Predator-induced morphological defences in a freshwater snail

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Abstract

The ability of a certain genotype to express different phenotypes depending on variations in the environment is called phenotypic plasticity. Predator induced defences are among the most studied and wide spread examples of phenotypic plasticity. For example, in freshwaters, predators are constantly shaping communities with their presence and a plethora of defensive adaptations in prey have evolved.

In this study, I have analyzed if the freshwater snail *Radix balthica* show any induced morphological defence when exposed to the molluscivorous fishes, crusian carp (*Carassius carassius*) and tench (*Tinca tinca*). Two treatments were used; without predator and with a non-lethal predator (fish cues), to examine if the snails exposed to predators changed their shells in size, thickness and shape. The study contained snails from both northern Sweden (Umeå) and from southern Sweden (Lund).

The experiment conducted on the northern snails of *R. balthica* did not confirm my hypothesis that snails threatened by a predator (fish cues) express inducible defences. However, there were tendencies pointing in that direction. In contrast, the *R. balthica* snails from southern Sweden, exposed to fish cues from tench, showed a strong response. Shells were thicker and showed a more rounded shape, i.e. a wider shell and lower apex in the presence of fish compared to in the absence of fish. The rounder shells are more resistant to crushing by molluscivorous fish, due to the fact that the crushing force is more evenly spread over the shell. Thickness and size of the shell also influences the crushing resistance. Shell crushing fish obviously have a strong effect on gastropod communities and are therefore likely to drive the evolution of induced morphological defence in their prey.

Sammanfattning

Förmågan att en särskild genotyp kan uttrycka olika fenotyper beroende på variationer i miljön kallas för fenotypisk plasticitet. Predator-inducerat försvar hos bytesorganismer är ett av de mest välstuderade och spridda exemplen på fenotypisk plasticitet. Till exempel, i sötvattensystem, formar predatorer ständigt samhällen genom sin närvaro och en mängd försvarsanpassningar hos bytesdjur har selekterats fram.


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1.0 Introduction

Variation in organisms can have different sources: genetic or environmental (Gotthard and Nylin, 1995). Charles Darwin also noticed that phenotypes could depend on both genetics and environment and posted this in his book, The Origin of Species, in 1859: “When a variation is of the slightest use to any being, we cannot tell how much to attribute to the accumulative action of natural selection, and how much to the definite action of the conditions of life. Thus, it is well known to furriers that animals of the same species have thicker and better fur the further north they live; but who can tell how much of this difference may be due to the warmest-clad individuals having been favoured and preserved during many generations, and how much to the action of the severe climate? For it would appear that climate has some direct action on the hair of our domestic quadrupeds” (Darwin, 1859).

The ability for a certain genotype to express different phenotypes depending on variations in the environment is called phenotypic plasticity (Gotthard and Nylin, 1995). Expressions in phenotypic plasticity can range from changes in behaviour to changes in morphology, and almost all biological disciplines have to deal with some aspects of phenotypic plasticity (Pigliucci, 2001).

Phenotypic plasticity is common across many taxa and the ability of phenotypic flexibility may increase the fitness of an organism living in a heterogeneous environment (Hollander and Butlin, 2010). The trait may also facilitate for expansions of populations in novel environments as phenotypic plasticity enables for natural selection to advance (Price et al., 2003). However, there are costs associated with phenotypic plasticity. For example, a phenotype produced among predators may not be the optimal phenotype in other environments without predators. This has lead to a hypothesis called the “Adaptive phenotypic plasticity hypothesis” which states that if a phenotype has higher fitness in the “induced environment” than alternative phenotypes, but lower fitness in other environments, phenotypic plasticity will be favoured (Gotthard and Nylin, 1995). The fact that these induced traits are not expressed in the absence of predators indicates costs and trade-offs connected to phenotypic plasticity. In short, plastic defences are favoured when the predator pressure is highly spatial or temporal varying, when the inducible defence leads to a higher survival probability and when there are reliable cues for the prey to detect the presence of predators (Tollrian and Harvell, 1999).

Predator induced defences are among the most studied and wide spread examples of phenotypic plasticity (Brookes and Rochette, 2007). For example, in freshwaters, predators are constantly shaping freshwater communities with their presence and a plethora of defensive adaptations in prey have been selected for (Palmer, 1990). Prey species in aquatic systems often use waterborne chemical substances to sense predator risk (McCarthy and Fisher, 2000). These substances may come from the predator itself or by an injured prey (Lakowitz, 2008). The general response to predator cues are for the prey to hide, which results in a lower growth rate due to less activity and foraging (Lewis, 2001), but they may also develop some morphological defenses such as thicker shells, changed body shape, spines etc. (Trussell, 2000).

In this study I have examined if the freshwater snail Radix balthica show any changes in morphology when threatened by a predator (in this case fish cues), i.e. if they express phenotypic plasticity in shell shape and thickness. According to Vermeij and Covich (1978) it is reasonable to believe that the main selective force of the evolution of morphology in snails, in general, may be through predation by shell-crushing predators. Shell-crushing predators, such as fish and crayfish, have according to Rundle and Brönmark (2001) been shown to
prefer thin and small shells. My hypothesis is that snails that are held together with a non-lethal predator will change the morphology of their shells in a way that makes it harder for the predator (fish) to crush the shell. Hence, a thicker shell and a more rounded shape are expected. Support for this has been found in a study by DeWitt et al. (2000), where more rounded shells were harder to crush for the fish, and therefore more often rejected. Further, the shells in the fish treatment should be smaller than the shells not threatened by a predator. This should occur because of differences in allocations of resources, where snails threatened by fish allocate more resources into shell thickness relative to shell size (Lewis, 2001). However, it has also been discussed whether predator induced changes in gastropod shell morphology is caused by an active physiological response, or if it is a by-product due to behaviour, i.e. reduced feeding (Palmer, 1990).

In many earlier studies the focus has been on marine systems. However, there are differences between marine and freshwater systems that may affect the expression of phenotypic plasticity, for example, the lower availability of calcium in freshwater. Calcium is important for growth in gastropods. Low levels increase the importance of adaptations not requiring calcium, like adaptive architectural innovations as shell shape or microstructure (DeWitt et al., 2000). It has also been shown that phenotypic plasticity may vary with different environmental conditions, and some marine snails have shown latitudinal variation in induced defenses (Trussell, 2000). In some populations predators induce little or no defence, while in others that have been subjected to predators for a longer time a strong predator induced morphological defense can be observed (Trussell, 1999). Therefore, it is also interesting to compare predator induced shell shape changes between populations from different latitudes. I used snails from southern and northern Sweden to compare differences in induced defense between populations.

There is a wide range in shell morphology and size in freshwater snail species and they are prey for many different predators (for example, fish, crayfish, water bugs and leeches), making them a good model organism for studying defensive traits (Lakowitz, 2008). In this study I used the common freshwater snail *Radix balthica*, which is known for its great variation in shell morphology. *R. balthica* is a pulmonate snail, i.e. as long as it has access to moisture it is able to breathe air. It can therefore stay out of the water to avoid predators, which is a common behavior in pulmonate snails (Turner, 1996). Further, *R. balthica* is a hermaphrodite, breeding all year around and has short generation time, making it suitable as a study organism (Lakowitz, 2008).

Crucian carp (*Carassius carassius*) and tench (*Tinca tinca*) were used as predators in my experiments. Crucian carp and tench belong to the family Cyprinidae and are molluscivores. They have highly modified jaws and teeth which facilitates the crushing of gastropods shells (Osenberg and Mittelbach, 1989).
2.0 Material and methods

2.1 Experimental setup – Umeå

In October of 2009, snails of *R. balthica* were collected from a pond with fish (Sofiehems-dammen) in Umeå, northern Sweden. These snails were held in a large indoor tank without fish, and the snails reproduced frequently. At the start of the experiment, July 23, 2010, snails from the second generation were collected from this tank and put in the experimental tubs.

Twelve large tubs (height 33 cm, diameter 53; 43 cm at the bottom) were used in the experiment with two different treatments: 6 with fish and 6 without fish. The tubs were held in a greenhouse, with the light conditions following the natural light cycle. The tubs were put in a random arrangement with regard to treatments.

The tubs were filled up to three quarters with non-chlorinated tap water and air supply was provided from aquaria air pumps. A net cage (diameter 19 cm, height 53 cm and mesh size 0.5 cm), allowing water exchange between the cage and the water outside the cage was placed in all 12 tubs. To provide nutrients ten fresh birch leaves were added to each tub (Figure 1.). Eight snails of different sizes were added to each tub. Two Crucian carp (*C. carassius*), were added as a non lethal predators to the 6 tanks with the fish treatment. Fifteen snails from the holding tanks were put in a freezer as controls.

Twenty-five % of the water was exchanged twice during the experiment, and every other week new water was added to the tanks up to a level corresponding to the initial water level. The fish were fed five to six days a week with frozen *Chironomidae* larvae bought from a commercial dealer. Temperature varied between 18 and 27°C during the experimental period.

On October 26 dead snails were collected from the tubs and preserved in vials with 70% alcohol. The experiment ended November 2, when all remaining snails were put into a freezer. November 10 water samples were collected for analyses of P and N. All nutrient analyses were done at the Department of Ecology and Environmental Science, Umeå University with a spectrophotometer (SIS-method (SS-EN 1189)).

![Figure 1. Experimental tubs with net cages for the fish.](image-url)
2.2 Experimental setup - Lund

For the second experiment *R. balthica* snails were received from a colleague in Lund, who conducted the snail rearing experiment. The snails were collected from a small pond about 50 km east from Lund, in the province of Skåne in southern Sweden. The pond is fish free but has a high presence of *R. balthica* and *Lymnaea stagnalis* snails. The adult snails collected were then held until they laid eggs in the laboratory, and the juveniles from this second generation were then used in the experiment.

Twenty large (70 liters) plastic tanks were used in the experiment. Snails were stocked in the tanks; half of the tanks contained no fish (control) and half of the tanks contained two tench (*T. tinca*) (fish cue). The snails were divided into smaller containers (ten snails/2 liter containers) that were submerged into the larger tanks. To allow water exchange, the small containers had two holes (10 cm in diameter) with net (0,5 mm mesh size) on each side. For maximum effect, the fish were fed with *R. balthica* snails in its container, giving not only a predator cue but also an alarm cue. The light:dark cycle was 12:12 h and the water temperature varied between 19-21° C. The snails were collected after 12 weeks and put in a freezer.

2.3 Shell measurements

After freezing the snails were thawed and the soft tissue removed from the shells, and all shells were given an individual number. The shells were scanned with a scanner (hp ScanJet 6300 C) and digital images were saved of each scanned snail. To facilitate further analyzes, Adobe Photoshop CS2 was used to draw lines. First a line was drawn from the top of the snail to the bottom that captured the maximal length of the snail. Then a rectangle was drawn around the outermost edges of the shell. The rectangle was placed parallel to the line of the maximal length (Figure 2). Length was estimated as the length of the maximum length line with the program ImageJ (ImageJ, http://rsbweb.nih.gov/ij/download.html). The shells were also weighted (mg), and then the length and weight were used to get the approximate thickness (mg/mm) of each shell.

![Figure 2. A Radix balthica shell, with a drawn rectangle at the outermost edge and a line from the top to the bottom of the shell. The line and the rectangle were added to facilitate measurements of the landmarks. The dots are the 8 landmarks collected with the program TpsDig2.](image-url)
To analyze shell shape the digital images were copied into the program TpsDig2 (Rohlf, 2009). In this program landmarks can be collected and these landmarks can then be used for shape interpretations and statistics. Eight landmarks were collected (Figure 2.), which captures the outlines of the shells. Thereafter TpsRelw (Rohlf, 2005) was used. This program uses the landmarks to generate relative warps which are principal components of the shape variation in the sample. To illustrate the shape differences in the samples TpsSplin (Rohlf, 2004) was used.

2.4 Data analysis

T-tests were used to analyze if there was any difference in thickness and length between shells from the two treatments. MANCOVAs were used to test for differences in shape among treatments using predator treatment as factor and size as covariate. ANCOVAs, with size as a covariate, were used to see if there were any significant effects of treatment and size on Relative warp 1. ANOVAs were used to examine if there were any difference in nutrient levels between the two treatments, with and without fish. Regressions were used to examine if there were any correlation between the nutrients and Relative warp 1.

3.0 Results

3.1 Results - shells from Umeå

A t-test comparing if there was any difference in size (length) between the two treatments showed no significant difference between shells from tanks containing fish (mean= 8.34 mm, S.E. of mean= 0.31 ) and shells from tanks without fish (mean= 8.86 mm, S.E. of mean= 0.50) (p= 0.59, t= 0.53, df= 112). A t-test comparing the thickness of the shells showed a trend towards a significant difference between the two treatments (p=0.07, t= 1.80, df= 112), with shells from the fish treatment being almost significant thicker (mean= 1.50, S.E. of mean= 0.15) than shells from the without fish treatment (mean= 1.24, S.E. of mean= 0.09). However, since some of the shells were collected after the snails had died and had therefore to some extent started to dissociate, which might affect thickness of the shells, separate t-tests on only dead-collected shells and shells alive when collected were also done. The t-test for dead-collected shells did show a significant difference between the treatments (p= 0.03, t= 2.17, df= 50), with shells from the fish treatment being thicker (mean with fish= 1.55, S.E. of mean= 0.12, mean without fish= 1.08, S.E. of mean= 0.12). In contrast shells collected alive did not show any significant difference between with and without fish (p= 0.77, t= 0.29, df= 60).

The MANCOVA used to examine the effect of the two treatments, with and without fish, on all Relative warps with size as a covariate, showed no significant difference, i.e. no significant effect of predators on shell shape (Table 1). The same MANCOVA showed a significant effect of the covariate size (Table 1), i.e. size has an effect on shell shape. It is hard to see any direct difference in shape between the two treatments from the illustrated shells (Figure 3), which also confirm the non significant results in the MANCOVA. The interaction effect between treatment and size did not show any significant difference and it was therefore excluded.

The shell shape extracted from all shells and the shape of the shells from the treatment without fish are very similar. However, there is a tendency of change in shape in the predator treatment. It has had a somewhat lower apex, a slightly wider shell opening making the shell
looking a bit rounder than the one without a predator (Figure 3). An ANCOVA on Relative warp 1 (which explained 31 percent of the variation), with size as a covariant, showed a significant effect of treatment and size on the shape of the shell (Table 2). This result indicates a slight tendency of differences between the shells from the different treatments. The positive relative warp 1 values indicates a lower apex, a wider body whorl and a larger shell opening, whereas a negative value indicates a higher apex, a more narrow body whorl and a smaller shell opening (Figure 4). There is a slight tendency that shells from the predator treatment show more positive values and shells from the predator free treatment show more negative values on Relative warp 1. Shell shape (Relative warp 1) overlapped with regard to size suggesting that Relative warp1 was not strongly associated with size (Figure 4).

Table 1. Results from a MANCOVA on all relative warps testing for the effects of the treatment (with and without fish) on the relative warps (shell shape) and for the effects of size (covariate) on the relative warps (shell shape). The p-value for the treatment effect show no significant difference (>0.05), whereas the p-value for size effect show a strong significant difference (<0.05).

<table>
<thead>
<tr>
<th>Multivariate Test Statistics</th>
<th>Value</th>
<th>F-ratio</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
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<tr>
<td>Fish effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wilks's Lambda</td>
<td>0.84426</td>
<td>1.52183</td>
<td>12, 99</td>
<td>0.12889</td>
</tr>
<tr>
<td>Size effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wilks's Lambda</td>
<td>0.44384</td>
<td>10.33773</td>
<td>12, 99</td>
<td>0.00000</td>
</tr>
</tbody>
</table>

Table 2. An ANCOVA testing for the effects of treatment (Fish) and length (Size) (covariate) on Relative warp 1. The p-values indicates that there is a significant effect of both treatment and size on Relative warp 1 (<0.05).

<table>
<thead>
<tr>
<th>ANCOVA Source</th>
<th>Type III SS</th>
<th>df</th>
<th>Mean Squares</th>
<th>F-ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
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<td>0.02357</td>
<td>18.6647</td>
<td>0.00003</td>
</tr>
<tr>
<td>Fish</td>
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<td>1</td>
<td>0.00509</td>
<td>4.02979</td>
<td>0.04713</td>
</tr>
<tr>
<td>Error</td>
<td>0.14011</td>
<td>111</td>
<td>0.00126</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To examine if there were any difference in nutrients between the tubs with and without fish, which might have an effect on the size and shape of the shells between the treatments, F-tests were performed. The tests showed significant higher nutrient content in treatments with fish for all nutrients tested: nitrogen p <0.001, phosphorus p <0.001 and nitrite+nitrate p <0.001. This indicates that nutrients potentially could have an affect on shell size and thereby also on shell shape. However a regression on size against nutrients showed a non-significant relationship (nitrogen p = 0.93, phosphorous p = 0.67, nitrite+nitrate p = 0.77). In addition regressions between nutrients and Relative warp 1 were also run and showed no correlation (with low R-square values and non significant p-values: Table 3).
Figure 3. Grid plots illustrating three shell shapes on snails from Umeå; a) the average shell shape of all groups, b) the average shell shape of the treatment without fish, control excluded, c) the average shell shape of the treatment with fish.

Figure 4. The size distribution of the two treatments in the relation to Relative warp 1, on snails from Umeå. Pictures visualizing the shell shapes for the positive and negative relative warp values are shown on the y-axis.
3.2 Results - shells from Lund

A T-test showed that the thickness of the shells were significant different between the two treatments: with fish (mean= 1.42, S.E of mean= 0.23) and without fish (mean= 0.95, S.E of mean= 0.07), with thicker shells from the fish treatment tubs (p < 0.001, t = 5.29, df= 93). A t-test on size (length) showed a non-significant difference between the two treatments (mean with fish= 8.08 mm, S.E of mean= 0.69, mean without fish= 8.45 mm, S.E of mean= 0.28) (p = 0.26, t=1.14, df= 161).

Shells from the predator treatment had a different shape than shells from the non-predator treatment (Figure 5) and this was supported by the MANCOVA. Treatment and size (co-variant) had a significant effect and also the interaction term treatment*size was significant (Table 4). The change in shape in shells from the predator treatment was seen as a lower apex, a wider body whorl and a larger shell opening, giving a more rounded shell (Figure 5).

Plotting the size distribution and Relative warp 1 of the shells from the different treatments showed an overlap of the shell size from the two treatments (Figure 6). The positive relative warp 1 values indicates a lower apex, a wider body whorl and a larger shell opening, whereas negative value indicates a higher apex, a more narrow body whorl and a smaller shell opening (Figure 6). There is a clear pattern showing that shells from the predator treatment have more positive values and shells from the predator free treatment have more negative values. This supports the significant values from the MANCOVA, that there is a difference in shell form between the two treatments.

Table 3. The results from linear regressions between nitrogen and Relative warp 1, phosphorous and Relative warp 1 and nitrite+nitrate and Relative warp 1. All regressions showed non significant p-values and very low R-square indicating no relationship between nutrients and Relative warp 1.

<table>
<thead>
<tr>
<th>Linear Regression</th>
<th>Standard error</th>
<th>t Stat</th>
<th>p-value</th>
<th>R-square</th>
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<tr>
<td>N and RW1</td>
<td>0.031333</td>
<td>-0.27522</td>
<td>0.788748</td>
<td>0.000936</td>
</tr>
<tr>
<td>P and RW1</td>
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<td>-0.18655</td>
<td>0.855745</td>
<td>0.019323</td>
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<td>NO2+NO3 and Rw1</td>
<td>0.014439</td>
<td>-0.12328</td>
<td>0.904327</td>
<td>0.008648</td>
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</tbody>
</table>

Table 4. Results from MANCOVA on all relative warps, testing for the combined effect of the treatment (with and without fish) and size (covariant), for the effects of size, and for the effects of the two treatments, on the relative warps (shell shape). The p-values showed significant differences (<0.05).

<table>
<thead>
<tr>
<th>Multivariate Test Statistics Statistic</th>
<th>Value</th>
<th>F-ratio</th>
<th>df</th>
<th>p-value</th>
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<td>Size effect Wilks's Lambda</td>
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<td>2.11614</td>
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<tr>
<td>Fish effect Wilks's Lambda</td>
<td>0.81488</td>
<td>2.80184</td>
<td>12, 148</td>
<td>0.00178</td>
</tr>
</tbody>
</table>
Figure 5. Gridplots illustrating three shell shapes on snails from Lund; a) the average shell shape of all groups, b) the average shell shape of the treatment without fish, c) the average shell shape of the treatment with fish.

Figure 6. The size distribution of the two treatments in the relation to Relative warp 1 on snails from Lund. Pictures visualizing the shell shapes for the positive and negative relative warp values are shown on the y-axis.
4.0 Discussion

The experiment conducted using Umeå snails of *R. balthica* did not confirm my hypothesis that snails threatened by a predator (fish cues by *C. carassius*) could express inducible defences i.e. rounder shape of the shells, thicker shell and also smaller shell size due to trade-offs. However, there were tendencies pointing in that direction. In contrast, the *R. balthica* snails from Lund exposed to fish cues from tench (*T. tinca*), showed a strong response with thicker shells and a more rounded shape, i.e. a wider shell and a lower apex compared to shells in the fish free treatment. The snails from Lund did not show a significant difference in shell size between the two treatments.

4.1 Size

I did not find any significant difference in size between the two treatments, still, there was a tendency that mean length of the shells where somewhat bigger in the treatment without fish for both snails in Umeå and snails from Lund. This might indicate some trade-off in allocation of resources where the snail put more resources into shell thickness than on shell size. However, it might also be due to changed behaviour of the snails. If snails try to avoid predators they have less time available for foraging. In support of this suggestion, DeWitt (1998) found that there was a strong negative selection gradient for growth rate, which in turn was associated with plasticity in the shell shape of the freshwater snail *Physa heterostropha*. The most likely explanation for the result found in the study by DeWitt was that the snails showed a changed behaviour in the presence of fish. The snails crawled out of the water, which reduced feeding opportunities; the response is therefore likely to lead to decreased growth.

I did not examine the behaviour of the snails in my experiment. However, I did observe some snails above the waterline. DeWitt et al. (1998) showed that small snails, thus more vulnerable to predators, tend to have a stronger response and antipredator behaviour than larger snails, i.e. a behavioural compensation for the morphological vulnerability. This should imply that small snails, which are more likely to change their behaviour in the presence of a predator, thereby also reduce their ability to increase in size due to reduced feeding. Size is often connected to reproduction, and therefore this could mean that there is a trade-off between survival and growth/reproduction (Crowl and Covich, 1990). DeWitt et al. (2000) has shown that handling time for pumpkinseed sunfish (*Lepomis gibbosus*) on *Physa* snails, were greater for larger snails compared to small snails, which further support that size is important for fitness.

4.2 Shell thickness

The thickness (weight/size) of all shells did not show any significant difference between the two treatments in the Umeå snails. However, when I separated the dead collected snails from the snails being alive when collected, I found a significant difference in shell thickness in the dead collected snails with those exposed to predator having a thicker shell. This might be due to that the snails being alive may be the youngest snails (new generation) and might therefore not have had enough time to develop the inducible defences as the dead snails had. The shells from Lund showed a strong significant difference between fish treatment and fish free treatment, with snails exposed to fish cues having clearly thicker shells than snails held in tanks without fish. This result is in accordance with other studies. Brookes and Rochette (2007) found that the shell thickness of the freshwater snail *Littorina obtusata* was increased
when the snails were exposed to predator cues. In addition, Lakowitz et al. (2008) found that shells of *R. balthica*, exposed to fish cues, were thicker than shells exposed to no predator or to crayfish. The increased shell thickness seems to be the result of an active calcification for protection against shell crushing predators (Brookes and Rochette, 2007).

Interestingly, Bourdeau (2009) who conducted a study where he examined the cause of shell thickening, induced by predators, in the marine snail *Nucella lamellose*, found that the predator, in this case a crab (*Cancer productus*), induced thicker shells. However, that response was likely a passive by-product of reduced feeding and somatic growth and not due to an active physiological response to the risk of predation. His study showed that the significant stronger shells exposed to predators were not different from those snails that had limited access to food. According to Bourdeau (2009), the increased shell strength is likely due to an increase in the energetically inexpensive microstructure layer of the shell rather than to material property changes in the shell. He suggests that this may indicate that shell defences induced by predators might be neither developmentally nor energetically costly for the snail.

### 4.3 Shell shape

I did not find as clear differences in shell shape between the two treatments in Umeå snails as I had expected. The MANCOVA using Relative warps suggested that the predator had a non-significant effect on shape. However, the ANCOVA did show a significant effect of treatment on Relative warp 1. Since the Rw1 explained 31 percent of the variation in shape, it is reasonable to think that there is a tendency for a difference in shell shape between the two treatments. Looking at the illustrated shapes, one can see that the shells from the fish treatment had a slightly wider shell opening making the shells appear a bit rounder. They also had a somewhat lower apex compared to the shells from the fish free treatment. The snails from Lund, on the other hand, did show strong induced morphology change in their shells. The snails held with fish, had shells with lower apex, wider body whorls and larger shell openings, which resulted in a rounder in shell shape.

The shell shape changes I observed might provide protection from fish predators. DeWitt et al. (2000) demonstrated that pumpkinseed sunfish (*Lepomis gibbosus*) had a longer handling time on the freshwater snail *Physa* on shells having a rotund shell morphology. They also found that the rounder shells were more resistant to crushing, due to the fact that the crushing force is more evenly spread over the shell. A rotund shell, with a greater handling time may facilitate escape behaviour and also reduce the value of the prey (greater handling time gives reduced energetic payoff). In their study, few snails were rejected by fish, but the once that were rejected were rotund compared to the consumed snails. Moreover, they found in a field survey, that snails collected from habitats where fish was common had more rounded shells compared to snails from habitats with few or no fish. There are also many studies conducted on snails with crayfish as predator, which has shown changed shell morphology of the snails when exposed to crayfish (DeWitt et al., 1999, DeWitt et al., 2000, Krist, 2002, Brookes and Rochette, 2007). When exposed to crayfish, which attacks snails by entry and sometimes clipping away the margin of the aperture, a more elongated snail with a narrow apertures is induced (DeWitt et al., 1999). Hence the optimal shell shape to this kind of predator is in the opposite direction compared to fish predators.

### 4.4 Nutrients

I found that the nutrient levels were different, with higher levels of phosphorous, nitrogen and nitrite+nitrate in the treatments with fish. However, there was a clear overlap of the size
and shell shapes of the shells showing that the different nutrient levels probably did not have any effects on shell shape and size.

### 4.5 Umeå and Lund

One aspect that might have influenced the snails differently in Umeå and Lund experiment was that the tench in the Lund experiment were fed with crushed snails, whereas the crucian carp in the Umeå experiment were only fed with *Chironomidae* larvae. When the fish is fed with crushed snails, the waterborne chemical cues become stronger for the snails in that treatment, i.e. both alarm cues from crushed snails and predator cues, and the snails may therefore show a stronger response (Palmer, 1990).

I have not done any tests to explore if there are any statistical geographical differences between the shells from Umeå and the shells from Lund. The main reasons are that the experimental conditions differed. Nevertheless, my results indicate that there are some differences, with a stronger response in the snails from Lund. This, might however, depend on differences in the experiment setup, see above. However, it might also be due to some geographical factors. Trussell has in several studies analyzed phenotypic plasticity in snail populations from different latitudes. In a study from 2000, Trussell found that marine snails of *Littorina obtusata* from northern habitats, in the Gulf of Maine, had thinner shells that weighted less and were weaker in compression (high-spired shape) than snails from habitats 400 km south of the northern localities, but the weight of the snails (body size i.e. soft tissue mass) was greater than for southern snails. He also found, by reciprocal transplant experiments, that southern snails moved to northern habitats produced thinner and lighter shells, and more body mass than the snails raised in their native habitat. Northern snails moved to southern habitats did, in contrast, produce thicker and heavier shells and less body mass than the controls in the north. Trussell found that the snails from north responded differently to the effects of transplanting and the northern snails also showed greater changes in trait means compared to the southern snails. For example, the increase in shell mass was 136% and the increase in thickness was 43% for northern snails whereas it was 44% respectively 18% for southern snails, indicating that the northern snails were more plastic in these traits. This suggests an among-population variation in plasticity. The differences are probably mainly caused by lower water temperature in the northern habitats which leads to less calcium since calcium becomes more saturated and soluble in lower temperature giving thinner and weaker shells. However, it may also be due to the abundance of an invading crab predator (*Carcinus maenas*). The longer historical contact with *C. maenas* and the predictability of the presence of the crab in the southern Maine Gulf, might have reduced the evolution of plasticity in the southern populations.

My snails did show a stronger plasticity in the southern snails which is reversed to Trussells results. However, there were no clear difference in water temperature between the Umeå and Lund experiments, it is therefore unlikely that water temperature had any direct effect on the different results. Further, since these snails were held in laboratory and not surveyed in field, and because I did not do any further analyze on this part, it is not possible to know how the geography might have affected the result. Again, most likely some differences in experimental setup, such as lower chemical cues in the Umeå experiment, had the biggest effects on the different outcomes between the two locations.

### 4.6 Future studies

If the experiment was to be repeated, the fish in Umeå should be fed with crushed snails to get stronger cues. This might result in a stronger response in predator induced snail shape. It
would also be interesting to study the behaviour of the snails. A field study to investigate if there were any differences between snails in similar habitats in Umeå and in Lund would also be interesting. Furthermore, I would have tested responses to different types of predators, e.g. fish (crushing) and crayfish (shell opening entry and clipping). For example, including a treatment with only fish, a treatment with only crayfish and a treatment with both fish and crayfish would be interesting. It is important to study the combined effect of different predators since the natural habitats seldom have only one type of predator. Such combined effects have been investigated by Hoverman and Relyea (2009), who found that induced defences by a predator will, for that particular predator, reduce the risk of predation but, it may also increase the risk of predation by another, functionally different, predator. DeWitt et al. (2000) have demonstrated a trade-off where rotund shells were resistant to crushing by fish but at higher risk to shell entry by crayfish. Lakowitz et al. (2008) conducted a study where they found that *R. balthica* snails were able to fine-tune different elements of morphology as a response to predator-specific foraging modes. In a combined predator treatment, with both fish (*T. tinca*) and crayfish (*P. leniusculus*), the snails responded with an intermediate shell morphology compared to the fish treatment and the crayfish treatment. The snails reduced their shell thickness in response to the crayfish but maintained the high crushing resistance (shell thickness) to meet the threat from the fish.

## 4.7 Conclusion

There have been many studies conducted on phenotypic plasticity in recent years, however, few studies have combined surveys on the different aspect as morphological defences and behaviour defences, geographical differences, trade-offs when exposed to many different predators and so on. To get the whole picture of the phenomenon phenotypic plasticity in freshwater gastropods, I think that it is important to look at all aspects, combined, in the future.

*Radix balthica* is shown to be a phenotypically plastic species. It has been found by e.g. Ekologgruppen (2000) that *R. balthica* often is one of the first species colonizing new water bodies and that it is an effective dispersing organism. This is likely the reason why this species has a strong induced defence. Plasticity in shell morphology and shape is an adaptive trait since the environment of the snail is unpredictable and the predator composition varies between different water bodies making it important to be able to adapt to different environments and predators. My results from Umeå was not as clear as I thought they would be but the snails from Lund showed clear defensive changes in morphology when threatened by a predator (fish). Shell crushing fish obviously have a strong effect on gastropod communities and are therefore likely to drive the evolution of induced defence traits in this prey.
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6.0 References

ImageJ; http://rsbweb.nih.gov/ij/download.html


