Reproductive activity and spatial behavior of common voles (*Microtus arvalis* Pallas, 1778) in response to simulated mustelid predation risk

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Chapter 1

Introduction

1.1 PREDATOR-PREY INTERACTIONS

In the broadest sense of predation, most animals, if not all, are predators. At the same time, most of them are other predators' prey. Every animal must balance its life between hunting (or foraging), and evading a hunter; finding food and not getting caught when doing so constitutes two basic animal instincts. These two activities are mutually exclusive, to a certain degree. The more time an animal spends foraging, the higher the chances of an encounter with a predator; active predator avoidance, in turn, limits foraging possibilities (Lima and Dill 1990).

According to the 'life-dinner' principle (Dawkins and Krebs 1979), a failed hunting attempt equals missed dinner; failure at avoiding predator, however, equals loss of life. The stakes are incomparably higher for prey than they are for predators. What animals invest into more efficient predator avoidance (in case of prey), or more efficient hunting (in case of predators), is proportional to the potential loss. The effort that prey put into anti-predatory adaptations pays off: they remain one step ahead of the predators. This fact is reflected in the dynamics of predator-prey systems. The co-dependence of prey and predators generates stable, perpetuating cycles of changes in their densities; as a rule, the density of predators lags behind the density of prey (e.g., the classic case of snowshoe hares and lynx; Keith 1990). If prey would not be leading in the 'arms race' against predators, both parties would fall into extinction. Having only poor adaptations, prey would have been killed off; devoid of source of food, predators would have followed soon after. In reality, some individuals successfully evade the predator, in one way or another. Efficient anti-predatory adaptations are what gives predator-prey systems such remarkable stability.

Predator avoidance comes at a price. The investment in anti-predatory tactics has to be spared in other areas of activity. Becoming harder to detect and catch requires the animal to change its behavior. Consequences of anti-predatory behavior reach further than just the

trade-off between avoiding predators and foraging. To adjust to the risk of predation, animals alter not only their feeding behavior, but also space use, diel activity and reproduction (Lima and Dill 1990). Thus, predation risk affects animal life histories.

1.2 OLFACTORY CUES OF PREDATION RISK

The ability to recognize presence of the predators is crucial for effective predator avoidance. Animals may use auditory, visual and tactile cues to assess the risk of predation. Yet, chemical signals are potentially the most reliable. Sound and vision may fail if the predator hunts by stealth or ambush. In contrast, chemical cues are relatively persistent and, most importantly, may be detected at a distance or in predator's absence.

Semiochemical signals are probably the most commonly used means of communication between animals. They are involved in many interactions, such as maintenance of social structure or finding mates, thus organizing the animal kingdom. Chemical signals mediate communication within one species, but also between species (Wyatt 2003, Sbarbati and Osculati 2006). Furthermore, these signals can be intercepted by species for which the information was not intended. Particularly in predator-prey interactions, olfactory cues act as a double-edged sword. Both prey and predators may take advantage of their presence. On one hand, reproduction-related odors of prey attract predators, such as snakes (Amo et al. 2004) or weasels (Cushing 1984). On the other hand, prey may perceive the presence of predators by detecting their odor (scent marks, excreta, etc.; Ferrero et al. 2011), and thus assess the risk of predation (Sih 1980, Ylönen et al. 2007). Naturally, high concentrations of predator odor indicate high predation risk; having detected it, the animal may adjust its behavior in order to avoid being killed by the predator. Adaptive changes in behavior may include habitat shifts, avoidance of the areas containing predator odor, decreased general activity, mobility, and space use, as well as altered diel activity (for a detailed review, see Apfelbach et al. 2005).

1.3 SEXUAL DIMORPHISM OF PREDATION RISK

Mating behaviors are in particular conflict with predator avoidance (Lima and Dill 1990). Depending on the level of involvement in the production of offspring, the sexes differ in individual predation risk. Territorial disputes, courtship displays or bird calls attract both females and predators, but are short-lasting. Contrary, maternal care is a full-time task. Maternity is so absorbing that predator avoidance may lose priority. This creates an excellent opportunity for predators, as prey off guard is easier to catch. In consequence, mothers are probably easier, and hence, preferred prey (Klemola et al. 1997).

1.4 BREEDING SUPPRESSION

Facing higher risk of predation, females require more rigorous adaptations to predation risk than males. In case of several species of microtine rodents, females may alter their breeding behavior to avoid predation. When challenged with a cue of predation risk, either direct (live predator), or indirect (predator odor), some female voles will suppress their reproductive activity; suppression of breeding should, by some margin, increase the chances of survival (breeding suppression hypothesis, BSH; Ylönen 1989, Ronkainen and Ylönen 1994, Ylönen and Ronkainen 1994, Koskela and Ylönen 1995). BSH assumes that suppressing females 'lie low' through the period of high risk, waiting for more favorable breeding conditions. Suppressed reproduction is a particular response of female voles to specialist predation risk from small mustelids (e.g. weasels and stoats), which in fact prefer female prey (Norrdahl and Korpimäki 1998). Conversely, suppressed reproduction is not evoked by increased avian risk of predation (Klemola et al. 1998).

Breeding suppression is a direct effect of predation risk, elicited not only by mere presence of the predator, but also by its semiochemical signals. At the same time, breeding suppression is a sexually dimorphic response—it has not been demonstrated in male voles. The division between the sexes reaches even deeper, however. The response of the females is binary: a certain portion of the population resorts to this adaptive behavior, while remaining females maintain reproduction, as if predation risk was low. It is an open question why these two behaviors coexist, and what mechanism drives breeding suppression .

BSH was subject to critique on grounds of methodological inconsistencies. Opponents of this hypothesis argued that the evidence in its favor was obtained mainly in laboratory experiments, characterized by artificial conditions and lack of control for novelty effect (Lambin et al. 1995, Mappes et al. 1998, Wolff 2003). Additionally, convincing evidence for breeding suppression in natural conditions is scarce, as few field studies on this topic exist. Despite a substantial research effort to date, BSH remains a matter of debate.

1.5 AIMS

Predator odors, conveying information about risk of predation, may affect prey in several ways. The effects of predation risk on voles are very complex, and not yet well understood. Evidence shows that reproduction and spatial behavior are affected, although these effects, especially regarding reproduction, are controversial. Additionally, very little is known about how female's breeding condition determines the response to predation risk. The aim of my doctoral project was to shed light on the effects of mustelid predation risk, simulated with

olfactory cues, on common voles (*Microtus arvalis*). Since female voles are under higher threat from mustelid predators than males (Norrdahl and Korpimäki 1998), I laid the emphasis on female reproductive activity. The aspect of reproduction was investigated on population level, as well as on individual level.

On population level, I tested if predation risk affected population size. Since breeding suppression affects reproductive output, the populations facing high risk should be smaller in numbers, relatively to a low-risk situation (Norrdahl and Korpimäki 2000). Further, I measured the effect of predation risk on reproductive activity of voles, both males and females. With regard to female reproductive activity, my aim was to verify the breeding suppression hypothesis in controlled field conditions. As a consequence of breeding suppression, the proportion of reproductively active females should be lower under high predation risk. However, this proportion is affected not only by predation risk, but also other factors. These factors include density of conspecifics, which on its own strongly influences vole reproduction (Marchlewska-Koj 1997). Few studies attempted to resolve breeding suppression induced by predation risk from suppression induced socially. Various concepts of the concurrence of those two effects were proposed (Hansson 1995, Ylönen et al. 1995). To resolve this issue, I inquired whether predation risk and vole density interactively shape reproductive activity of females.

In a population exposed to predators, some females would resort to breeding suppression, while others would not. Hence, in common opinion, breeding suppression divides the female population into two subpopulations: breeders and non-breeders. How predation risk induces suppression in non-breeders is still unknown. In contrast, the effects of predation risk in breeders are well studied and, apparently, intricate. On individual level, reproductive effort, maternal effects, and physiology of breeding under predatory stress received most attention (e.g., Heikkilä et al. 1993, Mappes and Ylönen 1997, Fuelling and Halle 2004, Bian et al. 2005b). Yet, the frequency with which females reproduce was overlooked. A single study on voles revealed that predation risk disturbs the estrous cycle (Koskela et al. 1996), thus suggesting a possible mechanism of breeding suppression. My aim was to verify this effect indirectly under field conditions, by measuring the frequency of litters in females breeding in spite of predation risk. For the sake of comparability with earlier studies, I also examined the females' reproductive output.

In addition to breeding suppression, voles may respond to predation risk by modifying their spatial and temporal behavior (Borowski 1998b, Jonsson et al. 2000, Borowski and Owadowska 2001, Eccard et al. 2008). So far, these effects were studied on population level, not always with distinction between sexes. Moreover, individual reproductive status

was rarely taken into account; some insight into the role of reproductive status was provided by Jedrzejewski and Jedrzejewska (1990) who revealed that, compared to other reproductive statuses, immediate response to a cue of predator's presence is different in breeding females and juveniles. In attempt to reduce this gap, I examined spatial and temporal responses to predation risk in breeding females.

To fulfill the aims of my doctoral project, I carried out an intensive, large-scale capture-mark-recapture (CMR) experiment, and a radio-tracking study. In chapter 2, I will present the results of the CMR experiment, with focus on the effects of simulated predation risk on population level. Chapter 2 will reveal how predation risk affects population density and reproductive activity of both male and female common voles. It will also answer the questions whether breeding suppression occurs in female common voles, whether BSH is valid for this species, and whether predator-induced breeding suppression depends on vole density.

Drawing on the individual records of reproductive history from the CMR experiment, chapter 3 will focus on the effects of predation risk on breeding females. It will show how predation risk affects the number of recruits per litter and the length of the litter interval. Chapter 4 will combine a spatial aspect of the CMR experiment with results of the radio-tracking study. It will reveal whether reproductively active females respond to predation risk in more conventional ways. Chapter 4 will show how mustelid risk of predation affects long-term space use (home range), short-term space use (activity range), mobility, and diel activity. Additionally, it will reveal whether breeding females avoid mustelid odor. Finally, chapter 5 will describe a pilot study on neurological processing of predator odor in common voles, an attempt carried out in the early stage of my doctoral project (unpublished).

Overview of manuscripts

Manuscript 1

Title: To breed, or not to breed? Predation risk induces breeding suppression in common voles (*Microtus arvalis*)

Authors: Mateusz Jochym and Stefan Halle

Summary: This manuscript presents the results of a capture-mark-recapture (CMR) experiment on the response of common vole populations to simulated mustelid risk of predation. We investigated how predation risk affects population size, reproductive activity of males and females. We also verified the breeding suppression hypothesis, and examined whether breeding suppression in females is density-dependent.

Author contributions: M. J. organized and carried out the field work, processed and analyzed the capture data, and wrote the manuscript (contribution of 90%); S. H. contributed the basic research idea, provided the facilities for the CMR experiment, supervised it, and revised the manuscript (contribution of 10%). The design of the experiment was a collaborative effort of both authors. This manuscript includes some of the suggestions made by S. H. during the preparation for submission.

Current status: in review for *Oecologia*.

Manuscript 2

Title: Influence of predation risk on recruitment and litter intervals in common voles (*Microtus arvalis*)

Authors: Mateusz Jochym and Stefan Halle

Summary: In this manuscript, we report the effects of simulated mustelid risk of predation on reproductive output of breeding females. We estimated the following parameters from the CMR data: the number of recruits per litter, and the interval between litters. Our results suggest the possible mechanism of predator-induced breeding suppression.

Author contributions: M. J. organized and carried out the field work, conceptualized the

measurements of litter intervals, processed and analyzed the capture data, and wrote the manuscript (contribution of 95%); S. H. provided the facilities for the CMR experiment, supervised it, and revised the manuscript (contribution of 5%). The design of the experiment was a collaborative effort of both authors.

Current status: in review for *Journal of Animal Ecology*.

Manuscript 3

Title: Voles in space: on the effects of predation risk on *Microtus arvalis* breeding females

Authors: Mateusz Jochym and Stefan Halle

Summary: This manuscript describes the effects of simulated mustelid risk of predation on space use and temporal activity of breeding female common voles. We used CMR data to estimate the long-term space use (home range). Using radio-tracking data, we examined the avoidance of predator odor (distance to odor source), and estimated short-term space use (activity range), mobility (mean relocation distance), and diel activity (indexes of crepuscularity and diurnality).

Author contributions: M. J. organized and carried out the field work, processed and analyzed the capture and radio-tracking data, and wrote the manuscript (contribution of 95%); S. H. provided the facilities for the CMR and radio-tracking experiments, supervised those experiments, and revised the manuscript (contribution of 5%). The design of the experiments was a collaborative effort of both authors.

Current status: in review for *Chemical Signals in Vertebrates, Volume XII*.

Chapter 2

To breed, or not to breed? Predation risk induces breeding suppression in common voles (*Microtus arvalis*)

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Abstract. Females of several vole species suppress breeding in response to high risk of predation. Compared with breeding females, suppressing females gain higher chances of survival. This phenomenon is known as the breeding suppression hypothesis (BSH). While breeding suppression was frequently demonstrated in the lab, replicating it under field conditions was less successful. BSH remains ambiguous, probably due to methodological inconsistencies. The response of breeding females to both high predation risk and high densities of conspecifics is similar. Since these two conditions often coincide in nature, their effects may be difficult to resolve. Additionally, these factors likely interact in regulation of the reproductive activity of female voles.

We tested if breeding suppression occurs in common voles (*Microtus arvalis*). We also explored the combined effect of population density and predation risk on female reproductive activity. Mustelid predation risk was simulated with odors of domestic ferrets (*Mustela putorius furo*), in a large-scale enclosure experiment near Jena, Germany. The study was conducted over two breeding seasons, in 2007 and 2008.

We found that female common voles suppress reproductive activity when faced with high predation risk. Males did not show this response. The size of the populations was not affected during the high predation risk period. Modeling of the interaction of predation risk and population density revealed that predator-induced breeding suppression depends on the density of conspecifics. We provide arguments why this adaptation is viable only at low vole densities. To improve the consistency of future studies, we identify the key issues of experimental design.

Key words: predation risk; predator odor; breeding suppression; density dependence; population dynamics; arvicoline rodents; *Microtus arvalis*

2.1 INTRODUCTION

Odors of predators modify the behavior of many mammals (for an extensive review, see Apfelbach et al. 2005). Small rodent prey—voles in particular—received much attention. Responses of voles fall into several categories: suppressed feeding (Calder and Gorman 1991, Borowski 1998a), decreased activity (Gorman 1984), and changes in spacing (Jedrzejewski et al. 1993). Some interesting sex-specific effects were found: males or non-breeding females fled in reaction to the cues of predator proximity, whereas breeding females and juveniles did not (Jedrzejewski and Jedrzejewska 1990).

Responses of voles to predation risk are not limited to obvious behavioral effects. Ylönen (1989) was first to show that presence of predators or predator odors suppresses females'

reproductive activity. This reaction inspired further studies, which discovered such intricate effects as copulation avoidance (Ronkainen and Ylönen 1994, Ylönen and Ronkainen 1994, Koskela and Ylönen 1995), delayed maturation and hindered development of gonads (Heikkilä et al. 1993), and decreased frequency of estrus (Koskela et al. 1996). Except the study of Heikkilä et al., no clear effects of predation risk were found in male voles. Intriguingly, female responses were induced only by specialist predators (i.e., mustelids, namely weasels and stoats). By contrast, generalists and avian predators did not cause these effects (Wolff and Davis-Born 1997, Klemola et al. 1998, Jonsson et al. 2000).

Based on the accumulated evidence, breeding suppression hypothesis (BSH) was coined. BSH predicts that, when faced with high predation risk from specialist predators, the females will attempt to minimize it by suppressing reproduction (Ronkainen and Ylönen 1994). Females are preyed upon more than males (Norrdahl and Korpimäki 1998). Moreover, females in estrus are particularly attractive to mustelid predators (Cushing 1985). This indicates that in a population of voles, breeding females are at the highest risk of predation. Females resorting to breeding suppression are rewarded with increased chances of survival. Still, it is unclear why only part of the population employs this adaptation, while some females continue to breed in spite of predatory threat (Fuelling and Halle 2004). For them, unfulfilled breeding opportunities may be too high a price for the relative safety.

BSH still remains controversial, despite a fair amount of research. Initial studies revealed acute behavioral or physiological effects of predator odor, but their validity was criticized on the grounds of artificial conditions (Mappes et al. 1998, Norrdahl and Korpimäki 2000, Wolff 2003) and lack of control for novelty (Lambin et al. 1995). The controversy prompted experiments under natural conditions. The attempts to verify breeding suppression in the field brought inconsistent results: some studies contradicted breeding suppression (e.g. Wolff and Davis-Born 1997, Mappes et al. 1998, Sullivan et al. 2004), but others confirmed it (e.g. Mappes and Ylönen 1997, Fuelling and Halle 2004). In contrast to laboratory studies, field studies are burdened with uncontrollable factors, which may hamper the traceability of breeding suppression. We think that carefully designed experiments will bring conclusive evidence for BSH. We address the key methodological issues in this paper.

Female reproduction is not only affected by predation risk, but also by factors such as photoperiod and population density. Photoperiod limits breeding to the long daylight season (Lecyk 1962, Breed and Clarke 1970), while high population density induces social breeding suppression (Saitoh 1981, Ostfeld et al. 1993). In autumn, coinciding high densities of vole populations and shortening length of day negatively influence reproductive activity. Predation risk has a similar effect; regardless of the root cause, the resulting numbers of breeding

females are reduced. The analogy between density and predation risk was emphasized earlier. Kaitala et al. (1997) concluded that both population density and predation risk determine the proportion of reproductively active females; Ylönen et al. (1995) proposed that these two factors act additively; Hansson (1995) implied that they may be additive, but only at high vole densities. We aim to verify these statements.

We present results of an experimental study with enclosed populations. The study was carried out in Central Europe under controlled, semi natural conditions. We tested whether common voles (*Microtus arvalis*) suppress breeding when challenged with mustelid odor. To differentiate the influences of population density and predation risk, we modeled the effect of the concurring factors on reproductive activity of females.

2.2 METHODS

2.2.1 Field site and time frame

The experiment was carried out at Remderoda Field Research Station, located in central Germany (50° 56' N, 11° 32' E; 320 m a.s.l.) near the city of Jena. The field site was protected from terrestrial predators with a 2 m high wire-mesh fence; the access of avian predators was unrestricted. The study area comprised six enclosures, organized as shown in figure 2.1. Each plot was a square of 0.25 ha area (50 × 50 m), delimited by a vole-proof barrier made of sheet metal. In the growing season, the interior of the plots was covered with grasses and tall herbs. To emulate habitat edge at perimeter of each plot, a 2.5 m wide strip was mown regularly. Within the habitat, 25 live traps (Oos trap with shelter box; Halle 1994) were placed in a five-by-five square grid, at distances of 10 m. We arranged 16 odor sources in a four-by-four square grid, at equal distances from the traps (figure 2.1). To let the voles habituate to the

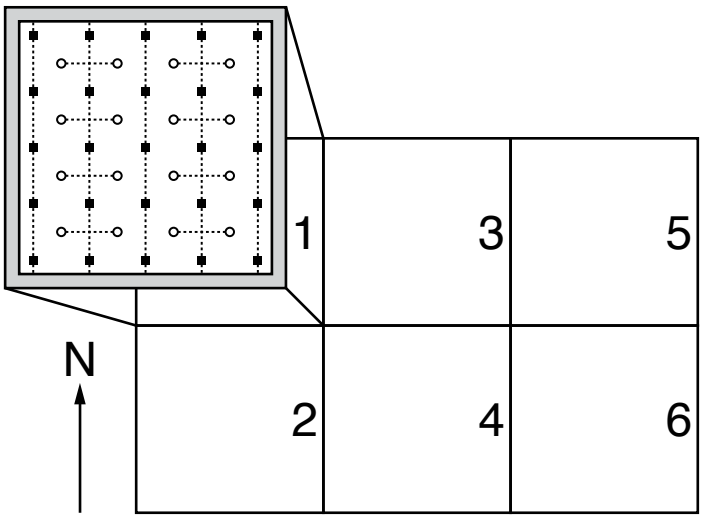

FIGURE 2.1: Diagram of the study site at the Remderoda Field Research Station. Traps are marked with filled squares. Odor sources are marked with open dots. Dotted lines denote access paths to traps and odor sources. The gray zone indicates the area devoid of vegetation. During the treatment phase in 2007, eight additional multiple-capture traps were used at the perimeter of each plot (not shown).

set-up, the odor sources were installed four weeks before the odor application period.

We ran the experiment during vole breeding season, following the same schedule in 2007 and 2008. In early spring, the plots were mown to bare ground. We trapped out the voles remaining from the previous year before the start of the experiment. Some voles were kept to repopulate the plots in mid-May. In 2007, we released 4 males and 7 non-pregnant females in each plot except plot 1, where 6 females were released. Due to low abundance in spring 2008, we limited the number of released females to 6 per plot. To imitate spring population structures, we included both young and overwintered individuals, with majority being in their first season. By the end of June we had started the first phase of the experiment, wherein we monitored the development of the populations. The monitoring phase was followed by the treatment phase, during which we manipulated the perceived risk of predation with olfactory cues.

2.2.2 Population monitoring

Each year, we sampled the six populations throughout both monitoring and treatment phases—on a weekly basis. We trapped each plot on two days a week, usually with one day in between. Traps were checked twice a day: in the morning and in the late afternoon. We activated the traps at sunset on the preceding day to allow trapping overnight. The traps were baited with pieces of fresh apple, barley, and commercial rodent chow. Upon first capture, individuals with body mass of 15 g or higher were sexed and marked under light anesthesia with a passive integrated transponder (PIT, Trovan); the tags allowed future identification of recaptured voles. We regarded individuals weighing less than 15 g as too small for marking; we classed them as juveniles and released. Since juveniles are not part of the reproductively active subpopulation, we excluded them from later calculations. Upon recapture of a tagged individual, we recorded its identity, body mass, and reproductive status. Based on these observations, we qualified each adult vole as either reproductively active or inactive in a given week.

Males with small or withdrawn testes were recorded as reproductively inactive; males with enlarged testes were recorded as reproductively active (Reichstein 1964). To count reproductively active females, we invented a simple scoring system based on their reproductive biology. Gestation in the common vole typically lasts for 20–21 days (Reichstein 1964). In the last 3–5 days preceding parturition, body mass of the female increases and the abdomen is enlarged. With regard to breeding suppression, reproductive activity materializes in ongoing gestations; hence, we deemed a female in antepartum state captured in week t_0 as reproductively active in weeks t_0, t_{-1}, and t_{-2}. The three weeks corresponded to the gestation period. In cases of

missing capture data or overlooked gestations, we inferred the female's reproductive status from sudden drops of body mass coinciding with a record of lactation in subsequent weeks. Reproductive activity was only interpolated when the individual's capture record contained sufficient information, and never for gaps longer than two weeks. Non-pregnant females counted as reproductively inactive; females with non-perforated vaginas were classified as juveniles, or as reproductively inactive if already marked. In cases of multiple captures within a week, the most frequent reproductive status was decisive, in both males and females.

2.2.3 Odor application

We applied olfactory cues to simulate two levels of perceived predation risk during the second phase of the experiment (i.e., the treatment phase). We used the odor of domestic ferrets (*Mustela putorius furo*) as a cue of high mustelid predation risk. Ferrets are not specialized in hunting for voles, but the composition of scent marks of species in the genus *Mustela* is very similar (Brinck et al. 1983, Zhang et al. 2003b). Furthermore, ferret body odor acts as a very potent stressor in rodents (Masini et al. 2005). Each year, we applied the ferret odor in three out of the six plots: plots 3, 5 and 6 in 2007, and plots 1, 2 and 4 in 2008 (figure 2.1). With this arrangement, we aimed to minimize the odor carryover to the adjacent plots. Plots receiving ferret odor are referred to cumulatively as 'predator treatment', for both phases of the experiment. The remaining plots served as controls with low level of predation risk ('non-predator treatment'). Here we applied two non-predator odors: of a herbivore, i.e. the European rabbit (*Oryctolagus cuniculus*), and of fresh cage bedding. We used these odors to control for the effect of novelty. Rabbit odor was applied in plots 1 and 4 in 2007, and in plot 3 in 2008; cage bedding odor was applied in plot 2 in 2007, and plots 5 and 6 in 2008 (figure 2.1). In the analyses, we tested for differences in the effect of the control odors.

The odors were prepared as water extracts of cage bedding. Both ferret and rabbit material was acquired from captive male-female pairs. It comprised a mixture of feces, urine, and fur. Fresh cage bedding was obtained commercially. To prepare the extracts, one part of bedding was soaked for 24 hours in five parts of water. Afterwards, the liquid fraction was strained, filtered, and frozen at -18 °C until use. To human nose, the ferret extract had a pungent smell. The smell of the rabbit extract was milder and clearly different, while the extract of fresh cage bedding smelled pleasantly of wet wood. As odor sources we used 0.5 L glass jars, placed on the ground amid vegetation. Jar lids were perforated to allow continuous odor release and refilling. We used manually operated, pressurized spray bottles to distribute the thawed extracts among the odor sources. We applied about 30 ml of the extract per odor source, totaling to approx. 500 ml per plot. The odors were applied sequentially, starting with the

non-predator plots and finishing in the predator plots. We used separate spraying equipment for each odor. As an extra precaution, we rinsed the impermeable protective clothing after each spraying session. The odors were applied throughout the whole treatment phase, three times a week at two-day intervals. In 2007, the treatment phase started in week 36 (early September) and lasted for 12 weeks; in 2008 it started in week 32 (early August) and lasted for 13 weeks.

2.2.4 Population parameters

Capture data were used to estimate population densities and reproductive activity. We estimated population densities with the Minimum Number Alive (MNA; Krebs 1999). This estimate was based solely on the marked portion of the population, i.e., individuals with body mass of at least 15 g. However, it was improbable to capture every single individual at exactly the 15 g threshold; in fact, some voles were much heavier at first capture. To account for this, we modeled the mean development of vole's body mass over time with our own capture data. The model assumed age of 5 weeks at the 15 g threshold. Using the curves of mean body mass development, we approximated the age at first capture and the time elapsed since the individual had passed the 15 g threshold. We added each such individual to the MNA estimate for improved accuracy. To minimize bias, data for females in advanced gestation were excluded from the model. Reproductive activity was measured as the proportion of reproductively active individuals to the total of all adults of the given sex. To minimize overlap between the two experimental phases, female activity derived from gestations conceived near the end of the monitoring phase was excluded from the analyses of treatment effects.

2.2.5 Data analysis

We analyzed the data using R, version 2.10.1 (R Development Core Team 2009) with package *lme4* (Bates and Maechler 2009) installed. Unless otherwise noted, we used linear mixed-effects models (LMM) for population densities, and generalized linear mixed-effects models (GLMM) with binomial error family and logit link function for proportions of reproductively active individuals. Likelihood ratio tests (LR) were used to determine the significance of model terms. To validate the models, we examined the graphical output for patterns in the residuals. We pooled the 2007 and 2008 datasets to test the interaction of population density and predation risk. The model included predation risk and experimental phase as binary factors, population density as continuous variable, and proportion of reproductively active females as the response variable.

2.3 RESULTS

2.3.1 Basic data

The analyses are based on 16,219 captures, 12,251 in 2007 and 3,968 in 2008. We have captured and marked 1,035 individuals in total, i.e., 366 females and 327 males in 2007, and 164 females and 178 males in 2008. The mean number of captures per individual was 19.0 (median 13), but females (mean = 20.2, median = 14) were captured more often than males (mean = 17.8, median = 10; t-test on square-root transformed data: t = 2.42, df = 1,000, p = 0.016). There was no difference in trappability between the two years (t = 0.26, df = 679, p = 0.79).

2.3.2 Population dynamics

The populations showed different dynamics in the two years. In 2007, vole numbers increased rapidly in the summer and peaked in mid-autumn, with maximum densities in range of 260–480 voles/ha. The seasonal increase in population size was not nearly as evident in 2008, when maximum densities reached only 120–200 voles/ha (figure 2.2). In the analysis

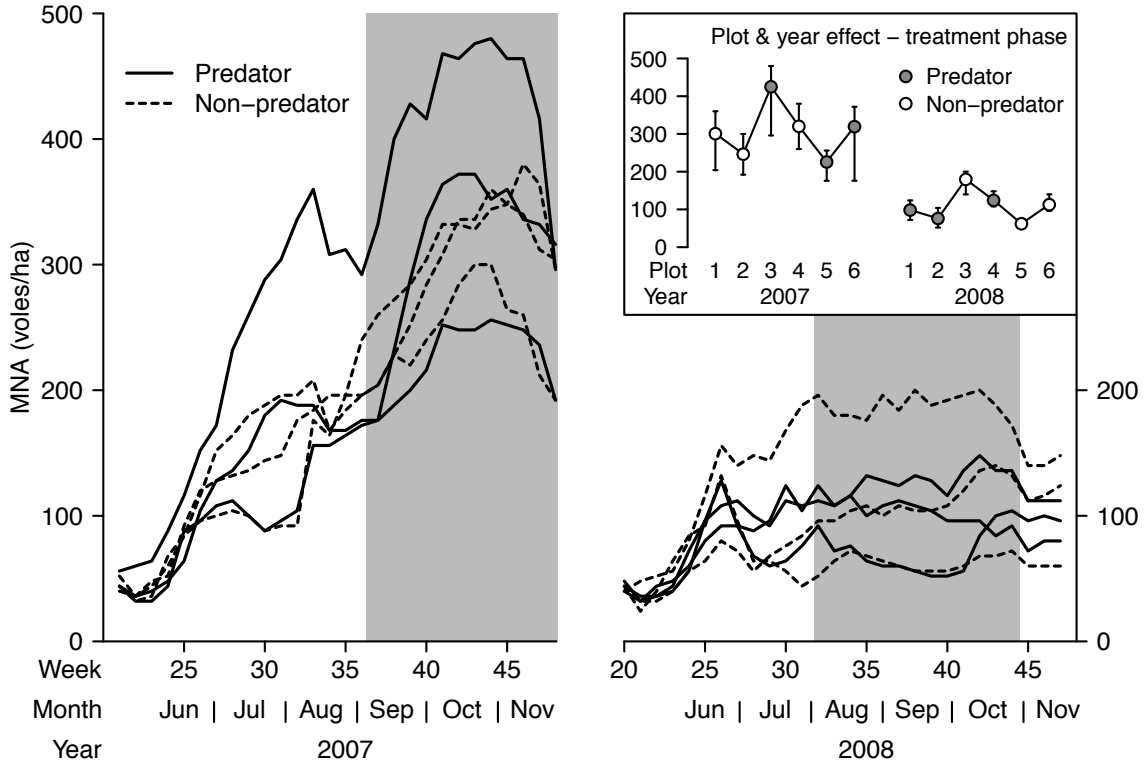

FIGURE 2.2: Population density over time (calendar weeks) during the two study years. Gray areas indicate the treatment phase. Solid lines represent plots assigned to the predator treatment; dashed lines represent non-predator treatment plots. The inset shows treatment phase means of population densities in each plot. Points mark the means, point shading denotes the kind of treatment, and whiskers indicate the range of values. Lines connecting the points visualize the consistent pattern of population densities across the two years. Statistics are given in the text.

(LMM), we allowed for a random intercept and random slope over time for each plot. Initially, we tested for the differences between the two non-predator treatments during the treatment phase, but we found none significant (LR: $\chi^2 = 1.57$, df = 1, p = 0.21).

Predator treatment had no significant effect on population density (predation risk $\times$ experimental phase interaction; LR: $\chi^2 = 0.78$, df = 2, p = 0.68). The difference between 2007 and 2008 in the rate of weekly density change was confirmed (LR: $\chi^2 = 224.00$, df = 1, p < 0.001). In addition to the year effect, we tested for a plot effect. We fit a model with a common intercept, corresponding to similar initial population sizes, and linear, quadratic and cubic terms for time. The latter two accounted for non-linear pattern of the population density changes. This analysis revealed significant differences between the plots (LR: $\chi^2 = 392.63$, df = 9, p < 0.001) with a strikingly consistent pattern in both years (figure 2.2 inset). The linear estimates of mean weekly change in population density (MNA) ranged from 3.0 ± 1.5 (SE) in plot 1 to 7.1 ± 1.1 (SE) in plot 3. Additionally, we found significant differences in both cubic (LR: $\chi^2 = 20.42$, df = 4, p < 0.001) and quadratic terms (LR: $\chi^2 = 17.03$, df = 4, p = 0.002).

2.3.3 Proportion of reproductively active males

The model (GLMM) for the proportion of reproductively active males included a random effect for the plots, with random intercept and random slope over time. We found no effect of the predator treatment on the proportion of reproductively active males during the treatment phase in both 2007 (LR: $\chi^2 = 1.48$, df = 2, p = 0.48) and 2008 (LR: $\chi^2 = 2.07$, df = 2, p = 0.36).

2.3.4 Proportion of reproductively active females

The model for females was structured analogically to the model for males. Reproductive activity of females was generally lower later in the season, which coincided with the application of odors. Still, the decrease of the proportion of reproductively active females was significantly stronger in the predator treatment, as compared with the non-predator treatment (predation risk $\times$ experimental phase interaction; LR: $\chi^2 = 25.56$, df = 1, p < 0.001); this effect was consistent in both years (figure 2.3 on the next page). In addition, we found a significant difference in the effect size between the two years (LR: $\chi^2 = 61.75$, df = 1, p < 0.001). No significant difference was found between rabbit and fresh cage bedding during the treatment phase (LR: $\chi^2 = 3.16$, df = 2, p = 0.20), justifying the integration of the two olfactory cues into one non-predator control.

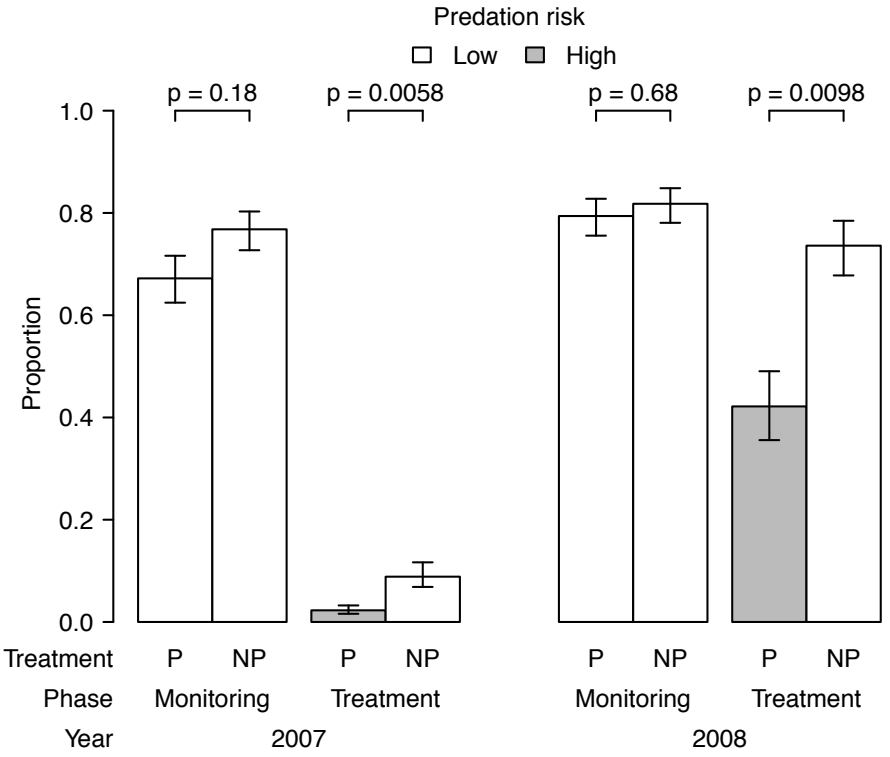

FIGURE 2.3: Mean proportion of reproductively active females. Grey bars indicate the values for increased predation risk. Error bars mark ± 1 binomial standard error of the means; p-values apply to comparisons between predator (P) and non-predator (NP) treatments within a phase, separately for each year.

2.3.5 Interaction of population density and predation risk

We tested the interaction between predation risk and population density in both phases of the experiment (figure 2.4 on the following page). The model (GLMM) included a random effect for the plots. We found that model slopes for predator and non-predator treatments (predation risk $\times$ population density) were not significantly different during both the monitoring phase (LR: $\chi^2 = 0.45$, df = 1, p = 0.50) and the treatment phase (LR: $\chi^2 = 1.15$, df = 1, p = 0.28). Model intercepts (simple treatment effect) for the predator and non-predator treatments were not different in the monitoring phase (LR: $\chi^2 = 3.30$, df = 1, p = 0.070). In the treatment phase, however, the intercept for the predator treatment was significantly smaller than the intercept for the non-predator treatment (LR: $\chi^2 = 23.54$, df = 1, $p < 0.001$).

In the non-predator treatment, model intercepts did not change between monitoring and treatment phases (LR: $\chi^2 = 0.37$, df = 1, p = 0.54), while in the predator treatment, the intercept decreased significantly (LR: $\chi^2 = 22.27$, df = 1, $p < 0.001$). In the non-predator treatment, the model slope was only marginally steeper during the treatment phase than during the monitoring phase (LR: $\chi^2 = 4.08$, df = 1, p = 0.043); in contrast, this difference was evident in the predator treatment (LR: $\chi^2 = 13.96$, df = 1, $p < 0.001$).

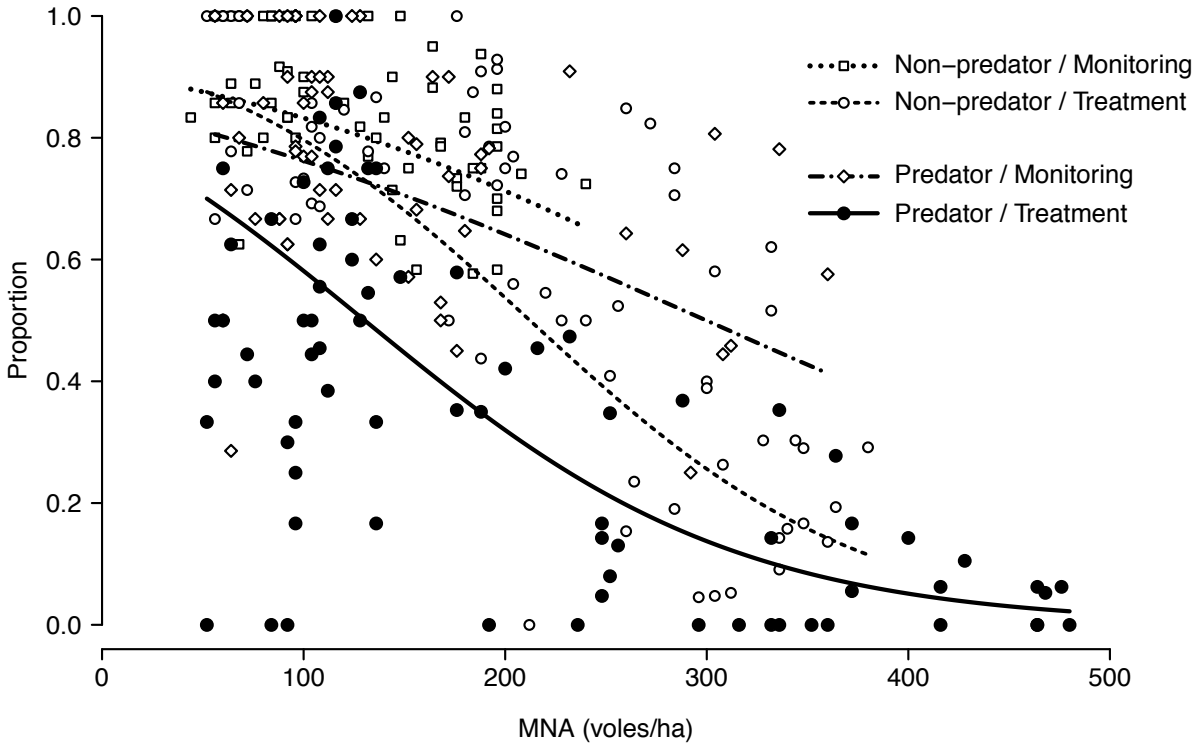

FIGURE 2.4: Relationship between the proportion of reproductively active females and population density at two levels of predation risk. Points mark weekly proportions in each plot. Lines represent the GLMM models' fits back-transformed from a logit scale. Line and point styles correspond to the combinations of treatment and experimental phase, according to the legend. Significance of model estimates is given in the text.

2.4 DISCUSSION

2.4.1 Population dynamics

Frequent recaptures provided us with a detailed dataset covering the demography and reproductive activity of twelve common vole populations. The populations developed in a similar way, starting from low initial size, increasing rapidly during the summer, and peaking in early autumn. After the annual peak, the densities declined slowly. Such pattern corresponds to the typical density development of free-living populations (Nabagło 1981).

Regardless of similar course of development, the densities of our study populations varied substantially. Despite almost identical initial sizes, the populations reached much higher maximum densities in 2007 than in 2008. Interestingly, the same difference was apparent in free-living common vole populations in the area (personal observation). In addition to the year effect, the mean densities differed markedly between the plots. These differences were consistent across years, but independent of the experimental treatment. Factors like variable habitat quality, soil structure, vegetation cover or food supply could explain the effect of the plot. However, the deviations of plot properties were not apparent on site. Altogether, variation of populations density reflected the differences between years and between plots,

rather than the differences in simulated predation risk.

According to BSH, an increase in predation risk suppresses breeding, and consequentially, should result in relatively lower population size (Norrdahl and Korpimäki 2000). Yet, we found no differences in population size between predator and non-predator treatments. Breeding females may increase reproductive effort under high predation risk (Norrdahl and Korpimäki 1995, Mappes and Ylönen 1997). Increased effort could, at least in part, compensate for the litters unrealized by the suppressing females. Alternatively, the overall reproductive output may have indeed decreased. Since recruitment substantially lags behind reproduction (six to twelve weeks between conception and recruitment of the resultant individual; personal estimation), a treatment period of 12 or 13 weeks could be too short for vole numbers to reflect the lower reproductive output.

2.4.2 Proportion of reproductively active males

We found no effect of increased predation risk on the proportion of reproductively active males, which is in accord with existing evidence (e.g. Ronkainen and Ylönen 1994, Klemola et al. 1997, Mappes and Ylönen 1997). It is commonly agreed that predator olfactory cues induce stress in male murids (for some contradicting evidence, see Fletcher and Boonstra 2006). For instance, stress response was reported in laboratory rats exposed to fox odor (Thomas et al. 2006). In male meadow voles (*Microtus pennsylvanicus*), fox odor reduced their general activity (Perrot-Sinal et al. 2000). The anal gland scent of Siberian weasel (*Mustela sibirica*) resulted in elevated stress hormone levels in rat-like hamsters (*Cricetulus triton*) and golden hamsters (*Mesocricetus auratus*), but did not affect reproductive physiology (Zhang et al. 2003a). Only acute exposures to predator odors affect reproduction in male rodents (Vasilieva et al. 2000, Wang and Liu 2002). It seems that this reaction is part of a general physiological or behavioral complex evoked by severe stress. Very high intensities of predatory stimuli are unlikely to imitate natural levels of predation risk. Therefore, occurrence of breeding suppression in male rodents is probably restricted to a laboratory setting.

In contrast to females, breeding suppression in males is not a viable adaptation to predation risk. Males merely escape from areas tainted with weasel odors, whereas reproductively active females do not (Jedrzejewski and Jedrzejewska 1990). Escape is a simple yet effective response to predation risk, but is not available to pregnant or pup-rearing females. Moreover, predators prefer to hunt females (Norrdahl and Korpimäki 1998). In consequence, males face lower mustelid predation risk; breeding suppression would be inadequate in their situation.

2.4.3 Proportion of reproductively active females

In the monitoring phase, the mean proportion of reproductively active females did not differ between populations. Yet in the treatment phase, it was distinctly lower in the populations with predator treatment. This finding is in agreement with the predictions of BSH.

Studies on captive voles demonstrated clear effects of predation risk on female reproduction. Ylönen (1989) reported that none of the four bank vole (*Myodes glareolus*) females kept in presence of a weasel (*Mustela nivalis*) had been in breeding condition, while three out of four females unexposed to predator had reproduced. In a scaled-up experiment, Ylönen and Ronkainen (1994) exposed breeding pairs of bank voles to stoat (*Mustela erminea*) odor. Only 6 out of 34 females were reproductively active under predation risk, whereas 23 out of 34 females reproduced in the control group. The same effect was found in field voles (*Microtus agrestis*). In pairs challenged with mixed weasel and mink (*Mustela vison*) odor, only 2 out of 16 females bred; by contrast, 14 out of 17 females bred in control pairs (Koskela and Ylönen 1995).

In opposition to the laboratory studies, experiments under more natural conditions brought less clear-cut results. Mappes and Ylönen (1997) placed cages with pairs of bank voles in outdoor enclosures and simulated predation risk with stoat odor. In the predator treatment, only 16 out of 51 females reproduced, while in the control, the corresponding figure equaled 25 out of 49. Here, the effect of simulated predation risk was not as strong as in laboratory trials, but still quite clear. However, subsequent study failed to produce the same effect in the field, despite the use of an acute predatory stimulus (Mappes et al. 1998). Several experiments with North American *Microtus* species produced further evidence against breeding suppression. Wolff and Davis-Born (1997) exposed grey-tailed voles (*Microtus canicaudus*) to mink feces and urine over a period of four weeks. Authors found no differences in the proportion of pregnant and lactating females between treatment and control areas. Jonsson et al. (2000) replicated this experiment, reaching the same conclusion. More recently, Sullivan et al. (2004) simulated predation risk with a mixture of two synthetic components present in the anal gland secretion of several mustelids. The treatment had no effect on the number of pregnant females of montane vole (*Microtus montanus*) and meadow vole (*M. pennsylvanicus*).

As accumulating negative reports increased the uncertainty of BSH, a field study in Scandinavia brought surprising evidence in its favor. Over three breeding seasons, Fuelling and Halle (2004) simulated predation risk by applying weasel odor in an open field. Three populations of gray red-backed voles (*Myodes rufocanus*) were exposed to the odor; three

others served as controls. Here, the average proportion of reproductively active females was lower in areas with predator treatment (0.79), as compared to control (0.92). Despite considerable variation—inherent in field experiments—the reduction of female reproductive activity under predation risk was clear.

Existing data indicate substantial geographic and taxonomic variation in responses of female voles to predation risk. The majority of Scandinavian studies on breeding suppression was positive, but these experiments were limited to voles of the genus *Myodes*. Conversely, North American studies focused on *Microtus* species, and produced no evidence for breeding suppression. Our study is the first to reveal breeding suppression in *Microtus arvalis*, an Eurasian species absent in most of Scandinavia (for distribution range, see Haynes et al. 2003). Geographical differences between Europe and North America seem to go deeper than differences between genera. Besides, co-existing species of the same genus may vary in response to predator odors (Heikkilä et al. 1993); breeding suppression among vole species could thus be less than a common strategy.

2.4.4 Methodological issues

Breeding suppression was never rejected in a laboratory study. Clear effects obtained in the laboratory may have been due to the proximity of the predator or high concentrations of its olfactory cues, but the consistency of the findings is striking. Attempts to reproduce the effects of predator odor in field conditions either revealed much weaker responses, or altogether failed. It may seem that traceability of breeding suppression decreases with increasingly natural conditions. In reality, field experiments varied in design and protocol, making definite generalizations difficult. To ensure comparability of data, future field studies should apply more consistent methodology. We identify some of the key issues below.

Wherever predation risk is simulated temporarily, care should be taken to avoid bias of reproductive activity estimates. Occurrence of breeding suppression in field studies is inferred from the reproductive states of females, so it is not instantly detectable. Females mating soon before the start of treatment period will be recorded as pregnant during thereof. If predation risk is simulated for a relatively short time, the counts of reproductively active females will biased. Additionally, treatment period spanning little over the duration of a single gestation (e.g. Wolff and Davis-Born 1997, Jonsson et al. 2000) could be too short for suppressed breeding to become apparent. Experimenters should allow enough time to account for the delay.

Studying breeding suppression requires an accurate definition of reproductive activity. Some of the existing studies (e.g. Wolff and Davis-Born 1997, Jonsson et al. 2000, Fuelling

and Halle 2004) regarded lactating females as reproductively active. In common voles, dams normally nurse the sucklings for over three weeks (personal observation); in other species, the length of the lactation period is probably similar. Predator-induced breeding suppression may take effect already during this period, postponing further litters. Hence, counting lactating but not parturient females as reproductively active will cause positive bias.

The origin and composition of predator odors are an important aspect of studies on breeding suppression. Apfelbach et al. (2005) emphasized that odors of a generalist predator (e.g. mink) may not be as significant for voles as odors of a specialist (i.e., weasel or stoat). Mink odor may be too weak an impulse to affect reproduction. In fact, trials using mink odors (Wolff and Davis-Born 1997, Jonsson et al. 2000) failed to induce breeding suppression. Contrary, studies using weasel or stoat odor brought positive results (reviewed in Apfelbach et al. 2005)—except for a case where two synthetic components were involved (Sullivan et al. 2004). In our experiment, breeding suppression was induced with the full body odor of the ferret—a generalist predator. Ferret body odor is a potent stress agent in rats (Masini et al. 2005), marking its relevance for rodents. Further, odor of European polecat (*Mustela putorius*, feral form of the ferret), modifies vole behavior (Jedrzejewski et al. 1993). Even though ferrets and polecats are generalists, their odors may affect voles in a similar way as odors of specialists. The age of the olfactory cues could play an additional role. Chemical composition of the cue may change along as it gradually decays, thus affecting its intensity and significance. However, we are not aware of studies dealing with this aspect. Existing evidence indicates that only ecologically significant, complete cues of predation risk induce breeding suppression, but the understanding of their function is limited.

Manipulation of the actual predation pressure is alternative to simulated predation risk. Klemola et al. (1997) surveyed the reproductive performance of sibling voles (*Microtus rossiaemeridionalis*) and field voles (*M. agrestis*) under reduced density of weasels and stoats. The proportion of reproductively active females decreased in 2 out of 6 study areas after predators had been removed, while it decreased in 5 out of 6 areas where predation risk remained high; here, the numbers of reproductively active females declined sharply. This outcome was interpreted as a consequence of selective predation, rather than suppressed breeding. Incidentally, this interpretation supports BSH: if most reproductively active females were indeed killed off by the predators, then surviving non-breeders would have gained the benefit of relaxed competition. Nonetheless, the study of Klemola et al. shows that coexisting predators may interfere with population dynamics of prey. Selective killing of reproductively active females changes the age structure, sex ratio, and particularly, the proportion of reproductively active females. Besides, the result of selective killing could be

mistaken for breeding suppression, as Klemola et al. already pointed out. To avoid this pitfall and afford control over apparent predation risk, actual predators should have no access to the study sites.

Apart from ambient predator pressure, also unrestrained vole migration may disturb field studies. Since voles avoid predator odors (Jedrzejewski and Jedrzejewska 1990, Jedrzejewski et al. 1993), some individuals could escape from the study area. In addition, immigration may dilute the study population, thus weakening any potential effects. In our opinion, outdoor enclosures provide an optimal balance between controlled and uncontrolled environment. Large enclosures offer almost natural conditions, while they keep away ground predators and unsolicited cues of predation risk, as well as eliminate vole migration.

2.4.5 Interaction of population density and predation risk

In natural vole populations, the proportion of reproductively active females decreases in the course of the breeding season. This is a result of two main factors: photoperiod and population density. The length of daylight controls breeding of *Microtus* voles (Lecyk 1962, Breed and Clarke 1970), limiting bulk of reproduction to the growing season. In Central Europe, reproduction of common voles begins in March and ceases in November; in wintertime, the voles remain reproductively inactive (Reichstein 1964). Hence, the proportion of reproductively active females decreases towards winter. We found a hint of seasonal breeding in our data: in the monitoring phase, spanning from late June till early August or September, the mean proportions of reproductively active females ranged between 0.65 and 0.80. In the treatment phase (lasting till November), the corresponding figures were always lower—regardless of the level of simulated predation risk.

The second factor affecting reproductive activity—population density—increases with advancing breeding season. Reproductive activity in a vole population is inversely related to its density: at high density, social breeding suppression occurs (Ostfeld et al. 1993). In presence of reproductively active females, maturation of young, lower-ranking females is suppressed (Saitoh 1981). A detailed account of density dependence of female reproductive activity in the common vole was provided by Reichstein (1964). Our data are in accord with Reichstein's findings. Mean densities in our populations were higher in 2007 than in 2008, while the reverse was true for the coinciding proportions of reproductively active females. We suppose that in the first year, some density-dependent breeding suppression occurred; this effect was weaker in the second year. Additionally, low densities of the early populations concurred with high proportions of reproductively active females; the proportions decreased as the populations grew in numbers.

The effect of increasing density is inseparable from the effect of shortening photoperiod; in the course of the breeding season, both factors result in decreased reproductive activity. Photoperiod follows a fixed annual pattern and, to some extent, it pre-determines reproductive activity. Fluctuations of population density add further variability—both within and between years, as indicated in our data. Kaitala et al. (1997) put forward that predation risk and population density may collectively determine female reproductive activity. Like population density, predation risk has a negative impact. Since both factors can occur simultaneously, one can easily confuse their effects.

To resolve this issue, we modeled the combined effect of population density and predation risk on the proportion of reproductively active females. As anticipated, the response variable was inversely related to vole density, regardless of treatment and experimental phase (figure 2.4). Moreover, high predation risk negatively affected the proportion of reproductively active females, but this effect was much weaker than the effect of high density. Finally, the effect of predation risk was evident only at low population densities. We conclude that predator-induced breeding suppression is conditional on population density. The effects of predation risk and population density are not additive at high vole densities, where social suppression is dominant. Our conclusion is in partial agreement with the concept of general additivity (Ylönen et al. 1995), but it contradicts the claim of additivity at high densities (Hansson 1995).

Not to breed is a rewarding strategy under high predation risk, albeit only at low densities of conspecifics. At high densities, voles are abundant prey. Resulting *per capita* predation risk is low, making breeding suppression devoid of purpose. Conversely, breeding females become most conspicuous when prey are scarce. For a non-breeding female, the chances of survival increase to a level comparable with male voles. Therefore at low vole density, the benefit of breeding suppression may outweigh the costs of reduced reproductive output.

Relative effects of population density and predation risk on vole reproductive activity were investigated in the past. Based on long-term field data, Norrdahl and Korpimäki (1995) inferred that the effect of predator pressure was stronger. The contradiction with our finding may be caused by the difference in perceived predation risk. Norrdahl and Korpimäki measured the actual density of mustelid predators, which varied over a range of values. By contrast, we mimicked the presence of predators with olfactory cues. We cannot rule out our stimulus was weak, compared with real risk of predation. Mappes and Ylönen (1997) found a minimal effect of population density on reproductive activity in bank voles. Again, the effect of predation risk was strong, regardless of vole abundance. The authors simulated high density with pre-collected vole odors, but vole pairs had no physical contact with conspecifics.

Inaccurate perception of density could partially explain the overwhelming effect of predation risk. In light of contrasting evidence, the interaction of predation risk and population density is open to further research.

Mappes and Ylönen (1997) observed that mean litter size increased both under high predation risk and high abundance of conspecifics. Apparently, females breeding in spite of poor survival perspectives or fierce competition try to make the most of a bad situation. This indicates a universal, dichotomous adaptation to unfavorable conditions: either maximize the breeding effort, or put it on hold in hope of better tomorrow. It is intriguing what individual trait determines the female's choice of strategy.

Chapter 3

Influence of predation risk on recruitment and litter intervals in common voles (*Microtus arvalis*)

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Abstract. Carnivores from the genus *Mustela*, particularly weasels, are specialist vole hunters. Breeding suppression hypothesis (BSH) predicts that, when faced with mustelid risk of predation, female voles will suppress breeding to increase their chances of survival. This phenomenon was reported in several species, but the exact mechanism has not yet been established. In general opinion, predation risk induces breeding suppression in a certain part of the female population. The response of the remaining females is unclear. To improve its understanding, we measured the efforts of females that maintain reproduction in spite of predation risk.

We used capture-mark-recapture data to test the effect of simulated predation risk on two parameters of reproductive output: the number of recruits per litter, and the interval between litters. The number of recruits per litter was not affected. We argue that, in field conditions, it may be impossible to detect the effect of predation risk on recruitment.

Compared to control populations, females under mustelid risk of predation had less frequent litters. This result indicates that the effects of predation risk on females are more complex than originally proposed. We conclude that predation risk not only induces breeding suppression in some females, but also influences the frequency of litters in others. Decreased litter frequency suggests that perception of predation risk is linked with regulation of estrus. Earlier laboratory experiments revealed that predation risk elongates voles' estrous cycle. We think that longer litter intervals, as observed in the field, are related to that effect. Elicited with a cue of predatory threat, delayed estrus could be the mechanism of breeding suppression. Alternatively, it could manifest a physiological stress response, of which increased chances of survival are only a by-product. Further studies are required to verify this.

Key words: litter size, litter interval, recruitment, reproduction, predation risk, breeding suppression, *Microtus arvalis*

3.1 INTRODUCTION

Females of several vole species adapt to predation risk by means of breeding suppression. This adaptation offers higher chances of survival, compared to normally breeding females. So far, several hypothetical mechanisms of breeding suppression have been proposed, of both behavioral and physiological nature. Predation risk may decrease foraging activity, leading to malnutrition and infertility (Ylönen and Ronkainen 1994, Koskela and Ylönen 1995). Effects on breeding behavior include decreased breeding activity and mate avoidance (Ylönen 1989, Ronkainen and Ylönen 1994, Ylönen and Ronkainen 1994, Koskela and Ylönen 1995). Physiological effects list delayed reproductive maturity (Ylönen et al. 1992, Heikkilä et al.

1993) and abnormal estrous cycles (Koskela et al. 1996). Additionally, breeding suppression may involve deeper physiological mechanisms, as more recent studies on rats (Naidenko et al. 2003, Voznessenskaya et al. 2003) and male voles (Bian et al. 2005a) may suggest. Given that predatory cues activate both endocrine and neural pathways in rodents (Heale et al. 1994, Morrow et al. 2000), the effects of predation risk on voles are not surprising.

Population studies on several vole species have shown that simulated predation risk negatively affects the proportion of reproductively active females (Mappes and Ylönen 1997, Fuelling and Halle 2004, chapter 2). In light of this evidence, breeding suppression is viewed as a binary strategy: in a given population, the females will either suppress breeding, or maintain it. This approach assumes that breeding females remain unaffected. However, some indication for the effects of predation risk on reproductively active females exists already, mostly with regard to reproductive success. Reported effects include decreased litter size (Korpimäki et al. 1994, Klemola et al. 1997), increased litter size (Mappes and Ylönen 1997), or decreased number of recruits per reproducing female (Fuelling and Halle 2004). Contrary to litter size, investigations of litter frequency in voles facing predation risk are scant. Koskela et al. (1996) conducted the seminal study on this topic. This laboratory experiment on bank voles *(Myodes glareolus)* concluded that risk of predation lengthens the estrous cycle, potentially decreasing the frequency of litters.

With this paper, we attempt to verify the effects of predation risk on breeding females. We carried out a capture-mark-recapture study in semi-natural conditions. We measured the effect of simulated predation risk on two parameters of reproduction: the number of recruits per litter, and the litter interval. Since reproductive output was often estimated in earlier studies on breeding suppression, we included the first parameter for the sake of comparability. Later in this paper, we critically discuss the evidence for the effects or predation risk on recruitment and litter size. With the second parameter, litter interval, we measured the frequency of litters. Knowing that predation risk affect the estrous cycle of bank voles, we tested for this effect in a different species in its natural environment, assuming that litter intervals reflect the estrous cycle. Our results provide further insight into the mechanism of breeding suppression.

3.2 MATERIALS AND METHODS

3.2.1 Study site and data acquisition

We carried out this experiment in 2007 and 2008. The study area was located in our field station near Jena, Germany (50° 56’ 17” N, 11° 31’ 45” E). The station was secured from terrestrial predators. The study site comprised six plots, separated by a vole-proof fence.

Each plot housed a population of common voles *(Microtus arvalis)*. We released 6–7 females and 4 males per plot in May. The majority of voles were in their first season. The experiment was split into two phases. In the first phase, we allowed the populations to develop naturally. We recorded evident gestations and changes in population size. In the present paper, we refer to this phase as 'monitoring phase'. In the second phase, in addition to population monitoring, we manipulated perceived risk of predation. We refer to the second phase as 'treatment phase'. Monitoring phase began in early July. It was scheduled for 12 weeks 2007 and 6 weeks in 2008. The length of the treatment phase was 12 and 13 weeks, respectively. Population monitoring followed a fixed regime. Each plot contained 25 live single-capture traps, placed 10 m apart in a square grid. The traps were set the day before, in order to allow overnight trapping. We used apple chunks, rodent chow and barley grain for bait. We trapped on two days a week, separated by one day. We checked the traps twice a day: in the morning and late in the afternoon. Captured voles were identified and weighed. Females were examined for signs of gestation. Unmarked individuals were anesthetized and tagged with a PIT (passive integrated transponder, Trovan). Juveniles (body mass below 15 g) were released without a tag. Further details of the experimental design are described in chapter 2.

3.2.2 Manipulation of predation risk

We simulated predation risk as a binary factor. In 3 out of 6 plots, presence of mustelid predators was simulated with odors of captive ferrets *(Mustela putorius furo)*. We refer to these plots as the 'predator treatment'. The alternative treatment simulated absence of mustelid predation risk. Here we applied two non-predator odors to control for the effect of novelty. Odor of captive European rabbits *(Oryctolagus cuniculus)* was applied in 2 plots in 2007 and in 1 plot in 2008. Second control odor was derived from unsoiled cage bedding. Contrary to the previous two, this was a non-animal odor. We applied it in the remaining plots. We combined both control odors into one treatment, referred to as 'non-predator treatment'. We assigned the treatments to the plots so as to minimize any carry-over effect. Each year, the arrangement of the plots was different. The odors were applied three times a week throughout the treatment phase. We sprayed the odors into glass jars with perforated lids, installed permanently in the field. Each plot contained 16 jars placed equidistantly from the four adjacent traps. We allowed voles to habituate to the odor sources for one month before the treatment phase.

3.2.3 Calculations and analyses

We partitioned the data according to phase and treatment. It served to measure two parameters related to vole reproduction. First, we calculated the mean number of recruits per litter. In order to tally the recruits, we defined the point of recruitment as the first capture at body mass of 15 g. We corrected the count of recruits for individuals weighing more than 15 g at the time of first capture. Using a model of body mass development (unpublished data), we estimated when the given individual had reached the recruitment threshold. The total number of litters was obtained from the record of females' reproductive activity. We identified the occurrence of a litter from evident gestations. In common voles, 74% of gestations are 19–21 days long, with mean gestation length equaling 20.6 days (n = 300; Reichstein 1964). Litters occurring in the first 21 days of the treatment phase were conceived in the monitoring phase. Hence, they counted for the first phase of the experiment. A certain time must elapse until a newborn vole qualifies as a recruit. Further on in this paper, we refer to this time as birth-to-recruitment delay, or 'delay' for short. We estimated that the average length of the delay equals 6 weeks. To account for some individual variation, we analyzed the recruitment data with delays of 5, 6, and 7 weeks.

As the second parameter, we calculated the mean litter interval (LI). Time span between two consecutively occurring advanced gestations of a given female constituted a single observation. The dates of parturitions were estimated from capture data. We identified gestations directly in the field (visually evident or birth in trap) or indirectly form individual patterns of change in body mass. Some pregnant females occasionally evaded capture. This rendered several gestations undetectable, inflating particular LI observations. In common voles, 79% of LIs fall below 45 days (n = 714; Reichstein 1964). To prevent the overestimation of mean LI, we ignored intervals exceeding 45 days, as they may have resulted from undetected gestations. Gestations concluding within the first 3–4 weeks of the treatment phase were attributed to the monitoring phase, since they had started before the treatments began. In this text, we refer to the initial period of the treatment phase as 'lag'. We performed two alternative analyses: with lags of 3 weeks (mean gestation length) and 4 weeks (mean gestation length + 1 week). The second analysis was intended to exclude some overdue gestations, which would have been falsely attributed to the treatment phase, and to account for the decreasing pace of reproduction towards the end of the breeding season.

We analyzed the data in R version 2.10.1 (R Development Core Team 2009) with package *lme4* (Bates and Maechler 2009). We used linear mixed-effects models (LMM). Significance of model terms was determined with likelihood ratio tests (LR). Models were validated by

examination of graphical output.

3.3 RESULTS

3.3.1 Number of recruits per litter

Despite identical initial population size, total reproductive output differed between the years. In 2007, we recorded 689 recruits and 428, 419, and 398 litters (for 5, 6, and 7-week long delay, respectively). The corresponding figures for 2008 were 252 recruits and 251, 233 and 225 litters. We compared the mean numbers of recruits per litter in predator and non-predator treatments within each phase, separately for the two years. The models included a random intercept for the plots. We analyzed the data for each value of delay separately. In each case, we found no differences between the predator and non-predator treatment (table 3.1 on the next page). We tested for the year effect and differences between the phases in pooled datasets. In the model with a 5-week delay, the year $\times$ phase interaction was not significant (LR: χ^2 = 2.96 at 1 df, p = 0.085). There was no significant difference between monitoring and treatment phases (LR: χ^2 = 0.39 at 1 df, p = 0.53), and a nearly significant year effect (χ^2 = 3.75 at 1 df, p = 0.053). In the model with a 6-week delay, the year $\times$ phase interaction was also not significant (LR: χ^2 = 2.51 at 1 df, p = 0.11). Again, there was no significant difference between monitoring and treatment phases (LR: χ^2 = 0.57 at 1 df, p = 0.45), and no significant year effect (χ^2 = 2.18 at 1 df, p = 0.14). In the model with a 7-week delay, the year $\times$ phase interaction was significant (LR: χ^2 = 4.52 at 1 df, p = 0.034).

3.3.2 Litter interval

The two study years differed with regard to the numbers of breeding females, and length of the breeding season. As a result, we observed twice as many LIs in the first year as in we did in the second year (205 vs. 101). In 2007, 39 females yielded a single observation each (from two consecutive litters); 26, 15, 11 and 5 females produced 2, 3, 4 and 5 observations (from three to six litters) respectively. The corresponding values for 2008 were 27, 18, 10 and 2 females; that year, we did not record females that had 5 or 6 litters.

We carried out two separate analyses of the LI data: with a 3-week and 4-week long lag. In the LMM models, we included a year factor and random intercept for the plot ID. Preliminary analysis showed no difference between the two non-predator controls: both in the 3-week lag model (χ^2 = 1.70 at 4 df, p = 0.79), and the 4-week lag model (χ^2 = 1.69 at 4 df, p = 0.79). Hence, unifying the two control odors into one non-predator treatment was justified. Estimates of the 3-week lag model are shown in figure 3.1 on page 33. In the full

TABLE 3.1: Number of recruits per litter

Delay	Year	Phase	Estimate		Standard error		χ^2	p
			P	NP	P	NP		
5 weeks	2007	M	2.64	1.36	1.372	1.393	0.86	0.35
		T	4.66	2.63	1.125	1.338	0.17	0.68
	2008	M	1.83	1.68	0.263	0.610	1.70	0.19
		T	1.39	0.65	0.523	0.622	1.96	0.16
6 weeks	2007	M	2.72	1.41	2.381	1.810	0.90	0.34
		T	6.87	2.84	1.619	1.925	0.52	0.47
	2008	M	1.91	1.85	0.291	0.641	1.48	0.22
		T	1.47	0.65	0.574	0.683	2.21	0.14
7 weeks	2007	M	2.88	1.60	1.255	1.332	0.11	0.74
		T	3.70	3.90	1.034	1.229	0.88	0.34
	2008	M	2.08	1.95	0.276	0.624	1.04	0.31
		T	1.22	0.61	0.493	0.587	1.67	0.20

This table summarizes the three models (LMM) with 5, 6, and 7-week delay. The mean number of recruits per litter is estimated; approximated standard errors of the estimates are given. P – predator treatment; NP – non-predator treatment. Phase: M – monitoring; T – treatment. Last two columns show the LR statistics for the P:NP comparisons (each at 1 degree of freedom).

model, year $\times$ phase interaction was not significant (LR: $\chi^2 = 0.48$ at 1 df, p = 0.49). There was no significant difference between years (LR: $\chi^2 = 0.09$ at 1 df, p = 0.77). The effect of predator treatment (treatment $\times$ phase) was non-significant by a small margin (LR: $\chi^2 = 2.99$ at 1 df, p = 0.084), and was thus dropped from the model. Finally, the difference between monitoring and treatment phases was significant ($\chi^2 = 8.00$ at 1 df, p = 0.0047). Comparable pattern appeared in the model with a 4-week lag, with mean litter intervals being similar in the monitoring phase, but differing in the treatment phase (figure 3.1 on the next page). The year $\times$ phase interaction was not significant (LR: $\chi^2 = 0.98$ at 1 df, p = 0.32), and there was no significant difference between years (LR: $\chi^2 = 0.20$ at 1 df, p = 0.66). In this model, the effect of predator treatment (treatment $\times$ phase) was significant (LR: $\chi^2 = 6.02$ at 1 df, p = 0.014).

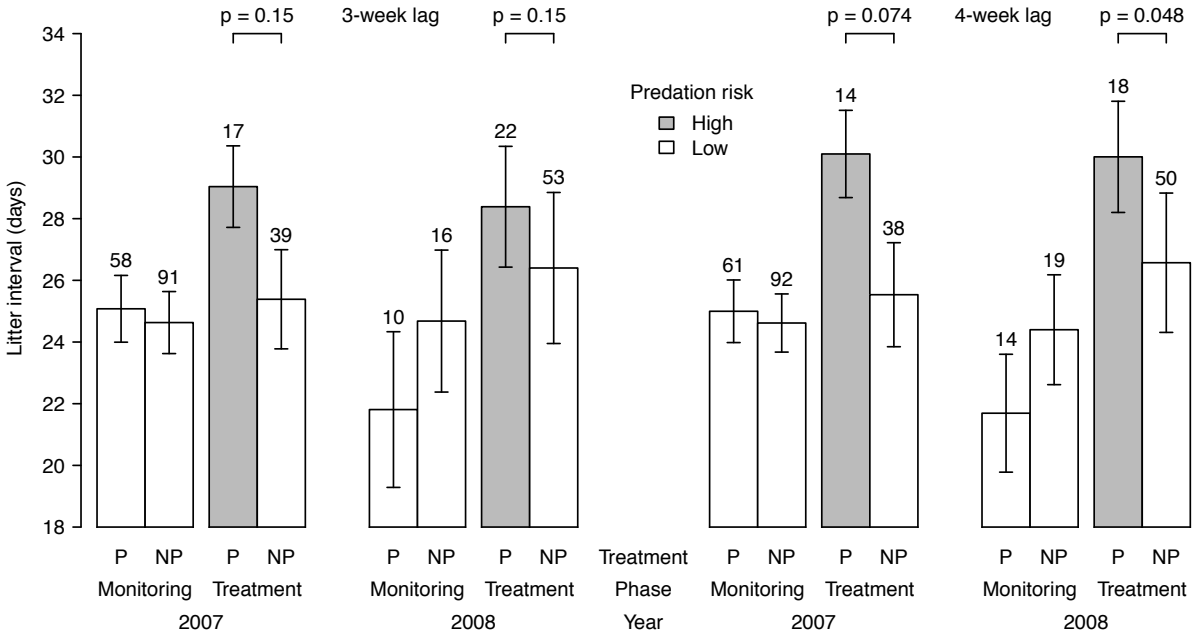

FIGURE 3.1: Mean litter intervals. *Left panel:* model with a 3-week lag. *Right panel:* model with a 4-week lag. Grey bars indicate the values for increased predation risk. Error bars mark $\pm$ 1 standard error of the mean; values above upper bars show numbers of observations; p-values refer to comparisons of predator (P) and non-predator (NP) treatments in the treatment phase, separately for each year. There were no statistically significant differences in the monitoring phase.

3.4 DISCUSSION

3.4.1 Number of recruits per litter

Regardless of delay length, predation risk had no effect on the number of recruits per litter. According to our assumption, emergence of voles should correspond to litter occurrences. We expected recruits to emerge after a fixed period, counting from the estimated litter they had been associated with. Instead, we encountered much variability in individual delay. We suspect that it was caused by various individual traits and environmental factors (such as weather or population density), which we did not control for.

In the first year of the experiment, the number of recruits per litter showed a weak tendency to increase from monitoring to treatment phase. This may be explained by the fact that litter size gradually increases with the age of a female (Reichstein 1964). Additionally, some of the less frequently trapped individuals fell behind the expected delay, despite the applied correction. In particular, belated emergence of several individuals born in the monitoring phase could be the cause of positive bias. In 2008, however, this pattern was reversed: the number of recruits per litter was lower in treatment phase. This difference is attributable to the lower overall reproductive output in 2008, and a shorter breeding season—despite the earlier beginning of the treatment phase. A nearly significant year effect could be due to the different rates of population growth. Peak population sizes reached in 2008 were lower than in 2007,

despite equivalent initial sizes (chapter 2). Our data showed roughly 1⅔ recruits per litter in 2007, versus just under 1 in 2008. The cause of the difference in reproductive output is unknown. We cannot exclude that in 2008, the rate of juvenile mortality was higher.

The evidence for the influence of predation risk on voles' reproductive output remains inconclusive, even though negative effects were demonstrated by a number of investigators. Klemola et al. (1997) manipulated the ambient predation risk from weasels *(Mustela nivalis nivalis)* and stoats *(Mustela erminea)*. The authors recorded a lower index of reproductive performance under high predation risk in field voles *(Microtus agrestis)* and sibling voles *(Microtus rossiaemeridionalis)*. Fuelling and Halle (2004) reported a similar result. They found a lower number of recruits per reproducing female in populations of grey-sided vole *(Myodes rufocanus)* exposed to weasel odors. In two vole species, Korpimäki et al. (1994) compared litter size (estimated from the number of embryos) with incident predation risk. The authors found a negative correlation of predation risk and litter size in bank voles, but only in spring litters. If field voles *(Microtus agrestis)* , the correlation was negative only for late summer litters. A solitary, positive effect of predation risk was communicated by Mappes and Ylönen (1997). They exposed caged pairs of bank voles to stoat odor. Here, estimated litter size was larger in females in the predator treatment, but this result was not replicated.

Most of the studies to date, including ours, denied the effect of predation risk on reproductive output. Weasel odors did not affect the actual litter size in bank voles (Mappes et al. 1998). In two related American experiments, populations of grey-tailed voles *(Microtus canicaudus)* were exposed to mink *(Mustela vison)* odors. Both studies concluded that odor-simulated predation risk does not affect the number of juvenile recruits per adult female (Wolff and Davis-Born 1997, Jonsson et al. 2000). In another American study, Sullivan et al. (2004) exposed montane voles *(Microtus montanus)* and meadow voles *(Microtus pennsylvanicus)* to synthetic mustelid odor. Here, too, no effect of predation risk on recruitment was found.

Being unable to track voles from birth in conventional CMR studies, the experimenters assume an arbitrary, fixed period from birth to recruitment. This period varies depending on the recruitment criterion (e.g. 15 g body mass in this study) and length of the reproductive cycle of the species in question. Earlier studies defined different delays: 4 weeks for grey-tailed voles (Wolff and Davis-Born 1997, Jonsson et al. 2000), 3 or 6 weeks for grey-sided voles (Fuelling and Halle 2004), and 3 weeks for juvenile recruits in montane and meadow voles (Sullivan et al. 2004). This is an idealized approach, however. In reality, the emergence of recruits is largely stochastic. In most species, the time from insemination to weaning can be estimated with reasonable precision. In common voles, 3 weeks of gestation are followed by 3 weeks until the newborn pups wean (Reichstein 1964). Contrary, the time until

the newborn vole's recruitment is much less predictable, and individually variable. Hence, common measures of recruitment can yield only a crude estimate of reproductive output.

Because of the birth-to-recruitment delay, the effects of a manipulation (such as simulated predation risk) are not instantly detectable. The delay limits the amount of available data, and long trapping campaigns are required to compensate for it. To ensure sound conclusions, the trapping period should cover at least the length of a single birth-to-recruitment cycle, plus the span of one gestation. In our study, we trapped for 21–22 weeks each year. We assumed delays of 5 to 7 weeks: between ¼ and ⅓ of the trapping period. To account for the delay, we rejected 11%–19% of observed litters and 19%–27% of recruits. The average loss of data per week of delay equaled 2.5% of observations for litters and 3.9% for recruits, which is a high price to pay for an estimate of reproductive output.

In light of current evidence, it is most reasonable to assume that predation risk has no effect on reproductive output, despite a number of studies on this topic. Some variability is noticeable; the responses vary across vole species or populations. The difference between American and European studies indicates taxonomic or geographic differences. Some indication of seasonality exists. Furthermore, the applied measures of reproductive output are inconsistent across studies. Although measures like the number of pups or embryos per litter, recruits per reproducing or adult female, or general recruit counts all gauge reproductive output, they are not directly comparable. In addition, reliability of these methods in natural environment is questionable. Due to limited precision, it might not be possible to measure the effects of predation risk on litter size or recruitment in field conditions. Perhaps future studies will bring conclusive evidence.

3.4.2 Litter interval

Mean LI recorded in the non-predator treatment and in the monitoring phase resembled the values observed in natural conditions (Reichstein 1964). In predator treatment, however, mean LI was clearly higher; this pattern was consistent for models with both the 3-week and 4-week lag. For most comparisons, the effect size approximated 4 days. We ascribe this effect to simulated mustelid predation risk. There was no difference between the 3-week and 4-week models with regard to the monitoring phase and non-predator treatment. In the predator treatment, however, the effect sizes were larger in the 4-week model (figure 3.1). The difference between the two models suggests that the lag of three weeks, spanning exactly one gestation, was too short to filter out the females that had been fertilized soon before the beginning of the treatment phase (3 observations in 2007, and 4 observations in 2008). Those observations negatively biased the means for the predator treatment, thus limiting the

significance of the effect. The significance was decreased even further due to substantial individual variability in length of LI (Reichstein 1964). The results of Koskela et al. (1996) suggest that risk of predation amplifies individual variability. On one hand, variation between females may affect significance, but on the other hand, it points to an unknown individual trait determining perception of predation risk in female voles.

Significance of effects was limited by yet another factor—the number of observations. We recorded relatively few observations in the area of main interest, the predator treatment. In these populations, fewer females were reproductively active—as a result of predator-induced breeding suppression (chapter 2). Since suppressing females postponed reproduction *ad infinitum*, they were not included in the measure of litter frequency. In addition, we excluded all unusually long LIs to increase precision of the estimates. Lastly, the breeding season of 2008 (a year of low to moderate vole density) afforded relatively few observations in the monitoring phase, probably due to the earlier beginning of the treatment phase.

According to Reichstein (1964), litter interval of the common vole ranges from 17 to 89 days. Contrary, length of gestation itself shows less variability: almost 60% of gestations are 20 or 21 days long; 90% are in the range of 19–22 days (Reichstein 1964). Thus, litter interval may depend mainly on the length of postpartum anestrus. Unpaired females show very irregular estrous cycles; cycle lengths fall within 6–18 days (Dobrowolska and Gromadzka 1978). In mating pairs, however, estrus occurs immediately postpartum (Kudo and Oki 1982). Since mechanical stimulation of reproductive organs suffices to induce ovulation (Dobrowolska and Gromadzka 1978), copulation almost invariably results in a new litter. This creates the capacity for perpetual reproduction, regardless of the irregularity in solitary females. New litters are produced at intervals of 20–21 days (the span of a single gestation). When conditions are optimal, common voles breed continuously (Walkowa and Bujalska 1977).

High risk of predation, an unfavorable condition, disturbs the breeding behavior of female voles (Ronkainen and Ylönen 1994). Hence, it may break the cycle of continuous reproduction. In our data, simulated risk of predation resulted in increased litter intervals. As gestation length in the common vole is fairly constant, we interpret this outcome as a result of reduced estrus frequency. The difference in behavior of estrous and anestrous females indicates that the former are at a higher risk of predation (Cushing 1985). Furthermore, urine of estrous prey attracts mustelid predators (Cushing 1984). Delaying estrus would therefore increase the chances of female's survival, thus constituting an adaptation to predation risk.

The effect of predation risk on estrous cycle was investigated by Koskela et al. (1996) in bank voles. Despite repeated exposures to a live weasel, estrus was observed in almost all

females during the 20-day study period. Compared to the control group, however, exposed females had fewer estrous cycles. Furthermore, some of the cycles were unusually long or altogether absent, while the cycles of remaining females were comparable with the control group. This result shows that females are affected differently. Analogically, increased litter intervals of some females in our study may have reflected a response to mustelid predation risk which was indirect, and which effected via delayed estruses.

According to the prevailing opinion, female voles challenged with predation risk either suppress breeding, or continue unaffected. Our evidence shows that breeding suppression is more complex. Predation risk not only renders some females reproductively inactive, but also affects those that maintain reproductive activity. We postulate that, instead of a binary response, predation risk may induce a wide range of effects. The responses of females may vary: from complete lack of reaction on one end of the scale (breeding at maximum frequency) to breeding suppression on the opposite end. In between there is room for intermediate responses, resulting in various estrus frequencies—and consequently, litter intervals.

The actual mechanism of breeding suppression in female voles has not yet been established. Speculations include physiological and behavioral responses. Certainly, predator odor changes breeding behavior of female bank voles (Ronkainen and Ylönen 1994). However, animal's behavior is often shaped by stress. In fact, ferret odor acts as a potent stressor in rats (Masini et al. 2005). In rats, acute stress affects levels of the primary sex hormone, estrogen (Shors et al. 1999). Additionally, induced increase of stress hormones results in asynchronous estruses (Pollard et al. 1975). Predatory stress could have a similar effect in voles, reducing females' receptivity. We cannot rule out that altered breeding behavior, as reported by Ronkainen and Ylönen (1994), stemmed from the disturbance of the estrous cycle. Delayed estrus is, potentially, the mechanism of breeding suppression. Whether it is an adaptation to predation risk, or a result of severe stress, is open to debate.

3.5 CONCLUSIONS

In this study, we investigated how predation risk affects the number of recruits per litter, and litter intervals. The first parameter was not affected; however, some females facing high risk of predation had less frequent litters. In breeding female common voles, longer litter intervals may constitute an adaptation to predation risk, or indicate a stress response to a cue of predatory threat. A physiological link might exist between perceived predation risk and regulation of estrus. Predatory stress may delay estrus and, consequently, hold up mating; breeding suppression would ensue in case of long-lasting stimulus. Further studies are required to confirm whether estrous cycle is involved in this process.

Chapter 4

Voles in space: on the effects of predation risk on *Microtus arvalis* breeding females

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Abstract. Voles show an array of adaptations to predation risk. The chances of encountering a specialist, mustelid predator are best minimized by reducing mobility and limiting the use of space. Potentially, voles may also shift their activity in time. We inquired whether, given their role in breeding, reproductively active females employ these adaptations.

We used capture location data and radio-tracking to examine long-term (home range) and short-term (activity range) space use under odor-simulated mustelid predation risk. The areas of the ranges were computed with two methods: minimum convex polygon (MCP) and local convex hull (LoCoH). Using radio-tracking data, we also measured movement distances and distances to the sources of predator odor. Our study species, the common vole (*Microtus arvalis*) is predominantly diurnal, with activity peaks at dusk and dawn. Using two indexes of circadian activity, we investigated whether the dams change their temporal behavior in response to mustelid odors. Non-predator odors served as controls.

We found no effect of mustelid predation risk on the size of the home range and activity range. Additionally, predation risk did not affect movement distances and temporal behavior. However, the females clearly avoided the sources of mustelid odor. We argue that reproductively active females cannot afford spatial and temporal adaptations, as these limit foraging opportunities and ultimately, female fecundity. Yet, avoidance of ferret odor confirms its significance for breeding female voles.

Key words: home range, activity range, circadian activity, predation risk, anti-predatory adaptation, reproduction, *Microtus arvalis*

4.1 INTRODUCTION

Different prey organisms face various levels of predator pressure. Voles, for that matter, are under constant predatory threat (Halle 1993). Vegetation cover provides a refuge from avian predators, but it offers no protection against carnivores, especially mustelids (e.g. weasels, stoats and polecats). Hence, voles are particularly vulnerable to mustelid predation.

Voles show various responses to mustelid predation risk, of which predator avoidance is possibly the most basic. Successful avoidance means that the chances of an encounter with a predator are minimized, which is best achieved by limiting one's spatial activity. In fact, foraging ranges of voles shrink when risk of predation increases; this effect was found in several species: field voles (*Microtus agrestis*; Borowski and Owadowska 2010), root voles (*Microtus oeconomus*; Borowski 1998b), and southern red-backed voles (*Myodes gapperi*; Anderson 1986). The decrease of the activity range results from the reduction of mobility, because the former is a function of the latter (Madison 1985). In *Microtus* voles, individuals

covering longer distances are more likely to be killed by the predator; in turn, a reduction of predation risk increases vole mobility (Norrdahl and Korpimäki 1998). Thus, restricted mobility constitutes voles' basic adaptation to mustelid predation, also demonstrated in field voles and common voles from Orkney Islands (*Microtus arvalis orcadensis*; Gorman 1984).

Adaptations to predation risk may not be equally accessible to both sexes. Females are under higher pressure from mustelid predators than males (Norrdahl and Korpimäki 1998). Therefore, females may need to go to greater lengths to efficiently avoid predation. However, this difference may reach even further—vole's response to predation risk may be determined by its reproductive status. This was shown by Jedrzejewski and Jedrzejewska (1990): when challenged with a weasel or its odors, reproductively active females remained within their ranges, whereas inactive females and males fled. Due to their role in production of offspring, breeding females are in a difficult situation. Cushing (1985) demonstrated in prairie deer mice (*Peromyscus maniculatus bairdii*) that females in estrus attract weasels—predators specializing in rodents of the *Cricetidae* family. Being so conspicuous, and hence, more vulnerable to predation, female voles have developed a complex adaptation: the predator-induced breeding suppression (Ylönen and Ronkainen 1994). In our earlier work, we verified the occurrence of breeding suppression in common voles (chapter 2), as well as explored the effects of predation risk on breeding females (chapter 3). In this paper, we investigate whether reproductively active females employ more conventional adaptations to predation risk, such as reduction of spatial activity.

Predator evasion may take place not only in space, but also in time. Some indication exists that voles can shift their circadian activity to the time of day when the prevailing predator is least active (Eccard et al. 2008). However, this response could depend on many factors, including the usual modes of activity of prey and predators, as well as on habitat properties (e.g. availability of cover). Our study species, the common vole (*Microtus arvalis*) is predominantly diurnal, with intensive activity at dusk and dawn (Lehmann and Sommersberg 1980). In addition to the use of space, we investigated whether this mode of circadian activity changes in response to mustelid odors.

In this study, we used capture location data to verify if female common voles adjust their long-term space use (home ranges) under odor-simulated mustelid predation risk. Further, we examined short-term space use (activity ranges and relocation distances) of reproductively active females with radio-tracking. The sizes of the long-term and short-term ranges were computed with two methods: the more common, minimum convex polygon (MCP), and the more accurate, local convex hull (LoCoH). Using the radio-tracking data, we tested whether reproductively active females avoid mustelid odor (mean distance to the odor source) and

whether the odor affects the dams' temporal behavior (mode of circadian activity).

4.2 METHODS

4.2.1 General setup

The work presented here was completed in Remderoda field station near Jena, central Germany (50.938 N, 11.529 E). During the breeding seasons of 2007 and 2008, we carried out an intensive capture-mark-recapture (CMR) experiment focusing on the effects of increased predation risk on vole reproduction (chapters 2 and 3). Capture location data served to estimate the sizes of long-term home ranges. In addition to the CMR experiment, we also carried out a radio-tracking study on spatial and diel activity of reproductively active females. The setup of the CMR experiment was described in greater detail in chapter 2; below, we provide a brief summary.

Each year, we monitored six separate populations of common voles; every population was enclosed in 50 by 50 m outdoor plot. To allow future identification, adult individuals were marked at first capture passive integrated transponders (PIT, Trovan). We trapped the voles using 25 live traps, placed 10 m apart on a regular square grid within each plot. We checked the traps twice a day, on two days a week. The trapping campaign was carried out from the beginning of the summer until mid-autumn. This period was divided into two phases: during the first phase, later referred to as the monitoring phase, the level of ambient predation risk remained unchanged in all plots; during the second phase, the treatment phase, we manipulated the perceived risk of predation. In 2007, the monitoring phase was 12 weeks long, while in 2008 it was shorter by 5 weeks. The treatment phase spanned at least 12 weeks in both years.

We simulated different levels of predation risk using animal and non-animal odors. The odors, in form of water extracts, were distributed in equal amounts between 16 permanent odor sources per plot. The odor sources were refilled every 2 days, for the total of 3 applications per week, throughout the whole treatment phase. Each odor source was located in the center of a square demarcated by the four adjacent traps. The arrangement of traps and odor sources was identical in each plot. Each of the six plots was pre-assigned to a level of predation risk; this assignment alternated between the two years. In three out of six plots, we simulated high predation risk with fecal odors of captive ferrets (*Mustela putorius furo*). These plots are hereafter referred to as the predator treatment. Remaining three plots served as reference and control for the effect of novelty. Those plots received either fecal odors of captive rabbits (*Oryctolagus cuniculus*), thus constituting the herbivore treatment, or the odor of

clean sawdust cage bedding (the neutral treatment). We assumed that neither of the two control odors would affect the level of predation risk as perceived by the voles. Whenever this assumption was met, we treated the herbivore and neutral treatments collectively as the non-predator treatment.

4.2.2 Capture location data

We used the capture records of the CMR experiment to estimate the effect of predation risk on females' home ranges. The minimum number of captures necessary to reliably estimate the home range of *M. arvalis* equals 7–8 (Reichstein 1960). Hence, we limited the calculations of home range area to females captured at least eight times in a given phase, regardless of their reproductive status at capture. To facilitate the computation of home range areas, we added generated data to each individual dataset. The number of added observations was equal to the number of actual observations; the added observations were randomly scattered within $\pm$ 0.5 m relative of the original barycenter, thus simulating the center of activity. In case of individuals captured in the same trap more than once, the overlapping coordinates were randomly shifted within $\pm$ 0.5 m relative of the trap location.

We calculated the areas of individual home ranges using two methods: adaptive Local Convex Hull (Getz et al. 2007), returning the area bounded by an isopleth containing 95% of observations (LoCoH 95%), and Minimum Convex Polygon, returning the area generated excluding the outermost 5% of observations (MCP 95%). Despite its poor precision (Burgman and Fox 2003, Börger et al. 2006), we included the MCP method for the sake of comparability with earlier studies. In the LoCoH 95% calculations, the applicable argument was set to 30 for all individuals. Prior to statistical analysis, the areas obtained with both methods were square-root transformed to approximate normal distribution of the data.

4.2.3 Radio-tracking

The radio-tracking study was carried out in August 2008. It comprised two 48 h sessions. The sessions started at 20:00 CEST. The first session (1–3 August) was conducted in the last three days of the monitoring phase—before the beginning of odor application. The second session (15–17 August) was held two weeks into the treatment phase—after the odors had been applied six times. This study included only resident, reproductively active females, of which all had given birth at least once. We chose 3–4 females from each of the five study populations; the sixth, least populated plot did not contain enough females meeting the selection criteria. The females were captured 48–72 h prior to the beginning of each

session, equipped with radio-collars (Pip, Biotrack), and released in place of capture. We followed 18 females in each session, equally divided between the predator, herbivore and neutral treatments.

The females were located manually at 30-minute intervals using Yaesu FT-290RII 2m receivers (Yaesu Musen Co., Ltd.), fitted with 5 m long handheld antennas. The antennas consisted of a screened coaxial cable with an exposed tip, mounted onto a fishing rod. Sufficient length of the antennas allowed us to minimize the disturbance of the voles' habitat. Fixes were recorded on a Cartesian grid with a resolution of 1 m.

Activity range area

Areas of the 48 h activity range were calculated using the same methods applied earlier to the capture location data. Each area was computed based on 96 observations at maximum. Adaptive LoCoH 95% argument (Getz et al. 2007) was set individually to the maximum distance between any two given fixes within session. The individual per-session estimates were square-root transformed prior to analysis.

Mean distance to odor source

For every female, separately for each session, we identified a 'core area' (a square two by two meters, comprising 9 possible locations) containing most of the fixes. We assumed that the core area contained the nest; the fixes falling within the core area were excluded from the calculations. For each of the remaining fixes, we calculated the Euclidean distance the nearest odor source (DOS). Due to the arrangement of odor sources in an array, the possible values of DOS were limited at 0 (at odor source) and $\sim$7.07 m (at trap, halfway between two diagonally adjacent odor sources). The theoretical mean value of DOS equaled $\sim$3.85 m.

Mean relocation distance

We calculated the Euclidean distances between subsequent locations of the females. Fixes falling within a square two by two meters relative to the previous fix were ignored. We assumed that these relocations were either below the resolution of our radio-tracking method, or that they were due to the female's movements in the nest. Relocations greater than $\sim$2.82 m were grouped according to the time of day. The 'time of day' factor contained three levels: day, night, and twilight (dusk and dawn). The twilight grouping included relocations nearest to the time of sunrise or sunset, plus the ones occurring within $\pm$ 30 minutes; out of 48 individual relocations possible in a diel cycle, the twilight grouping contained up to six. Remaining

relocations were attributed to either daytime or nighttime. The length of day differed between the radio-tracking sessions by approximately 1 hour, so the number of possible relocations in the daytime or nighttime categories varied accordingly.

Crepuscularity and diurnality indexes

We used the relocation distances (grouped by time of day) to compute two individual indexes of diel activity: crepuscularity index I_C, and diurnality index I_D (Halle and Lehmann 1987, Halle 1995). Positive values of I_C show that the animal's activity is increased at twilight, relatively to the remaining part of the diel cycle; negative values show the opposite. The second index compares daytime activity with nighttime activity, excluding relocations occurring at twilight. Positive values indicate a relative increase in the former, and vice versa. The numbers of observations were corrected for each individual to account for missing data.

4.2.4 Statistical analysis

Statistical analysis and all computations were carried out in R, version 2.12.1 (R Development Core Team 2010). Areas of activity and home ranges were calculated using the package *adehabitat*, version 1.8.3 (Calenge 2006). Statistical analysis employed linear mixed-effects models (LMM) implemented in the *lme4* package, version 0.999375-37 (Bates and Maechler 2010). We included a random intercept for plot ID in models fitted to capture location data. All models based on the radio-tracking data included a random intercept for female ID and plot ID. Significance of model terms was estimated using likelihood ratio tests (LR) in the course of backward model selection.

In the analyses, 'phase' factor corresponded to the monitoring and treatment phases of the CMR experiment (capture location data); in the radio-tracking experiment, 'session' factor corresponded to particular sessions. Assignments of the plots to the treatments ('a priori' groupings hereafter) were identified as levels of the 'treatment' factor. As such, this differentiation was only effective when predation risk was manipulated—in the second radio-tracking session, as well as in the treatment phase of the CMR experiment. To test for the effect of increased predation risk, we estimated the significance of treatment $\times$ session interaction (or treatment $\times$ phase interaction in case of capture location data). Where additional factors (e.g., year or time of day) were considered, we tested for the significance of applicable three-way interactions.

4.3 RESULTS

4.3.1 Capture location data

The total number of female captures equaled 6155. The mean number of individual captures in the monitoring phase 2007 equaled 15.8 (median = 14; maximum = 32), while in the treatment phase it equaled 18.0 (median = 15.5; maximum = 45). The respective figures for 2008 came to 9.9 (median = 9; maximum = 16) and 17.9 (median = 16; maximum = 39). In total, the dataset afforded 359 observations of home range area. As much as 40% of the observations were paired: 67 out of 292 females were present in both phases of the experiment. In 2007, as obtained with the MCP 95% method, there were 83 observations in the monitoring phase and 148 observations in the treatment phase. In 2008, the observations amounted to 12 and 82, respectively. Relatively to the MCP 95%, the LoCoH 95% algorithm failed to compute 34 out of 359 observations. Of those, 30 occurred in the treatment phase of 2007, while 2 occurred in the monitoring phases of both years.

Home range area

Home range areas calculated with the LoCoH 95% method are shown in figure 4.1. We did not find significant differences between herbivore and neutral groupings (LR: χ^2 = 9.34 at 4 df, p = 0.053); hence, we merged them into one (non-predator) for further analysis. The year-dependent effect of predator treatment (treatment $\times$ phase $\times$ year interaction) was non-

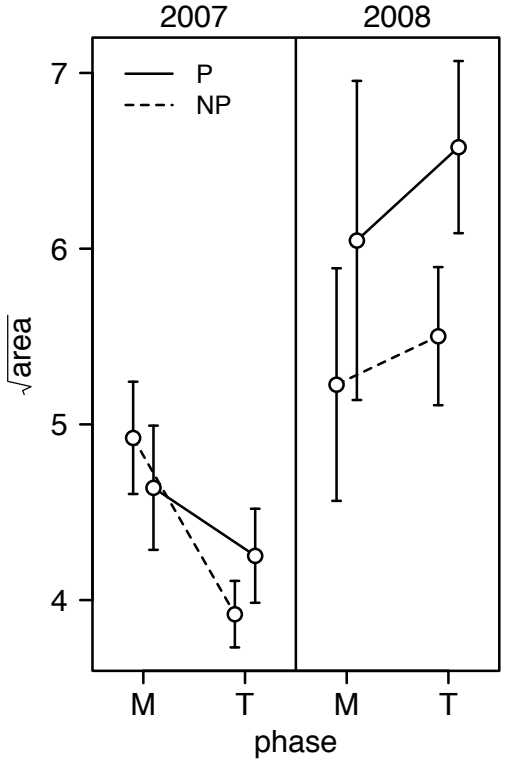

FIGURE 4.1: Square-root transformed home range areas (LoCoH 95%) of female common voles, computed from capture location data. Connected dots indicate model estimates of the means; whiskers indicate $\pm$ 1 SE of the mean. Significance of model terms is given in the text. A priori groupings: P – predator; NP – non-predator. Phases of the CMR experiment: M – monitoring; T – treatment.

significant (LR: χ^2 = 0.046 at 1 df, p = 0.83). So was the interaction between the a priori grouping and year (treatment × year; LR: χ^2 = 0.71 at 1 df, p = 0.40). Our main interest, the effect of predator treatment, was non-significant (treatment × phase interaction; LR: χ^2 = 1.28 at 1 df, p = 0.26). Likewise, the phase × year interaction was non-significant (LR: χ^2 = 2.09 at 1 df, p = 0.15). The difference between the phases was marginally non-significant (LR: χ^2 = 3.48 at 1 df, p = 0.062). The differences between the a priori groupings were significant (LR: χ^2 = 4.01 at 1 df, p = 0.045). Finally, the year effect was very highly significant (LR: χ^2 = 34.72 at 1 df, p < 0.001).

Areas calculated with the MCP 95% method displayed a similar pattern, but the estimates were consistently higher than those obtained with the LoCoH 95% method. Merging of the two non-predator treatments was justified (LR: χ^2 = 7.65 at 4 df, p = 0.11). There was no significant year-dependent effect of predator treatment (treatment × phase × year interaction; LR: χ^2 = 0.67 at 1 df, p = 0.41). The interaction of the a priori grouping and year (treatment × year) was non-significant as well (LR: χ^2 = 0.19 at 1 df, p = 0.66). Again, the effect of predator treatment (treatment × phase interaction) was not significant (LR: χ^2 = 0.68 at 1 df, p = 0.41). There were no significant differences between the a priori groupings (LR: χ^2 = 2.24 at 1 df, p = 0.13). As estimated by the simplified model, mean square root of home range area in the monitoring phase (2007) equaled 7.96 ± 0.393 (SE), while in the treatment phase it decreased to 5.86 ± 0.306. This pattern was reversed in 2008: the estimates equaled 9.34 ± 1.260 and 9.69 ± 0.488, respectively. The phase × year interaction was marginally non-significant (LR: χ^2 = 3.79 at 1 df, p = 0.052). Both phase effect (LR: χ^2 = 12.321 at 1 df, p < 0.001) and year effect (LR: χ^2 = 47.006 at 1 df, p < 0.001) were very highly significant.

4.3.2 Radio-tracking

We started each session with 18 females. During the first session, we lost track of one. Of 17 females followed in the first session, 14 were recaptured for the second session. We replaced the four missing females with similar ones from respective plots. In the second session, we lost track of two females, both of which were successfully tracked in the first session. The losses during sessions were either due to malfunction or loss of a radio-collar. Additionally, we have failed to detect 17.7% of the fixes in the first session (individual range 6%–42%) and 2.6% in the second session (individual range 0%–8%). Ultimately, the radio-tracking study supplied two datasets: a full one and a paired one. The full dataset contained all radio-tracked females, including those present in one session only. The paired dataset was restricted to the 12 individuals present in both sessions (4 females per treatment), allowing pairwise comparisons. We indicate which dataset was used for the calculations.

Activity range area

We calculated the activity range areas using the full dataset. We present the LoCoH 95% estimates in figure 4.2. We found no significant differences between the two non-predator groupings (LR: χ^2 = 5.12 at 2 df, p = 0.077), and merged them into one. Predator treatment (treatment × session interaction) had no effect (LR: χ^2 = 0.22 at 1 df, p = 0.64). Additionally, there were no differences between the radio-tracking sessions (LR: χ^2 = 1.08 at 1 df, p = 0.30) or a priori groupings (LR: χ^2 = 0.035 at 1 df, p = 0.85).

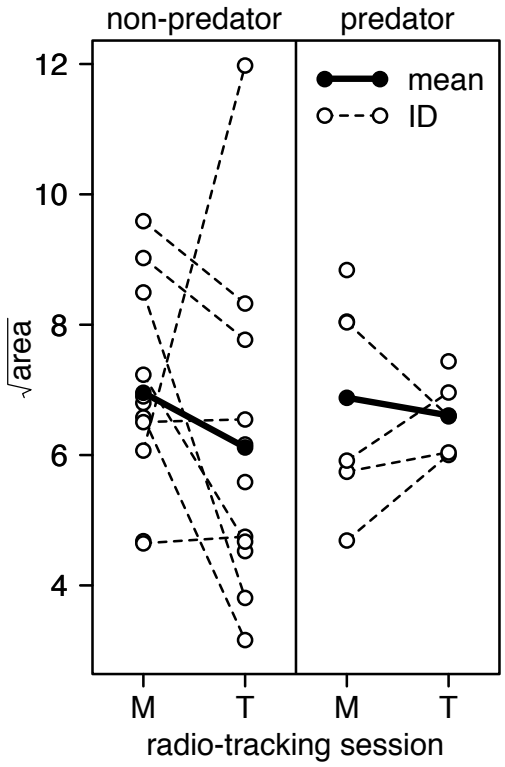

FIGURE 4.2: Square-root transformed activity range areas (LoCoH 95%) of reproductively active females, computed from radio-tracking data (full dataset). Connected dots indicate model estimates for females present in both sessions (dashed lines; ID), and of session means (solid lines). Significance of model terms is given in the text. Radio-tracking sessions: M – monitoring; T – treatment.

The course of the analysis of the MCP 95% output was analogous. There were no significant differences between the two non-predator groupings (LR: χ^2 = 5.95 at 2 df, p = 0.051), thus justifying the merging. Again, predator treatment (treatment × session interaction) had no effect (LR: χ^2 = 0.25 at 1 df, p = 0.62). Furthermore, there were no differences between the radio-tracking sessions (LR: χ^2 = 0.09 at 1 df, p = 0.76) or a priori groupings (LR: χ^2 = 0.07 at 1 df, p = 0.79). The MCP 95% estimate (mean square root of activity range area) for the non-predator treatment equaled 9.57 ± 0.606 (SE) in the first session and 9.63 ± 1.209 in the second session. In predator treatment, the respective values equaled 10.17 ± 1.153 and 9.28 ± 0.637.

Capture location data vs. radio-tracking

For 15 females (of which 5 were present in both phases and sessions), we estimated both the home range (capture locations from 2008) and activity range (radio-tracking). For the MCP 95% method, the individual ratio of 'capture location' area to 'radio-tracking' area fell within the range 0.16-2.48 (mean 1.18)—except for one individual, where 'capture location' estimate was almost 4 times greater than the 'radio-tracking' estimate. The ratios for the LoCoH 95% method ranged within 0.17-1.56 (mean 0.76); here, the exceptionally high ratio equaled 6.70.

Mean distance to odor source

We calculated the mean distance to the nearest odor source (DOS) using the paired dataset. The proportion of fixes contained within the core area amounted to 41% in the first session and 47% in the second session; these locations were excluded from the analysis. Individual and mean estimates of DOS are shown in figure 4.3. We found no significant differences between the two non-predator groupings (LR: χ^2 = 0.07 at 2 df, p = 0.97). Hence, a merged grouping was used in further analysis. In the second session, DOS was significantly higher in

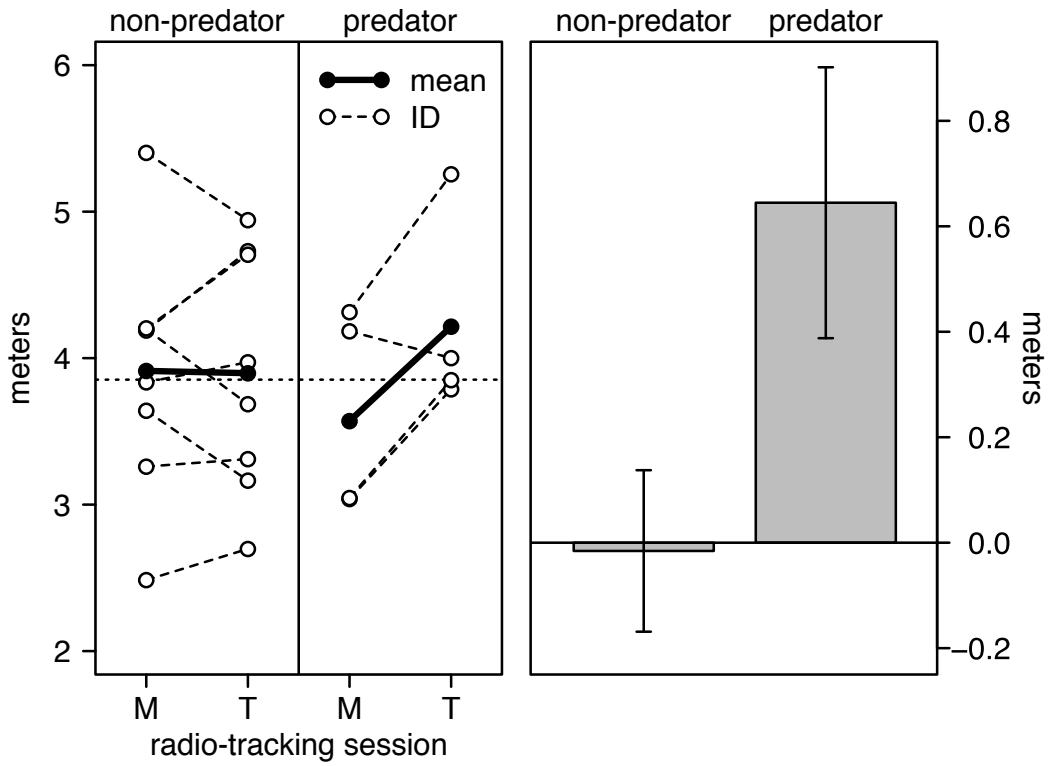

FIGURE 4.3: *Left panel:* Mean distance to the odor source (DOS), calculated with the paired dataset. Connected dots indicate model estimates for the individual (dashed lines; ID) and mean DOS (solid lines). Horizontal dashed line marks the theoretical mean value of DOS. Radio-tracking sessions: M – monitoring; T – treatment. *Right panel:* Change in the individual DOS between the radio-tracking sessions. Bars indicate the mean; whiskers indicate ± 1 SE of the mean. Significance of model terms is given in the text.

the predator treatment (treatment × session interaction; LR: χ^2 = 9.06 at 1 df, p = 0.0026).

Mean relocation distance

We estimated the relocation distances from the paired dataset. The total number of observations exceeding 2.82 m amounted to 628, obtained over two 48 h sessions from 12 individuals in three groups. 63% of the observations were collected during the day, 12% at twilight, and 25% at night. Estimates of the mean relocations are shown in figure 4.4. Due to significant differences, the distinction between herbivore and neutral groupings was retained. With regard to the time of day, the effect of the predator treatment was not significant (treatment × session × time of day interaction; LR: χ^2 = 3.44 at 4 df, p = 0.49). Likewise, the sole effect of predator treatment (regardless of time of day) was not significant (treatment × session interaction; LR: χ^2 = 5.07 at 2 df, p = 0.079). The remaining two-way interactions were non-significant: a priori grouping × time of day (LR: χ^2 = 1.28 at 4 df, p = 0.87), as well as session × time of day (LR: χ^2 = 1.63 at 2 df, p = 0.44). Time of day as a simple effect was not significant (LR: χ^2 = 1.28 at 2 df, p = 0.53). Similarly, there were no differences between the sessions (LR: χ^2 = 0.73 at 1 df, p = 0.39). Finally, the differences between a priori groupings were significant (LR: χ^2 = 6.79 at 2 df, p = 0.033).

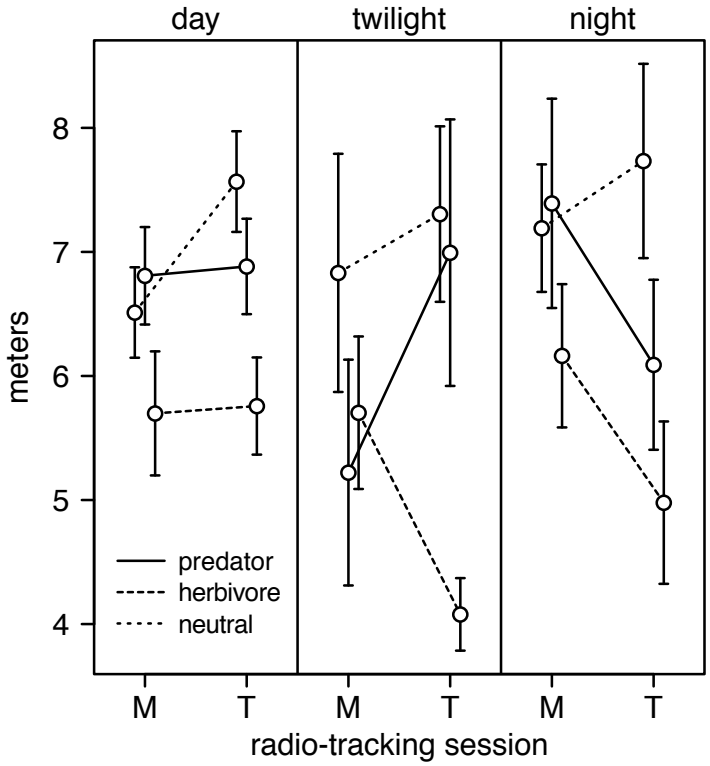

FIGURE 4.4: Mean relocation distance (paired dataset). Connected dots indicate model estimates of the means; whiskers indicate ± 1 SE of the mean. Labels at the top correspond to the levels of the 'time of day' factor. Significance of model terms is given in the text. Radio-tracking sessions: M – monitoring; T – treatment.

Crepuscularity and diurnality indexes

Both I_C and I_D were calculated from the paired data. Due to the lack of observations in the second session at twilight, two individuals (one in each of the predator and neutral treatments) were excluded from the calculation of I_C. Estimates of the I_C model are shown in figure 4.5. Merging of the two non-predator groupings was justified (LR: χ^2 = 0.44 at 2 df, p = 0.80). The effect of predator treatment (treatment × session interaction) was not significant (LR: χ^2 = 0.19 at 1 df, p = 0.67). Lack of significance was found for both of the simple effects: session (LR: χ^2 = 0.01 at 1 df, p = 0.92), and a priori grouping (LR: χ^2 = 0.01 at 1 df, p = 0.92). Estimates of the I_D model are shown in figure 4.6. Merging of the two non-predator groupings was justified (LR: χ^2 = 4.43 at 2 df, p = 0.11). Again, the effect of predator treatment (treatment × session) was not significant (LR: χ^2 = 0.62 at 1 df, p = 0.43). So was the a priori grouping (LR: χ^2 = 1.09 at 1 df, p = 0.30). The increase of I_D between sessions was highly significant (LR: χ^2 = 7.48 at 1 df, p = 0.0063).

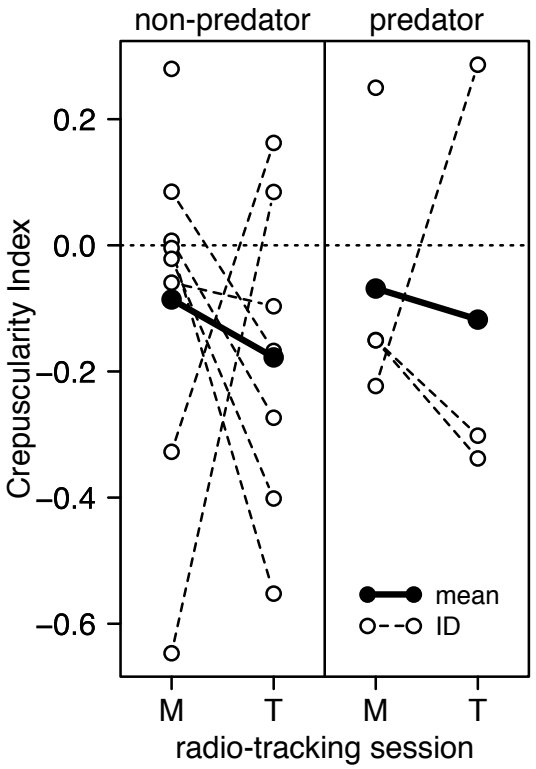

FIGURE 4.5: Crepuscularity index (I_C; paired dataset). Connected dots indicate model estimates for the individual (dashed lines; ID) and mean I_C (solid lines). No movements were recorded in two females (one in each treatment) during twilight periods of the second session (stray dots). Horizontal dashed line marks equal activity during twilight and the rest of the diel cycle. Significance of model terms is given in the text. Radio-tracking sessions: M – monitoring; T – treatment.

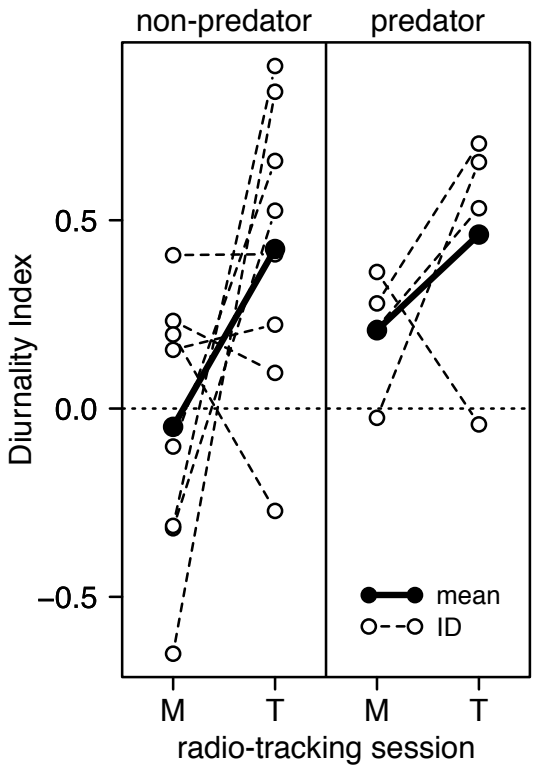

FIGURE 4.6: Diurnality index (I_D; paired dataset). Connected dots indicate model estimates for the individual (dashed lines; ID) and mean I_D (solid lines). Horizontal dashed line marks equal activity during day and night. Significance of model terms is given in the text. Radio-tracking sessions: M – monitoring; T – treatment.

4.4 DISCUSSION

4.4.1 Home range area

Predation risk did not affect home range size, as estimated with both MCP 95% and LoCoH 95% methods. The estimates of the former were clearly higher than those of the latter, but this difference was as expected. MCP overestimates home range size (Burgman and Fox 2003, Börger et al. 2006), while LoCoH is more accurate (Getz and Wilmers 2004, Huck et al. 2008). Regardless of the computational method, home range data revealed substantial individual variability. Still, the estimates of both methods revealed an evident year effect, as well as a noticeable effect of the experiment phase. Additionally, we encountered differences between the a priori groupings in the LoCoH 95% dataset, which may have resulted from high individual variability, or low resolution of capture location data.

Home range size in microtines is inversely related to population density (Madison 1985). Apparently, the pattern of home range areas (figure 4.1) corresponded to the changes of population densities revealed by the CMR experiment (chapter 2). Despite identical initial size, the study populations in 2007 grew faster than in 2008, producing much higher densities in the first year; the difference between the years was most obvious in the treatment phase. Thus, changes in density of the studied populations would explain the year effect and the difference between the phases. Changing density also resulted in an unbalanced number of observations. The first year yielded more data points, with a larger proportion of young individuals. Since newer recruits have smaller home ranges (Mazurkiewicz 1971), numerous individual observations at higher densities may have decreased the means. In the second year, with low to moderate densities, we obtained relatively few observations; females with large home ranges may have increased the overall means.

4.4.2 Activity range area

Analogically to capture location data, radio-tracking MCP estimates were consistently higher than LoCoH estimates; the same was true with regard to the variability of the estimates. Irrespective of the method, we found no effect of the predator treatment on the size of activity ranges in reproductively active females. Lack of difference between sessions showed that the sole manipulation of predation risk with odors had no effect, either. Instead, we found considerable variation between individuals under equal conditions. Furthermore, in females with paired observations, the size of activity ranges varied randomly between sessions. This indicates that short-term activity ranges are very labile, which is in agreement with conclusions of Madison (1985). It also shows that activity ranges of reproductively active

females are shaped by factors other than mustelid predation risk (e.g., population density, vegetation cover), which were not controlled for in our study.

We think that the lack of effect of mustelid odors on space use is attributable to the reproductive status of the voles. All of the radio-tracked females were in breeding condition. Lactating and pregnant females have a very high demand for energy: it exceeds the demand of non-breeding females by 133% and 32%, respectively (Migula 1969). Even though voles in general adapt to mustelid predation risk by reducing spatial activity (Gorman 1984, Borowski 1998b, Borowski and Owadowska 2010) and foraging (Bolbroe et al. 2000), this adaptation might not be feasible for breeding females. Reduction of foraging ranges could limit their food intake and consequentially, negatively affect their fitness. Furthermore, it could give more leeway to intraspecific competitors. It seems that such costs of reduced predation risk are too high for the breeding females. This conclusion is supported by the fact that upon exposure to a mustelid predator, breeding females do not abandon their ranges, contrary to voles with a different reproductive status (e.g., inactive individuals or males; Jedrzejewski and Jedrzejewska 1990). Sexual dimorphism in anti-predatory reactions would also explain why mustelid predators kill more females (Norrdahl and Korpimäki 1998), while avian predators kill more males (Halle 1988).

4.4.3 Capture location data vs. radio-tracking

The average size of the activity ranges and the home ranges of 2008 were comparable. For the 15 radio-tracked females that also provided capture location data, the mean activity range was smaller than the mean home range when estimated with MCP method; this relation was reversed when the LoCoH method was applied. The individual proportions varied greatly in both instances, but less so for the LoCoH estimates. Madison (1985) predicted that long-term ranges overestimate space use. The home range constitutes of locations visited over an extended period, becoming a union of discrete activity ranges enclosed within the polygon marked by the outermost locations. It seems that the estimates obtained with MCP reflect this difference. In contrast, the output of the LoCoH method is restricted to locations where the animal had actually been trapped. Avoided traps are excluded, often resulting in oblong, ragged or non-convex ranges. Furthermore, spatial resolution of radio-tracking data was 100 times better than the resolution of capture location data. Generated radio-tracking ranges possibly included areas that would have remained undetected with capture location data, explaining why in the LoCoH analysis, the mean activity ranges were larger.

4.4.4 Mean distance to odor source

The difference in mean distance to odor source (DOS) between the monitoring and treatment sessions indicated a repelling effect of ferret odor. In the predator treatment, the increase of DOS was observed in 3 out of 4 females. In the non-predator treatment, the females were not affected by the introduction of odors: Between the sessions, the individual means of DOS varied randomly, but the overall mean remained virtually unchanged. Furthermore, the overall mean of DOS approximated the theoretical mean, reflecting a random distribution of females in relation to the source of odor. The values of DOS were strictly bound at 0 m and $\sim$7 m; the extremes of this scale reflected an unrealistic situation where a vole remained at one location (at odor source or at trap, respectively) throughout the radio-tracking session. Taking that into account, the effect size of 0.65 m clearly indicates that reproductively active female *M. arvalis* avoid ferret odors. Since we had relatively few females in the predator treatment, this result should be reinforced with a larger sample.

Our result is in accord with existing evidence. Gorman (1984) demonstrated that common voles and field voles were repelled by stoat anal gland scent, both in laboratory trials (scented paper sheets) and in the field (soiled traps). Such response may be species-specific: the distance to odor source was not affected in wild root voles (Borowski 1998b). Furthermore, avoidance may occur under laboratory conditions but not in the field, as shown by Parsons and Bondrup-Nielsen (1996) in meadow voles (*Microtus pennsylvanicus*). Despite the general evidence for avoidance of predator odor, the role of voles' reproductive condition remains unclear. Regardless of their reproductive status, bank voles (*Myodes glareolus*) avoided pens visited by a weasel; breeding females responded differently only when weasel entered their vicinity (Jedrzejewski and Jedrzejewska 1990). We suppose that, in contrast to activity ranges, avoidance of predator odor is independent of reproductive status. Further, our result confirms the significance of ferret odor in breeding females. This odor is avoided, but need not limit the use of space.

4.4.5 Mean relocation distance

The distances traveled by females were independent of the time of day, neither alone, nor in interaction with predation risk. *Microtus arvalis* is active throughout the diel cycle, with periods of high mobility at dusk and dawn (Lehmann and Sommersberg 1980). Interestingly, we found no indication of this pattern, regardless of other factors. This may be due to the relatively poor temporal resolution of radio-tracking. Given the 30 min fix interval and the 2.82 m distance cut-off, the distribution of resulting movements throughout the sessions

was random and relatively sparse. Containing the fewest observations, the twilight phase was burdened with highest error; higher frequency of fixes may have revealed the fine crepuscular pattern. Instead, we encountered substantial individual variability. It was reflected in the significance of a priori groupings, particularly that each grouping contained only four individuals.

Radio-tracking studies have shown that in *Microtus*, mobility is decreased in response to predation risk (Borowski 1998b, Borowski and Owadowska 2010); reduction of the risk evokes an increase of mobility (Norrdahl and Korpimäki 1998). However, no clear differences with regard to reproductive status were demonstrated. Since movement distances and activity ranges are closely related, the arguments provided in the discussion of activity ranges also apply here. We suppose that reproductively active females roam their ranges in search for food. Disregarding the risk of predation, they remain very mobile. Since very mobile voles are more likely to be killed by mustelid predators (Norrdahl and Korpimäki 1998), a lack of response of reproductively females to mustelid predation risk would explain why carnivorous predators kill more females than males—as demonstrated by Norrdahl and Korpimäki.

4.4.6 Crepuscularity and diurnality indexes

The two indexes of circadian activity, calculated from the movement distances, showed no effect of ferret odors. The individual changes of both I_C and I_D appeared random, and varied greatly between the sessions. On average, the females seemed to be less active during the twilight phase, as indicated by I_C. This result is somewhat surprising, since peak activity of common voles usually takes place at twilight (Lehmann and Sommersberg 1980). Still, such outcome may have resulted from a short twilight window with infrequent fixes, yielding few movement observations. I_D revealed that the females were more diurnal than nocturnal, with pronounced diurnality in the treatment session. The prominent shift to diurnality in some of the radio-tracked females may be resultant of the random nature of recorded movements. However, given better detection of vole locations in the second session (98% versus 82%), the increased diurnality may, in fact, reflect the activity pattern typical of common voles.

Although voles may, under certain circumstances, response to predation risk with a shift of the diel activity (e.g., Eccard et al. 2008), it is generally agreed that circadian rhythms are independent of predation risk. Voles have to face constantly high risk of predation, from one type of predator or another (Halle 1993). Instead of a flexible circadian rhythm, voles developed a polyphasic pattern, consisting of bouts of foraging activity interlaced with periods of rest. As such, this type of activity is an adaptation to predation risk (Gerkema and Daan 1985, Reynolds and Gorman 1994). Due to high energetic demands, it would be impossible

for voles to entirely shift activity to a period when the predator is inactive. This applies to reproductively active females, especially.

4.5 CONCLUSIONS

Our evidence suggests that reproductively active females differ form voles with other reproductive statuses (e.g., males or inactive females) with regard to their spatial and temporal adaptations to predation risk. Specifically, reproductively active females show no response, neither adjusting their long- and short-term space use, nor shifting the mode of circadian activity. These adaptations are undesirable, as they would translate into limited foraging opportunities, leaving the energy demand of breeding females unsatisfied. If employed, such adaptations would ultimately curtail reproductive output and limit voles' fitness. Still, reproductively active females remain sensitive to predator odors—avoidance of ferret odor confirms its biological significance. To verify the lack of spatial and temporal adaptations, further studies should compare reproductively active females with inactive females. Larger groups and more frequent sampling should be used to account for high individual variability and uncertainty of vole movements.

Chapter 5

Neurological processing of cues of predation risk in common voles

As a tool for perception of environment, olfaction in mammals performs multiple functions (Eisenberg and Kleiman 1972); mediation of sexual selection may be its central role (Blaustein 1981). Small rodents use scents not only to find mates, but also to assess the risk of predation (see Introduction). The importance of olfaction for reproduction and for survival makes it indispensable for the perpetuation of species. On the level of central nervous system, the role of chemical cues in reproduction was revealed in a study using functional magnetic resonance imaging (fMRI; Toftegaard et al. 2002). Using the same method, I attempted to show how small rodents perceive chemical cues of predation risk.

Functional MRI is a non-invasive, *in vivo* method used primarily in medical diagnostics. It takes advantage of the diamagnetic properties of oxygenated hemoglobin; fMRI detects increased flow of oxygenated blood in certain areas of the brain, which is correlated with their activation (Ogawa et al. 1990). Activation of the brain is a result of stimulation, and the response to different stimuli may be compared. This method produces quantitative visual data. The obtained image is subsequently compared with the data acquired during absence of the stimulus (baseline), or with the data obtained for a different stimulus. A "map" of brain activation is provided as a final effect.

Toftegaard et al. successfully implemented this technique to study activation of the brain with an olfactory stimulus related to reproduction. The authors investigated the neurological response in a marsupial mouse, the brown antechinus (*Antechinus stuartii*), exposed to urinary pheromones of conspecifics (figure 5.1 on the following page). I intended to use fMRI to investigate the neurological processing of an interspecific olfactory signal, namely the odor of the predator. The scent of predator's urine, feces, fur, and glandular secretions simulates the presence of the predator in the environment, and constitutes a signal perceived by an individual of prey species. I planned to examine the patterns of brain activity of common voles

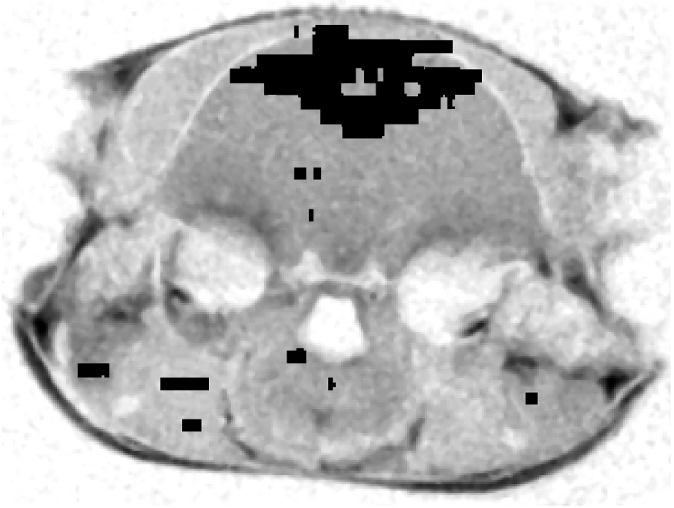

FIGURE 5.1: Anatomical image of the brain of a female *Antechinus stuartii* (background); functional response to male urine (overlay). Note the difference in resolution between anatomical and functional images. Adapted from Toftegaard et al. (2002).

(*Microtus arvalis*), typical prey of mustelid predators. As a cue of predation risk, I used the odor of domestic ferret (*Mustela putorius furo*). Odor of a herbivorous animal, the European rabbit (*Oryctolagus cuniculus*), and odor of clean cage bedding were used as controls for the effect of novelty, and as reference stimuli. In case a vole would perceive predator odor in a different way than control odors, the activation of the brain upon olfactory stimulation should be distinguishable. Given the differences between the sexes in behavioral or physiological responses to predator odor (e.g., breeding suppression), I also planned to compare the patterns of brain activity of female and male voles.

The initial trials were carried out in February 2007, in cooperation with the Medical Physics Group at the Institute of Diagnostic and Interventional Radiology I, Jena University Hospital. The MRI scanner, intended primarily for the use on humans, was equipped with a 3 Tesla magnet (figure 5.2); a rodent coil was added to adapt the scanner to voles (figure 5.3).

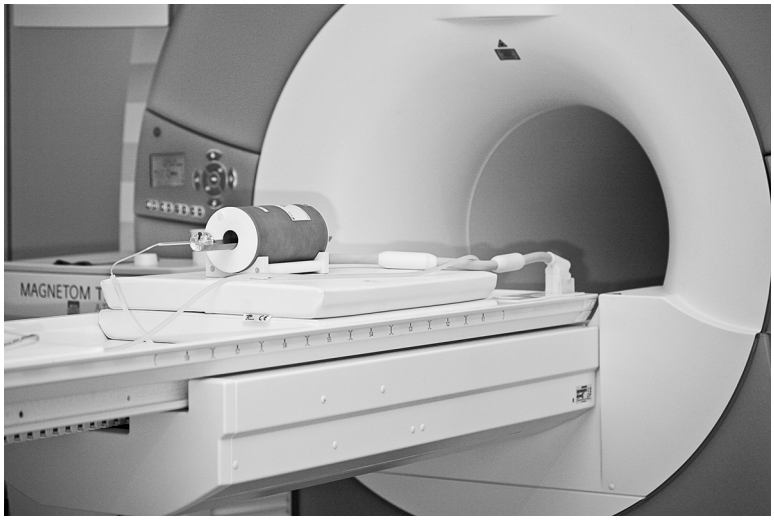

FIGURE 5.2: 3 Tesla scanner equipped with a rodent-dedicated coil (in the front). Institute of Diagnostic and Interventional Radiology I, Jena University Hospital.

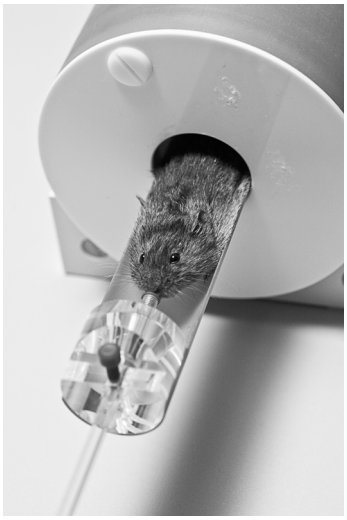

FIGURE 5.3: Anesthetized vole in the coil cradle.

We obtained good anatomical images of vole brains (figure 5.4), but the resolution provided by the 3 Tesla scanner was too low to detect a functional response.

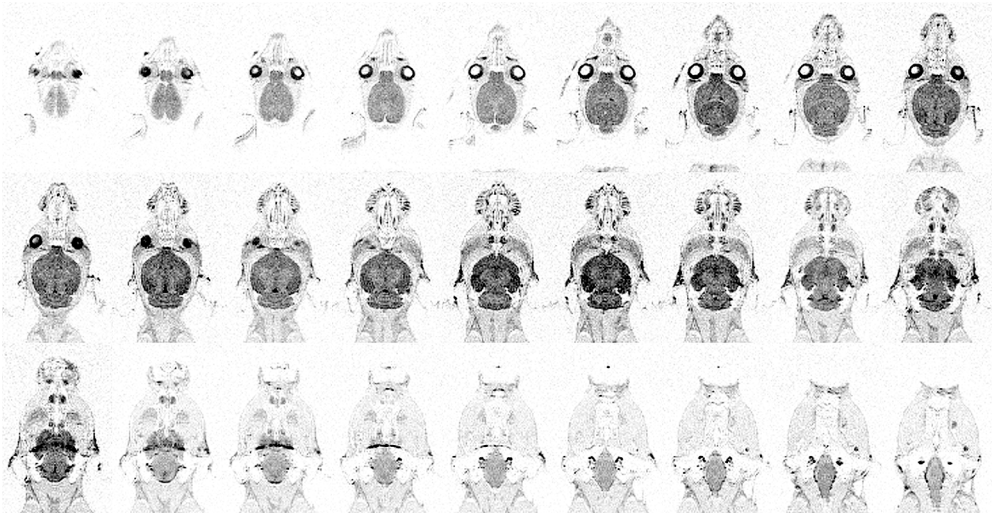

FIGURE 5.4: Anatomical images of a female common vole's head, obtained with a 3 Tesla scanner (coronal slices; 0.5 mm slice interval). Images were inverted for clarity.

The second trial was carried out in October 2007. The Non-invasive Brain Imaging laboratory at the Leibniz Institute for Neurobiology in Magdeburg kindly provided a rodent-dedicated 4.7 Tesla scanner (figure 5.5). This equipment had been used in functional studies on rats (e.g. Angenstein et al. 2007). Anatomical images of the cranial area (figure 5.6) obtained with this scanner were improved. However, the resolution of functional measurement was still too low to detect a signal above baseline.

FIGURE 5.5: Rodent-dedicated 4.7 Tesla scanner. Leibniz Institute for Neurobiology, Magdeburg.

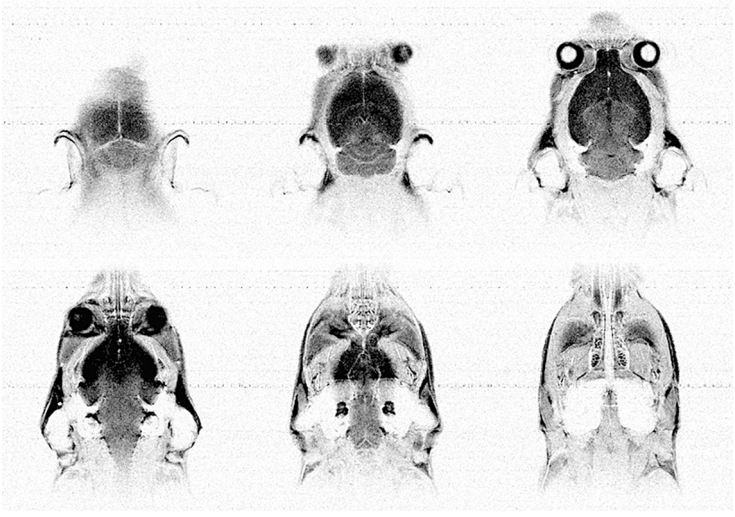

FIGURE 5.6: Anatomical images of a female common vole's head, obtained with a 4.7 Tesla scanner (coronal slices; 2 mm slice interval). Images were inverted for clarity.

The study of Toftegaard et al. shows that fMRI can successfully visualize the processing of biologically meaningful odors in small rodents. Nonetheless, olfactory stimulation in fMRI

studies poses some challenges. The brain of a brown antechinus is larger than that of a common vole; a smaller brain requires a higher resolution. Furthermore, odors of predators are probably milder stimuli than urinary pheromones. Hence, they may produce a weaker response.

The fMRI technique may allow us to directly measure the qualitative and quantitative response of prey to odors of predators. It has the capacity for confirming or denying the biological relevance of a given odor in a matter of several imaging sessions. Obtaining a comparable result in a behavioral bioassay may require an entire field season, and an immense amount of labor. In future studies on neurological processing of olfactory cues in rodents, the use of stronger magnets or contrast agents should provide the necessary increase of resolution. The patterns of brain activation may almost immediately provide an understanding of the mechanisms of behavioral and physiological responses of prey to predator odors.

Chapter 6

Discussion

6.1 MY APPROACH

Existing literature shows that the effects of direct or indirect cues of predator presence on arvicoline rodents are intricate. However, our understanding of those effects is partial. In attempt to deepen our knowledge on this topic, I investigated the influence of simulated predation risk on vole behavior by means of CMR and radio-tracking techniques. I simulated the risk of predation with odors of a terrestrial predator, a domestic ferret, member of the *Mustelidae* family. The experiments were performed in Central Europe, where common voles, the species under investigation, are abundant. I made the following assumptions: (1) voles can detect the odors of the ferrets, and (2) they associate those odors with a threat of predation.

My efforts were focused on the female segment of a vole population, as well as on individual females. I investigated several aspects of their behavior, which included reproductive activity, reproductive output, litter intervals, space use, and diel activity. Some of those aspects did change in response to mustelid odors, while others did not. In this chapter I will explore the possible causes of the effects of predation risk on vole behavior, and also the implications of particular findings.

6.2 OVERVIEW OF RESULTS

The CMR experiment revealed that some female voles suppress their reproductive activity when exposed to a mustelid odor; this result confirms the breeding suppression hypothesis (BSH; chapter 2). It is the first account of predator-induced breeding suppression in common voles, a species which received relatively little attention in this matter. Even though this phenomenon was demonstrated earlier in laboratory experiments, very few studies confirmed it under natural conditions. Therefore, the result of my field experiment with semi-natural enclosures reinforces the BSH. It also denies that breeding suppression is geographically limited to Scandinavia, and taxonomically to voles of genus *Myodes*.

As far as reproductive activity is concerned, the effects of predation risk on common voles are apparent only in females. I will explore the differential response of the sexes in sections dedicated to the sexually dimorphic response to predation risk (6.3.1 and 6.3.2). In fact, the result of the CMR experiment denied that reproductive activity of male common voles is sensitive to olfactory cues of predation risk (chapter 2); this result is largely in agreement with existing research (see section 6.3.1 for details).

Some females become reproductively inactive as a result of predator-induced breeding suppression; other females, however, maintain breeding. I was interested if those females, while still reproductively active, are resistant to risk of predation. It turned out that they are in some ways, but not in others. In section 6.4, I will discuss why litter frequency is affected by predator odors (subsection 6.4.1), while space use is not (subsection 6.4.2). I will also discuss possible traits and habitat properties which determine the effect of predation risk on females (section 6.5)

The CMR experiment was carried out over two breeding seasons. In addition to the parameters mentioned already, it provided detailed information on population size (chapter 2) and recruitment (chapter 3) in twelve populations of common voles. Neither population size nor recruitment was affected by the simulated risk of predation. However, the two years differed with regard to maximum population sizes (chapter 2). This may have been a symptom of a 'vole cycle', but the time window was nonetheless too short to verify this; besides, vole cycles are outside the scope of this thesis. Regardless, the 12 populations of voles supplied a broad range of observations of population density. Its combination with two levels of simulated risk of predation (high risk and baseline) and a sudden onset of the high-risk period (treatment phase) furnished an indication of density-dependent breeding suppression. Density dependence of suppressed breeding has been a matter of debate. My explanation of its potential mechanism, provided in chapter 2, may settle the differences. In addition, I will discuss how predation risk and population density shape voles' reproductive activity—but from an altogether different perspective (section 6.6).

The final sections of this chapter are devoted to methodological aspects of studies on behavioral responses to predation risk. In section 6.7, I will explain why measurements of some of the parameters reported herein are challenging in field conditions; I will also share some practical remarks on the methodology applied in my study. Finally, I will comment on the interpretation of behavioral responses to simulated risk of predation (section 6.8).

6.3 SEXUALLY DIMORPHIC RESPONSE TO PREDATION RISK

6.3.1 Males

Mustelid predators kill more females than males (Norrdahl and Korpimäki 1998). Because of the differential risk of predation, males and females should employ different adaptations to predation risk. My results confirm this presumption: the proportion of reproductively active males was not affected by increased risk of predation, while the proportion of females decreased (chapter 2). For males, the comfort of lower predator pressure comes at a price. Being less susceptible to mustelid predators is balanced on a different front: male voles, particularly dispersing ones, are being picked out by avian predators (Halle 1988).

In response to odors of terrestrial predators, male *Microtus* voles may reduce activity (Perrot-Sinal et al. 2000), or alter their mating behavior (Bian et al. 2005a). Even though some effects of predation odors on male reproductive physiology were revealed in laboratory experiments (Vasilieva et al. 2000, Wang and Liu 2002), it is unlikely that in natural conditions predation risk will induce an effect comparable with female breeding suppression.

The reason for a sexually dimorphic response to predation risk is rooted in the separate roles that the two sexes play in reproduction. Male investment is limited to guarding of mating territories, production of sperm, and insemination of females. Compared to the burden which the females must carry, male investment is obviously much lower, and requires less resources. In addition, sexually active males are more mobile than females, and occupy larger territories (Ostfeld 1990); in common voles, a territory of a single reproductively active male encompasses the territories of a few breeding females (Reichstein 1960). Being less attractive to a terrestrial predator, males enjoy a lower risk of encountering it. This further increases their advantage over females. Finally, males have access to an immediate and most effective adaptation to predation risk: escape. In contrast, dams do not flee when put face to face with a weasel (Jedrzejewski and Jedrzejewska 1990), again leaving them more vulnerable to predation than males. With straightforward adaptations on hand, males have no need to resort to breeding suppression.

6.3.2 Females

In contrast to males, reproduction and predator avoidance in females stand in conflict (Lima and Dill 1990). Breeding in common voles is unique in its scale and intensity. The females reach puberty very early, as early as in their fourteenth day of age (Reichstein 1964). In adults, estrus occurs immediately postpartum (Kudo and Oki 1982). Under optimal conditions, this facilitates very frequent litters, separated only by length of gestation. This

cycle perpetuates throughout a lifetime: in the wild, a female may have as many as five litters within a lifespan of under a year; in captivity, the number of litters per year rises to twelve (Reichstein 1964). Apparently, reproduction is at the center of life history of a female common vole. Compared with non-breeding females, breeding at such rate generates a huge energy demand (Migula 1969). Longer foraging makes breeding females more exposed to predation. Additionally, odors of reproductively active females attract predators (Cushing 1984). Chasing a female vole in her burrow, a weasel may expect to feed on the pups, too. Taking all of the above factors into account, it is clear that breeding females face a much higher risk of mustelid predation than non-breeding females or males. Thus, their adaptations have to be more rigorous.

6.4 EFFECTS OF PREDATION RISK ON BREEDING FEMALES

6.4.1 Litter interval

I found that the litter interval in continuously breeding females increased in response to predation risk (chapter 3). This result shows that, in addition to suppressing females, predation risk also affects breeding ones: some of them reproduce less often. In a laboratory experiment, predation risk was shown to lengthen the estrous cycle of bank voles (Koskela et al. 1996). I assumed that longer litter interval, as observed in field conditions, reflects a longer estrous cycle. Given that this assumption is true, the conclusion that predation risk interferes with the estrous cycle may have broad implications. In light of evidence obtained both in the laboratory in the field, a new interpretation of breeding suppression emerges.

Previous studies on suppressed breeding assigned the females to two categories: suppressing or breeding. However, this view may have been oversimplified. I think that breeding suppression is not a binary, but a heterogeneous response, as implied by elongated estrous cycles and litter intervals. Should all of possible litter intervals or lengths of the estrous cycle fall on a scale, females breeding at an uninterrupted rate would occupy its near end; suppressing females would fall on the opposite, far end of the scale. The gap between the two extremes would be filled by females breeding despite predation risk, but at various, decreased frequencies. A potential trait determining the place of a given female on this scale discussed in section 6.5.

Potential advantage of estrus suppression

Much of my research draws on the assumption that reproductively active females attract predators. Originally, Cushing (1984) found that urine of estrous prairie deer mice did, in

fact, draw weasels' attention. This effect was studied in greater detail by Ylönen et al. (2003): to a predator, the odors of estrous bank voles and odors of pregnant and lactating dams were equally attractive. In continuously breeding voles, estrus occurs soon after parturition. Hence, estrous females are at the same time postpartum, lactating or rearing pups—except for virgin females. Perhaps predators cannot distinguish the odors of breeding females at different stages of the reproductive cycle. Given that dams and estrous females attract predators equally, a female delaying estrus (and resultant litter) could remain undetected. Hiding in the shadow of estrous, pregnant or lactating females would give a suppressing female a huge advantage in terms of survival.

6.4.2 Space use and diel activity

Due to lowered energy intake and decreased body condition, living under pressure from predators may lead to limited fecundity (Lima 1998). This statement certainly does apply to suppressing females, but it is less clear if it also applies to females breeding in spite of the pressure. Reduction of foraging ranges or limiting diel activity to a certain time of day would dramatically limit females' food supply. As a consequence, their fitness in terms of reproductive success would decrease. Given their unique reproductive biology, I suspect that female voles cannot afford spatial or temporal adaptations. Should a female breed in spite of the risk, she would require unrestricted access to her resources, both in space and time. Lima's (1998) concept of trade-off between benefits and costs of predator avoidance may not apply to breeding females.

In addition to suppressed breeding, predation risk may reduce space use and mobility of female voles. This hypothesis can be verified by comparing spatial and temporal behavior of breeding and suppressing females, under the condition of increased risk of predation. Suppressing females, having temporarily given up reproduction, would have a lower energy demand. This should give way to further adaptations to predation risk, such as decreased space use, reduced mobility, and potentially, shifted diel activity. It seems probable that these responses would emerge in parallel with breeding suppression.

My radio-tracking study revealed that home range, activity range, mobility and diel activity of breeding females were not affected by ferret predation risk (chapter 4). However, breeding females unambiguously avoided the sources of predator odor. Repelling effect of ferret odor confirmed its biological significance for common voles. Furthermore, the fact that avoidance was the only effect of predator odor recorded in the radio-tracking study implies that breeding females cannot adapt the remaining aspects of their spatial and temporal behavior. Given breeding females' demand for a steady supply of food to support gestation or

lactation, avoidance of predator odor might be the sole adaptation which they can resort to.

6.5 WHAT DETERMINES FEMALE'S RESPONSE TO PREDATION RISK?

6.5.1 Traits affecting reproductive activity

In the experiment on female reproductive activity, I investigated the combined effect of vole population density and risk of predation. In reality, more factors shape the proportion of breeding females. Some of them depend on long-term population dynamics, and include sex ratio and age structure. Due to relatively low intrasexual competition, higher levels of reproductive activity should occur at sex ratios shifted in favor of males (e.g. in spring; Bryja et al. 2005). It is reasonable to expect that sex ratio would interact with population density: at high densities, competition would increase and conditions would no longer be favorable.

The response to predation risk could also depend on the age of individual females. In words of Kaitala et al. (1997), breeding suppression is 'either an age-structured combination of pure breeding behaviors (old females breed and young delay maturity) or a mixed breeding behavior within age-classes (a fraction of females breed and the rest of the age class postpones breeding)'. According to Ylönen and Ronkainen (1994), young females would suppress reproduction under high predation risk, whereas old females would continue to breed. Young females would realize their fitness in the future, having survived the high-risk period, which would not be possible in old females. However, Fuelling and Halle (2004) reported a contradicting result. They found that high predation risk suppressed breeding in young as well as in old females. The question whether female's age determines the choice of strategy remains open.

The implications of female age potentially reach further. In voles, some females may overwinter and reproduce in the second season. The capacity for reproduction decreases as the females grow old. Under certain circumstances, the proportion of older females may increase, constituting and 'old' age structure. With senescent females dominating in the population, the potential for reproduction may became exhausted. This mechanism was proposed as an explanation for the puzzle of microtine cycles ('senescence hypothesis'; Boonstra 1994). According to the senescence hypothesis, the aging of a population is closely interwoven with its density: at low population density and young age structure, reproductive potential is high. What follows is high density which, in turn, induces social breeding suppression. Successive litters are delayed, and females progressively grow old. At some point, senescent females are no longer capable of breeding and die off. The circle closes in a sparse population consisting of young individuals.

Boonstra's elegant argument implies that density and age structure are key in determining reproductive activity of females. It seems that predation risk is a facultative factor in this cycle. My experiment revealed that predation risk interacts with density. It is quite likely that it also interacts with the age structure of the population. The susceptibility to stress should change with age (Reeder and Kramer 2005). Perhaps the predisposition of a female to suppress breeding also changes throughout life. If so, the effect of predation risk on female reproduction would depend on both the density of the population, and the age of the females. This effect could change with time, as both population growth and aging are dynamic processes. It seems likely at this point that the females' age determines their response to predation risk. In my experiment, the age structure was reset in the beginning of each breeding season. Hence, I could not verify the role of age. Perhaps future studies will.

6.5.2 Habitat properties affecting space use

Vegetation cover is one of the most important factors affecting perception of predation risk and thus, determining space use (Lima 1998). As an efficient refuge from avian predators, cover determines space use in several species of *Microtus* voles (e.g., Anderson 1986, Getz et al. 2005). This fact suggests that voles primarily avoid winged predators. Yet, mustelids should pose a higher threat. Owing to their slender body shape, small mustelids (particularly female weasels) are able to hunt for voles in burrows. This should leave voles without refuge, rendering mustelid predation risk ubiquitous ('uniform' *sensu* Eccard et al. 2008)—as opposed to avian predators.

Paradoxically, voles may adapt their use of space to the pressure of terrestrial predators, but only when some protection from avian predation risk is available. For instance, most flying predators are excluded in woodland habitats. Mustelid predation risk takes priority and induces anti-predatory space use (Jedrzejewska and Jedrzejewski 1990, Borowski and Owadowska 2001). This situation is reversed in grasslands. Common voles, a typical grassland species, are heavily preyed upon by birds (Halle 1988). In the Townsend's vole *Microtus townsendii*, another species occupying the same type of habitat, spatial response to a mustelid odor was only apparent when cover was completely absent (Merkens et al. 1991). This indicates that avian predators are indeed a higher threat then carnivores.

In grasslands, due to predominantly avian predation risk, shape and size of vole activity ranges under mustelid predation risk should depend on the distribution and availability of cover. I suspect that in non-homogenous habitats, increase of mustelid predation risk will reduce voles' use of space in areas with sparse cover, while it will have no effect in areas with dense cover.

In my experiments, natural avian predation risk may have been more prominent than mustelid predation risk. The vegetation in my study plots varied somewhat, with patches of mugwort (*Artemisia vulgaris*) and tall grasses. Overall, it was quite dense. In areas with thinner vegetation, burrow entrances of some of the females were concentrated under burdock (*Arctium sp.*) canopy (personal observation). This suggests that those females did use vegetation as cover against avian predators. Other than that, cover had little or no impact on spatial activity of breeding females, despite presumably high risk of avian predation. It is understandable, given the breeding condition of radio-tracked females (see arguments in sections 6.3.2 and 6.4.2). Apparently, the dependence of breeding females on resources excludes a spatial response to avian predation risk, as it does in case of mustelid predation risk.

6.6 VOLE DENSITY, PREDATION RISK AND STRESS

Stress can have a detrimental effect on animal reproduction (Dobson et al. 2003). This effect was proposed as the driving force for the cyclic changes of snowshoe hare populations (Boonstra et al. 1998). In natural populations, the sources of stress may be multiple. However, two seem most important: high density of conspecifics, and high predation risk.

The accounts of breeding disturbance as a result of high population density are numerous in literature on rodent reproduction. Some examples include density-dependent changes in estrus frequency in caged mice (Whitten 1959), decreased ovulation frequency in prairie deermice at high population density (Terman 1973), and abnormal estrous cycles in female *Mus musculus* at high densities (Massey 1986). In the last study, Massey found that mean length of the estrous cycle increased threefold; the females showed different susceptibility to crowding (Massey 1986).

Changes of reproductive physiology under predation risk are less well documented. Heikkilä et al. (1993) found that immediate predation risk suppressed the development of gonads in two out of the three studied species of voles. As far as the estrous cycle is concerned, Koskela et al. (1996) discovered that it was lengthened in bank voles exposed to the presence of a weasel; here, too, the females responded differently. I found a similar pattern in longer litter intervals of common voles (chapter 3) and interpreted it as a result of a disturbed estrous cycle.

Curiously, the response of rodents to high density of conspecifics closely resembles their response to high predation risk. This is an interesting analogy. It seems that these factors, both of them being potent stressors, cause the same effect: they interfere with the estrous cycle of female rodents. In some females, this leads to what we view as socially- or predator-

induced breeding suppression. Distortion of the estrous cycle suggests some kind of endocrine response. Perhaps those two circumstances activate a common physiological pathway. Hence, predator-induced breeding suppression would not constitute a specific effect of predation risk, but rather part of a universal response, a mere result of living in a stressful environment.

6.7 REMARKS ON METHODOLOGY

6.7.1 Laboratory or field?

Due to variation in experimental design, broad generalizations drawing on the existing body of evidence should be made with caution. Future studies should aim for more methodological consistency. Researcher's choice between a laboratory and field setting is often difficult. A laboratory setting limits or altogether eliminates the uncontrollable factors, but it is at the same time far from natural environment. Unfenced study sites allow mustelid predators not only to leave cues of predation risk, but also to prey on study animals. This situation is close to natural, but causes the risk of serious interference due to unsolicited predation and prey migration. In contrast to open field, large-scale enclosures offer semi-natural conditions, eliminate migration, and exclude ground predators, thus providing a good balance between a controlled and uncontrolled environment. Studies performed in those conditions complement laboratory experiments, because they can reliably verify the ideas emerging in small-scale, controlled trials.

6.7.2 Reproductive activity of females

The conclusions from existing studies on breeding suppression are not directly comparable, due to variation in study species, geographical location, and experimental design. Generally, studies from Northern America denied breeding suppression (e.g., Wolff and Davis-Born 1997, Jonsson et al. 2000, Sullivan et al. 2004), while most European studies confirmed it (e.g., Ronkainen and Ylönen 1994, Koskela and Ylönen 1995, Mappes and Ylönen 1997, Fuelling and Halle 2004). Among studies measuring reproductive activity on population level, fewer have been done in the field than in the laboratory. The effect of the predator odor is weaker in natural conditions. Strong effects obtained in the laboratory are probably due to extreme proximity of the predator or its cues. Interestingly, most of those studies focused on a single vole species, namely the bank vole. They were also restricted to one geographical region, i.e., Scandinavia. I am not aware of laboratory experiments neither denying the effect of predation risk on female reproductive activity, nor focusing on New World species. Furthermore, it is still uncertain if conclusions drawn for the bank vole apply

to other species, especially those of the New World. Results of Heikkilä et al. (1993) show that even within one area, predator odors may have a different effect on co-existing species. Breeding suppression might not be common among all voles. What determines if a species (or given population) shows this effect has yet to be discovered. For the sake of improved comparability of the data, there is a need for both laboratory experiments on North American voles, and field studies on a broader range of species.

Choice of predator is another important issue in experimental design. Existing studies used various predators, with different relevance to prey species. The ecological relationship between predator and voles should be very close. Cues of generalist pressure may not be meaningful enough, as emphasized by Apfelbach et al. (2005). Some studies employed minks or their odors, mainly with to negative outcomes. Minks are generalists, and not the greatest threat to voles. In contrast, weasels and stoats specialize in *Microtus* rodents (Erlinge 1975, Brzeziński and Żurowski 1992, McDonald et al. 2000). Hence, mink odor may be a weaker stimulus than odors of smaller mustelids.

In addition to different predator species, various types of predator body odor were used to simulate the risk of predation. Masini et al. (2005) reported that out of possible ferret odors, only odor of fur had produced a hormonal stress response in rats, while odors of anal gland secretions, urine and feces failed. This suggests that the type of body odor may be a key factor in eliciting breeding suppression. I used soiled cage bedding as the raw material, assuming that it should contain a full array of ferret body odors: from the odor of urine, feces, and glandular secretions, to the odor of fur. This mixture induced breeding suppression (chapter 2), and lengthened litter intervals in breeding females (chapter 3). Additionally, sources of the ferret odors were avoided (chapter 4). Even though chemical composition of ferrets' anal gland secretion is quite similar to that of weasels and stoats (Brinck et al. 1983, Schildknecht and Birkner 1983, Crump and Moors 1985, Zhang et al. 2003b), little is known of the composition of the remaining body odors. Still, in light of my results, ferret odor appears as a meaningful cue of predation risk, at least as perceived by common voles.

6.7.3 Population density

Predation risk should, by induced breeding suppression, reduce the size of a population (Norrdahl and Korpimäki 2000). My CMR experiment failed to confirm this effect (figure 4.2 (inset) on page 47). Lagging of recruitment behind reproduction is a potential explanation, considering the reproductive cycle of the common vole. In this species, gestation is three weeks long; after parturition, at least three weeks must elapse until the newborn pups wean (Reichstein 1964). This amounts to a minimum of six weeks from conception. After weaning,

a period of uncertain length must pass for the pup to reach the threshold body mass of 15 g, required by the measure of population density. The length of this period depends on several factors, including season, food availability, pup's sex, population size, social rank, and the definition of the population density measure itself. Additionally, new recruits may not be captured instantly, depending on the environmental conditions, population density, and the individual's traits. This causes further delay and introduces negative bias in population size measures. I estimated that a period of six to twelve weeks elapses from the moment a female is fertilized until her pups are recorded as recruits. Despite a span of nearly three months, the treatment phase in my experiment was probably still too short to show the limiting effect of high predation risk on population size. I suggest that in similar future studies, the length of the treatment period should be even longer, with respect to reproductive biology of studied species.

Uncontrollable factors are another possible cause of the lack of differences in density between treatment and reference populations in my study. Both year and plot factors (see figure 4.2 inset) explained enough variability to reach statistical significance. Despite identical initial sizes, the populations reached different maxima in 2007 and 2008. Furthermore, an unknown property of the experimental plots resulted in pronounced differences in densities, notwithstanding the treatments applied. Remarkably, this effect was consistent in both years. It cannot be ruled out that, relatively to risk of predation, other environmental factors have much stronger influence on population size. Additionally, the conditions for reproduction under predation risk may actually improve, at least from the perspective of breeding females. Since some of the females suppress reproduction, the intraspecific competition is lower. Hypothetically, breeding females could take advantage of this situation by increasing their reproductive output. In consequence, they would compensate for the litters unrealized by the suppressing females.

6.7.4 Individual reproductive output

Effects of predation risk on litter size and litter frequency seem to result from different physiological mechanisms. Regardless of the actual physiological response, the overall reproductive output of a female is affected. However, this effect is difficult to quantify in conventional CMR studies. Hence, few of the reproduction-related effects have been replicated in natural conditions. In case of a diminishing effect of predation risk on reproductive output, it might often be difficult to resolve smaller litter size from lower litter frequency. Additionally, the methods for estimation of litter size and litter frequency are entirely different.

In field studies on breeding suppression, the most commonly used estimates of repro-

ductive output included litter size and number of recruits per litter (or reproducing female). Unfortunately, these measures are not directly comparable. Litter size is individual-based, while the number of recruits per litter is usually averaged over the whole population. Furthermore, the measurement of litter size in field experiments is very limited. When voles are held captive, litter size is taken either directly at parturition, or indirectly from the number of embryos. In traditional field studies, early identification of pups with the mother is necessary to assess litter size. Normally, the observers have no access to the nests, so the pups can only be tallied by frequent trapping. Even though the count may be fairly accurate for litters of solitary females, it is useless in case of communally nesting females, where determining kin from trapping data is impossible. Direct methods (e.g. abdomen palpation, ultrasound scans, genetic identification of kin or nest inspections) may facilitate the evaluation of litter size in natural populations. Still, these methods restrict the scale of the experiment. Additionally, some of them require regular trapping of females in late stage of gestation. As long as trapping is the only method on hand, litter size is an unreliable measure of reproductive output.

As an alternative, the number of recruits per litter approximates litter size. It uses well-established and simple techniques, making it suitable for large-scale field experiments. However, this measure lacks precision. Since exact attribution of a recruit to a litter is impossible, the experimenters are forced to take the total number of recruits over the total number of litters. The pooling of the data averages out all individual variability. Wherever polymorphism of female responses is expected, as in case of breeding suppression (chapter 3), the number of recruits per litter should not be used. Furthermore, the number of recruits per litter is resultant not only of litter size, but also of juvenile mortality. In field conditions, controlling for juvenile mortality is difficult (see Millar 2007). This difficulty alone may confound the effect of predation risk. Additionally, estimates of recruitment are influenced by other factors, such as trappability of recruits. Predation risk can independently affect both juvenile mortality, and trappability. Hence, the resulting number of recruits per litter departs even further from the actual litter size. Given the complex interaction of factors, a change of the reproductive output estimate can be falsely attributed to predation risk. Hence, the reliability of the number of recruits per litter is unsatisfactory for studies on natural populations.

Contrary to measures based on litter size, determining litter frequency is relatively straightforward in field conditions. In its simplest form, it can be expressed as the interval between two consecutive litters. This figure is derived directly from the temporal pattern of gestations observed in a given female; in polycyclic animals, it yields several observations per individual. It does neither require identification of recruits with litters, nor is burdened

with unknown juvenile mortality. Hence, it is more reliable then measures based on litter size. Nonetheless, estimation of litter intervals also requires frequent trapping. Additionally, litter intervals may be insensitive to predation risk, depending on the actual mechanism of breeding suppression. As part of a physiological response to predation risk, resorption of embryos in an on-going gestation may occur (Voznessenskaya et al. 2003). In case predation risk affects litter size only, litter intervals will fail to detect its effect. Otherwise, litter interval may prove useful in studies on breeding suppression.

6.7.5 Spatial activity

Home range

The mean female home ranges in my study varied between 34 and 94 m^2 (regardless of treatment or experiment phase). In contrast, Reichstein (1960) estimated that home ranges of reproductively active *M. arvalis* fall within 300-400 m^2 (MCP 100%). These values are not directly comparable, however. Five factors may have caused this discrepancy: (1) my female populations mainly consisted of reproductively inactive females, having relatively small home ranges; (2) the outermost 5% of locations, which strongly influence area estimates, were excluded; (3) I used a sparser array of traps; (4) the movements in my populations were restricted to the experimental plots; (5) the densities of my study populations were relatively high, possibly limiting individual space use. The first four factors were purely methodological, but the fifth was independent from the observers. The deviation from Reichstein's measurements shows that, in addition to the applied methodology, the size of the home range may be affected to a high degree by environmental factors other than predation risk; of those, population density is probably the most influential.

Capture location data

It appears that the resolution of capture location data may be too crude to reveal subtle or short-term factors affecting the size of the home range. Compared to radio-tracking, spatial resolution of the data is rather low, depending on the density of the trap array, and the expected home range size. Moreover, it can be strongly influenced by the spatial relation of capture locations: in a regular grid of traps, four hypothetical captures may form a rectangular, triangular or linear home range. Furthermore, the traps alone may be a source of undeterminable bias. Bait and conspecific scent marks may attract or repel some individuals, decreasing both accuracy and precision of the estimates. Still, capture location data prove useful in revealing relatively strong effects, such as food addition (Taitt and Krebs 1981),

sex and reproductive status (Swihart and Slade 1989), or the density effects (chapter 4). Capture records yield large sample sizes, somewhat balancing the poor precision of individual observations. With little extra effort, capture location data may allow a spatial aspect in some CMR studies.

Radio-tracking

Compared to capture location data, radio-tracking methods acquire information over short periods. Because individual space use within a population is a dynamic process (Madison 1985), radio-tracking should have a relatively high sensitivity to its changes. However, the behavioral effects of mustelid odors are almost immediate (Eccard et al. 2008), and may also be short-lived, as suggested by Parsons and Bondrup-Nielsen (1996). At some point, habituation to simulated predation risk may occur. If spatial adaptations of voles to mustelid odors were momentary, neither capture data nor radio-tracking could detect them. The radio-tracking sessions in my study were separated by 12 days, with six applications of odor in the meantime; this may have been long enough for the females to to habituate. In laboratory rats, however, any physiological habituation to ferret odor was denied (Masini et al. 2005), indicating the significance of ferret odor for rodents. Despite that, some chance exists that in my system, the lack of effect of predator odor on size of short-term ranges (as well as long-term ranges) was a result of habituation. This possibility should be taken into account whenever the effect of predation risk is investigated over an extended period.

6.8 ALTERED BEHAVIOR: ADAPTATION OR RESULT OF STRESS?

The interpretations of the effects of predation risk on small rodent behavior pose some challenges. These interpretations may vary, depending on the research background. While anatomical features do constitute an adaptation to animal's environment (e.g., re-growing incisors in rodents facilitate gnawing on large amounts of plant food), this may not necessarily hold true for all behavioral traits. With regard to behavior, an 'adaptation' would imply deliberate action on the side of the animal, or prey in case of an adaptation to predation risk. Altered behavior, however, is a result of an endocrine or neurological response to stress.

Reduced general activity or mobility, observed in voles exposed to predatory stimulus (e.g. Gorman 1984), is remarkably similar to observations made on laboratory rodents (including voles) exposed to stress-inducing stimuli; those stimuli often include predator odor (e.g. Perrot-Sinal and Petersen 1997, Perrot-Sinal et al. 1999b). Neurological studies interpret such responses as 'fear', 'anxiety', or 'defensive behavior' (Dielenberg and McGregor 2001,

Takahashi et al. 2005, Fendt 2006), while ecologists are often inclined to view a certain behavior as an 'adaptation'. Physiological studies confirm the notion that predation risk causes stress (e.g. Chabot et al. 1996). In fact, the link between predator odor and neurological and endocrine mechanism of stress was demonstrated in laboratory rats (Perrot-Sinal et al. 1999a). Results of a stress response may, as a side effect, minimize exposure to predation. Hence, mere fear may lead to what can be viewed as an adaptive response to predation risk.

A thin line separates a general response (e.g. fear) from an adaptive behavior. Breeding suppression, often viewed as an adaptation to predation risk, may simply be a result of stress (section 6.6). The same effect may be produced by various other stressful situations. Therefore, interpreting behavioral observations as adaptations should be made with consideration. Perhaps endocrine or neurological studies will verify whether responses of voles to predation risk are specific.

* * *

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Summary

Small mammals perceive much of their environment by means of olfaction. This also applies to predation risk: when detected, olfactory cues of predators convey information on their presence. To microtine rodents, pressure of mustelid predators (e.g. weasels) is highly relevant. Mustelids can hunt for voles inside their burrows, posing an ubiquitous threat. The ability to assess the risk of predation allows by its olfactory cues allows prey to adjust their behavior in advance, and thus adapt.

Voles respond to mustelid odors in several ways. General responses may include reduced mobility and space use, altered diel activity, as well as avoidance of the odor. Sex-specific responses include breeding suppression: when challenged with a cue of predation risk, some female voles will suppress their reproductive activity; suppression of breeding should, by some margin, increase the chances of survival ('breeding suppression hypothesis'; BSH). This hypothesis was questioned, because evidence in its favor was obtained in artificial conditions, and only a few field studies supported it. Furthermore, studies on breeding suppression were almost exclusively limited to Scandinavia, and were mostly performed on voles of genus *Myodes*. Finally, it is still unclear whether breeding suppression is a density-dependent effect.

In my doctoral project, I investigated the effects of predation risk, simulated with odors of specialist predators, on several aspects of vole behavior. Over two breeding seasons, I exposed twelve populations (six each year) of common voles *Microtus arvalis* to either olfactory cues of mustelid risk of predation, or control odors. The experiments were carried out in semi-natural enclosures, using capture-mark-recapture and radio-tracking techniques. I measured the effect of predation risk on reproductive activity of both male and female voles. With regard to female reproductive activity, my aim was to verify BSH in controlled field conditions. On individual level, I studied the effects of predation risk on reproductive output and litter intervals in breeding females. I also investigated if they show general responses to predation risk, including changes in space use, mobility and diel activity. On population level, I tested if predation risk affected population size, and if breeding suppression was a density-dependent effect. In addition to the field experiments, I attempted to study the neurological response of female brains with functional magnetic resonance imaging (fMRI). The aim of the fMRI trials

was to better understand how olfactory cues of predation risk affect prey.

I found that female voles suppressed breeding in response to mustelid predation risk. Further, my results provided an indication of density dependence of predator-induced breeding suppression. The effect of predation risk was pronounced at low densities, but negligible at high densities. In male voles, predation risk had no effect. Additionally, simulated risk of predation had no effect on population density—despite suppressed breeding. Reproductive output of females breeding in spite of predation risk, measured as the number of recruits per litter, was also not affected. However, the length of the litter interval increased.

Simulated predation risk did not induce general anti-predatory responses, such as reduced long-term space use (home range), reduced short-term space use (activity range), decreased mobility, or shifted diel activity. Nonetheless, breeding females unambiguously avoided mustelid odor, thus confirming its ecological relevance. The pilot study with fMRI revealed that, despite having obtained good anatomical images of vole brains, the sensitivity of the available equipment was too low to detect a significant functional response.

Much as expected, the response of common voles to mustelid predation risk was sexually dimorphic. Decreased female reproductive activity confirmed BSH in a field experiment in Central Europe, in a species which has not yet been investigated in this regard. The fact that breeding suppression occurred primarily at low vole densities suggests a new interpretation of the density dependence of this effect: at high densities, when females are numerous potential prey, suppressed breeding is no longer beneficial (cf. 'safety in numbers'). Because of a delay in recruitment of subadult voles, longer experiments are needed to demonstrate the effect of simulated predation risk on population density.

Increased litter intervals indicated that predation risk not only induces breeding suppression, but also affects breeding females. Until now, breeding suppression was viewed as a binary effect: a female either suppressed breeding, or was not affected. My results revealed that female voles may respond to predation risk with various outcomes: from arrested breeding, via lengthened litter intervals, to no response (normal breeding). Increased litter intervals also suggest that breeding suppression is mediated hormonally: hypothetically, predation risk interferes with the estrous cycle. This may be the mechanism of breeding suppression.

Since different factors may affect reproductive behavior in microtines (e.g. population density), it is still unclear whether breeding suppression is a specific adaptation to predation risk, or a universal response to unfavorable or stressful conditions (cf. social breeding suppression). The effects of predator odors on breeding females may vary with individual's age or another, yet undetermined trait.

Zusammenfassung

Kleinsäuger nehmen einen großen Teil ihrer Umgebung mit Hilfe ihres Geruchssinns wahr. Das gilt besonders für die Abschätzung des Prädationsrisikos, da der Geruch von Räubern die Tiere über deren Anwesenheit informiert. Für Nager der Gattung *Microtus* ist die Bedrohung durch wieselartige Räuber (Musteliden) von großer Relevanz. Musteliden können Wühlmäuse innerhalb ihrer Höhlen jagen und stellen dadurch eine allgegenwärtige Gefahr dar. Die Fähigkeit, das Prädationsrisiko über olfaktorische Signale abzuschätzen ermöglicht eine Verhaltensänderung der Beute vor einem Zusammentreffen mit dem Räuber und damit eine Anpassung an diese Bedrohung.

Wühlmäuse reagieren auf den Geruch von Räubern auf verschiedene Art und Weise. Generelle Reaktionen beinhalten eine verringerte Mobilität in Verbindung mit einem reduzierten Bewegungsradius und führen außerdem zu Veränderungen der zirkadianen Aktivität. Außerdem versuchen die Tiere den Duft des Räubers generell zu meiden. Geschlechtsspezifische Reaktionen auf die Anwesenheit einer Duftmarke eines Räubers beinhalten beispielsweise die Unterdrückung der Reproduktion. Bei Konfrontation mit dem Geruch eines Räubers unterdrücken einige Weibchen alle Aktivitäten im Zusammenhang mit der Fortpflanzung und steigern damit ihre Überlebenswahrscheinlichkeit (‘breeding suppression hypothesis’; BSH). Die Gültigkeit dieser Hypothese wird in Frage gestellt, da sie stützende Resultate aus Versuchen in künstlicher Umgebung stammen und nur wenige Feldstudien zu positiven Ergebnissen kommen. Darüber hinaus sind entsprechende Studien zur Unterdrückung der Reproduktion bei Wühlmäusen fast ausschließlich auf den skandinavischen Raum beschränkt und wurden meist an Tieren der Gattung *Myodes* durchgeführt. Schließlich ist zurzeit auch noch nicht geklärt, ob die Unterdrückung der Reproduktion von der Populationsdichte abhängt.

In der vorliegenden Arbeit wurden die Effekte eines, durch die Applikation von Duftmarken spezialisierter Räuber simulierten, erhöhten Prädationsrisikos auf verschiedene Aspekte des Verhaltens von Wühlmäusen untersucht. Über zwei Fortpflanzungsperioden wurden zwölf Populationen der Feldmaus (*Microtus arvalis*) (sechs pro Jahr) entweder mit Geruchsproben von Frettchen (*Mustela putorius furo*), einem Musteliden, oder einem Kontrollduft

konfrontiert. Die Experimente wurden in einer naturnahen Anlage mit sechs voneinander getrennten Gehegen mit einer Fläche von jeweils 2500 m^2 durchgeführt. Dabei wurden Tiere wiederholt gefangen, markiert und vermessen. Außerdem wurden Tiere mit Sendern versehen und mit Hilfe radiotelemetrischer Methoden deren Bewegungsmuster aufgenommen. Die Wirkung des erhöhten Prädationsrisikos auf die reproduktive Aktivität wurde sowohl für weibliche als auch für männliche Tiere aufgenommen. Ziel war es, die BSH für weibliche Tiere unter Feldbedingungen zu testen. Dazu wurden auf individueller Ebene der Fortpflanzungserfolg und die Wurfintervalle bestimmt. Darüber hinaus wurden generelle Reaktionen auf das erhöhte Prädationsrisiko untersucht, indem die Mobilität, die zirkadiane Rhythmik und die Raumnutzung verschiedener Tiere gemessen wurden. Auf Populationsebene wurde untersucht, ob das erhöhte Prädationsrisiko einen Einfluss auf die Größe der Population hat bzw. ob die Unterdrückung der Reproduktion ein Effekt der Populationsgröße ist. Zusätzlich zu den Freilanduntersuchungen wurde der Versuch unternommen, mit Hilfe der funktionellen Magnetresonanztomographie (fMRI) die neurologischen Reaktionen im Gehirn weiblicher Tiere auf die Applikation des Räuberduftes hin zu untersuchen. Ziel dieser Untersuchung war ein besseres Verständnis der neuronalen Wirkung räuberischer Duftmarken auf die Beuteorganismen.

Es konnte gezeigt werden, dass weibliche Wühlmäuse tatsächlich ihre Reproduktion unter Anwesenheit eines Räuberduftes unterdrücken. Desweiteren deuten die Ergebnisse darauf hin, dass die Unterdrückung der Reproduktion zusätzlich von der Populationsdichte beeinflusst wird. Der Einfluss des erhöhten Prädationsrisikos auf die Unterdrückung der Reproduktion war besonders ausgeprägt in Populationen mit geringeren Dichten und bei Populationen mit hohen Dichten nahezu vernachlässigbar. Es konnten keine Effekte des erhöhten Prädationsrisikos bei männlichen Tieren nachgewiesen werden.

Desweiteren zeigten sich keine Effekte erhöhten Prädationsrisikos auf die Populationsdichte, trotz der Unterdrückung der Fortpflanzung. Zwar war der Fortpflanzungserfolg weiblicher Tiere, gemessen an der Anzahl neu hinzugekommener Adulte pro Wurf, nicht beeinflusst durch das erhöhte Prädationsrisiko, aber die Abstände zwischen den einzelnen Würfen vergrößerten sich unter der Behandlung mit Räuberduft.

Es konnten darüber hinaus keine Effekte des simulierten Räuberdruckes auf das generelle räubervermeidende Verhalten der Wühlmäuse gezeigt werden. Weder die Größe der genutzten Reviere (home range) noch die kurzzeitliche Größe des Aktionsraumes (activity range) änderten sich unter der Anwesenheit des Räuberduftes. Auch konnten weder Änderungen in der Mobilität oder der zirkadianen Aktivität verzeichnet werden. Trotzdem vermieden weibliche Tiere zweifellos den applizierten Frettchengeruch, was deutlich dessen ökologische

Relevanz bestätigt.

In der fMRI-Teststudie konnten neben guten anatomischen Aufnahmen von Wühlmaushirnen keine signifikanten funktionalen Reaktionen auf die Applikation von Räuberduft nachgewiesen werden. Vermutlich besaß die zur Verfügung stehende Apparatur nicht die notwendige Sensitivität um die neuronalen Veränderungen sichtbar machen zu können.

Wie erwartet war die Reaktion der Feldmäuse auf das erhöhte Prädationsrisiko stark sexuell dimorph. Die verringerte Reproduktion weiblicher Tiere bestätigt die BSH in einem Feldexperiment in Zentraleuropa am Beispiel einer Art die vorher noch nicht bezüglich dieses Effektes getestet worden ist. Außerdem deutet die Tatsache, dass die Unterdrückung der Reproduktion hauptsächlich in Populationen mit geringen Dichten eine Rolle spielt, auf eine neue Beurteilung der Dichteabhängikeit dieses Phänomens hin: bei hohen Dichten, bei denen weibliche Tiere eine häufige potentielle Beute darstellen, ist die Unterdrückung der eigenen Reproduktion nicht länger von Vorteil (vgl. 'safety in numbers').

Weitere Langzeit-Experimente sind nötig, um die tatsächlichen Effekte eines simuliert erhöhten Prädationsrisikos auf die Populationsdichte zu bestimmen, da die Rekrutierung subadulter Feldmäuse zeitlich verzögert zum Tragen kommt.

Die Vergrößerung der Wurfintervalle deutet darauf hin, dass ein erhöhtes Prädationsrisiko nicht nur zu einer Unterdrückung der Reproduktion führt sondern darüber hinaus auch die reproduktionsaktiven Tiere selbst beeinflusst. Bis jetzt wurde die Unterdrückung der Reproduktion als ein ausschließlich binäres Phänomen betrachtet: entweder unterdrückt ein weibliches Tier seine Reproduktion oder es ist in keiner Weise beeinflusst. Die vorliegenden Ergebnisse relativieren diese Sichtweise indem sie zeigen, dass weibliche Tiere innerhalb eines Spektrums von unterbrochener Reproduktion über einen vergrößerten Zeitraum zwischen zwei Würfen bis zu einer normalen Fortpflanzung auf das erhöhte Prädationsrisiko reagieren können. Der vergrößerte Zeitraum zwischen den Würfen deutet außerdem darauf hin, dass die Unterdrückung der Reproduktion hormonell, vermutlich durch eine Störung des Östrogen-Zykluses, gesteuert sein könnte. Vielleicht ist das der Mechanismus der unterdrückten Reproduktion.

Da verschiedene Faktoren das Fortpflanzungsverhalten von Tieren der Gattung Microtus beeinflussen (z.B. Populationsdichte) ist es noch immer unklar, ob die Unterdrückung der eigenen Reproduktion eine Anpassung an ein erhöhtes Prädationsrisiko darstellt oder eher eine generelle Reaktion auf 'unwirtliche Bedingungen' (vgl. social breeding suppression). Der Einfluss von Räuberduft auf reproduktionsaktive Weibchen könnte auch mit dem Alter der Tiere oder einer anderen noch ungeklärten Eigenschaft variieren.

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Ehrenwörtliche Erklärung

Hiermit erkläre ich, Mateusz Jochym, geboren am 19. August 1979 in Warschau (Polen) an Eides statt,

- dass mir die geltende Promotionsordnung der Biologisch-Pharmazeutischen Fakultät bekannt ist;
- dass ich die vorliegende Dissertation mit dem Titel 'Reproductive activity and spatial behavior of common voles (*Microtus arvalis* Pallas, 1778) in response to simulated mustelid predation risk' selbst angefertigt habe, keine Textabschnitte eines Dritten ohne Kennzeichnung übernommen habe und alle von mir benutzten Hilfsmittel, persönlichen Mitteilungen und Quellen in meiner Arbeit angegeben habe;
- dass die Hilfe eines Promotionsberaters nicht in Anspruch genommen wurde und dass Dritte weder unmittelbar noch mittelbar geldwerte Leistungen von mir für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen;
- dass ich die Dissertation noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht habe;
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- dass ich weder während meiner Zeit an der Friedrich-Schiller-Universität Jena, noch davor ein anderes Promotionsverfahren beantragt oder durchlaufen habe.

Jena, März 2012 .....

Mateusz Jochym