

Survival trade-offs associated with inducible defences in snails: the roles of multiple predators and developmental plasticity

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Summary

1. For decades, organisms with inducible defences have been used extensively as model systems for addressing the ecology and evolution of phenotypic plasticity. However, our current understanding of inducible defences comes largely from studies that have examined the survival benefits of an inducible defence in a predator environment vs. the growth or reproductive costs in a no-predator environment. Moreover, this work has focused on a single point in prey development. Unfortunately, this approach neglects the role that functionally different predator species (with divergent selection pressures) may play in maintaining inducible defences in prey populations and overlooks the dynamic nature of prey responses and prey vulnerability over development.

2. In this study, we used freshwater snails and a subset of their predators to address the survival costs and benefits of predator-induced plasticity in response to functionally different predators at multiple points in development.

3. We found that snails altered unique suites of traits over development in response to three different caged predator environments. Moreover, short-term predation trials demonstrated that the phenotype induced by one predator had higher survival with that predator than any alternative phenotype but lower survival with a different predator species (i.e. survival trade-offs). However, the trade-off was only strongly detected midway in development before a size refuge was attained from predation and after morphological defences had formed in the snails.

4. Our study suggests that functionally different predators favour inducible defences in freshwater snails, and that incorporating development into tests of the adaptive plasticity hypothesis can provide valuable information about the dynamics of trait induction and variation in fitness trade-offs over time.

Key-words: developmental plasticity, fitness, functional trade-off, gastropod, predator-specific defence, size refuge

Introduction

Organisms with inducible defences have served as excellent model systems for studying the ecology and evolution of phenotypic plasticity. Within natural communities, prey encounter temporal and spatial variation in predation risk and use a variety of visual, tactile and chemical cues to evaluate this risk (Dodson *et al.* 1994; Chivers & Smith 1998; Kats & Dill 1998; Brönmark & Hansson 2000). In response to predator cues, prey express a diversity of phenotypic

changes in behaviour, life history, and morphology (Lima & Dill 1990; Kats & Dill 1998; Tollrian & Harvell 1999). While these responses are readily formed in the presence of predators, their conditional nature suggests that the optimal phenotype produced with predators is not the optimal phenotype expressed across all other environments the organism encounters. This basic premise has led to the formulation of the adaptive plasticity hypothesis, which posits that phenotypic plasticity (e.g. inducible defences) will be favoured if a phenotype has higher fitness in the inducing environment than alternative phenotypes, but lower fitness in other environments (Gotthard & Nylin

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1995; Kingsolver 1995a,b; Dudley & Schmitt 1996). Thus, as studies accumulate documenting the existence of predator-induced plasticity, it will be critical to test for the underlying fitness trade-offs that drive these conditional responses and, subsequently, favour phenotypic plasticity.

Traditionally, studies compare the survival benefits of a predator-induced response in a predator environment vs. the growth or reproductive costs in a no-predator environment to assess fitness trade-offs (Krueger & Dodson 1981; Kuhlmann & Heckmann 1985; Dodson & Havel 1988; Brönmark & Miner 1992; Kusch 1995; McCollum & Van Buskirk 1996; Van Buskirk & Schmidt 2000; Relyea 2001). This approach has demonstrated that predator-induced responses typically reduce the likelihood of predation compared to phenotypes produced in the absence of predators. However, individuals that form these same predator-induced responses typically display reduced growth or reproductive investment. In addition to such survival vs. growth trade-offs, trade-offs can occur between two predator-specific responses. When prey form defences against one predator species, those responses can lead to greater susceptibility to other predator species (i.e. survival trade-offs). This is likely to occur when there is mechanistic interference among defences such that formation of one defence has a negative effect on the formation of a second defence (Poitrineau, Brown & Hochberg 2003). Indeed, predator species typically differ in their foraging strategies and can favour different suites of traits in prey populations. Studies have demonstrated that prey express predator-specific suites of defences to cope with the predator regime encountered (Karban & Baldwin 1997; McPeck 1998; McIntosh & Peckarsky 1999; Dewitt, Robinson & Wilson 2000; Relyea 2001; Schmitz & Sokol-Hessner 2002). Given that prey within natural communities coexist with a diversity of predator species that often differ functionally (Sih, Englund & Wooster 1998; Chalcraft & Resetarits 2003), it is critical to assess whether responses induced by different predators lead to survival trade-offs. Such information can help elucidate whether the selective pressures exerted by functionally different predators favour the maintenance and evolution of predator-induced plasticity (Tollrian & Dodson 1999; Poitrineau, Brown & Hochberg 2003; Kishida & Nishimura 2005; Benard 2006).

Our current understanding of predator-induced plasticity comes largely from studies focused on a single point in prey development. While this approach can provide a wealth of information, it overlooks the dynamic nature of prey trait formation and, subsequently, how developmental plasticity influences fitness trade-offs among different induced phenotypes. For example, prey that can reach a size refuge from predators might only express predator-induced plasticity while they are within a vulnerable size range to predation, to reduce costs associated with continued allocation to the predator-induced phenotype. Alternatively, prey could simply allocate resources to growth into a size refuge rather than altering their morphological phenotype to defend against predation (Pettersson, Nilsson & Bronmark 2000; Relyea 2003). Also, since most predator-induced responses have associated

time lags (i.e. time from cue detection to the formation of the response), the fitness benefits of trait induction might not be realized until later in development after the phenotype has fully formed (Tollrian 1995; Laforsch & Tollrian 2004). Thus, the defensive strategies used by prey over development may depend both on the ability to reach a size refuge from predation and the speed of phenotypic induction. By assessing survival trade-offs at multiple developmental points, we can identify when phenotypic responses are effective against a predator, how long they are effective, and how effective they are compared to phenotypes produced over development in different environments.

Over the past several years, we have used the freshwater snail *Planorbella (Helisoma) trivolvis* and two common snail predators [water bugs (*Belostoma flumineum*) and crayfish, (*Orconectes rusticus*)] that use functionally different feeding techniques to investigate the effects of predation risk on phenotypic expression (Hoverman, Auld & Relyea 2005; Hoverman & Relyea 2007a,b, 2008). *P. trivolvis* are perennial with overlapping generations; adults begin reproducing in May, hatchlings are typically abundant in June and July, and adulthood is typically reached between August and September of that year depending on local resource levels (J.T. Hoverman, C.J. Davis, E. E. Werner, D. K. Skelly, R. A. Relyea, & K. L. Yurewicz, unpublished data). Water bugs and crayfish are a significant threat to snail populations in semi-permanent habitats that lack fish especially when predator populations are high (Kesler & Munns 1989; Alexander & Covich 1991; Turner, Fetterolf & Bernot 1999; Hoverman & Relyea 2007b). Moreover, these two predators and snails overlap extensively in aquatic habitats. In ponds sampled from 2004 to 2006 (May, June, and July of each year) on the E. S. George Reserve in Michigan, 94% and 67% of the ponds containing *P. trivolvis* ($n = 18$) also contained water bugs or crayfish, respectively (J.T. Hoverman, C.J. Davis, E. E. Werner, D. K. Skelly, R. A. Relyea, & K. L. Yurewicz, unpublished data). In addition, the densities of these two predators varied substantially within any particular pond but did not show any seasonal patterns in abundance suggesting that *P. trivolvis* encounters variation in predation risk with these two predators over development.

When cues of water bug predation are detected, snails form wider shells in just 7 days (Hoverman, Auld & Relyea 2005; Hoverman & Relyea 2007a,b, 2008). This response appears to facilitate their ability to retract into the shell and increase the distance water bugs must reach inside the shell with their proboscis (an elongated sucking mouthpart that is tubular and flexible) before contacting the body (Fig. 1). There is a strong positive correlation between shell width and the distance snails can retract into their shells (Pearson's correlation = 0.985, $P < 0.001$, $n = 60$). In contrast, crayfish either chip the snail's aperture with their first set of walking legs to gain access to the snail's body or crush the shell against their bodies. While previous work has shown that crayfish induce *P. trivolvis* to form thicker shells that are expected to confer greater resistance to crayfish attack, this response takes more than 14 days to appear (Hoverman & Relyea



Fig. 1. Image of the giant water bug (*Belostoma flumineum*) feeding on the freshwater snail *Planorbella (Helisoma) trivolvis*. Water bugs are shell invaders that use a modified sucking mouthpart to pierce the body of the snail and inject digestive enzymes while holding the snail with the forelimbs.

2007b, 2008). Studies have shown that snails can reach a size refuge from these two predators near adulthood (Alexander & Covich 1991; Hoverman unpublished data) but the size refuge requires 1–3 months to reach. While we have a firm understanding of the phenotypic responses of *P. trivolvis* to water bugs and crayfish, we have not explored the effectiveness of the responses against each respective predator. We also do not know whether the responses result in survival trade-offs, or how developmental plasticity affects the outcome of predation, which is necessary for understanding predator-prey dynamics in the system.

In this experiment, we induced three different phenotypes (no-predator, water bug-induced, and crayfish-induced) and examined their survival with lethal water bugs and crayfish using pair-wise combinations of each phenotype. Due to the dynamics of trait induction, we conducted three sets of predation trials during key points in development. In general, we predict that the responses induced by a predator will reduce the risk of predation with that particular predator but increase the risk of predation by the other predator (i.e. survival trade-off). However, we expect that this outcome will depend on when in development survival is examined due to time lags in trait development and the existence of a size refuge from predation. Given that snails rapidly form wider shells with water bugs (within 7 days), we predict that snails induced by water bugs will have higher survival with this predator compared to the other two phenotypes over most of development. In contrast, snails take longer (> 14 days) to form thicker shells with crayfish. Thus, we predict that snails induced by crayfish will not have higher survival with this predator compared to the other treatments until mid-way in development. Additionally, snails should be able to reach a size refuge against both predators. Thus, we predict that all three phenotypes will have high survival with lethal predators

late in development. Finally, we predict that survival will be similar between the no-predator phenotype and the crayfish-induced phenotype when paired with a lethal water bug and between the no-predator and water bug-induced phenotype when paired with a lethal crayfish. We expect these results because in both cases the snails in these treatments will not have formed the appropriate defences (wider shells and thicker shells, respectively) to escape predation.

Materials and methods

The experiment was conducted at the University of Pittsburgh's Aquatic Research Facility in Linesville, PA in the summer of 2005. On 14 May, we collected 400 adult *P. trivolvis* from Geneva Pond #3 (41° 35' N; 80° 14' W), a semi-permanent pond that contains both water bugs and crayfish. We placed 40 adults into each of 10 pools filled with 200-L of well water to oviposit. Egg deposition began immediately and continued until the adults were removed from the culturing pools on 22 May. The eggs began to hatch on 7 June and hatchlings were fed rabbit chow *ad libitum* until the start of the experiment.

On 14–15 June, we filled 90 pools with 100-L of well water to serve as our experimental units for phenotypic induction. The pools were placed on tables in an open field at the research facility. To each pool, we added 5 g of rabbit chow as an initial nutrient source and an aliquot of pond water containing algae (i.e. phytoplankton and periphyton) and zooplankton. We also added a single predator cage to each pool that was constructed from a 10 × 10 cm section of corrugated polyethylene pipe and capped with shade cloth on both ends. Predator cages allow chemical cues of predation to diffuse throughout the pools without killing the focal snails in the pools (Chivers & Smith 1998; Kats & Dill 1998). We covered each pool with shade cloth to prevent colonization by unwanted animals during the experiment. On 27 June, 60 juvenile snails obtained from a mixture of snails from the culturing pools were added to each experimental pool (mean mass \pm 1 SE = 42 \pm 5 mg).

The first objective in the experiment was to use caged predators to induce different phenotypes that could be used in predation trials at three times in development. This induction phase of the experiment was a randomized design with three temporal units. Each temporal unit contained three caged-predator treatments replicated 10 times for a total of 30 experimental units. The three caged-predator treatments were the following: (1) no predator (i.e. an empty cage), (2) one caged water bug, and (3) one caged crayfish. The caged predators were fed 400 mg of snail biomass (i.e. 2–4 snails) three times per week. Cages in the no-predator treatment were lifted briefly out of the water and replaced to equalize disturbance.

We destructively sampled 30 pools of the induction experiment on each of three dates [6 July (day 9 of the experiment), 13 July (day 16), and 1 August (day 35)] and used the recovered snails to conduct predation trials. Given that the pools were destructively sampled, the three sample dates are independent sampling units. The first two sampling dates were based on our previous experience with the formation of morphological responses over development. Snails are capable of forming wider shells in response to water bugs in just 1 week while the formation of thicker shells generally takes over 2 weeks. Thus, we sampled on day 9 and 16 to assess the dynamics of trait formation with these two predators and the impacts on survival. The last sample date was based on our estimate of when snails may reach a size refuge from predation. Our previous work has shown that snails reach adult size around 7 weeks post-hatching and growth rate tends to decrease

at this time (Hoverman & Relyea 2007a). Moreover, adult snails are expected to have reached a size refuge from predation (Alexander & Covich 1991, Hoverman unpublished data). On day 35 of our experiment, the snails were over 8 weeks post-hatching and assumed near adulthood although reproduction had not started in the pools.

For one pool sampled on day 35, snail survival was extremely low (31%) with caged water bugs. This pool was excluded from the experiment. In the remaining pools, final survival in the induction phase of the experiment was > 95% and did not differ among induction treatments or time periods. Ten snails, from each of the destructively sampled pools, were randomly selected and preserved in 10% formalin to assess morphological induction. For each phenotype, the remaining snails were then pooled together. To minimize mass differences among the phenotypes and place the focus on relative shape differences, we sorted the phenotypes into groups of similar mass prior to the predation trials. Mean snail mass \pm SE for the no-predator, crayfish-induced, and water bug-induced phenotypes was 46 ± 5 mg, 49 ± 5 mg, and 52 ± 5 mg on day 9 ($F_{2,42} = 0.4$, $P = 0.665$), 74 ± 3 mg, 80 ± 3 mg, and 72 ± 3 mg on day 16 ($F_{2,42} = 3.1$, $P = 0.055$), and 105 ± 3 mg, 114 ± 3 mg, and 111 ± 3 mg on day 35 ($F_{2,42} = 0.9$, $P = 0.429$), respectively. Importantly, the sorting process did not change the mean shape differences among the induced phenotypes.

We used a marking scheme to identify the phenotypes during the predation trials. For each phenotype, the snails were randomly divided into two groups and were blotted dry. To one group, we applied non-toxic paint to the shell and allowed the shells to dry for 30 min (Henry & Jarne 2007). The other group remained unmarked but experienced the same drying time as the painted group. To control for our marking scheme, the phenotype receiving the mark was switched for half of the replicates within each treatment. Since our analyses indicated that the marks had no effect on predation rates (predators were not repulsed or attracted by the marks), there will be no further discussion of the marking scheme. For each phenotype, 15 snails from the unmarked and marked group were set aside to assess mortality due to handling and marking; 48-h survival was 100%.

On each sample date, we examined the survival trade-offs associated with the induced phenotypes by conducting predation trials. For each predation trial, we used a completely randomized design consisting of two lethal predator treatments (lethal water bugs or crayfish) crossed with three pair-wise phenotypic combinations: (1) no-predator phenotype combined with water bug-induced phenotype, (2) no-predator phenotype combined with crayfish-induced phenotype, and (3) crayfish-induced phenotype combined with water bug-induced phenotype. Thus, each phenotype was paired separately with the other two phenotypes. However, since the set of background phenotypes was different for each phenotype, we treated this as a nested-experimental design (see Statistical analysis of survival). To equalize predation rates between the two lethal-predator treatments, we used two water bugs or one crayfish (Hoverman & Relyea 2007b). After sorting the phenotypes for mass, our sample size permitted four, six, and five replicates of the six treatments on day 9, 16, and 35, respectively.

The predation trials were conducted in 10-L plastic tubs ($35 \times 23 \times 15$ cm) filled with 3 L of water to eliminate the possibility of snails moving higher in the tub and out of reach of the predators. To each tub, we added 10 snails from each assigned phenotype (e.g. 10 no-predator phenotype + 10 water bug-induced phenotype) for a total of 20 snails per tub. Within each tub, one phenotype was marked while the other was unmarked. The lethal predators were added to the tubs 30 min after the snails were added and were removed after half of the snails were consumed or after 48 h had passed (whichever

occurred first). After the predators were removed, the surviving snails were identified by their markings.

To assess phenotypic induction by the caged predator treatments on snail morphology, the preserved snails were dried at 80 °C for 24 h, weighed to the nearest milligram, and measured for shell width using digital imaging software (Optimus Co., Bothell, WA). We then used digital calipers to measure shell thickness at the leading edge of the aperture. Based on our previous work, shell width and thickness are the most important and reliable shell traits to be induced by these two predators (Hoverman, Auld & Relyea 2005; Hoverman & Relyea 2007a,b, 2008).

STATISTICAL ANALYSIS OF PREDATOR-INDUCED MORPHOLOGY

We first analysed the effects of caged predators on the shell width, shell thickness, and mass of the snails. For each pool, we calculated the mean shell width, shell thickness, and mass of the 10 individuals measured for use in our analyses. When analyzing morphological plasticity, it is important to address the allometric relationship between morphological traits and mass. Using each pool's mean, we found that shell width was positively correlated with log-transformed mass (Pearson correlation ≥ 0.618 , $P \leq 0.001$) within each sample date. However, there was not a significant correlation between shell thickness and log-transformed mass (Pearson correlation ≤ 0.186 , $P \geq 0.994$). To account for size variation in our analysis of shell width, we used analysis of covariance (ANCOVA) with log mass as our covariate (Hoverman, Auld & Relyea 2005). A critical assumption in the ANCOVA procedure is that the treatments share a common slope of their regression lines (i.e. a similar allometric relationship). While this assumption was satisfied within each sample date (i.e. predator treatment-by-log mass interaction; $F_{2,26} \leq 2.7$, $P \geq 0.089$), the slopes were not parallel across the sample dates and this issue could not be resolved by additional transformations. Therefore, we conducted a separate ANCOVA for each sample date with the caveat that direct comparisons could not be performed across dates. For shell thickness and mass, we performed analysis of variance (ANOVA) to examine the main effect of predator treatment. Given that our three sample dates were independent of each other (i.e. destructively sampled) but the three traits measured within each sample date were not independent, we performed a Bonferroni correction for performing multiple tests ($\alpha = 0.017$). When univariate tests were significant, we conducted mean comparisons using Fisher's LSD test.

STATISTICAL ANALYSIS OF SURVIVAL

The second analysis examined the survival of the snail phenotypes with lethal predators. Survival was calculated as the proportion of surviving snails of each phenotype (number alive/10) in a tub and the data were arcsine-square root-transformed to meet the assumptions of normality. The predation trials used a nested design in which each induced phenotype was paired individually with one of the other two induced phenotypes and the pair was then exposed to lethal predators. Consequently, the set of background phenotypes was different for each focal phenotype. Thus, we conducted a nested ANOVA using lethal-predator treatment, date, induced phenotype, and phenotypic background (nested within induced phenotype) as our main effects. Date was used as a categorical factor because we did not expect linear responses over time. In the analysis, we found several interactions involving the lethal predator treatment (Table 2). To reveal more detailed treatment effects, we conducted subsequent nested ANOVAs

within each lethal-predator treatment using date, induced phenotype, and phenotypic background (nested within induced phenotype) as our main effects.

Results

TRAIT INDUCTION BY CAGED PREDATORS

The caged predator treatments affected the shell width, shell thickness, and mass of the snails. There was a significant effect of our treatments on shell width on all sample dates (Table 1, Fig. 2a). Snails had 5.7–8.8% wider shells in the water bug treatment compared to the no-predator and crayfish treatments ($P \leq 0.001$) but there was no difference in shell width between the latter two treatments ($P \geq 0.146$). There was a significant effect of our treatments on shell thickness on days 16 and 35 but not day 9 (Table 1, Fig. 2b). On days 16 and 35, shells were 23–24% and 45–47% thicker in the crayfish treatment compared to the no-predator treatment and the water bug treatment, respectively ($P \leq 0.005$). Shells were 15–16% thinner in the water bug treatment compared to the no-predator treatment ($P \leq 0.054$). Our treatments also had a significant effect on snail mass on all sample dates (Table 1, Fig. 2c). Snails were 14–33% larger in the water bug and crayfish treatments compared to the no-predator treatment ($P \leq 0.054$). There was no difference in mass between the water bug and crayfish treatments on days 9 and 16 ($P \geq 0.090$) but the crayfish treatment was larger than the water bug treatment on day 35 ($P = 0.005$). Thus, as previously observed, snails rapidly formed wider shells with water bugs while snails required over 2 weeks to form thicker shells with crayfish.

SURVIVAL OF THE DIFFERENT PHENOTYPES AGAINST LETHAL PREDATORS

In the overall analysis of snail survival, we found significant interactions involving the lethal-predator treatment (Table 2) that lead us to conduct separate analyses for each lethal predator (Table 3). When we examined snail survival with lethal water bugs, the nested ANOVA detected significant main effects of phenotype, phenotypic background nested within phenotype and the interaction of date-by-phenotype (Table 3). Across the three dates, the water bug-induced phenotype had higher survival with lethal water bugs than the no-predator and the crayfish-induced phenotype in the phenotypic pairwise combinations ($P \leq 0.003$, Figs. 3a and c). However,

when the no-predator and crayfish-induced phenotypes were combined, there were no differences in survival across the three dates ($P \geq 0.123$, Fig. 3b). Given that survival was generally lower than 50% for these two phenotypes across the three sample dates, there does not appear to be a size refuge from water bugs. Within each phenotype, we examined survival across the three dates to determine the cause of the date-by-phenotype interaction. While survival of the no-predator and crayfish-induced phenotypes did not differ across the

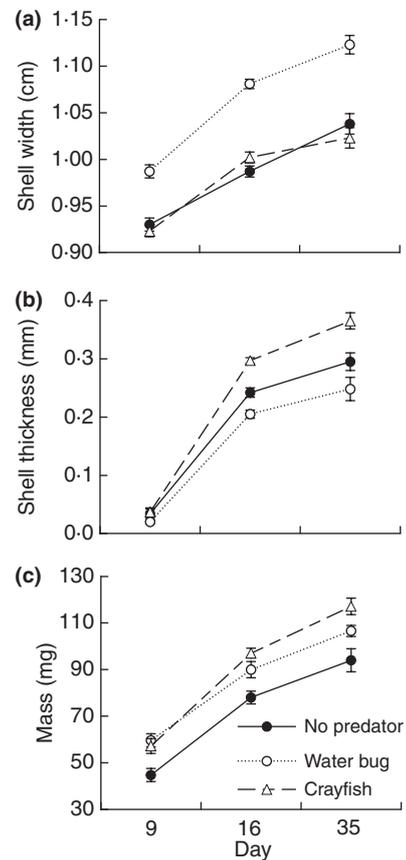


Fig. 2. The effects of caged predators on the size-adjusted shell width (a), shell thickness (b), and mass (c) of snails on day 9, 16 and 35 (means \pm 1 SE). Closed circles, open circles, and open triangles represent the no-predator, caged water bug, and caged crayfish treatments, respectively. Comparisons were not made across the sample days due to the limitations of our analyses (see Statistical analysis of predator-induced morphology). However, we have used lines to connect each treatment through time to aid in visualization of the treatments.

Table 1. Univariate tests (within each sample date) of the effects of caged predator treatment on the shell width, shell thickness, and mass of snails. The univariate test for shell width is from an ANCOVA using log mass as a covariate

Univariate tests	Day 9			Day 16			Day 35		
	d.f.	F	P	d.f.	F	P	d.f.	F	P
Shell width	2,26	27.1	0.001	2,26	89.7	0.001	2,25	30.8	0.001
Shell thickness	2,27	2.0	0.155	2,27	46.3	0.001	2,26	12.7	0.001
Mass	2,27	7.4	0.003	2,27	11.4	0.001	2,26	9.3	0.001

Table 2. Results of a nested ANOVA testing the effects of lethal predators, induced phenotype, and phenotypic background (nested within induced phenotype) on the survival of snail phenotypes in predation trials over time (i.e. sample dates)

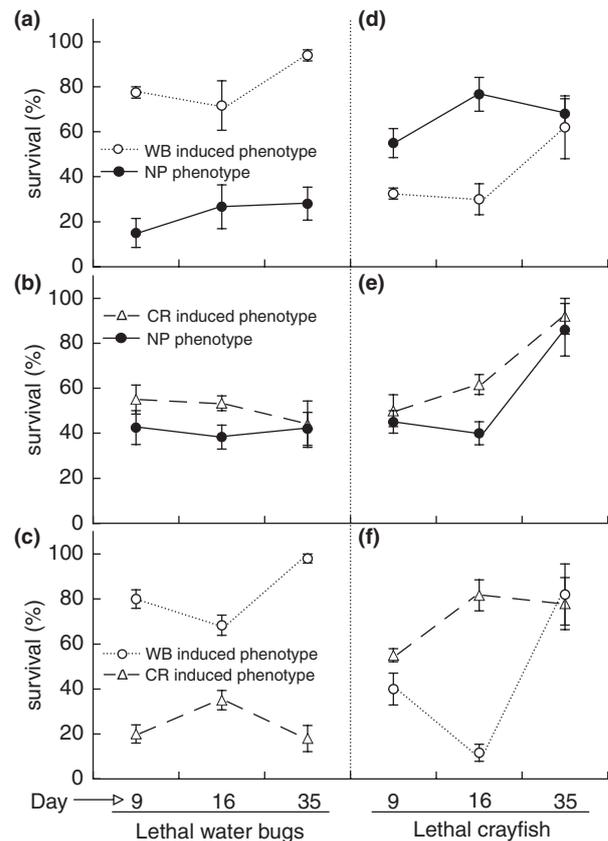
Overall ANOVA	d.f.	F	P
Phenotype	2	4.7	0.119
Day	2	6.7	0.030
Predator	1	2.3	0.229
Day * Phenotype	4	2.0	0.210
Predator * Phenotype	2	14.9	0.028
Background (Phenotype)	3	0.4	0.781
Day * Predator	2	5.6	0.043
Day * Background (Phenotype)	6	2.1	0.192
Day * Predator * Phenotype	4	0.7	0.635
Predator * Background (Phenotype)	3	2.4	0.163
Day * Predator * Background (Phenotype)	6	1.8	0.093
Error	144		

Table 3. Results of nested ANOVAs testing the effects of induced phenotype and phenotypic background nested within induced phenotype on the survival of snail phenotypes in predation trials over time (i.e. sample dates) within each lethal predator treatment

ANOVA	d.f.	Lethal water bugs		Lethal crayfish	
		F	P	F	P
Day	2	3.2	0.113	6.6	0.030
Phenotype	2	9.4	0.050	63.9	0.003
Day * Phenotype	4	8.6	0.012	0.8	0.555
Background (Phenotype)	3	11.6	0.006	0.1	0.985
Day * Background (Phenotype)	6	0.8	0.605	4.2	0.001
Error	72				

three dates ($P \geq 0.081$), survival of the water bug-induced phenotype was similar on days 9 and 16 ($P = 0.414$) but higher on day 35 ($P < 0.001$). The significant effect of phenotypic background suggested that the survival of each phenotype depended on the presence of the other two phenotypes. For the water bug-induced phenotype, the phenotypic background did not affect survival across the three dates ($P \geq 0.521$). For the no-predator and crayfish phenotype, survival was higher when combined with each other than when combined with the water bug-induced phenotype ($P \leq 0.050$). In sum, the water bug-induced phenotype had high survival with lethal water bugs over development regardless of the background phenotype. For the no-predator and crayfish-induced phenotypes, survival was lower when paired with the water bug-induced phenotype than when paired with each other.

When we examined snail survival with lethal crayfish, the nested ANOVA detected significant main effects of phenotype, sample day, and the interaction of day-by-phenotypic background nested within phenotype (Table 3). The crayfish-induced phenotype experienced higher survival than the other two phenotypes on day 16 ($P \leq 0.022$, Figs. 3e and f) but not on day 9 or 35 ($P \geq 0.070$). When the no-predator and water bug-induced phenotypes were combined, the no-predator

**Fig. 3.** The effects of lethal water bugs (a, b, c) and crayfish (d, e, f) on the survival of predator-induced phenotypes on day 9, 16, and 35 (means \pm 1 SE). The predators were presented with pair-wise combinations of phenotypes as follows: snails with a no-predator phenotype combined with snails with a water bug-induced phenotype (a, d), snails with a no-predator phenotype combined with snails with a crayfish-induced phenotype (b, e), and snails with a crayfish-induced phenotype combined with snails with a water bug-induced phenotype (c, f). Predator-induced phenotype labels are as follows: no-predator (NP), water bug (WB), and crayfish (CR). Symbols are as described in Fig. 2.

phenotype had higher survival on day 9 and 16 ($P \leq 0.010$), but not on day 35 ($P = 0.709$, Fig. 3d). To examine the effect of day-by-phenotypic background nested within phenotype, we compared the survival of each phenotype in the presence of the other two phenotypes across sample days. On days 9 and 35, the phenotypic background did not affect survival for any of the three phenotypes ($P \geq 0.215$). However, phenotypic background did affect survival on day 16. Survival was higher for the crayfish-induced phenotype and the no-predator phenotypes when combined with the water bug-induced phenotype than when combined with each other ($P \leq 0.033$). In contrast, survival was higher for the water bug-induced phenotype when combined with the no-predator phenotype than when combined with the crayfish-induced phenotype on day 16 ($P = 0.049$). In sum, the crayfish-induced phenotype only had a clear survival advantage over the other two phenotypes midway in the experiment and all phenotypes had relatively high survival in the last sample providing evidence for a size refuge.

Discussion

To explore survival trade-offs associated with predator-induced morphological plasticity in freshwater snails, we exposed combinations of three induced phenotypes (i.e. no-predator, water bug-induced, and crayfish-induced) to either lethal water bugs or crayfish. We found general support for our prediction that responses induced by a predator will reduce the risk of predation with that particular predator but increase the risk of predation by a functionally different predator. However, it was clear that the outcome of the predation trials was dependent on when in development the predation trials were conducted due to different rates of trait formation between the caged predator treatments. There was equivocal support for our prediction that snails can reach a size refuge from both predators. While snails appeared to reach a size refuge from crayfish, there was no evidence that a size refuge can be reached from water bugs. We found support for our prediction that survival will be similar between the no-predator phenotype and the crayfish-induced phenotype when paired with a lethal water bug, which was driven by similarities in shell width between these two treatments. Interestingly, the no-predator phenotype had higher survival than the water bug-induced phenotype when paired with a lethal crayfish. This result is explained by the thinner shells expressed by the water bug-induced phenotype compared to the no-predator phenotype. Below, we synthesize these dynamic results and discuss their significance in the broader framework of inducible defences.

SURVIVAL WITH LETHAL WATER BUGS

Water bugs are shell-invaders that insert their probosces into the shell opening and pierce the snail body. Consequently, snails that build wider shells would increase the coiled distance of tube available for retracting their body from water bugs to escape predation (see Fig. 4 in Hoverman & Relyea 2007a). With lethal water bugs, we found that the water bug-induced phenotype, which was formed early in development, performed better (i.e. higher survival) than the other two phenotypes on all three sample dates. Indeed, survival of the water bug-induced phenotype ranged from 68–98% with lethal water bugs. However, the no-predator and crayfish-induced phenotypes were equally vulnerable to predation by water bugs when paired together. This suggests that even relatively small snails that form wider shells are able to reduce their risk of predation by the water bug, and verifies that phenotypes induced in other environments are highly susceptible to water bug predation. Curiously there does not seem to be an attainable size refuge from water bug predation within the range of the sizes tested, given that snails lacking relatively wider shells were highly susceptible to water bug predation throughout all stages of development. The survival benefits associated with relatively wider shells corroborate recent findings that snails rapidly form relatively wider shells when water bugs are encountered throughout their pre-reproductive development (Hoverman & Relyea 2007a, 2008). Collectively,

these data provide evidence that the formation of a relatively wider shell is an effective defensive strategy against water bugs over development and that relatively narrow shells increase the risk of predation.

SURVIVAL WITH LETHAL CRAYFISH

Crayfish are shell-chippers/crushers that break the aperture or entire shell to gain access to the snail body (Alexander & Covich 1991). Thus, snails that build thicker shells would withstand the attempts of crayfish to break the aperture (opening of the shell). With lethal crayfish, we found that the outcome of the predation trials was dependent on development. Early in development, all three induced phenotypes were generally equally vulnerable to crayfish. While there were differences in shell morphology among the induced phenotypes (i.e. wider shells in the caged water bug treatment), there were no detectable differences in shell thickness. This suggests that relatively small and thin-shelled snails are highly susceptible to crayfish predation early in development. Mid-way in development (i.e. day 16), we found significant differences among the phenotypes in their susceptibility to predation. The crayfish-induced phenotype had a clear survival advantage over the no-predator and water bug-induced phenotypes and the no-predator phenotype was more effective at escaping the lethal crayfish than the water bug-induced phenotype. Moreover, survival of the no-predator and crayfish-induced phenotypes was 20–36% higher when combined with the water bug-induced phenotype than when combined with each other. These results parallel treatment differences in shell thickness at day 16; shells were thick, intermediate, and thin in the crayfish-induced, no-predator, and water bug-induced treatment, respectively (Fig. 1b). Given that we ensured that the three different phenotypes were similar in mass during the predation trials, the survival differences among the phenotypes on day 16 can be attributed largely to differences in shell thickness. Interestingly, snails were also larger on day 16 compared to day 9. As a result, the survival advantage of the crayfish-induced phenotypes may be a function of both an increase in size and shell thickness. Additional support for the role of size in reducing predation rates by crayfish came on day 35. On this date, we found that there were no differences in survival between the phenotypes and survival was relatively high (i.e. > 62%) regardless of the snail phenotype, suggesting that snails were approaching a refuge from crayfish predation. This refuge is probably the result of overall size and shell thickness combined since both increased in absolute value during the experiment. Alexander & Covich (1991) found similar results when they examined the ability of the crayfish *Procambarus simulans* to consume *P. trivolvis* of different sizes; handling time increased while ingestion probability decreased with increasing shell size. Given that shell thickness and size are confounded over development, additional studies that test snails that are similar in shell thickness but differ in overall size will help to elucidate the relative importance of these two traits in evading crayfish predation. Together, our results demonstrate that

thicker shells are deterrents to crayfish predation but given that thicker shells require longer to form they are primarily effective midway in development before snails reach a size refuge from crayfish predation.

Our predation trials were focused on understanding the importance of morphological responses for predator defence. Indeed, shell attributes (e.g. morphology, thickness) are important for deterring many marine and freshwater predators of snails (Vermeij & Covich 1978; Appleton & Palmer 1988; Dewitt, Robinson & Wilson 2000; Trussell 2000a,b; Trussell & Smith 2000; Rochette, Doyle & Edgell 2007). However, there has been substantial work addressing the behavioural responses of snails to their predators. Many snails spatially avoid predators including crayfish especially when they are small and vulnerable to predation (Alexander & Covich 1991; Dewitt, Sih & Hucko 1999; Hoverman, Auld & Relyea 2005). By spatially avoiding crayfish (i.e. movement to the surface), snails can dramatically reduce encounter rates with crayfish that use benthic habitats. Snails also have been observed to compensate for morphological vulnerability to predators with behaviour responses (Dewitt, Sih & Hucko 1999). Thus, behavioural responses to crayfish may provide snails with additional time to morphologically respond to the predator, which would be particularly valuable early in development when snails are highly vulnerable to predation. Given the importance of behavioural and morphological responses to predators, future studies that examine the relative importance of both defensive traits for reducing predation rates would be valuable.

FITNESS BENEFITS OF INDUCIBLE DEFENCES

The benefits of inducible defences have been confirmed in a number of systems for a variety of different traits. For behavioural defences, prey that reduce their foraging activity or spatially avoid predators can reduce their risk of predation (Sih 1987; Dill 1987; Werner & Anholt 1993; Lima & Dill 1990; Kats & Dill 1998). For morphological defence, some prey are more difficult to capture while others are more difficult to consume after being captured (Krueger & Dodson 1981; Kuhlmann & Heckmann 1985; Dodson & Havel 1988; Brönmark & Miner 1992; Kusch 1995; Nilsson, Brönmark & Pettersson 1995; McCollum & Van Buskirk 1996; Spitz & Sadler 1996; Van Buskirk & Schmidt 2000; Relyea 2001). However, there are several studies that have not found strong evidence for the benefits of induced morphological responses. For example, the predator-induced phenotypes of amphibian larvae occasionally do not decrease predation rates with lethal predators relative to individuals not previously exposed to predators (Van Buskirk & Relyea 1998; Van Buskirk & McCollum 2000). Such results may be explained by addressing the rate of trait formation over prey development. If prey defences have not completely formed at the time of the performance test (due to time lags), the predicted performance benefit may not be observed. For example, the inducible defences in *Daphnia* are not effective at reducing predation rates until late in development (Tollrian 1995; Laforsch & Tollrian

2004). We found a similar time lag in the formation of a thicker shell with crayfish, which limited the effectiveness of the response to mid-way in development. While such results clearly underscore the importance of development in addressing the benefits of inducible defences, they also provide evidence for the limitations of phenotypic plasticity. Indeed, time lags for trait induction can pose limitations to the benefits of inducible defences because they decrease the accuracy of matching the environment, which may result in selection against phenotypic plasticity (Moran 1992; Padilla & Adolph 1996; Tollrian & Harvell 1999; Gabriel *et al.* 2005). Given that time lags can significantly limit the benefits of inducible defences, studies that explore the adaptive value of induced defences at multiple developmental stages will contribute substantially to our understanding of the factors that shape their evolution.

FITNESS COSTS OF INDUCIBLE DEFENCES

The prediction that predator-induced phenotypes will have lower fitness in alternative environments has received equivocal support. Commonly, these results are from fitness tests associated with predator-induced phenotypes placed into predator-free environments after induction (e.g. growth costs; Van Buskirk & Relyea 1998; Van Buskirk & Schmidt 2000). However, for prey species that routinely encounter different predator species and form predator-specific defences, it is also important to test for fitness trade-offs in alternative predator environments. Indeed, the few studies that have examined survival trade-offs of induced defences with different predators have found that phenotypes formed with one predator increased susceptibility to a different predator (Tollrian & Dodson 1999; Kishida & Nishimura 2005; Benard 2006). Other studies examining fitness trade-offs have taken a functional approach in which laboratory-reared or wild-caught prey are exposed to different predators and the direction of selection is analyzed and compared to existing data on predator-induced plasticity. For example, DeWitt and colleagues have shown that selection by fish and crayfish favour opposing shell morphologies (i.e. round vs. elongate apertures), which is consistent with the phenotypes induced by each predator (Dewitt, Robinson & Wilson 2000; Dewitt & Langerhans 2003). However, predator-specific responses may not always lead to fitness trade-offs in different predator environments. Some studies have shown that responses to one predator can also reduce predation rates with other functionally similar predators (Van Buskirk & McCollum 2000; Traw & Dawson 2002; Laforsch & Tollrian 2004). Thus, although prey may form predator-specific responses, if these responses are in a similar direction, they may provide general defences against multiple predator species especially if the predators use similar foraging tactics. Together these studies show that identifying the predators that prey encounter and the foraging tactics of each predator will be necessary to rigorously test for fitness trade-offs. Even if certain predator environments are rare, theory has shown that they can play important

roles in the evolution of phenotypic plasticity (Van Tienderen 1991; Donohue *et al.* 2000; Sultan & Spencer 2002).

Conclusions

Our results demonstrate the importance of incorporating functionally different predator species and different developmental stages into studies that address the fitness costs and benefits of predator-induced responses. Indeed, obtaining evidence for the adaptive value of plasticity is a critical step in answering ecological and evolutionary questions about phenotypic evolution in heterogeneous environments. A substantial body of theory exists to predict how phenotypes should evolve in response to environmental heterogeneity (Via & Lande 1985, 1987; Gavrillets 1986; Lively 1986; Via 1987; Van Tienderen 1991, 1997; Moran 1992; Tufto 2000; Sultan & Spencer 2002; Poitrineau, Brown & Hochberg 2004). Collectively, this theory predicts that populations should evolve specialized, non-plastic phenotypes in constant environments but generalized, plastic phenotypes in variable environments (given some qualifying assumptions). Using organisms with inducible defences as model systems, researchers can manipulate a variety of factors including the degree of spatial vs. temporal variation in predation risk, the strength of selection, and the timing of the selective agent to empirically test the predictions of plasticity models. Not only will this information provide valuable insights into the evolution of plasticity within populations, but it will also help guide studies interested in the divergence of populations experiencing different local selective pressures. Consequently, as studies accumulate showing that environmental heterogeneity can affect phenotypic expression, it will be critical to test for the adaptive value of phenotypic plasticity in order to inform future efforts aimed at understanding phenotypic evolution in heterogeneous environments.

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