

Environmental calcium modifies induced defences in snails

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Inducible defences are adaptive phenotypes that arise in response to predation threats. Such plasticity incurs costs to individuals, but there has been little interest in how such induced traits in animals may be constrained by environmental factors. Here, we demonstrate that calcium availability interacts with predation cues to modify snail shell growth and form. Small snails increased their growth and were heavier when exposed to fish chemical cues, but this response was calcium limited. There was also an interactive effect of fish cues and calcium on the shell growth of larger snails, but shell strength and aperture narrowness were affected by calcium alone. For small snails, behavioural avoidance was greatest for snails exhibiting least morphological plasticity, suggesting a trade-off. There was no trade-off of somatic growth with plasticity. We suggest that the expression of defensive traits in molluscs can be constrained by calcium availability, which has implications for molluscan ecology and evolution.

Keywords: morphological defence; avoidance behaviour; plasticity; trade-off

1. INTRODUCTION

Inducible defences have been documented for a wide range of taxa (Tollrian & Harvell 1999) where they have major ecological implications, allowing species to exploit broader environmental ranges (Spitze & Sadler 1996) and altering food web interactions (Turner *et al.* 2000; Peacor & Werner 2001). Such plastic traits for defence are often thought of as secondary, alternative phenotypes that are expressed only in the presence of predation threat. This implies the notion of a cost–benefit trade-off, otherwise the expressed version of the trait would be fixed in the genome (Tollrian & Harvell 1999). There are several examples of costs associated with induced defences (e.g. DeWitt 1998; Van Buskirk & Saxer 2001), but other potential constraints have received little attention. In particular, little is known of how abiotic factors might modify induced defences, for example where biotic inducement and abiotic factors act, interactively, on defensive traits. In plants, herbivore-induced defences are modified by factors such as CO₂ and light (Lindroth & Kinney 1998; Nabeshima *et al.* 2001) but, for animals, examples of environmental effects on induced defences are limited (Hansson 2000). An understanding of the interaction

between biotic and abiotic control of phenotypic plasticity is critical for understanding how such traits evolve.

Aquatic molluscs are excellent model organisms for examining the interaction between induced defences and environmental parameters. The evolution of molluscan diversity in the oceans has been linked to an increase in the diversity of crushing predators in the Mesozoic and Cenozoic (Vermeij 1987), and there are clear present-day examples of plastic responses in shell morphology to predation (DeWitt 1998; Trussell & Smith 2000). At the same time, there are relationships between environmental parameters and molluscan shells. In particular, calcium availability is thought to have played a major role in the evolution of molluscan shell diversity (Marin *et al.* 1996) and correlates with shell morphology in present-day populations (Lewis & Magnuson 1999). In fresh waters, calcium concentration can vary dramatically between habitats, influencing species physiology, reproduction and distributions (Dillon 2000). As freshwater molluscs depend on their shells for protection, interactions between calcium availability and induced changes in shell structure could have important implications, but as yet are unknown.

As well as using shells for defence, many aquatic molluscs exhibit behavioural avoidance in the presence of predation risk, responses that are often mediated by chemical cues (Rundle & Brönmark 2001). Since behavioural avoidance also incurs a cost to individuals (Turner *et al.* 2000), it might be predicted that there would be a trade-off between morphological and behavioural defences.

Here, we investigate whether induced plasticity in shell growth in the freshwater snail *Lymnaea stagnalis* (L.) is modified by the availability of environmental calcium, whether observed responses are size dependent, and if there are any trade-offs associated with such plasticity.

2. METHODS

(a) General experimental set-up

We employed an orthogonal experimental design to investigate plastic responses in shell morphology to chemical cues for predation risk and environmental calcium concentration ([Ca²⁺]). *L. stagnalis* individuals were obtained from egg batches produced by adults collected from Exminster Marshes, UK (50°39.04' N, 03°27.25' W). Snails were maintained in artificial pond water (ASTM 1980) with [Ca²⁺] adjusted to 45 mg l⁻¹ (low) and 90 mg l⁻¹ (high) in the presence and absence of fish chemical cues obtained from low [Ca²⁺] water in which tench (*Tinca tinca* (L.)) had been maintained for 24 h previously (five individuals of 10 cm length in 20 l of ASTM). This water was added to header tanks at the beginning of the trials, and in all subsequent top-up water at a concentration of 10% (Rundle & Brönmark 2001). There were two, randomly interspersed, replicate header tanks per treatment. All trials were run for 7 days at 16 °C (± 0.5°) with a 12 L : 12 D regime.

We used a gravity-fed, flowthrough system to ensure a constant supply of [Ca²⁺] and chemical cues to, and elimination of wastes from, experimental individuals. Experimental units comprised polypropylene header tanks (volume of 20 l each), which each supplied ten 25 ml polypropylene pots, via 20 cm lengths of Nalgene tubing (diameter of 0.4 cm). Each pot contained an individual snail fed *ad libitum* on a zero-calcium, gelatin-based feed. Inlet tubes supplied water at just above the bottom of the pots, with outlets in the pot lids, ensuring complete mixing within the chambers. Outlet tubing was 15 cm long and terminated in an airline valve, adjusted to produce a constant flow (0.3 ml min⁻¹). Stability in water flow was ensured by maintaining, on a daily basis, a volume of 20 l in the header tanks. The water in each header tank was continuously aerated.

(b) Morphological measurements

We measured several shell parameters that relate to predation risk (DeWitt 1998). Shell strength was measured as the compressive force (in newtons) required to fracture shells, using an Instron 5 kN dynamic testing system 4301 (Instron Corporation, MA). Shells (soft parts excised) were placed aperture-down and the load plate was set

Table 1. Response of *Lymnaea stagnalis* shell morphological parameters to fish chemical cues (presence versus absence) and calcium (low versus high) in (a) 5 mm long snails and (b) 10 mm long snails. (MS, mean square; n.s., not significant.)

(a) source	treatment	d.f.	MS	F	p
percentage length increase	cue	1	3.1×10^{-3}	10.3	< 0.005
	calcium	1	3.3×10^{-3}	11.0	< 0.005
	cue \times calcium	1	1.0×10^{-5}	0.0	n.s.
	residual	76	3.0×10^{-4}		
mass	cue	1	6.0×10^4	6.5	< 0.05
	calcium	1	4.7×10^4	5.2	< 0.05
	cue \times calcium	1	1.1×10^3	11.9	< 0.005
	residual	76	9.2×10^5		
(b) source	treatment	d.f.	MS	F	p
percentage length increase	cue	1	5.0×10^{-4}	1.7	n.s.
	calcium	1	1.0×10^{-5}	0.0	n.s.
	cue \times calcium	1	1.9×10^{-3}	6.3	< 0.05
	residual	76	3.0×10^{-4}		
percentage aperture ratio change	cue	1	1.2×10^{-2}	2.7	n.s.
	calcium	1	3.3×10^{-2}	7.5	< 0.01
	cue \times calcium	1	1.2×10^{-2}	2.6	n.s.
	residual	76	4.6×10^{-3}		
crush resistance	cue	1	8.0×10^{-2}	0.6	n.s.
	calcium	1	1.4	10.1	< 0.01
	cue \times calcium	1	6.5×10^{-2}	0.5	n.s.
	residual	76	1.3×10^{-1}		

to move 1 mm min⁻¹ downwards until the shell was crushed. The precise force at which the shell broke was determined from load deformation curves. Snail bodies and shell fragments were then freeze-dried for 24 h and weighed (on a microbalance Sartorius 2001 MP2).

Linear dimensions were measured (± 0.1 mm) using a calibrated eyepiece graticule under low power ($\times 40$). Shell growth was estimated by expressing the increase in shell length after 7 days as a proportion of the initial length. The shape of the shell and of the shell aperture, both of which relate directly to predation risk (DeWitt 1998), were expressed as the aspect ratio (shell width : length) and the aperture ratio (aperture length : width). The ratio of shell : body mass was used to investigate whether there was a trade-off of somatic growth with any plasticity observed.

Differences in morphology in relation to the interaction of environmental effects (calcium) and predation risk (cue) were tested for by ANOVA followed by separation of factors, where appropriate, by Student–Newman–Keuls tests.

(c) Behavioural avoidance

On completion of growth trials, experimental individuals were exposed to a pulse of predation cues and their short-term avoidance response recorded. Groups of snails were split between two trials, which were performed 3 h apart in one tank per treatment. Each arena was a circular, polypropylene pot (diameter of 16 cm, height of 6 cm) containing 400 ml ASTM with 45 mg l⁻¹ calcium. Snails were placed in the centre of the base of these arenas and their position noted every 5 min for a period of 30 min following the addition of 40 ml fish cue water prepared as for growth trials. We used ‘crawl out’, i.e. a position at or above the water surface, as an avoidance response measure (Rundle & Brönmark 2001). In analyses, we used two variables: latency (percentage of the total trial before showing an avoidance) and time in avoidance (total time spent in avoidance). Differences in behavioural avoidance were tested using ANOVA, as above.

3. RESULTS

(a) Shell morphology

Small snails grew more in length in the presence of fish cues, but this growth was limited by [Ca²⁺] (table 1;

figure 1a). There was also an interactive effect of fish cues and [Ca²⁺] on shell mass; at low [Ca²⁺], shells were lighter in the absence of fish cues (table 1; figure 1b). For larger snails, there was an interactive effect of fish cues and [Ca²⁺] on length increase (table 1; figure 1c). There was also a significant effect of [Ca²⁺] on shell strength and percentage change in aperture ratio: shells were stronger and had narrower apertures at high [Ca²⁺] (table 1; figure 1d,e). There were no significant differences between treatments in shell : body mass ratios.

(b) Behavioural avoidance

For small snails, there was an interactive effect of chemical cues and [Ca²⁺] on latency, and of cues on total avoidance (table 2; figure 2). Latency was lower for snails grown at low [Ca²⁺] in the presence of fish cues and snails grown in the presence of cues spent less time in avoidance (table 2). This result occurred despite a significant trial effect, which suggested that snails exhibited consistent between-treatment differences in behaviour, despite temporal variability in activity. There were no significant effects of fish cues or [Ca²⁺] on the behaviour of large snails.

4. DISCUSSION

Here, we show that calcium availability acted alongside predation cues to change the expression of induced defences in *L. stagnalis*. There was evidence for both independent and interactive effects of chemical cues and [Ca²⁺] in inducing plastic responses in shell morphology for both small and large snails. For small snails, there were also independent and interactive effects of chemical cues

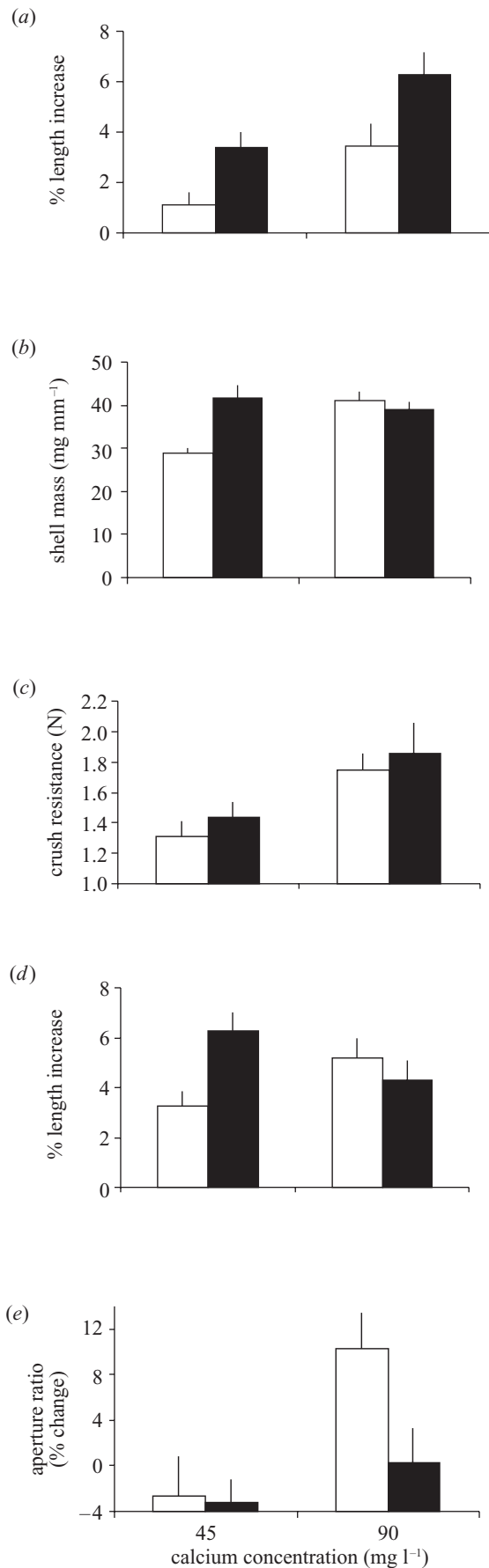


Figure 1. Shell morphological parameters (mean + s.e.) of *Lymnaea stagnalis*: (a,b) length 5 mm; and (c-e) 10 mm, grown in the presence (closed bars) or absence (open bars) of fish cues at two concentrations of Ca²⁺.

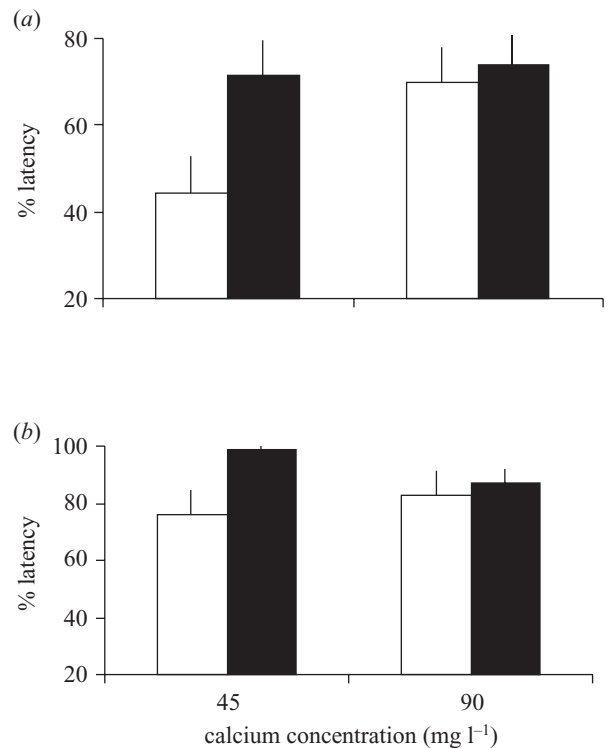


Figure 2. Behavioural avoidance (percentage latency, mean + s.e.) of small (5 mm) *Lymnaea stagnalis* grown in the presence (closed bars) or absence (open bars) of fish cues at two concentrations of Ca²⁺. (a) Trial 1, (b) trial 2.

and [Ca²⁺] on behavioural avoidance. Consequently, we suggest that, as for plants, interactive effects between induced defences and natural environmental parameters may also be of fundamental significance for animals. [Ca²⁺] may play an important role in the expression of both morphological and behavioural inducible defences in molluscs, with significant ecological and evolutionary implications. Molluscs in low-[Ca²⁺] habitats may be unable to optimize protection against predators, making them more vulnerable and needing to invest more in avoidance. This could have knock-on effects for fitness and on predator-prey interactions. Over larger time-scales, the rate at which anti-predator defences can evolve might also vary between high and low [Ca²⁺] environments.

The interaction between induced defences and calcium varied with snail size. Small snails responded to predation cues by investing more in shell growth, but this process was [Ca²⁺] limited. For larger snails, there was an interactive effect of fish cues and [Ca²⁺] on growth, but [Ca²⁺] was more important for anti-predator morphology as snail shells were stronger and had narrower apertures at high [Ca²⁺]; long thin apertures in gastropods reduce vulnerability to shell-entry predators (DeWitt *et al.* 2000). These results suggest: (i) that anti-predator adaptation may arise through environmental influences other than predation pressure, an example of a hitchhiking side-effect trait (West-Eberhard 2003); and (ii) a general induced defence rather than a predator-specific response (Reimer & Harms-Ringdal 2001).

As well as variation in morphological plasticity with size, behavioural avoidance responses following growth trials were also size dependent. Large snails showed no differ-

Table 2. Behavioural avoidance activities of small (5 mm) *Lymnaea stagnalis* from growth trials. (n.s., not significant.)

source	d.f.	MS	F	p
percentage time to avoidance (latency)				
cue	1	4.5×10^{-1}	7.7	< 0.01
calcium	1	7.8×10^{-2}	1.3	n.s.
trial	1	8.3×10^{-1}	14.0	< 0.001
cue × calcium	1	2.4×10^{-1}	4.1	< 0.05
calcium × trial	1	1.3×10^{-1}	2.2	n.s.
cue × trial	1	1.0×10^{-4}	0.0	n.s.
cue × calcium × trial	1	4.6×10^{-3}	0.1	n.s.
residual	72	6.0×10^{-2}		
percentage time in avoidance				
cue	1	2.2×10^3	4.5	< 0.05
calcium	1	4.3	0.0	n.s.
trial	1	5.7×10^3	11.4	< 0.001
cue × calcium	1	6.2×10^2	1.3	n.s.
calcium × trial	1	6.6×10^2	1.3	n.s.
cue × trial	1	1.4×10^2	0.0	n.s.
cue × calcium × trial	1	3.7×10^3	0.7	n.s.
residual	72	4.9×10^2		

ence in behavioural avoidance between treatments, whereas those small snails that showed the least morphological plasticity, i.e. those with no experience of fish cues, which were maintained in a low calcium environment showed the most rapid avoidance behaviour. The lack of avoidance in larger snails may be due to a lower vulnerability owing to increased shell size and/or strength (Rundle & Brönmark 2001). Alternatively, the ability to detect cues may develop only in early life stages or snails may be acclimatized following exposure to chemical cues. For small snails, there may be a trade-off between morphological and behavioural defences, with those snails that invested more resources in growing more quickly or in producing a more resistant shell morphology, exhibiting reduced avoidance activities (DeWitt 1998; Rundle & Brönmark 2001).

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