



Temporal environmental variation and phenotypic plasticity: a mechanism underlying priority effects

Jason T. Hoverman and Rick A. Relyea

J. T. Hoverman (jhoverma@utk.edu) and R. A. Relyea, Dept of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260, USA. Present address for JTH: 274 Ellington Plant Sciences Building, Dept of Forestry, Wildlife, and Fisheries, Univ. of Tennessee, Knoxville, TN 37996-4563, USA.

Understanding the role of history in the formation of communities has been a major challenge in community ecology. Here, we explore the role of phenotypic plasticity and its associated trait-mediated indirect interactions as a mechanism behind priority effects. Using organisms with inducible defenses as a model system, we examine how aquatic communities initially containing different predator environments are affected at the individual and community level by the colonization of a second predator. Snails and tadpoles were established in four different caged-predator environments (no predator, fish, crayfish or water bugs). These four communities were then crossed with three predator colonization treatments (no colonization, early colonization, or late colonization) using lethal water bugs as the predator. The snails responded to the caged predator environments with predator-specific behavioral and morphological defenses. In the colonization treatments, snails possessing the wrong phenotype attempted to induce phenotypic changes to defend themselves against the new risk. However, snails initially induced by a different predator environment often suffered high predation rates. Hence, temporal variation in predation risk not only challenged the snail prey to try to track this environmental variation through time by adjusting their defensive phenotypes, but also caused trait-mediated interactions between snails and the colonizing predator. For tadpoles within these communities, there was little evidence that the morphological responses of snails indirectly effected tadpole predation rates by colonizing water bugs. Unexpectedly, predation rates on tadpoles by colonizing water bugs were generally higher in the three caged-predator treatments, suggesting that water bugs elevated their foraging activity in response to potentially competing predators. In summary, we demonstrate an important priority effect in which the initial occurrence of one species of predator can facilitate predation by a second predator that colonizes at a later date (i.e. a TMII) suggesting that phenotypic plasticity can be an important driver behind priority effects (i.e. historical exposure to predators).

Across a variety of systems, ecologists have demonstrated that the historical sequence of species additions can facilitate or inhibit the establishment of other species in communities and, thereby, affect community assembly (i.e. priority effects; Connell and Slatyer 1977, Alford and Wilbur 1985, Menge and Sutherland 1987, Robinson and Dickerson 1987, Lawler and Morin 1993). Logically, the ability of a species to inhibit or facilitate the establishment of subsequent species is associated with the traits of the species (e.g. competitive ability, degree of predator avoidance). Using a functional trait-based approach, a number of assembly rules have been established to help elucidate the underlying mechanism associated with patterns in communities (reviewed by Belyea and Lancaster 1999). While trait-based approaches to community assembly have been useful, an implicit assumption is that species' traits are static. However, many taxa express adaptive phenotypic plasticity such that they can produce different phenotypes in different environments (Schlichting and Pigliucci 1998, Pigliucci 2001, West-Eberhard 2003). Since phenotypic plasticity

affects the outcome of species interactions, it may be important to understand how phenotypic plasticity affects the development of communities.

Phenotypic plasticity has the potential to affect community development through trait-mediated indirect interactions (i.e. TMII, Abrams 1995, Werner and Peacor 2003). TMII occur when a species alters its traits in different environments (e.g. predation, competition) and these trait changes can subsequently alter how that species interacts with other members of the community. Priority effects may be of importance because as new species colonize a community, the species currently in the community experience temporal variation in competition, predation, or parasitism. While we expect organisms to developmentally track temporal changes in the environment (e.g. competition, predation) to reduce the costs of expressing the wrong phenotype in a particular environment (Gabriel 1999), environmental history may determine if an organism is capable of responding to future environmental changes. For example, historical phenotypic responses could place an organism on a developmental

trajectory that constrains future phenotypic responses to environmental changes and, thereby, affects interactions within the changed community (i.e. a TMII). Thus, an understanding of phenotypic plasticity and any associated TMIIIs may be important for assessing whether a species persists in a community as other species are added.

Organisms with inducible defenses are excellent model systems for assessing the role of phenotypic plasticity in the development of communities. Many prey species change their behavior, morphology, and life history in predator environments (Sih 1987, Karban and Baldwin 1997, Tollrian and Harvell 1999). These changes not only affect direct interactions with predators (e.g. per-capita predation rates) but they can also affect interactions, indirectly, with other species in the community (i.e. TMIIIs). Accordingly, priority effects may be important for understanding the TMIIIs initiated by predator-prey interactions. For example, temporal variation in predation risk can occur when predators colonize habitats that were previously either predator-free or contained different predator species. Recent research has shown that prey can express defenses throughout much of development when predators colonize predator-free habitats (i.e. wide developmental windows, Kuhlmann et al. 1999, Tollrian and Dodson 1999, Hoverman and Relyea 2007). However, if predators colonize habitats that already contain other predators, prey are placed in a potentially precarious situation. If the colonizing predator favors different prey defenses than the existing predators, the subsequent phenotypic responses of prey to the colonizing predator may be constrained and predation rates may increase (i.e. a TMII in the form of predator facilitation, Charnov et al. 1976, Soluk and Collins 1988). Whereas we have some insight into how prey respond to combinations of predators (Relyea 2003, Hoverman and Relyea in press), we currently have no insights as to how prey adjust already induced phenotypes following colonization by a second predator and the consequences, if any, for prey fitness (e.g. survival).

Because of the reticulate nature of communities, TMIIIs can be transmitted through communities via a number of pathways. For example, a colonizing predator has the potential to also affect other species in a community including alternative prey. If a given prey species is well defended against the colonizing predator, predation pressure may increase on other prey species. In contrast, if a given prey species is poorly defended against the colonizing predator, predation pressure may decrease on other prey species (Abrams 1987). In such cases, changes in predation rates would be mediated by the defensive traits expressed by the focal prey rather than by the densities of the focal prey (i.e. a TMII). While prey nutritional quality, handling time, and abundance play important roles in understanding the prey preferences of predators (Murdoch 1969, van Baalen et al. 2001), the anti-predator defenses of prey within the community may be equally important (Abrams 1987, Bolker et al. 2003). Within the context of priority effects, we can determine how temporal variation in predation risk affects the transmission of TMIIIs.

We examined priority effects using simple freshwater communities composed of two herbivores (planorbid snails *Helisoma trivolvis* and green frog tadpoles *Rana clamitans*) and one of three common predators (pumpkinseed sunfish

Lepomis gibbosus, crayfish *Orconectes rusticus* and water bugs *Belostoma flumineum*). *H. trivolvis* expresses predator-specific behavioral and morphological defenses in response to cues from caged predators (Hoverman et al. 2005, Hoverman and Relyea in press) and these defenses have relatively wide developmental windows combined with the ability to reverse defensive phenotypes early in ontogeny (Hoverman and Relyea 2007). Whereas many tadpoles also have predator-specific defenses (Relyea 2001a), previous work has shown that tadpoles do not respond to caged predators consuming snails (Schoeppner and Relyea 2005) and green frog tadpoles do not respond to predatory water bugs eating tadpoles (Relyea 2001b). Thus, we could examine whether tadpole growth and survival were indirectly affected by caged predator treatments that solely affect snail phenotypes. To incorporate temporal variation, we simulated the migration of predators using lethal (i.e. uncaged) water bugs. Ponds and wetlands experience large monthly and annual fluctuations in water bugs density as a result of seasonal migrations of adult water bugs and within habitat reproduction during the spring and summer (J. Hoverman, R. Relyea, E. Werner, D. Skelly and K. Yurewicz unpubl.). Moreover, *H. trivolvis* is a rather long-lived snail (1- to 2-year life span) and green frogs have a 1-year larval period. Thus, these taxa can experience substantial temporal variation in predation risk from water bugs. Our specific questions were the following: 1) how do the three predators affect the behavior, morphology, and growth of snails and tadpoles? 2) Does the initial induction by different caged predators affect the interaction between snails and colonizing water bugs? 3) Does initial exposure to caged predators affect tadpole predation by colonizing water bugs?

Methods

We examined the effects of caged predators and lethal water bug colonization on a simple aquatic community by conducting a mesocosm experiment at the Univ. of Pittsburgh's Aquatic Research Facility in Linesville, PA. On 27 March 2004, we collected 350 adult snails from a local pond and placed 25 into each of 14 pools filled with 100 l of well water to oviposit. Egg deposition began in April and continued until May, at which time the adults were removed from the pools. Snails began hatching on 9 May and were fed rabbit chow ad libitum until the start of the experiment. A mixture of four green frog *Rana clamitans* egg masses were collected from a nearby pond on 18 July and placed into four pools. Tadpoles began hatching after one week and were fed rabbit chow ad libitum.

On 19 July, 48 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 from nearby ponds to simulate a simple aquatic community. A single clay tile platform (32 × 32 cm tile supported by a 10 × 10 cm tile) was placed in the center of each tank to serve as artificial structure. We also added three predator cages to each tank. One cage, designed to house fish, was constructed from 30 × 30 cm corrugated pipe capped with fiberglass window screen on each end. The other two cages, designed to house crayfish and water bugs, were made from 10 × 10 cm corrugated pipe capped with shade cloth. We

placed a shade cloth lid over each tank to prevent colonization by insects and amphibians during the experiment. On 30 July, we added 50 randomly selected tadpoles (mean mass ± 1 SE = 12 ± 4 mg) from a mixture of the four original egg masses and 50 randomly selected juvenile snails (73 ± 4 mg) from a mixture of the 14 wading pools to each tank. These densities (23 snails or tadpoles m^{-2}) are within natural densities for the two species (Hoverman unpubl.).

We designed a completely randomized experiment with 12 treatments and 4 replicates. The experiment was a factorial combination of caged predators crossed with lethal water bug colonization. Our caged predator treatments were the following: 1) no predator, 2) two water bugs, 3) two crayfish, and 4) one fish. Our lethal water bug colonization treatments were the following: 1) no water bug colonization, 2) early colonization by two water bugs (on day 10), and 3) late colonization by two water bugs (on day 20). Caged predators emit water-borne chemical cues, which provide the opportunity to examine trait induction without a reduction in target prey density (Chivers and Smith 1998). All caged predators were fed three times per week. To equalize the total amount of prey biomass consumed, each caged fish was fed 1 g of snail biomass while each caged water bug and crayfish was fed 0.5 g of snail biomass at each feeding (i.e. 1 g total).

Tadpole and snail behavior were observed on day nine of the experiment before the initiation of the water bug colonization treatments. We assessed tadpole behavior by quantifying the number of tadpoles that could be observed and the proportion of observed tadpoles that were moving (i.e. active). For each tank, we conducted four observations and calculated the mean number of tadpoles seen and the mean percent activity to serve as our response variables. We assessed snail behavior by counting 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 surface by dividing our counts by 50 (i.e. the initial number of snails added to each tank). These proportions were used as our two response variables for snail behavior. Since our observations required that we lift the tile platform and disturb the snails, we only conducted a single observation. These protocols have been used successfully in previous experiments examining tadpole and snail behavior (Turner 1996, Relyea 2001b). We decided not to observe snail and tadpole behavior after the addition of water bugs because we did not want the disturbance associated with lifting the tile to affect predation rates. Consequently, we were unable to assess the effects of colonizing water bugs on snail and tadpole behavior.

The experiment was terminated on day 28 and all surviving snails and tadpoles were counted to assess survival and preserved in 10% formalin to later quantify mass and morphology. The tadpoles and snails from each tank were weighed and then mean tadpole and snail mass were used as our mass response variables. Because one of our major goals was to examine treatment effects on snail shell shape, 20 randomly selected snails from each tank were weighed to the nearest mg and measured for shell height and width using digital imaging software. We also measured the shell thickness (at the leading edge of the aperture) of each snail using digital calipers.

When studying morphological plasticity, it is important to account for the allometric relationships between linear dimensions and mass (i.e. size). Analysis of covariance (ANCOVA) has frequently been used as a method to account size variation in studies examining morphological plasticity (Dahl and Peckarsky 2002, Hoverman et al. 2005, McCoy et al. 2006, Hoverman and Relyea 2007). In our data, shell width and height positively covaried with snail mass (cube-root-transformed to make the relationship linear). Thus, we used ANCOVA with mass as our covariate to correct for size. 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. 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. By using all the measured individuals in the ANCOVA, we had ample power to capture the allometric relationship between our traits and mass. For each morphological trait, we then calculated the mean mass-adjusted shell dimensions for each experimental unit and used the means from each experimental unit as our morphological response variables. Shell thickness did not covary with mass even after various transformations, which is consistent with our previous work in this system (Hoverman et al. 2005, Hoverman and Relyea 2007). Thus, we simply calculated the mean shell thickness for the snails from each tank and this served as our shell thickness response variable.

We conducted two analyses with the data. First, we used a multivariate analysis of variance (MANOVA) to analyze the effect of caged predators on tadpole behavior (number seen and percent active) and snail behavior (percent at the water surface and percent using structure) on day 9, which was just prior to applying the water bug colonization treatments. The proportional data was binomially distributed and arcsine-transformed prior to analysis. Second, we used a factorial MANOVA to examine the effects of caged predators, water bug colonization, and their interaction on snail morphology, snail and tadpole growth, and snail and tadpole survival. Prior to the analysis, we conducted Levene's test for homogeneity of variances and found heteroscedasticity for mass and survival of tadpoles and snails. Thus, snail and tadpole mass were arcsine- and log-transformed, respectively whereas snail and tadpole survival were arcsine-transformed to meet the assumptions of equality of variances. For all the analyses, significant multivariate effects were followed by univariate tests. When univariate tests were significant, we conducted mean comparisons using Fisher's LSD test.

Results

Prey behavior

In the first analysis, we examined the effects of caged predators on the behavior of tadpoles and snails and found a significant multivariate effect (Wilks' $\lambda_{12,109} = 8.9$, $p < 0.001$). While caged predators did not affect tadpole behavior (univariate $p \geq 0.194$; Fig. 1A), the predators did affect snail behavior (univariate $p \leq 0.001$; Fig. 1B). Compared to the no-predator treatment, caged water bugs and crayfish had no effect on snail behavior, whereas caged

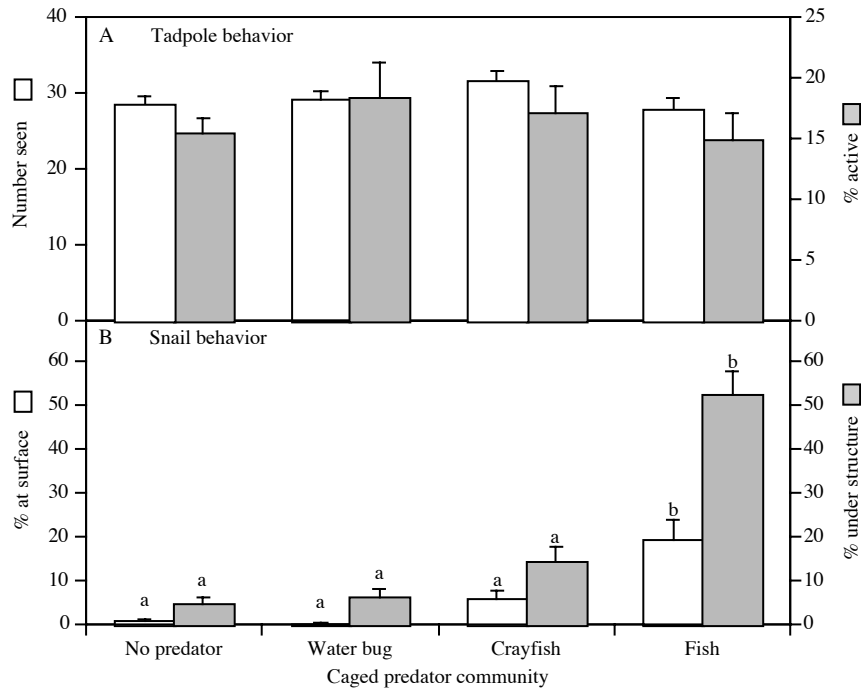


Fig. 1. The effects of different caged predator treatments on: (A) green frog tadpole behavior (the number of tadpoles seen and percent active) and (B) snail behavior (the use of surface and structure). Observations were taken prior to the colonization of lethal water bugs. Data are means \pm 1 SE. For snail behavior, treatments sharing lower case letters are not significantly different from each other based on pairwise comparisons using Fisher's LSD test ($p > 0.05$).

fish induced 20% greater use of the surface and 48% greater use of structure.

Snail morphology

In the second analysis, we examined the treatment effects on snail morphology, snail and tadpole mass, and snail and tadpole survival. There was a significant multivariate effect of caged predators, lethal water bug colonization, and their interaction. Below, we examine each of the univariate response variables.

Shell height was affected by caged predators but not by water bug colonization or the interaction (Table 1, Fig. 2A). Compared to the no-predator treatment, snails reared with

crayfish and water bugs were not different while snails reared with fish had 3% lower shells. Snails reared with water bugs also had higher shells than snails reared with fish but snails reared with crayfish or fish did not differ.

Shell width was affected by caged predators, water bug colonization, and their interaction (Table 1, Fig. 2B). In the treatments lacking colonization, caged water bugs induced 4–6% wider shells than the other three treatments and the other three did not differ from each other. Within the no-predator environments, early and late colonization by water bugs induced 8–9% wider shells than the no-colonization treatment. Within the caged-water bug environments, shell width was not affected by early or late colonization. Within the caged-crayfish environments, late colonization induced marginally wider shells whereas early colonization induced

Table 1. Results of a MANOVA (Wilks' λ with F approximations) on the effects of different caged predator treatments and water bug colonization on snail morphology, snail and tadpole mass, and snail and tadpole survival. Univariate tests (p -values) are shown for both main effects and their interaction.

A. Multivariate	DF	F	p
Caged predator	21,86	7.9	<0.001
Water bug colonization	14,60	19.8	<0.001
Caged predator \times colonization	42,144	3.2	<0.001

B. Univariate	Caged predator	Colonization	Caged predator \times colonization
Shell height	0.009	0.450	0.120
Shell width	<0.001	0.001	0.023
Shell thickness	<0.001	<0.001	<0.001
Snail mass	<0.001	0.111	0.753
Tadpole mass	0.025	0.103	0.923
Snail survival	<0.001	<0.001	0.027
Tadpole survival	0.349	<0.001	0.020

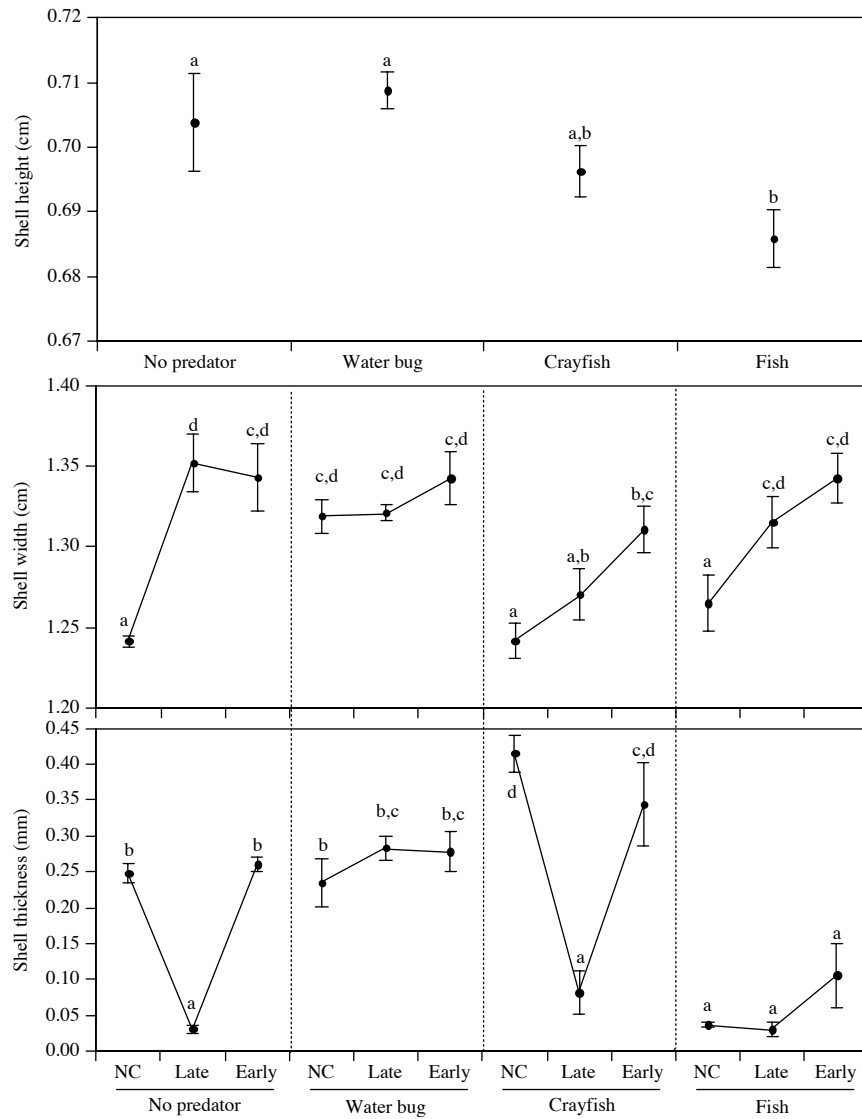


Fig. 2. The effects of different caged predator treatments and water bug colonization on the final shell height, width, and thickness of snails. The lethal water bug colonization treatments were either no colonization, early colonization (i.e. two lethal water bugs added at day 10), or late colonization (i.e. two lethal water bugs added at day 20). Shell height and width were corrected for size by regressing the linear dimensions against cube-root transformed mass (using analyses of covariance) and saving the residuals for each tank. The data for shell height are averaged over water bug colonization treatments (see text for details). Data are means \pm 1 SE. Treatments sharing lower case letters are not significantly different from each other based on pairwise comparisons using Fisher's LSD test ($p > 0.05$).

6% wider shells compared to the no-colonization treatment. Within the caged-fish environments, late- and early-colonization induced 4–6% wider shells than the no-colonization treatment.

Caged predators, water bug colonization, and their interaction also affected shell thickness (Table 1, Fig. 2C). In the treatments lacking colonization, caged water bugs induced no change, caged crayfish induced 67% thicker shells, and caged fish induced 85% thinner shells compared to the no-predator treatment. Within the no-predator environments, shell thickness in the no-colonization and early-colonization treatments was 88% thicker than the late-colonization treatment but there was no difference between no colonization and early colonization. Within the caged-water bug environments, shell thickness was not affected by colonization. Within the caged-crayfish environments, shell thickness in the no-colonization and early-colonization

treatments were 76–80% thicker than the late-colonization treatment; there was no difference between no-colonization and early-colonization. Within the caged-fish environments, shell thickness was not affected by colonization.

Prey mass

Snail mass was affected by caged predators but not by water bug colonization or the interaction (Table 1, Fig. 3). Compared to the no-predator environment, caged water bugs had no effect, caged crayfish induced 15% larger mass, and caged fish induced 22% smaller mass. Snails reared with caged water bugs and caged fish were 12% and 33% smaller, respectively, than snails reared with caged crayfish. Snails reared with caged fish were 24% smaller than snails reared with caged water bugs.

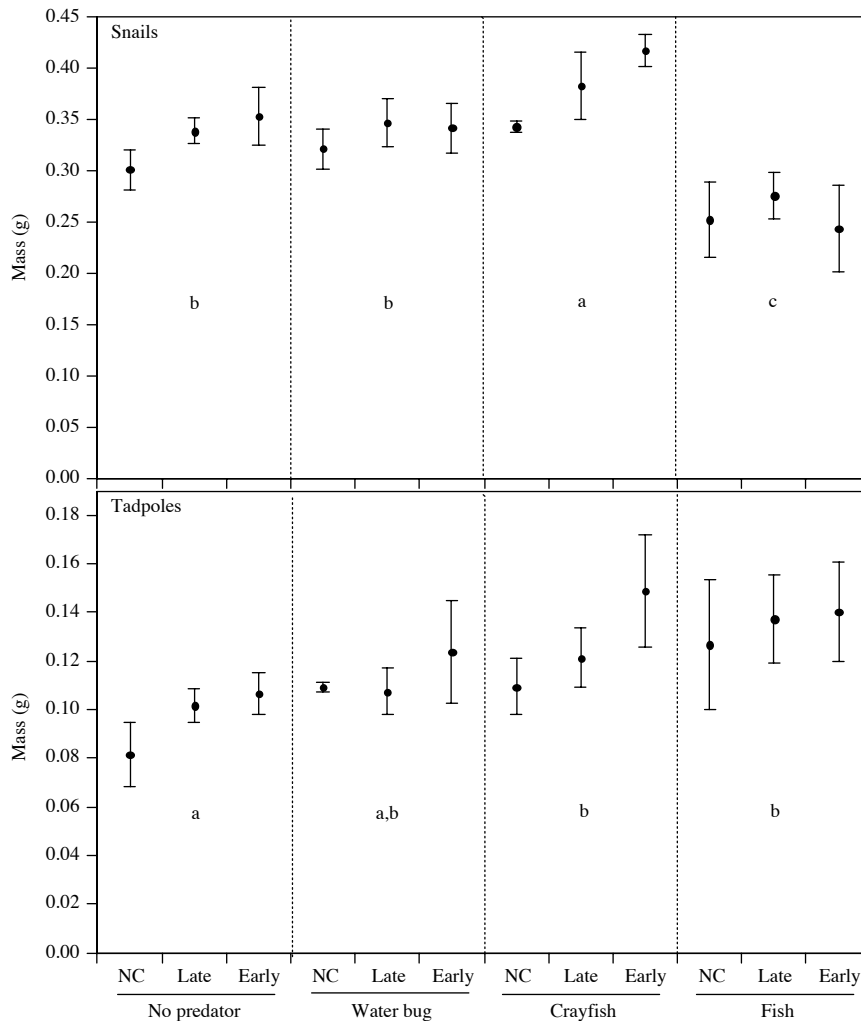


Fig. 3. The effects of different caged predator treatments and water bug colonization on the final mass of tadpoles and snails. See Fig. 2 for treatment details. Data are means \pm 1 SE. For both variables, there was only a significant effect of the caged predator treatments. Thus, caged predator treatment sharing lower case letters are not significantly different from each other based on pairwise comparisons using Fisher's LSD test ($p > 0.05$).

Tadpole mass was affected by caged predators but not water bug colonization or their interaction (Table 1, Fig. 3). Compared to the no-predator environment, caged water bugs had no effect while caged crayfish and caged fish induced 31–40% larger mass. There were no differences among the three caged predator treatments.

Prey survival

Caged predators, water bug colonization, and the interaction affected snail survival (Table 1, Fig. 4). Within all of the caged-predator environments, snail survival declined from no colonization to early colonization reflecting the long exposure time to lethal water bugs. By examining survivorship within each colonization treatment, we can assess how existing defenses affected snail survival against colonizing water bugs. When there was no colonization, survival was high across all caged-predator environments (mean = 98%). When there was late colonization, there was only one significant comparison; snails reared with caged water bugs survived 10% better than snails reared with

caged fish. However, when there was early colonization, snails reared in no-predator environments experienced survival that was similar to snails reared in caged-water bug environments but 14% and 36% higher than snails reared with caged-crayfish and caged-fish environments, respectively.

Tadpole survival exhibited no main effect of caged predators but there was an effect of water bug colonization and a predator-by-colonization interaction (Table 1, Fig. 4). In the no-predator environments, an increased duration of lethal water bugs (from no colonization to early colonization) had no effect on tadpole survival. However, the other three caged-predator environments all experienced a decline in tadpole survival. Within the no-colonization treatments, tadpoles living with non-induced snails experienced 9–10% lower survival than tadpoles living with water bug- and crayfish-induced snails but similar survival as tadpoles living with fish-induced snails. Within the late colonization treatments, tadpole survival was similar among caged-predator environments. However, within the early colonization treatments, tadpoles living with non-induced snails

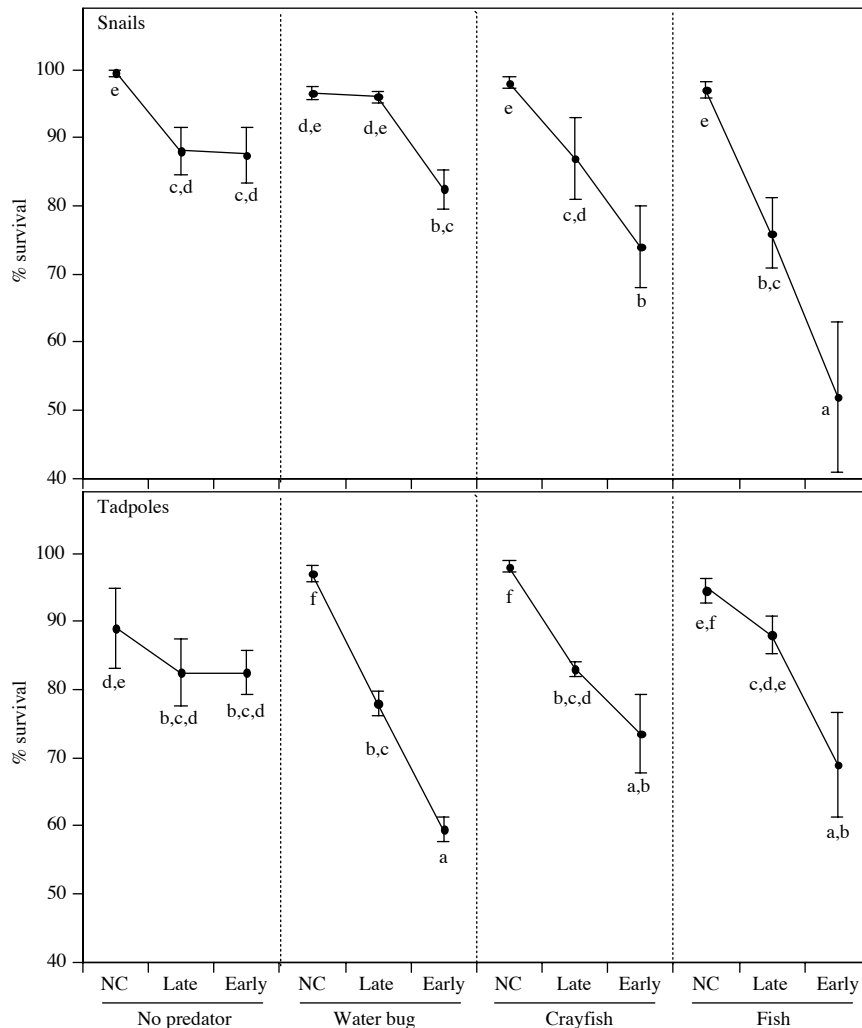


Fig. 4. The effects of different caged predator treatments and water bug colonization on snail and tadpole survival. Data are least-squares means ± 1 SE. Treatments sharing lower case letters are not significantly different from each other based on pairwise comparisons using Fisher's LSD test ($p > 0.05$).

experienced 24% higher survival than tadpoles living with water bug-induced snails but similar survival as tadpoles living with crayfish- and fish-induced snails.

Discussion

The results of this study demonstrate that phenotypic plasticity is important for understanding priority effects. Consistent with our previous work with water bugs and crayfish (Hoverman et al. 2005), we found that *Helisoma trivolvis* formed predator-specific defenses that reflect the differences in how the predators consume snails. Water bugs induced snails to form wider shells that allow snails to pull deep inside the shell and escape the probing stylet of the water bug. While wider shells reduce the risk of predation by water bugs, they come at the cost of delayed reproduction. Crayfish induced snails to grow larger and form thicker shells. Thicker shells are more resistant to aperture chipping by crayfish and larger snails are more difficult for crayfish to handle, but these responses come at the cost of reduced fecundity (Hoverman et al. 2005,

Hoverman and Relyea unpubl.). Based on selection experiments (Hoverman and Relyea unpubl.), the responses to water bugs and crayfish appear to be adaptive. Also consistent with our previous work, fish strongly induced snails to avoid the middle of the water column (Hoverman and Relyea in press). This movement should reduce encounter rates with fish but it clearly comes at the cost of substantially reduced growth. Overall, our results parallel those in other systems in which prey form predator-specific defenses as a consequence of the functional differences among their predators (Karban and Baldwin 1997, Tollrian and Dodson 1999, Relyea 2001b, DeWitt et al. 2000). These functional differences among predators can lead to fitness tradeoffs for different phenotypes, which should favor the evolution of inducible rather than constitutive defenses (Kingsolver 1995, Dudley and Schmitt 1996).

While our experiment was not specifically designed to assess whether snails and tadpoles were competing, we can obtain some insights into this potential interaction. If the snails and tadpoles were competing for periphyton in the mesocosms, any predator-induced negative effects on snail mass should be associated with indirect positive effects on

tadpole mass. While snail growth was greater with caged crayfish but lower with fish, tadpoles had greater growth in both of these treatments. As expected from previous research, tadpoles did not change their behavior when the caged predators were fed snails, either because tadpoles do not recognize the cues generated by predation on snails or because predation on snails communicates a low level of predation risk to tadpoles (Lefcort et al. 1999, Schoeppner and Relyea 2005, Persons et al. 2001). Therefore, the changes in tadpole growth were not due to direct changes in any tadpole behaviors. In the case of caged-crayfish environments, the most parsimonious explanation for the increase in tadpole growth is that both the snails and the tadpoles were benefiting from the fertilization effect of crayfish digestion. Increases in prey growth with predators present (but no change in prey density) are typically attributed to the nutrients that predators add to the water via prey digestion which fertilize algal growth (i.e. consumer-mediated nutrient recycling, Vanni and Layne 1997). Indeed, predators can alter the abundance and structure of producer communities via increased nutrient inputs or altered nutrient stoichiometry (McCollum et al. 1998, Elser and Urabe 1999). On the other hand, in the case of the caged-fish environments, the most parsimonious explanation for the increase in tadpole growth is that the tadpoles were benefiting from the fertilization effect of fish digestion as well as (perhaps) the 22% decline in snail growth that should increase overall periphyton abundance (Bernot and Turner 2001). While these results provide equivocal evidence for competition, past studies have shown that tadpoles and snails can feed on different groups of periphytic algae (diatoms and green algae, respectively; Werner and Peacor 2006). Thus, at the densities employed in our study, the low diet overlap may have precluded any competitive effects. Additional evidence for no competitive effects comes from a general lack of strong increases in growth in either snails or tadpoles following the addition of lethal water bugs, despite experiencing up to 50% snail mortality and 40% tadpole mortality. While the snails and tadpoles did experience changes in growth in the caged predator treatments, there was little evidence of competition but additional experiments are necessary to assess this conclusion.

Priority effects played an important role in understanding predation rates on snails with colonizing water bugs. We found that initial exposure to caged predators affected the morphology and behavior of snails and, consequently, affected the ability of snails to escape predation by colonizing water bugs. Snails living in no-predator and caged-water bug environments had similar survival following early water bug colonization, but snails living with caged-crayfish and caged-fish suffered 14–36% higher predation. These changes in predation rates are tied to the defenses that the snails possessed at the time of water bug colonization and the ability to form defenses against the colonizing water bugs. Based on inferences from the final phenotypes, snails exposed to caged water bugs had wide shells and did not form wider shells after water bug colonization, suggesting that these snails were already maximally induced and well defended against water bugs. On the other hand, fish-induced snails initially had narrow shells and smaller overall mass, making these snails

particularly vulnerable to a water bug predator that preferentially kills such phenotypes (Hoverman and Relyea unpubl.). When water bugs colonized tanks containing no predators or caged crayfish, the snails subsequently exhibited relatively wider shells. Although the wider shells could be the consequence of either selection for or induction of wider shells by lethal water bugs, induction likely played the dominant role because previous work on *H. trivolvis* has found that when non-lethal (i.e. caged) water bugs are added to predator-free habitats the snails quickly develop wider shells, but these wider shells are initially built using very thin shell material. However, within three weeks, shell thickness increases and resembles the thickness of snails that had continuous exposure to caged water bugs (Hoverman and Relyea 2007). We saw a similar pattern when lethal water bugs were added to either no-predator or caged-crayfish environments (Fig. 2); in both cases, the short-duration addition of lethal water bugs (i.e. late colonization) was associated with very thin shells whereas the long-duration addition of water bugs (i.e. early colonization) was associated with a shell thickness that resembled those snails induced by water bugs throughout the entire experiment. Why the non-induced snails would have similar survival as the water bug-induced snails after early colonization is unclear, although the lack of behavioral responses by the non-induced snails may have allowed them to respond more rapidly to the colonizing water bugs and quickly become invulnerable.

In communities with multiple prey species, the level of defense expressed by different prey species may affect the predation rates on alternative prey (i.e. a TMII; Abrams 1987, Matsuda et al. 1994, Bolker et al. 2003). In our experiment, there was limited evidence that the morphology of snails indirectly affected predation rates on tadpoles by colonizing water bugs. For example, snails reared with any of the three caged predators exhibited different morphologies but survival of the tadpoles was generally indistinguishable among these treatments. Alternatively, the caged-predator environments appeared to affect the predatory behavior of the colonizing water bugs. Water bugs consumed approximately twice as many total prey (within the early colonization treatment) when there were caged predators present than when no caged predators were present. Increasing the density of predators in the tanks (detectable via visual or chemical cues) may have caused an increase in water bug foraging (a common response to competition by animals; Stephens and Krebs 1986, Relyea 2002). Thus, tadpole predation rates would be mediated by the historical presence of predators that altered the foraging behavior of a colonizing predator (i.e. a TMII). As previous authors have suggested, many more studies are needed to determine whether changes in predation rates are mediated by prey traits or by other predators in the system (Lima 2002). These results demonstrate the impact of a colonizing species will depend on community composition prior to colonization.

Together, these results demonstrate an important priority effect in which the initial occurrence of one species of predator can facilitate predation by a second predator that colonizes at a later date (i.e. a TMII). This suggests that phenotypic plasticity can be an important driver behind priority effects (i.e. historical exposure to predators).

Indeed, as species are added to communities, the number of potential interactions increases dramatically. Consequently, whether or not species are able to adjust their phenotypes as a consequence of temporal environmental variation will likely play an important role in structuring the community (i.e. determine species extinction or persistence in the community). While the duration of the experiment was short and the mesocosm conditions rather simplistic, our results suggest that phenotypic plasticity can be a mechanism driving priority effects. However, experiments conducted within natural communities and over multiple generations will be valuable for assessing whether our results are applicable to natural communities. Second, our results suggest that the common approach of lumping species into functional groups may be problematic for understanding community assembly. The existence of predator-specific defenses and consequently predator-specific TMIs suggests that predator identity can provide important insights into the transmission of TMIs because different predators have the potential to transmit predator-specific TMIs (Polis and Strong 1996, Chalcraft and Reseratis 2003). Here we demonstrate that the magnitude of the TMI (i.e. prey survival) depended on the specific caged predator present before the second predator colonized. Thus, although functional groups may simplify our understanding of community assembly, we should be cautious when applying this approach in all systems as a consequence of species-specific interactions and responses.

Acknowledgements – We thank Josh Auld, Kerry Edwards, Helena Rosenlew, Dani Rosenberger and Nancy Schoeppner for their assistance with the experiment. Josh Auld, Nancy Schoeppner, Jonathan Shurin and Andy Turner provided many helpful comments. The Conchologists of America, the Pennsylvania Academy of Science, Sigma Xi Grants-in-Aid of Research, the Univ. of Pittsburgh McKinley Research Award, and the National Science Foundation provided support for this work.

References

- Abrams, P. A. 1987. Indirect interactions between species that share a predator: varieties of indirect effects. – In: Kerfoot, W. C. and Sih, A. (eds), *Predation: direct and indirect impacts on aquatic communities*. Univ. Press of New England, pp. 38–53.
- Abrams, P. A. 1995. Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. – *Am. Nat.* 146: 112–134.
- Alford, R. A. and Wilbur, H. M. 1985. Priority effects in experimental pond communities: competition between *Bufo* and *Rana*. – *Ecology* 66: 1097–1105.
- Belyea, L. R. and Lancaster, J. 1999. Assembly rules within a contingent ecology. – *Oikos* 86: 402–416.
- Bernot, R. J. and Turner, A. M. 2001. Predator identity and trait-mediated indirect effects in a littoral food web. – *Oecologia* 129: 139–146.
- Bolker, B. et al. 2003. Connecting theoretical and empirical studies of trait-mediated interactions. – *Ecology* 84: 1101–1114.
- Chalcraft, D. R. and Reseratis, W. J. 2003. Predator identity and ecological impacts: functional redundancy or functional diversity? – *Ecology* 84: 2407–2418.
- Charnov, E. L. et al. 1976. Ecological implications of resource depression. – *Am. Nat.* 110: 247–259.
- Chivers, D. P. and Smith, R. J. F. 1998. Chemical alarm signalling in aquatic predator-prey systems: a review and prospectus. – *Ecoscience* 5: 338–352.
- Connell, J. H. and Slatyer, R. O. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. – *Am. Nat.* 111: 1119–1141.
- Dahl, J. and Peckarsky, B. L. 2002. Induced morphological defenses in the wild: predator effects on a mayfly, *Drunella coloradensis*. – *Ecology* 83: 1620–1634.
- DeWitt, T. J. et al. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. – *Evol. Ecol. Res.* 2: 129–148.
- Dudley, S. A. and Schmitt, J. 1996. Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. – *Am. Nat.* 147: 445–465.
- Elsler, J. J. and Urabe, J. 1999. The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. – *Ecology* 80: 735–751.
- Gabriel, W. 1999. Evolution of reversible plastic responses: inducible defenses and environmental tolerance. – In: Tollrian, R. and Harvell, D. (eds), *The ecology and evolution of inducible defenses*. Princeton Univ. Press, pp. 286–305.
- Hoverman, J. T. and Relyea, R. A. 2007. How flexible is phenotypic plasticity: developmental windows for trait induction and reversal. – *Ecology* 88: 693–705.
- Hoverman, J. T. and Relyea, R. A. in press. The rules of engagement: how to defend against combinations of predators. – *Oecologia*.
- Hoverman, J. T. et al. 2005. Putting prey back together again: integrating predator-induced behavior, morphology, and life history. – *Oecologia* 144: 481–491.
- Karban, R. and Baldwin, I. T. 1997. *Induced responses to herbivory*. – Univ. of Chicago Press.
- Kingsolver, J. G. 1995. Fitness consequences of seasonal polyphenism in western white butterflies. – *Evolution* 49: 942–954.
- Kuhlmann, H. W. et al. 1999. Predator-induced defenses in ciliated protozoa. – In: Tollrian, R. and Harvell, C. D. (eds), *The ecology and evolution of inducible defenses*. Princeton Univ. Press, pp. 142–159.
- Lawler, S. P. and Morin, P. J. 1993. Temporal overlap, competition, and priority effects in larval anurans. – *Ecology* 74: 174–182.
- Lefcort, H. et al. 1999. Ramifications of predator avoidance: predator and heavy-metal-mediated competition between tadpoles and snails. – *Ecol. Appl.* 9: 1477–1489.
- Lima, S. L. 2002. Putting predators back into behavioral predator-prey interactions. – *Trends Ecol. Evol.* 17: 70–75.
- Matsuda, H. et al. 1994. Effects of predator-specific defense on community complexity. – *Evol. Ecol.* 8: 628–638.
- McCollum, E. W. et al. 1998. Complex interactions of fish, snails, and littoral zone periphyton. – *Ecology* 79: 1980–1994.
- McCoy, M. W. et al. 2006. Size correction: comparing morphological traits among populations and environments. – *Oecologia* 148: 547–554.
- Menge, B. A. and Sutherland, J. P. 1987. Community regulation: variation in disturbance, competition, and predation in relation to environmental stress and recruitment. – *Am. Nat.* 130: 730–757.
- Murdoch, W. W. 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. – *Ecol. Monogr.* 39: 335–354.
- Persons, M. H. et al. 2001. Wolf spider predator avoidance tactics and survival in the presence of diet-associated predator cues (Araneae: *Lycosidae*). – *Anim. Behav.* 61: 43–51.
- Pigliucci, M. 2001. *Phenotypic plasticity: beyond nature and nurture*. – Johns Hopkins Univ. Press.

- Polis, G. A. and Strong, D. R. 1996. Food web complexity and community dynamics. – *Am. Nat.* 147: 813–846.
- Relyea, R. A. 2001a. Morphological and behavioral plasticity of larval anurans in response to different predators. – *Ecology* 82: 523–540.
- Relyea, R. A. 2001b. The relationship between predation risk and antipredator responses in larval anurans. – *Ecology* 82: 541–554.
- Relyea, R. A. 2002. Competitor-induced plasticity in tadpoles: consequences, cues, and connections to predator-induced plasticity. – *Ecol Monogr* 72: 523–540.
- Relyea, R. A. 2003. How prey respond to combined predators: a review and an empirical test. – *Ecology* 84: 1827–1839.
- Robinson, J. F. and Dickerson, J. E. 1987. Does invasion sequence affect community structure? – *Ecology* 68: 587–595.
- Schlichting, C. and Pigliucci, M. 1998. Phenotypic evolution: a reaction norm perspective. – Sinauer.
- Schoepfner, N. M. and Relyea, R. A. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. – *Ecol. Lett.* 8: 505–512.
- Sih, A. 1987. Predators and prey lifestyles: an evolutionary and ecological overview. – In: Kerfoot, W. C. and Sih, A. (eds), *Predation: direct and indirect impacts on aquatic communities*. Univ. Press of New England, pp. 203–224.
- Soluk, D. A. and Collins, N. C. 1988. Synergistic interactions between fish and stoneflies: facilitation and interference among stream predators. – *Oikos* 52: 94–100.
- Stephens, D. and Krebs, J. 1986. *Foraging theory*. – Princeton Univ. Press.
- Tollrian, R. and Dodson, S. I. 1999. Inducible defenses in cladocera: Constraints, costs, and multipredator environments. – In: Tollrian, R. and Harvell, C. D. (eds), *The ecology and evolution of inducible defenses*. Princeton Univ. Press, pp. 177–202.
- Tollrian, R. and Harvell, D. 1999. *The ecology and evolution of inducible defenses*. – Princeton Univ. Press.
- Turner, A. M. 1996. Freshwater snails alter habitat use in response to predation. – *Anim. Behav.* 51: 747–756.
- van Baalen, M., Krivan, V. et al. 2001. Alternative food, switching predators, and the persistence of predator-prey systems. – *Am. Nat.* 157: 512–524.
- Vanni, M. J. and Layne, C. D. 1997. Nutrient recycling and herbivory as mechanisms in the “top-down” effect of fish on algae in lakes. – *Ecology* 78: 21–40.
- Werner, E. E. and Peacor, S. D. 2003. A review of trait-mediated indirect interactions in ecological communities. – *Ecology* 84: 1083–1100.
- Werner, E. E. and Peacor, S. D. 2006. Lethal and nonlethal predator effects on an herbivore guild mediated by system productivity. – *Ecology* 87: 347–361.
- West-Eberhard, M. J. 2003. *Developmental plasticity and evolution*. – Oxford Univ. Press.