Predator induced phenotypic plasticity in a freshwater snail

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32485 snails, 81940 litres of water, 18 barrels, 40 large plastic tanks, 170 small plastic tanks, 20 aquarium pumps, 4 air pumps, 1 drainage pump, 238 meter hose, 87 air dispensers, 576 reference printouts, 12483 sheets of paper, 28 pencils, 34 pens, 2 computers, 512 litres of gasoline and 416 pints of beer was necessary for the completion of this thesis.
AKADEMISK AVHANDLING

som avläggande av filosofie doktorsexamen
vid Naturvetenskapliga fakulteten vid Lunds Universitet
kommer att offentligen försvaras i Blå Hallen, Ekologihuset, Sölvegatan 37, Lund,
torsdagen den 30 oktober, klockan 09.30.

Fakultets opponent: Prof. Simon Rundle, University of Plymouth, UK.

Avhandlingen kommer att försvaras på engelska.

Dissertation
Lund 2008
A doctoral thesis at a university in Sweden is produced either as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis, which summarizes the accompanying papers. These have either already been published or are manuscripts at various stages (in press, submitted or in ms).
Predator induced phenotypic plasticity in a freshwater snail

Predator recognition - non-lethal predator cues

**Predator recognition - non-lethal predator cues**

For prey, information on predator presence and identity is of great importance. In aquatic systems prey commonly use waterborne chemical substances, as these are effective information carriers independent of season or time of day. Prey organisms use this information to assess predation risk (McCarthy and Fisher 2000; Sih and McCarthy 2002), in order to adjust their behaviour accordingly. Generally, prey react to predator cues by hiding and reducing their activity and this will in turn have negative effects on prey growth rates as the time spent foraging is reduced (see e.g. Kats and Dill 1998). Prey react on these signals with great accuracy and snails, for example, have been shown to have avoidance behaviours that are predator specific.

Fish hunting in the water column cause snails to seek refuge under structures at the bottom or crawl out of the water, while snails respond to crayfish that hunt on the bottom by moving away from the bottom area or crawl out of the water (Turner et al. 1999). We have learned about the importance of chemical predator cues not least through behavioural studies on plankton (Tollrian and Harvell 1999), and the desire to understand the exact chemical nature of these compounds have been great. As ecologists, however, we may be satisfied by knowing the effect of these compounds in an ecological context. Further, many attempts to isolate and purify the active substances have yielded different results, why it seems that many substances may be responsible for the effects they have on prey (Burkes and Lodge 2002). The substances may either originate from the predator itself, for example as metabolites that the predator excretes after consuming the prey, or they may be alarm substances elicited by the prey when attacked and injured. For an organism, associating cue with predation is important and this may occur through learning, for example it has been shown for a range of different prey species that predator naïve prey after an exposure to injured conspecifics in combination with predator odour learn to associate predator odour alone with predation risk (Chivers and Smith 1994; Wisenden and Millard 2001; Dalesman et al. 2006). The evolution is favouring prey to correctly match defensive measures with predator cue as a maladaptive defence may be costly, either through unnecessary defensive reactions, or failure to detect the predator (Langerhans and DeWitt 2002).

The physical and/or chemical properties of alarm substances limit their use to prey. Turner and Montgomery (2003) showed that while snails displayed anti-predator behaviour up to a meter away from the predator their growth rates were affected up to almost five times that distance. The behavioural response to predator cue disappeared about four days after the removal of the predator, suggesting a rather rapid degradation of the chemical substances.

In the laboratory, predator chemical cues are very useful, as it is possible to isolate the effects of a predator on a prey without having the consumptive effects of predation.

Using snails for studying prey defences

Why study snails? Freshwater snails are ideal model organisms for studying defensive traits as they are prey for a range of different predators, including fish, crayfish, leeches, water bugs and flatworms (Townsend & McCarthy 1980; Brönmark et al. 1992; Nystöm et al. 1999; Hoverman et al. 2005; Hoverman & Relyea 2007). This shows predation pressure have resulted in the evolution of many different anti-predator responses, including behaviour (Brönmark & Malmqvist 1986; Turner et al. 1999; Rundle & Brönmark 2001), morphology (Dewitt et al. 2000; Hoverman et al. 2005) and/or life-history strategies (Crowl & Covitch 1990). freshwater snails are very variable in body size and in shell morphology, both of which are traits that relate...

An important part of ecology is the interaction between predator and prey. This is because predators have a strong impact on the system they occupy. Predation is a strong structuring force that affects density and species composition in prey assemblages. In freshwater, predation has a dramatic impact on the invertebrate fauna (Brönmark 1994; Nystöm et al. 2001). An illustrative example is that, depending on if the predator regime is dominated by invertebrates or fish, will have different structuring effects.

 Dominance of invertebrate predators often results in a community characterized by large and active invertebrates, whereas fish predators result in a community dominated by small inactive invertebrates (Wellborn 1996). This is because invertebrate predators are often slow moving, relying on tactile organs to detect their prey, while fish are actively hunting size selective predators, relying on vision to locate prey (Werner and Hall 1974; Osenberg and Mittlebach 1989).

Given the strong impact of predation there should be a high benefit of evolving efficient defence adaptations in prey organisms. The predation process can be described as a cycle where the predator repeats the same pattern for every prey item it takes. It starts with the search for prey, followed by encounter, attack, capture and finally ingestion. Prey are able to escape predation at each step of the predation cycle and naturally prey would benefit the most by having defence adaptations that break this cycle as early as possible. For example, through behaviour, prey may reduce their encounter rates with predators, both spatially and temporally. If the prey is encountered and attacked it may escape by having a morphological defence that reduce the capture success or prolong handling times. For the predator, feeding on defended prey becomes less profitable and the predator may shift to an alternative food source.

The defence may be either constitutive, i.e. always present in the prey organism, or prey may have evolved a phenotypically plastic defence. That is, to have the ability to change a trait, morphologically or physiologically to increase fitness in the presence of a predator. This is triggered when the prey is sensing the presence of a predator and reacts by expressing an alternative phenotype, i.e. behavior, morphology or life-history that is specific for defending against that predator.

**Introduction**

Predator recognition - non-lethal predator cues

Predator induced phenotypic plasticity in a freshwater snail

Predator induced phenotypic plasticity in a freshwater snail
to predation risk. One of the most important predators on snails and of primary interest in this thesis is fish. They may severely reduce snail population densities (Brönmark 1994). In European freshwaters, mainly fish of the family Cyprinidae has specialized in feeding on snails. Molluscivorous fish has developed a special feeding apparatus, pharyngeal teeth, to crush the snail shell. The species belonging to this family are among the most common in freshwater systems, including species like roach (Rutilus rutilus), tench (Tinca tinca), crucian carp (Carassius carassius), rudd (Scardinius erythrophthalmus) and bream (Abramis brama) that are benthivorous and may include snails in their diet to a high degree (Stein et al. 1975; Brönmark 1994).

Another important predator of snails, and partly in focus of this thesis, is crayfish, which have been shown to have dramatic negative impacts on snail density (Nyström et al. 1999; Dorn & Wojdak 2004). In Sweden, two species exists, the noble crayfish (Astacus astacus) and the introduced signal crayfish (Pacifastacus leniusculus). Crayfish forage on snails by clipping off the shell at the aperture with their mandibles. Thus, the effectiveness of the snail shell as a defence organ is highly specific to the predator and may call for completely different characteristics in morphology or shell thickness (Brown 1998; Trussel 2000; Hoverman et al. 2005).

The study organism
I have studied one of the most common European freshwater snails, Radix balthica (Glover 2002, formerly Lymnaea peregra L.). It is a pulmonate snail and is able to breathe air providing it has access to moisture. This means that it can stay out of the water for long periods, a common predator avoidance behaviour in pulmonate snails (Turner 1996; Rundle and Brönmark 2001). It is one of the first species to appear in newly created wetlands, often as soon as within six months (Ekologgruppen 2002), a paradox perhaps as snails lacks obvious means of dispersal like legs or wings. There have been explanations to snail dispersal that involves waterfowl as dispersing vectors, showing they can survive aerial transportation for quite some distance (Boag 1986). Nevertheless, R. balthica is very successful when established and is often the dominating snail species in ponds and lakes (Ekologgruppen 2002). These aquatic systems may be quite diverse in respect to species composition and contain different predator regimes varying from invertebrate predators to molluscivorous fish.

R. balthica has been known for a long time to be very variable in shell shape (Fig. 1., Hubendick 1951), from elongated shells with narrow apertures to more round shells with wider apertures. Previous studies have related shell shape to differences in abiotic factors among habitat (Hubendick 1951; Lam and Calow 1988; Wulfschleger and Ward 1998).

In the laboratory, pulmonate snails make excellent study organisms for several reasons. They are small and can be easily handled, breed all year and they are hermaphrodites with fast maturation and short generation times. Further, they are durable and easy to monitor and have easily quantifiable trait expressions.

Methods
Snails build their shell continuously (Wilbur and Saleuddin 1983) and as I aimed at studying morphological changes and, in some studies, life history changes, it was necessary to raise snails from eggs, letting the development of the snail occur in the designated treatment already from its earliest days. The approach was taken in all the experiments I conducted. Except for the study in paper 1, I used snails that originated from the same pond in Frihult 40 km southeast of Lund, southern Sweden. It is a ground water fed pond and contains no fish or crayfish (Picture 1). The approach was to take adult snails and letting them reproduce in the laboratory. Young from several egg batches were then used to reduce the chance of having the whole study population consisting of siblings.

To induce a reaction in the snails, I exposed them to chemical cues from fish and/or crayfish. The predators were feeding on conspecific snails and, for most experiments, they were separated from the snails either by a mesh or by pumping water from a predator holding tank into the snail rearing tank (Picture 2).

One main focus in this thesis was to quantify phenotypic change in different shape characteristics from eggs, letting the development of the snail occur in the designated treatment already from its earliest days.
Predator induced phenotypic plasticity in a freshwater snail

Fig. 2. Example of visualized shape through inverted Fourier transformation. On the left is the snail outline contour with overlapping contours showing the mean shape as a dotted line and shape represented by +2 SD (middle) and +2 SD (right). Compare the outline shapes with those from Hubendick.

Fixed and plastic defence

of the snail shells. To do this a morphometric method has to be used and for snails perhaps the simplest approach is to measure different ratios of the shell, for example the height: width ratio. Another approach is to use geometric morphometrics; defining shape as “all the geometric information that remains when location, scale and rotational effects are filtered out from an object” (Kendall 1977). One of the most frequently used methods is landmark morphometry where landmarks are placed on homologous points. However, snails have very few homologous points and those that exist tend to show small differences, why I instead used elliptic Fourier analysis. This is an object outline analysis and it captures the curved shapes of the shell that I was interested in quantifying. For the measurements one needs an image of the object that is replicable for all individuals. To capture the image from the same angle, I scanned the snails with the opening facing down on a flatbed scanner. Images were then analyzed using an image analyzing program (SHAPE, Iwata & Ukai 2002). The program calculates elliptic Fourier descriptors that in turn are used by the program to generate shape characteristics as principal components. To interpret the morphological meaning of each principle component, shape has to be visualized by inverting the Fourier transformation. In this way it is possible to detect the biologically significant meaning of each component (Fig. 2).

The loadings of the principal component analysis can then be analyzed with traditional significance statistics. This method was used consistently in all studies presented in this thesis.

In the papers II, III and IV additional shell characteristics were obtained by using another image processing program (Image J) for measuring shell length as the length along the length axis from the top of the spire to the bottom of the snail. This program was also used to calculate the outline area of the shell. Combined with the shell weight I was able to obtain a relative estimate of the shell thickness as the weight: outline area ratio. Shell crushing resistance was the final parameter measured. The shell was placed at the bottom of a glass beaker with the opening facing down. Another glass beaker was then placed on top inside the first one and was filled with sand until the shell was crushed (Osenberg and Mittelbach 1989; Rundle and Brönmark 2001). When the shell is crushed it occurs instantaneously and the whole shell collapses. The sand and beaker is then weighed and the crushing resistance is calculated, correcting for shell size.

In paper IV a part of the study aimed at assessing the effects fish had on primary production, periphyton and macrophyte growth, through direct predation and through non-lethal predator cues. I planted saplings of watermilfoil (Myriophyllum spicatum), that were later sampled and I assessed macrophyte growth from the dry weight. To quantify periphyton growth the experiment contained plastic ribbons that stretched from the surface to the bottom. Periphyton growth was quantified by analyzing Chlorophyll a.

Fixed and plastic defence

Since R. balthica has been known to exhibit a great variability in shell shape (Hubendick 1951), in paper I I investigated whether this had to do with fish selecting for differences in shape. When molluscivorous fish ingest the snail and crush the shell with their pharyngeal teeth, resisting crushing forces on the shell may reduce the risk of predation by fish (Osenberg & Mittelbach 1989; DeWitt et al. 2000). Rounder shells are through their architecture harder to crush as the forces are spread more evenly over the surface and this reduces the need to invest in extra shell material (DeWitt et al. 2000). For example, DeWitt et al. (2000) found fish that attacked snails had a higher rejection rate for rounder shaped snails. I therefore expected fish to have selected for rounder snails in fish ponds, while snails in ponds with other predators would have evolved a different shape. Many invertebrate predators, like water bugs and leeches, are shell entry predators and, thus, an elongated shell shape was expected in ponds void of fish (Langerhans and DeWitt 2002). I collected snails from ponds with and without fish and analyzing their shape. I found a strong association between shell shape and pond type, as snails from fishless ponds had elongated shells with a long spire, while fish ponds contained snails that were rounder, had a large aperture and a short spire. However, when nesting populations within pond categories I found that there was an effect of population suggesting that selection from other predators than fish may act in the evolution of shell shape in R. balthica. This may be due to local differences in the predator assemblages including predation threat from invertebrate predators that may select for other shape characteristics than fish (DeWitt et al. 2000; Hoverman et al. 2005).

Although the field survey in paper I showed significant differences in shell shape that could be ascribed to specific predator regimes in the ponds, it was not possible to determine if differences in shell morphology were genetically fixed, or if R. balthica is a phenotypically plastic species. Selection from predation may favour prey to have a constitutional defence or it may favour development of a plastic defence. Environmental variability drives the evolution of phenotypically plastic defences, but associative cues must be reliable and the phenotype must match the environment so that fitness is superior in that environment compared to alternative phenotypes (Via and Lande 1985; Harvell 1990; Moran 1992; Gomulkiewicz and Kirkpatrick 1992), while costs constrains the evolution of extreme phenotypes (Van Buskirk 2000). The degree of plasticity, the slopes of the reaction norms, will be influenced by predictability of the environment and the evolution of a more plastic response will follow with increasing environmental uncertainty.

Picture 3. An example of Radix balthica raised with fish (left) and without fish (right).

To be able to establish if shape variation was a result of phenotypic plasticity, I conducted a common garden experiment. I used snail offspring from fish and fish free populations from a subset of ponds in the field survey in paper I. These were raised in the presence or absence of fish and when tested for shape they showed similar slopes of their reaction norms in the presence or absence of fish no matter their origin (Fig. 3). Thus, the induction of different shell mor-
Phenotypic plasticity in response to fish predation threat is a
phenotypically plastic response. I also calculated a plasticity index and tested it for differences between categories of pond origin. The index did not show any difference between pond categories, which further indicates that the snails react to the same extent on fish cue irrespective of their origin. Further, the slopes were similar to those from the field study indicating fish to be the selective factor for plasticity in *R. balthica* (Fig. 3). Similar results have been found in *Phyllidia* snails in response to predator cues from fish (DeWitt et al. 2000).

An alternative evolution of the reaction norm between populations of different origin is that they occupy different parts of the reaction norm, thus having the same slopes but differing in intercept. There was no significant effect in induced shape between snails of fish or fish free origin suggesting they have evolved the same reaction norm intercept. However, when nesting population within pond category (fish versus fishless ponds) there was a significant effect of population. This showed there were population specific differences in the reactions to fish that may reflect genetic components evolving from differences in local conditions like snail density, resource availability, predator density and predator assemblage.

In freshwater, prey defences may vary on a spatial or temporal scale between ponds or lakes. Differences between populations may mirror local predation regimes differing in selection pressure on prey. Local trait adaptation evolves if specific environmental conditions are met that are advantageous in that environment causing genetic differentiation of populations through divergent selection by the local predator assembly, provided there is limited dispersal among prey populations (Kawecki and Ebert 2004). Limited dispersal also suggests increases of local gene frequencies that further will enforce a local adaptation. However, increased gene flow, i.e. through migration, will restrict development of locally adapted populations and favour plasticity over locally adapted defences (Sultan and Spencer 2002; Sultan and Spencer 2002). Further, high dispersal rates increases temporal variation in a population that should favour plasticity over locally adapted defences (Sultan and Spencer 2002). The shape of the incidence function (Fig. 4) indicates that *R. balthica* is a rapidly dispersing species. This is further supported by studies from Ekologgruppen (2002) who in a survey of newly created ponds that ranged in age from 6 months to 6 years found that *R. balthica* had colonized all of them. Thus, it may be that *R. balthica* maintains genetic variation and variation in predation regime through rapid dispersal. Being a hermaphroditic further influences the potential of establishing in newly created waters.

### Multiple predators

For a species like *R. balthica* dispersing into new habitats means that the predation regime it encounters may be very different. Although prey is commonly exposed to many different predators that each differs in their foraging strategies, the need to defend against all may not be equally great. Thus, the effects of multiple predators are not just the sum of the single predator species (Sih et al. 1998). However, evolution has favoured a range of specific anti-predator responses that can be very different in isolation (DeWitt and Langerhans 2003). In paper II I quantified several shell parameters, shell shape, shell thickness and crushing resistance, for two predators that differ in their foraging modes. One predator was molluscivorous fish, represented by tench (*Tinca tinca*), a thus a shell crushing predator, while the other was signal crayfish (*Pacifastacus leniusculus*), that feeds on snails via shell entry. The functional morphology of the shell needs to differ as fish predation selects for a round shell with high crushing resistance (DeWitt et al. 2000) whereas crayfish predation demands a narrow shell difficult to enter and clip open (Brown 1998; DeWitt et al. 2000; Hoverman et al. 2005). This may create problems as a defence directed towards one predator increases the risk of predation by another (Sih et al. 1998; Krupa & Sih 1998). In most studies on multiple predator effects on prey there is a priority effect of predator defences, i.e., when exposed to a combination of predators prey develop a defence directed towards the predator posing a higher mortality risk (Relyea 2003).

In my study, the snails raised in the presence of tench developed a rounder shell with a large aperture and a short apex (Fig. 5a). Although round shells should reduce the need to invest in extra shell material (DeWitt et al. 2000), this was not the case for *R. balthica*, which add on extra shell material in the presence of tench (Fig. 5d). However, when regressing crushing resistance with shape and shell thickness I found that shape contributed more to crushing resistance than shell thickness.

For the other predator, the crayfish, feeding efficiency on snails should be reduced for elongated shells with narrow apertures and thicker shells (DeWitt et al. 2000; DeWitt and Langerhans 2003). However, snails from the crayfish treatment did not differ from the control in any way (Fig. 5a, b). This contrasts with other pulmonate snails, such as physids, where narrower shell morphology is induced in the presence of crayfish (DeWitt et al. 2000; Krist 2002) or for the snail *Helisoma trivolvis* that increases shell thickness to reduce the effectiveness of chipping at the aperture (Hoverman et al. 2005; Hoverman & Relyea 2007). The lack of a response in *R. balthica* to crayfish could be explained by that the shells are already elongated and that there may be structural constraints to what can be achieved by the snails with regards to narrowing the body. Shell thickness in the crayfish treatment was intermediate to the control, tench treatment, i.e. there seem to be a tendency of adding on extra shell material. Interestingly, when studying the combined response of the two predators, the shells became slightly less round in overall shape (comparing PC1 and PC2, ...
Fixed and plastic defence

Costs of plasticity

tors that may be the evolutionary result of the ease with which it spreads to new waters and the possibility of encountering various predators.

Selection, vulnerability

In paper IV I performed a study on competitive interactions among three snail species differing in shell morphology and, thus, potentially in vulnerability to predation. To test their vulnerability I made a separate experiment to determine fish selectivity on these species. These were, besides *R. balthica*, *Bithynia tentaculata*, a hard shelled snail that is very common in lakes with fish and *Physa fontinalis*, a thin shelled species. As I did not know anything about the other species reactions to fish, they were tested for morphological characters. There was a significant increase of crushing resistance in *B. tentaculata* with lethal fish treatment, but no morphological difference. The reason behind the stronger shell was extra investment in shell material. *P. fontinalis* only showed a minor change in morphology (represented only by 7.23% of the explained shape) and no major change to improve roundness or extra shell material was detected. Consequently, there was no increased crushing resistance in the presence of fish. An overall comparison of the strengths of the three species showed that *B. tentaculata* had the strongest shell, while *R. balthica* is intermediate and *P. fontinalis* have the weakest shell (paper IV). It seems that shell strength is the factor determining snail consumption by fish, independent of if this is achieved by having a constitutive defence or an alternative phenotype is affected under two constraints, increased intraspecific competition and predation threat. I hypothesized that differences in trait expression between a control and predator treatment would be greater at low intraspecific competition where resource avail-

Costs of plasticity

Evolution should constrain predator induced phenotypic plastic traits through costs. The evidence comes from the notion that these traits are not expressed in the absence of predators. Measuring costs of plasticity is very hard in reality since selection should also act to minimizing them why they may be hard to detect (Tollrian 1995; Van Buskirk 2000). DeWitt (1998) lists five types of costs: maintenance costs for keeping a sensory system for detecting cues and the physiological mechanisms for expressing alternative phenotypes, production cost for the alternative phenotype, information acquisition cost through increased risks when sampling the environment for cues, developmental inutility in the population resulting in reduced fitness and genetic costs when plasticity genes have negative effects on other genes. The study in paper III is focused on the first two, the maintenance and production cost of plasticity.

Predator induced defences means that the organism has to reallocate resources into defensive structures compared to the same genotype in a predator free environment. Costs of producing alternative phenotypes involves allocation shifts and trade offs that in turn will affect survival probability and fecundity (Arendt 1997). Fitness costs emerge from needs to allocate resources to defensive- or life-history traits that come in conflict with growth and reproduction (Van Buskirk 2000; Relyea 2002; Teplitsky and Laurila 2007). I investigated how production of an alternative phenotype is affected under two constraints, increased intraspecific competition and predation threat. I hypothesized that differences in trait expression between a control and predator treatment would be greater at low intraspecific competition where resource avail-

**Fig. 5.** Shell parameters from the study of multiple predators (control, tench, crayfish, tench and crayfish combined). Shape variation represented by PC 1 (a) and PC 2 (b) with the visualized outline shell shape on the y-axis. Size-corrected crushing resistance (c) and a relative measure of shell thickness (d). Error bars represent ± 1 SE

**Fig. 5a, b**, which should result in reduced crushing resistance. Instead shell material was added to a much higher degree (Fig. 5d) than in the tench treatment so that the main function, crushing resistance, was maintained (Fig. 5c). In other words, the snails were indeed reacting on the crayfish and reduced the roundness of the shell while maintaining a high crush resistance force needed to meet the predation threat from the fish. In a review by Relyea (2003) he observed that the change observed in inducible defences is most often the same as that to the more risky predator alone, sometimes an intermediate response occurs, but the change is never greater than that shown to the more risky predator. For *R. balthica*, most changes in shell parameters are induced in the presence of tench, indicating it to be the more risky predator. However, here the snails in the combined predator treatment had a higher shell thickness in the presence of tench alone, but this was done when lowering the defensive efficiency of shape to tench. It seems *R. balthica* has evolved a very flexible shell response to different predators that may be the evolutionary result of the ease with which it spreads to new waters and the possibility of encountering various predators.
ability is high, while the differences would be smaller as intraspecific competition increases and the resource availability decreases. This relation has been documented for growth rates where large differences between control and predator environments at low densities fades out at higher densities (Van Buskirk and Yurewicz 1998; Luttbeg et al. 2003; Relyea 2004; Turner 2004). Prey should respond adaptively and adjust activity to balance growth and risk to maximize fitness (Werner and Anholt 1993). Thus, I expected to see differences between control and predator treatments that were density dependent in the presence of costs through allocation shifts and trade-offs.

In paper III I found support for a density dependent growth-rate reduction that was stronger for the predator treatment at low densities. The reduction in food intake rates due to suppression of activity in the presence of a predator is greatest when resources are abundant, i.e. when conspecific density is low (Van Buskirk and Yurewicz 1998). At higher densities the effects were equalized probably because then low resource levels does not influence food intake rates as much; it does not matter if they spend time feeding or not when resources are scarce (Werner and Anholt 1993; Luttbeg et al. 2003).

**Trade-offs**

Several trade-offs can be made by an organism when under the threat of predation; i.e. how to allocate resources for growth and defence, how to respond to risk and how to grow and reproduce. The growth allocation model states that low competition and abundant resources allows defensive structures to be produced as all necessary somatic functions can be maintained, while defensive production is reduced when competition increases and resources are lower. An opposing model suggests that missed opportunity to feed in a resource rich environment should decrease investment in defence and favour fast growth (Van Buskirk 2000).

In paper III I found strongest support for the growth allocation theory as the main defence towards fish, the induction of rounded shape, showed density dependence. Snails exposed to fish cues at high densities were less rotund then at low densities (Fig. 6a). There was a significant interaction effect in shell shape, indicating a cost of changing shell shape. However, low investment in defence, may also mirror a relatively lower predation risk at high densities (McCoy 2007). The second principal component shows stronger density dependence in the control (Fig. 6b). It seems the snails in the presence of fish are much more restricted in shape, suggesting a cost in deviating from a particular shape.

Another trade-off involves the behavioural effects. Resources should influence risk taking behaviour as reduced activity and lower feeding rates that follows from behavioural alterations should be strongest at low competition and high resource availability, whereas at high competition and low resources the risk of starvation should result in a weaker response (Anholt and Werner 1998; Van Buskirk 2000; Van Buskirk et al. 2002). In the study in paper III there was no such effect of density on behaviour. Rundle and Brönmark (2001) showed a stronger behavioural reaction in snails with increasing shell strength. In this study I did not find a density effect of crushing resistance. Behaviour, responding to the crushing resistance, may therefore have shown a similar response and no density effect. The lack of density dependent reaction implies these trait alterations come with relatively low cost.

The third trade-off I studied in paper III was for the organism to grow or to reproduce. An organism may favour growth to size refugia if the adult predation risk is relatively lower than for juveniles (Crowl and Covich 1990), while low resource availability favours large size at adulthood (Stibor 1992). Further, high resource availability will favour a growth to size refugia and then start of reproduction, while low resource availability will favour reproduction as soon as possible as growth rates are low (Chase 1999).

*R. balthica* reproduced later irrespective of density in the presence of fish, indicating a relatively high adult survival but no trade-off between growth and reproduction. With increasing snail density, snails grew slower and laid fewer eggs as growth and reproduction is strongly correlated (paper IV). The reproduction was almost an order of magnitude lower at low densities in the presence of fish compared to the control. For higher densities there was no difference and differences were equal and the predator treatment even exceeded egg production in the control. However, the egg production at the highest densities was only a fraction of the production at low density.

I made correlations between the measured traits to explore how the different traits are linked at high and low densities respectively. Negative correlations are likely to be found where allocation of resources is mainly affecting the traits and positive correlations when traits are affected mainly by resource acquisition (Glazier 2002). Size seems to be costly for a rounder shape, negative scores in PC 1; while size, low growth rates and egg production are costly for positive scores in PC 2 that represents high snail densities and a slightly rounded shape.

In conclusion, *R. balthica* show some costs, for altering shell shape in the presence of fish. The other defensive traits like crushing resistance and shell thickness show low costs. Defensive traits seem to be traded-off with fitness related traits like reduced growth rate and reduced egg production. Additional costs may be associated to reduced feeding, probably through activity suppression. It seems altering shell shape is costly for *R. balthica*, but to understand the complexity of adaptive defences we may need to further investigate the role of activity and food intake rate.

**Competition and predation**

In paper III I showed that *R. balthica* is strongly negatively affected by intraspecific competition that severely reduced growth, final size and fecundity. What about competition with other snail species? Snails of different species are likely to have a strong impact on each other as they are sharing the same resources. Since *R. balthica* is morphologically plastic it may affect its com-
Petitive ability with other snails that differs in their degree of protection.

**Paper IV** aimed at studying the effects of trait mediated indirect interactions (TMII) as well as density mediated indirect interactions (DMII) between *R. balthica* and two other snail species. TMII are the indirect effects a predator have in a food chain by affecting the traits, e.g. behaviour, and not the density, of its prey (Relyea 2000; Peacock and Werner 2001; Werner and Peacock 2003), while DMII arises through a density reduction of prey which in turn affects its resources through cascading effects (Brönmark et al. 1992). The effects of TMII may be relatively larger at high resource levels because at high resource levels foragers are prone to take fewer risks (Luttge et al. 2003). However, the setup in **Paper IV** initially contained an equal number of snails per species and the same resource level, not allowing for studying the relative contribution of each effect separately (Peacock and Werner 2001; Luttge et al. 2003). Instead, focus was on differential vulnerability to the predator and the resulting effects of competition across generations. For instance, in a study by Kohler (1989), more vulnerable *Baetis* mayfly larvae responded adaptively to fish presence by hiding, while the less vulnerable caddisfly *Glossosoma* main response was a shift in food quality. As a consequence there is a potential for the less vulnerable prey to increase in density when growth of the competitor is suppressed by antipredator avoidance behavior (Miyasaka et al. 2003). In a study of natural ponds Chase (2003) found an increase in less vulnerable species with increased productivity. Such a system represents the typical pond or lake community that contains both molluscivorous fish and many snail species.

The experiment in **Paper IV** was run as a long time experiment over two generations of the snails. It involved two other snail species besides *R. balthica*, namely *Physa fontinalis*, and *Bithynia tentaculata*. *P. fontinalis* is also a pulmonate snail and relatively thin shelled, while *B. tentaculata* is a prosobranch snail with a thick shell. Neither species are known to change shell shape in response to fish predation. Both *R. balthica* and *P. fontinalis* are effective grazers on periphyton while *B. tentaculata* may switch to filter feeding, thus it reduces the need to be active in order to forage and may utilize an alternative food source (Brendelberger and Jürgens 1993; Brendelberger 1997; T. Lakowitz unpublished material).

Snails were exposed to three treatments: a control with no fish, a non-lethal fish treatment (fish cues only) and a treatment were fish were allowed to feed on snails ("lethal"). The rational behavior setup was that plasticity constraints through altered resource acquisition abilities may have negative effects in the non-lethal presence of fish, influencing the competitive interactions with other species. In the lethal predation treatment snails with inducible shells like *R. balthica* and hard shelled snails like *B. tentaculata* that have a high crushing resistance would benefit and have increased survival. In parallel, I performed a separate experiment to obtain a detailed picture of the competitive effects between combinations of the different snail species at different densities with and without fish predator cue. I found that *R. balthica* was the stronger competitor. The other two species showed no impact on each other. *P. fontinalis* and *R. balthica* were negatively affected by intraspecific competition.

The control and non-lethal snail assemblages developed from having consisted of equal numbers of *R. balthica* and *P. fontinalis* to a dramatic dominance of *R. balthica*. However, biomass and density was the same for *R. balthica* in both treatments (**Fig. 7a, b**). *P. fontinalis* showed a increased density in the control and a reduced density in the non-lethal treatment, although total biomasses was similar. Thus, the population of *R. balthica* went from few large to many small snails in both treatments, while *P. fontinalis* went from few large to many small snails in the control and few large in the non-lethal treatment. The relatively higher vulnerability in *P. fontinalis* may have led to a greater degree of behavioural suppression (Rundle and Brönmark 2001). This in turn may have had a positive effect on *R. balthica*, and may further explain the non significant difference in density for *R. balthica* between the control and non-lethal treatments (Werner and Anholt 1996). Another explanation of the negative density effect in *P. fontinalis* in the non-lethal treatment may be that the negative effect of TMII on prey density is stronger with increased competition. These two species showed competitive effects and *R. balthica* was the numerically dominant species, which may further explain the negative effects in *P. fontinalis* (Bolnick and Preisser 2005). *B. tentaculata* remained at low numbers but developed positively in both numbers and biomass with time, showing it initially to be a relatively poor competitor (**Fig. 7c**). However, relatively high mortality rates have been reported among juveniles that may have contributed to low numbers already from the initial stocking of the juvenile snails (Richter 2001).

In the lethal treatment, fish had a profound impact on the community. As was evident from the selection trial, *P. fontinalis* was the most vulnerable species, followed by *R. balthica* and *B. tentaculata*. *P. fontinalis* was eradicated from the lethal treatment and the number of *R. balthica* was severely reduced. The total biomass of *R. balthica* was equal to the other two treatments and the community consisted of a few large in-vulnerable specimens with an even more pronounced induced shape, shell thickness and crushing resistance. However, the impact on
B. tentaculata was equal to that in the other treatments initially, but with time B. tentaculata did better in the lethal environment than in the other treatments. This confirms its poor competitive ability with the other species even further since only here, when it was released from competition, it could increase in density.

It seems that trade-offs between vulnerability, determined through the shell strength, and behaviour determines community structure (Rundle and Brönmark 2001). The most protected species are persistent, but poor competitors, probably through the need for building an energetically costly shell. The weakest protected species rely most on trait compensation that will affect them negatively in the competition with other snails through spending more time in refuge (DeWitt et al. 1999). A species like R. balthica may not be as strongly affected as it alters shape and reduces the need for behavioural suppression, remaining a strong competitor.

**Trophic cascades**

The cascading effect of predation in an ecosystem is a result of the structuring role predators have on their prey (Brönmark 1985; Brönmark et al. 1992). In fact, lentic aquatic ecosystems, ponds and lakes, show perhaps the greatest effects of predators on trophic cascades, the indirect effect the predators have on plants through the herbivores, of any system (Shurin et al 2002). Further, one of the strongest cascades in the lentic system is caused by the predator-snail interaction, which is playing a particularly forceful role through snails being highly susceptible to predators and at the same time having a large impact on their resources (Shurin et al 2002). Their positive effect on the growth of submerged macrophytes has been well documented, as snails are very effective grazers on periphyton, they will increase the availability of light and nutrients for the macrophytes (Brönmark 1985; Brönmark et al. 1992; Turner et al. 2000). In *paper IV*, part of the study aimed at determining the role snails have on macrophytes through the indirect effect of periphyton grazing, both through a direct lethal predation effect (fish-snail) and a non-lethal effect resulting from exposure to predator cues only. I found that through direct predation macrophyte biomass was reduced where fish had a negative effect on snail density (Picture 3) and that there was a tendency, however not significant, for decreased macrophyte growth in the presence of non-lethal cues from fish.

Periphyton growth mirrored that for macrophytes, although inverted. These effects emerge in the non-lethal treatment from suppression of snail activity resulting in a reduction of feeding rates on periphyton and in the lethal treatment both through suppressed activity and through fish reducing snail population density. However, an interesting relation between snails and grazing effects emerged when snail density and snail biomass was correlated to macrophyte biomass and periphyton Chl-a content. While there was no correlation of snail biomass with either macrophyte biomass or periphyton Chl-a content, snail density showed a strong positive correlation with macrophyte biomass and a strong negative correlation with periphyton Chl-a content. The snail communities in the control and non-lethal treatments consisted of many smaller snails, while in the lethal treatment there were fewer large snails. None of the treatments differed in biomass, showing snail number to be an important driver of the cascading effects of fish predation on snails. These results parallel studies under natural conditions where reduced snail density by fish had a strong positive effect on periphyton growth (Brönmark et al. 1992).

**Conclusions**

*R. balthica* is a phenotypically plastic species with a spectrum of traits that change in the presence of predation threat. Fish is one on the most important predators on snails as and as they have a great impact on snail communities, they have the potential of driving the evolution of defensive mechanisms in their prey. Local conditions have influenced these reactions in *R. balthica* through differences in selection pressure. However, fish has selected for the same degree of plasticity, reaction norms of similar slopes and intercept. By being a species that disperses rapidly, *R. balthica* encounters environmental variability both temporally and spatially. This is probably what has led to the evolution of a phenotypically plastic defence in this species.

Being able to specifically alter shape is of great importance in an organism relying on the shell for protection. Different foragers demand different adaptations and this may cause a dilemma when a reaction to counter the attack from one predator is favouring another predator. *R. balthica* showed considerable ability in changing different shell parameters to fine tune the shell in a way that would meet the conflicting demands for a differently protected shell.

When responding phenotypically to meet a predation threat, *R. balthica* alters shape, behaviour and life history. A phenotypically plastic defence means that fitness of that phenotype should be superior averaged across populations. Costs of expressing a plastic defence in *R. balthica* is manifested primarily through slow growth and fewer eggs that are deposited relatively later than in a fish free environment. Shape change in itself shows some cost, but it seems relatively small in relation to costs associated with reduced activity levels. Additionally, maintaining defences, even if resources are constraining the trait expressions, seem to be very important for this species.

In competition with other snail species sharing the same resources, *R. balthica* is a very strong competitor that has an advantage of induced morphology in the presence of fish. Increasing shell strength through changed morphology may enable this species to increase feeding activities relative to more vulnerable species. Further, the ability to alter shell shape saves the costs of building a thick and energetically costly shell. *R. balthica* rapidly establish in new environments and often become the dominant species, irrespective if fish is present or not (Ekologgruppen 2002). The key lies no doubt in its versatility in the trait alterations themselves and in the ability to alter a range of traits to meet the local predator assembly.
Predator induced phenotypic plasticity in a freshwater snail

References


My contribution to the papers

**Paper I:** My supervisor Christer Brönmark and I discussed the idea for the field study and the common garden experiment. C. B. and Per Nystöm provided data for the ponds and I also used data from Ekologgruppen (2002). C. B. and I collected snails in the field. I set up and conducted the experiment. Fish were collected by Jakob Brodersen. I analyzed the data and performed the statistics in collaboration with C. B. I wrote an earlier version of the manuscript that was later improved by C. B. and by comments from two anonymous referees.

**Paper II:** The idea was discussed by C. B., P. N., and I. I conducted the experiment. Fish were provided by J. B. and Erika Nilsson. I analyzed the data, performed the statistics, and wrote the paper with valuable comments from C. B., Susanne Gustafsson and Samuel Hylander.

**Paper III:** The experiment was planned by C. B. and me. It was performed under my supervision by Charlotte Lennartsdotter as her degree project. She analyzed the data and performed initial statistical tests for her project under my supervision. Later I extracted new data from the original data set. I performed new and additional statistical tests with advice from C. B., Jörgen Ripa and Anders Nilsson. I wrote the paper with valuable comments from C. B., Susanne Gustafsson and Samuel Hylander.

**Paper IV:** The study was planned by C. B. and me. I set up the experiment with help from Mikael Jönsson. Sampling was made by C. B. and me. The competition and selection experiments were made with help from Per Hallgren. I extracted the majority of the data with help from P. H. I performed the statistics with advice from A. N. and wrote the paper with valuable comments from C. B. and P. N.
De stora likheterna mellan reaktionerna tyder på att fisk är en drivande evolutionär kraft för bildandet av den rundare formen.

Detta är en art som sprids snabbt till nya vattendrag trots att den saknar självklara spridningsmöjligheter som vingar eller ben. Troligtvis utgör fisken en spredningsvektor för snäckor. Snabb spridning är även en trolig förklaring till att den här arten utvecklat ett plastiskt försvar eftersom sannolikheten är stor att hamma i olika habitat som skiljer sig i t.ex. predator regim.


Predator induced phenotypic plasticity in a freshwater snail

Thanks!

Chrisrer! This work would never have surfaced without your supervision over the years. I remember when we set up the first experiment partly on chance as we were not sure what to expect from the snails. I guess we were equally excited when we saw, with the naked eye, the shape changes the snails had undergone in the fish treatments. I remember you said: "with these guys we will have great fun in the future", and I think we have, with many interesting results emerging from the studies. But it has not only been supervision, as I have had a great teacher in you all the time. The initiation of the writing course by you and Lasse was one of the best initiatives taken from a PhD-students point of view. Further on you have been firmly a member when we set up the first experiment and you helped bring in the fishes. You have been away. I had excellent help from you on everything from practicalities about the experiments to statistics and the writing process. We also share the same interest in frogs and it was great fun when you finally showed me the elusive spadefoot toad (Sw. Lökgroda) that I so far only saw as tadpoles abroad.

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MikÅl, thank you for the summer we spent at 14 °C in the basement looking down into grey mud. You and I had a great collaboration during the bioturbation side project. You learned me a lot about your speciality, plankton, and for that I am very grateful. I think it was a great time when we did the experiment, but it got more extreme towards the end when you brought my attention to German trucker music. That was certainly an unexpected experience.

Charlotte who did her degree project for me on the costs and trade-offs of plasticity. Thank you for your endurance and accuracy when working with the snails. It was always interesting discussing and sharing your results that finally led to a very interesting paper.

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I may have bothered you much Anders N, when asking the statistical questions, but it was worth it (for me)! We have also had interesting discussions on everything from important things like snails to unimportant issues like fish biology. Thank you!

In no particular order I thank: Samuel and Susanne, for our discussions in the inducible defence group and help with ideas for projects and proofreading. Geraldine, thank you for the nice collaboration we had when reading for the literature exam. Leo, it has been a privilege to accompany you to Aneboda, where I relived the Limnology course once again. It was perhaps even more educating now when I didn’t have the pressure to write the exam. Your teaching experience has also been inspiring when I have been teaching. Marie, you have been great help with analysis, you are always kind and take your time to help out. Per H, you have become a good friend, helping me much at the end of my time as PhD-student when time is a scare commodity. It has been really nice to see the development of your own results as well. Perhaps it is not surprising as you have been working with such interesting organisms as snails. Mikael, thank you for helping me out when we started up the large long term experiment in the greenhouse, I really needed that help. Erika, you helped me with my fishes and we have had good discussions concerning our subjects and multiple predators. Kerstin, as the department secretary you have been invaluable and I have been troubling you much, but I am very thankful for all your help. Lynn, my room mate, thank you for sharing the mush room interest and I think we have interesting discussions all the time, although I may have become more asocial lately as the stress level increased, sorry for that. Kullberg, you take your time helping computer illiterates like me, thank you. Marika, you helped me a great deal in New Zealand with that wash away project, great fun, thank you. Anders P, thank you for stimulating discussions and for letting me play a marginal role in the oestrogen project. It has been really fascinating to see the results and hopefully this was just the beginning. Pia H, thank you for exchanging material and we share a common interest in the snails that has led...
to interesting comparisons with your results, not least the competition experiments. **Kajsa**, thank you for the valuable comments you have had on my various projects. Lately, I discovered your interest in garden plants and exchanging garden seeds with you, that is also valuable, but for other reasons, thank you! Finally, a big ‘Thank you!’ to all new and old limnologists who make, and have made, the Limnology department such a nice place!

Posthumously I would like to thank **Håkan Turesson** who encouraged me to get a position as a PhD-student at the limnology department. I really miss the many conversations we had during the time I did my degree project. Now I occupy your old room and chair and will not forget you, thank you.

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**Leo** and **Victor**, I hope you will pursue your own interests in due time as I did and find it’s never too late to do something else. Maybe you will get inspiration from this thesis, not through the contents, but from the work itself.

I would also thank my parents, **Christl** and **Karl-Heinz** for helping our family so much. Your support is priceless and I we are so grateful for your help.

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As an undergraduate student I met many people that I will always remember as you are part of my education leading up to this point. **John** and **Charlotte**, we started as a good team over at the Chemistry centre and without you the studies there would have been really dull. **Markus** and **Stefan** came later and we supported each other through the studies, thank you.