

HOW FLEXIBLE IS PHENOTYPIC PLASTICITY? DEVELOPMENTAL WINDOWS FOR TRAIT INDUCTION AND REVERSAL

JASON T. HOVERMAN¹ AND RICK A. RELYEA

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260 USA

Abstract. Inducible defenses allow prey to modulate their phenotypic responses to the level of predation risk in the environment and reduce the cost of constitutive defenses. Inherent in this statement is that prey must alter their phenotypes during development in order to form these defenses. This has led many ecologists and evolutionary biologists to call for studies that examine developmental plasticity to provide insights into the importance of development in controlling the trajectories of trait formation, the integration of phenotypes over ontogeny, and the establishment of developmental windows for trait formation and reversal. By moving away from studies that focus on a single point in development, we can obtain a more complete understanding of the phenotypic decisions and limitations of prey. We exposed freshwater snails (*Helisoma trivolvis*) to environments in which predatory water bugs (*Belostoma flumineum*) were always absent, always present, or added and removed at different points in development. We discovered that snails formed morphological defenses against water bugs. Importantly, after the initial induction of defenses, snails showed similar developmental trajectories as snails reared without predators. Further, the snails possessed wide developmental windows for inducible defenses that extended past sexual maturity. However, being induced later in development appeared to have an associated cost (i.e., decreased shell thickness) that was not found when water bugs were always present. This epiphenotype (i.e., new shell formation as an extension of the current shell) suggests that resource limitation plays an important role in responses to temporal variation in predation risk and may have critical ecological costs that limit the benefits of the inducible defense. Lastly, the ability of snails to completely reverse their defenses was limited to early in ontogeny due to the constraints associated with modular growth of shell material. In sum, we demonstrate that taking a developmental perspective is extremely valuable for understanding the ecology of inducible defenses.

Key words: developmental constraints; developmental windows; epiphenotype; gastropod; *Helisoma trivolvis*; modular growth; ontogenetic contingency; phenotypic trade-offs; reversibility.

INTRODUCTION

Predator-induced plasticity has received a great deal of attention due to the astounding diversity of prey responses to their predators including changes in behavior, morphology, and life history (Kats and Dill 1998, Tollrian and Harvell 1999). While interest in predator-induced plasticity has increased, our studies have often overlooked the importance of development in the formation of inducible defenses during an individual's lifetime (West-Eberhard 2003). The link between development and phenotypic plasticity is clear; environmentally induced phenotypes require time to form and adaptive strategies can change over ontogeny (Schlichting and Pigliucci 1998, Gilbert 2001, West-Eberhard 2003). Research in non-predator prey systems has led the way in documenting developmental plasticity within and across species (Hensley 1993, Pigliucci and Schlichting 1995, Gedroc et al. 1996, Smits et al. 1996, Winn

1996, Huber et al. 1999, Thompson 1999). Our challenge is to determine how a developmental perspective can improve our understanding of the ecology and evolution of inducible defenses.

Ecologists are increasingly aware that organisms can alter suites of traits in the face of environmental variation (Boersma et al. 1998, Pigliucci and Preston 2004). Given that most traits have a developmental component, several authors have called for more integrative studies that include multiple developmental stages, multiple traits, and multiple environments (Schlichting and Pigliucci 1998, Pigliucci 2003, West-Eberhard 2003, Relyea 2004, Boege and Marquis 2005). Such integrative studies are necessary to understand prey defensive strategies. For example, as prey grow into size refuges, they may no longer employ costly behavioral or morphological defenses (e.g., energetic and maintenance costs [Havel and Dodson 1984, Brönmark and Pettersson 1994]). Further, ontogeny plays an important role in how plants induce and allocate secondary chemicals after herbivory (Zangerl and Rutledge 1996, Karban and Baldwin 1997, Ohnmeiss and Baldwin 2000). In short, a developmental approach

Manuscript received 28 October 2005; revised 3 September 2006; accepted 8 September 2006. Corresponding Editor: A. Sih.

¹ E-mail: jthst21@pitt.edu

permits a more complete understanding of prey defensive strategies.

A developmental perspective is also needed to examine how organisms respond to temporal environmental variation. During an individual's lifetime the environment may change state at any time in development (including reverting back to an earlier state). If alternative phenotypes are adaptive solutions to different environments, theory predicts that individuals that track environmental change will be favored by selection (Gabriel 1999, Gabriel et al. 2005). Therefore, when organisms encounter temporal environmental variation, we might expect wide developmental windows for trait formation (Karban and Baldwin 1997, Ohnmeiss and Baldwin 2000) and trait reversal (Stenson 1987, Brönmark and Pettersson 1994, Piersma and Lindström 1997, Trussell 1997, Yamada et al. 1998, Kuhlmann et al. 1999, Marchinko 2003, Rohde et al. 2004) where a developmental window is defined as the length of time during ontogeny in which a phenotype can be expressed in response to a changing environment (i.e., a wide developmental window implies that a trait is inducible over most of ontogeny). However, if organisms are unable to respond to frequent environmental changes during development (i.e., narrow developmental windows) and there are large costs of displaying a suboptimal phenotype (i.e., strong phenotypic trade-offs), then selection may operate against attempts to track environmental change. The inability to respond to such fine-grained variation can occur in a number of ways including ontogenetic contingency, developmental constraints, and unresponsive sensory systems (Newman 1992, Diggle 1994, Leips and Travis 1994, Novoplansky et al. 1994, Trussell 1997, Emerson 2000). In predator-prey systems, we know that many prey defenses are phenotypically plastic, but the developmental windows associated with the formation and reversal of these responses are relatively unexplored (Harvell 1991, Kats and Dill 1998, Kuhlmann et al. 1999, Tollrian and Dodson 1999, Ohnmeiss and Baldwin 2000, Van Buskirk 2002, Relyea 2003). Thus, there is a clear need to examine how development affects suites of inducible defenses when individuals experience fine-grained environmental variation.

To address changing phenotypic strategies over ontogeny and the importance of developmental windows, we examined predator-induced plasticity in a system of freshwater snails (*Helisoma trivolvis*) and predatory water bugs (*Belostoma flumineum*). Water bugs are a major snail predator in fishless habitats (Kesler and Munns 1989) and previous work in this system (using the constant presence and absence of caged predators) has found that water bugs have no effect on snail habitat use (use of the water's surface) but do induce snails to develop changes in shell shape that reduce vulnerability to water bug predation (Hoverman et al. 2005; J. T. Hoverman and R. A. Relyea, *unpublished data*). While the defenses reduce predation

rates with water bugs, they come at the cost of delayed reproduction (Hoverman et al. 2005) and increased vulnerability to attack by crayfish (J. T. Hoverman and R. A. Relyea, *unpublished data*). Thus, variation in the predator environment may play an important role in favoring plasticity in snails. This system is also excellent for examining how prey respond to temporal variation in predation risk because water bug densities are generally low in May (<0.5 adults/m²) but can increase dramatically by July (e.g., 14 adults/m²) due to reproduction and migration from permanent overwintering ponds to more ephemeral ponds (J. T. Hoverman, E. E. Werner, D. K. Skelly, K. L. Yurewicz, and R. A. Relyea, *unpublished data*). The short generation time of snails also allows us to examine developmental windows that may extend into sexual maturity. Last, snails provide a unique opportunity to examine the flexibility and constraints associated with phenotypic responses in a species with accretionary (i.e., modular) growth, which should constrain morphological responses to temporal variation to early in ontogeny when the potential for shape change is maximal. In this experiment, we quantified developmental trajectories of snails exposed to the constant presence and absence of caged water bugs as well as water bug colonization and emigration at different times in development. We hypothesized that (1) the anti-predator phenotypic strategies of snails will change over ontogeny, (2) predator induction of snails will be restricted to early stages of development (i.e., narrow developmental windows), and (3) the reversal of predator-induced phenotypes will be restricted to early stages of development.

METHODS

The experiment was conducted in an open field at the University of Pittsburgh's Aquatic Research Facility in Linesville, Pennsylvania, USA. We began by collecting 120 adult snails on 28 March 2003 from Geneva Pond #1 (a permanent pond located in northwestern Pennsylvania). We placed 20 adults into each of six 100-L wading pools filled with well water to oviposit. Egg deposition began in mid-April and continued until 1 May, at which time the adults were removed from the pools. Snails hatched from 15–29 May and were fed rabbit chow ad libitum until the start of the experiment.

We designed a completely randomized experiment that simulated four conditions: (1) predators never present (i.e., constant no-predator), (2) predators always present (i.e., constant predator), (3) predators colonizing at four different times, and (4) predators emigrating at four different times. Although we use the phrase "constant-predator treatment" to describe snails reared with caged predators throughout the experiment, it is important to note that these snails did not experience predators since hatching. Predator colonization and emigration occurred on days 7, 14, 21, and 28 of the experiment and the experiment was terminated on day

35. During these five weeks, we repeatedly observed (i.e., once per week) snail phenotypes during ontogeny. Our design resulted in 10 treatments that were replicated six times for a total of 60 experimental units. The experimental units were 100-L pools that were filled with well water on 13 June. We added 5 g of rabbit chow as an initial nutrient source and an aliquot of pond water containing zooplankton, phytoplankton, and periphyton. We also added a 16×16 cm clay tile platform in the center of each pool to serve as structure. On 19 June, 60 hatchling snails (mass 30 ± 3 mg, mean \pm SE) were added to each pool when they were approximately four weeks old. At this size (30 mg), young snails can be safely handled without crushing yet they have only grown to 10% of their adult mass.

Each pool contained a single predator cage constructed of 10 cm of sewer pipe capped with fiberglass window screen on each end to permit the chemical cues from predation to diffuse throughout the pool without allowing the predators to kill our target animals. Using predator cages allows ecologists to examine the induction effect of predators separate from the thinning effects of predators (Chivers and Smith 1998, Kats and Dill 1998, Tollrian and Harvell 1999, Relyea 2002). For the treatments assigned a predator, one adult water bug was added to the cage. Each predator was fed 400 mg of snail biomass (approximately three snails) three times per week. To equalize disturbance across treatments, cages in the no-predator treatments were lifted and immediately replaced. Every seven days, we simulated predator colonization and emigration by removing and adding predators to the appropriate pools. Although we removed the predators from the emigration treatments, there is the potential for predator kairomones to remain in the pools. However, chemical cues released by predators tend to break down quickly (i.e., one to two days) after predators are removed (Van Buskirk 2002, Relyea 2003, Turner and Montgomery 2003). After the predators were switched, all predators were fed.

We observed two behavioral responses (i.e., habitat use) every seven days before the cages were switched between treatments. For each pool, we counted the number of snails seen, the number that were under structure (i.e., the clay tile or predator cage), and the number within 3 cm of the water's surface. We then calculated the fraction of snails using structure and the surface as our two response variables. After inspecting the data, less than 1% of the snails were observed at the surface across the experiment. Therefore, this variable was excluded from the analysis.

After habitat use was recorded, we removed 10 individuals from each pool and preserved them in 10% formalin for morphological analysis. We decided to use a repeated sampling method instead of destructive sampling to reduce the number of experimental units (60 vs. 300 experimental units, respectively). Sampling snails over time without replacement causes an increase

in per-capita resources over time, which induces shells that are relatively narrow and high but does not affect the formation of predator-induced defenses (Hoverman et al. 2005). The preserved snails were blotted dry, weighed to the nearest milligram, and measured using digital imaging software (Optimas Co., Bothell, Washington, USA). 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.

On day 21 of the experiment (seven weeks post-hatching), the pools were monitored for egg masses. Unfortunately, 46 of the 60 pools already contained egg masses at this time. Therefore, we were unable to collect adequate data to assess time to reproduction, but we were able to count the number of egg masses deposited in the experiment. Roughly every third day we counted the number of egg masses and then removed them. The total number of egg masses oviposited in each pool was used as our response variables. Because the number of egg masses is strongly correlated with the number of eggs (Hoverman et al. 2005), counting egg masses provided an unbiased assessment of snail fecundity. After 35 days, we terminated the experiment and preserved all surviving snails. Snail survival was high across all the predator treatments (mean \pm SE = $97\% \pm 1\%$). Because the experiment was terminated before reproduction had ended, the fecundity data represent only the initial reproductive efforts of the snails in each treatment.

Statistical analysis

When examining morphological plasticity, one needs to account for differences related to overall size (i.e., mass). While shell thickness showed no relationship to mass, the shell and aperture dimensions were positively related to mass. To improve the linearity of the relationships, we conducted transformations that were specific to each trait. Mass was raised to the power of 0.14 to improve the relationship with shell width while mass was raised to the power of 0.25 with aperture width. Both shell height and aperture height showed saturating relationships with mass. Thus, we used Michaelis-Menten equations to provide transformations of snail mass that improve its linearity with shell and aperture height. To account for size variation, we regressed the four linear measurements of all individuals against their transformed mass and we saved the residuals. We then calculated the mean residual for each pool within each time period. After inspecting the data, it was clear that the four size-adjusted linear traits showed similar trends. Thus, to reduce the number of variables, we conducted a principal components analysis with our four size-adjusted traits using the pool means across the five time periods ($n = 300$). The first principal component (i.e., PC-1) had an eigenvalue of 2.4 and explained 61% of the variation. The remaining PCs had

TABLE 1. Results of repeated-measures MANOVA on the effects of predator colonization and emigration on the behavior, mass, shell and aperture shape (PC-1), and shell thickness of snails (*Helisoma trivolvis*) over time.

Effects	Multivariate tests			Univariate tests (<i>P</i> values)			
	df	<i>F</i>	<i>P</i>	Use of structure	Mass	PC-1	Shell thickness
Predator	36, 178	4.9	<0.0001	0.259	0.231	<0.0001	<0.0001
Time	16, 35	274.7	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Predator × time	144, 292	2.4	<0.0001	0.028	0.028	<0.0001	<0.0001

Note: Univariate tests of time and predator × time are conducted using the Huynh-Feldt degrees of freedom correction factor because the assumption of sphericity was violated.

eigenvalues smaller than one and were not extracted. All four traits loaded strongly positive on PC-1 (i.e., loading ≥ 0.677). Thus, the PC-1 score for each pool across the time periods was saved and used as our response variable for shell and aperture shape.

We conducted two analyses on the data. First, because pools were sampled repeatedly, we conducted a repeated-measures multivariate analysis of variance (MANOVA) using snail behavior, mass, PC-1 (i.e., shell and aperture shape), and shell thickness to test for the effects of time, predator treatment, and their interaction. Significant multivariate tests were followed by univariate tests of significance using the Huynh-Feldt degrees of freedom correction (because the sphericity assumption was violated). If a response variable was significant following the univariate test, we conducted mean comparisons using Fisher's LSD test.

We had several objectives with our comparisons. First, we wanted to determine how each trait changed over time (i.e., developmental trajectories). Second, we wished to determine if and when the no-predator and constant-predator treatments differed. Our third objective was to determine whether predator colonization caused a divergence from the constant no-predator treatment and a convergence to the constant predator treatment. Our final objective was to determine whether predator emigration caused a divergence from the constant predator treatment and a convergence to the constant no-predator treatment.

For the second analysis, we used an ANOVA to examine the effects of the predator treatments on the total egg production of the snails.

RESULTS

Our MANOVA on snail behavior, mass, shell shape (i.e., PC-1), and shell thickness detected significant multivariate effects of predator treatment, time, and their interaction (Table 1). For each trait, we present the results in light of our four objectives. First, we considered how snails used structure. Univariate tests indicated that there was no main effect of predators but there was an effect of time and a treatment-by-time interaction (Table 1, Fig. 1A–C). Within the no-predator treatment, snails spent more time under structure in week 1 compared to the other four weeks ($P < 0.001$). Snails also spent more time under structure

in week 2 compared to weeks 3–5 ($P \leq 0.002$). There were no differences in snail behavior from weeks 3 to 5 ($P \geq 0.450$). Within the constant predator treatment, snails spent more time under structure in week 1 compared to the other four weeks ($P \leq 0.035$). Snail behavior was not different in week 2 compared to weeks 3 and 4 ($P \geq 0.302$), but snails spent more time under structure in week 2 compared to snails in week 5 ($P = 0.013$). There was no difference in snail behavior from weeks 3 to 5 ($P \geq 0.075$). In comparing the two constant treatments within each week, the predator treatment induced snails to decrease their use of structure by 15% in week 1 ($P < 0.001$), but not over the remainder of the experiment ($P > 0.12$). In sum, predator effects on snail behavior were limited to early in ontogeny.

We next examined the effects of predator colonization on snail behavior (Fig. 1B, Appendix A). After every colonization event (i.e., weeks 1, 2, 3, and 4), snail behavior simply followed the trajectory of the constant no-predator treatment ($P > 0.2$). For example, snails experiencing predator colonization and no colonization in week 1 both reduced their use of structure by week 2 ($P = 0.001$), and the magnitudes of these reductions were similar between treatments ($P = 0.706$). In short, the four colonization treatments never diverged from the constant no-predator treatment. Moreover, the four colonization treatments were never different from the constant predator treatment. Thus, predator colonization had no effect on snail behavior.

We then examined the effects of predator emigration on snail behavior (Fig. 1C, Appendix B). After nearly every emigration event, snail behavior followed the trajectory of the constant predator treatment. There was only one exception to this pattern; when snails experienced predator emigration in week 1, snail use of structure was greater than the constant predator treatment by week 2 ($P = 0.038$) but not during any of the subsequent weeks ($P > 0.3$). In the other emigration treatments, snail behavior never differed from the constant predator treatment ($P > 0.05$). Moreover, the four emigration treatments were never different from the constant no-predator treatment. Thus, predator emigration had minor effects on snail behavior.

Snail mass exhibited no main effect of predators but was affected by time and the treatment-by-time interaction (Table 1). Within each constant treatment, snail

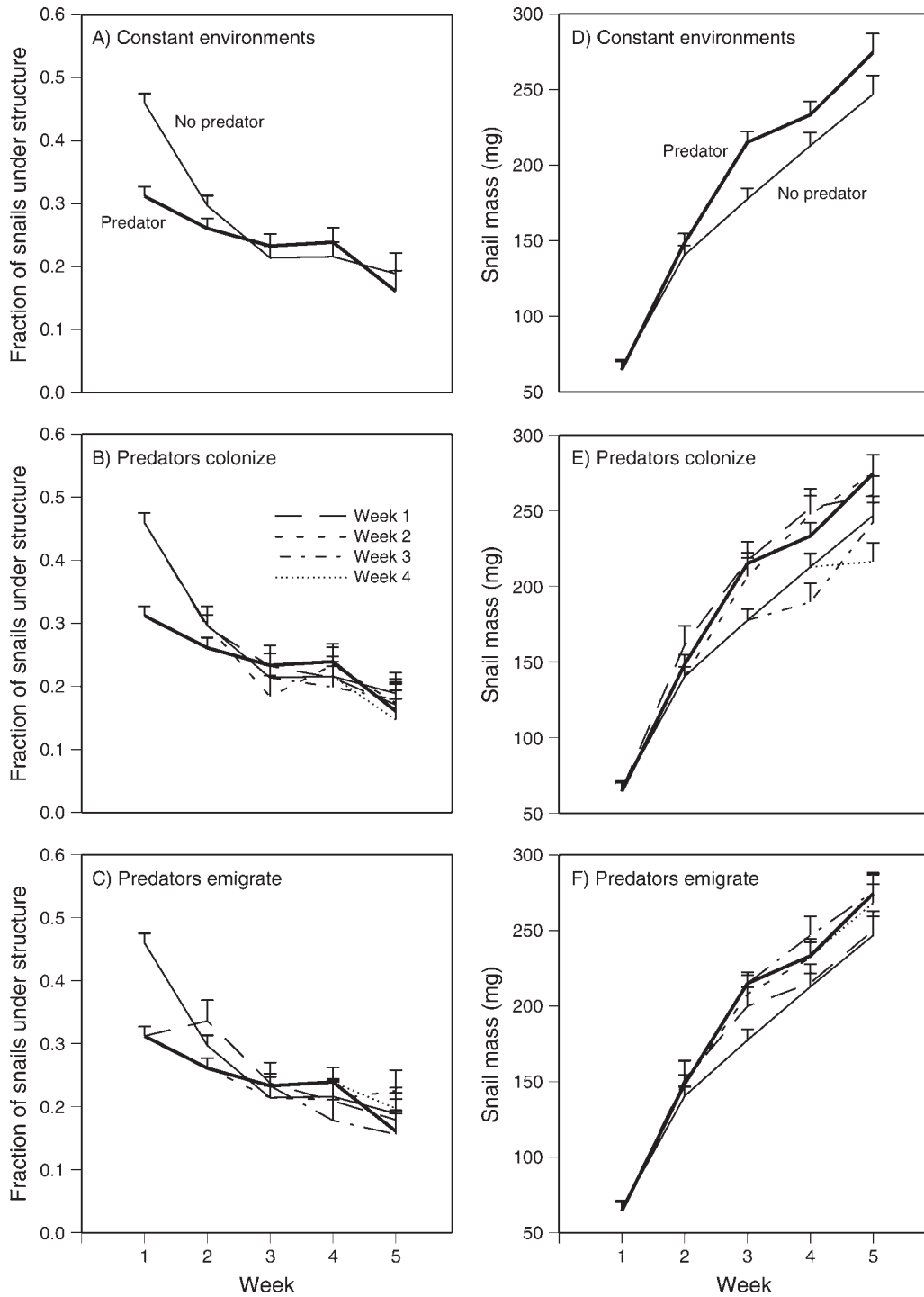


FIG. 1. The effects of different predator treatments on snail behavior (use of structure, A–C) and mass (D–F) over time, for the freshwater snail *Helisoma trivolvis*. The treatments simulated the constant presence or absence of the predatory water bug *Belostoma flumineum* (A, D), water bug colonization at different times (B, E), or water bug emigration at different times (C, F). Water bug colonization and emigration occurred at four different times and are represented by dashed lines. The constant presence or absence of water bugs is shown in each panel for comparison. Data are least-square means \pm SE.

mass increased significantly each week ($P \leq 0.030$). Averaged across both constant treatments, snail mass increased fourfold during the experiment. In comparing the two constant treatments at each week, snails reared in constant-predator and constant-no-predator treatments only differed in mass during week 3 (when snails in the former treatment were more massive; $P = 0.004$; Fig. 1D). Thus, predators did not have strong effects on snail mass.

We next examined the effects of predator colonization on mass (Fig. 1E, Appendix A). After one week of living without predators, snails experiencing either predator colonization or no colonization exhibited increased mass the following week ($P < 0.001$), but the magnitude of the increase was similar between treatments ($P = 0.134$). In subsequent weeks, these snails had greater mass than the constant no-predator treatment during weeks 3–4 ($P < 0.04$) but this difference eroded by week 5 ($P = 0.469$). After two to four weeks of living without predators, snails experiencing predator colonization exhibited a mass that was always similar to the mass of snails in the constant no-predator treatment ($P > 0.06$). Compared to the constant predator treatment, snail mass eventually converged on the constant predator treatment if colonization occurred during weeks 1–3 ($P > 0.09$) but not if colonization occurred during week 4 ($P = 0.003$). Thus, predator colonization had limited (and often ephemeral) effects on snail mass.

We then examined the effects of predator emigration on mass (Fig. 1F, Appendix B). For all four emigration treatments, snail mass always followed the same trajectory as snails that experienced a constant predator environment ($P > 0.19$). Moreover, the increase in snail mass over time in the four emigration treatments was never different from the constant no-predator treatment ($P > 0.07$). Thus, predator emigration had no effect on snail mass.

When we conducted a PCA on snail morphology, we found that higher scores on PC-1 were associated with relatively higher and wider shells and apertures (for simplicity, we will refer to higher PC-1 scores as relatively larger shells). Snail shape (i.e., PC-1) was affected by predators, time, and the treatment-by-time interaction (Table 1). Within the constant no-predator treatment, shell size increased from week 1 to week 2 ($P = 0.001$). By week 3, shells became relatively smaller compared to week 2 ($P = 0.002$) and similar to week 1 ($P = 0.593$). In weeks 4 and 5, shells were smaller than in weeks 1–3 ($P < 0.001$) but not different from each other ($P = 0.984$). Within the constant predator treatment, relative shell size increased from week 1 to week 2 ($P = 0.001$). At week 3, shells were similar to week 2 ($P = 0.410$) but larger than week 1 ($P = 0.001$). In weeks 4 and 5, shells were relatively smaller than weeks 2–3 ($P < 0.001$), similar to week 1 ($P \geq 0.197$), and not different from each other ($P = 0.269$). Comparing the two constant treatments within each time period (Fig. 2A),

snails were similar in shell size at week 1 ($P = 0.354$), but the constant predator treatment induced larger shells during weeks 2–5 ($P \leq 0.048$). An additional repeated-measured ANOVA using only the data from weeks 2–5 confirmed the effects of predators ($F_{1,10} = 15.9$, $P = 0.003$) and time ($F_{3,8} = 36.8$, $P < 0.001$) but no interaction ($F_{3,8} = 1.3$, $P = 0.331$). Hence, after the initial induction of relatively larger shells, the developmental trajectories of the two treatments were similar.

We next examined the effects of predator colonization on shell size (Fig. 2B, Appendix A). After living without predators for one week, snails experiencing either predator colonization or no colonization exhibited increased shell size the following week ($P = 0.001$), but the magnitude of the increase was greater in the colonization treatment ($P = 0.002$). In subsequent weeks, this colonization treatment followed the trajectory of snails in the constant predator treatment ($P > 0.3$) and remained different from the constant no-predator treatment ($P \leq 0.005$). After living without predators for two weeks, shell size remained similar the following week ($P = 0.176$) in the colonization treatment but decreased in the constant no-predator treatment ($P = 0.055$), producing a difference in shell shape between these two treatments in week 3 ($P = 0.015$). The subsequent trajectory followed the trajectory of snails experiencing constant predators ($P > 0.08$) and remained different from the constant no-predator treatment ($P \leq 0.002$). After living without predators for three weeks, shell size declined the following week in both the colonization and constant no-predator treatments ($P \leq 0.008$) and the two treatments did not differ ($P = 0.183$) at week 4. By week 5, however, snails in this colonization treatment diverged from the constant no-predator treatment ($P = 0.002$) and converged onto the constant predator trajectory ($P = 0.232$). After living without predators for four weeks, shell size did not change in either the colonization or constant no-predator treatments by week 5 ($P > 0.6$). However, this colonization treatment was not different from the constant predator treatment ($P = 0.108$). In summary, predator colonization in weeks 1–3 led to the formation of relatively larger shells that diverged from the constant no-predator trajectory and converged upon the constant predator trajectory.

We then examined the effects of predator emigration on shell size (Fig. 2C, Appendix B). After living with predators for one week, snails experiencing emigration or constant predators increased their shell size the following week ($P \leq 0.005$). However, the increase in shell size was smaller in the emigration treatment than in the constant predator treatment ($P = 0.012$) and converged upon the constant no-predator treatment ($P = 0.157$). Throughout the subsequent weeks, snails in the week-1 emigration treatment followed the same trajectory as snails in the constant no-predator treatment ($P > 0.5$) and were always smaller than snails in the constant predator treatment ($P < 0.03$). After living

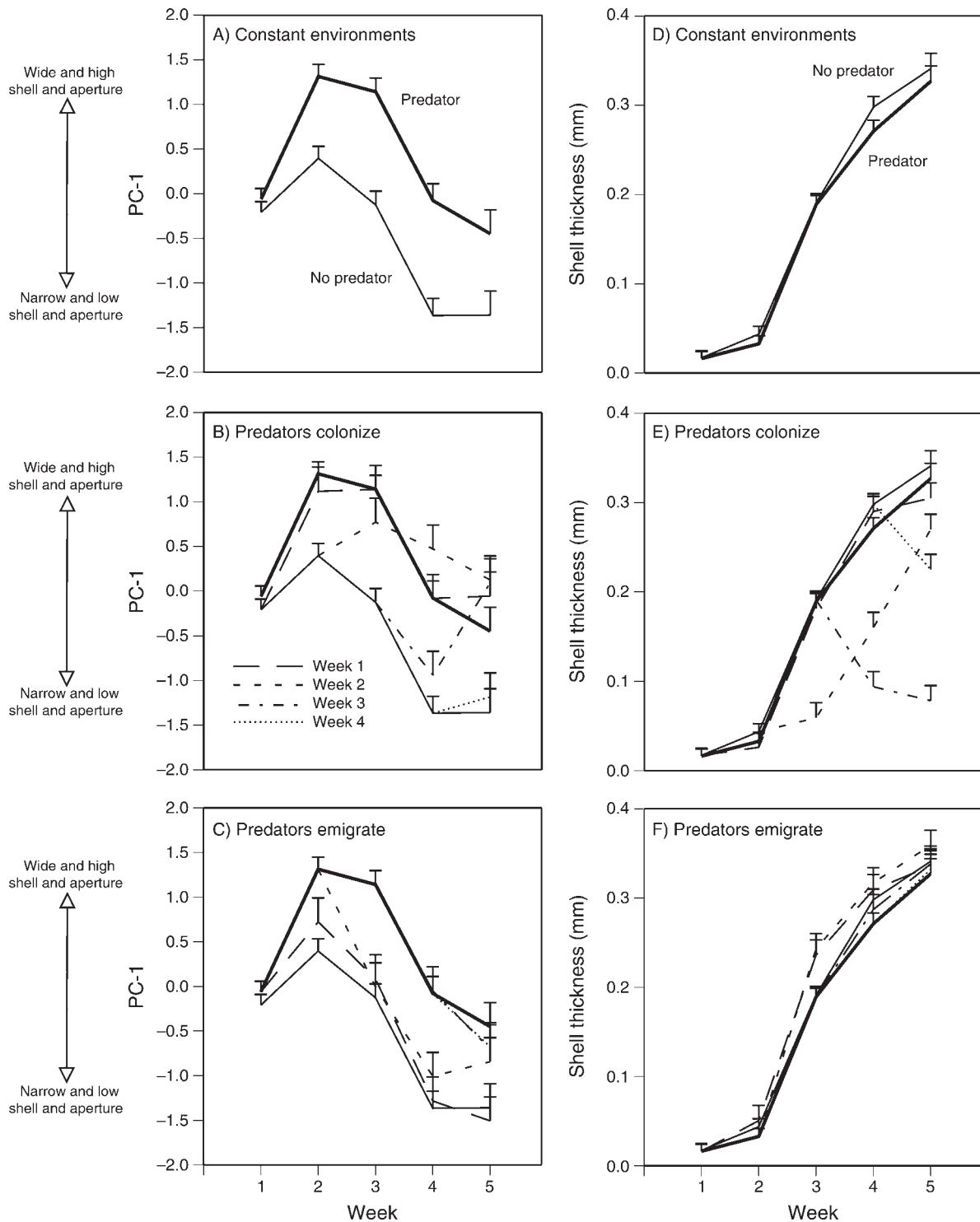


FIG. 2. The effects of different predator treatments on PC-1 (i.e., shell and aperture shape, A–C) and shell thickness (D–F) over time. See Fig. 1 for a description of the treatments. Data are least-square means \pm SE.

with predators for 2 weeks, snails experiencing predator emigration exhibited a decline in shell size the following week ($P = 0.002$), snails experiencing constant predators exhibited no change ($P = 0.410$) and the two treatments

were different from each other by week 3 ($P = 0.002$). In the subsequent weeks, snails in the week-2 emigration treatment were always similar to the constant no-predator treatment ($P > 0.25$) and remained different

from the constant predator treatment at week 4 ($P = 0.005$) but not at week 5 ($P = 0.385$). After living with predators for three weeks, snails experiencing either predator emigration or constant predators exhibited a decline in shell size the following week ($P \leq 0.001$) and the declines followed a similar trajectory through weeks 4 and 5 and were of similar magnitude ($P > 0.5$). The week-3 emigration treatment was different from the constant no-predator treatment on week 4 ($P = 0.001$) but not on week 5 ($P = 0.148$). After living with predators for four weeks, snails experiencing predator emigration did not change shell size the following week ($P = 0.130$) and did not differ in shell size from either of the two constant treatments ($P > 0.1$). In summary, reversal of the relatively larger shell was constrained to early in development.

Univariate tests indicated that predators, time, and the treatment-by-time interaction affected shell thickness (Table 1). Within each constant treatment, shell thickness increased significantly each week ($P \leq 0.057$). Averaged across both constant treatments, thickness increased by 23-fold. In comparing the two constant treatments within each week (Fig. 2D), snails reared with predators were slightly thinner than snails reared without predators in week 2 ($P = 0.011$) but not during the other weeks ($P \geq 0.181$). Thus, the constant predator environment had minor effects on the shell thickness.

We then examined the effects of predator colonization on shell thickness (Fig. 2E, Appendix A). After living without predators for one week, snails experiencing either no predators or predator colonization exhibited increased shell thickness the following week ($P < 0.03$), but the increase was smaller in the colonization treatment ($P = 0.01$). In subsequent weeks, this colonization treatment was never different from the trajectories of the two constant treatments ($P > 0.2$). After living without predators for two weeks, shell thickness increased the following week if snails continued to experience no predators ($P = 0.001$) but not if the snails experienced predator colonization ($P = 0.122$). In subsequent weeks, this colonization treatment always had thinner shells than the constant no-predator treatment ($P < 0.04$) and generally had thinner shells than the constant predator treatment (week 4, $P = 0.001$; week 5, $P = 0.084$). After living without predators for three weeks, shell thickness increased the following week if snails continued to experience no predators ($P = 0.001$) but decreased if the snails experienced predator colonization ($P = 0.004$). The colonization treatment was different from the constant no-predator treatment ($P = 0.001$). In the subsequent week, this colonization treatment had thinner shells than either constant treatment ($P = 0.001$). After living without predators for four weeks, shell thickness increased the following week if snails continued to experience no predators ($P = 0.021$) but decreased if the snails experienced predator colonization ($P = 0.019$). The colonization treatment induced thinner shells than both of the constant

treatments ($P \leq 0.003$). In summary, when predators colonized midway to late in ontogeny, snails developed much thinner shells.

Next, we examined the effects of predator emigration on shell thickness (Fig. 2F, Appendix B). After living with predators for one week, shell thickness increased the following week if snails experienced constant predators or predator emigration ($P < 0.001$). The increase in shell thickness was greater in the emigration treatment than in the constant predator treatment during week 2 ($P = 0.009$) but not during the subsequent weeks ($P > 0.09$). Shell thickness remained similar between this emigration treatment and the constant no-predator treatment throughout the experiment ($P > 0.09$). After living with predators for two weeks, shell thickness increased the following week if snails experienced constant predators or predator emigration ($P < 0.001$), but the emigration treatment induced a slightly thicker shell ($P = 0.050$). In the subsequent weeks, this emigration treatment was not different from either of the two constant treatments ($P \geq 0.07$). After living with predators for three weeks, shell thickness increased the following week if snails experienced constant predators or predator emigration ($P \leq 0.003$) and the increase was similar in magnitude ($P = 0.507$). The emigration treatment was not different from the constant no-predator treatment ($P = 0.663$). In the subsequent week, this emigration treatment was not different from either of the two constant treatments ($P > 0.7$). After living with predators for four weeks, shell thickness increased the following week if snails experienced constant predators or predator emigration ($P \leq 0.047$) and the increase was similar in magnitude ($P = 0.888$). The emigration treatment was not different from the constant no-predator treatment ($P = 0.789$). In summary, predator emigration always induced a thickness that was similar to or greater than the two constant treatments.

Our last analysis examined the total egg production of snails. Egg production was not significantly affected by the predator treatments ($F_{9,50} = 1.2$, $P = 0.293$, Fig. 3). However, there was a trend in which the constant predator treatment produced fewer egg masses than the constant no-predator treatment ($P = 0.072$).

DISCUSSION

We discovered that snails are amazingly flexible in the formation of anti-predator defenses over ontogeny. By observing phenotypic responses across development, one can obtain an understanding of how prey integrate suites of defensive traits (Brönmark and Pettersson 1994, Arnqvist and Johansson 1998, Relyea 2003). In our study, water bugs induced a short-lived behavioral response in snails. During the first week of the experiment, water bugs induced a 15% decrease in the use of structure by snails but this response did not persist. The use of structure by snails is known to be predator specific. For example, predatory crayfish spend much of

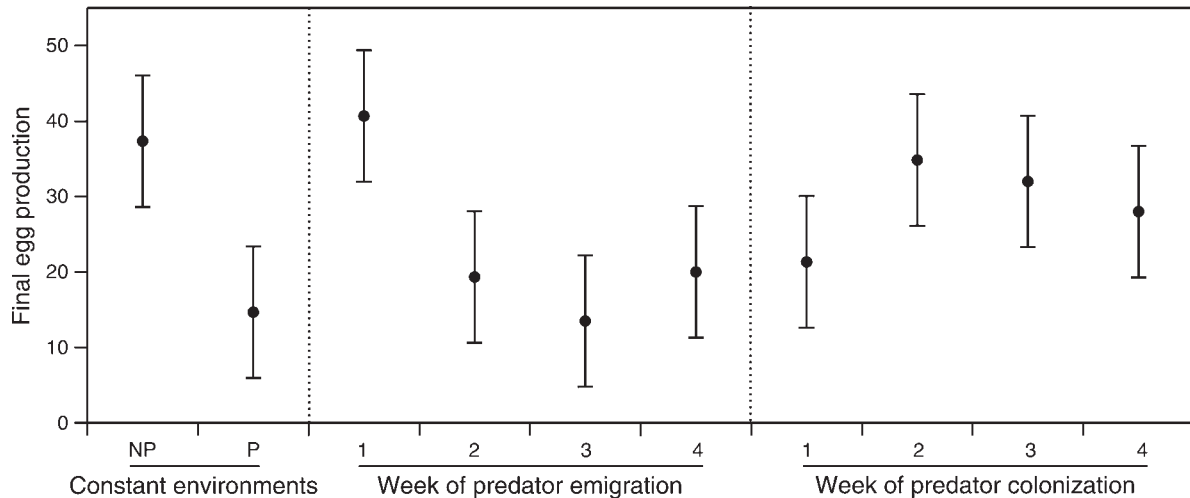


FIG. 3. The effects of different predator treatments on the total egg production of snails. The constant no-predator (NP) and predator treatment (P) are shown in the first panel. The predator emigration and colonization treatment are displayed in the next two panels, respectively, according to the week the treatment was applied. Data are least-square means \pm SE.

their time under structure and induce snails to move away from structure whereas fish spend much of their time in the water column and induce snails to move into structure (Turner et al. 1999). In contrast, water bugs in natural ponds feed throughout the water column (i.e., at the surface and under refuges). Therefore, it is unlikely that the avoidance of structure is an adaptive strategy that enables snails to escape predation by water bugs.

Snails also expressed morphological changes with water bugs that included larger shells and larger apertures. Although predators affected the overall developmental trajectory of shell size, snails that were induced by predators followed a similar developmental trajectory as the uninduced snails after the initial induction. This suggests that snail defenses are induced early in development and the relative magnitude of the defense is maintained over time. The formation of larger shells is generally consistent with laboratory and mesocosm experiments that examined how the constant presence of water bugs affected snail final morphology (Hoverman et al. 2005; J. T. Hoverman and R. A. Relyea, *unpublished manuscript*). We have recently tested the adaptive value of water bug-induced traits and found several clear trends: (1) water bugs impose strong selection for relatively larger shells, (2) snails with relatively larger shells escape predation over a wide range of body sizes, and (3) large body size alone is not an effective strategy for escaping predation from water bugs (J. T. Hoverman and R. A. Relyea, *unpublished manuscript*). Thus, for snails to escape predation by water bugs, they must form and maintain a relatively larger shell as they develop. In addition to morphology, we also found that snails were 21% larger in week 3 when reared with water bugs. Previous work has shown that snails will delay reproduction and reach a larger size at reproduction in the presence of water bugs (Hoverman et

al. 2005). However, once reproduction begins, snails induced by water bugs exhibit a higher rate of egg production that quickly converges on the egg production of the no-predator phenotype (Hoverman et al. 2005). While we did not find significant differences in egg production, there was a trend for snails exposed to water bugs to lay fewer egg masses, suggesting that reproduction was somewhat delayed in the predator treatment.

Recently, ecologists have become more aware that phenotypic plasticity can occur in multiple traits and follow different developmental trajectories. For example, some prey use behavioral defenses early in ontogeny that allow immediate avoidance of predators and provide the time necessary to form morphological defenses. After morphological defenses are formed, prey can rely less on costly behavioral defenses (e.g., reduced growth rates [DeWitt et al. 1999, Rundle and Brönmark 2001, Relyea 2003, Cotton et al. 2004]). Such results underscore the importance of quantifying developmental trajectories because we can identify the changing strategies of organisms (Pigliucci and Schlichting 1995, Boersma et al. 1998, Baldwin 1999, Donohue and Schmitt 1999, Sultan 2000). Because there can be genetic variation for developmental trajectories, we also need to consider the potential for natural selection to operate on the shape of the trajectory rather than simply the phenotype at a single point in time (Gedroc et al. 1996, Pigliucci 1997, Cheplick 2003). In sum, the explicit inclusion of development into plasticity research will be imperative to understanding how organisms integrate their phenotypic responses in the face of environmental variation.

Numerous studies have investigated predator-induced plasticity by exposing prey to the constant presence or absence of predators, an approach that adequately represents spatial variation in predation risk but

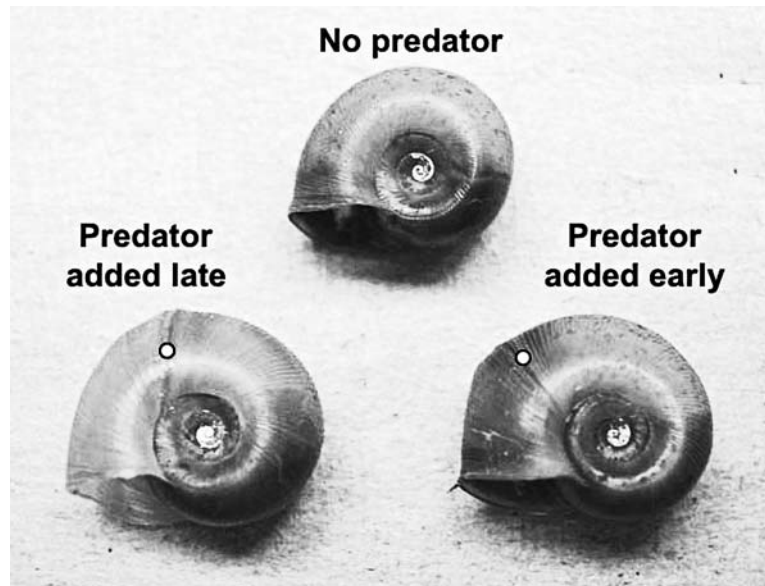


FIG. 4. Morphological responses of *Helisoma trivolvis* to water bug colonization. The top snail was reared in the absence of water bugs while the bottom two snails experienced water bug colonization early (bottom right) and late (bottom left) in the experiment. The dots illustrate the approximate point in shell formation where the water bug was added. Note the difference in shell thickness (dark vs. light gray regions) between the snails exposed to water bug colonization early compared to late in the experiment.

overlooks the importance of temporal variation in predation risk. For many organisms, the state of the environment can change during development (i.e., within-generation or fine-grained variation). If possessing the wrong phenotype in a given environment is costly, then selection should favor individuals with wide developmental windows that allow induction throughout ontogeny (Schlichting and Pigliucci 1998, Gabriel 1999, Gabriel et al. 2005). In our system, water bugs colonize semi-permanent ponds in mid-May to early-June. Given that snails can develop into a reproductive adult within two to three months, they can experience water bug colonization at any time from hatching through adulthood. We found that as long as water bugs colonized before the fourth week of the experiment, snails could produce larger shells (Fig. 4). Interestingly, the speed of trait induction was more rapid when water bugs colonized early. For example, snails experiencing water bug colonization in weeks 1 and 2 formed shells that were as large as the constant-predator treatment within one week. In contrast, snails experiencing water bug colonization in week 3 required two weeks to converge onto the constant-predator phenotype. Importantly, the lack of significant growth during the week following water bug colonization may underlie the delayed induction of relatively larger shells. Such a slow rate of defensive trait induction may lead to higher rates of predation until the defense is formed. More work is needed in this system to determine how effective these defenses are at reducing predation rates at different points in trait development. Overall, snails possess relatively wide developmental windows for their induc-

ible defenses, suggesting that there are substantial costs of possessing a no-predator phenotype in an environment containing predatory water bugs (i.e., a high probability of mortality).

While developmental windows for induced defenses have frequently been examined in plants (Karban and Baldwin 1997, Ohnmeiss and Baldwin 2000), the few existing studies in animal systems have found that developmental windows can be either narrow (Harvell 1991) or wide (Kats and Dill 1998, Kuhlmann et al. 1999, Tollrian and Dodson 1999, Van Buskirk 2002, Relyea 2003). Likewise, in non-predatory systems, the size of developmental windows varies for both plant and animal systems (Newman 1992, Diggle 1994, Leips and Travis 1994, Novoplansky et al. 1994, Trussell 1997, Emerson 2000, Weinig and Delph 2001, Sachs 2002). Evolutionarily, narrow developmental windows pose possible limitations to the benefits of phenotypic plasticity because they decrease the accuracy of matching the environment and this may result in selection against phenotypic plasticity (Moran 1992, Padilla and Adolf 1996, DeWitt et al. 1998, Tollrian and Harvell 1999, Gabriel et al. 2005). The incorporation of natural patterns of temporal environmental variation into our experiments will continue to provide valuable insights into the ecology and evolution of phenotypic plasticity.

Our inclusion of temporal variation in predation risk also allowed us to document a unique effect on shell thickness that would have been masked by simply examining phenotypes in constant predator and no-predator treatments. When water bugs colonized after week 1, snails developed 7–77% thinner shells (Fig. 2E).

Interestingly, snails were not constrained to have extremely thin shells throughout the remainder of development. When water bugs colonized in week 2, snails produced a large shell by week 3 (i.e., similar to the constant-predator treatment) and in weeks 4 and 5 their shell thickness increased. Another important result occurred when water bugs colonized in week 4. While we did not observe significant induction of larger shells in week 5, snails did produce shells that were thinner. This suggests that given more time (i.e., an additional week) snails may have formed larger shells since the shells were becoming thinner. Overall, these results demonstrate that complete induction in one trait at different points in ontogeny does not imply complete induction of all traits. Moreover, these findings may be explained by resource limitation that frequently affects induced defenses in plants and animals (Karban and Baldwin 1997, Tollrian and Harvell 1999). Snails directly absorb the majority of the calcium needed for shell formation from their environment and produce thicker, larger shells with higher calcium availability (Russell-Hunter 1978, Brodersen and Madsen 2003). Given that our mesocosms are closed systems, calcium availability was likely more reduced late in our experiment, preventing snails from simultaneously forming a thicker shell and a larger shell when water bugs colonized. While further work is needed in our system, resource limitation may play a critical role in the ability for organisms to respond to temporal environmental variation.

When selection favors wide developmental windows, there can be fitness costs associated with phenotypes induced later in ontogeny (DeWitt et al. 1998, West-Eberhard 2003). The production of larger, thinner shells when water bugs colonized late in ontogeny may represent an epiphenotype (i.e., new shell formation as an extension of the current shell; DeWitt et al. 1998). Such add-on phenotypes can have two possible consequences for prey. First, the epiphenotype may be less effective at defense against water bugs than phenotypes induced early in ontogeny. In this case, the epiphenotype would have lower fitness compared to phenotypes produced in constant environments. Second, the epiphenotype may make prey more vulnerable to other predators. For example, background responses to one predator may render prey easier to handle and consume by a second predator or place prey on developmental trajectories that constrain future responses to predators. Thus, prey may find themselves in a precarious situation in which they must balance their responses to predators that kill by using different strategies. In our system, crayfish attack snails by chipping the aperture or crushing the shell. Thus, snails with thinner shells as a result of water bug colonization may be extremely vulnerable to attack by crayfish. While possessing wide developmental windows allows flexibility in phenotypic responses, organisms may incur additional costs that are not associated with phenotypes produced in constant environments.

The removal of inducing cues can provide insights into the size of developmental windows for trait reversibility. Theory predicts that the evolution of reversibility is favored when individuals experience multiple environmental states within a generation, when the fitness cost of not matching the phenotype to the environment is large (i.e., strong phenotypic trade-offs), and when the response lag time is shorter than the duration of the environmental state (Gabriel 1999). We found relatively narrow development windows for the complete reversal of larger shells, which is in contrast to the wide developmental windows found for defensive trait formation. While a complete reversal of shell size occurred when water bugs were removed in week 1, an incomplete reversal occurred when water bugs were removed in weeks 2-4.

The lack of reversibility later in ontogeny could be explained in several ways. First, snails may be reluctant to reverse defenses if they are uncertain that the predator has left the habitat (Sih 1992, Van Buskirk 2002). Delaying the reversal of defensive phenotypes would be an adaptive strategy that reduces the probability of attack from an undetected predator (Van Buskirk 2002). Alternatively, snails may experience developmental constraints associated with shell formation because the shape of previously deposited shell cannot be altered (Stone 1995). For example, if we examine the raw data (i.e., before size correction) from our study, final shell width in the no-predator treatment was 1.13 ± 0.02 cm. When water bugs emigrated late in the experiment, shell width was already >1.2 cm, prohibiting any reversal to the no-predator shell width. In contrast, snail shells were quite small early in development (week 1 shell width = 0.7 cm) allowing snails experiencing predator emigration to reverse to the no-predator phenotype. Developmental constraints for trait reversal may also be linked to how the organism produces the trait. For example, tadpoles are able to reverse their morphological defenses (i.e., smaller bodies and deeper tails) because they simply shunt more resources to the growth of the body and thereby reduce the relative size of their tail (Van Buskirk 2002, Relyea 2003). Modular traits such as plant stems or gastropod shells may be more developmentally constrained because the shape of previously formed structures cannot be altered. Thus, there may be limits on the reversibility of inducible defenses resulting from how the defenses were developed. Overall, the reversibility of defensive traits will depend upon the past history of phenotypic responses, the assessment of predation risk, the fitness benefit of reversing, and the mode of trait production (Sih 1992, Diggle 1994, Weinig and Delph 2001, Sachs 2002, Kurashige and Agrawal 2005).

Conclusions

Evolutionary biologists are aware of the developmental component of inducible defenses, yet few studies have rigorously incorporated a developmental perspective.

Such studies are imperative for understanding developmental trajectories of different traits, allocation trade-offs, developmental windows, and phenotypic reversibility. By taking a developmental approach, we discovered that freshwater snails possess wide developmental windows for trait induction but narrow windows for trait reversibility due to the constraints of modular growth. Moreover, incorporating temporal variation in the predator environments illuminated important responses that could not be observed in traditional experiments incorporating only spatial variation in predator environments. Through the incorporation of both spatial and temporal heterogeneity, we can obtain a more complete understanding how organisms have evolved to make their phenotypic decisions and how these decisions affect the ecology of the system.

ACKNOWLEDGMENTS

We thank Josh Auld, Elizabeth Kennedy, Adam Marko, Stacy Phillips, and Nancy Schoeppner for their assistance with the experiment. Josh Auld, Nancy Schoeppner, and several anonymous reviewers provided many helpful comments on the manuscript. Financial support was provided by the Conchologists of America, the Pennsylvania Academy of Science, Sigma Xi, the University of Pittsburgh McKinley Research Award (J. T. Hoverman), and the National Science Foundation (R. A. Relyea).

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APPENDIX A

Results from analysis of the effects of predator colonization at four times on snail traits (*Ecological Archives* E088-046-A1).

APPENDIX B

Results from analysis of the effects of predator emigration at four times on snail traits (*Ecological Archives* E088-046-A2).