



LUND UNIVERSITY

Predator induced phenotypic plasticity in a freshwater snail

Lakowitz, Thomas

2008

[Link to publication](#)

Citation for published version (APA):

Lakowitz, T. (2008). *Predator induced phenotypic plasticity in a freshwater snail*. [Doctoral Thesis (compilation), Department of Biology].

Total number of authors:

1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

- I Lakowitz, T. and Brönmark, C. 2008. Predator induced morphological plasticity in geographically isolated populations of a freshwater snail.
- *Submitted.*
- II Lakowitz, T., Brönmark, C. and Nyström, P. 2008. Tuning in to multiple predators: conflicting demands for shell morphology in a freshwater snail.
- *Freshwater Biology*, DOI: 10.1111/j.1365-2427.2008.02045.x
- III Lakowitz, T., Lennartsdotter, C. and Brönmark, C. Cost of predation threat and phenotypic plasticity.
- *Manuscript.*
- IV Lakowitz, T. and Brönmark, C. Prey vulnerability and phenotypic plasticity effects on competition and resources in three freshwater snails.
- *Manuscript.*



THOMAS LAKOWITZ • PREDATOR INDUCED PHENOTYPIC PLASTICITY IN A FRESHWATER SNAIL

Predator induced phenotypic plasticity in a freshwater snail

Thomas Lakowitz

ISBN 978-91-7105-284-1

2008

Department of Ecology
Limnology
Lund University, Sweden
Lund 2008



LUND
UNIVERSITY

*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.*

Organization LUND UNIVERSITY		Document name DOCTORAL DISSERTATION	
Department of Ecology, Limnology Ecology Building SE-223 62 Lund Sweden		Date of issue October 30, 2008	
		Sponsoring organization	
Author(s) Thomas Lakowitz			
Title and subtitle Predator induced phenotypic plasticity in a freshwater snail			
Abstract In this thesis I examined a phenotypically plastic defence in a common freshwater snail, <i>Radix balthica</i> . Adaptive prey defences may involve behavioural, life-history or morphological changes. Snails are important species in freshwater systems. Their role as grazers on periphytic algae makes them particularly important as they have a positive impact on macrophyte growth in lakes and ponds. The structuring role of predators, especially fish, on snails may therefore have a great impact on lake ecosystems. I have studied the occurrence of different shell shapes in this snail in nature. In laboratory studies, I have determined the degree of shell plasticity over many populations, the morphological reaction to multiple predators, costs of expressing plastic traits and the role of plasticity in a community context with other snails. My results show this snail to have different shell shapes in nature that can be attributed to the presence or absence of molluscivorous fish. In the laboratory, I established it to be a phenotypically plastic species with identical reaction norms irrespective of the population originated from a fish or fish free pond. In a test with multiple predators, fish and crayfish that differs in foraging strategies, I showed that this species produces different shell shapes, but that the main defensive change was directed to defend against fish predation and that shape determined the crushing resistance of the shell. I also showed it to be able to compensate differences in shell shape with shell thickness to maintain crushing forces of the shell that are an important factor for defence against fish. The costs for adaptive trait changes like morphology, shell thickness or behaviour seems to be relatively low in this species. Instead, costs mainly emerge from reduced growth rates and lower fecundity. In the competition with other snail species, <i>R. balthica</i> is a strong competitor that dominates the system with or without the presence of predator cues only. Under direct predation from fish, it depends on the higher crush resistance of a morphologically altered shell shape and persists together with another hard shelled species in this environment. The main conclusions from my thesis is that this species is plastic over a large geographical range, probably through high gene flow and that fish has been a key predator in the evolution of a plastic defence. It shows great versatility in many traits in the presence of fish and at relatively low costs. It is a strong competitor and due to the morphologically plastic shell, persistent in many environments.			
Key words: <i>Radix balthica</i> , dispersal, shell morphology, reaction norm, multiple predators, cost of plasticity, intraspecific competition, interspecific competition			
Classification system and/or index terms (if any):			
Supplementary bibliographical information:		Language English	
ISSN and key title:		ISBN 978-91-7105-284-1	
Recipient's notes		Number of pages	Price
		Security classification	

Distribution by (name and address)

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature 

Date September 22, 2008

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 opponenter: *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).

Contents

Introduction.....	8
Predator recognition - non-lethal predator cues.....	9
Using snails for studying prey defences.....	9
<i>The study organism</i>	10
<i>Methods</i>	10
Fixed and plastic defence.....	12
<i>Multiple predators</i>	14
<i>Selection, vulnerability</i>	16
Cost of plasticity.....	17
<i>Trade-offs</i>	18
Competition and predation.....	19
<i>Trophic cascades</i>	22
Conclusions.....	23
References.....	24
Contributions.....	28
Swedish abstract.....	29
Thanks!.....	32
Papers.....	36
I Lakowitz, T. and Brönmark, C. 2008. Predator induced morphological plasticity in geographically isolated populations of a freshwater snail. - <i>Submitted</i> .	36
II Lakowitz, T., Brönmark, C. and Nyström, P. 2008. Tuning in to multiple predators: conflicting demands for shell morphology in a freshwater snail. - <i>Freshwater Biology</i> , DOI: 10.1111/j.1365-2427.2008.02045.x	52
III Lakowitz, T., Brönmark, C. and Lennartsdotter, C. Cost of predation threat and phenotypic plasticity. - <i>Manuscript</i> .	62
IV Lakowitz, T. and Brönmark, C. Prey vulnerability and phenotypic plasticity : effects on competition and resources in three freshwater snails. - <i>Manuscript</i> .	80

Cover Photo: Per Hallgren

*Layout: Peter Lakowitz
Proofreading: Thomas Lakowitz
Font: Adobe Garamond Pro 11 / 12,5
Photos by Per Hallgren and Thomas Lakowitz
Printed by E-huset, Lund*

ISBN 978-91-7105-284-1

INTRODUCTION

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; Nyström 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 will 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 Mittelbach 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 capture 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.

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 reacts 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, 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; Nyström 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; Wulschleger and Ward 1998).



Fig. 1. Natural variation in shell shape of *Radix balthica* (from Hubendick 1949).

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



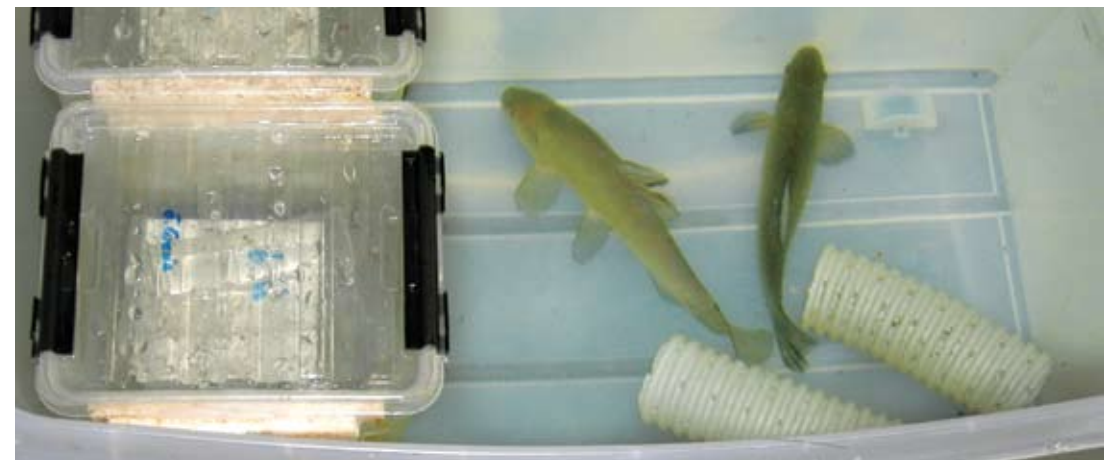
Picture 1. The ground water fed pond from which most snails in the experiments originated.

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).

One main focus in this thesis was to quantify phenotypic change in different shape characteristics



Picture 2. An example of an experimental setup with predator cues. Here, tench (*Tinca tinca*) swim in the larger box. The snails are confined within the smaller box that has mesh on the sides to allow water to circulate through.

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



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.

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 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.



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-

phology 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 showed no 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 survey indicating fish to be the selective factor for plasticity in *R. balthica* (Fig. 3). Similar results have been found in *Physella* 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

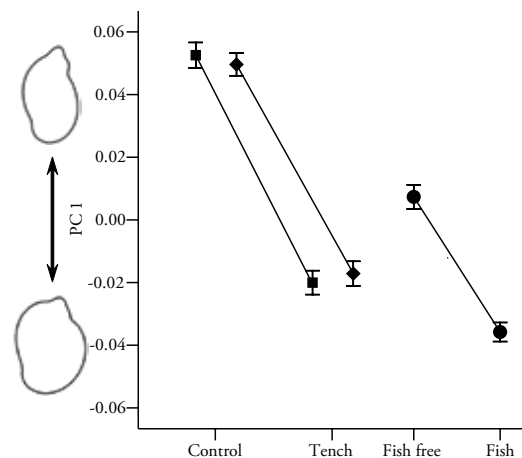


Fig. 3. The shape results from the common garden experiment and the field study. The visualized outline shell shape is shown on the y-axis. Snails from fish free ponds (squares) and fish ponds (diamonds) raised in the absence and presence of fish. For comparison of the relative position in the reaction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

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

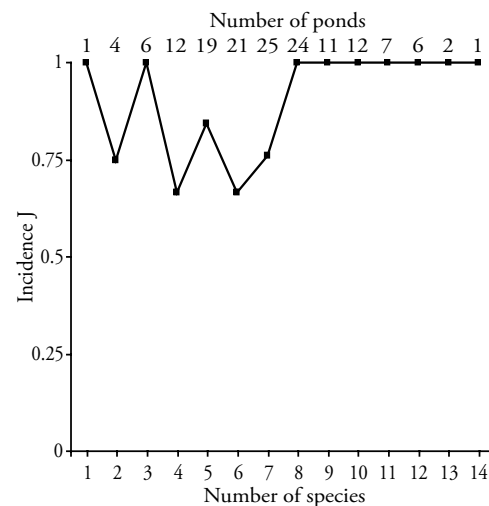


Fig. 4. Incidence function for *R. balthica* showing the probability of occurrence (J) in ponds with increasing number of other snail species. Number of ponds per category is shown on top of the graph.

(Sultan and Spencer 2002; Lenormand 2002). Further, high dispersal rates increase 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 (*Pascifastacus 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 prob-

lems 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, 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,

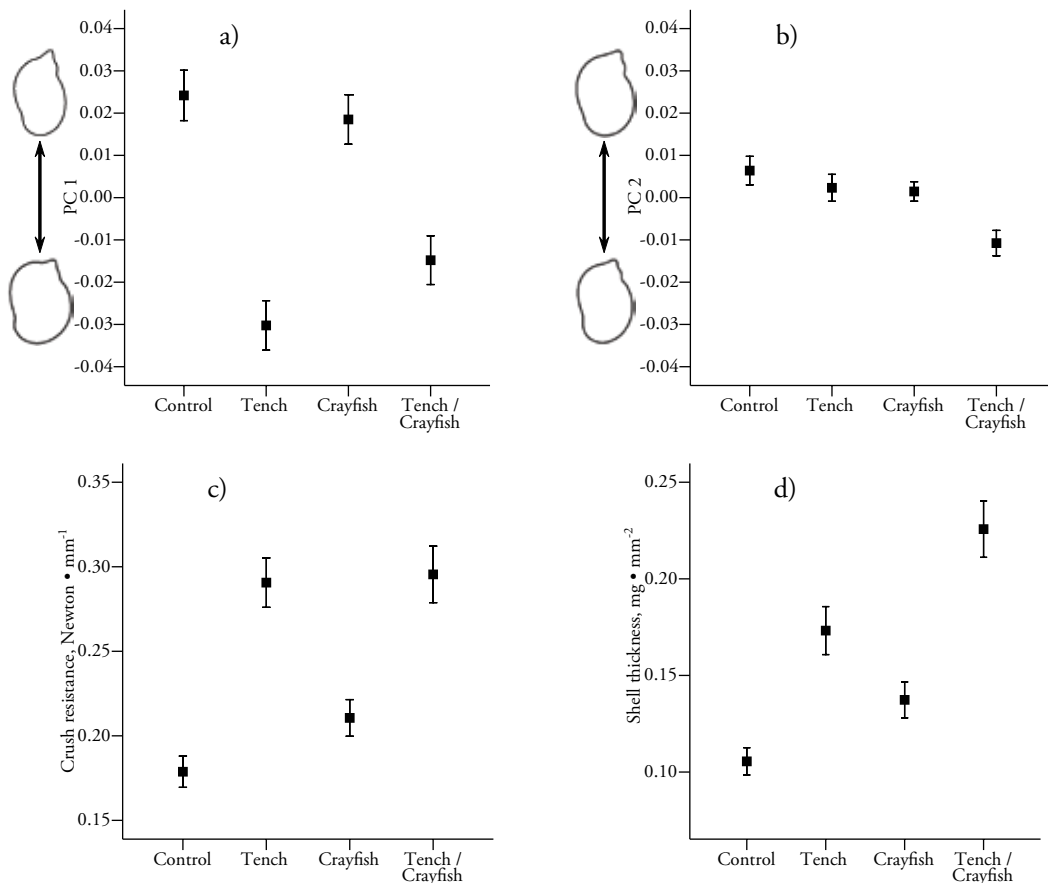


Fig. 5. Shell parameters from the study of multiple predators (control, tench, crayfish, tench and crayfish combined). Shape variation represented by PC 1 (a) and PC 2 (b) with the visualized outline shell shape on the y-axis. Size-corrected crushing resistance (c) and a relative measure of shell thickness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

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, 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 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-

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 intakes rate 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 than 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 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 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-

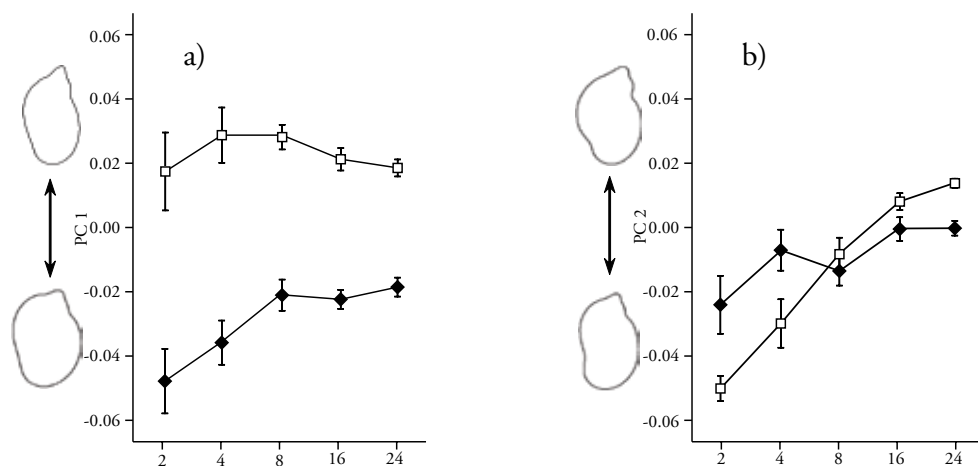


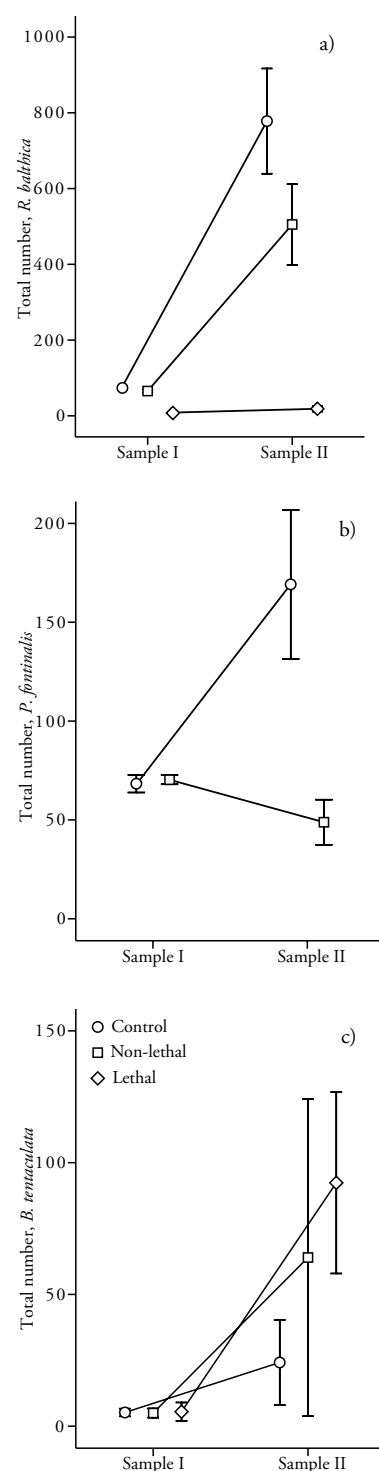
Fig. 6. The effects of shell shape in increasing density (2, 4, 8, 16 and 24 snails on the x-axis), in treatments with no fish (open squares) and in the

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.

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; Peacor and Werner 2001; Werner and Peacor 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 (Peacor 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 typi-

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 where fish were allowed to feed on snails (diamonds). Error bars represent ± 1 SE.



cal 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 where fish were allowed to feed on snails (“lethal”). The rationale 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 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 an increased density in the control and a reduced density in the non-lethal treatment, although total biomass 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 invulnerable 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



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.

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

Radix balthica is a phenotypically plastic species with a spectrum of traits that change in the presence of predation threat. Fish is one of 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.

REFERENCES

- Anholt, B. R., and Werner, E. E. 1998. Predictable changes in predation mortality as a consequence of changes in food availability and predation risk. *Evolutionary Ecology* **12**:729-738.
- Arendt, J. D. 1997. Adaptive intrinsic growth rates: an integration across taxa. The quarterly review of biology **72**:149-176.
- Boag, D. A. 1986. Dispersal in pond snails: potential role of waterfowl. *Canadian journal of zoology* **64**:904-909.
- Bolnick, D. I., and Preisser, E. L. 2005. Resource competition modifies the strength of trait-mediated predator-prey interactions: a meta analysis. *Ecology* **86**:2771-2779.
- Brendelberger, H. 1997. Contrasting feeding strategies of two freshwater gastropods, *Radix peregra* (Lymnaeidae) and *Bithynia tentaculata* (Bithyniidae). *Archiv für Hydrobiologie* **140**:1-21.
- Brendelberger, H., and Jürgens, S. 1993. Suspension feeding in *Bithynia tentaculata* (Prosobranchia, Bithyniidae), as affected by body size, food and temperature. *Oecologia* **94**:36-42.
- Brown, K. M. 1998. The role of shell strength in selective foraging by crayfish for gastropod prey. *Freshwater Biology* **40**:255-260.
- Brönmark, C. 1985. Interactions between macrophytes, epiphytes and herbivores: an experimental approach. *Oikos* **45**:26-30.
- Brönmark, C. 1994. Effects of tench and perch on interactions in a freshwater benthic food chain. *Ecology* **75**:1818-1828.
- Brönmark, C., Klosiewski, S. P. and Stein, R. A. 1992. Indirect effects of predation in a freshwater, benthic food chain. *Ecology* **73**:1662-1674.
- Brönmark, C., and Malmqvist, B. 1986. Interactions between the leech *Glossiphonia complanata* and its gastropod prey. *Oecologia* **69**:268-276.
- Burks, R. L., and Lodge, D. M. 2002. Cued in: advances and opportunities in freshwater chemical ecology. *Journal of Chemical Ecology* **28**:1901-1917.
- Chase, J. M. 1999. To grow or to reproduce? The role of life-history plasticity in food web dynamics. *The American Naturalist* **154**:571-585.
- Chase, J. M. 2003. Experimental evidence for alternative stable equilibria in a benthic pond food web. *Ecology Letters* **6**:733-741.
- Chivers, D. P., and Smith, R. J. F. 1994. The role of experience and chemical alarm signalling in predator recognition by fathead minnows, *Pimephales promelas*. *Journal of Fish Biology* **44**:273-285.
- Crowl, T. A., and Covich, A. P. 1990. Predator-induced life-history shifts in a freshwater snail. *Science* **247**:949-951.
- Dalesman, S., Rundle, S. D., Coleman, R. A. and Cotton, P. A. 2006. Cue association and antipredator behaviour in a pulmonate snail, *Lymnaea stagnalis*. *Animal Behaviour* **71**:789-797.
- DeWitt, T. J. 1998. Costs and limits of phenotypic plasticity: tests with predator-induced morphology and life history in a freshwater snail. *Journal of Evolutionary Biology* **11**:465-480.
- DeWitt, T. J., and Langerhans, B. R. 2003. Multiple prey traits, multiple predators: keys to understanding complex community dynamics. *Journal of Sea Research* **49**:143-155.
- DeWitt, T. J., Robinson, B. W. and Wilson, D. S. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. *Evolutionary Ecology Research* **2**:129-148.
- DeWitt, T. J., Sih, A. and Hucko, J. A. 1999. Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behaviour. *Animal Behaviour* **58**:397-407.
- Dorn, N. J., and Wojdak, J. M. 2004. The role of omnivorous crayfish in littoral communities. *Oecologia* **140**:150-159.
- Ekologgruppen. 2002. Biologisk mångfald i dammar. Bottenfauna. (in Swedish).
- Glazier, D. S. 2002. Resource-allocation rules and the heritability of traits. *Evolution* **56**:1696-1700.
- Glöer, P. 2002. Die Süßwassergastropoden Nord- und Mitteleuropas. ConchBooks, Hackenheim
- Gomulkiewicz, R., and Kirkpatrick, M. 1992. Quantitative genetics and the evolution of reaction norms. *Evolution* **46**:390-411.
- Harvell, D. C. 1990. The ecology and evolution of inducible defenses. *The quarterly review of biology* **65**:323-340.
- Hoverman, J. T., Auld, J. R. and Relyea, R. A. 2005. Putting prey back together again: integrating predator-induced behavior, morphology, and life history. *Oecologia* **144**:481-491.
- Hoverman, J. T., and Relyea, R. A. 2007. How flexible is phenotypic plasticity? Developmental windows for trait induction and reversal. *Ecology* **88**:693-705.
- Hubendick, B. 1949. Våra snäckor; Snäckor i sött och bräckt vatten, illustrerad handbok. Bonniers, Sweden.
- Hubendick, B. 1951. Recent Lymnaeidae, their variation, morphology, taxonomy, nomenclature and distribution. *Kongliga Svenska Vetenskaps-Akademiens Handlingar* **4**.
- Iwata, H., and Ukai, Y. 2002. Shape: a computer program package for quantitative evaluation of biological shapes based on elliptic fourier descriptors. *The Journal of Heredity* **93**:384-385.
- Kats, L. B., and Dill, L. M. 1998. The scent of death: Chemosensory assessment of predation risk by prey animals. *Ecoscience* **5**:361-394.
- Kawecki, T. J., and Ebert, D. 2004. Conceptual issues in local adaptation. *Ecology Letters* **7**:1225-1241.
- Kendall, D. 1977. The diffusion of shape. *Advances in Applied Probability* **9**:428-430.
- Kohler, S. L. 1989. Predation risk and the foraging behavior of competing stream insects. *Ecology* **70**:1811-1825.
- Krist, A. C. 2002. Crayfish induce a defensive shell shape in a freshwater snail. *Invertebrate Biology* **121**:235-242.
- Krupa, J. J., and Sih, A. 1998. Fishing spiders, green sunfish, and a stream-dwelling water strider: Male-female conflict and prey responses to single versus multiple predator environments. *Oecologia* **117**:258-265.
- Lam, P. K. S., and Calow, P. 1988. Differences in the shell shape of *Lymnaea peregra* (Müller) (Gastropoda: Pulmonata) from lotic and lentic habitats, environmental or genetic variance? *The Journal of molluscan studies* **54**:197-207.
- Langerhans, B. R., and DeWitt, T. J. 2002. Plasticity constrained: over-generalized induction cues cause maladaptive phenotypes. *Evolutionary Ecology Research* **4**:857-870.
- Lenormand, T. 2002. Gene flow and the limits to natural selection. *Trends in Ecology and Evolution* **17**:183-189.
- Luttbeg, B., Rowe, L. and Mangel, M. 2003. Prey state and experimental design affect relative size of trait- and density-mediated indirect effects. *Ecology* **84**:1140-1150.
- McCarthy, T. M., and Fisher, W. A. 2000. Multiple predator-avoidance behaviours of the freshwater snail *Physella heterostropha pomila*: responses vary with risk. *Freshwater Biology* **44**:387-397.
- McCoy, M. W. 2007. Conspecific density determines the magnitude and character of predator-induced phenotype. *Oecologia* **153**:871-878.

- Miyasaka, H., Genkai-Kato, M., Kuhara, N. and Nakano S. 2003. Predatory fish impact on competition between stream insect grazers: a consideration of behaviourally- and density-mediated effects on an apparent coexistence pattern. *Oikos* **101**:511-520.
- Moran, N. A. 1992. The evolutionary maintenance of alternative phenotypes. *The American Naturalist* **139**:971-989.
- Nyström, P., Brönmark, C. and Granéli, W. 1999. Influence of an exotic and a native crayfish species on a littoral benthic community. *Oikos* **85**:545-553.
- Nyström, P., Svensson, O., Lardner, B., Brönmark, C. and Granéli, W. 2001. The influence of multiple introduced predators on a littoral pond community. *Ecology* **82**:1023-1039.
- Osenberg, C. W., and Mittelbach, G. G. 1989. Effects of body size on the predator-prey interaction between pumpkinseed sunfish and gastropods. *Ecological Monographs* **59**:405-432.
- Peacor, S. D., and Werner, E. E. 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. *Proceedings of the National Academy of Sciences of the United States of America* **98**:3904-3908.
- Relyea, R. A. 2000. Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. *Ecology* **81**:2278-2289.
- Relyea, R. A. 2002. Costs of phenotypic plasticity. *The American Naturalist* **159**:272-282.
- Relyea, R. A. 2003. How prey respond to combined predators: a review and an empirical test. *Ecology* **84**:1827-1839.
- Relyea, R. A. 2004. Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. *Ecology* **85**:172-179.
- Richter, T. 2001. Reproductive biology and life history strategy of *Bithynia tentaculata* (Linnaeus, 1758) and *Bithynia leachii* (Sheppard, 1823). Universität Hannover, Hannover.
- Rundle, S. D., and Brönmark, C. 2001. Inter- and intraspecific trait compensation of defence mechanisms in freshwater snails. *Proceedings of the Royal Society of London. Series B* **268**:1463-1468.
- Shurin, J. B., Borer, E. T., Seabloom, E. W., Anderson, K., Blanchette, C. A., Broitman, B., Cooper, S. D. and Halpern, B. S. 2002. A cross-ecosystem comparison of the strength of trophic cascades. *Ecology Letters* **5**:785-791.
- Sih, A., Englund, G. and Wooster, D. 1998. Emergent impacts of multiple predators on prey. *Trends in Ecology and Evolution* **19**:350-355.
- Sih, A., and McCarthy, T. M. 2002. Prey responses to pulses of risk and safety: testing the risk allocation hypothesis. *Animal Behaviour* **63**:437-443.
- Stein, R. A., Kitchell, J. F. and Knezevic, B. 1975. Selective predation by carp (*Cyprinus carpio* L.) on benthic molluscs in Skadar Lake, Yugoslavia. *Journal of Fish Biology*:391-399.
- Stibor, H. 1992. Predator induced life-history shifts in a freshwater cladoceran. *Oecologia* **92**:162-165.
- Sultan, S. E., and Spencer, H. G. 2002. Metapopulation structure favors plasticity over local adaptation. *The American Naturalist* **160**:271-283.
- Teplitsky, C., and Laurila, A. 2007. Flexible defense strategies: competition modifies investment in behavioral vs. morphological defenses. *Ecology* **88**:1641-1646.
- Tollrian, R. 1995. Predator-induced morphological defenses: costs, life history shifts, and maternal effects in *Daphnia pulex*. *Ecology* **76**:1691-1705.
- Tollrian, R., and Harvell, D. C. 1999. *The ecology and evolution of inducible defences*. Princeton University Press, Princeton, New Jersey.
- Townsend, C. R., and McCarthy, K. T. 1980. On the defence strategy of *Physa fontinalis* (L.), a freshwater pulmonate snail. *Oecologia* **46**:75-79.
- Trussel, G. C. 2000. Predator-induced plasticity and morphological trade-offs in latitudinally separated populations of *Littorina obtusata*. *Evolutionary Ecology Research* **2**:803-822.
- Turner, A. M. 1996. Freshwater snails alter habitat use in response to predation. *Animal Behaviour* **51**:747-756.
- Turner, A. M. 2004. Non-lethal effects of predators on prey growth rates depend on prey density and nutrient additions. *Oikos* **104**:561-569.
- Turner, A. M., Bernot, R. J. and Boes, C. M. 2000. Chemical cues modify species interactions: the ecological consequences of predator avoidance by freshwater snails. *Oikos* **88**:148-158.
- Turner, A. M., Fetterolf, S. A. and Bernot, R. J. 1999. Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. *Oecologia* **118**:242-247.
- Turner, A. M., and Montgomery, S. L. 2003. Spatial and temporal scales of predator avoidance: experiments with fish and snails. *Ecology* **84**:616-622.
- Van Buskirk, J. 2000. The costs of an inducible defense in anuran larvae. *Ecology* **81**:2813-2821.
- Van Buskirk, J., Müller, C., Portman, A. and Surbeck, M. 2002. A test of the risk allocation hypothesis: tadpole responses to temporal change in predation risk. *Behavioural Ecology* **13**:526-530.
- Van Buskirk, J., and Yurewicz, K. L. 1998. Effects of predators on prey growth rate: relative contributions of thinning and reduced activity. *Oikos* **82**:20-28.
- Via, S., and Lande, R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**:505-522.
- Wellborn, G. A., Skelly, D. K. and Werner, E. E. 1996. Mechanisms creating community structure across a freshwater habitat gradient. *Annual review of ecology and systematics* **27**:337-363.
- Werner, E. E., and Anholt, B. R. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *The American Naturalist* **142**:242-272.
- Werner, E. E., and Anholt, B. R. 1996. Predator-induced behavioral indirect effects: consequences to competitive interactions in anuran larvae. *Ecology* **77**:157-169.
- Werner, E. E., and Hall, D. J. 1974. Optimal foraging and size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). *Ecology* **55**:1042-1052.
- Werner, E. E., and Peacor, S. D. 2003. A review of trait-mediated indirect interactions in ecological communities. *Ecology* **84**:1083-1100.
- Wilbur, K. M., and Saleuddin, A. S. M. 1983. Shell formation. *The Mollusca* **4**:235-287.
- Wisenden, B. D., and Millard, M. C. 2001. Aquatic flatworms use chemical cues from injured conspecifics to assess predation risk with novel cues. *Animal Behaviour* **62**:761-766.
- Wulschleger, E. B., and Ward, P. I. 1998. Shell form and habitat choice in *Lymnaea*. *The Journal of molluscan studies* **64**:402-404.

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. and two anonymous referees.

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.

ETT FLEXIBELT FÖRSVAR – svensk sammanfattning

En av de viktigaste ekologiska interaktionerna är den mellan predatorer och bytesdjur. Predatorer formar, genom sin påverkan på bytesdjuren, indirekt hela ekosystemet. Som en konsekvens av samexistensen med predatorer har bytesdjuren evoluerat en lång rad försvarsmekanismer. Predatoren upprepar samma mönster när den konsumerar byte; den söker, träffar på, attackerar, hanterar, konsumerar och slutligen absorberar den bytesdjuret. Därmed kan bytet genom olika typer av försvar bryta den här kedjan vid olika tillfällen. Ett förändrat beteende kan göra att risken att träffa på en predator minskar. Det kan vara att vara aktiv vid andra tidpunkter eller byta habitat. Om bytesdjuret blir attackerat kan det försvara eller förlänga hanteringen för predatoren. Detta kan ske genom morfologiska karaktärer, som gör att predatoren antingen förkastar bytesdjuret eller spenderar så lång tid med att hantera det att det inte är energetiskt lönsamt och därför söker alternativa bytesdjur istället.

Många organismer i akvatiska system, använder sig av kemiska signaler som härstammar från predatoren, för att kunna göra en

riskbedömning. Signalerna kan till exempel 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 predatoren är närvarande, utan också vilken art det rör sig om. Eftersom predatorer av olika arter har olika strategier för att söka, fånga och äta upp ett visst bytesdjur på kan då bytesdjuret, genom informationen om predatoren, använda sig av försvarsmekanismer 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 responser gynnas när det är stor men förutsägbar variation i omgivningen. Predator specifika förändringar innefattar till exempel beteende, livs-historie strategi eller kroppsform.

I sötvattenssystem är snäckor en utomordentligt viktig organism grupp. Som betare av påväxtalger reducerar de dessa på undervattensväxter och gör ljus och näringsämnen tillgäng-

liga, vilket gynnar undervattensvegetationen. En predator som konsumerar snäckor bidrar därmed indirekt till en sämre tillväxt av undervattensväxter.

En av de viktigaste predatorerna på snäckor är fisk. Det finns fiskar som specialiserat sig på att äta snäckor och har utvecklat svalgtänder, med vilka de krossar snäckan. Kan inte fisken klara att krossa snäckan spottas den ut igen. Därmed är skalstyrka en viktig del av försvarsmekanismen hos snäckor.

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 känd 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 statistiskt formen på snäckorna och fann att snäckor från dammar utan fisk var avlånga med en tydlig spira och avlång mynning. I fiskdammar däremot, var snäckorna rundare med kort spira och stor mynning. Syftet med ett rundare skal är att kraften att krossa det fördelas jämnare över ytan vilket reducerar behovet av att göra skalet tjockare.

Men var formskillnaderna ett resultat av fenotypisk plasticitet, eller hade snäckorna evoluerat fram fixa former i sina respektive habitat? För att svara på det samlade jag in snäckor från en del av dessa populationer med och utan fisk och födde upp ungar från t ex en fisk damm i en miljö utan respektive med lukt från fisk och samma sak för ungar från en fiskfri damm. Resultatet visade att oavsett vilken dammtyp föräldrarna kom från utvecklades ungar på liknande sätt med avlånga former i fiskfri miljö och rundare former i fiskmiljö. Riktningen och magnituden av dessa förändringar var identiska. Det fanns mindre skillnader mellan populationerna som indikerade att även andra, lokala förhållanden, har viss påverkan på formutvecklingen, men

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 fåglar en spridningsvektor för snäckor. Snabb spridning är även en trolig förklaring till att den här arten utvecklat ett plastiskt försvar eftersom sannolikheten är stor att hamna i olika habitat som skiljer sig i t ex predator regim.

Mötet med olika predatorer utgör en konfliktsituation eftersom ett försvar riktat mot en predator kan förenkla för en annan. Oftast blir reaktionen hos bytesdjuret riktad mot predatorn som utgör det starkaste hotet eller en intermediär reaktion mellan de båda. För snäckorna, om responsen mot fisk är ett rundare skal och stor mynning, så förenklar den formen predation från kräfta, eftersom de öppnar skalet den vägen. För att testa förändringen i skalmorfologi i en sådan konfliktsituation födde jag upp *R. balthica* i miljöer med fisk, kräfta och en kombination av fisk och kräfta. Den största formförändringen kom med fisk och snäckorna blev rundare, men de byggde även ett tjockare skal. Jag kunde visa att formen på skalet hade större betydelse för krossbarheten än skaltjockleken. Hos kräfta skedde ingen formförändring, men en svag antydning till ett tjockare skal. Det intressanta skedde vid kombinationen av de två predatorerna. Där blev snäckornas form något mer avlång vilket tydde på en reaktion mot kräfta. Eftersom en avlång form gör avkall på skalstyrka kompenserade snäckorna med ett ännu tjockare skal än när de endast var med fisk. På så sätt kunde de bibehålla krossbarheten även om formen var annorlunda.

Snäckorna förändrar morfologi endast som en reaktion när en predator är närvarande. Därför antar man att det är förknippat med en kostnad att göra förändringen i frånvaro av en predator.

För att ta reda på var kostnaderna med ett predator försvar kommer när de uttrycks, gjordes ett experiment där snäckorna utsattes för kemiska signaler från fisk i en gradient av ökande täthet och därmed minskade resurser. Skillnader mot samma gradient utan fisk gör att man kan bedöma var en kostnad uppstår. Jag fann att kostnader för förändring av skalform, skaltjocklek och krossbarhet är relativt små. Däremot fanns kostnaderna i en kraftigt reducerad tillväxt och ännu kraftigare reducerad och fördröjd fortplantning. Att ha ett kraftigt försvar 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 förändringar i skalmorfologi. Påverkar detta konkurrensen med andra snäckor där de andra arterna inte har den här förmågan? Hur klarar sig *R. balthica* med den förändrade formen där fisken har möjlighet att konsumera snäckorna direkt? Jag gjorde ett långtids experiment med två andra snäckarter som inte är kända att ändra form i närvaro av fisk. De skilde sig åt i graden av skydd deras skal erbjöd, där en art var tunnskalig och en hårdskalig. Man har visat att styrkan av antipredator beteende, som hos snäckor leder till minskad aktivitet och skifte av habitat, är korrelerat till krossbarheten. Den tunnskaliga arten

minskade kraftigt i antal tillsammans med *R. balthica* i närvaro av fisk. Fisknärvaron fick den förmodligen att minska aktivitet och därmed tillväxt och mängden avkomma. Den tjockskaliga arten visade sig vara en sämre konkurrent vilket kan vara relaterat till att den allokerar mycket energi för uppbyggnaden av det tjocka skalet. Där fisken hade möjlighet att direkt konsumera snäckorna skedde en dramatisk nedgång av antalet, samt ett skifte mot större, osårbara, individer. Den tunnskaliga arten försvann helt och kvar fanns bara den tjockskaliga arten och *R. balthica*. Dessa hade en mer extrem morfologi i denna behandling och krossbarheten var snarlik den hos den tjockskaliga arten. Detta kan ha sin orsak i den selektiva effekten fisk har där de helst konsumerar snäckor som är lätta att krossa.

Sammanfattningsvis är *R. balthica* en snäcka som utvecklat ett effektivt skydd mot predatorer, i huvudsak fisk, troligen som ett resultat av snabb spridning. Den har även möjligheter att finjustera sitt morfologiska försvar. Skyddet kommer till en relativt låg kostnad men på bekostnad av minskad fortplantning. Emellertid, är den vid fiskpredation inte utsatt för konkurrens från mer sårbara arter. Detta sammantaget bidrar troligen till att den är en av de vanligaste och dominerande snäckarterna i många typer av akvatiska system.

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's point of view. Further on you have been firmly teaching me the writing process for which I am extremely grateful (I must have given you gray hair sometimes). Looking back in the learning and developing process as a PhD-student it feels like (I know you like biological patterns, or we all do perhaps...) it has been exponential at least the last one and a half years, but I don't think it had been possible without the very

constructive talks we have had. Now when the work is finished I sincerely thank you for the collaboration over these few years.

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. Lökgröda) that I so far only saw as tadpoles abroad.

Lasse, you have also been a great support and you have been very enthusiastic and encouraging. I find you are always happy to help and I am very grateful for that. In our little side project on bioturbation I learned a lot from

you about the plankton world. We had great discussions all four on that project that kind of took off in another direction than first intended. Now it seems to be bearing fruit as well, which brings good hope for publishing the remaining material.

MikÄl, 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.

I must mention **Jakob.** The trip to New Zealand would never have been the same without you. We have also had great fun when not partying, especially on that little Lake Krankesjön and you helped bring in the fishes. You have made an everlasting impression on my son Leo by bringing him along at times when we electrofished. Actually his term for going fishing is not "let's go fishing" but "let's go test-fishing". These future biologists...

I may have bothered you much **Anders N,** when asking the statistical questions, but it was worth it (for me)! We have also had 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 scarce 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 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 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.

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.

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.