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Secondhand horror: effects of direct and indirect predator cues on behavior and reproduction of the bank vole

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Abstract. Risk recognition by prey is of paramount importance within the evolutionary arms race between predator and prey. Prey species are able to detect direct predator cues like odors and adjust their behavior appropriately. The question arises whether an indirect predation cue, such as the odor of scared individuals, can be detected by conspecifics and subsequently affects recipient behavior. Parents may also transfer their experience with predators to their offspring. In two experiments, we assessed how direct and indirect predation cues affect bank vole (*Myodes glareolus*) foraging behavior, reproduction, and pup fitness. Weasel (*Mustela nivalis*) odor served as the direct cue, whereas the odor of weasel-scared conspecifics, alarm pheromones, was used as an indirect cue and both of those were compared to a control odor, clean wood shavings. Alarm pheromones attracted female voles, measured as time in proximity to the treatment and foraging. Both predator odor and alarm pheromones enhanced reproduction compared to the control odor. Females treated with alarm pheromone had significantly higher pregnancy rates, and pups from predator-treated mothers were significantly heavier at birth. Our study provides two novel ideas. First, the impact of a predator can be socially transmitted. Second, predation risk likely triggers terminal investment in reproduction.

Key words: alarm pheromone; ecology of fear; *Mustela nivalis*; *Myodes glareolus*; odor; stress response; terminal investment.

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INTRODUCTION

Predators decrease an individual's survival probability (Sih et al. 1985, Murdoch et al. 2003). Predation, and the indirect effects of predator presence, has been recognized as strong life-history determinants across different taxa (Sih 1994, Ylönen and Ronkainen 1994, Werner and Peacor 2003, Nelson et al. 2004, Ylönen and Brown 2007, Sheriff et al. 2009). Historically, ecological research has focused on the aforementioned direct predation effects (Paine 1966, Taylor 1984, Krebs et al. 1995). However, in the last decades,

the focus has shifted more and more toward the indirect effects of predation (see reviews by Lima 1998, Creel and Christianson 2008), and it has been recognized that perceived predation risk alone can have large fitness or survival effects on the population level as direct mortality by predators (Schmitz et al. 1997, Nelson et al. 2004, Preisser et al. 2005, Pangle et al. 2007).

Co-evolution of predator and prey species suggests prey evolved a number of sensory and behavioral adaptations in order to recognize and avoid predators. In many mammalian prey species, this includes behavioral changes such as

freezing, avoidance, and heightened vigilance, but also the ability to detect and correctly recognize the odors emitted by predators, from here on predator odor (PO), which serve as triggers for the adaptive behaviors previously mentioned (Kats and Dill 1998, Dielenberg and McGregor 2001, Sundell and Ylönen 2004, Conover 2007, Osada et al. 2014, Apfelbach et al. 2015, Sievert and Laska 2016). Indirect effects of predation, for example, decreased reproduction (Ylönen and Ronkainen 1994, Sheriff et al. 2009, 2015), as well as the interaction of risk and competition, are drawing increasing attention in current literature (e.g., Apfelbach et al. 2005, Parsons et al. 2017).

In a natural environment, the odor of a predator might be abundant in the form of excrement or markings. It is therefore not surprising that several studies have reported rapid habituation to predator-borne odor in a natural environment (Cox et al. 2010, Elmeros et al. 2011, Bytheway et al. 2013). This leads to the assumption that prey, while detecting PO, considers it as an ambient risk (Brown et al. 2015). A study by Bleicher et al. (2018) showed that vole's reaction to predator odor returns to baseline levels after being confronted with a live predator. This indicates that actual predator presence outweighs the information content of an olfactory cue alone and that there is no increase in perceived risk toward a predator odor cue. Prey species then need different means to convey actual threatening or acute predation risk, allowing them to dynamically adjust their behavior to different threat levels (Duffield et al. 2017). This role is most likely covered by intra-species communication.

Intra-species communication and signaling about increased risk, for instance, through Schreckstoff (Frisch 1938) or alarm pheromones (henceforth AP), are evolutionarily widespread in many taxa (Bowers et al. 1972, Howe and Sheikh 1975, Stowe et al. 1995, Boissy et al. 1998, Beale et al. 2006, Gutiérrez-García et al. 2007). In several social species of fish, insects, and mammals, AP secretions are recognized as a signal to protect their colony, group, or family when in danger (Breed et al. 2004, Kiyokawa et al. 2004a, Gomes et al. 2013). Despite some papers raising concern about the categorization of APs, arguing that these chemicals cannot be classified as real pheromones (Magurran et al. 1996, Viney and

Franks 2004), the behavioral response is the same, given the correct context (Magurran et al. 1996). While for most mammals, the chemical structure of APs is still unknown, it has been identified in, for example, aphids (Bowers et al. 1972, Beale et al. 2006), sea anemones (Howe and Sheikh 1975), and several insects (Crewe and Blum 1970, Heath and Landolt 1988, Kuwahara et al. 1989). To fulfill their sensory warning role, APs should be volatile or hydrophilic (Kiyokawa et al. 2005, Inagaki et al. 2009). Given the majority of experiments on mammalian APs have been done on lab animals, their chemical structure has been described only for mice (C57BL/6J and OMP-GFP strains; Brechbühl et al. 2013) and Wistar rats (Inagaki et al. 2014). Brechbühl et al. (2013) state that both mouse APs and mammalian predator olfactory cues share structural similarities, specifically sulfur-containing molecules. In this paper, we utilize the concept of APs similarly as in previous studies, although we acknowledge that in most studies the chemical or biological nature of the different odors of stress is not yet properly determined.

High predation risk affects mating behaviors and reproductive success (Sih 1994, Ruxton and Lima 1997, Kokko and Ruxton 2000). There is strong support for the notion that predation risk negatively affects breeding success (Sih 1994). This is manifested as delayed breeding in bank voles and gray-sided voles (*Myodes rufocanus*; Mappes and Ylönen 1997, Fuelling and Halle 2004), hindering copulations in bank voles (*Myodes glareolus*; Ronkainen and Ylönen 1994), elevating stress levels in snowshoe hares (*Lepus americanus*; Sheriff et al. 2009), or decreasing weights of breeding individuals or their offspring in snowshoe hares and bank voles (Sheriff et al. 2009, Trebatická et al. 2012). However, the mechanisms and adaptive value of delayed or suppressed breeding under risk are not clear and continue to be debated (Ruxton and Lima 1997, Kokko and Ruxton 2000). Several publications have already explored the effects of increased risk of predation in parents' environment on offspring behavior and fitness, finding altered learning behavior in three-spine sticklebacks (*Gasterosteus aculeatus*; Roche et al. 2012, Feng et al. 2015), altered stress reaction in C57BL/6 mice and Long Evans rats (St-Cyr and McGowan 2015, St-Cyr et al. 2017), or changed foraging

strategies in Sprague Dawley rats (Chaby et al. 2015).

An alternative explanation suggests that parents will maximize reproductive efforts at all costs in risky conditions (henceforth terminal investment). In this scenario, individuals breeding in a risky environment will enhance, or speed up, reproduction in order to maximize fitness by producing a number of strong offspring despite the high costs for the parents' or mother's survival. If offspring survive and reach a fertile age, this then compensates for parental disappearance from the reproductive pool (Kokko and Ranta 1996, Kokko and Ruxton 2000). This strategy of bet-hedging or terminal investment has been shown in experimental studies in passerine birds breeding under increased predation risk (Mönkkönen et al. 2009) as well as in crickets (Adamo and McKee 2017). Additionally, it has been shown as a reaction to infections in ants and sparrows (Bonneaud et al. 2004, Giehr et al. 2017).

The relationship between weasels and voles has been intensively studied as the weasel is a specialist predator of rodents and is the major cause of mortality in boreal voles, especially during a population's decline (Korpimäki et al. 1991, Norrdahl and Korpimäki 1995, 2000). As an adaptation against the dramatic predation pressure by weasels, voles are able to detect the odor of mustelids as an antipredator measure and change their behavior accordingly. Bank voles decrease their movement and foraging when exposed to weasel odor (Ylönen 1989, Sundell and Ylönen 2004, Bleicher et al. 2018). They shift their activity times and spatial use to avoid weasels (Jędrzejewska and Jędrzejewski 1990, Jędrzejewski and Jędrzejewska 1990, Sundell et al. 2008) and use more arboreal escape under predation risk (Jędrzejewska and Jędrzejewski 1990, Mäkeläinen et al. 2014). In the study by Mäkeläinen et al. (2014), weasels rarely followed bank voles into a tree, if the bank vole climbed one, showing the efficiency of bank voles' antipredator responses.

Here, we studied in two experiments how increased predation risk, either direct risk in the form of least weasels (*Mustela nivalis nivalis*) odor, or indirect risk in the form of odor emitted by weasel-scared conspecifics, influenced behavior and reproductive investment in bank voles.

The effect was assessed in both behavioral trials and a breeding experiment with cue exposure of parents and monitoring subsequent offspring performance. Social cues, such as pheromones, have previously been shown to be sufficient to trigger cross-generational changes (Koyama et al. 2015). In order to differentiate between the effects of PO, AP, and mere social odor, we also used non-stressed conspecific bedding as a second control in addition to clean wood shavings.

In the behavioral experiment, we predicted that:

1. Voles would feel safer in control and social odor treatments and spend more time in boxes containing those treatments. This would lead to increased foraging in those treatments and foraging to be lowest in PO treatment and second lowest in AP treatment. This would be in accordance with previous studies (Osada et al. 2014, Sánchez-González et al. 2017).

In the fitness experiment, we predicted, based on the existing body of research, that predation cues have a detrimental effect on reproduction. Specifically, we predicted that:

2. The direct predation cue, PO, would decrease the breeding success of parent voles (measured as number of breeding females and litter size) more than AP.
3. Both predation cues, PO and AP, will decrease the number of breeding females and cause the production of smaller litters (Kokko and Ruxton 2000, Fuelling and Halle 2004).
4. Both PO and AP treatments will cause pups to be smaller (Sheriff et al. 2009, 2015).
5. There would be no effect of social odor or control odor on condition, breeding of parent voles, or size of offspring.

MATERIALS AND METHODS

Study species

Bank voles are common rodents in boreal forest areas. Vole populations cycle in Scandinavia and specialist predators have a large role in causing this cyclicity (Hanski et al. 2001). Regular high predation pressure in the wild maintains

bank vole antipredator behavior at a high level. The breeding season of the bank vole in central Finland usually begins at the end of April and lasts until September. During the breeding season, breeding female bank voles are strictly territorial and male territories overlap with several female territories (Bujalska 1973). The gestation period is about 20 d, after which 3–6 pups are born. These pups mature after 30 d.

The least weasel is a specialist predator of small mammals and lives in the same habitat as its prey. Due to its small size, the weasel is able to hunt in tunnels and burrows of voles during both summer and winter, leaving only a few safe places for the voles (Norrdahl and Korpimäki 1995, 2000). Weasels are adapted to the harsh winter conditions by a coat change in late autumn. The weasel, like all small mustelids, uses strong odors in its intraspecific communication, giving the prey a means to evaluate the current predation risk.

The studies were conducted in the laboratory at Konnevesi Research Station in Central Finland (62°37' N, 26°20' E). In the laboratory, the voles are kept in light and climate-controlled husbandry rooms with a 12-L:12-D daily cycle. The animals were kept individually in 42 × 26 × 15 cm transparent cages with wire mesh lids with ad libitum water and food supply. Each cage had wood shavings and hay as bedding. Males and females were kept in the same room. Study animals were the F1 generation of individuals housed in the lab during the winter months. The average initial weight of the voles was 16.3 g ± 2.8 g (mean ± SD). All animals were individually marked with ear tags (#1005-1L1, National Band & Tag Company, Newport, Kentucky, USA).

Weasels for the odor treatment were housed individually in 60 × 160 × 60 cm cages in an outdoor shelter. Each cage had a nest box and wood shavings and hay as bedding. During the experiment, weasels were fed dead bank voles.

Odor cues

For this experiment, the following odor cues were used:

Predator odor (PO): 1 mL of odor solution. The PO was obtained by collecting 6 dL of weasel bedding (wood shavings soiled with urine and fecal matter) and mixing it with 6 dL of

diethyl phthalate (CAS 84-66-2), a solvent for a broad variety of chemical substances and often used for fragrances (Api 2001). The mix was left overnight in a refrigerator, and the liquid phase was extracted after 24 h (±2 h). The odor solution was renewed every 7 d and stored in a stable temperature of +4°C (±0.2°C) in a refrigerator in-between application. The use of extracted olfactory cues allowed for even exposure to all animals and reduced the stress to our captive weasels. Alarm pheromone (AP): 1 dL of vole beddings from individuals directly exposed to a predator. To obtain AP, two male voles were individually exposed to a weasel for 1 min every other day. Each individual was placed in a wire mesh cage, which was then put directly into the weasel cage. The animal was immediately returned to its cage afterward. When the treatments were applied, all the bedding of both animals was thoroughly mixed together. If the voles were scared on the same day the treatments were applied, the bedding was collected at the earliest 1 h after the animal returned to its cage. Social odor (SO): 1 dL of vole beddings collected from two male voles that were not handled before collection nor exposed to weasel. The bedding of both animals was carefully mixed before application. The control (C) odor consisted of clean vole bedding, that is, fresh wood shavings changed between each trial. The odor cues were renewed for each trial.

Experimental design—behavioral assays

For the first experiment, we used 50 bank voles (28 males, 22 females). We applied two behavioral measures to study the response to olfactory cues in the voles: The first was a test measuring the individual's perceived risk using optimal patch use (Brown 1988, Lima and Dill 1990) and the other investigating spatial avoidance or preference.

Brown (1988) framed the harvest rate an animal makes at a given patch as a balance of the energetic gains and costs attributed to foraging effort, predation, and missed opportunity costs. The density of food remaining in a patch after the forager stops foraging is called a giving-up density (GUD; Brown 1999) and reflects the point where the energy remaining in the patch is equal to or outweighed by the combined costs to the forager. The GUD, as a method, has been

adapted to test a large variety of elements affecting the strategic decisions animals take (Bedoya-Perez et al. 2013) and has been widely applied as a measure for habitat use (Ylönen et al. 2002, Orrock et al. 2004, Bleicher 2017, Bleicher et al. 2018).

Each individual was placed in a 190 × 190 cm cross-shaped system (Appendix S1: Fig. S1) for three hours. At the center of the cross is a release cage (20 × 20 cm). Going outward in the four horizontal directions, the odor chamber is connected via an opaque tube (10 cm long, 4 cm diameter) to an antechamber (30 × 20 cm) with a metal grid lid. This prevented the odors entering and mixing in the central area of the maze and minimized the chances of an odor contamination. From there, going outward, an opaque tube (5 cm long, 4 cm diameter) led to a closed and opaque odor chamber (40 × 25 cm). This tube was considered as part of the odor chamber for the analysis. Each odor chamber contained one of four odor treatments together with a box acting as a foraging patch (henceforth patch). The odor cues were attached to the lid of the chamber to avoid contaminations by the vole and renewed for each trial. PO was applied to filter paper (article no. 120002, grade 1001; Munktell Filter AB, Falun, Sweden). The spatial orientation of the odors was randomly changed for each trial to avoid a spatial bias. Two mazes were used simultaneously, both were located in a dimly lit room 2 m apart. The ventilated experimental room was 7.5 × 7.5 m with the height of 4 m, allowing a large overhead space to dilute escaping odors from the systems. The experiment was performed during day time. Two trials were run simultaneously for a total of four to six trials per day. After each trial, every segment of the maze was cleaned with denatured ethanol (70%) and dried, to avoid odor contamination between trials.

The design of the patches was a lidless box (19 × 19 × 6 cm) containing 8 dL of sand into which 20 husked sunflower seeds were mixed. Each animal was allowed to forage in the system for three hours (henceforth trial). The optimal trial length was determined beforehand with pilot trials. After each trial, the sand was sieved and the remaining untouched seeds were counted to obtain the GUD. To avoid cross-contamination of olfactory treatments, the sand was

left to air out for 3 d between trials. Bytheway et al. (2013) showed that even though predator odor still elicited increased investigative behavior after 24 h, it no longer elicited a change in foraging behavior. Based on this, it seems reasonable to assume that if the voles were still able to detect the odor after 72 h, the information conveyed drastically changed. To encourage foraging in the novel systems, the animals were starved for three hours prior to each trial.

Each trial was recorded using a GoPro4 for later analysis. During the video analysis, the following parameters were measured for each of the four arms: choice of the first odor box entered, time spent in the connection tubes, and time spent in the odor box. The first hour of each trial was analyzed separately from the whole duration to account for a possible habituation effect.

Experimental design—trans-generational effects

The 240 bank voles (120 males, 120 females) were divided equally into four treatment groups for the second experiment. Prior to grouping the animals, every individual was weighed and the dominance of the male individuals was assessed following the urine marking of males as described by Horne and Ylönen (1996) and Klemme et al. (2006). The males were placed in the urine marking arena for 4 h and had access to a small amount of food and water. The urine markings were analyzed twice by two observers independently and the average score was recorded. Each individual received a dominance score from 1 (no marking, a subordinate male) to 6 (markings all over the arena, a dominant male).

During the group assignment, we made sure that all treatment groups consisted of an equal number of males and females, the weight distribution for each sex was similar and that the dominance distribution for each treatment was similar. Within these constraints, the animals were assigned randomly into four different husbandry rooms.

The voles were kept in the rooms for seven days to acclimate to their new husbandry rooms.

The treatments consisted of the following four odor cues (measurements per cage). Predator odor (PO): 1 mL of odor solution on filter paper (article no. 120002, grade 1001; Munktell Filter AB, Falun, Sweden), Alarm pheromone (AP):

1 dL of male vole beddings from scared individuals, social odor (SO): 1 dL of male vole beddings and control (C): 1 dL of dry, aired wood shavings. Each treatment was directly applied through the lid of the cage, without handling the animal or the cage itself. Treatments were applied three times per week for a total of seven weeks. The treatments were collected and prepared identically to what was outlined before. All animals were moved to clean cages after the mating phase. This is the standard procedure in our laboratory. It allows the pregnant female to build a nest in a cage free of the odor of a male conspecific. Furthermore, it reduces the need to disturb the female to clean its cages during pregnancy/lactation.

After the first week, the animals within the treatment were randomly paired for mating, avoiding pairing of first-degree siblings. For pairing, the animals were housed in a joined cage for seven days. From 18 d on after the beginning of the pairing, female cages were checked for pups twice per day. When litters were found each pup was weighed one day after birth and the size of the litter was recorded. The treatments were stopped as soon as all pregnant individuals had given birth. All individuals were weighed again and the dominance of the males was reassessed. The females were weighed again 5 d after giving birth. The experiment and all measurements ended at this point.

At the end of the habituation, prior to the odor treatment, fecal samples were collected from all voles for stress analysis. The voles were put individually in smaller cages without bedding for a maximum of three hours, after which all fecal pellets not contaminated with urine were collected into Eppendorf tubes then stored at -20°C . This procedure was repeated for all individuals, including nursing females, after the treatment was stopped. Corticosterone metabolites in the samples were analyzed following the method outlined by Sipari et al. (2017) at the University of Veterinary Medicine in Vienna.

Statistical analyses

All statistical analyses were performed in R (R Core Team 2018). Plots were generated with ggplot2 (Wickham 2009) and ggsignif (Ahlmann-Eltze 2017). To analyze the directional choice of voles as they entered the behavioral assays, a

multinomial log-linear regression (MLM), package nnet (Venables and Ripley 2002), was run. This was combined with a Wald z-test to determine *P*-values, package AER (Kleiber and Zeileis 2008). In order to analyze not only the distribution of litter sizes between treatments but also the differences in successful pregnancies, and GUDs, zero augmented generalized linear models, from the package pscl (Zeileis et al. 2008) were used. The time spent in each compartment, the differences in weight, the weight of the pups, and the difference in stress metabolites were analyzed with a linear model (LM) or linear mixed model (LMM) for repeated measurements, packages lme4 (Bates et al. 2014) and lmerTest (Kuznetsova et al. 2017). Other measurements were analyzed with linear or generalized (mixed) models, depending on the measurement in question. Data points with missing observation were excluded from the data set, resulting in an effective sample size for the statistical tests of 93 breeding pairs for the breeding success part of the experiment.

For each analysis, the most complex model included an interaction between Treatment and Sex. Other factors, such as litter size and weight, were added to the most complex model if appropriate, but never in interaction with other factors. To achieve the best model fit, first the interaction was removed, then other factors, only leaving Treatment for the simplest model. The individual animal was always included as a random factor in analyses with repeated measurements. Each treatment was compared to the C (control) treatment. For each analysis, the most fitting distribution and model were chosen based on AICc, package MuMIn (Barton 2018). A model was considered the best if the difference in AICc from the next model was greater than 2.5. In the cases where there was no clear best model, all models within a ΔAICc of 2.5 were weighed based on their differences to the best fitting model and weighed averages of the parameter estimates were reported. The tables with all fitted models for each statistical test can be found in Appendix S1: Tables S1–S14.

RESULTS

Foraging behavior and giving-up densities

The first choice of animals did not show a significant preference for or avoidance of entering

any specific odor compartment. However, we found a tendency (MLM, $P = 0.056$, $df = 3$, $n = 46$) that voles were 2.25 times more likely to enter the SO compartment first. Otherwise, it seems likely that the sex of the individual had a negligible role in the decision to enter either odor compartment of the maze, as it was not included in the best model.

In contrast to the first choice for odors, we found that over the full experimental duration of three hours there was a significant interaction between the sex of the vole and the time spent in the AP tube (LMM, $P = 0.031$, $df = 10$, $n = 50$, Fig. 1). When both sexes were analyzed together, female voles spent on average 2.5 min (147.5 s) longer in the AP tubes compared to the males. When the two sexes were analyzed separately, male voles did not show a preference or avoidance for the tubes (LMM, $P > 0.05$, $df = 6$, $n = 28$), but females spent two minutes (121.4 s) longer in the tubes connecting the AP compartment (LMM, $P = 0.027$, $df = 6$, $n = 22$) compared to the tubes leading to C compartment (close to four minutes, 225.2 s). For the time spent in the odor compartment, there are no significant differences for the whole trial (LMM, $P > 0.05$, $df = 10$, $n = 50$).

The analysis of the GUD showed that about 1.1 seeds more (weighted average) were

harvested from the AP compartment compared to control independent of the animal's sex (GLMM, Poisson, $P = 0.019$, $df = 5$, 6 , $n = 50$, Fig. 2).

Effect on parents and offspring

Weight change in parental generation.—On average, female voles gained 0.92 g in weight during the experiment. However, the weight gain of females was solely dependent on the number of pups born, as for every additional pup the females gained 1.2 g of weight (LM, $P < 0.001$, $n = 93$, $df = 3$) and was not affected by the treatments (Appendix S1: Fig. S2).

The two best models show a significant (LM, $P = 0.023$, $n = 120$, $df = 5$, 6 , $n = 93$) weight increase for the males in the SO treatment. The weighted average of those models indicates that the male voles in the SO treatment gained about 1.49 g more weight than males in the control group (Fig. 3). Change in male dominance may also play a role in the weight change since it was included in the second best model. Our treatments, however, did not affect male dominance (LM, $P > 0.05$, $df = 5$, 6 , $n = 93$).

Breeding success and offspring weight.—During the experiment, a total of 74 litters were born from 120 breeding pairs. The analysis of the litter

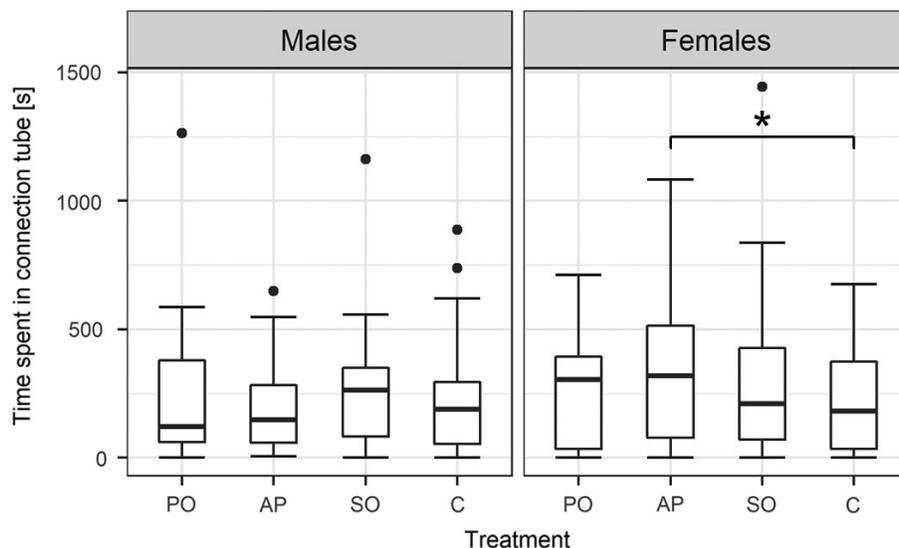


Fig. 1. Time spent in the connective tube by sex and treatment. Females reacted significantly different from males to AP ($P < 0.05$). Asterisks indicate a significant difference from C at $P < 0.05$.

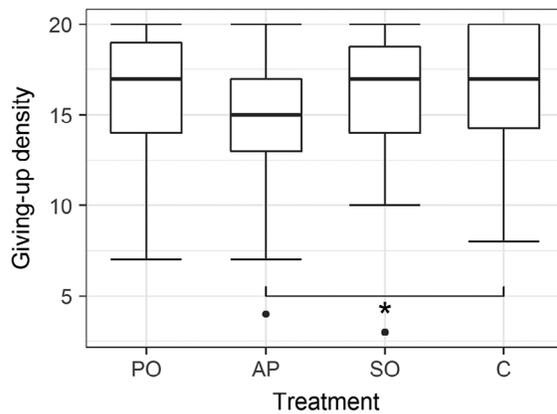


Fig. 2. Giving-up density by treatment. Asterisk indicates a significant difference from control at $P > 0.05$.

rate per treatment showed a clear best model. Significantly, more female voles gave birth under the AP treatment compared to those in the control treatment (Hurdle GLM, Poisson & Binomial, $P = 0.0095$, $df = 8, 10$, $n = 93$). About 36.8% of the females in the control treatment successfully gave birth, whereas about 84.5% of those under AP treatment gave birth (weighted averages, Fig. 4).

Seventy-four litters resulted in a total of 290 pups across all treatments. There were no significant differences in litter sizes between the treatment groups (Hurdle GLM, Poisson & Binomial, $P > 0.05$, $df = 8, 10$, $n = 93$). However, pups

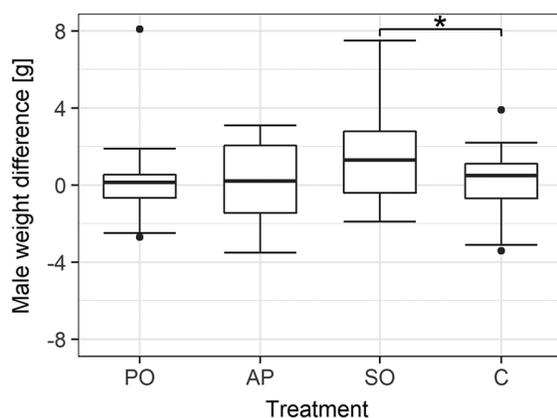


Fig. 3. Boxplots of the weight change of male voles separated by treatment. Asterisks symbolize a significant difference from the control group at $P > 0.05$.

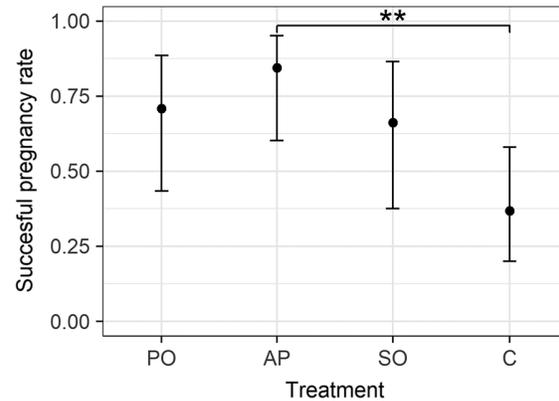


Fig. 4. Female average pregnancy rates for each treatment weighted by AICc. Whiskers show the 95% confidence intervals. Asterisks symbolize a significant difference from the control group at $P > 0.01$.

from the PO group weighed about 2.81 g and were significantly heavier one day after delivery than the control pups which weighed about 2.44 g (LMM, $P = 0.026$, $df = 6, 7$, $n = 262$ pups, 64 mothers, Fig. 5). This was independent of litter size.

Fecal corticosterone metabolite levels.—From the 240 experimental animals, we non-invasively collected pre- and post-exposure fecal samples from 230 individuals. The difference of stress metabolites per 50 mg of fecal matter between the two measurements was assessed with a LM. No clear

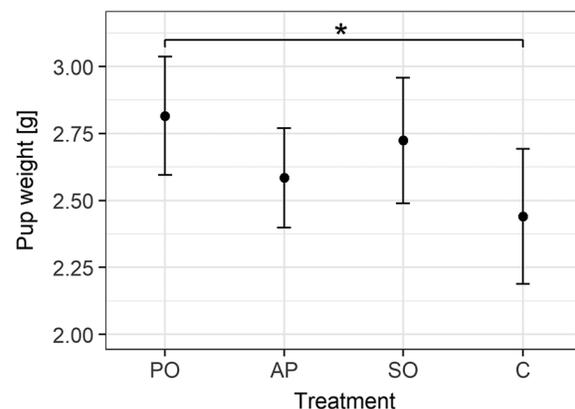


Fig. 5. Weighted (by AICc) model averages of the individual pup weight at day 1. Whiskers show 95% confidence intervals. Asterisk indicates a significant difference at $P < 0.05$.

best model was found; however, both models within the AICc range show a significant difference between the C and SO treatments (LM, $P = 0.037$, $n = 225$). In the control treatment stress metabolites rose by 82.7 ng/50 mg but in the SO treatment the metabolites only rose by 32.5 ng/50 mg (weighted averages by AICc, Fig. 6).

DISCUSSION

Our study brings new insights into the complex system of predator–prey interactions. We propose a novel way how prey can determine predator presence and how prey change their behavior in response to olfactory cues. First, we show that the voles were able to distinguish between an ambient or conservative level risk, that is, PO (Duffield et al. 2017) and a cue of an acute or reliable risk signaling life-threatening imminent possibility of predator attack, that is, AP. Second, we also show how these different

perceived threat levels differently affected reproductive investment and success. In the AP treatment, most of the females were breeding, and in the PO treatment, voles were investing in larger pup size.

Compared to the information that a direct predator odor presents a more long-lasting habitat level risk, the acute risk information in the form of odor of a recently scared conspecific vole, seemed to outweigh in importance the weasel odor. Actually, the conspecific carried cues may include both predation risk levels, as the predator must have been close enough to scare a prey individual, who carries then the immediate threat cue to other conspecifics. Non-olfactory conspecific cues, for example, vocalizations, have already been known to convey information about predator presence (Blanchard et al. 1991, Barati and McDonald 2017, Forti et al. 2017), the perceived risk, or even the identity of the predator (Manser et al. 2002, Ouattara et al. 2009, Barati and McDonald 2017, Collier et al. 2017). Thus, in the total assessment of predation risk both cues may well be complimentary: Predator odor increases vigilance from the base level and a scared conspecific vole having survived a close encounter with a predator may signal more accurately and more rapidly for a group of conspecifics that the real danger is acute and near.

We found females treated with both PO and AP showed a positive response in their reproductive states compared with control females. This manifested itself in two major ways: (1) AP-treated females had a higher successful insemination rate, and (2) PO-treated parents had heavier pups shortly after birth. This further indicates that both predator presence cues and alarm cues from conspecifics can work at the same time, both to increase vigilance but also to trigger enhancement of reproduction in the form of terminal investment. Contradictory to our expectations derived from previous experiments (Ylönen and Ronkainen 1994, Fuelling and Halle 2004, Haapakoski et al. 2012), voles' breeding effort increased under elevated predation risk. However, our results are in accordance with Haapakoski et al. (2018) where female bank voles had larger litters in the AP-treated field enclosures compared to social odor treated females.

We cannot rule out that AP produced by our voles exposed to predator might communicate

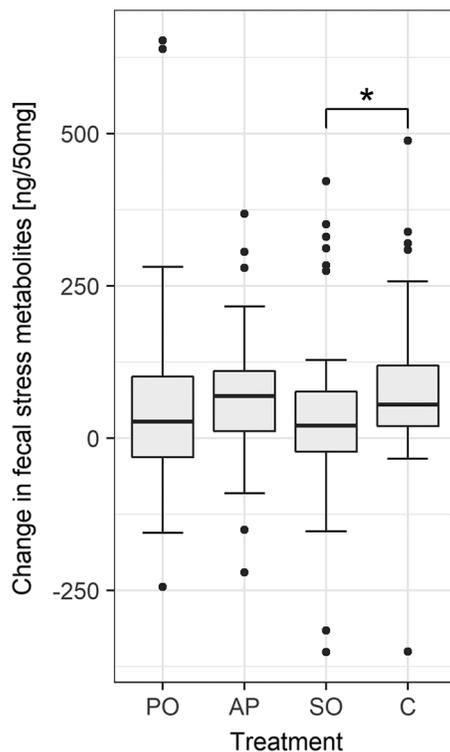


Fig. 6. Difference in fecal stress metabolites pre- and post-treatment per 50 mg fecal matter. Asterisk indicates a significant difference at $P < 0.05$.

more than just alarm. We do not know yet what kind of physiological processes are involved in the odor production of a scared individual; that is, the odor may be a combination of being scared but also relief due to being able to escape predation. This issue needs further studying. In fact, at least two studies show that male mice exposed to competitors or predators are more attractive to females. In the first one, chronic exposure of cat odor enhanced aggression, urinary attractiveness, and sex pheromones in mice (Zhang et al. 2008). In the second one, chronic co-housing with rats increased the competitiveness of male mice and their urines were more attractive to females (Liu et al. 2017). Liu et al. (2017) also found that the levels of major urinary proteins (MUP) and some volatile pheromones were increased in the co-species-housed mouse urine, along with their serum testosterone levels. It is known that MUP functions as a pheromone and stimulates sexual attraction (Roberts et al. 2010) and estrus in female mice (Marchlewska-Koj et al. 2000). We have an ongoing bioassay study for clarifying and analyzing the body odor compounds of AP voles compared to non-disturbed voles. After this information, we hope to find more answers to the role of MUP and AP on the vole reproduction.

The behavioral experiment suggested that the voles were more likely to inspect the maze arm containing the AP cue than the arm containing the control cue. In Haapakoski et al. (2018), vole females also preferred the AP odor compared to SO while males preferred SO over AP odor. This is also partly reflected in our result that female voles spent significantly more time in the maze arm leading to the AP compartment compared to males and significantly more time compared to the control. We attribute this to exploring the arms to gain information from the signal (Barocas et al. 2016, Parsons et al. 2017). In contrast, AP cue enhanced foraging compared to C, causing lower GUDs in the experimental patches containing the AP, which is suggestive of lower vigilance (Embar et al. 2011). Further, this could be a result of a heightened energetic need (Arenz and Leger 2000) and the first indicator of terminal investment.

Increasing reproductive investment despite severe negative changes in the breeding environment seems maladaptive at first glance.

However, Duffield et al. (2017) propose a new dynamic model for adaptive reproductive strategies. At low-to-medium perceived risk levels, reproduction is affected negatively, as parents invest in own survival. Above a certain threshold a coping mechanism, that is, terminal investment, would be triggered to compensate for the loss in an individual's own reproductive value. A similar idea is described with the insurance hypothesis, where individuals increase their reproductive investment in anticipation of an unfavorable environment (Promislow and Harvey 1990, Forbes 1991, Houston et al. 2012). The increased number of offspring or increase in fertility is designed to counteract expected low survival chances of offspring. While this has been mainly shown in birds (Anderson 1990a, Forbes 1990), there is also evidence in humans (Anderson 1990b, Strassmann and Gillespie 2002). As the majority of studies about terminal investment in mammals focuses on the aspect of senescence (e.g., Ericsson et al. 2001, Hoffman et al. 2010, Weladji et al. 2010), it is difficult to assess the benefits of this strategy on an evolutionary scale considering high predation pressure. We invite others to investigate this phenomenon further and to incorporate it into existing evolutionary frameworks.

For our study species, the bank vole, Eccard et al. (2011) describe a similar pattern for a dramatic increase in breeding effort after a critical threshold vole density was surpassed. Breeding bank vole females require a breeding territory and if the breeding habitat is occupied, the surplus females cannot breed. In the Eccard et al. (2011) study, the number of females was gradually increased from normal to four times the sustainable number of territory owners. As the density of females became far too high and no opportunities for an individual's own breeding territory existed anymore, all females started to breed regardless of costs (Eccard et al. 2011). This was explained by incomplete control of a social behavior (Reeve et al. 1998). Similarly, Ylönen et al. (2002) found in their study with Australian house mouse during a plague, that despite an extremely high predation risk, mice were taking high risks in exploiting food sources in open habitats with a diverse guild of predators including mammals, birds, and snakes. As the number of competitors becomes intolerably high

and food becomes scarce, risk-taking is the only solution. The authors described the desperate behavior of the mice as Stalingrad effect, after the behavior of desperate soldiers during the siege of Stalingrad in World War 2. While those two studies do not investigate the effect of predator odor exposure, they investigate the results of extreme stress situations due to crowding and social cues or crowding and direct predation risk. It is conceivable that the underlying mechanism is similar to what we found in our study in giving up a conservative strategy for risky strategies under high risk.

Levels of stress hormone metabolites rose significantly less in the SO, compared to C, while no change was observed for the two predation cues. This was unexpected as the SO, signaling competition environment, was affecting male weights as discussed below. It also contradicts our hypothesis as we expected to see a strong increase in those metabolites under both predator cues. However, as the treatment period lasted a total of seven weeks, it is possible that the treatments elicited an initial spike in corticosterone metabolites, but then adapted to the new perceived stress level, causing only mildly elevated levels due to new environment and handling. With this in mind, it was interesting to see that the SO treatment, the odor of an unstressed conspecific male, reduced the increase in stress hormone metabolites. This could be caused by social buffering, the decreased impact of stress by a social interaction. It has been shown before in rodents that the presence of an unfamiliar conspecific is sufficient to cause social buffering (Terranova et al. 1999, Kiyokawa et al. 2004b, Klein et al. 2015, Kiyokawa and Hennessy 2018). A potential explanation is that voles are potentially able to adapt to a prolonged period of stress, while the benefits of social interactions do not vanish with prolonged exposure.

The weight of the voles varied significantly throughout the experiment. The changes we observed suggest that there is a difference in how the sexes respond to different cues. In females, the strongest effect was offspring care—the larger the litter, the greater the weight increase. However, we also found an effect of conspecific cues. Only the odor cue of conspecific rival males caused an increase of weight for the males. The SO treatment possibly simulated a

high-density environment with increasing intrasexual competition in males. So it might be favorable for the males to invest in growth in order to outcompete competitors, as shown in Kalahari meerkats (*Suricata suricatta*; Huchard et al. 2016). It is therefore interesting that the alarm pheromone treatment, which is the odor of a stressed/anxious male vole, failed to elicit a similar response. Thus, it is possible that the addition of the external risk pheromone cancels out the effect of the social cue, as alarm pheromones are only excreted in extreme situations.

Our study contributes to the picture of how mammals with highly developed olfactory senses can interpret the information carried in olfactory signals correctly and are able to differentiate between ambient level predation risk and socially validated risk. We collected evidence that voles are able to gather information about acute risk levels from conspecific odor cues, which in turn triggered a higher successful insemination rate and heavier offspring. This is in accordance with terminal investment ideas. If a female takes the risk of investing in producing offspring (Ylönen and Ronkainen 1994 vs. Kokko and Ranta 1996), then it pays to put all efforts into breeding (Mönkkönen et al. 2009). As predator odor and alarm pheromones yield such different results, we propose that both odors provide the voles with different information, that is, the predator odor simulates a low-to-medium threat environment, but the alarm pheromone clearly represents a high and immediate threat.

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LITERATURE CITED

Adamo, S. A., and R. McKee. 2017. Differential effects of predator cues versus activation of fight-or-flight behaviour on reproduction in the cricket *Gryllus texensis*. *Animal Behaviour* 134:1–8.

- Ahlmann-Eltze, C. 2017. ggsgnif: significance Brackets for “ggplot2”. <https://cran.r-project.org/web/packages/ggsgnif/>
- Anderson, D. J. 1990a. Evolution of obligate siblicide in boobies. 1. A test of the insurance-egg hypothesis. *American Naturalist* 135:334–350.
- Anderson, D. J. 1990b. On the evolution of human brood size. *Evolution* 44:438–440.
- Apfelbach, R., C. D. Blanchard, R. J. Blanchard, R. A. Hayes, and I. S. McGregor. 2005. The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neuroscience & Biobehavioral Reviews* 29:1123–1144.
- Apfelbach, R., M. H. Parsons, H. A. Soini, and M. V. Novotny. 2015. Are single odorous components of a predator sufficient to elicit defensive behaviors in prey species? *Frontiers in Neuroscience* 9:263.
- Api, A. M. 2001. Toxicological profile of diethyl phthalate: a vehicle for fragrance and cosmetic ingredients. *Food and Chemical Toxicology* 39:97–108.
- Arenz, C. L., and D. W. Leger. 2000. Antipredator vigilance of juvenile and adult thirteen-lined ground squirrels and the role of nutritional need. *Animal Behaviour* 59:535–541.
- Barati, A., and P. G. McDonald. 2017. Nestlings reduce their predation risk by attending to predator-information encoded within conspecific alarm calls. *Scientific Reports* 7:11736.
- Barocas, A., H. N. Golden, M. W. Harrington, D. B. McDonald, and M. Ben-David. 2016. Coastal latrine sites as social information hubs and drivers of river otter fission–fusion dynamics. *Animal Behaviour* 120:103–114.
- Barton, K. 2018. MuMIn: multi-Model Inference. <https://cran.r-project.org/web/packages/MuMIn/>
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1–48.
- Beale, M. H., et al. 2006. Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behavior. *Proceedings of the National Academy of Sciences* 103:10509–10513.
- Bedoya-Perez, M. A., A. J. R. Carthey, V. S. A. Mella, C. McArthur, and P. B. Banks. 2013. A practical guide to avoid giving up on giving-up densities. *Behavioral Ecology and Sociobiology* 67:1541–1553.
- Blanchard, R. J. J., D. C. C. Blanchard, R. Agullana, and S. M. Weiss. 1991. Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. *Physiology & Behavior* 50:967–972.
- Bleicher, S. S. 2017. The landscape of fear conceptual framework: definition and review of current applications and misuses. *PeerJ* 5:e3772.
- Bleicher, S. S., H. Ylönen, T. Kämpylä, and M. Haapakoski. 2018. Olfactory cues and the value of information: Voles interpret cues based on recent predator encounters. *Behavioral Ecology and Sociobiology* 72:187–199.
- Boissy, A., C. Terlouw, and P. Le Neindre. 1998. Presence of cues from stressed conspecifics increases reactivity to aversive events in cattle: evidence for the existence of alarm substances in urine. *Physiology and Behavior* 63:489–495.
- Bonneaud, C., J. Mazuc, O. Chastel, H. Westerdahl, and G. Sorci. 2004. Terminal investment induced by immune challenge and fitness traits associated with major histocompatibility complex in the house sparrow. *Evolution* 58:2823–2830.
- Bowers, W., L. Nault, and R. Webb. 1972. Aphid alarm pheromone: isolation, identification, synthesis. *Science* 177:1–2.
- Brechbühl, J., et al. 2013. Mouse alarm pheromone shares structural similarity with predator scents. *Proceedings of the National Academy of Sciences* 110:4762–4767.
- Breed, M. D., E. Guzmán-Novoa, and G. J. Hunt. 2004. Defensive behavior of honey bees: organization, genetics, and comparisons with other bees. *Annual Review of Entomology* 49:271–298.
- Brown, J. S. 1988. Patch use as an indicator of habitat preference, predation risk, and competition. *Behavioral Ecology and Sociobiology* 22:37–47.
- Brown, J. S. 1999. Vigilance, patch use and habitat selection: foraging under predation risk. *Evolutionary Ecology Research* 1:49–71.
- Brown, G. E., C. K. Elvidge, I. Ramnarine, M. C. O. Ferrari, and D. P. Chivers. 2015. Background risk and recent experience influences retention of neophobic responses to predators. *Behavioral Ecology and Sociobiology* 69:737–745.
- Bujalska, G. 1973. The role of spacing behavior among females in the regulation of reproduction in the bank vole. *Journal of Reproduction and Fertility Supplement* 19:465–474.
- Bytheway, J. P., A. J. R. Carthey, and P. B. Banks. 2013. Risk vs. reward: how predators and prey respond to aging olfactory cues. *Behavioral Ecology and Sociobiology* 67:715–725.
- Chaby, L. E., M. J. Sheriff, A. M. Hirrlinger, and V. A. Braithwaite. 2015. Does early stress prepare individuals for a stressful future? Stress during adolescence improves foraging under threat. *Animal Behaviour* 105:37–45.
- Collier, K., A. N. Radford, S. W. Townsend, and M. B. Manser. 2017. Wild dwarf mongooses produce general alert and predator-specific alarm calls. *Behavioral Ecology* 28:1293–1301.

- Conover, M. 2007. Predator-prey dynamics. CRC Press, Boca Raton, Florida, USA.
- Cox, T. E., P. J. Murray, G. P. Hall, and X. Li. 2010. Pest responses to odors from predators fed a diet of target species conspecifics and heterospecifics. *Journal of Wildlife Management* 74:1737–1744.
- Creel, S., and D. Christianson. 2008. Relationships between direct predation and risk effects. *Trends in Ecology and Evolution* 23:194–201.
- Crewe, R. M., and M. S. Blum. 1970. Identification of the alarm pheromones of the ant *Myrmica brevinodis*. *Journal of Insect Physiology* 16:141–146.
- Dielenberg, R. A., and I. S. McGregor. 2001. Defensive behavior in rats towards predatory odors: a review. *Neuroscience & Biobehavioral Reviews* 25:597–609.
- Duffield, K. R., E. K. Bowers, S. K. Sakaluk, and B. M. Sadd. 2017. A dynamic threshold model for terminal investment. *Behavioral Ecology and Sociobiology* 71:185.
- Eccard, J. A., K. Fey, B. A. Caspers, and H. Ylönen. 2011. Breeding state and season affect interspecific interaction types: indirect resource competition and direct interference. *Oecologia* 167:623–633.
- Elmeros, M., J. K. Winbladh, P. N. Andersen, A. B. Madsen, and J. T. Christensen. 2011. Effectiveness of odour repellents on red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*): a field test. *European Journal of Wildlife Research* 57:1223–1226.
- Embar, K., B. P. Kotler, and S. Mukherjee. 2011. Risk management in optimal foragers: the effect of sightlines and predator type on patch use, time allocation, and vigilance in gerbils. *Oikos* 120:1657–1666.
- Ericsson, G., K. Wallin, J. P. Ball, and M. Broberg. 2001. Age-related reproductive effort and senescence in free-ranging moose, *Alces alces*. *Ecology* 82:1613–1620.
- Feng, S., K. E. McGhee, and A. M. Bell. 2015. Effect of maternal predator exposure on the ability of stickleback offspring to generalize a learned colour-reward association. *Animal Behaviour* 107:61–69.
- Forbes, L. S. 1990. Insurance offspring and the evolution of avian clutch size. *Journal of Theoretical Biology* 147:345–359.
- Forbes, L. 1991. Insurance offspring and brood reduction in a variable environment: the costs and benefits of pessimism. *Oikos* 62:325–332.
- Forti, L. R., A. B. B. S. Forti, R. Márquez, and L. F. Toledo. 2017. Behavioural response evoked by conspecific distress calls in two neotropical treefrogs. *Ethology* 123:942–948.
- Frisch, K. v. 1938. Zur Psychologie des Fisch-Schwarmes. *Naturwissenschaften* 26:601–606.
- Fuelling, O., and S. Halle. 2004. Breeding suppression in free-ranging grey-sided voles under the influence of predator odour. *Oecologia* 138:151–159.
- Giehr, J., A. V. Grasse, S. Cremer, J. Heinze, and A. Schrempf. 2017. Ant queens increase their reproductive efforts after pathogen infection. *Royal Society Open Science* 4:170547.
- Gomes, L. A. P., P. M. P. Salgado, E. N. Barata, and A. P. P. Mira. 2013. Alarm scent-marking during predatory attempts in the Cabrera vole (*Microtus cabreræ* Thomas, 1906). *Ecological Research* 28:335–343.
- Gutiérrez-García, A. G., C. M. Contreras, M. R. Mendoza-López, O. García-Barradas, and J. S. Cruz-Sánchez. 2007. Urine from stressed rats increases immobility in receptor rats forced to swim: role of 2-heptanone. *Physiology and Behavior* 91:166–172.
- Haapakoski, M., A. A. Hardenbol, and K. D. Matson. 2018. Exposure to chemical cues from predator-exposed conspecifics increases reproduction in a wild rodent. *Scientific Reports* 8:17214.
- Haapakoski, M., J. Sundell, and H. Ylönen. 2012. Predation risk and food: opposite effects on overwintering survival and onset of breeding in a boreal rodent: predation risk, food and overwintering. *Journal of Animal Ecology* 81:1183–1192.
- Hanski, I., H. Henttonen, E. Korpimäki, L. Oksanen, and P. Turchin. 2001. Small-rodent dynamics and predation. *Ecology* 82:1505–1520.
- Heath, R. R., and P. J. Landolt. 1988. The isolation, identification and synthesis of the alarm pheromone of *Vespula squamosa* (Drury) (Hymenoptera: Vespidae) and associated behavior. *Experientia* 44:82–83.
- Hoffman, C. L., J. P. Higham, A. Mas-Rivera, J. E. Ayala, and D. Maestripieri. 2010. Terminal investment and senescence in rhesus macaques (*Macaca mulatta*) on Cayo Santiago. *Behavioral Ecology* 21:972–978.
- Horne, T. J., and H. Ylönen. 1996. Female bank voles (*Clethrionomys glareolus*) prefer dominant males; but what if there is no choice? *Behavioral Ecology and Sociobiology* 38:401–405.
- Houston, A. I., P. C. Trimmer, T. W. Fawcett, A. D. Higginson, J. A. R. Marshall, and J. M. McNamara. 2012. Is optimism optimal? Functional causes of apparent behavioural biases. *Behavioural Processes* 89:172–178.
- Howe, N., and Y. Sheikh. 1975. Anthopleurine: a sea anemone alarm pheromone. *Science* 189:386–388.
- Huchard, E., S. English, M. B. V. Bell, N. Thavarajah, and T. Clutton-Brock. 2016. Competitive growth in a cooperative mammal. *Nature* 533:532–534.
- Inagaki, H., Y. Kiyokawa, S. Tamogami, H. Watanabe, Y. Takeuchi, and Y. Mori. 2014. Identification of a

- pheromone that increases anxiety in rats. *Proceedings of the National Academy of Sciences* 111:18751–18756.
- Inagaki, H., K. Nakamura, Y. Kiyokawa, T. Kikusui, Y. Takeuchi, and Y. Mori. 2009. The volatility of an alarm pheromone in male rats. *Physiology and Behavior* 96:749–752.
- Jędrzejewska, B., and W. Jędrzejewski. 1990. Antipredatory behaviour of bank voles and prey choice of weasels – enclosure experiments. *Annales Zoologici Fennici* 27:321–328.
- Jędrzejewski, W., and B. Jędrzejewska. 1990. Effect of a predator's visit on the spatial distribution of bank voles: experiments with weasels. *Canadian Journal of Zoology* 68:660–666.
- Kats, L. B., and L. M. Dill. 1998. The scent of death: chemosensory assessment of predation risk by prey animals. *Écoscience* 5:361–394.
- Kiyokawa, Y., and M. B. Hennessy. 2018. Comparative studies of social buffering: a consideration of approaches, terminology, and pitfalls. *Neuroscience and Biobehavioral Reviews* 86:131–141.
- Kiyokawa, Y., T. Kikusui, Y. Takeuchi, and Y. Mori. 2004a. Alarm pheromones with different functions are released from different regions of the body surface of male rats. *Chemical Senses* 29:35–40.
- Kiyokawa, Y., T. Kikusui, Y. Takeuchi, and Y. Mori. 2004b. Partner's stress status influences social buffering effects in rats. *Behavioral Neuroscience* 118:798–804.
- Kiyokawa, Y., T. Kikusui, Y. Takeuchi, and Y. Mori. 2005. Alarm pheromone that aggravates stress-induced hyperthermia is soluble in water. *Chemical Senses* 30:513–519.
- Kleiber, C., and A. Zeileis. 2008. *Applied econometrics with R*. Springer-Verlag, New York, New York, USA.
- Klein, B., V. Bautze, A. M. Maier, J. Deussing, H. Breer, and J. Strotmann. 2015. Activation of the mouse odorant receptor 37 subsystem coincides with a reduction of novel environment-induced activity within the paraventricular nucleus of the hypothalamus. *European Journal of Neuroscience* 41:793–801.
- Klemme, I., J. A. Eccard, and H. Ylönen. 2006. Do female bank voles (*Clethrionomys glareolus*) mate multiply to improve on previous mates? *Behavioral Ecology and Sociobiology* 60:415–421.
- Kokko, H., and E. Ranta. 1996. Evolutionary optimality of delayed breeding in voles. *Oikos* 77:173.
- Kokko, H., and G. D. Ruxton. 2000. Breeding suppression and predator-prey dynamics. *Ecology* 81:1178.
- Korpimäki, E., K. Norrdahl, and T. Rinta-Jaskari. 1991. Responses of stoats and least weasels to fluctuating food abundances: Is the low phase of the vole cycle due to mustelid predation? *Oecologia* 88:552–561.
- Koyama, S., et al. 2015. Cross-generational impact of a male murine pheromone 2-sec-butyl-4,5-dihydrothiazole in female mice. *Proceedings of the Royal Society of London B: Biological Sciences* 282:2015.1074.
- Krebs, C. J., S. Boutin, R. Boonstra, A. R. E. Sinclair, J. N. M. Smith, M. R. T. Dale, K. Martin, and R. Turkington. 1995. Impact of food and predation on the snowshoe hare cycle. *Science* 269:1112–1115.
- Kuwahara, Y., W. S. Leal, Y. Nakano, Y. Kaneko, H. Nakao, and T. Suzuki. 1989. Pheromone study on astigmatid mites: XXIII. Identification of the alarm pheromone on the acarid mite, *Tyrophagus neiswanderi* and species specificities of alarm pheromones among four species of the same genus. *Applied Entomology and Zoology* 24:424–429.
- Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest package: tests in linear mixed effects models. *Journal of Statistical Software* 82.
- Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions. *BioScience* 48:25–34.
- Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68:619–640.
- Liu, Y. J., L. F. Li, Y. H. Zhang, H. F. Guo, M. Xia, M. W. Zhang, X. Y. Jing, J. H. Zhang, and J. X. Zhang. 2017. Chronic co-species housing mice and rats increased the competitiveness of male mice. *Chemical Senses* 42:247–257.
- Magurran, A. E., P. W. Irving, and P. A. Henderson. 1996. Is there a fish alarm pheromone? A wild study and critique. *Proceedings of the Royal Society of London Series B: Biological Sciences* 263:1551–1556.
- Mäkeläinen, S., L. Trebatická, J. Sundell, and H. Ylönen. 2014. Different escape tactics of two vole species affect the success of the hunting predator, the least weasel. *Behavioral Ecology and Sociobiology* 68:31–40.
- Manser, M. B., R. M. Seyfarth, and D. L. Cheney. 2002. Suricate alarm calls signal predator class and urgency. *Trends in Cognitive Sciences* 6:55–57.
- Mappes, T., and H. Ylönen. 1997. Reproductive effort of female bank voles in a risky environment. *Evolutionary Ecology* 11:591–598.
- Marchlewska-Koj, A., A. Cavaggioni, C. Mucignat-Caretta, and P. Olejniczak. 2000. Stimulation of estrus in female mice by male urinary proteins. *Journal of Chemical Ecology* 26:2355–2366.
- Mönkkönen, M., J. T. Forsman, T. Kananoja, and H. Ylönen. 2009. Indirect cues of nest predation risk

- and avian reproductive decisions. *Biology Letters* 5:176–178.
- Murdoch, W. W., C. J. Briggs, and R. M. Nisbet. 2003. *Consumer-resource dynamics*. Princeton University Press, Princeton, New Jersey, USA.
- Nelson, E. H., C. E. Matthews, and J. A. Rosenheim. 2004. Predators reduce prey population growth by inducing changes in prey behavior. *Ecology* 85:1853–1858.
- Norrdahl, K., and E. Korpimäki. 1995. Mortality factors in a cyclic vole population. *Proceedings of the Royal Society B: Biological Sciences* 261:49–53.
- Norrdahl, K., and E. Korpimäki. 2000. The impact of predation risk from small mustelids on prey populations. *Mammal Review* 30:147–156.
- Orrock, J. L., B. J. Danielson, and R. J. Brinkerhoff. 2004. Rodent foraging is affected by indirect, but not by direct, cues of predation risk. *Behavioral Ecology* 15:433–437.
- Osada, K., S. Miyazono, and M. Kashiwayanagi. 2014. Pyrazine analogs are active components of wolf urine that induce avoidance and fear-related behaviors in deer. *Frontiers in Behavioral Neuroscience* 8:276.
- Ouattara, K., A. Lemasson, and K. Zuberbuhler. 2009. Campbell's monkeys concatenate vocalizations into context-specific call sequences. *Proceedings of the National Academy of Sciences* 106:22026–22031.
- Paine, R. T. 1966. Food web complexity and species diversity. *American Naturalist* 100:65–75.
- Pangle, K. L., S. D. Peacor, and O. E. Johannsson. 2007. Large nonlethal effects of an invasive invertebrate predator on zooplankton population growth rate. *Ecology* 88:402–412.
- Parsons, M. H., et al. 2017. Biologically meaningful scents: a framework for understanding predator-prey research across disciplines. *Biological Reviews*.
- Preisser, E. L., D. I. Bolnick, and M. F. Benard. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology* 86:501–509.
- Promislow, D. E. L., and P. H. Harvey. 1990. Living fast and dying young: a comparative analysis of life-history variation among mammals. *Journal of Zoology* 220:417–437.
- R Core Team. 2018. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Reeve, H. K., S. T. Emlen, and L. Keller. 1998. Reproductive sharing in animal societies: Reproductive incentives or incomplete control by dominant breeders? *Behavioral Ecology* 9:267–278.
- Roberts, S. A., D. M. Simpson, S. D. Armstrong, A. J. Davidson, D. H. Robertson, L. McLean, R. J. Beynon, and J. L. Hurst. 2010. Darcin: a male pheromone that stimulates female memory and sexual attraction to an individual male's odour. *BMC Biology* 8.
- Roche, D. P., K. E. McGhee, and A. M. Bell. 2012. Maternal predator-exposure has lifelong consequences for offspring learning in threespined sticklebacks. *Biology Letters* 8:932–935.
- Ronkainen, H., and H. Ylönen. 1994. Behaviour of cyclic bank voles under risk of mustelid predation: Do females avoid copulations? *Oecologia* 97:377–381.
- Ruxton, G. D., and S. L. Lima. 1997. Predator-induced breeding suppression and its consequences for predator-prey population dynamics. *Royal Society Proceedings: Biological Sciences* 264:409–415.
- Sánchez-González, B., I. Barja, and Á. Navarro-Castilla. 2017. Wood mice modify food intake under different degrees of predation risk: influence of acquired experience and degradation of predator's faecal volatile compounds. *Chemoecology* 27:115–122.
- Schmitz, O. J., A. P. Beckerman, and K. M. O'Brien. 1997. Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. *Ecology* 78:1388–1399.
- Sheriff, M. J., C. J. Krebs, and R. Boonstra. 2009. The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. *Journal of Animal Ecology* 78:1249–1258.
- Sheriff, M. J., E. K. McMahon, C. J. Krebs, and R. Boonstra. 2015. Predator-induced maternal stress and population demography in snowshoe hares: the more severe the risk, the longer the generational effect. *Journal of Zoology* 296:305–310.
- Sievert, T., and M. Laska. 2016. Behavioral responses of CD-1 mice to six predator odor components. *Chemical Senses* 41:399–406.
- Sih, A. 1994. Predation risk and the evolutionary ecology of reproductive behaviour. *Journal of Fish Biology* 45:111–130.
- Sih, A., P. Crowley, M. McPeck, J. Petranka, and K. Strohmeier. 1985. Predation, competition, and prey communities: a review of field experiments. *Annual Review of Ecology and Systematics* 16:269–311.
- Sipari, S., H. Ylönen, and R. Palme. 2017. Excretion and measurement of corticosterone and testosterone metabolites in bank voles (*Myodes glareolus*). *General and Comparative Endocrinology* 243:39–50.
- St-Cyr, S., S. Abuaish, S. Sivanathan, and P. O. McGowan. 2017. Maternal programming of sex-specific responses to predator odor stress in adult rats. *Hormones and Behavior* 94:1–12.
- St-Cyr, S., and P. O. McGowan. 2015. Programming of stress-related behavior and epigenetic neural gene regulation in mice offspring through maternal

- exposure to predator odor. *Frontiers in Behavioral Neuroscience* 9:145.
- Stowe, M. K., T. C. Turlings, J. H. Loughrin, W. J. Lewis, and J. H. Tumlinson. 1995. The chemistry of eavesdropping, alarm, and deceit. *Proceedings of the National Academy of Sciences of the United States of America* 92:23–28.
- Strassmann, B. I., and B. Gillespie. 2002. Life–history theory, fertility and reproductive success in humans. *Proceedings of the Royal Society of London Series B: Biological Sciences* 269:553–562.
- Sundell, J., L. Trebatická, T. Oksanen, O. Ovaskainen, M. Haapakoski, and H. Ylönen. 2008. Predation on two vole species by a shared predator: antipredatory response and prey preference. *Population Ecology* 50:257–266.
- Sundell, J., and H. Ylönen. 2004. Behaviour and choice of refuge by voles under predation risk. *Behavioral Ecology and Sociobiology* 56:263–269.
- Taylor, J. D. 1984. A partial food web involving predatory gastropods on a Pacific fringing reef. *Journal of Experimental Marine Biology and Ecology* 74:273–290.
- Terranova, M. L., F. Cirulli, and G. Laviola. 1999. Behavioral and hormonal effects of partner familiarity in periadolescent rat pairs upon novelty exposure. *Psychoneuroendocrinology* 24:639–656.
- Trebatická, L., P. Suortti, J. Sundell, and H. Ylönen. 2012. Predation risk and reproduction in the bank vole. *Wildlife Research* 39:463–468.
- Venables, W. N., and B. D. Ripley. 2002. *Modern applied statistics with S*. Fourth edition. Springer, New York, New York, USA.
- Viney, M. E., and N. R. Franks. 2004. Is dauer pheromone of *Caenorhabditis elegans* really a pheromone? *Naturwissenschaften* 91:123–124.
- Weladji, R. B., Ø. Holand, J.-M. Gaillard, N. G. Yoccoz, A. Mysterud, M. Nieminen, and N. C. Stenseth. 2010. Age-specific changes in different components of reproductive output in female reindeer: Terminal allocation or senescence? *Oecologia* 162:261–271.
- Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. *Ecology* 84:1083–1100.
- Wickham, H. 2009. *ggplot2: elegant graphics for data analysis*. Springer-Verlag, New York, New York, USA.
- Ylönen, H. 1989. Weasels *Mustela nivalis* suppress reproduction in cyclic bank voles *Clethrionomys glareolus*. *Oikos* 55:138.
- Ylönen, H., and J. S. Brown. 2007. Fear and the foraging, breeding, and sociality of rodents. Page 610 in J. O. Wolff and P. W. Sherman, editors. *Rodent societies: an ecological & evolutionary perspective*. University of Chicago Press, Chicago, Illinois, USA.
- Ylönen, H., J. Jacob, M. J. Davies, and G. R. Singleton. 2002. Predation risk and habitat selection of Australian house mice, *Mus domesticus*, during an incipient plague: desperate behaviour due to food depletion. *Oikos* 99:284–289.
- Ylönen, H., and H. Ronkainen. 1994. Breeding suppression in the bank vole as antipredatory adaptation in a predictable environment. *Evolutionary Ecology* 8:658–666.
- Zeileis, A., C. Kleiber, and S. Jackman. 2008. Regression models for count data in R. *Journal of Statistical Software* 27:1–25.
- Zhang, J. X., L. Sun, K. E. Bruce, and M. V. Novotny. 2008. Chronic exposure of cat odor enhances aggression, urinary attractiveness and sex pheromones of mice. *Journal of Ethology* 26:279–286.

DATA AVAILABILITY

Data and statistical code, respectively, are available from Figshare: <https://doi.org/10.6084/m9.figshare.7064390> and <https://doi.org/10.6084/m9.figshare.7064381>.

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.2765/full>