

Predator-induced defences in offspring of laboratory and wild-caught snails: prey history impacts prey response

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ABSTRACT

Questions: Do behavioural, morphological, and life-history responses to predators differ among offspring of laboratory and newly captured snails from the same field site? The risk allocation hypothesis states that prey should balance predator avoidance and feeding time according to the degree of predation risk. Are there patterns in behavioural, morphological, and fitness traits that provide insight into predictions of this hypothesis?

Organism: We used offspring from two parentage lines of the freshwater gastropod, *Physa pomilia*, obtained from the exact same stream location but either (1) maintained in the laboratory for 3–4 generations or (2) newly field captured.

Methods: We employed a caged-snail design in which offspring obtained from the laboratory culture and the newly field-captured snails were both exposed to a crayfish predator cue plus alarm cue for 40 days. We assessed behavioural, morphological, and fitness-related traits and conducted a short-term predator avoidance assay at the end of the 40-day experiment.

Results: Over 40 days, offspring of newly caught and laboratory snails displayed similar proportions of predator avoidance behaviour on average. However, offspring of newly caught snails decreased their predator avoidance behaviour over time, as predicted by the risk allocation hypothesis, while offspring of laboratory snails did not. These differences were confirmed with a short-term behavioural assay. Offspring of newly caught snails also developed thicker shells with longer and narrower apertures than the offspring of laboratory snails, making them more predator resistant, which may have explained differences in behavioural responses. Predator cues strongly reduced total egg mass production. Egg mass production was greater in the offspring of laboratory snails under predator-free conditions but greater in the offspring of newly caught snails when exposed to predator cues. The results highlight the importance of population history in studies of phenotypic plasticity.

Keywords: non-consumptive effect, phenotypic plasticity, Physid, predator–prey interaction, risk allocation hypothesis.

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INTRODUCTION

Predator–prey interactions are important in the structure and function of communities (Abrams *et al.*, 1996; Chase, 1999; Peckarsky *et al.*, 2008; Boeing and Ramcharan, 2010). Predators can reduce prey abundance directly or indirectly through non-consumptive effects (Lima, 1998). Non-consumptive effects of predators can manifest in prey as alterations in morphology, physiology, life history, and/or behaviour, all of which can impact prey energy expenditures. Aquatic invertebrates from a number of taxa show a wide variety of inducible predator defences, including defensive spine formation in freshwater rotifers (Stemberger and Gilbert, 1984), neck teeth and helmets in cladocerans (Dodson, 1989), vertical migration in zooplankton (Bollens and Frost, 1991), changes in fish body shape (Brönmark and Pettersson, 1994), avoidance and altered habitat use in snails (Turner *et al.*, 1999; Lewis, 2001), and changes in shell shape and thickness in snails (DeWitt, 1998; DeWitt *et al.*, 1999; Covich, 2010).

For inducible predator defences to be most effective, prey must be good risk assessors, as induced defences can and do incur a cost (Agrawal *et al.*, 1999). The risk allocation hypothesis (Lima and Bednekoff, 1999) states that prey should weigh competing risks under conditions of temporally varying predation threat to balance the allocation of energy and time to predator avoidance and feeding. In addition, prey in consistently high-risk environments should display relatively less anti-predator behaviour as interactions with predators become more frequent or lengthy (Lima and Bednekoff, 1999). A recent review of the risk allocation hypothesis found mixed empirical support (Ferrari *et al.*, 2009). However, when prey were given more time to assess risk via relatively longer exposure to predators or when prey were energy limited, the hypothesis was more generally supported. Although Ferrari *et al.* (2009) pointed to elements of experimental design (e.g. duration, resource limitation) that impact whether experiments show support for the risk allocation hypothesis, other biological and environmental factors can alter how prey perceive and/or respond to environmental cues. These factors likely contribute to the wide variation in responses seen in the literature.

Population of origin, for example, affected the expression of inducible defences in marine snails threatened with predatory crab cues. Snails from predator-free environments showed a diminished response compared with snails from habitats with predators (Trussell and Nicklin, 2002). In Physid snails, Turner *et al.* (2006) found that wild-caught individuals responded more strongly to predator and alarm cues than captive reared snails. Even animals from the same source population, however, can display differences in predator-induced defences. Agrawal *et al.* (1999) found that offspring from adult *Daphnia magna* exposed to predator cues displayed stronger predator-induced defences via longer helmet lengths than offspring from unexposed adults. More recently, Storm and Lima (2010) found that grasshopper mothers exposed to predatory wolf spiders imparted an enhanced response to predators to their offspring.

Several species of freshwater snails show behavioural, morphological, and life-history changes in response to predator cues and are excellent models for studying predator-induced defences. Physid snails, for example, alter foraging activity and habitat use in response to predator and alarm cues (Alexander and Covich, 1991) and can also display a delayed time to reproduction (Crowl and Covich, 1990). Furthermore, Physids can increase shell thickness and alter shell morphology in response to predators (DeWitt *et al.*, 1999; Auld and Relyea, 2008).

In the present study, we were interested in the expression of predator-induced defences among freshwater *Physa pomilia* snails from the same source population and location that were either (1) maintained in the laboratory under constant, predator-free conditions for

several generations, or (2) recently obtained from wild-caught parents. We employed a relatively long-term predator cue exposure (40 days) followed by a short behavioural assay to evaluate the responses of prey to predator cues. We hypothesized that snails from wild-caught parents would display stronger predator-induced defences than laboratory-reared snails but that both would show a diminished predator avoidance response through time in support of the risk allocation hypothesis.

METHODS

Physa pomilia is a freshwater habitat generalist, pulmonate snail found in North America and recently confirmed as a species separate from the common *Physa acuta* (Dillon *et al.*, 2007). *Physa pomilia* were collected from the North Fork of the Double Mountain Fork of the Brazos River (NFD MF-BR) near Lubbock, Texas and the species was confirmed by penile examination (R. Dillon, personal communication). Crayfish, which prey on snails, occur in the same stream. The laboratory *P. pomilia* line was started from approximately 100 egg masses collected in March 2009. Hatchling snails from these egg masses were mixed and reared in the laboratory in glass aquaria at approximate densities of 1 snail per 100–200 mL laboratory water with water changes once or twice per week. Snails were housed in laboratory water (3.0 g CaSO₄, 3.0 g MgSO₄, 0.2 g KCl, and 4.9 g NaHCO₃ to 50 L deionized water) and fed cooked romaine lettuce *ad libitum*. The laboratory temperature was 22 ± 1°C with a 14:10 light-to-dark cycle. The laboratory snail culture, once established, was maintained with about 300–400 freely breeding adults in 8–12 aquaria at any one time. Snails were propagated for 3–4 generations before use as parents of experimental animals, hereafter referred to as ‘LAB snails’, in this study. During laboratory culturing, snails were never exposed to predators, predator cues or conspecific alarm cues (crushed snails). In June 2010, approximately 50 egg masses were collected from the LAB snails and placed in a single aquarium separate from the laboratory snail culture for hatching and rearing before use in the experiment.

In June 2010, a total of 70 adult *P. pomilia* snails were collected from the same exact location in the NFD MF-BR used to obtain LAB snails. These adults were maintained for approximately one week under the laboratory conditions described above before collection of egg masses for experimentation. Over the same 2-day period in which egg masses were collected from the LAB snails, approximately 50 egg masses were collected from adult field-collected snails and maintained in a single aquarium for hatching and rearing. These snails are hereafter referred to as offspring of field-caught or ‘OFC snails’. Both LAB and OFC snails were reared for approximately one month after hatching under predator-free laboratory conditions prior to use in the experiments.

Experimental design

The LAB and OFC snails were exposed either to crayfish (*Procambarus clarkii*) predators plus alarm cues (crushed snail) or predator-free conditions in a factorial design. All snails were maintained in 40-L glass aquaria using a ‘caged-snail’ design for 40 days. The experimental duration encompassed juvenile and adult snail life stages. Snail cages were 500-mL plastic, BPA-free containers with two 6 × 4 cm mesh-covered openings, which allowed water flow into and out of the cages. Snail cages were loosely covered to prevent snails from escaping. Crayfish in the aquaria with snail cages were free to roam but could

not access snails and were fed by crushing three adult snails in the tanks, three times per week at 10 am. The combination of predator (crayfish) plus alarm (crushed snail) has been shown to elicit strong predator-induced defences in Physid snails (Alexander and Covich, 1991; Turner *et al.*, 1999) and is hereafter referred to as 'predator cues' for brevity. Aquaria were filled to a depth of 5 cm, which left approximately 1.5 cm of space in the snail cages above the water line. Laboratory water was used throughout with complete water changes every week. Two cages were placed in each aquarium, one of which contained five OFC snails and the other five LAB snails. Snails had a shell length of 3–4 mm before addition to cages. Twelve aquaria were used, six with crayfish and six without, for a total of 120 snails (30 per treatment). Aquaria were maintained in the laboratory at $22 \pm 1^\circ\text{C}$ with a 14:10 light-to-dark cycle and were covered with opaque plastic sheeting on three sides and the top to minimize visual disturbance of crayfish. Snails were fed cooked romaine lettuce *ad libitum*.

Behavioural and life-history observations

Three times per week, all cages were observed and the number of snails at the water line or above was noted. Crawl-out behaviour among Physid snails is a common avoidance behaviour, particularly in response to crayfish predators (Turner *et al.*, 1999). Observations were taken 2 h after crayfish were each fed crushed adult snails. Once per week, during water changes, egg masses were counted and removed to estimate the onset of reproductive maturity and reproductive output (DeWitt, 1998; Auld and Relyea, 2008).

We also conducted a short behavioural assay to provide additional insight into how the LAB and OFC snails from the 40-day experiment responded to a spike in predator cues. According to the risk allocation hypothesis, snails exposed to predator cues for 40 days would be expected to show less of a predator avoidance response than control (predator-free) snails. Upon completion of the 40-day caged-snail predator exposures, snails were removed from cages and placed individually in 425-mL glass jars with 300 mL of laboratory water. Individual snails were taken from all cages in a uniform but haphazard manner such that 20 individual snail replicates were generated for the LAB and OFC snails for each predator treatment for a total of 80 snails housed individually. Snails were maintained in these conditions and fed cooked romaine lettuce for 2 days prior to the behavioural assay. After 2 days, the water was changed and snails acclimated for 2 h before the addition of predator cues. Predator cues were created by crushing 16 snails in 450 mL of water obtained from three crayfish tanks used in the 40-day experiment in which crayfish were still present and being fed crushed snails. The behavioural assay began with a 5-mL addition of the predator + alarm cue solution to each individual snail container. Observations on snail location were recorded every 5 min for 2 h after the addition of predator cues (Dalesman *et al.*, 2006). As in the behavioural observations above, snails occupying the water line or the space above were considered to exhibit anti-predator behaviour. We did not include a sham control because previous studies in our laboratory have shown a lack of snail avoidance behaviour when only laboratory water was added to jars.

Morphological responses

Morphological changes in response to predator cues in Physid snails can include increased shell thickness (Auld and Relyea, 2008) and alterations in shell shape (DeWitt, 1998; DeWitt *et al.*, 1999). At

completion of the caged snail predator exposure and the short-term behavioural assay, all experimental snails were individually weighed to the nearest 0.1 mg (total wet mass) after blotting dry and sitting out of the water for 1 min. Total shell length, height, and shell thickness at the leading edge of the shell aperture were measured with digital calipers to the nearest 0.01 mm. Shell height was measured by placing the snail aperture flush to one side of the caliper. In addition, each snail was digitally photographed using a Leica MZ9.5 dissecting scope. Images were later analysed using ImageJ software version 1.44 (National Institutes of Health, 2010). Shell length, width (longest distance perpendicular to length with aperture down), and height, as well as aperture length (longest axis measurement) and width (perpendicular to length) were measured from digital images. Both calipers and digital image analysis for length and height were used as a comparison of the two methods, which have both been used in previous studies. Specifically, we wished to determine if caliper measurements that can be conducted with limited equipment are suitable for capturing predator-induced morphological changes for future studies.

Statistical analyses

Crawl-out behaviour during the 40-day experiment was analysed as the proportion of snails per cage observed at or above the waterline per observation period. We used a repeated-measures analysis of variance (ANOVA) to determine effects of line (OFC or LAB), predator cue (present or not) and time, and their interactions. The proportion of snails per cage displaying anti-predator behaviour was arcsine square root transformed before analysis, although all figures were back-transformed to proportions for presentation. Results from the 2-h behavioural assay were analysed as the proportion of observation periods in which crawl-out behaviour was observed for each individual snail using ANOVA (Dalesman *et al.*, 2006), with line (OFC or LAB) and predator cue (present or not during 40-day experiment) as factors. Proportions were arcsine square root transformed for analysis but figures represent actual proportions. The number of egg masses produced was analysed using a repeated-measures ANOVA on the number of egg masses produced per snail per cage, with time, snail line, and predator cue as factors.

Shell thickness at the end of the 40-day experiment was analysed using a linear mixed effect model with line (OFC or LAB) and predator cues (present or absent) as fixed effects and tank as a random effect (Zuur *et al.*, 2009). Interactions between terms were also considered. Shell thickness was analysed by first regressing shell thickness on total mass and saving residuals for the analysis, thereby controlling for the effects of mass (Relyea, 2002; Mandrillon and Saglio, 2009). To evaluate size differences at the end of the 40-day experiment, shell length, width, height, and total mass were analysed using a mixed effect model with predator cue and line as fixed effects and tank as a random effect, but not controlling for mass as above. Also, to evaluate the effect of predator cues and snail line on shell and aperture shape, we used the square root of the ratio of length-to-width for both the shell and aperture by first controlling for mass and using a mixed effect model as above. The ratio of shell length-to-width is referred to as the aspect ratio, which provides a reasonable estimate of shell shape and relates positively to crushing resistance (DeWitt, 1998; DeWitt *et al.*, 1999). We tested the difference between caliper measurements and measurements from digital images for shell length using ANOVA with line and predator cue as factors. Measurements taken by calipers and from digital images for shell length ($F = 0.001$, $P = 0.975$) were not different, so all measurement analyses except shell thickness (no image data available) were performed using

digital image data. All statistical analyses were conducted in R (R Development Core Team, 2010) with $\alpha = 0.05$.

RESULTS

Behavioural observations

In the 40-day experiment, a greater proportion of predator-exposed snails displayed crawl-out behaviour than controls ($F = 196.959$, $P < 0.001$; Fig. 1). While line alone was not significant ($F = 0.395$, $P = 0.530$), there was a significant predator cue $\times$ line $\times$ time effect ($F = 7.322$, $P = 0.007$; Fig. 1). In the absence of predator cues, OFC and LAB snails rarely crawled out. In contrast, when predator cues were present, OFC snails' initially strong crawl-out response declined over time, while LAB snails' crawl-out behaviour was consistent or slightly increased over time (line $\times$ time in predator-exposed snails only:

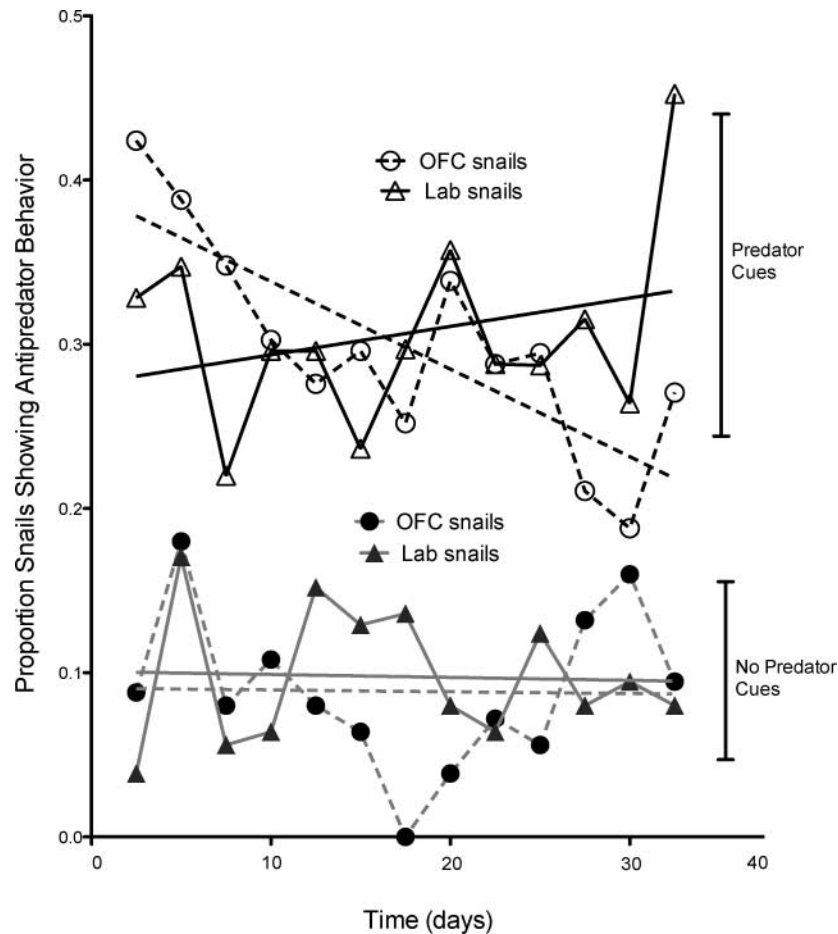


Fig. 1. Proportion of snails displaying predator avoidance behaviours in response to predator cues (crayfish + alarm) for 40 days. Regression lines show trends.

$F = 15.713$, $P < 0.001$). To further evaluate these results, we conducted a repeated-measures ANOVA on the first three time points and the last three time points to determine the effects of line and time. Initially, OFC snails responded more strongly to predator cues than did LAB snails (first three time points: $F = 4.942$, $P = 0.033$) but towards the end of the experiment, OFC snails showed less predator avoidance than LAB snails (last three time points: $F = 12.342$, $P = 0.001$; Fig. 1).

The diminished predator avoidance behaviour in predator-exposed OFC snails observed towards the end of the 40-day experiment was also seen in the 2-h behavioural assay (line $\times$ predator cue: $F = 4.213$, $P = 0.044$; Fig. 2). OFC snails exposed to predator cues for 40 days showed less avoidance behaviour than control (predator-free) OFC, control LAB, and predator-exposed LAB snails.

Morphology

At the end of the 40-day experiment, OFC snails had greater total wet mass, shell length, shell width, shell height, and mass-corrected shell thickness than LAB snails (Table 1, Fig. 3a, b). OFC snails also had longer, narrower apertures than LAB snails (aperture length-to-width ratio, Table 2, Fig. 3d). Both OFC and LAB snails exposed to predator cues had thicker (Table 2, Fig. 3b), rounder (shorter and wider; shell length-to-width ratio, Table 2, Fig. 3c) shells with longer and narrower apertures (aperture length-to-width ratio, Table 2, Fig. 3d) compared with control snails.

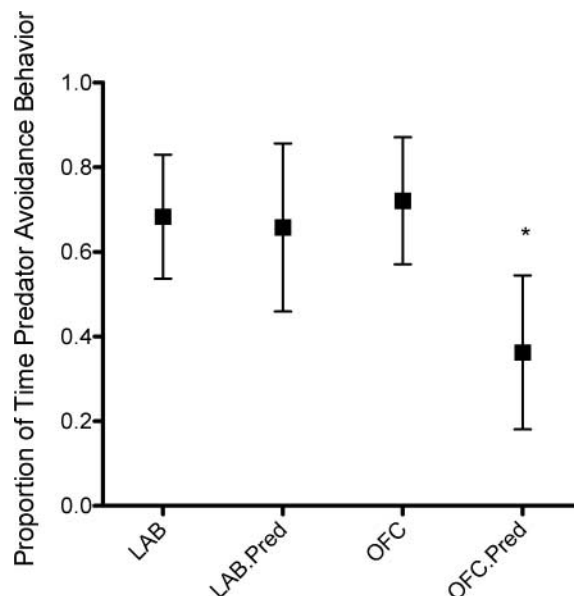


Fig. 2. Proportion of snails displaying predator avoidance behaviour during a 2-h behavioural assay in response to predator cues. Proportions represent averages for 20 snails from each treatment where, for each snail, we calculated the number of observation periods in which the individual displayed an avoidance behaviour by the total number of observation periods. LAB.PRED and OFC.PRED represent the snails exposed to predator cues during the 40-day experiment from the LAB and OFC populations, respectively. Error bars represent 95% confidence limits.

Table 1. Results from mixed effect model analysis of size characteristics of *P. pomilia* for OFC and LAB snails exposed to predator cues

Morphological characteristic	Mean square	<i>F</i>	<i>P</i>
<i>Total wet mass</i>			
Line	0.003	6.736	0.011
Predator cues	0.001	1.609	0.207
Line × Predator cues	0.0000	0.053	0.819
<i>Shell length</i>			
Line	9.043	7.481	0.007
Predator cues	0.365	0.302	0.584
Line × Predator cues	0.060	0.0500	0.824
<i>Shell width</i>			
Line	4.281	11.046	0.001
Predator cues	1.424	3.673	0.058
Line × Predator cues	0.015	0.04	0.843
<i>Shell height</i>			
Line	3.037	11.271	0.001
Predator cues	0.876	3.250	0.074
Line × Predator cues	0.006	0.022	0.883

Note: Population and predator cues (present or not) were fixed factors while tank was a random factor. Degrees of freedom for factors = 1.

Reproduction

Egg mass production increased with time ($F = 202.386$, $P < 0.001$; Fig. 4) because snails were not reproductively mature at the start of the experiment. Predator-exposed snails produced fewer egg masses than unexposed snails ($F = 58.349$, $P < 0.001$). Reproductive output increased more quickly in control (predator-free) snails than predator-exposed snails (predator cue × time: $F = 10.368$, $P = 0.001$). In the presence of predator cues, OFC snails produced more egg masses per snail per cage (mean ± s.d.: 1.38 ± 1.07) than LAB snails (1.19 ± 1.16). The reverse was true in the absence of predator cues; LAB snails produced more egg masses (2.43 ± 1.83) than OFC snails (2.10 ± 1.45) (predator cue × line: $F = 4.129$, $P = 0.043$).

DISCUSSION

In general, LAB and OFC snails showed similar and predictable responses to predator cues, although there were important differences between the lines. Snails from both lines exposed to predator cues for 40 days showed an increased frequency of predator avoidance behaviour (crawl-out) compared with predator-free snails. OFC snails exposed to predator cues showed a strong initial avoidance response that diminished with time in accord with the risk allocation hypothesis (Lima and Bednekoff, 1999). In contrast, LAB snails showed a more consistent avoidance response to predator cues that exceeded that of OFC snails by the end of the 40-day exposure. Furthermore, in the short-term behavioural assay, predator cue

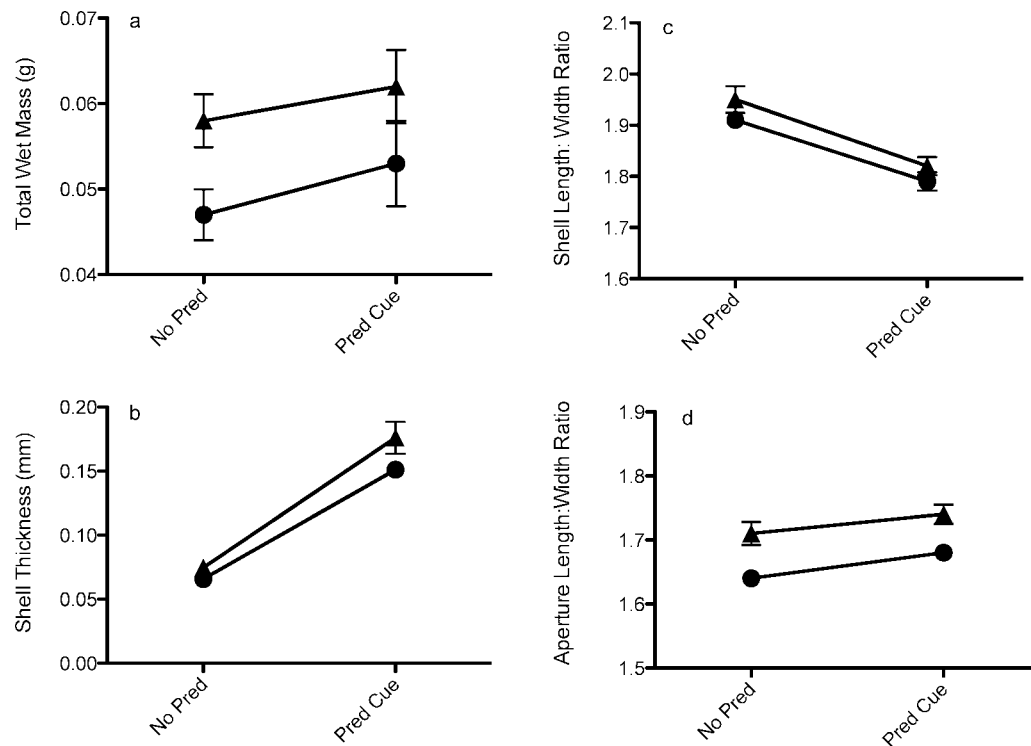


Fig. 3. Morphology of OFC (triangle) and LAB (circle) snails exposed to predator cues. All figures represent measurements obtained from digital images of snails for (a) total wet mass, (b) shell thickness, (c) aspect ratio (shell length-to-width), and (d) the ratio of aperture length-to-width. Statistical analyses of shell thickness and shell and aperture shape were conducted on mass-corrected morphological measurements. Error bars represent standard deviation.

exposed OFC snails displayed less avoidance behaviour in response to predator cues than control (predator-free) OFC, control LAB, and predator cue exposed LAB snails. This indicates that the combination of prey line (OFC or LAB) and prey experience (40-day predator cues or not) impacted how prey responded to predators. The diminished behavioural response of OFC snails exposed to predator cues in both short- and long-term experiments suggests that these snails adjusted their risk perception as a result of long-term exposure to predator cues whereas LAB snails did not.

Predator-exposed snails of both lines also produced fewer egg masses than snails in predator-free conditions, probably because they spent less time foraging (Trussell *et al.*, 2003; Turner, 2004). However, OFC snails produced more egg masses than LAB snails when exposed to predator cues, which may have been the result of diminished predator avoidance seen in the latter part of the 40-day experiment. Improved fitness (egg mass production) would lend further support to the risk allocation hypothesis, as diminished predator avoidance should allow for increased foraging and reproduction.

LAB and OFC snails exposed to predator cues had thicker and rounder (shorter and wider) shells, both of which increase the force needed to crush shells (DeWitt, 1998; DeWitt *et al.*, 1999, 2000; Lakowitz *et al.*, 2008). Snails exposed to predator cues also produced longer and

Table 2. Results from mixed effect model analysis of mass-corrected morphological characteristics for OFC and LAB snails exposed to predator cues

Morphological characteristic	Mean square	<i>F</i>	<i>P</i>
<i>Shell thickness</i>			
Line	0.018	9.021	0.003
Predator cues	0.218	107.689	<0.001
Line × Predator cues	0.001	0.987	0.405
<i>Shell length-to-width ratio</i>			
Line	0.003	2.333	0.130
Predator cues	0.052	35.648	<0.0001
Line × Predator cues	0.000	0.005	0.944
<i>Shell aperture length-to-width ratio</i>			
Line	0.013	14.66	<0.001
Predator cues	0.007	7.815	0.006
Line × Predator cues	0.000	0.052	0.819

Note: Line and predator cues (present or not) were fixed factors while tank was a random factor. Degrees of freedom for factors = 1.

narrower apertures, which defend against shell-entry predators such as crayfish (DeWitt *et al.*, 2000). OFC snails produced relatively thicker shells with longer and narrower apertures compared with LAB snails, indicating that the OFC snails produced a more predator-resistant morphology. These morphological changes may reduce predation risk, which would have allowed OFC snails to spend less time avoiding predators compared with LAB snails. In other experiments, Physids have shown similar trait compensation in which larger snails displayed less of an avoidance response to crayfish predator cues than smaller, more vulnerable snails (DeWitt *et al.*, 1999). In addition, whereas smaller larval ringed salamanders (*Ambystoma annulatum*) displayed anti-predator behaviours, larger larvae did not because gape-limited newt predators could not consume them (Mathis *et al.*, 2003).

There are several possible explanations for the observed differences in LAB and OFC snails. We cannot entirely discount that selection in the LAB or OFC snails occurred over the year between collections, especially since OFC snails seemed to have grown better in our experimental conditions versus the LAB snails in predator-free conditions. Previous studies have also noted effects of laboratory conditions on specific traits of populations maintained for many generations (Quintana and Prevosti, 1990). However, while the change in conditions from the field to the laboratory was potentially stressful, laboratory conditions were kept as benign as possible based on our previous experience raising *P. pomilia*. As a result, there was not strong selection for the 3–4 generations that LAB snails were maintained before experimentation (Huey and Rosenzweig, 2009). Hence, we do not think that selection played a significant role in the differences between LAB and OFC snails. Genetic drift was also likely not a factor because we initiated our LAB culture from a large number of egg masses and maintained a large number (300–400) of individuals.

The differences in predator avoidance behaviours, morphology, and reproduction observed in the 40-day experiment might perhaps be explained by trans-generational effects

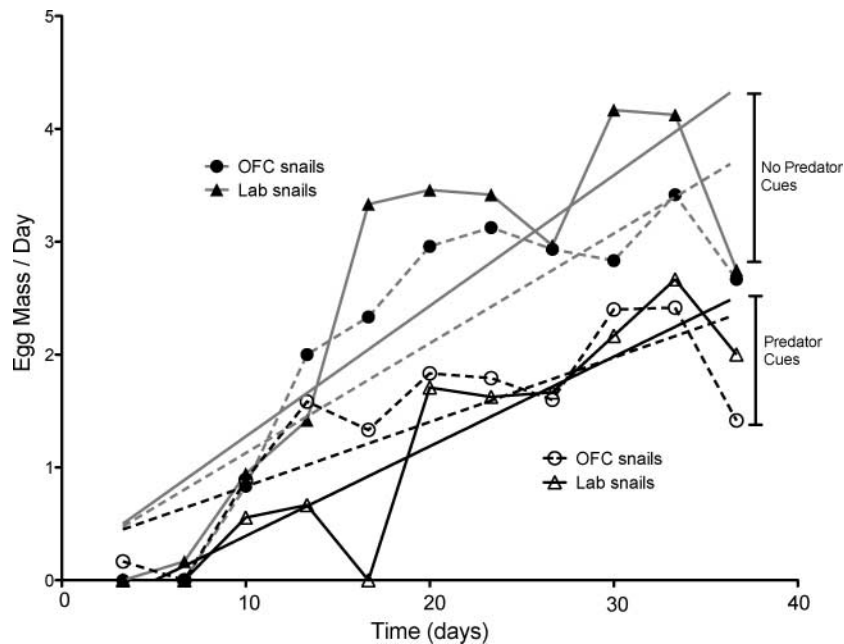


Fig. 4. Effect of predator + alarm cues on reproductive output through time for OFC (circles, dashed lines) and LAB (triangles, solid lines) snails from the 40-day experiment. Reproductive output is number of egg masses per container. Linear regression was used to plot trend lines.

(Agrawal *et al.*, 1999; Storm and Lima, 2010). Several studies have shown differences in predator-induced defences between populations of snails with different predator environments in the field (Trussell, 2000; Åbjörnsson *et al.*, 2004). These differences in defence may be caused by both genetic differences and maternal experiences, which impact offspring responses to stressors such as predators (Agrawal *et al.*, 1999; Storm and Lima, 2010; Giesing *et al.*, 2011) and abiotic factors (Räsänen *et al.*, 2003; Marshall, 2008). In our experiment, parents exposed to predators in the field may have imparted differential abilities to respond to predators, both behaviourally and via shell growth, compared with mothers from the constant condition, predator-free LAB snails.

Few studies have directly compared responses to predators in laboratory-reared and field-isolated prey and none has evaluated the response of their offspring as here. Turner *et al.* (2006) reported that field-caught Physid snails showed stronger anti-predator behaviour than snails housed in predator-free laboratory conditions for 10 months, similar to our results at the start of the 40-day experiment. Gallie *et al.* (2001) did not find a difference in anti-predator behaviour among laboratory-reared and wild-caught American toad tadpoles (*Bufo americanus*). However, predator-experienced tadpoles had lived under natural conditions for only 10 days longer than the laboratory-reared tadpoles and both were presumably from the same cohort of mothers. Increasing the length of time prey are in predator-free conditions may increase the likelihood of observing differences between field and laboratory prey in response to predators, although this hypothesis remains to be tested.

In conclusion, our results suggest that while offspring of snails from laboratory cultures and freshly caught snails responded similarly to predator cues, they displayed clear and

important differences related to prey history and potentially the result of trans-generational effects. Therefore, caution is warranted in interpreting results from studies of predator-induced defences in laboratory stocks of prey species. Lastly, the current model system may prove useful in improving our understanding of how historical effects impact offspring responses to environmental conditions and may offer the opportunity to explore the potential adaptive value of trans-generational effects.

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REFERENCES

- Abrams, P.A., Leimar, O., Nylin, S. and Wiklund, C. 1996. The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. *Am. Nat.*, **147**: 381–395.
- Åbjörnsson, K., Hansson, L.-A. and Brönmark, C. 2004. Responses of prey from habitats with different predator regimes: local adaptation and heritability. *Ecology*, **85**: 1859–1866.
- Agrawal, A.A., Laforsch, C. and Tollrian, R. 1999. Transgenerational induction of defences in animals and plants. *Nature*, **401**: 60–63.
- Alexander, J.E. and Covich, A.P. 1991. Predator avoidance by the freshwater snail *Physella virgata* in response to the crayfish, *Procambarus simulans*. *Oecologia*, **87**: 435–442.
- Auld, J.R. and Relyea, R.A. 2008. Are there interactive effects of mate availability and predation risk on life history and defence in a simultaneous hermaphrodite? *J. Evol. Biol.*, **21**: 1371–1378.
- Boeing, W.J. and Ramcharan, C.W. 2010. Inducible defences are a stabilizing factor for predator and prey populations: a field experiment. *Freshw. Biol.*, **55**: 2332–2338.
- Bollens, S.M. and Frost, B.W. 1991. Diel vertical migration in zooplankton: rapid individual response to predators. *J. Plank. Res.*, **13**: 1359.
- Bronmark, C. and Pettersson, L.B. 1994. Chemical cues from piscivores induce a change in morphology in crucian carp. *Oikos*, **70**: 396–402.
- Chase, J.M. 1999. Food web effects of prey size refugia: variable interactions and alternative stable equilibria. *Am. Nat.*, **154**: 559–570.
- Covich, A.P. 2010. Winning the biodiversity arms race among freshwater gastropods: competition and coexistence through shell variability and predator avoidance. *Hydrobiologia*, **653**: 191–215.
- Crowl, T.A. and Covich, A.P. 1990. Predator-induced life-history shifts in a freshwater snail. *Science*, **247**: 949.
- Dalesman, S., Rundle, S.D., Coleman, R.A. and Cotton, P.A. 2006. Cue association and antipredator behaviour in a pulmonate snail, *Lymnaea stagnalis*. *Anim. Behav.*, **71**: 789–797.
- DeWitt, T.J. 1998. Costs and limits of phenotypic plasticity: tests with predator-induced morphology and life history in a freshwater snail. *J. Evol. Biol.*, **11**: 465–480.
- Dewitt, T.J., Sih, A. and Hucko, J.A. 1999. Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behaviour. *Anim. Behav.*, **58**: 397–407.
- DeWitt, T.J., Robinson, B.W. and Wilson, D.S. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. *Evol. Ecol. Res.*, **2**: 129–148.
- Dillon, R.T., Jr., Robinson, J.D. and Wethington, A.R. 2007. Empirical estimates of reproductive isolation among the freshwater pulmonate snails *Physa acuta*, *P. pomilia*, and *P. hendersoni*. *Malacologia*, **49**: 283–292.

- Dodson, S.I. 1989. The ecological role of chemical stimuli for the zooplankton: predator-induced morphology in *Daphnia*. *Oecologia*, **78**: 361–367.
- Ferrari, M.C.O., Sih, A. and Chivers, D.P. 2009. The paradox of risk allocation: a review and prospectus. *Anim. Behav.*, **78**: 579–585.
- Gallie, J.A., Mumme, R.L. and Wissinger, S.A. 2001. Experience has no effect on the development of chemosensory recognition of predators by tadpoles of the American toad, *Bufo americanus*. *Herpetologica*, **57**: 376–383.
- Giesing, E.R., Suski, C.D., Warner, R.E. and Bell, A.M. 2011. Female sticklebacks transfer information via eggs: effects of maternal experience with predators on offspring. *Proc. R. Soc. Lond. B*, **278**: 1753–1759.
- Huey, R.B. and Rosenzweig, F. 2009. Laboratory evolution meets catch-22: balancing simplicity and realism. In *Experimental Evolution: Concepts, Methods, and Applications of Selection Experiments* (T. Garland, Jr. and M.R. Rose, eds.), pp. 671–701. Berkeley, CA: University of California Press.
- Lakowitz, T., Brönmark, C. and Nystrom, P. 2008. Tuning in to multiple predators: conflicting demands for shell morphology in a freshwater snail. *Freshw. Biol.*, **53**: 2184–2191.
- Lewis, D.B. 2001. Trade-offs between growth and survival: responses of freshwater snails to predacious crayfish. *Ecology*, **82**: 758–765.
- Lima, S.L. 1998. Nonlethal effects in the ecology of predator–prey interactions. *Bioscience*, **48**: 25–34.
- Lima, S.L. and Bednekoff, P.A. 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *Am. Nat.*, **153**: 649–659.
- Mandrillon, A.L. and Saglio, P. 2009. Effects of single and combined embryonic exposures to herbicide and conspecific chemical alarm cues on hatching and larval traits in the common frog (*Rana temporaria*). *Arch. Environ. Contam. Toxicol.*, **56**: 566–576.
- Marshall, D.J. 2008. Transgenerational plasticity in the sea: context-dependent maternal effects across the life history. *Ecology*, **89**: 418–427.
- Mathis, A., Murray, K.L. and Hickman, C.R. 2003. Do experience and body size play a role in responses of larval ringed salamanders, *Ambystoma annulatum*, to predator kairomones? Laboratory and field assays. *Ethology*, **109**: 159–170.
- National Institutes of Health. 2010. *ImageJ: Image Processing and Analysis in Java*. Bethesda, MD: National Institutes of Health.
- Peckarsky, B.L., Abrams, P.A., Bolnick, D.I., Dill, L.M., Grabowski, J.H., Luttbeg, B. *et al.* 2008. Revisiting the classics: considering nonconsumptive effects in textbook examples of predator–prey interactions. *Ecology*, **89**: 2416–2425.
- Quintana, A. and Prevosti, A. 1990. Genetic and environmental factors in the resistance of *Drosophila subobscura* adults to high temperature shock. *Theor. Appl. Genet.*, **80**: 847–851.
- R Development Core Team. 2010. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Räsänen, K.R., Laurila, A. and Merilä, J. 2003. Geographic variation in acid stress tolerance of the moor frog, *Rana arvalis*. II. Adaptive maternal effects. *Evolution*, **57**: 363–371.
- Relyea, R.A. 2002. Local population differences in phenotypic plasticity: predator-induced changes in wood frog tadpoles. *Ecol. Monogr.*, **72**: 77–93.
- Stemberger, R.S. and Gilbert, J.J. 1984. Spine development in the rotifer *Keratella cochlearis*: induction by cyclopoid copepods and *Asplanchna*. *Freshw. Biol.*, **14**: 639–647.
- Storm, J.J. and Lima, S.L. 2010. Mothers forewarn offspring about predators: a transgenerational maternal effect on behavior. *Am. Nat.*, **175**: 382–390.
- Trussell, G.C. 2000. Predator-induced plasticity and morphological trade-offs in latitudinally separated populations of *Littorina obtusata*. *Evol. Ecol. Res.*, **2**: 803–822.
- Trussell, G.C. and Nicklin, M.O. 2002. Cue sensitivity, inducible defense, and trade-offs in a marine snail. *Ecology*, **83**: 1635–1647.

- Trussell, G.C., Ewanchuk, P.J. and Bertness, M.D. 2003. Trait-mediated effects in rocky intertidal food chains: predator risk cues alter prey feeding rates. *Ecology*, **84**: 629–640.
- Turner, A.M. 2004. Non lethal effects of predators on prey growth rates depend on prey density and nutrient additions. *Oikos*, **104**: 561–569.
- Turner, A.M., Fetterolf, S.A. and Bernot, R.J. 1999. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. *Oecologia*, **118**: 242–247.
- Turner, A.M., Turner, S.E. and Lappi, H.M. 2006. Learning, memory and predator avoidance by freshwater snails: effects of experience on predator recognition and defensive strategy. *Anim. Behav.*, **72**: 1443–1450.
- Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A. and Smith, G.M. 2009. *Mixed Effects Models with Extensions in Ecology with R*. New York: Springer.