

The rules of engagement: how to defend against combinations of predators

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Abstract Studies of inducible defenses have traditionally examined prey responses to one predator at a time. However, prey in nature encounter combinations of predators that should force them to produce phenotypic compromises. We examined how snails (*Helisoma trivolvis*) alter their phenotype in the presence of three different predator species that were presented alone and in pairwise combinations. When snails were exposed to each predator alone, they formed predator-specific defenses that reflected the differences in each predator's foraging mode. When snails were exposed to pairwise combinations of predators, their phenotype was dependent on their ability to detect each predator, the risk posed by each predator, and the effectiveness of a given defense against each predator. Consequently, responses to combined predators were typically biased towards one of the predators in the pair. This suggests that prey facing combined predators do not form simple intermediate defenses and, as a result, may experience enhanced mortality risk when they encounter natural predator regimes.

Keywords Combined predators · Functional trade-offs · Inducible defenses · Multiple predators · Multiple traits · Predator density · Predator identity · Predation risk · Trait integration

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Introduction

Prey rarely encounter a single predator in natural communities but rather coexist with a diversity of predator species that often differ functionally (Sih et al. 1998; Chalcraft and Reserits 2003; Griffen 2006). Indeed, predator species typically differ in their foraging location or capture technique, which can lead to opposing selective pressures on prey phenotypes. Consequently, many prey species have evolved the ability to form predator-specific defenses that are integrated across morphology, behavior, and life history to cope with each predator species (Krupa and Sih 1998; Kats and Dill 1998; McIntosh and Peckarsky 1999; Tollrian and Harvell 1999; Relyea 2001). Moreover, environmental variation in the presence of functionally different predators can favor inducible over constitutive defenses (permanent responses produced regardless of predator presence; Tollrian and Harvell 1999). While research on inducible defenses has grown steadily over the years, research on induced defenses with combinations of predators has not kept pace. By addressing the effects of combined predators on prey phenotypes, community ecologists can obtain insights into the effects of predation on prey populations within natural communities as well as their indirect effects on other species in the community.

Ecologists have clearly established that the inducible defenses documented in a diversity of groups can be adaptive strategies for reducing the risk of predation with a given predator (Karban and Baldwin 1997; Tollrian and Harvell 1999). However, prey encounter a more complex situation when two or more predator species are present simultaneously. Not only must prey be able to detect the presence of each predator, an optimal response requires the integration of their defensive traits based both on the risk posed by each predator and on the relative effectiveness of

each defense against each predator. Over the last decade, several studies have addressed prey responses to combined predators (Lima 1992; Matsuda et al. 1993, 1994, 1996; Peckarsky and McIntosh 1998; McIntosh and Peckarsky 1999; Turner et al. 2000; Relyea 2003; Wiackowski et al. 2003; Teplitsky et al. 2004). When predator species induce responses in the same direction but with different magnitudes, prey respond to combined predators by simply responding to the most risky predator in the combination (reviewed in Relyea 2003). This decision rule appears to work well because a defense against the most risky predator automatically defends the prey against less risky predators (Relyea 2003). When individual predator species induce prey defenses in opposite directions, prey generally produce intermediate phenotypes in response to multiple predators (McIntosh and Peckarsky 1999). This compromise decision with combined predators is assumed to represent a balance in the overall risk of predation. Thus, while combined predators may present a more complex situation for prey compared to encounters with a single predator, we have clear expectations of how prey should respond to combined predators.

In this study, we utilized the snail *Helisoma trivolvis* and three of its most common predators: (1) water bugs (*Belostomatia flumineum*), (2) crayfish (*Orconectes rusticus*), and (3) pumpkinseed sunfish (*Lepomis gibbosus*). These three predators are of particular interest because they differ in both their foraging locations and tactics and, consequently, favor unique defenses in *H. trivolvis*. Water bugs forage throughout the water column and consume snails by invading the shells using a modified mouthpart that pierces the snail's soft body. To escape predation by water bugs, *H. trivolvis* forms relatively larger shells (e.g., relatively wider shells and wider apertures) that increase the distance water bugs must reach inside the shell before contacting the body (Hoverman et al. 2005; Hoverman and Relyea 2007; J.T. Hoverman and R.A. Relyea, unpublished data). Crayfish typically forage in the benthos and consume *H. trivolvis* by chipping the shell aperture. While *H. trivolvis* alters a variety of traits in response to crayfish, they appear to rely on the formation of thicker shells that increase resistance against aperture chipping by crayfish (Hoverman et al. 2005; J.T. Hoverman and R.A. Relyea, unpublished data). Lastly, fish generally forage in the middle of the water column and use their pharyngeal jaw muscles to crush snail shells. While there is limited data available on the responses of *H. trivolvis* to fish, physid snails are known to avoid the middle of the water column and form relatively round shells that increase the snail's resistance to shell crushing (DeWitt 1998; Turner et al. 2000; DeWitt and Langerhans 2003). Given these functional differences among the predators, the next logical step is to address how snails will respond to combinations of these predator species.

Indeed, because *H. trivolvis* has such a broad distribution, it not only encounters habitats with different predator species but also habitats that contain multiple predator species (J.T. Hoverman, unpublished data). In sum, we seek to expand on previous work that has examined snail responses to single predators by investigating responses to predator combinations.

In this study, we conducted two experiments. The first examined the predation risk (i.e., mortality rate) associated with each of the three predator species. Crayfish and sunfish are generally large predators that occur at low densities (≤ 2 adults/m²; J.T. Hoverman, unpublished data), whereas water bugs are small predators that can reach high densities (40 adults/m²; Kesler and Munns 1989). Since fish and crayfish are relatively large predators, we expected snail mortality rates to be much higher with these two predators than with water bugs. The second experiment addressed the phenotypic responses of snails to separate and combined predators. We can make several predictions about how snails should respond to separate and combined predators. First, snails will form predator-specific defenses that reflect the differences in predator foraging locations and feeding tactics (see above). Second, higher densities of each predator (i.e., higher risk) should lead to more extreme defenses. Third, given that each predator induces a unique suite of traits, we expect pairwise combinations of predators to induce intermediate phenotypes that potentially balance the risk of predation from both predators.

Methods

In the first experiment, we conducted predation trials to quantify the risk of predation associated with the three predator species (water bugs, crayfish, and sunfish) for snails of two different sizes. The experiment was conducted in nine 800-l cattle tanks containing 700 l of well water at the University of Pittsburgh's Aquatic Research Facility in Linesville, PA. Each tank contained two 30 × 30-cm corrugated pipes capped with a fiberglass window screen on each end. The experiment was a completely randomized split-plot design with predator species as the whole-plot factor and snail size as the split-plot factor. Snail size was broken into small [mean mass ($n = 20$) ± 1 SE = 39.1 ± 2.1 mg] and large (68.1 ± 2.9 mg) size classes. The snails in each size class were reared from eggs in outdoor wading pools in the absence of predators (i.e., predator-naïve) until the start of the predation trials. Within each tank, for each predator species we placed ten snails of the appropriate size class and one predator in the cages. Each whole-plot factor was replicated three times. Our main interest was the mortality rate of snails (i.e., number consumed/h) with each predator species. Based on previous observations, crayfish and sunfish

consume snails rapidly (i.e., several snails/h). To accurately assess mortality rates, we stopped the crayfish and fish treatments after 1 h to ensure that a few snails remained in the cages. Because water bugs require several hours to consume a single snail, water bugs were allowed to feed for 24 h, and the mortality rate was calculated as the number of snails consumed divided by 24 h. Mortality rate was log-transformed prior to conducting an analysis of variance (ANOVA) to satisfy the assumption of homogeneous errors.

In the second experiment, we examined the effects of combined predators on snail phenotypes using pond mesocosms. On 27 March 2004, we collected 350 adult snails from a nearby pond and placed 25 adults into each of 14 wading pools filled with 100 l of well water to oviposit. Egg deposition began in April and continued into early May, at which time the adults were removed from the pools. Snails began hatching on 9 May and were fed rabbit chow *ad libitum*.

On 25 May, 50 cattle tanks (800 l) were filled with 700 l of well water. To each tank we added 15 g of rabbit chow as an initial nutrient source and an aliquot of pond water containing periphyton, phytoplankton, and zooplankton to simulate a simple aquatic community. We provided structure for the snails by placing a clay tile platform (32-cm² tile supported by a 10-cm² tile) in the center of each tank. We also added three predator cages to each tank. Predators that feed inside cages release chemical cues that diffuse throughout the tank without allowing the predators to kill the focal animals (Chivers and Smith 1998; Kats and Dill 1998; Tollrian and Harvell 1999). One cage, designed to house fish, was constructed from 30 × 30-cm corrugated pipe capped with a fiberglass window screen on each end. The other two cages, designed to house water bugs or crayfish, were made from 10 × 10-cm corrugated pipe and were capped with shade cloth. Although the predator species were placed in different cages, the chemical cues from each predator readily diffuse and mix within the tank (Relyea 2003). We placed a shade cloth lid over each tank to prevent colonization by insects and amphibians during the experiment. On 10 June, 50 hatchling snails were added to each tank (mean mass ± 1 SE = 19.7 ± 1.1 mg). This density (23 snails/m²) is well within natural densities of juvenile snails (J.T. Hoverman, unpublished data).

We designed a completely randomized experiment with ten treatments and five replicates. Our goal was to examine the responses of snails to different caged predator environments: (1) a no-predator control (i.e., empty cages), (2) each predator species at low density (×), (3) each predator species at high density (2×), and (4) pairwise combinations of the predator species with each predator at a low density. With our additive-plus-substitutive design, we were able to compare prey responses to combined predators to both

single and double densities of each predator alone to address responses to changes in predator composition and predator density (Sih et al. 1998; Relyea 2003; Vance-Chalcraft et al. 2004; Griffen and Byers 2006).

Based on the results from our first experiment, it was clear that snail consumption was greater with a single crayfish or fish than with a single water bug (see “Results”). Therefore, we had to devise a way to equalize the consumption rates without starving the larger crayfish and fish or using a large number of water bugs. We accomplished this by manipulating predator density and controlling the amount of prey given to each predator. First, we used two water bugs for every one crayfish or fish. Hence, for the three low-density predator treatments, we added one fish, one crayfish, or two water bugs to the cages. All of the predators were placed into a single cage in the tanks. For the three high-density predator treatments, we placed two fish into a single cage, two crayfish into separate cages, or four water bugs split into two cages. The final three treatments were the three possible pairwise combinations of the predators at a low density, and each predator species was placed into a separate cage. Second, we equalized prey consumption among predators by feeding each fish and crayfish 1 g of snail biomass while each water bug was fed 0.5 g. Therefore, predators in the low-density treatments consumed a total of 1 g of snail biomass per feeding while predators in the high-density treatments consumed a total of 2 g per feeding. Predators were fed three times per week. This food ration was used because it provided enough food for the larger fish and crayfish while not overwhelming the water bugs (i.e., a single water bug cannot consume 3 g of snail biomass in a week but can easily consume 1.5 g). No uneaten snails were observed in the predator cages when the predators were fed, confirming that prey consumption was equal among predators and not affected by the presence of other predators.

Snail behavioral responses to the predator treatments were observed on 21 and 22 June (one observation per day). For each tank, we counted the number of snails that were under the tile platform (i.e., using structure) and the number of snails at the water’s surface. We calculated the proportion of snails using structure and the proportion using the surface by dividing our counts by the final number of surviving snails in each tank. For each behavior, we averaged the responses over the 2 days for each tank and used these means as our response variables.

The experiment was terminated on 25 June. While the experiment lasted for just 15 days, there was, on average, a 13-fold increase in snail mass. Given this substantial increase in snail mass during the experiment, snails had sufficient opportunity to respond to the predator treatments. All surviving snails were counted and preserved in 10% formalin. Survival was greater than 90% in all treatments.

To examine treatment effects on shell characteristics, we dried 25 randomly selected snails from each tank at 80°C for 24 h. The dried snails were weighed to the nearest milligram and measured using digital imaging software (Optimas Co, Bothell, WA). We measured four linear shell dimensions: shell width and height, and aperture width and height (see Fig. 1 in Hoverman et al. 2005). We also measured shell thickness at the leading edge of the aperture using digital calipers. After the snail was measured, we determined shell crushing-resistance by using a piston apparatus (Osenberg and Mittelbach 1989). The shell was placed on its side in a beaker, and a smaller glass jar was placed flat on the shell and perpendicular to the aperture. The jar was then slowly filled with sand until the shell was crushed. The jar and sand were weighed to the nearest 0.01 g to estimate the crushing resistance of the shells. While a dry shell may be weaker than a wet shell, the relative differences in shell crushing-resistance should be maintained.

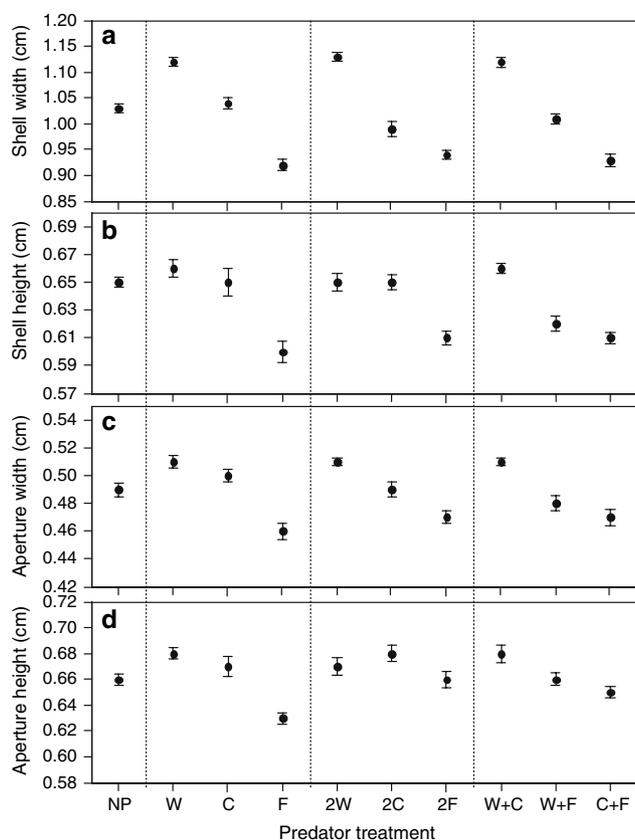


Fig. 1 The effects of different predator environments on the size-corrected shell width (a), shell height (b), aperture width (c), and aperture height (d) of *Helisoma trivolvis* (mean \pm 1 SE). Size-corrected morphology was obtained through an analysis of covariance (ANCOVA) using snail mass as the covariate (see Methods for details). The predator treatments are: no predator (NP), water bugs (W), crayfish (C), and fish (F). The high density of each predator is represented with a 2 before the letter

When studying morphological plasticity, it is important to account for the allometric relationships between linear dimensions and mass (i.e., size). In our data, shell width and height, aperture width and height, and shell crush-resistance (natural-log transformed) had positive relationships with snail mass (shell thickness did not scale with size). Thus, we used analysis of covariance (ANCOVA) with log-transformed mass as our covariate to correct for size (Hoverman et al. 2005). A critical assumption in the ANCOVA procedure is that the treatments share a common slope of their regression lines, and our data met this assumption (McCoy et al. 2006). From the ANCOVA, we used the mass-adjusted treatment means and residuals from the within-treatment regressions to calculate each individual's mass-adjusted value. The residuals were not significantly correlated with mass, suggesting that the regressions effectively accounted for size variation. For each morphological trait, we then calculated the mean mass-adjusted shell dimensions for each experimental unit and used these means as our morphological response variables. Since shell thickness was not corrected for size, we used the tank means as our response variable.

Our data set consisted of nine response variables (the five size-adjusted morphological variables, shell thickness (log-transformed), two behavioral responses, and mass). Since we were interested in how our predator treatments affected the multivariate response of the snails, we conducted a principal components analysis (PCA) using the tank means for each of the nine response variables (univariate responses are presented in Figs. 1, 2). With this ordination technique, we reduced our multivariate data set into a smaller set of composite variables (principal components) with a limited loss of information (McGarigal et al. 2000). The first two principal components had eigenvalues greater than one and were extracted for analysis. The PC-1 and PC-2 scores for each tank were then subjected to univariate analysis of variance (ANOVA). Because PC-1 and PC-2 are uncorrelated by definition, we conducted separate ANOVAs. When univariate tests were significant, we conducted mean comparisons using Fisher's LSD test.

Results

In the first experiment, we examined the mortality rates of the three predator species on small and large size classes of snails. We found a significant effect of predator species on snail mortality rates ($F_{2,6} = 80.1$, $P = 0.001$) but no effect of snail size ($F_{1,6} = 0.3$, $P = 0.629$) or the predator-by-size interaction ($F_{2,6} = 2.5$, $P = 0.166$). Averaged across snail sizes, crayfish consumed 8.67 ± 0.42 snails/h (mean \pm 1 SE), fish 7.67 ± 1.12 snails/h, and water bugs 0.18 ± 0.03 snails/h. Based on mean comparisons, mortality rates were

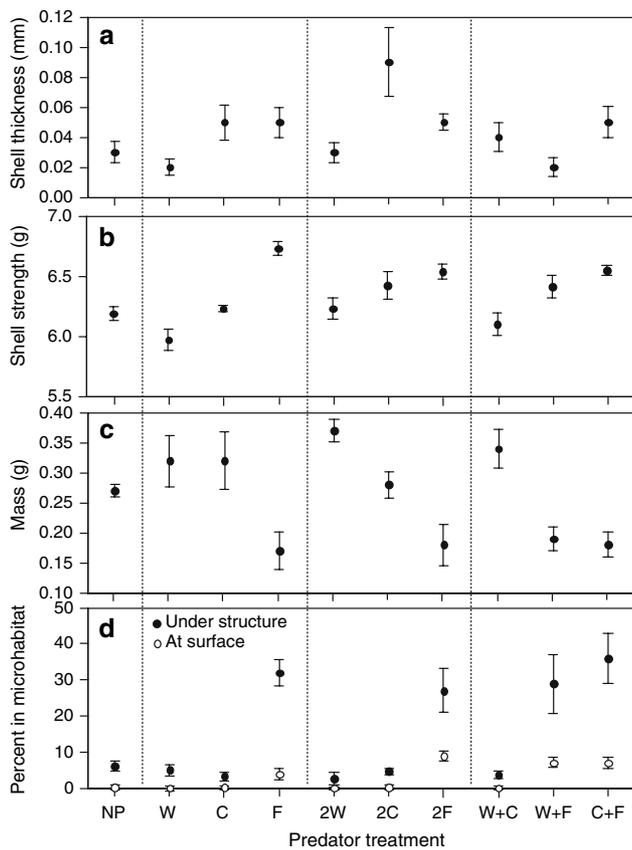


Fig. 2 The effects of different predator environments on the size-corrected shell thickness (a), shell strength (size corrected) (b), mass (c), and predator avoidance behavior (d) of *H. trivolvis* (mean \pm 1 SE). Size-corrected shell strength was obtained through an ANCOVA using snail mass as the covariate (see Methods for details) and is presented on a natural log scale. The predator treatments are: no predator (NP), water bugs (W), crayfish (C), and fish (F). The high density of each predator is represented with a 2 before the letter

similar between crayfish and fish ($P = 0.422$), but both of these were greater than those of water bugs ($P < 0.0001$)

In the second experiment, we examined how the snails altered their phenotypes in different predator environments. The univariate responses for the nine response variables are presented in Figs. 1 and 2. Although treatment effects for each individual trait were not analyzed, we examined the percentage differences between some of the treatments to provide a sense of the magnitude of the treatment effects. For size-adjusted shell width, water bugs induced 9% wider shells and fish induced 10% narrower shells compared to the no-predator treatment. For size-adjusted shell height, aperture width, and aperture height, water bugs induced small effects (2–4% increases) while fish induced relatively large effects (5–8% decreases) compared to the no-predator treatment. Two crayfish had the largest effect on shell thickness, inducing a twofold increase compared to the no-predator treatment. For size-adjusted shell strength, fish induced

9% stronger shells than the no-predator treatment. Compared to the no-predator treatment, snails living with two water bugs were 37% larger, while those living with two fish were 33% smaller. In terms of behavior, fish had the greatest effect, inducing a 17- to 41-fold increase in the use of the surface and a three- to fourfold increase in the use of structure compared to the no-predator treatment.

Using PCA, we were able to condense our nine response variables into two PCs. PC-1 accounted for 65% of the variation in the data and had an eigenvalue of 5.8. Positive loadings on PC-1 were associated with relatively larger shells and apertures, a greater final mass, relatively weaker shells, and less use of the surface and structure (i.e., eight of the nine response variables; Table 1). Although many of the shell traits and mass loaded together on PC-1, our size-correction procedure accounted for differences in mass prior to the PCA. Thus, differences the shell traits are independent of differences in mass. PC-2 accounted for 14% of the variation and had an eigenvalue of 1.3. Positive loadings on PC-2 were associated with thicker shells (Table 1). Importantly, the variables exhibited good agreement with the extracted components (i.e. communalities >0.6). Based on ANOVA using the scores from PC-1 and PC-2, there were significant predator effects for PC-1 ($F_{9,40} = 33.1, P < 0.001$) and PC-2 ($F_{9,40} = 3.1, P = 0.006$). The responses to each predator at low and high density are summarized first followed by the responses to combined predators.

Our first hypothesis was that each predator would induce unique responses. We tested this hypothesis by comparing snail responses among the no-predator and low-density predator treatments. Water bugs induced higher scores on PC-1 compared to snails reared in the other three treatments. In contrast, fish induced lower scores on PC-1 compared to snails reared in the other three treatments. Snails

Table 1 Principal component structure for the first two principal components (PC-1, -2) from a principal components analysis on the mass, shell and aperture shape, shell thickness and strength, and behavior of *Helisoma trivolvis*. Final communalities are shown for each variable

| Variable | PC-1 | PC-2 | Final communality |
|------------------|--------|--------|-------------------|
| Mass | 0.828 | 0.325 | 0.791 |
| Shell width | 0.907 | -0.243 | 0.881 |
| Shell height | 0.892 | 0.135 | 0.814 |
| Aperture height | 0.830 | 0.061 | 0.693 |
| Aperture width | 0.921 | -0.012 | 0.849 |
| Shell thickness | -0.213 | 0.954 | 0.956 |
| Shell strength | -0.820 | 0.319 | 0.774 |
| Use of surface | -0.755 | -0.035 | 0.602 |
| Use of structure | -0.829 | -0.276 | 0.764 |

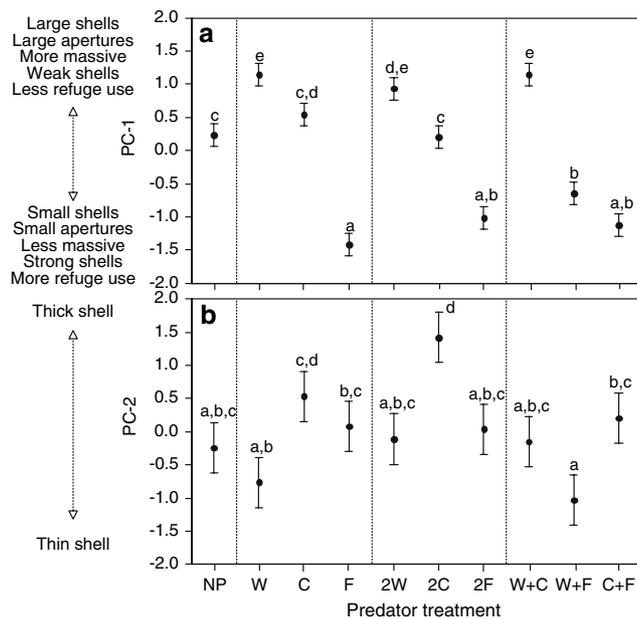


Fig. 3 The effects of different predator environments on the first (PC-1; **a**) and second (PC-2; **b**) principal components generated from a principal components analysis (PCA) on *H. trivolvis* relative morphology, behavior, and final mass (least-squares means \pm 1 SE). PC-1 and PC-2 accounted for 65 and 14% of the variation in the data set, respectively. For PC-1, relative shell size (height and width), relative aperture size (height and width), and mass loaded positively, while snail refuge use (i.e., use of surface and structure) and relative shell strength loaded negatively. For PC-2, shell thickness loaded positively. The predator treatments are: no predator (NP), water bugs (W), crayfish (C), and fish (F). The high density of each predator is represented with a 2 before the letter. Treatments sharing *lowercase letters* are not significantly different from each other based on pairwise comparisons using Fisher's LSD test ($P > 0.05$)

reared with crayfish were not different from snails reared without predators. There were no differences among the treatments on PC-2. To summarize, low densities of water bugs and fish induced unique and opposing responses, while crayfish had no effect.

We also tested this hypothesis by comparing snail responses among the no-predator and high-density predator treatments. As above, water bugs induced higher scores on PC-1 than snails reared in the other three treatments, and fish induced lower scores. Snails reared with crayfish were not different from snails reared without predators. On PC-2, the no-predator, water bug, and fish treatments were not different from each other, but all had lower scores compared to the crayfish treatment. To summarize, high densities of each predator induced the formation of unique responses.

Our second hypothesis was that doubling the densities of conspecific predators would induce more extreme responses than the low-density treatments. However, doubling the density of predators did not lead to significantly more extreme PC-1 or PC-2 scores.

Our third hypothesis was that prey should produce intermediate responses to pairwise combinations of predators to balance the overall risk of predation. In addressing this hypothesis, the responses with combined predators must be compared to each predator alone at low and high density to assess whether snails are responding to the change in predator composition or to the increase in total predator density.

We first considered the water bug-plus-crayfish treatment. On PC-1, snails in the water bug-plus-crayfish treatment were similar to snails reared with water bugs at low and high densities, but they had significantly higher scores than snails reared with crayfish at either low or high densities. On PC-2, snails in the water bug-plus-crayfish treatment were similar to snails reared with water bugs and crayfish at low densities. However, snails in the water bug-plus-crayfish treatment were similar to snails reared with water bugs at high densities but had lower PC-2 scores than snails reared with crayfish at high densities. Because neither predator induced changes on PC-2 at low density (i.e., the density of each predator in the combined predator treatment) compared to the no-predator treatment, the PC-2 phenotype produced in the water bug-plus-crayfish treatment is interpreted as a lack of response to either predator – and not as a risk-balancing strategy. In summary, snails experiencing water bugs plus crayfish responded solely to water bugs.

We next considered the water bug-plus-fish treatment. On PC-1, snails in the water bug-plus-fish treatment were intermediate to snails reared with water bugs and fish at low densities. While snails in the water bug-plus-fish treatment had lower PC-1 scores than snails reared with water bugs at high densities, they had similar scores to snails reared with fish at high densities. On PC-2, snails in the water bug-plus-fish treatment had lower scores than snails reared with fish at low densities, but these were similar to the scores of snails reared without predators or with water bugs at low densities. In contrast, snails in the water bug-plus-fish treatment were similar to snails reared with water bug or fish at high densities. Since neither predator alone at low nor high density affected PC-2 scores compared to the no-predator treatment, the thinner shells produced in the water bug-plus-fish treatment may be the consequence of a non-additive phenotypic response to the combined predator species. In summary, snails altered a suite of PC-1 traits when experiencing water bugs plus fish, and the intermediate phenotype was biased toward the fish-induced phenotype.

Finally, we considered the crayfish-plus-fish combination. On PC-1, snails in the crayfish-plus-fish treatment were similar to snails reared with fish at low and high densities but had significantly lower scores than snails reared with crayfish at either low or high densities. On PC-2, snails in the crayfish-plus-fish treatment were similar to snails reared with crayfish and fish at low densities.

However, snails in the crayfish-plus-fish treatment were similar to snails reared with fish at high densities but had lower PC-2 scores than snails reared with crayfish at high densities. Because neither predator induced changes on PC-2 at low density compared to the no-predator treatment, the phenotype produced in the crayfish-plus-fish treatment is interpreted as a lack of response to either predator – and not as a risk-balancing strategy. In summary, snails altered a suite of PC-1 traits when experiencing the crayfish-plus-fish combination, and the phenotype was biased toward the fish-induced phenotype.

Discussion

One of the major goals in community ecology is to understand predator–prey interactions within natural communities, and the information gained by examining the inductive effects (i.e., non-lethal presence) of single and combined predators on prey phenotypes is a critical step toward reaching this goal. We discovered that snails display an astounding diversity of responses to different predator environments. In our single predator treatments, each of the predators induced unique responses in *Helisoma trivolvis*. Consistent with previous experiments, fish had a large impact on snail traits (Turner 1996; DeWitt et al. 2000; Turner et al. 2000). In behavioral terms, snails increased their refuge use and spent more time at the water's surface in the presence of fish. While both of these behaviors can reduce encounter rates with fish that predominantly use the middle of the water column during feeding, they were associated with slower growth. In addition to behavior and growth, fish also induced relatively smaller shells. While smaller shells have a greater resistance to shell crushing, more work is necessary to determine their adaptive value. In contrast, water bugs induced phenotypes that opposed those formed with fish. For example, the snails formed relatively large shells that allow them to pull deep inside their shells (Hoverman and Relyea 2007). While larger shells reduce predation rates with water bugs, they come at the cost of increased susceptibility to crayfish predation and reduced ability to resist crushing forces (J.T. Hoverman and R.A. Relyea, unpublished manuscript). Crayfish only induced changes in shell thickness in snails and only at the highest density of crayfish. Thicker shells reduce predation rates with crayfish but come at the cost of increased susceptibility to water bug predation and increased time to reproduction and reduced fecundity (Hoverman et al. 2005, J.T. Hoverman and R.A. Relyea, unpublished manuscript). Interestingly, thicker shells were not associated with stronger shells, possibly because shell thickness was measured at the leading edge of the aperture, while shell strength was tested on the entire shell.

We found little support for our hypothesis that high predator densities induced more extreme responses than the low predator densities. The general lack of more extreme responses to increased water bug or fish density despite greater amounts of predation cues (i.e., additional predators and more consumed prey) suggests that the phenotypic responses were maximized at low predator densities. While our prediction was not supported in water bugs or fish, our results are consistent with observations in other systems that inducible defenses often saturate at low predator densities (e.g., zooplankton, tadpoles, and bryozoans; Tollrian 1993; Harvell 1998; Van Buskirk and Arioli 2002; Relyea 2004). Nonetheless, our results are particularly striking considering the large difference in mortality risk posed by fish and water bugs (7.67 snails/h and 0.18 snails/h, respectively). Given the size of and mortality risk posed by fish, our mesocosms were most likely saturated with chemical cues at low fish densities. Thus, snails probably did not perceive an increase in fish density as a more risky situation. However, water bugs also induced maximized responses at a low density despite their associated low risk of mortality. This is consistent with previous work in which increased water bug densities did not induce more extreme responses under laboratory conditions using 10-l tubs (Hoverman et al. 2005). Thus, although the size of the experimental units was 100-fold greater in the current study, snails still did not respond to higher water bug densities. Moreover, previous experiments with water bugs have shown that snails can form defenses that reduce the risk of predation within 1 week with limited long term reproductive costs (i.e., fecundity; Hoverman et al. 2005; Hoverman and Relyea 2007; Hoverman and Relyea, unpublished data). Accordingly, snails may have induced maximal defenses at low water bug densities because the defenses are highly effective and have limited reproductive costs. While the magnitude of antipredator defenses in prey is generally positively correlated with the amount of risk posed by predators (Kusch 1993; McKelvey and Forward 1995; Anholt et al. 1996; Relyea 2001), our results and those in other systems suggest that this generalization may not hold (Relyea 2003). If prey encounter discrete environments (e.g., absence vs. presence of predators) and there is little benefit to more extreme responses, the evolution of discrete phenotypes may be favored over continuous phenotypes (Lively 1986; Moran 1992). In our system, dose-response experiments that manipulate predation risk more precisely will be valuable in determining if the phenotypic responses of snails are continuous or discrete.

In contrast, more extreme responses were found with crayfish. For shell thickness (i.e., PC-2), we observed no responses to low densities of crayfish, but there were significant responses to high densities, suggesting that snails were responding to either the higher predator density or the

higher total amount of prey consumed. In a previous laboratory experiment using 10-l tubs, we found that one and two crayfish induced phenotypic responses in snails that were similar to each other (Hoverman et al. 2005). Importantly, the amount of prey fed to a predator (an index of chemical cue concentration) was much greater in the laboratory experiment (12 and 24 mg/l per feeding, respectively) than the current mesocosm experiment (1.4 and 2.8 mg/l per feeding, respectively). This suggests that snails require a critical threshold of chemical cue(s) before forming phenotypic responses against crayfish. While this result was surprising given the high risk of mortality posed by crayfish (8.67 snails/h), similar thresholds have been documented in snails and other prey species when these have been exposed to predator cues (Snyder 1967; Brown et al. 2004; Mirza and Chivers 2003). Our results demonstrate that the induction threshold for crayfish-specific defenses was greater than that for the fish- and water bug-specific defenses.

The formation of predator-specific defenses in prey suggests that predators are not functionally identical entities that can be lumped together into a single mortality factor for prey (Polis and Strong 1996; Chalcraft and Reserants 2003). If prey experience variation in the presence of various predators and there are fitness trade-offs associated with responses to each predator, inducible defenses will be maintained over constitutive defenses (Gotthard and Nylin 1995; Kingsolver 1995a, b; Dudley and Schmitt 1996). Although less frequently studied than fitness trade-offs between non-induced and predator-induced responses, a number of studies have found that functionally different predators will favor opposing phenotypic responses in prey (Tollrian and Harvell 1999; DeWitt and Langerhans 2003; Kishida and Nishimura 2005). Given that prey will face different predator species across their geographic distributions, studies that specifically address the fitness trade-offs associated with predator-specific responses to different predators will provide valuable insights into the ecology and evolution of inducible defenses.

Whereas prey in natural communities frequently encounter combinations of predators, our knowledge of inducible prey defenses comes largely from studies focused on single predator species. By examining different predator combinations and multiple prey traits, ecologists can obtain an excellent understanding of how prey respond to more complex predator regimes. Because the predators in our experiment induced unique defenses, we expected snails to form intermediate responses that integrated their defensive strategies against the predator combination. However, we found that the responses of snails to predator combinations were strongly biased towards one predator in the combination. These results suggest that the phenotypic responses of snails might have important consequences for prey mortality rates.

Traditionally, ecologists have attempted to predict prey mortality rates with combined predators based on mortality rates with single predators. Interestingly, the prediction that prey mortality rates by different predators will lead to additive effects when the predators are combined is often not supported. Indeed, Sih et al. (1998) reviewed the literature on multiple predator effects (MPEs) and found a variety of reported effects on prey survival, including risk reduction and risk enhancement. For example, risk reduction can occur in a system when there are predator–predator interactions (e.g., intraguild predation) that alter per-capita predation rates or predator densities. In contrast, risk enhancement typically occurs when prey defenses against one predator conflict with defenses against another predator. Sih et al. (1998) concluded that risk reduction was more common than risk enhancement because many prey switch to compensatory defenses with multiple predators, thereby reducing the overall risk of predation despite possible conflicting responses to different predators. By combining data on the non-lethal effects of combined predators on prey defenses and an understanding of the fitness trade-offs associated with different phenotypes, we can generate predictions about prey survival with combined predators.

In the treatments containing crayfish and either fish or water bugs, snails did not respond to the crayfish. While crayfish are efficient predators on snails, it appears as though the snails were unable to detect the crayfish at the low density used in our experiment. Therefore, the snails may experience high mortality from crayfish when snails focus their defensive traits (e.g., behavior and morphology) towards other predators (i.e., risk enhancement). One would also predict that snails which experience water bugs plus fish and bias their responses towards fish (e.g., relatively small shells) may experience increased predation rates by water bugs compared to when they experience water bugs alone. Risk enhancement would occur in this case because snails that possess relatively small shells are more vulnerable to attack by water bugs (J.T. Hoverman and R.A. Relyea, unpublished data). Alternatively, fish in natural communities may indirectly benefit snails by affecting water bugs in two ways. First, fish may consume water bugs, thereby reducing their density as well as the risk the latter pose to snails (i.e., a density-mediated indirect interaction). Second, fish may induce behavioral changes in water bugs (e.g., foraging location) that reduce their encounter rates with snails (i.e., a trait-mediated indirect interaction). In both cases, predation rates on snail populations by water bugs would be reduced. Thus, snail populations that bias their responses towards fish when water bugs are present may, in fact, not experience an increase in predation rate from water bugs. In summary, an understanding of the inducible defenses of prey will greatly benefit our interpretation of future experiments that address prey survival with combined predators.

Predator–prey interactions are embedded within complex natural communities that can alter the predictions of experiments conducted under more simplistic conditions. Our study has taken a simplified approach by examining prey responses to two different predator species encountered simultaneously. Of course, natural systems may contain more diverse predator assemblages (i.e., more than two predator species) that include predators with different foraging techniques and locations and population densities. There is a dearth of information on the effects of more than two predators on the phenotypic responses of prey. Future work that examines how prey respond to three or more predators will be invaluable for understanding prey defensive decisions in nature. As research on multiple predators progresses, we will make significant strides towards understanding how larger communities affect the evolution of inducible defenses.

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