The long-term impacts of predators on prey: inducible defenses, population dynamics, and indirect effects

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Despite the amount of research on the inducible defenses of prey against predators, our understanding of the long-term significance of non-lethal predators on prey phenotypes, prey population dynamics, and community structure has rarely been explored. Our objectives were to assess the effects of predators on prey defenses, prey population dynamics, and the relative magnitude of density- versus trait-mediated indirect interactions (DMIIs and TMIIs) over multiple prey generations. Using a freshwater snail and three common snail predators, we constructed a series of community treatments with pond mesocosms that manipulated trophic structure, the identity of the top predator, and whether predators were caged or uncaged. We quantified snail phenotypes, snail population size, and resource abundance over multiple snail generations. We found that snails were expressing inducible defenses in our system although the magnitude of the responses varied over time and across predator species. Despite the expression of inducible defenses, caged predators did not reduce snail population size. There also was no evidence of TMIIs throughout the experiment suggesting that TMIIs have a minimal role in the long-term structure of our communities. The absence of TMIIs was largely driven by the lack of predator-induced reductions in resource consumption and the lack of consistent reductions in population size with predator cues. In contrast, we detected strong DMIIs associated with lethal predators suggesting that DMIIs are the dominant long-term mechanism influencing community structure. Our results demonstrate that although predators can have significant effects on prey phenotypes and sometimes cause short-term TMIIs, there may be few long-term consequences of these responses on population dynamics and indirect interactions, at least within simple food webs. Research directed towards addressing the long-term consequences of predator–prey interactions within communities will help to reveal whether the conclusions and predictions generated from short-term experiments are applicable over ecological and evolutionary timescales.
The interactions between predators and their prey have important implications for larger communities where species interact directly and indirectly with each other. The direct effect of predators on prey density can lead to density-mediated indirect interactions (DMIIs). While ecologists have traditionally focused on changes in species density, there is a growing appreciation that changes in the traits of species can indirectly affect communities (i.e. trait-mediated indirect interactions, TMIIs; Abrams 1995). The challenge is to determine the conditions under which TMIIs will play an important role in structuring communities. The few studies that have been explicitly designed to examine the relative contributions of DMIIs and TMIIs in structuring communities have shown that although TMIIs vary in magnitude they can be just as strong as DMIIs (Werner and Peacor 2003). However, these studies were conducted over relatively short time scales (i.e. <1 generation) preventing an assessment of the long-term importance of DMIIs versus TMIIs (Werner and Peacor 2003). The importance of TMIIs over the long term will largely depend on the types of traits prey change in response to predators, the influence of trait changes on resource consumption and population growth, and the structure of the food web. Empirical and theoretical research on TMIIs has shown that behavioral responses are an important driver of TMIIs due to the impacts on prey resource acquisition or demand (Bolker et al. 2003, Werner and Peacor 2003, Schmitz et al. 2004, Belovsky et al. 2011). However, the initiation of TMIIs via morphological plasticity is expected to depend on the topology of the food web (Werner and Peacor 2003). In simple linear food chains, changes in morphology alone are not expected to transmit TMIIs unless resource consumption rates are simultaneously altered (Abrams 2008). However, predator-induced changes in morphology can lead to TMIIs within more complex food webs (Relyea 2000, Trussell et al. 2002). Ultimately, for TMIIs to be important over the long term, population growth rate must change, which may be mediated by changes in resource acquisition, resource demand, or a suite of physiological stress mechanisms (Abrams 2008, Sheriff et al. 2009, Hawlena and Schmitz 2010a, b). Unfortunately, short-term studies prevent an assessment of life history responses and their impacts on population dynamics. Thus, TMIIs could be quite different over the long term, even to the point that they become unimportant relative to DMIIs (Persson and De Roos 2003). The few long-term investigations that exist suggest that TMIIs may exist over longer time periods (Chase 1999, Raimondi et al. 2000, Schmitz 2000), but we have too few data to draw any generalities. The objectives of our study were to assess the effects of predators on prey defenses, prey population dynamics, and the relative magnitude of DMIIs and TMIIs over multiple prey generations.

Study system

Our model system consisted of the freshwater snail *Helisoma trivolvis* and three common snail predators (water bugs *Belostoma flumineum*; crayfish *Orconectes rusticus*; sunfish *Lepomis gibbosus*). In response to the non-lethal presence of predators (i.e. caged predators), *H. trivolvis* form invasion-resistant shells with water bugs, crush-resistant shells with crayfish, and increase their use of refuges to avoid detection by fish (Hoverman and Relyea 2007, 2008, 2009). While water bugs do not appear to affect the egg production of *H. trivolvis*, crayfish induce a 45–68% reduction in total egg production over four months compared to predator-free environments (Hoverman et al. 2005). Snails are typically 22–33% smaller when reared with caged fish compared to no-predator treatments due to strong behavioral responses that reduce resource consumption (Hoverman and Relyea 2007, 2008). Although we have not examined egg production of snails reared with caged fish, slower growth is expected to reduce investment in egg production, which should lead to slower population growth. To date, we have limited information on how inducible defenses in snails alter long-term population dynamics and interactions within the community.

Based on our research in this system and existing theory (Abrams 2008), we expected that each predator would have different effects on prey trait induction, population dynamics, and indirect effects within our communities (i.e. top predator, snails, and an algal resource base). With caged water bugs, we expected an induction of morphological defenses, no induction of behavioral defenses that would affect resource consumption, and no effect on total egg production; therefore, we predicted no effect of caged water bugs on snail population size (i.e. no TMII). Given the low predation rate of water bugs (<5 snails d⁻¹; Hoverman and Relyea 2007), we expected lethal water bugs to cause a weak DMI. With caged crayfish, we expected an induction of morphological defenses, no induction of behavioral defenses that would affect resource consumption, and no effect on total egg production; therefore, we predicted no effect of caged crayfish to cause a reduction in snail population size leading to a long-term TMI. Given the high predation rate of crayfish (>150 snails d⁻¹; Hoverman and Relyea 2007), we expected lethal crayfish to cause a strong DMII. With sunfish, we expected a minimal induction of morphological defenses, but a strong induction of behavioral defenses that reduces resource consumption, and a reduction in total egg production; therefore, we expected caged fish to cause a reduction in snail population size leading to a TMII over both the short- and long-term. The high predation rate of lethal sunfish on snails should cause a strong DMII.

Methods

We employed a modified version of the experimental design of Peacor and Werner (2001), which uses a combination of hand-thinning, caged-predator, and lethal-predator treatments to estimate the relative magnitude of density- and trait-mediated effects within pond communities. The experiment was a completely randomized design with 12 treatments replicated four times for 48 experimental units (Table 1). The first treatment represented the base community and consisted solely of periphyton, phytoplankton, and zooplankton (i.e. no snails). This treatment provided an estimate of the periphyton biomass (i.e. resource abundance) without herbivores and predators. The next two treatments contained the herbivore trophic level (i.e. snails). We examined the role...
of density-mediated effects without predators by randomly hand-thinning snails at two rates (no hand thinning and hand thinning). For the remaining treatments, we added the predator trophic level using three different predator species (fish, water bugs, and crayfish) crossed with three predator manipulations: 1) a lethal predator that can produce predation cues and thin the snail population, 2) a caged predator that can produce predation cues but not thin the snail population, and 3) a caged predator that can produce predation cues combined with experimenter applied hand-thinning. Together, these treatments provided multiple ways to examine the separate and combined effects of trait-mediated and density-mediated effects on snail traits, snail population size, and resource abundance in the system (Table 1).

The experiment was conducted at the Univ. of Pittsburgh’s Pymatuning Lab of Ecology in Linesville, PA. On 14 May 2005, we collected 400 adult *Helisoma trivolvis* from a population known to express inducible defenses against our three predators (Hoverman and Relyea 2007). 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 pools on 22 May. The snails began to hatch on 7 June and were fed rabbit chow ad libitum until the start of the experiment.

On 7–10 July, we filled 48 cattle tanks with 700-l of well water to serve as our experimental units. To provide natural substrate, we added 45 kg of soil (29% sand, 21% clay, and 50% silt) to each tank. The tanks were left unmanipulated for two weeks to allow the soil particles to settle to the tank bottom. We then added a 300 ml aliquot of pond water containing phytoplankton, periphyton, and zooplankton obtained from surrounding ponds. Nutrients (e.g. nitrogen, phosphorus) were not added to the tanks and no measurements of nutrients were made during the experiment. We provided structure for the snails by placing a clay tile platform (32 × 32 cm tile supported by a 16 × 16 cm tile base) in the center of each tank. The platform rested approximately 3 cm above the substrate to provide space for snails to move under the tile. A periphyton sampler was added to the south-facing corner of each tank. The periphyton sampler was a weighted structure composed of eight plastic specimen cup lids (7.6 cm diameter) pinned in a linear fashion to 80 cm of well pipe. The sampler rested 5 cm from the substrate bottom when submerged in the tank. While these mesocosms were relatively simple (i.e. lacking macrophytes or depth gradients), similar setups have frequently been used to examine snail–predator interactions in the context of inducible defenses (Turner et al. 1999, 2000, Turner 2004).

We added two predator cages to each tank. Caged predators that are fed snails release chemical cues that diffuse throughout the water without allowing the predators to kill the focal snails (Hoverman and Relyea 2007). One cage, designed to house fish, was constructed from 30 × 30 cm corrugated pipe capped with fiberglass window screen on each end. The other cage, designed to house water bugs or crayfish, was made from 10 × 10 cm corrugated pipe and was capped with shade cloth. We placed a shade cloth lid over each tank to prevent colonization by insects and amphibians during the experiment.

On 3 August, 100 snails were added to each tank (mean mass ± 1 SE = 90.5 ± 7.4 mg). The snail density (55 snails m⁻²) is near the upper limit of observed snail densities in natural ponds (Hoverman et al. 2011). However, this density was used to ensure sufficient snails for the lethal predators and to conduct our hand-thinning treatments during the first month of the experiment before egg production. On the following day, we added the caged and lethal predators. The lethal predators were caged for 1 d and fed 1 snail to provide the experimental snails the opportunity to acclimate to the predator’s presence. The caged predators were fed 1 g (∼6–12 snails) of snail biomass each week, which is sufficient to elicit phenotypic responses in snails (Hoverman
and Relyea 2007, 2009). All empty cages were lifted briefly out of the water to equalize disturbance. Predator survival was checked every three days during the experiment and any dead predators were immediately removed from the tanks and replaced. An important consideration in the experiment was the effects of winter conditions on our predators. During the winter months (December to March) in Pennsylvania, the upper portion of the water column in cattle tanks typically freezes, which would kill our caged predators. Moreover, we expected minimal snail feeding, growth, and reproduction due to the cold temperatures. Thus, we decided to remove all predators from the experiment from December to March.

Our goal with the hand-thinning treatment was to mimic the predation rates of our lethal predators. However, it was only feasible to implement a single hand-thinning treatment rather than three hand-thinning treatments to match each lethal predator. Given that fish and crayfish consume more snails than water bugs (Hoverman and Relyea 2007), we expected these two predators to dramatically reduce snail population size. Matching our hand-thinning rate with the rate of predation by crayfish and fish would bias the results towards detecting large DMIIs in the system rather than TMIs. Thus, we decided to mimic the predation rate observed in the lethal water bug treatment, which was expected to be lower than the other predators. To accomplish this goal, we visually monitored snail population size in the lethal water bug treatment over the first three weeks and observed a predation rate of 20 snails week⁻¹. Hand thinning was conducted until the predators were removed from the tanks in December. At the start of the spring 2006, we resumed hand thinning at 20 snails week⁻¹ until the end of the experiment. We randomly selected snails that were <1 cm in shell width during the thinning events. Snails ≥1 cm were assumed to be in a size refuge from predation and, consequently, not an appropriate target for the thinning treatment (Hoverman and Relyea 2009). After removing the snails, we obtained the total wet biomass of snails removed from each tank. The total biomass removed from the hand-thinning treatment did not differ between caged predator treatments (F3,12 = 1.5, p = 0.262).

On a monthly schedule from September to November 2005 and April to August 2006, we sampled periphyton, estimated snail population size, and quantified snail behavior. To sample periphyton biomass in each tank, we removed one lid from the periphyton sampler, and scraped it with a brush into a tub of water to remove all attached periphyton. We then filtered the water through a pre-weighed Whatman GF/C filter that had previously been dried at 80°C for 24 h. After filtration, the filters were dried for 24 h at 80°C and weighed to determine the biomass of attached periphyton on the lid.

Snail population size and snail behavior were estimated on the same dates that periphyton was sampled. We surveyed the number of snails that were within a 30-cm wide transect extending from the water’s surface, down the side of the tank, across the bottom of the tank, and to the tile platform in the center (i.e., 20% of the tank). In addition to visually surveying for snails within the transect, we used our hands to gently probe for snails buried in the substrate. Snails detected in the substrate were lifted briefly from the water to confirm survival and replaced. All observations were done on the west end of each tank. All hatchling and adult snails were recorded in the survey but egg masses were excluded. Although egg masses were not counted, we observed three distinct reproductive bouts in the tanks based on the presence of egg masses. In August 2005, April 2006 and June 2006, egg masses were present in all the tanks except the lethal crayfish and fish treatments. Few egg masses were observed in the latter two treatments throughout the experiment. Given that H. trivolvis has a perennial life history with overlapping generations, the snail population within each tank likely represented three snail generations by the end of the experiment. In addition to recording the number of snails in the transect, we recorded the number that were within 2.5 cm of the water’s surface and the number under the tile platform. We calculated the proportion of snails using structure and the proportion using the surface by dividing our counts by the total number of snails observed in the transect for each tank. Given that no more than three snails were ever found at the water’s surface, we did not analyze this response variable.

On five of the eight months (September, May, June, July and August) that we sampled periphyton, snail population size, and snail behavior, we also removed a sample of snails from each tank for morphological analysis (in addition to snails removed from the hand-thinning treatments). For the lethal fish and lethal crayfish treatments, there were generally fewer than 10 individuals available for sampling, which would lead to unreliable estimates of mass and morphology. Thus, these tanks were not sampled. On the dates that we did not sample morphology (November, October, and April), there were predominately hatchling snails in the tanks that would not have had time to morphologically respond to predators and are extremely difficult to measure due to their small size. However, behavioral responses were measured during these months. To avoid small snails, we selected 10 individuals from the entire tank that were ≥0.8 cm in shell width. We used digital calipers to ensure that the snails were within the size range and preserved them in 10% formalin. After the sampled snails were dried at 80°C for 24 h, they were weighed to the nearest milligram and measured for shell width (Fig. 1 in Hoverman et al. 2005) using digital imaging software. We also used digital calipers to measure shell thickness at the leading edge of the aperture.

**Statistical analyses**

Our response variables included snail traits (mass, behavior, and morphology), snail population size, and periphyton biomass. For snail traits, we excluded the lethal predator treatments from the analysis because there were too few snails to reliably estimate responses. To analyze snail behavior, we conducted a repeated-measures analysis of variance (rm-ANOVA) to test for the effects of time, caged predator treatment, hand-thinning treatment, and their interactions. The second analysis examined the effects of caged predators and hand-thinning on snail mass and relative morphology. When analyzing morphological plasticity, it is important to first address the allometric relationship between morphological traits and size. Shell width and shell thickness showed positive relationships with mass; mass was log₁₀-transformed to improve the linearity of the relationships. To account for
size variation, we used analysis of covariance (ANCOVA) with log_{10}-transformed mass as the covariate (Hoverman et al. 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; McCoy et al. 2006); this assumption was satisfied (F_{12,80} \leq 1.6, p \geq 0.118). From the ANCOVA, we used the mass-adjusted treatment mean and residuals from the within-treatment regression to calculate each individual’s size-adjusted trait value. We then calculated the mean response for each experimental unit within each sample and used these as our morphological response variables. While there are several different size-correction methods, this is a powerful approach for obtaining size-adjusted morphology based on our previous work (Hoverman et al. 2005, Hoverman and Relyea 2007). We then conducted three rm-ANOVAs for mass, shell width, and shell thickness to test for the effects of time, caged-predator treatments, hand-thinning treatments, and their interactions. Given that three simultaneous tests were conducted, we employed a Bonferroni correction prior to interpreting test significance. We conducted mean comparisons using Fisher’s LSD test if a response variable was significant.

We used rm-ANOVAs to assess treatment effects on snail population size over time. Due to the incomplete factorial design of the experiment, we conducted two analyses with the data to address our hypotheses. In the first analysis, we conducted a two-way rm-ANOVA with predator treatment (no predator and the three caged predator treatments) and thinning treatment (no thinning and hand thinning) as the main effects. Our main interests were whether the caged predators reduced snail population size, which would indicate a trait-mediated effect on population dynamics, and whether our thinning treatment was effective at reducing snail population size (i.e. density-mediated effect). In the second analysis, we used a one-way rm-ANOVA to assess the effects of the lethal predators on snail population size. We included the three lethal predator treatments and the no-predator, no thinning treatment in the analysis. Given that two tests were conducted, we employed a Bonferroni correction prior to interpreting test significance. Population size was log_{10}-transformed to meet the assumption of homogeneity of variance.

We used a one-way rm-ANOVA to test for treatment effects on periphyton biomass (log_{10} transformed). Given that there was not a significant treatment \times time interaction, we averaged across time for our subsequent tests to determine if TMIs and/or DMIIs occurred in the system (Table 1). We tested for TMIs in the system by comparing periphyton biomass among the four caged-predator treatments within the no-hand-thinning treatment and then within the hand-thinning treatment. We tested for DMIIs in the system by comparing the no-hand-thinning and hand-thinning treatment for each predator and by comparing the lethal predator treatments to the caged-predator plus no-hand-thinning treatments. We compared across the predator species to determine how each predator impacted DMIIs versus TMIs. We conducted mean comparisons using Fisher’s LSD test. To further explore DMIIs, we conducted a Pearson’s correlation (p) between log_{10}-transformed periphyton biomass and snail population size. The assumptions of linearity, normality, and homoscedasticity were satisfied. A negative correlation would suggest that larger snail populations were reducing periphyton biomass. For both variables, we used treatment means for each sample month (n = 8 treatment^{-1}) for a total n = 88 in the analysis. We excluded the no-snails treatment in the analysis because there were no snails present.

Results

Effects on snail behavior

For snail behavior, there were significant effects of time, caged predator, and the time \times caged predator interaction (Table 2, Fig. 1). The interaction was driven by caged predator effects early in the experiment (September, October 2005) and again in June 2006, but not in the other months (p \geq 0.17). In September, mean comparisons found no differences between the no-predator and each of the three caged-predator treatments (p \geq 0.12). However, caged fish induced a greater use of structure than caged crayfish and caged water bugs (p \leq 0.006). In October, caged fish induced greater use of structure compared to the other three treatments (p \leq 0.001). There were no differences among the remaining treatments (p \geq 0.21). In June, there were no differences between no-predator and the three caged-predator treatments (p \geq 0.10). However, there was greater use of structure with caged fish and caged water bugs compared to caged crayfish (p \leq 0.018). To assess whether snail population size could be influencing snail behavior in the caged-fish treatment, we conducted a Pearson’s correlation (p) between log_{10}-transformed population size and snail behavior size using the caged-fish treatments across all sample dates. There was a negative correlation between refuge use and population size within the fish treatments over time (p = –0.395, p < 0.001, n = 64), suggesting that when the snail population was large, a smaller proportion of the snails were found using the refuge.

Table 2. Results of repeated-measures ANOVA (Wilks’ \lambda) on the effects of caged predator and hand-thinning treatments on snail behavior (% using structure) over time.

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within subjects</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Time</td>
<td>7.18</td>
<td>5.6</td>
<td>0.001</td>
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<td>21.52</td>
<td>2.0</td>
<td>0.025</td>
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<td>Time \times Hand-thinning</td>
<td>7.18</td>
<td>2.1</td>
<td>0.086</td>
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<td>Time \times Predator \times Hand-thinning</td>
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<td>Between subjects</td>
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<tr>
<td>Predator</td>
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<td>Hand-thinning</td>
<td>1.24</td>
<td>0.3</td>
<td>0.613</td>
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<td>3.24</td>
<td>0.2</td>
<td>0.892</td>
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Effects on snail population size

In the first analysis of snail population size, we examined the effects of predator treatment (no predator and the three caged predator treatments) and thinning treatment (no thinning and hand thinning) over time. There were only effects of time ($F_{7,18} = 10.2, p \leq 0.001$) and hand thinning ($F_{1,24} = 5.8, p = 0.024$) on snail population size (Fig. 3a, b). Averaged across time and predator treatments, snail population size was 27% smaller in the hand-thinning treatment compared to the no-hand-thinning treatment. The lack of a predator effect suggests that the caged predators were not affecting snail population size despite the expression of inducible defenses by the snails.

In the second analysis, we compared the three lethal-predator treatments to the no-predator treatment without hand-thinning to determine the effects lethal predators on snail population size (Fig. 3c). There was an effect of treatment ($F_{3,12} = 65.5, p \leq 0.001$) on snail population size, but not time or the time-by-treatment interaction. Snail population size was lowest in the lethal fish treatment, intermediate in the lethal crayfish treatment, and highest in the no-predator treatment.

Effects on snail mass and morphology

Mass was significantly affected by time, caged predator, and the time $\times$ hand-thinning interaction (Table 3). Snails reared with caged crayfish and caged fish were 30% and 20% larger, respectively, compared to the no-predator treatment (Fig. 2a). In addition, snails reared with caged fish were 9% larger than snails reared with caged water bugs. The time $\times$ hand-thinning interaction was driven by a shift in the direction of the response to hand thinning over time. Averaged across all caged-predator treatments, snails in the hand-thinning treatment were $>24\%$ larger in September and May ($p = 0.023$), similar size in June ($p = 0.203$), and $>25\%$ smaller in July and August ($p \leq 0.015$) compared to the no-thinning treatment (data not shown).

Shell width and thickness were significantly affected by time and caged predators, but exhibited no time-by-treatment interactions (Table 3). Caged water bugs induced 7–9% wider shells compared to all other treatments (Fig. 2b), while caged crayfish induced 27–33% thicker shells compared to all other treatments (Fig. 2c).

Table 3. Results of repeated-measures ANOVAs (Wilks’ $\lambda$) on the effects of caged predators and hand-thinning treatments on snail traits (mass, shell thickness, and shell width) over time. p-values $\leq 0.017$ (indicated in bold) are significant following Bonferroni correction for conducting three simultaneous tests.

<table>
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<th></th>
<th>DF</th>
<th>Mass F</th>
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<th>Shell width p</th>
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<td>Time</td>
<td>4,21</td>
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<td>4.5</td>
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<td>Time $\times$ Predator</td>
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<td>0.195</td>
<td>1.9</td>
<td>0.054</td>
<td>1.6</td>
<td>0.128</td>
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<td>4,21</td>
<td>12.3</td>
<td>$&lt;0.001$</td>
<td>2.4</td>
<td>0.088</td>
<td>0.4</td>
<td>0.831</td>
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<tr>
<td>Time $\times$ Predator $\times$ Hand-thinning</td>
<td>12,56</td>
<td>0.7</td>
<td>0.790</td>
<td>1.0</td>
<td>0.446</td>
<td>0.8</td>
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<tr>
<td>Predator</td>
<td>3,24</td>
<td>4.6</td>
<td>$0.011$</td>
<td>31.1</td>
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<td>0.674</td>
<td>0.3</td>
<td>0.812</td>
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This suggests that the 27% reduction in snail population periphyton biomass did not differ for each predator treatment. Comparing the two thinning treatments, and without hand thinning, there was no evidence of TMIIs predator treatment to the caged-predators treatments (with an impact on the periphyton (Fig. 4). Comparing the no-thinning treatments), confirming that the snails were having prepared to the caged-predator treatments (averaged across times more abundant in the treatment lacking snails com
pared to the caged-predator treatments (i.e. express behavioral plasticity). Once hatchlings started to appear in the population, they would compensate for the movement of adults away from the refuge with movement into the refuge. However, we observed hatchling snails overlapping with the adults early in the experiment when behavioral responses to fish was not maintained; snails did not use refuges after the winter months. However, snails were consistently larger in the fish treatment over time compared to the control. Size-mediated predator avoidance behavior does not appear to be a mechanism driving this pattern (Alexander and Covich 1991). According to this mechanism, large adult snails may increasingly forage outside of refuges whereas younger cohorts predominately use refuges (i.e. express behavioral plasticity). Once hatchlings started to appear in the population, they would compensate for the movement of adults away from the refuge with movement into the refuge. However, we observed hatchling snails overlapping with the adults early in the experiment when behavioral responses to fish were still observed. Previous studies have observed the relaxation of behavioral defenses following the formation of morphological defenses (DeWitt et al. 1999, Relyea 2003, Hammill et al. 2010). However, snails were consistently larger in the caged fish treatment regardless of the observed behavioral responses, suggesting that trait compensation was not occurring. Another possibility is that increases in population size may have caused greater competition forcing snails to alter their behavioral decisions.

**Discussion**

We found clear evidence that snails were forming inducible defenses against caged predators in our study. The morphological responses were maintained over multiple generations whereas the behavioral responses were strong early in the experiment but not evident later in the experiment. Despite the ubiquity of predator-induced defenses, caged predators did not reduce snail population size during the experiment. As a result, we found no evidence of TMII in our experiment, but did find evidence of DMII; lethal crayfish and lethal fish dramatically reduced snail population sizes and this lead to increased periphyton biomass. Below, we discuss the effects of our community treatments on inducible defenses, population dynamics, and indirect effects over multiple snail generations.

The snails in the experiment expressed predator-induced defenses; water bugs induced wide snail shells, crayfish induced thick snail shells, and fish induced greater refuge use. These morphological and behavioral responses to predators are largely consistent with previous work (Alexander and Covich 1991, McCarthy and Fisher 2000, Hoverman and Relyea 2007). Importantly, this is one of the few experiments to track predator-induced defenses of prey over multiple generations (Altwegg et al. 2004, Verschoor et al. 2004). While the morphological responses to water bugs and crayfish were maintained over multiple generations, the behavioral response to fish was not maintained; snails did not use refuges after the winter months. However, snails were consistently larger in the fish treatment over time compared to the control. Size-mediated predator avoidance behavior does not appear to be a mechanism driving this pattern (Alexander and Covich 1991). According to this mechanism, large adult snails may increasingly forage outside of refuges whereas younger cohorts predominately use refuges (i.e. express behavioral plasticity). Once hatchlings started to appear in the population, they would compensate for the movement of adults away from the refuge with movement into the refuge. However, we observed hatchling snails overlapping with the adults early in the experiment when behavioral responses to fish were still observed. Previous studies have observed the relaxation of behavioral defenses following the formation of morphological defenses (DeWitt et al. 1999, Relyea 2003, Hammill et al. 2010). However, snails were consistently larger in the caged fish treatment regardless of the observed behavioral responses, suggesting that trait compensation was not occurring. Another possibility is that increases in population size may have caused greater competition forcing snails to alter their behavioral decisions.
Snails may be allocating resources to reach a size refuge from fish predation but due to behavioral responses that reduce foraging rates, attaining the size refuge requires at least a month to reach. Together, these results demonstrate that short-term observations of predator-induced plasticity may not reflect long-term dynamics for some predator–prey interactions.

Although caged predators altered the traits of the snails, there was no evidence that caged predators reduced snail population size. The lack of an impact on population size may be the consequence of the relatively high reproductive output of snails coupled with density-dependent population growth (Dillon 2000). Our laboratory experiments have shown that snails exposed to caged water bugs and crayfish are capable of producing 50–200 eggs per snail in just four months (Hoverman et al. 2005). Despite the high reproductive output of the snails, snail population size rarely exceeded 200 snails (i.e. log_{10}-population size of 2.3) and peaks in population size were not sustained suggesting that the snail populations

In support of this mechanism, there was a negative correlation between refuge use and population size within the fish treatments over time. However, alternative mechanisms include that: 1) snails, regardless of size, may have habituated to the presence of fish over time and increasingly foraged outside of the refuge, 2) later snail generations may have lost sensitivity to fish cues due to constant exposure starting in the egg, and 3) the removal of fish during the winter altered behavioral responses the following spring. Although our study cannot assess the importance of these mechanisms, it is clear that this shift in behavior has important implications for addressing the potential for indirect effects. We also found that caged fish and crayfish consistently induced larger snail size compared to the no-predator treatment. While we have previously observed this response to caged crayfish, the response to caged fish is in the opposite direction compared to our previous experiments (Hoverman and Relyea 2007, 2008). The main difference between our past research and the present study is the short time scale over which responses were observed (<1 month). Snails may be allocating resources to reach a size refuge from fish predation but due to behavioral responses that reduce foraging rates, attaining the size refuge requires at least a month to reach. Together, these results demonstrate that short-term observations of predator-induced plasticity may not reflect long-term dynamics for some predator–prey interactions.

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Figure 3. The effects of (A) no-hand-thinning, (B) hand-thinning, and (C) lethal predator treatments on log_{10}-transformed snail abundance (number of individuals per sample) over time. The no-predator treatment without hand thinning is shown in panel (C) for comparison. Data are least-squares means ± 1 SE. Symbols are as described in Fig. 1. Predators were removed from the tanks during the winter (December to March).
were displaying density-dependence. The negative correlation between periphyton biomass and snail population size further supports our assertion that high densities of snails were competing for limited resources. Also, while our previous experiments have observed delayed time to reproduction with caged predators, this delay was generally less than 13 days (Hoverman et al. 2005). Considering our experiment was conducted for 1 year, a relatively brief delay in time to reproduction appears to be insufficient to reduce long-term snail population size under mesocosm conditions.

Although many environmentally induced traits can affect the growth and reproduction of organisms, it has never been clear whether these changes affect population dynamics. The magnitude of life history costs associated with inducible defenses, the reproductive output of individual prey, and the carrying capacity of the environment will largely determine whether long-term population effects are observed. Investigations of the long-term effects of predator induction on population dynamics provide equivocal support for the induction of smaller population sizes (Gilbert 1980, Black 1993, Kusch and Kuhlmann 1994, Riessen 1999, Nelson et al. 2004). While short-term plasticity experiments often conclude that induced traits should affect future survival and reproduction, long-term studies are needed to determine whether such extrapolation is reasonable.

We found no evidence of TMIIs in our study system, which is counter to the growing body of literature demonstrating predator-induced TMIIs in a variety of different systems including streams (Huang and Sih 1991, Peckarsky and McIntosh 1998), ponds (Turner 1997, Relyea 2000), old fields (Beckerman et al. 1997, Gelwick 2000, Belovsky et al. 2011), tropical forests (Gastreich 1999), and intertidal communities (Trussell et al. 2002). While the absence of TMIIs within the water bug communities was expected, these results were not expected in the crayfish and fish communities. However, our results support theoretical models that have demonstrated the absence of TMIIs when inducible defenses do not reduce resource consumption or population size in three-species food chains (Abrams 2008). Based on the results from the lethal predator treatments, snail population size would have to be reduced by $>75\%$ for the threat of predation to initiate a TMII over the long term. Ultimately, the magnitude of TMIIs may jointly depend on the costs associated with altering foraging rates and resource availability (Van Buskirk 2000, Luttbeg et al. 2003, Wójdak and Luttbeg 2005). At low resource levels, the magnitude of TMIIs may be diminished due to increased risk taking behavior by prey faced with starvation (Luttbeg et al. 2003). Alternatively, prey may respond more to predation risk at low resource levels if opportunity costs associated with risk taking behavior are lower leading to strong TMIIs (Van Buskirk 2000). In support of this latter theoretical prediction, Wójdak and Luttbeg (2005) documented a negative relationship between the strength of a behaviorally-mediated TMII and initial resource levels in a freshwater food chain. While our experiment did not manipulate initial resource levels to assess the above theoretical predictions, resource levels may have been reduced to levels that minimized the likelihood of observing a TMII. Despite observations from short-term experiments that our predators impact the behavior, time to reproduction, or egg production of snails, it is clear that these responses have minimal effects on population dynamics and trophic cascades within our simple linear food chains.

We observed strong DMIIs associated with lethal crayfish and fish but not water bugs, which supports our predictions based on the differences in predation rate between the predators (Hoverman and Relyea 2007). Lethal crayfish and lethal fish reduced snail population size by $>95\%$ relative to the comparable caged-predator treatments that lacked hand thinning. These reductions in snail population size caused a 2- to 15-fold increase in periphyton biomass. Both predators may also have contributed to periphyton growth due to consumer-mediated nutrient recycling (Vanni and Layne 1997). By increasing nutrient inputs or altering nutrient stoichiometry, predators can alter the abundance and structure of producer communities (McCullum et al. 1998). The difference in the magnitude of the DMI between the lethal fish and lethal crayfish treatments is most likely a consequence of crayfish, which are omnivorous, consuming greater amounts of periphyton as snail population size decreased.

Figure 4. The effect of treatments on log$_{10}$-transformed periphyton biomass. The predator treatments are: no predator (NP), water bug (W), crayfish (C), and fish (F). Data (least-squares means ± 1 SE) are averaged over time. Treatments sharing lower case letters are not significantly different from each other based on pairwise comparisons using Fisher’s LSD test ($p > 0.05$).
We also assessed DMIIs in our system using hand-thinning treatments that were based on predation rates typical of water bugs. We observed a 27% reduction in snail population size with hand thinning across the predator treatments, yet no indirect effect on periphyton biomass was observed. The lack of a DMI was likely driven by compensatory growth of the remaining snails; early in the experiment snails in the thinning treatment were 25% larger than snails in the no thinning treatment. Due to logistical constraints, we could not apply additional hand-thinning rates to mimic the effects of lethal fish and crayfish, which would be expected to lead to significant DMIIs. Additional hand-thinning treatments also would provide insights into whether productivity (i.e. rate of periphyton growth) played a role in the lack of a DMI. Although the presence of snails in the tanks reduced periphyton biomass, high rates of periphyton productivity may have sustained snail populations due to feedbacks between producers, prey and predators (Vanni and Layne 1997). While the hand-thinning treatments provided little support for DMIIs in the system, we found support for DMIIs in the negative correlation between periphyton biomass and snail population size. This demonstrates that periphyton biomass was directly associated with fluctuations in snail population size.

Overall, DMIIs appear to be the dominant factor driving indirect interactions within our system. However, our ability to assess interactions between TMIIs and DMIIs was limited by our implementation of a single thinning treatment. Using a broader range of hand-thinning rates and caged predator densities, Peacock and Werner (2001) were able to demonstrate that the total indirect effect of lethal dragonflies on tadpole communities could be attributed to independent effects of density and trait changes as well as their interaction. While the absence of TMIIs in our system confirms that trait changes had no independent effect on the observed cascade in the lethal predator treatments, there is the possibility that TMIIs could manifest as snail densities are reduced. Thus, the implementation of a gradient in hand-thinning rates including rates that mimic lethal crayfish and fish will help to determine if TMIIs and DMIIs interact within our system.

Conclusions

It is clear that environmental variation can have dramatic effects on the phenotypes expressed by nearly all organisms. While a wealth of information regarding the ecological and evolutionary importance of phenotypic plasticity has been obtained from short-term experiments, there is a need for more long-term studies that explore its role in structuring communities. In particular, multi-generation, long-term studies that track population parameters (e.g. t) of the species within communities will make significant strides towards our understanding of whether phenotypic plasticity has long-term effects on community structure and its relative importance to changes in species density. Moreover, the emerging physiological stress framework may provide greater insight into the general mechanisms underlying the effect of predator-induced stress on prey populations and food web interactions (Sheriff et al. 2009, Hawlena and Schmitz 2010a, b). Studies that compare TMIIs and DMIIs across communities with different food web topologies also will be vital for addressing how indirect effects can be transmitted through different communities. Given that many species do not solely express behavioral plasticity, there also must be a greater emphasis on indirect effects that are transmitted by means other than behavior. This is especially important in more complex communities in which species may be interacting in a multitude of ways. In sum, research directed towards addressing the long-term consequences of predator–prey interactions within communities will help to reveal whether the conclusions and predictions generated from short-term experiments are applicable on ecological and evolutionary timescales.

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Appendix 1

The effects of predators and thinning treatments on log_{10}-transformed periphyton biomass over time (means ± 1 SE). Closed circles, open circles, open triangles, open diamonds represent the no-predator, water bug, crayfish and fish treatments, respectively. Predators were caged within the no-hand-thinning (A) and hand-thinning treatments (B). The no-snail treatment (open squares) is shown in each panel for comparison.