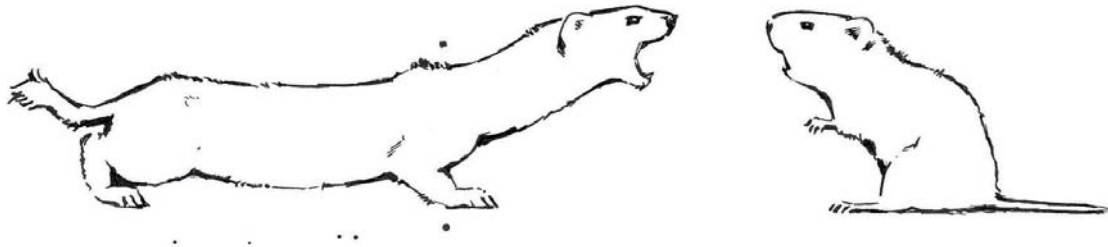Lenka Trebatická

Predation Risk Shaping Individual Behaviour, Life Histories and Species Interactions in Small Mammals

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ABSTRACT

Predation is one of the strongest selective forces affecting the life history parameters of its prey. Indirect non-lethal effects on prey are assumed to have as large a consequence as direct lethal effects. In this thesis I focus mainly on behavioural and physiological anti-predatory responses of a small mammal, the bank vole, *Myodes glareolus*. Risk of predation by the least weasel, the most common predator of voles, was studied in combination with other factors shaping individual life histories, such as gender, competing species, season, and female reproductive status. Using an experimental approach in three laboratory studies, and two field enclosure studies, I found that gender-specific anti-predatory responses to predation risk, and gender-specific characteristics in vulnerability to predation, were of greater importance than species-specific ones in two closely related species. Further, it seems that season alone effected on female reproductive success, whereas predation risk had only a season-dependent effect on spacing activity. This suggests that life time reproductive success is largely seasonally driven. Physiologically, measured as CO_2 production, females responded similarly to stress, whether it was harmless disturbance or predation threat. Behavioural responses to predation threat were clearly different, non-pregnant females reacted more strongly than pregnant ones, emphasizing the importance of the behavioural response with a clear survival benefit. I also show that, quite unexpectedly, that pregnancy and the entire breeding cycle do not increase the energetic costs of a female. The outcomes of this thesis will help us to understand better the complex processes that act in predator-prey interactions and potentially affect the whole community.

Keywords: Anti-predatory behaviour; bank vole; *Myodes (Clethrionomys) glareolus*; reproductive success; seasonality; vole community.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on five original papers, which will be referred to in the text by their Roman numerals I-V. I was involved in planning of the research in papers I-IV, conducting major part of laboratory or field work in I-V, and analyzing the data and writing the first versions of the manuscripts in I, III–V.

I   Trebatická L., Sundell J., Tkadlec E. & Ylönen H. 2008. Behaviour and resource use of two competing vole species under shared predation risk. Oecologia 157: 707-715**.**

II   Sundell J., Trebatická L., Oksanen T., Ovaskainen O., Haapakoski M. & Ylönen H. 2008. Predation on two vole species by a shared predator: antipredatory response and prey preference. Population Ecology 50: 257-266.

III   Trebatická L., Lampinen P., Sundell J. & Ylönen H. The effect of seasonality and predation risk on reproduction and behaviour in the bank vole. Manuscript.

IV   Trebatická L., Ketola T., Haapakoski M., Reckordt A. & Ylönen H. Is fear a factor in voles? Short term physiological and behavioural response to predation stress. Manuscript.

V   Trebatická L., Ketola T., Klemme I., Eccard J. A. & Ylönen H. 2007. Is reproduction really costly? Energy metabolism of bank vole (*Clethrionomys glareolus*) females through the reproductive cycle. Écoscience 14 (3): 306-313.

1 INTRODUCTION

1.1 General introduction

Predation has a large impact on ecological communities. The predator affects its prey populations either directly, by killing, consuming and reducing prey densities, or indirectly by its mere presence through changes in prey physiology and behaviour. Both can affect prey populations, their competitors, and other predators, and thus whole communities, leading potentially to top-down cascade effects (Preisser et al. 2005, Preisser & Bolnick 2008).

At the population level, these indirect effects are nowadays considered to be as large as, or even larger than direct effects, i.e. direct consumption by a predator (Preisser et al. 2005). It is known that olfactory and auditory cues released by a predator can induce responses in prey, since prey individuals are able to detect a predator's presence (for review see Apfelbach et al. 2005). On the other hand, prey species have evolved such behaviour which allows them to maximize their fitness, while minimizing costs associated with encountering predators (Sih 1997). The most typical anti-predatory behavioural responses is for example, reduced mobility (Lima & Dill 1990, Magnhagen 1991, Ylönen & Ronkainen 1991) associated with reduced foraging leading to reduced availability of energy for reproduction and growth (Lima & Dill 1990). Also, mating opportunities may decrease because of lower encounter frequency with the opposite sex (Magnhagen 1991). Further, shifts of the activity of a vulnerable prey species to safe microhabitats (Brown et al. 1998) or the use of refuges (e.g. Sih et al. 1992, Ylönen et al. 2003) are common responses. By balancing the costs and benefits of predator avoidance, individuals are expected to optimize their lifetime fitness, which has important consequences for their life history and hence population and community dynamics. Any allocation of resources into some other activity than anti-predatory behaviour increases the prey's vulnerability to predation (Lind & Cresswell 2005). These behavioural decisions might affect predator-prey interactions, and can have large-scale ecological consequences (e.g. Kokko & Ruxton 2000).

Predators do not only affect the behaviour of their prey, but also the physiological processes. The most common symptom of physiological stress, i.e. the physiological anti-predatory response seems to be reflected in higher levels of corticosteron (Siegel 1980, Eilam et al. 1999, Scheuerlein et al. 2001). The presence of stress proteins (Kagawa et al. 1999, Pauwels et al. 2005, Slos & Stocks 2008, see however, Ylönen et al. 2006), and anti-oxidant enzymes (Slos & Stocks 2008) are good indicator of stress. Despite these known metabolic changes to stress, only a few studies have focused on metabolic responses to predation risk. Elevated oxygen consumption in response to predation threat has been shown in a few species (Woodley & Peterson 2003, Beckerman et al. 2007, Slos & Stocks 2008). An even more profound effect could be seen in individuals of different functional groups exposed to predation risk, especially in females, since it is well known that pregnant females have a higher metabolic rate than non-pregnant females (Speakman & McQueenie 1996, Johnson et al. 2001).

However, predator cues affect not only a target prey individual, but can also modify interactions of the target prey with other individuals of the same species (intra-specific competition) or with individuals of other interacting species (inter-specific competition). Within both intra- and inter-specific competition, selection by a predator is focused mainly on more vulnerable individuals for example juveniles (Jedrzejewski & Jedrzejewska 1990), and depending on the type of the predator, on females (mammalian predators, Norrdahl & Korpimäki 1998) or males (avian predators, Mappes et al. 1993) and this is likely to alter the balance of the relationship.

Predators that cause their prey, to switch and forage in alternative microhabitats are likely to affect the competition of the prey species with other species (Preisser & Bolnick 2008). Such behavioural effects on prey animals can explain species coexistence as an interaction of predation and competition (Kotler & Holt 1989). In fact there are a number of studies concerning inter-specific competition, for example, on space use and habitat selection (e.g. Eccard & Ylönen 2002). However, predation effects were usually neglected, although predation is one of the factors believed to have a major impact on competitive interactions. Nevertheless, studies focusing on the interaction between predation and inter-specific competition show that predators can alter inter-specific competitive interactions substantially (see Chase et al. 2002 for review).

Multi-behavioural anti-predatory responses (Boyer et al. 2006, Lind & Cresswell 2006) or anti-predatory behaviours in a multi-predator environment (Kotler et al. 1992, Korpimäki et al. 1996, Eccard et al. 2008) have been seldomly studied. Paying attention to more complex issues is important, as the various anti-predatory behaviours may not be independent, and for instance the presence of both a competitor, and an individual of the opposite sex, could expose a prey individual to a conflicting situation. Moreover, since individuals live together with their competitors in the same environment, which vary spatially and temporally, seasonality is another factor which could bring individuals into conflicting demands. It is well known that especially in

iteroparous animals, living in highly seasonal environments, season has a strong effect on their life history traits (Tkadlec & Zejda 1998, Prevod-Julliard et al. 1999), which in turn affects reproduction and population growth rates. Thus, seasonality is, besides predation, another important factor in shaping individuals' life history (Bronson 1989), and should be considered, preferably in combination with predation.

1.2 Aims of the study

The main objective in this thesis was to study anti-predatory behaviour in small mammals on individual, population and community level. More specifically, I focused on indirect effects of mammalian predation risk on prey behaviour, reproduction, and its related traits (e.g. pregnancy rate, timing of mating, litter size) as well as prey physiology in rodent species.

In study I and II, I studied the effect of specialized vole predator, the least weasel (*Mustela nivalis nivalis*), on the behaviour of two competing *Myodes* species, the bank vole (*Myodes glareolus*) and the grey-sided vole (*M. rufocanus*). A gender-specific response was also investigated, thus allowing comparison of species versus gender effects. Additionally, a possible predator preference for prey was investigated in study II.

In study III, I focused on the anti-predatory responses of bank voles under a simulated predation risk in different seasons, under semi-natural conditions. Measured variables included female reproductive success and spacing activity.

In study IV and V, I explored physiologically mediated responses in bank vole females with regard to combined effects of stress (harmless disturbance and predation threat) and pregnancy (IV), and solely to pregnancy (V). CO_2 production was used as a measure of metabolic rate.

2 METHODS

2.1 Study species

The main actor in this thesis is the bank vole, *Myodes glareolus*. It is a common iteroparous boreal rodent species. As a microtine rodent it is an important prey for many predators. During evolution, an arms race between predators and prey, has seen the bank vole evolve elaborate specific anti-predatory behaviour in order to survive in the struggle of life. Due to its life in a highly seasonal environment, and short life-span, it produces seasonally distinct cohorts. For these reasons, the bank vole presents a good model species for studying the effects of predation and season on its life history parameters. Moreover, as it is a promiscuous species (Klemme 2008), with a relatively fast reproduction and post-partum oestrus, it is also a good model species to study the energetics of reproduction too.

The supporting actor in this play has been another *Myodes* species, the grey-sided vole (*M. rufocanus*), ecologically resembles more species of another common genus of voles, the *Microtu*s voles (Hanski & Henttonen 1996). It is generally slower, clumsier, and breeds in higher densities that other *Myodes* species. Similar requirements for food and shelter, common predators, and dominancy of the grey-sided vole over the bank vole make these two species a suitable pair for studying the effects of predation risk on a prey community.

"The bad guy" of this story has been the least weasel *Mustela nivalis nivalis.* It is assumed to be the world's smallest mammalian predator (Carnivora; King & Powel 2006), and it is common in whole Fennoscandia (Hellstedt et al. 2006). Because of its small size and elongated snake-like body form (King and Powel 2006), it leaves practically no refuge for its main prey species (Simms 1979), i.e. small rodents. It has been found to be the main cause of mortality in voles (Norrdahl & Korpimäki 1995), preferring females over males (Norrdahl & Korpimäki 1995). Although the least weasel is an efficient and specialized predator of voles, it is not a perfect one (Norrdahl & Korpimäki

2000). Unsuccessful attacks are required for the evolution of efficient anti-predatory behaviours.

2.2 Study designs

All experiments of this thesis have been conducted at the Konnevesi Research Station, and at the Animal Unit of University of Jyväskylä. A simplified overview of the study designs is given in Table 1.

2.2.1 Anti-predatory behaviour and species- and sex-dependent vulnerability to predation (I-II)

The effect of a shared predation risk for a prey community was studied in an indoor arena (I) and a field enclosure (II) experiment. More explicitly, I explored species- and sex-specific anti-predatory responses to predation risk (I, II), as well as the predator's preference for prey (II). The prey community and the predator were represented by two small mammal *Myodes* vole species and the least weasel. In both studies, predation risk was manipulated by the presence of live (enclosed in I) least weasels. The experimental animals came from wild populations (bank voles from Central Finland, grey-sided voles from Finnish, Swedish and Norwegian Lapland), or were first laboratory-born descendants. In both studies, adult individuals were used. In study I animals were not in breeding condition, while in study II they were mature. In the laboratory study (I) I followed the behaviour of the individuals by an automatic data-logging system, which was built in a 7.5 x 7.5 m^2 large indoor arena. This system allowed checking for species- and gender-specific response differences. The experimental vole community lived in an environment supplying food, refuges (allowing also an arboreal escape), and the possibility to choose between risky (with a predator presence), and non-risky (without a predator) areas. The activity, space use, use of refuges, and foraging of both voles species were investigated. In the field experiment (II), populations living under semi-natural conditions in outdoor enclosures (0.25 ha) were investigated. Two habitat types were created; one with trees and another one without trees. As in the laboratory experiment, the environment supplied populations with food, shelter and escape possibilities. Individuals were, however, followed by radio-tracking. Using this method, information about an individual's activity, space use and vulnerability to predation were provided.

2.2.2 The effect of seasonality and predation risk on reproductive success and behaviour (III)

The reproductive success of bank vole females, and vole spacing activity, under the risk of predation and seasonal changes were studied in a field experiment. It

was conducted in large outdoor enclosures (8 x 0.25 ha) during spring, summer and autumn. Enclosures were built on an old field with tall grasses and bushes. Multiple-capture live traps, and an automatic data-logging system allowed the monitoring of the studied populations. Predation risk (treatment enclosures) was manipulated by the presence of live caged weasels, and the presence of bedding material and faeces distributed systematically in the enclosures. In control enclosures, empty cages and clean bedding material were used and distributed in the same manner. Female reproductive success was measured in terms of pregnancy rate, timing of mating and litter size. Also the litters' sex ratio was followed. All these measures were taken in the laboratory after a period spent in outdoor enclosures. The animals used in the experiment were wild voles kept overwinter in a laboratory, or were the first generation descendants. In seasonal replicates, the age composition of populations mimicked the natural situation, i.e. only old individuals in spring, a mixture of adult and juveniles during summer and only juveniles in autumn.

2.2.3 Energy use under predation risk and pregnancy, and throughout the breeding cycle (IV-V)

The effect of stress (harmless disturbance and predation threat) and pregnancy on energy use and behavioural activity (IV), and the effect of pregnancy and lactation on energy use (V) were studied in bank vole females in two laboratory experiments. The production of carbon dioxide was used as a measure of energy use, i.e. metabolic rate and was measured by an open-flow respirometry system. The study procedure was similar in both experiments. After an acclimation period, the resting metabolic rate was measured (IV, V) and afterwards, a treatment (stress) was applied (IV). In study IV, harmless disturbance was simulated by an injection of pure air into the system, and threat by an injection of the least weasel smell, obtained from weasel faeces. A change in the CO_2 production, expressed as before-after stress application, was followed for harmless disturbance and threat treatments, in non-pregnant and pregnant females. In study V the changes in CO_2 production throughout the female breeding cycle, i.e. non-pregnancy, pregnancy, lactation and concurrent pregnancy and lactation were monitored. Females used in both studies were wild individuals (originally trapped in Central Finland) kept overwinter in the laboratory.

TABLE 1   Overview of the experimental setups of all studies (I-V). The table includes
information on the place, where the study was carried out, the main subject of
interest, manipulation conducted, measured variables and specific focus of
the study.

	I	II	III	IV	V
Place	indoor arena	field enclosure	field enclosure	laboratory	laboratory
Subject	APB	APB prey preference	APB	APB	Reprod cost
Manipulation	PR/C	PR/C/ Habitat	PR	PR	
Measures	activity space use refuge use foraging	space use activity prey preference	RS space use	CO_2 activity	CO_2
Specific focus	2 species gender	2 species gender	season PR	energetics behaviour	energetics reproduction

APB = antipredatory behaviour, PR = predation risk, C = competition,
RS = reproductive success

3   RESULTS AND DISCUSSION

3.1   Anti-predatory behaviour and vulnerability to predation

The effect of predation risk on two competing vole species, with predicted dominance and predator preference was studied in a large indoor arena and in a field enclosure study (I and II). To date, most of the anti-predator behavioural studies focused on simple systems, i.e. one predator-one prey systems, with gender differences examined separately. The use of two competing vole species, and an inclusion of gender into the same setup increases the ecological relevance of theses studies.

In an indoor arena study (I) the main focus was on anti-predatory behaviour to simulated predation risk. Predation risk varied temporally (time without vs. with a predator) and spatially (areas without vs. with a predator). Vole response to predation risk was largely similar to common anti-predatory behaviours found in a variety of species (see Lima & Dill 1990 for a review), such as shifts in microhabitat (in this study from risky to less risky area), the use of refuges (climbing of the trees) and changes in activity (lower activity under predation risk). What was not in concordance with previous studies was foraging activity. It is assumed that a reduction of activity itself lowers the probability of encounters with predators, but also reduces food intake (Brown et al. 1992, Koskela & Ylönen 1995, Norrdahl & Korpimäki 1998, but see Banks et al. 2000) consequently. This may then affect individual growth or developmental rate (e.g., Crowl & Covich 1990 for invertebrates, Remeš 2007 for birds). In this study, no effect of predation risk on foraging, in terms of seed consumption, was found. However, a species-specific difference in body mass was observed, probably as an indirect effect of the presence of both the predator, and a second species. The subdominant bank voles lost weight during the experiment, while grey-sided voles maintained their initial body mass.

The use of refuges, at least in the case of my study species, seems to be another species-specific case. Although both grey-sided voles and bank voles responded to predation risk by climbing trees, bank voles spent significantly

more time in trees. Similar results were also obtained in study II. This difference in tree use was predicted, since observations on grey-sided voles in trees are rather anecdotal, but bank voles are known to use trees as refuges when encountered by a small mustelid (Jedrzejewski & Jedrzejewska 1990). Probably less aggression, and reduced interference competition due to non-breeding condition of both species, could be the reason for the lack of species difference in the time spent in risky and less risky areas. This result was unexpected, since the grey-sided vole is considered to be dominant over the bank vole (e.g. Hanski & Henttonen 1996, Johannesen et al. 2002). Dominance in this experiment was probably expressed by a higher activity of grey-sided voles within the less-risky area, and by generally higher activity of the grey-sided vole vs. the bank vole.

More gender-specific, than species-specific, anti-predatory responses were found; males spent more time in the risky area compared to the less-risky one. Within risky areas, males were more active and foraged more than females. Moreover, there was a strong trend for spending more time in trees in males, compared to females, suggesting different escape strategies between males and females. Thus, although males are assumed to be more vulnerable mainly to avian predators, and females to mammalian ones, we found that males, regardless of species, seemed to expose themselves more to mammalian predation risk than females. However, this result could be skewed by the fact that the experiment was carried out in winter, and all individuals were in non-breeding condition. It is known that during the breeding season, females are preferred over males by small mustelids (Norrdahl & Korpimäki 1998, Sundell 2003), probably due to pregnancy.

The main differences between the field enclosure study (II) and the previous indoor arena study (I) was that the experiment was conducted outdoors. Individuals were in breeding condition, and the predation risk was real, as the predator was a free-ranging weasel. In addition the main focus, besides anti-predatory behaviour in the same two competing vole species, was the preference by the least weasel. Assumed different species vulnerability to the predator was based on species characteristics. For instance, higher vulnerability of the grey-sided vole arises from being clumsier and more like a *Microtus*-type vole species, the principal prey of small mustelids (Korpimäki et al. 1991). Moreover, since both prey species are assumed to have habitat-dependent vulnerability, two different habitat types (forest and open area) were created. Whereas bank voles are more vulnerable in open habitats, grey-sided voles are more vulnerable in forest habitats. This habitat-dependent vulnerability arises from assumed different escape strategies. The bank vole as a more agile species, is known for arboreal escape reaction when exposed to small mustelids (Jedrzejewski & Jedrzejewska 1990). In the grey-sided vole, observations on climbing the trees have been, in spite of its proven ability to climb (I, II), rarely documented.

Similar results on anti-predator behaviour were found in the enclosures and in the indoor arena study (I). Predation risk had an effect on vole activity. In an immediate response to weasel presence, species-specific differences were

observed; the grey-sided voles moved longer distances after a weasel was released, this was not observed in bank voles. Moreover, voles adjusted their activity according to the least weasel activity. High vole activity was observed during the low weasel activity. In females higher activity lead to higher mortality, whereas in males it led to lower mortality, suggesting an activity-dependent gender-specific vulnerability to predation. It seems that there are different strategies to avoid weasel predation. In general, intermediate activity would be the best strategy to avoid mustelid predators (see also Banks et al. 2000).

Weasels showed a preference for grey-sided vole males over females, but no gender-specific preference in bank voles. Similar to the indoor arena study (I), males were more vulnerable. In contrast to the previous study, animals used in this experiment (II) were all mature and in breeding condition. Therefore, the results of the first study cannot be explained purely by non-breeding condition of experimental individuals. This needs more research.

Both studies (I & II) lead to the conclusion that gender-specific anti-predatory responses, and gender-specific characteristics in vulnerability to predation are of larger importance than species- and habitat-specific characteristics in these closely related species.

3.2 Predation risk and seasonal reproductive strategies

A combined effect of predation risk and seasonality on female reproduction to my knowledge has never been studied before. Both factors strongly shape life history strategies of individuals (Bronson 1989). These strategies allow individuals to achieve successful reproduction in spatially and temporally varying environments. Therefore, one expects an especially clear response under the combined effect of such strong forces. In a field enclosure study (III), I focused on the effect of predation risk on female reproductive success and vole spatial activity during spring, summer and autumn. Although many laboratory studies have emphasized the possible impact of a predator on female reproductive success via suppression of reproduction (e.g. Ylönen & Ronkainen 1991), there is almost no field evidence for this (but see Fuelling & Halle 2004). However, our study is the only one to repeat during different seasons, which does not allow a real comparison of the studies. Another difference between the studies can be seen in the simulation of predation risk. So far, only weasel faeces have been used to imitate predation risk. Although, some recent studies suggest that skin and fur derived odours have longer lasting effects than urine and faeces (see Apfelbach et al. 2005 for review). In this study weasel faeces were used along with live caged weasels, both systematically distributed over enclosures, in order to make the effect of simulated predation risk even stronger. In spite of this, predation risk did not affect female reproduction, or its related traits, such as pregnancy rate, timing of mating, litter size or offspring sex ratio. Consequently, no combined effect of season and predation risk on

female reproduction was found, and only vole spacing activity was affected. Under predation risk, males tended to move over smaller areas compared to a no-risk situation. Therefore, it seems that season itself appears to be the major factor, which has an impact on female reproduction, affecting timing of mating, and pre-weaning body growth of offspring. There was a strong trend in timing of mating, females mated in the autumn earlier after initiation of experimental conditions compared to spring and summer. This was probably the consequence of a trade-off between timing of reproduction and quality of environmental conditions. The later breeding occurs, the harsher conditions that offspring face at birth. Or, since all "autumn" females were born in spring, i.e. were young-of-the-year individuals, it is also likely that they tried to reproduce as soon as possible. It was found that the survival rate of a young-of –the-year female, that breeds in year of the birth, is similar to that of an immature one (Millar 1994). Thus, by producing further litters, females maximize their reproductive success. Pre-weaning offspring body growth was faster during summer compared to spring and autumn, although offspring were born and grew under *ad libitum* conditions in the laboratory. Mother's condition at conception in the field and current environmental conditions could have an effect on her reproduction.

The lack of a combined effect of season and predation risk, or of predation risk only, may be due to short-term seasonal "constructed" replicates. It might be that negative effects of predation risk on reproduction are delayed and probably displayed as a higher/lower overwinter survival. More work is needed to explore this.

3.3   Energy use, predation risk and reproduction

The indirect effect of a predator on prey is behaviourally, but also physiologically mediated. In several species, one of the symptoms of stress under predation risk is seen in metabolic changes (longnose killifish Woodley & Peterson 2003, water flea Beckerman et al. 2007, damselfly larvae Slos & Stocks 2008). These studies show elevated oxygen consumption under predation threat. Individuals' physiological changes in metabolic rate, when studying the use of energy, can also be expressed in terms of carbon dioxide production, as in our two laboratory studies. In the laboratory study (IV), in which the effect of stress (harmless disturbance and predation threat) was manipulated, and female reproductive status (non-pregnant and pregnant females) was controlled for, behavioural response, rather than a physiological response was found. Females reacted to both types of the stress similarly. This is interesting, since the ability of the females to recognize the threat from harmless disturbance was expected. Prey individuals have been shown to be able to assess the severity of the cues (see Apfelbach et al. 2005 for review). On the other hand, non-pregnant and pregnant females responded to stress, but regardless of its type, differently. Non-pregnant females responded to stress more strongly, i.e. increased their

CO_2 production more, than pregnant ones. This, however, seems to be the consequence of lower metabolic rate (energy use) in non-pregnant females in contrast to pregnant females before stress application (IV). First, as a rule, it is easier for an individual to boost the level of a given parameter, when initial values are lower. This fact could explain the observed pattern, since the response of both non-pregnant and pregnant females to both stress types was similar. Second, it has been shown that pregnancy and lactation increases female metabolic rate (e.g., Speakman & McQueenie 1996, Johnson et al. 2001), i.e. that pregnant and lactating females have higher metabolic rate than non-pregnant ones. It is also assumed that a complete reproductive cycle of ovulation, conception, pregnancy and lactation is one of the most energetically expensive activities that a female mammal can undertake. But there is also evidence that an increase in energy use in females is limited. The outcomes of the fifth laboratory study show such physiological limitation, as concurrent pregnancy and lactation did not increase energy use more than found in lactating females (V). Therefore, it could also be speculated that already high energy use by pregnant females (IV) was reaching a limit above which another increase was not possible. However, interestingly, the results of the fifth study do show that non-pregnant females do not always necessarily have lower metabolic rates than pregnant ones, and females might not experience large energetic costs by allocating energy from their own maintenance to reproduction. On the other hand, energy consumption per gram of tissue decreased throughout the cycle, which suggests that females were able to sustain more tissue (V).

Behaviourally females, regardless of their status, reacted to different stress types differently (IV). The response towards predation threat was stronger than to harmless disturbance. Females were much more active after the simulated predation threat was applied than to harmless disturbance. This is in contrast to the physiological response, as females did not respond to predation threat by elevating CO_2 production (i.e. metabolic rate) more than to harmless disturbance. One of the possible reasons could be that some of the modes of the activity (sniffing, cleaning, licking, biting, climbing) are not really energy consuming, otherwise this discrepancy is difficult to explain.

For an individual to be able to detect and recognize a predator is of vital importance, since failure of a prey individual to respond to a predator increases the probability that it will be captured during an encounter with the predator. On the other hand, anti-predatory responses are costly (Lima & Dill 1990). Therefore, in the stress study (IV) the post-stress physiological response was also explored. A difference between stress types was not found, however, non-pregnant and pregnant females' dissimilarity in immediate response disappeared. A discrepancy between immediate and post-stress response could indicate an ability of females to be able to recognize the vanished threat.

4 CONCLUSIONS

In this thesis I focused on 1) anti-predatory responses of a small mammal, the bank vole, to indirect predation risk imposed by the least weasel and 2) the use of energy during the breeding cycle in bank vole females. I have mainly concentrated on behavioural and physiological responses to simulated predation risk. The aim of this research was approached experimentally, with both laboratory and field enclosure studies. Predator-prey interactions, as well as anti-predatory behaviour via changes in behaviour and physiology, have been studied already for decades in a wide variety of species, and the findings of this thesis result in similar conclusions to earlier studies. For instance, indirect predation risk effects prey, in terms of changes in prey activity, space use, foraging or the use of refuges. However, I found behavioural rather than a physiological response. Females did not react by changes in their carbon dioxide production, as a measure of metabolic rate and energy use in response to predation threat but instead responded by higher activity. Different reactions to stress (harmless disturbance and predation threat) between different functional groups, non-pregnant and pregnant females, seemed to be a consequence of lower energy use in non-pregnant females compared to pregnant ones. Based on the results of this thesis, pregnant and non-pregnant females might also use a similar amount of energy. Besides looking at the previous stress study just from physiological point of view, this study can be seen as a study of a combined effect of predation risk and female reproductive status. It is assumed that pregnant and lactating females use more energy and thereby have reproductive costs that affect females' life history parameters. This effect could be more profound under predation risk.

In general, few studies have focused on investigations of multi-behavioural anti-predatory responses, or anti-predatory behaviours in a multi-predator environment. This thesis partly contributes to this matter. A novelty of my work is in the combination of simulated predation risk with some other important factor influencing life histories of the prey species, such as i) intra-/inter-specific interactions, ii) seasonality and iii) female reproductive status. The results show the importance of these kinds of multi-factorial studies. For

instance, if two species and gender would not have been used in one study, a gender-specific response (with males more vulnerable to predation risk) over species-specific response would not have been recognized. Similarly, in the study on prey preference it was found that males of one species are preferred more than females. This was, however, not found in a competing species. As both the season and predation risk are assumed to shape life history parameters, a combined effect of the season and predation risk on reproduction was expected. However, it seems that predation risk has a season-dependent effect on spacing activity rather than on female reproduction, suggesting that life time reproductive success is rather seasonally driven.

Although, in all of the studies expected combined effects were weak, I believe that this kind of complicated designs like the studies in my thesis should be carried out more in future and could help us to understand better the complex processes going on within predator-prey interactions.

YHTEENVETO (RÉSUMÉ IN FINNISH)

Petoriskin vaikutus yksilön käyttäytymiseen, elinkiertopiirteisiin ja yksilöiden välisiin suhteisiin

Pedot ovat yksi merkittävimmistä eliöyhteisöjä, populaatioita ja yksilön elinkiertoja säätelevistä tekijöistä. Petojen vaikutus voi olla kahdenlainen: yksilöiden eliminoiminen populaatiosta suoran saalistuksen kautta tai epäsuora yksilön käyttäytymiseen ja elinkiertopiirteisiin vaikuttava saalistusuhka. Pedon läsnäolon tai siitä kertovien epäsuorien signaalien oletetaan vaikuttavan voimakkaasti yksilön käyttäytymiseen ja lisääntymiseen. Tuore lähes kaksisataa tutkimusta käsittänyt meta-analyysi osoitti, että petouhan epäsuorat vaikutukset voivat olla populaatiotasolla samaa suurusluokkaa kuin suorat saalistusvaikutukset: "Peto säikäyttää saaliin kuoliaaksi".

Oma tutkimukseni keskittyi saalistuksen ja petoriskin vaikutuksiin saaliin käyttäytymiseen, lisääntymiseen ja fysiologiaan, sekä monilajisen saalisyhteisön lajienvälisiin suhteisiin. Tutkin sekä pedon suoraa saalistusvaikutusta että epäsuoran petoriskin vaikutusta kolmen laboratoriotutkimuksen ja kahden laajan luonnonympäristössä, suurissa ulkotarhoissa toteutetun kenttätutkimuksen avulla. Pääasiallinen tutkimuskohteeni oli metsämyyrä (Myodes glareolus), joka on erittäin yleinen boreaalisten ja lauhkeiden metsäympäristöjen jyrsijä, ja yksi Suomen yleisimmistä nisäkkäistä peltomyyrän ja metsäpäästäisen ohella. Metsämyyrä on pääosin siemensyöjä. Lajin naaraat ovat lisääntymiskaudella territoriaalisia, ja sekä koirailla että naarailla saattaa olla useita kumppaneita yhden lisääntymissyklin aikana. Tutkimuksissa, joissa tarkasteltiin pedon vaikutusta monilajisessa saalisyhteisössä, metsämyyrän parina oli toinen saman suvun laji, harmaakuvemyyrä (Myodes rufocanus). Harmaakuvemyyrä on yleinen laji Pohjois-Suomessa, jossa se esiintyy yhdessä metsämyyrän kanssa. Se on metsämyyrää kookkaampi ja lajienvälisessä kilpailussa metsämyyrää vahvempi kilpailija.

Tutkimuksissa käytettiin petona lumikkoa (Mustela nivalis nivalis), joko vapaasti luonnonmukaisessa ympäristössä saalistavana tai epäsuorasti petouhkaa aiheuttavana. Pieniin näätäeläimiin kuuluva yleinen lumikko on ns. myyräspesialisti, eli myyrät muodostavat pääosan sen ravinnosta. Tämän vuoksi lumikon kannanvaihtelu seuraa tiiviisti myyräkantojen vaihtelua. Lumikko on myös ainoa nisäkäspeto, joka pystyy pienen kokonsa ansiosta seuraamaan myyriä niiden käytäviin ja koloihin kenttäkerroksessa ja kivien alla. Lumikon oma lajinsisäinen kommunikaatio perustuu paljolti voimakkaisiin hajuaineisiin, mistä johtuen lumikon hajun oletetaan olevan myyrille luotettava signaali saalistusriskin kohoamisesta niiden elinalueella.

Tutkimukseni jakautui kahteen kategoriaan, laboratorio- ja kenttätutkimuksiin. Laboratoriossa tutkin myyrien käyttäytymis- ja fysiologisia vasteita petoriskiin, ja suurissa ulkotarhoissa myyrien käyttäytymistä ja lisääntymistä petoriskin alla. Myyrät altistuivat joko lumikon suoralle saalistukselle tai epä-

suorille signaaleille, kuten lumikon hajulle ja läsnäololle. Petoriskin vaikutusta tutkittiin yhteydessä muihin yksilön elinkiertopiirteisiin vaikuttaviin tekijöihin, kuten sukupuolten välisiin eroihin, yksilön lisääntymistilaan, lajienvälisiin suhteisiin sekä lisääntymiskauden vaiheeseen.

Molemmat myyrälajit, metsä- ja harmaakuvemyyrä, käyttäytyivät pääosin samalla tavoin suhteessa petoriskiin tai saalistukseen, eikä odotettua lajien välisiin voimasuhteisiin perustuvaa eroa havaittu. Molemmat lajit, yllättäen myös harmaakuvemyyrä, kiipesivät lumikkoa pakoon puihin niin laboratoriossa kuin luonnonoloissakin. Kiipeämistä on pidetty tehokkaimpana pedonvälttämiskäyttäytymisenä normaalisti kenttäkerroksessa liikkuville myyrille. Syynä lajienvälisten käyttäytymiserojen puutteeseen saattaa olla kokeiden lisääntymiskauden ulkopuolinen ajankohta. Yksilöiden talviaikainen hormonaalinen tila on saattanut mahdollistaa lajien yhteiselon ja yhtenäisen käyttäytymisen lumikkouhan alla. Lajienvälisten käyttäytymiserojen sijaan suurimmat erot löytyivät sukupuolten väliltä: koiraat reagoivat petoon naaraita voimakkaammin.

Vuodenaikaisuuden vaikutusta yhdessä lumikkoriskin kanssa tutkittiin laajassa ulkotarhakokeessa. Petoriskiä manipuloitiin lumikon hajun ja läsnäolon avulla kolmena eri lisääntymiskauden vaiheen aikana: keväällä aivan lisääntymiskauden alussa, keskikesällä lisääntymiskauden aikana ja syksyllä lisääntymiskauden kääntyessä loppua kohti. Kontrollipopulaatiot saivat samanlaisen käsittelyn ilman petosignaalia. Tutkimus osoitti, että lyhyen lisääntymiskauden optimaalinen käyttö määräsi naaraan lisääntymisstrategiaa petoriskiä enemmän. Metsämyyrä lisääntyy elämänsä aikana useita kertoja, mutta pääosin ainoastaan peräjälkeen yhden lisääntymiskauden aikana. Niinpä lyhyen kesäkauden tehokas käyttö näytti olevan sille optimaalinen strategia siitä huolimatta, että uhka lumikon hampaisiin joutumisesta vaani kaiken lisääntymiseen liittyvän käyttäytymisen ja fysiologisten muutosten taustalla.

Epäsuoran lumikkoriskin vaikutusta metsämyyränaaraiden fysiologiaan ja erityisesti energian kulutukseen tutkittiin laboratoriossa erittäin pelkistetyissä olosuhteissa. Respirometrin mittauskammiossa rauhoittunut naaras sai ilmavirran mukana häiriöärsykkeen lumikon hajulla tai ilman. Tutkimus osoitti, että myyränaaras havaitsee tuloilman mukanaan kantaman pienenkin häiriön, mutta reagoi samoin vaarattomaan ja pedonhajun sisältävään ärsykkeeseen. Lyhytaikainen varokäyttäytyminen ei näytä olevan energeettinen kustannus, mistä johtuen myyrän kannattaa aina varuilta reagoida, ja vasta reaktion jälkeen tunnistaa ärsykkeen viestittämä mahdollinen uhka.

Samaa menetelmää kuin edellä käytettiin myös metsämyyränaaraan lisääntymisen energeettisten kustannusten selvittämiseen. Respirometritutkimuksella seurattiin naaraiden lisääntymissyklin aikaista energiankulutusta kiimaan tulemisesta tiineyteen ja imetykseen asti. Yllättävä tulos osoitti, että metsämyyränaaraan lisääntyminen on energeettisesti erittäin optimaalista, ja lisääntynyt energiankulutus lisääntymissyklin aikana heijasti pääosin ainoastaan naaraan painonmuutosta. Optimaalinen lisääntymisen energetiikka lienee yksi tekijä, joka mahdollistaa metsämyyrällä synnytyksen jälkeiseen kiimaan perustuvan ajallisesti optimaalisen lisääntymisen. Tällöin naaras on uudelleen tiineenä imettäessään vielä edellistä poikuettaan.

Kokonaisuutena väitöstutkimukseni osoittaa, kuinka monimutkainen ja hienosyinen on peto-saalissuhteen se puoli, joka ei perustu pedon suoraan kuolleisuusvaikutukseen saalispopulaatiossa. Pedonvälttämiskäyttäytyminen perustuu monivaiheiseen päätöksentekoon: signaaliin reagoimiseen, sen oikeaksi tunnistamiseen, ja oikean pakoreaktion tai muun käyttäytymisen valitsemiseen. Luonnonpopulaatiossa yksilö kuitenkaan harvoin reagoi petouhkaan yksin, vaan vaste riippuu myös oman lajin muista yksilöistä ja toisista samalla alueella elävistä lajeista. Tämä tekee peto-saalissuhteen evoluution tutkimuksen edelleen haastavaksi.

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