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 were 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.

Fakultetens opponent: Prof. Simon Ruddle, University of Plymouth, UK.

Avhandlingen kommer att försvars 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 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 accurately. 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 hunts 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, as they are prey for a range of different predators (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 & Covich 1990). Freshwater snails are very variable in body size and in shell morphology, both of which are traits that relate to 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 & Covich 1990). Freshwater snails are very variable in body size and in shell morphology, both of which are traits that relate...
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 (Glöer 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; Wulfschlegle 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 same approach was taken in all the experiments I conducted. Except for the study in paper I, 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).

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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 flaved 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 length 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). 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 analyzed 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.
Fixed and plastic defence

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.

Fixed and plastic defence

Fig. 3. The shape results from the common garden experiment and the field study. The visualized outline shell shape is shown on the y-axis. Snails from fish free ponds (squares) and fish ponds (diamonds) raised in the absence and presence of fish. For comparison of the relative position in the reaction norms are the snails from natural ponds (circles) without and with fish. Error bars represent ±1 SE.

Fig. 4. Incidence function for R. balthica showing the probability of occurrence (J) in ponds with increasing number of other snail species. Number of ponds per category is shown on top of the graph.

population within pond category (fish versus fishless ponds) there was a significant effect of population. This show 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.

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Fixed and plastic defence

(Sultan and Spencer 2002; Lenormand 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 hermaphrodite further influences the potential of establishing in newly created waters.

Multiple predators

For a species like R. balthica dispersing into new habitats mean 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), thus a shell crushing predator, while the other was signal crayfish (Paciifastacus 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,
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 of a strong shell or through induced shell morphology to improve shell crushing resistance (Stein et al. 1975; DeWitt et al. 2000). To test this, I made a feeding trial where crucian carp were allowed to feed on the three snail species. The results showed the hard shelled snail *B. tentaculata* was not consumed to any great extent, *R. balthica* was not consumed to any great extent, while the fish consumed all of the weak shelled *P. fontinalis*. Consumption on *R. balthica* was intermediate between the two species. It shows that crucian carp is a selective forager that has a directional effect on prey through difference in vulnerability and, further, it shows the importance of shell strength for protection and reducing predation rates in snails.

**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 instability 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 RELOCATE RESOURCES INTO DEFENSIVE STRUCTURES COMPARED TO THE SAME GENOTYPE IN A PREDATOR FREE ENVIRONMENT. COSTS OF PRODUCING ALTERNATIVE PHENOTYPES INCLUDES 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; RELYE 2002; TEPLITZKY AND LAURILA 2007). I INVESTIGATED HOW PRODUCTION OF AN ALTERNATIVE PHENOTYPE IS AFFECTED UNDER TWO CONSTRAINTS, INCREASED INTRASPECIFIC COMPETITION AND PREDATION THREAT. I HYPOTHESES THAT DIFFERENCES IN TRAIT EXPRESSION BETWEEN A CONTROL AND PREDATOR TREATMENT WOULD BE GREATER AT LOW INTRASPECIFIC COMPETITION WHERE RESOURCE AVAIL-
Predator induced phenotypic plasticity in a freshwater snail

Costs of plasticity

Predator induced phenotypic plasticity in a freshwater snail

Competitiveness and predation

Fig. 6. The effects of shell shape in increasing density (2, 4, 8, 16 and 24 snails on the x-axis), in treatments with no fish (open squares) and in the presence of fish (closed diamonds). Outline shell shape is visualized on the y-axis for PC 1 (a) and PC 2 (b). Error bars represent ± 1 SE.

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 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).

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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 deviation 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 early reproduction is favoured when adult survival is relatively lower than for juveniles (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 slow 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 where traits are affected mainly by resource acquisition (Gliazer 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.

Competitiveness 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 (Reylea 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 (Luttbeg 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; Luttbeg 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 behind the 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 inductive 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

**Fig. 7.** The total number of snails in the competition experiment at the first and second sampling. Snails are *R. balthica* (**a**), *P. fontinalis* (**b**) and *B. tentaculata* (**c**). The treatments are control (circles), predator cues from fish only (squares) and a treatment were fish were allowed to feed on snails (diamonds). Error bars represent ± 1 SE.
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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 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.

**Picture 4.** This illustrates the positive effect snails have on submerged macrophytes (left) and the indirect negative effect fish have on macrophytes by reducing the snail density through predation (right). A noticeable difference in periphyton growth is also visible in the two treatments.
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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 Nyström 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.


Många organismer i akvatiska system, använder sig av kemiska signaler som härstammar från predatorn, för att kunna göra en riskbedömning. Signalerna kan t ex uppstå genom avsöndringar från predatoren när den konsumerar bytesdjur av samma art. Man har visat att dessa signaler är väldigt användbara för bytesdjur och att de kan avgöra, inte bara om predatören är närvarande, utan också vilken art det rör sig om. Eftersom predatorer av olika arter har olika strategier för att söka, fanga och åta upp ett visst bytesdjur på kan då bytesdjuret, genom informationen om predatoren, använda sig av försvarsmechanismer som är specifika för att möta just det hotet.

Ett försvar kan vara fixerat och alltid uttryckas, men en del försvar är så kallat fenotypiskt plastiska och uttrycks bara som en reaktion på att bytesdjuret känner av närvaron av en viss predator. Evolutionen av plastiska resonerar gynnas när det är stor men förutsägbar variation i omgivningen. Predator specifika förändringar innefattar t ex beteende, livs-historie strategi eller kroppsform.

I sörvattensystem är snäckor en utomordentligt viktig organismgrupp. Som betare av påväxtalger reducerar de dessa på undervattensväxter och gör ljus och näringsämnen tillgäng-
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ligen, vilket gynnar undervattensvegetationen. En predator som konsumerar snäckor bidrar därmed indirekt till en sämre tillväxt av undervattensväxter.


Jag har studerat hur en av de vanligaste europeiska sötvattenssnäckorna, oval dammsnäcka (Radix balthica), förändrar olika egenskaper när den utsätts för hotet från bl.a. fisk. Den har sedan länge varit känt för sin stora formvariation mellan olika lokaler och jag undersökte om detta kunde ha samband med förekomsten av fisk.

Genom insamling av snäckor i dammar med eller utan snäckätande fisk, mätte jag statistisk formen på snäckorna och fann att snäckor från dammar utan fisk var avlånga än när snäckorna blev rundare, men de stora likheterna mellan reaktionerna tyder på att fisk är en drivande evolutionär kraft för bildandet av den rundare formen.


Mötes med olika predatorer utgör en konfliktsituation eftersom ett spridsnabbt risiko mot en predator kan förenkla för en annan. Oftast blir reaktionen hos bytestadjuret riktad mot predator som utgör det starkaste hotet eller en intermediär reaktion mellan de båda. För snäckorna, om risken mot fisk är ett rundare skal och stor mynning, så förenklar den formen predation från kräfta, eftersom de öppnar skälet för att för att prova förändringer i skalmorfologi och eftersom de öppnar skälet för att prova förändringer i skalmorfologi och eftersom de öppnar skälet för att prova förändringer i skalmorfologi och eftersom de öppnar skälet för att prova förändringer i skalmorfologi.

Ändringen i form och skäl påverkar snäckornas förmåga att krossa det. Snäckorna som har en rundare form har mindre energi för uppbyggnad av det tjocka skallet. Där fiskens förmåga att krossa är starkare, så krossar snäckorna snabbare för att undvika predation.


Sammanfattningsvis är R. balthica en snäcka som utvecklat ett effektivt skydd mot predatörer. I huvudsak fisk, troligen som ett resultat av snabb spridning. Detta kan ha sin orsak i de selektiva effekter fisk har där de helst konsumerar snäckor som är lätt att krossa.

För att ta reda på var kostnaderna med ett predatör försvar kommer när de utförs, gjordes ett experiment där snäckorna utsattes för kemiska signaler från fisk i en gradient av oändligt täthet och därmed minskade resurser. Skillnader mot samma gradient utan fisk gjort att man kan bedöma var en kostnad uppstått. Jag fann att krossneder för förändring av skalmorfologi och krossjock och krossbarhet är relativt små. Däremot fanns skillnaderna i en kraftigt reducerad tillväxt och annu kraftigare reducerad och förmoderj förplantning. Att ha ett kraftigt försvaret är viktigt för snäckorna, vilket går ut över tillväxten och mängden avkomma som produceras.

Det finns olika strategier hos snäckor och en del arter som lever med fisk har mindre utvecklade försvar för att förhindra predation. En predator som utgör det starkaste hotet kan förenkla för en annan predator, och därmed minskade kvaliteten av skälet. Fisknärvaron fick den tunnskaliga snäckan att försvinna, medan den förändrade formen där den finns minskades kraftigt i antal tillsammans med R. balthica i närvaro av fisk. Fisknärvaron fick den förmodligen att minska aktiviteten och därmed minskade kvaliteten på att försvaras mot predatörer.

Snäckorna förändrar formen på sina sköldar för att förhindra predation. De bildar en rundare form, vilket kan vara en försvarsmechanism för snäckorna. Detta kan ha sin orsak i de selektiva effekter fisk har där de helst konsumerar snäckor som är lätt att krossa.
Thanks!

Christer! 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-student. Not only saw as tadpoles abroad. 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.

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Per, as assisting supervisor I have had great help from you not least when we worked on the crayfish/fish project. You were also of great help in New Zealand although we all know how that experiment went literally down the drain at the farm of “Mr. Elbow”. You have been, invaluable at times when Christer 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.

Marika, you helped me a great deal with analysis, you are always kind 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 mushroom 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, 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, 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.

On the private side, I would first like to thank my wife, Charlotte, for being so understanding. It has been amazing what you have been put up to, taking care of the kids when I was away or lately when I spent so much time writing. I love you!

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.

A very important: ‘Thank you!’ goes to my younger brother Peter who is responsible for making the layout of this thesis. Your skills really rescued me, probably saving a lot of headaches and foremost: I didn’t get a thesis print branded; ‘Thomas Lakowitz’, which feels extra good.

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