvis from a pond in northwestern Pennsylvania (41°35′26.32″N, 80°14′31.27″W). We have previously documented inducible defenses against water bugs and crayfish in this snail population (Hoverman and Relyea 2009). We placed six adults into each of 30 10-l tubs filled with UV-irradiated, carbon-filtered well water to oviposit in our controlled laboratory facility. Egg deposition began immediately and continued until the adults were removed on 8 April. The eggs began to hatch on 30 April. The hatchlings were reared in the 10-l tubs at a density of ~15 snails l⁻¹. They were fed rabbit chow ad libitum and complete water changes were conducted every 4 d until the start of the experiment.

The experiment was a completely randomized design consisting of a factorial combination of four increasing levels of consumed biomass by caged water bugs crossed with four increasing levels of consumed biomass by caged crayfish. For each predator species, the consumed biomass treatments included a control (i.e. predator absent) and 25, 100 or 400 mg of snail biomass consumed by each predator per feeding. Thus, there were 16 treatments replicated four times for a total of 64 experimental units. This experimental design
is based on the knowledge that our predators induce snail phenotypes in opposing directions (e.g., wider shells versus narrower shells). Because of these opposing effects on snail phenotypes, we can assume that the predators are producing distinct chemical cues that are used by the snails to produce predator-specific phenotypes. When predators induce traits in the opposite direction, cues from predator A should represent a counteracting force of induction on responses to predator B’s cues. As a result of these expectations, we did equalize the total amount of consumed biomass between the single-predator (e.g., one predator fed 100 mg of snail biomass) and combined-predator treatments (e.g., water bugs fed 100 mg of snail biomass + crayfish fed 100 mg of snail biomass). If our predators induced traits in the same direction, an experimental design that accounted for changes in total biomass consumed and the identity of the predators in the combination would have been necessary (Relyea 2003).

Our experimental design is based on several assumptions regarding predator cues. First, starved predator treatments were not included in the experiment because previous studies have demonstrated that starved predators rarely induce responses in prey because the lack of digestion prevents or reduces the release of kairomones (Walls and Ketola 1989, Crowl and Covich 1990, McCellum and Leimberger 1997, Schoepnner and Relyea 2009). Second, we assume that the predators are producing similar amounts of cue within each of the consumed biomass treatments despite an order of magnitude difference in predator biomass. As is the case for most predator species, we have not identified the chemical composition of water bug or crayfish cues to quantify the amount of predator cue produced. However, predator cues are typically produced following the digestion of prey (Schoepnner and Relyea 2009). Given that the predators were fed an identical snail biomass within each consumed biomass treatment, we can assume that the amount of cue produced would be similar. Moreover, because our predators tend to induce opposing phenotypes, the amount of each unique cue from the predators is not the critical point. Our focus is on how the unique cues from one predator influence responses thresholds to the unique cues from a second predator. An alternative approach would have been to standardize predator biomass by using ~10 water bugs to equal the mass of a single crayfish. However, this approach would result in water bug densities that exceed field patterns (Hoverman et al. 2011) and confound predator number between the treatments, which could also influence the amount of predator cue generated.

Our experimental units were 100-l wading pools filled with well water on 27–28 April. We added 5 g of commercial rabbit chow to serve as an initial nutrient source and a 300-ml aliquot of pond water containing algae (i.e., phytoplankton and periphyton) and zooplankton obtained from four surrounding ponds. We added two predator cages to each pool consisting of 10 × 10 cm corrugated pipe capped with shade cloth covering each end. Predator cages allow the release of chemical cues of predation into the experimental units without allowing the predators to kill the focal animals (Hoverman and Relyea 2009). We placed a shade cloth lid over each pool to prevent colonization by insects and amphibians during the experiment.

On 24 May (day 0), we added 50 juvenile snails to each pool from a mixture of the culture tubs (mean mass ± 1 SE = 20.4 ± 1.2 mg). We set aside a sample of 25 snails to estimate survivorship due to handling; 24-h survival was 100%. For treatments assigned a water bug or crayfish, we placed a single adult water bug or crayfish into one of the cages. We added the caged predators to the pools 12 h after adding the snails. We fed the caged predators the appropriate snail biomass (1 to 2 snails for the 25 mg treatment, 3 to 4 snails for the 100 mg treatment, and 6 to 8 snails for the 400 mg treatment) three times per week. To equalize disturbance, we briefly lifted and replaced all empty cages.

To track phenotypic changes over ontogeny, we randomly selected 10 snails from each pool on days 10 and 30 and preserved them in 10% buffered formalin. On day 50, we terminated the experiment and preserved all remaining snails. Three experimental units from three different treatments had poor survivorship (0 to 76% survival). One of these experimental units (water bug 100 mg + crayfish 400 mg) lacked snails at the conclusion of the experiment and was excluded from the statistical analyses. Excluding these three experimental units, snail survival was high across all treatments (mean ± 1 SE = 98 ± 1%).

To assess changes in snail morphology, the preserved snails were dried at 80°C for 24 h, weighed to the nearest milligram, and measured for shell width (see Fig. 1 in Hoverman et al. 2005) using digital imaging software. We also used digital calipers to measure shell thickness at the leading edge of the aperture. We focused on shell width and thickness because they are consistently induced and are the most important traits in the predator–snail interaction (Hoverman and Relyea 2008, 2009, 2012, Hoverman et al. 2014).

Statistical analyses

To examine the morphological responses of snails to our predator treatments over time, we started by addressing the allometric relationships between the morphological traits and mass. There was not a significant relationship between shell thickness and log_{10}-transformed mass. However, shell width showed a positive relationship with log_{10}-transformed mass. To account for the effects of mass on shell width, we used analysis of covariance (ANCOVA) with log_{10}-transformed mass as the covariate (Hoverman and Relyea 2009, Hoverman et al. 2014). An assumption of this analysis is that the treatments shared a common slope for their regression lines (i.e., a similar allometric relationship; McCoy et al. 2006); this assumption was satisfied (F_{9,222} = 0.185, p = 0.996).

From the ANCOVA, we used the mass-adjusted treatment mean and residuals from the within-treatment regression to calculate each individual’s size-adjusted trait value. We then calculated the mean response for each experimental unit within each sample date and used these as our response variable. While there are a number of different size-correction methods, this is a powerful approach for obtaining size-adjusted morphology based on our previous work (Hoverman and Relyea 2007a, b, 2009, 2012, Hoverman et al. 2014).

We conducted a series of analyses to address response thresholds with each predator alone (objective 1) and how
prey integrate information on the amount of predation risk from combined predators to form inducible defenses (objective 2). We began by conducting a repeated-measures multivariate ANOVA to test for the effects of time, water bug treatment, crayfish treatment, and their interactions on log10-transformed mass, shell thickness, and relative shell width. To determine the source of multivariate effects, we conducted univariate tests using the Huynh–Feldt degrees of freedom correction because the sphericity assumption was violated for all three traits. Because only shell thickness and shell width were significantly affected by the predator treatments or interactions, we proceeded with additional analyses for just these two traits. Shell thickness was only affected by the time-by-crayfish interaction. Thus, we conducted univariate ANOVAs within each time period to determine the source of the interaction and response thresholds to crayfish (objective 1). Because there was no evidence that water bugs influenced shell thickness, we did not use this trait to address our second objective. In contrast, shell width was affected by both predators and in opposite directions, which allowed us to use this trait to determine whether each predator affected snail response to the other predator (objective 2). To examine responses thresholds to each predator alone (objective 1), we conducted pairwise comparisons among treatments containing only a single predator (i.e. control, 25, 100 and 400 mg of snail biomass consumed). To address objective 2, we conducted pairwise comparison among the 16 predator treatments (averaged across time). For all analyses, we conducted mean comparisons using Tukey’s HSD. All analyses were conducted in SPSS ver. 22.

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.jd6m0> (Hoverman and Relyea 2015).

Results

We found that the magnitude of predator-induced plasticity in snails was sensitive to time and the amount of prey biomass consumed by each predator (Table 1). Because we detected a diverse set of main effects and interactions on each of the measured traits, we will address each of the traits separately.

Snail mass increased over time but was insensitive to the amount of prey consumed by predators (Table 1). Averaged across the predator treatments, mass (untransformed) increased by 3.7-fold over the experiment (p < 0.001). Because there was no effect of crayfish or water bugs on mass, we did not explore our two objectives with this trait. Shell thickness increased and then decreased over time (Table 1). Averaged across treatments, shell thickness increased 22-fold from day 10 to day 30 (from 0.012 mm to 0.268 mm) but decreased by 45% from day 30 to day 50 (from 0.268 mm to 0.147 mm; p < 0.001). For the predator cue manipulations, there was no main effect of the water bug treatments or any interaction between the crayfish and water bug treatments. However, we found a marginal effect of the crayfish treatments and a significant time-by-crayfish interaction. In considering the crayfish effect over time, we found that early in ontogeny (day 10) the snails showed no change in shell thickness (p = 0.707) but by day 30 they exhibited increased shell thickness with crayfish cues (p = 0.007, Fig. 1). Later in life, as the snails grew larger, the thickness of the shells decreased and the consumed biomass treatments converged (p = 0.335). Using the data from day 30,

Table 1. Results of repeated-measures MANOVA on the effects of water bug and crayfish treatments on snail traits (mass, shell thickness, and shell width) over time.

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>F</th>
<th>p</th>
<th>Mass</th>
<th>Shell thickness</th>
<th>Shell width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>6.42</td>
<td>1083.5</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time × Water bug</td>
<td>18,119</td>
<td>0.5</td>
<td>0.964</td>
<td>0.627</td>
<td>0.748</td>
<td>0.984</td>
</tr>
<tr>
<td>Time × Crayfish</td>
<td>18,119</td>
<td>2.0</td>
<td>0.015</td>
<td>0.947</td>
<td>&lt;0.001</td>
<td>0.316</td>
</tr>
<tr>
<td>Time × Water bug × Crayfish</td>
<td>54,219</td>
<td>0.9</td>
<td>0.646</td>
<td>0.911</td>
<td>0.110</td>
<td>0.964</td>
</tr>
<tr>
<td>Between subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water bug</td>
<td>9,110</td>
<td>13.7</td>
<td>&lt;0.001</td>
<td>0.404</td>
<td>0.801</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Crayfish</td>
<td>9,110</td>
<td>1.7</td>
<td>0.090</td>
<td>0.058</td>
<td>0.066</td>
<td>0.437</td>
</tr>
<tr>
<td>Water bug × Crayfish</td>
<td>27,132</td>
<td>1.9</td>
<td>0.009</td>
<td>0.241</td>
<td>0.259</td>
<td>0.012</td>
</tr>
</tbody>
</table>

1Wilks’ Lambda.
2Univariate within-subjects tests were conducted using the Huynh–Feldt degrees of freedom correction factor because the assumption of sphericity was violated.
we found a main effect of the water bug treatments, no main effect of the prey treatments (p = 0.026). There was no difference in shell width among the other treatments (p = 0.929). Thus, the crayfish response threshold for shell width was between 100 and 400 mg of prey biomass. However, there was no evidence that the response plateaued.

We addressed our second objective by examining how the amount of consumed biomass by one predator influenced the response threshold to the other predator. As noted above, in the absence of crayfish, water bugs fed 100 and 400 mg of prey biomass induced 3 to 5% wider shells compared to the other treatments. When crayfish were fed 25 mg of prey, water bugs fed 25 and 100 mg of prey had no effect on shell width compared to the no-water bug control (p = 0.397, Fig. 2), but water bugs fed 400 mg of prey induced 5% wider shells (p < 0.001). When crayfish were fed 100 mg of prey, there was no difference in shell width between the no-water bug control and water bugs fed 25 mg of prey (p = 0.752), but water bugs fed 100 mg of prey induced 2% wider shells (p = 0.046) and water bugs fed 400 mg of prey induced 4-6% wider shells (p < 0.001). When crayfish were fed 400 mg of prey, water bugs fed 25, 100 and 400 mg of prey biomass induced 3 to 9% wider shells compared to the no-water bug treatment (p = 0.016). Additionally, water bugs fed 100 and 400 mg of prey biomass induced 5 to 6% wider shells than the 25-mg treatment (p < 0.001). There was no difference in shell width between the 100 and 400-mg treatments (p = 0.212).

We also examined the responses to crayfish within each water bug treatment. As noted above, in the absence of water bugs, crayfish fed 400 mg of prey biomass induced 3 to 4% narrower shells compared to the other treatments. When water bug cues were present, the crayfish consumption treatments never differed from the control (p = 0.124). There was only one significant comparison among the remaining treatments: when water bugs were fed 100 mg of prey, crayfish fed 400 mg of prey biomass induced 3% wider shells than the 25-mg treatment (p = 0.037). Together, these results suggest that the response threshold was contingent on the amount of prey consumed by each predator.

**Discussion**

Organisms within natural communities are challenged by a complex set of environmental factors that influence phenotypic expression. We examined how the freshwater snail *Helisoma trivolvis* integrated information on predation risk from two predators (water bugs and crayfish) that consumed increasing amounts of prey biomass to form inducible defenses. Our results demonstrate that snails have response thresholds that varied between the two predators and among the traits examined. For shell width, snails in the single predator treatments were more sensitive (i.e. lower response threshold) to the amount of consumed biomass by water bugs compared to crayfish. Additionally, we detected trait-level variation in sensitivity to amount of biomass consumed by crayfish; the shell thickness response threshold was lower than the shell width response threshold within the crayfish treatments. Importantly, response thresholds were dependent on whether the other predator species was present and the amount of prey the other predator consumed. In
particular, the sensitivity of snails to consumed biomass by the predators increased such that a lower amount of consumed prey was necessary to elicit a phenotypic response. Collectively, these results suggest that the phenotypic responses of prey can be sensitive to fine-scale variation in predation risk from multiple predators.

Studies that have expanded beyond simple environmental dichotomies frequently document graded responses to environmental gradients suggesting the organisms are capable of detecting and responding to fine-scale variation in environments (Harvell 1998, Kusch et al. 2004, Relyea 2004, Schoeppner and Relyea 2008, McCoy et al. 2012). In predator–prey systems, studies that have examined prey responses to predators fed increasing amounts of prey or responses to increases in predator densities have detected graded phenotypic responses suggesting a continuous fitness function (Relyea 2004, McCoy et al. 2012). While graded responses are common in many systems, reaction norms can display a wide range of shapes (Schlichting and Pigliucci 1998, Pigliucci 2001). Thus, our first objective was to assess how snails responded to predation risk gradients to visualize predator-specific reaction norms.

We found that there was a response threshold such that a certain amount of consumed prey biomass was necessary to elicit a response to each predator in the absence of cues from the other predator. For instance, the water bug response threshold for shell width was between 25 and 100 mg of consumed prey. However, the magnitude of the crayfish response threshold depended on the trait; while the threshold for shell thickness was between 25 and 100 mg of consumed prey, the threshold for shell width was between 100 and 400 mg of consumed prey. Importantly, there was limited evidence that the magnitude of the responses increased beyond the response threshold (i.e. the response magnitude plateaued). Indeed, we found relatively consistent evidence that the magnitude of the responses plateaued between 100 and 400 mg of consumed prey biomass despite the 4-fold difference in the amount of consumed prey. These results suggest that increases in the magnitude of the response with greater risk levels may offer minimal fitness benefits. Our previous work has found that phenotypic responses of the magnitude seen in the present study (5% increase in shell width and 44% increase in shell thickness) are effective at reducing the risk of water bug and crayfish predation (Hoverman and Relyea 2009). Thus, snails may gain few benefits from forming more extreme phenotypes but incur greater costs associated with possessing or forming the phenotype. For instance, snails may be faced with structural constraints that prohibit more extreme increases in shell width or thickness; increases in relative shell width could lead to shells that are structurally unstable and easily damaged (Hoverman et al. 2014).

Based on our previous research, the responses to each predator appear to be adaptive. Water bugs induced the formation of wider shells throughout the experiment. Wider shells allow snails to withdraw deeper inside the shell beyond the reach of the water bug’s mouthpart (Hoverman and Relyea 2009). The maintenance of the induced defense across the sampling dates is also consistent with our previous research (Hoverman and Relyea 2012). Snails can induce wider shells in as few as 7 days and maintain relatively wider shells throughout life (Hoverman and Relyea 2007a, Hoverman and Relyea 2012). Because *H. trivolvis* does not appear to reach a size refuge from water bugs, the maintenance of the defense over development is necessary to reduce predation risk (Hoverman and Relyea 2009).

With crayfish, we observed a diverse set of responses in shell thickness and width. Crayfish induced the formation of thicker shells but the responses were only observed mid-way through the experiment (day 30). Thicker shells provide greater resistance to the shell cracking and chipping tactics used by crayfish (Hoverman and Relyea 2009). However, the benefits of thicker shells are relatively short lived; *H. trivolvis* can reach a size refuge from crayfish (Hoverman and Relyea 2009). Moreover, snails face an allocation tradeoff between investing in shell coiling (i.e. growth) versus shell thickness (Russell-Hunter 1978, Brodersen and Madsen 2003, Hoverman and Relyea 2007a, 2008). Consequently, snails growing out of a high-risk category and reducing allocation to morphological defenses could have driven the temporal fluctuation that we observed in shell thickness. Crayfish also induced the formation of narrow shells at the highest amount of consumed prey. Although shell thickness appears to be the main defense against crayfish, narrow shells tend to be more crush resistant, which may enhance defenses against crayfish at higher risk levels (Hoverman et al. 2005, Hoverman and Relyea 2007b).

Our second objective was to assess how the amount of consumed prey influenced phenotypic response to combined predators. For shell width, which was the one trait that was induced in opposite directions by the two predators, we found that snail responses to one predator were dependent on the amount of prey consumed by the other predator and, moreover, that it altered the environmental response thresholds. For instance, crayfish induced narrower shells at the highest amount of consumed prey (400 mg). However, the presence of water bugs consuming 25, 100 or 400 mg of prey eliminated this response to crayfish. A similar pattern was observed when we examined the effect of crayfish cues on snail responses to water bugs. In the absence of crayfish and when crayfish were fed 400 mg of prey, snails responded to water bugs by forming wider shells when water bugs were fed ≥ 100 mg of prey. However, if crayfish were consuming 25 or 100 mg of prey, the magnitude of the response to water bugs fed 100 mg of prey was reduced or eliminated. Together, these results suggest that snails largely bias their phenotypic responses towards water bugs despite the presence of predatory crayfish. These results are consistent with our previous research examining snail responses to a single level of risk for each predator (Hoverman and Relyea 2007b). These patterns could be driven by the ability of snails to reach a size refuge from crayfish predation but not water bug predation. If the benefits (i.e. reduced predation by crayfish) of the crayfish-induced phenotype are short-lived because individuals can reach a size refuge, snails may select to bias their responses towards water bugs to ensure long-term defense against this predator. However, these intermediate levels of risk could have important implications for fitness because snails are not producing shells that are wide enough to reduce water bug predation. From our previous work, snails need to form > 5% wider shells to reduce water bug predation risk (Hoverman and Relyea 2009). Thus, while snails appear to bias
their responses towards water bugs, the level of induction may be insufficient to confer greater fitness. Future studies examining the fitness consequences of these responses to gradients in combined predators will help to elucidate the fitness benefits and costs of these responses.

Our results suggest that snails can detect both predators when they are consuming low amounts of prey, which influenced the magnitude of the response to combined predators. This also demonstrates that prey can detect predators at cue concentrations that are lower than the amount of cue necessary to elicit a response to a single predator. In other words, the detection threshold can be lower than the response threshold. To our knowledge, this is the first study to demonstrate the decline in the response threshold to one predator when a second predator is present. A perpetual challenge in plasticity research is determining whether the absence of an induced response is driven by detection limits or a choice by the organism (Moran 1992, Dewitt et al. 1998). Previous studies have shown that cue levels that are below response thresholds can still hold important information used in decision making (Brown et al. 2004). Given that induced responses have their associated costs and benefits (DeWitt et al. 1998, Schlichting and Pigliucci 1998), it seems likely that organisms have sensitive sensory systems that enable detection of low cue levels. Based on our results, one way to reveal the sensitivity of sensory systems and explore detection limits is through the manipulation of environmental gradients and multiple environmental factors.

Another result of the combined predator treatments was the detection of graded responses. When we examined snail responses to each predator in isolation, the magnitude of the phenotypic responses tended to plateau around the response threshold. However, graded responses were revealed when snails were exposed to certain combinations of the predators. More specifically, we found evidence for graded responses to water bugs when crayfish were consuming the two highest levels of prey biomass. This suggests that snails can shift from the expression of discrete responses to more continuous responses when combinations of predators are detected in the environment. This shift may provide snails with more flexibility in their phenotypic decisions in order to balance the relative risk posed by different predators in the environment. Ultimately, these results underscore the need for greater attention to how complex environmental gradients influence phenotypic expression.

While significant insights into the importance of phenotypic plasticity continue to accumulate, there is an urgent need to explore the complexity of these responses (Pigliucci and Preston 2004, Miner et al. 2005, Kasumovic 2013). In particular, a broader understanding of the ecological and evolutionary factors underlying plasticity can be obtained by extending beyond simple environmental dichotomies (Pigliucci 2001, Dewitt and Scheiner 2004). For example, the presence of a high-risk predator that is below the detection threshold for the prey could lead prey to focus defenses towards another predator in the environment. Consequently, prey could produce a phenotype that is poorly matched to the predator composition of the community. Additionally, we found that snails have the ability to detect predator cues at relatively low levels (i.e. consumption rates). However, our ability to observe this result was only possible when we included cue gradients from multiple predators. Moreover, it was clear that snails were using these relatively low cue concentrations to modulate their phenotypic responses to the level of predation risk posed by combined predators. By moving beyond environmental dichotomies, our study revealed the complexity associated with the phenotypic responses of prey to predator communities. Our results also have implications for interpreting field patterns of plasticity. For instance, a common approach to exploring inducible defenses in nature is to correlate predator abundance or biomass to the magnitude of phenotypic responses in prey populations (Van Buskirk 2009). Given that prey phenotypes depend on how much prey the predators are consuming and how prey integrate cue gradients from multiple predators, these relatively simple assessments of predation risk based on predator number or biomass may not accurately reflect the predation threat perceived by prey and their phenotypic decisions.

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