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Changing winter conditions in the boreal forest: the effects of fluctuating temperature and predation risk on activity and physiological stress level in bank voles

Saana Sipari[1] · Marko Haapakoski[1] · Ines Klemme[1] · Rupert Palme[2] ·
Janne Sundell[3] · Hannu Ylönen[1]

Abstract

Due to global climate change, the winter conditions in the North are predicted to change, as the time with an intact insulating snow cover gets shorter or disappears altogether. For small mammals, this could cause exposure to strong temperature fluctuations and increased predation risk, inducing severe stress and leading to alterations in the physical condition and behavior. To test this, we exposed bank voles (*Myodes glareolus*) to different temperature regimes and cues of predator threat under laboratory conditions. The test animals experienced either a stable but cool temperature regime resembling the stable conditions under snow cover, or an unstable temperature regime with cold nights and warmer days simulating the climate change scenario with unstable winter. After 3 weeks, the animals were additionally exposed to owl calls or sounds of non-predatory bird species. Stress levels, activity, food consumption, and body mass were monitored. We observed that the voles exposed to unstable temperatures adjusted their normal, mostly nocturnal, and circadian activity pattern towards a more diurnal rhythm without any significant responses in their stress level. Introducing the sound manipulation elevated the stress levels in females but not in males. The sound-induced stress levels did not differ between the temperature treatments. However, the temperature regime tended to affect anti-predator behavior as individuals experiencing unstable temperatures and a threatening sound decreased their overall activity, unlike individuals under stable temperature treatments. It seems that behavioral plasticity in bank voles may diminish the risk of accumulation of stress in cases of multiple simultaneous stressors.

Significance statement

The future course of many ecosystems in the northern hemisphere has raised many questions as climate change is predicted to have notable consequences especially in these regions due to alterations in winter conditions. Our results suggest that these environmental changes could alter the circadian activity pattern and possibly, even the anti-predator behavior of bank voles. However, the adaptability and behavioral plasticity in this species may diminish the risk of pathological accumulation of stress effects.

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✉ Saana Sipari
  saana.m.sipari@jyu.fi

1 Department of Biological and Environmental Science, Konnevesi Research Station, University of Jyväskylä, P.O. Box 35, 40014 Jyväskylä, Finland

2 Unit of Physiology, Pathophysiology and Experimental Endocrinology, Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria

3 Lammi Biological Station, University of Helsinki, Pääjärventie 320, 16900 Lammi, Finland

Introduction

At high latitudes, snow cover during winter is characteristic and plays an important role in ecosystem dynamics, as many northern animals and plants are strongly dependent on the protection the snow provides against cold and predators (Marchand 1996; Stien et al. 2012; Wolkovich et al. 2012; Mills et al. 2013). However, due to global warming, the winters in the North are about to go through some drastic changes

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as the time period with an intact snow cover is predicted to become shorter, or in the worst case, vanish altogether (Serreze et al. 2000; Lovejoy and Hannah 2005; Jylhä et al. 2008). For small ground-dwelling mammals, the lack of snow would expose them to severe temperature fluctuations and increase their susceptibility to many predators (Marchand 1996). This could lead to chronic stress and consequently, deteriorated winter survival (Huey and Bennett 1990; Cichon et al. 2002; McEwen and Wingfield 2003; Duchesne et al. 2011; Sheriff et al. 2011; Zimova et al. 2016).

Animals are capable of adapting to new environmental conditions but it often requires both behavioral and physiological responses to survive in suddenly deteriorating environmental circumstances (Wingfield and Sapolsky 2003; Boonstra 2004; Wingfield 2013; Boonstra et al. 2014). Under unfavorable external conditions, animals face trade-offs, which drive them to allocate resources from less vital functions, like reproduction or somatic growth to survival (McNamara and Buchanan 2005; Bronson 2009). For example, under elevated predation pressure, animals usually adjust their behavior accordingly, e.g., reduce their activity and the time spent for foraging (Preisser et al. 2005). This, in the long run, however, can lead to an insufficient energy gain. In the case of abiotic stressors, such as cold stress, maintaining homeostasis by thermoregulation is energy consuming (Marchand 1996; Wang et al. 1999; Schultz et al. 1999; Cichon et al. 2002; Moshkin et al. 2002). If the availability and quality of food resources is poor, it can eventually lead to the neglect of other physiological functions (e.g., immunocompetence) causing even pathological conditions (McEwen and Wingfield 2003; McEwen 2004; Beldomenico et al. 2008). When multiple stressful events occur simultaneously, the reallocation of resources becomes even more demanding (McNamara and Buchanan 2005). However, stress response is an adaptive response for restoring the physiological homeostasis after external or internal disturbance (i.e. stress), and thus, essential for the organism's survival. Only if it becomes chronic, the long-term elevation of stress hormone levels can become harmful for the organism (Viau 2002; Wingfield and Sapolsky 2003).

Here, we tested the hypothesis that environmental conditions predicted by climate change cause behavioral and physiological responses in a boreal small mammal, the bank vole (*Myodes glareolus*). To mimic the possible effects of climate change, we exposed test animals to different temperature regimes and cues of predators. Voles experienced either a stable but cool temperature regime resembling the stable conditions under snow cover, or an unstable diel temperature regime with cold nights and warm days simulating the climate change scenario with unstable winter and early snow melt. In addition, we exposed animals to owl calls representing cues of predation risk, or sounds of non-predatory bird species. Resident owls, which frequently call during late winter and early spring, are significant predators of small mammals (Laine 2009; Korpimäki and Hakkarainen 2012). We

expected that strong fluctuations in temperature would increase stress levels of voles and change their activity pattern towards a diurnal rhythm to avoid cold nights. Under natural conditions, becoming more diurnal would also allow them to exploit sun basking to reduce the costs of thermoregulation (Schultz et al. 1999; Schwaibold and Pillay 2006). We also predicted that the joint effect of unstable temperatures combined with a threat of predation would result in even higher stress levels and consequently reduce overall activity. Thus, we expected to see the highest stress levels and the lowest activity in treatments with variable temperature and predatory cues. In stable temperature treatments with non-predatory bird sounds, the activity was expected to stay species-typical and stress levels low (Eilam et al. 1999; Hettena et al. 2014).

Material and methods

Experimental animals

All animals used in this study (40 males and 32 females) were born in the laboratory in April–July 2012 at Konnevesi research station, Central Finland. Animals used in this study were from a colony of wild bank voles (originally 70 females and 70 males) derived from local populations in central Finland that had been outbred for five generations. To mimic the natural seasonal rhythm in the wild in Central Finland, voles were overwintered in climate chambers at 6 °C from November 2012 until the beginning of the experiment in April 2013. The light period was set to resemble the average light regime in winter time with 7 h of light and 17 h of darkness. As communal nesting is suggested to be common for *Myodes* voles during winter under natural conditions (Merritt 1984; Ylönen and Viitala 1985) we housed the voles in same-sex groups of four individuals in large cages (60 × 40 × 20 cm) until February. After that, they were separated into individual standard mouse cages (43 × 26 × 15 cm) to habituate for the experiment. We also gradually increased the day length. All voles had wood shavings and cotton wool as bedding and food (mice pellets, Labfor R36, Lantmännen) and water were provided ad libitum.

At the beginning of April 2013, all voles were weighed and injected with subcutaneous PIT tags for monitoring their activity. After this, voles were introduced to their experimental cages and divided evenly among four climate chambers according to sex and body mass. In each chamber, we housed 10 males and 8 females, and the mean body mass was approximately the same in all four treatments (mean 22.9 ± SE 0.35 g, $F_{1,68} = 0.188$, $p = 0.666$). Throughout the experiment, the light regime of the chambers was set to 12 L:12 D, resembling the day length of late winter/early spring in Central Finland (Finnish Meteorological Institute). The light regime was constant during the experiment.

Experimental design

The experiment was carried out in four temperature-adjustable laboratories, i.e., climate chambers at the Konnevesi Research Station, Central Finland during April–May 2013. To study the effects of fluctuating temperature and predation risk on the activity and stress levels in bank voles, we generated different treatments. Voles were experiencing either a stable but cool temperature, as are the conditions under intact snow cover in late winter/early spring, or fluctuating daily temperature with cold nights and warm days representing the climate change scenario with unstable snow cover and early melting snow, exposing them to strong fluctuation of ambient temperature. In the stable temperature, treatment the temperature was constant at 6 °C (±1 °C), whereas in the unstable temperature treatment, it fluctuated from 1 °C at night to 12 °C during the day (±1 °C). Thus, the average daily temperature was approximately the same in both treatments. Under natural conditions, the temperature would be lower, as the temperature under the snow cover stays generally around 0 °C, and the temperature fluctuations without the snow cover could be even more severe (Finnish Meteorological Institute). However, we want to emphasize that the ultimate purpose of the manipulations was to act as an indicator of a stable environment and unstable environment, rather than examine the effect of absolute temperatures per se. Temperature manipulation continued through the whole experiment as described.

To monitor daily activity, standard mouse cages were equipped with a separating wall across the middle of the cage made from plywood and a hole large enough for a vole to go through (Ø 5 cm). In 36 (out of 72) of these cages, there was a sensor (Trovan®, EID Aalten BV, Aalten, Holland) around the hole connected to a PC, which recorded each passage of the PIT-tagged individual through the hole (date and time). The activity sensing cages were distributed equally across all treatments (18 for stable temperature chambers and 18 for unstable temperature chambers). The remaining cages had an otherwise similar structure but without the sensor system. Food and water were provided only in one side of the plywood wall, and nest material in the other.

Predation risk was simulated by using tawny owl (*Strix aluco*) calls played from loudspeakers. Tawny owls are common nocturnal predators in forested areas, often occupying the same habitats as bank voles (Laine 2009). Songs of the redwing (*Turdus iliacus*), which is a non-predatory early arriving migrant thrush (Laine 2009), was used as a control. Depending on the treatment, either owl calls or thrush sounds were played three times per night (at 09:00 p.m., 01:00 a.m., and at 05:00 a.m.) 1 h at a time during two nights at standardized sound volume. However, to separate the possible stress reactions (elevated stress level or changes in behavior) caused by a new stimulus (i.e., new sound) and stress caused by recognized predation threat (owl calls), we performed a preceding sound treatment. One week before the actual predation manipulation, we exposed animals to a mixture of basic forest background sounds, e.g., distant birds, wind, water, etc. Sounds of tawny owl (or any predatory species) or redwing were not present in the background recording. The sound regime was the same as the above-mentioned, but instead of two nights, it was played each night during 1 week.

With these manipulations, we generated the following treatments: Unstable temperature with no sound treatment (UT/NS), unstable temperature/thrush song (UT/control), unstable temperature/owl calls (UT/owl). Also, stable temperature with no sound treatment (ST/NS), stable temperature/thrush song (ST/control), and stable temperature/owl calls (ST/owl). Temperature treatment remained the same for each individual through the whole experiment but sound manipulation alternated, so that each vole experienced both predator sound and control sound. To make the predator and control sound treatments more natural, the owl calls and thrush song were recorded on top of slightly muffled background sound. The experiment was carried out according to following time table (Table 1).

Table 1 The time table of the experiment

Time	Treatment	Experimental procedures
Week 1	Temperature (ST, UT), no sound	- no procedures, habituation period
Week 2	Temperature (ST, UT), no sound	- monitoring daily activity
		- measuring food consumption
		- collecting fecal samples
Week 3	Temperature + background sound	- no procedures, habituation, for sound
Week 4	Temperature + owl or control sound	- monitoring daily activity
		- measuring food consumption
		- collecting fecal samples
Week 5	Temperature + owl or control sound (reversed)	- monitoring daily activity
		- measuring food consumption
		- collecting fecal samples

Food consumption was measured twice per week (first and third morning of the week) during weeks 2, 4, and 5 (Table 1) by weighing the pellets given to the animals and weighing the leftovers. As an indicator of stress, we used the level of corticosterone metabolites detected from the feces (Touma et al. 2003; Ylönen et al. 2006; Eccard et al. 2011; SS, HY, RP unpublished results). The sound treatments (owl or control) were played during the first two nights of weeks 4 and 5 (Table 1). In the morning, after the second night, the pellet consumption was measured and fecal samples collected. The forest background sounds were always played during the rest of the week. Daily activity was monitored and recorded non-stop via the Trovan-system, but for activity analyses we selected 24-h periods from each week, the same for all individuals. In case of sound treatments (control or owl) recordings from the second night and next day (24 h) were used. At the end of the experiment, all animals were weighed again. To minimize observer bias, blinded methods were used when all behavioral data were recorded and analyzed.

Fecal sampling and analyzing

To collect fecal samples, each vole was temporarily moved to a clean and empty (no beddings) mouse cage. This was done at 10:00 am. After the vole defecated, it was returned to its home cage. This usually happened quickly and took maximum 1 h. After this, feces were collected to empty Eppendorf tubes (1.5 ml, one tube per individual per session) from the sampling cage using tweezers and the samples were stored at −20 °C. Samples soaked in urine were not used as it can distort the results (SS, HY, RP, unpublished results). Then, each fecal sample was homogenized and an aliquot of 0.05 g was mixed with 80 % methanol. After this, the suspension was centrifuged for 10 min at 2500×g. An aliquot of the supernatant was diluted (1:10) with assay buffer (Tris/HCl 20 mM, pH 7.5) and stored in freezer in −20 °C until analyzed. All fecal samples were analyzed by using 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay (EIA), which measures metabolites with 5α-3β,11β,-diol structure. For a more detailed description of the used methods and the assay (e.g. antibodies and inter- and intra-assay variance), see Touma et al. (2003). This specific EIA has been validated and proven suitable for measuring fecal corticosterone metabolites in bank voles (SS, HY, RP, unpublished results) by using an ACTH challenge test and by radiometabolism experiment including high-performance liquid chromatography (HPLC). In that particular validation experiment, it was shown that the intestinal gut passage time of corticosterone in bank voles is ca. 6–8 h. That is the time gap between hormone secretion and the excretion of its metabolites in feces. Further, males excrete on average 70 % of corticosterone metabolites via feces, whereas females excrete only around 50 %. Due to this significant difference, we decided to adjust the measured corticosterone metabolite levels to the estimated value of 100 % for both sexes for meaningful comparisons in our statistical analyses and illustrations (Sipari et al. 2016). All laboratory analyses were performed in the University of Veterinary Medicine in Vienna, Austria.

Statistical analyses

Statistical analyses were performed using R 3.0.3 and IBM SPSS Statistics 20. Corticosterone metabolite levels (stress), food consumption, and body mass were all analyzed using linear mixed models with Gaussian error structure, fit by REML. Activity was analyzed using a generalized linear mixed model with negative binomial distribution. Temperature treatment (ST and UT), sound treatment (NS, control and owl), and sex were set as fixed factors, and vole ID and climate chamber as random factors when analyzing stress levels and activity. Stress levels were also tested separately for the sexes (see "Material and methods" section, Fecal sampling and analyzing). To compare night and day activity within and between groups during different sound treatments, the model was extended by adding time (12D:12L, night = 8 p.m.–8 a.m. and day = 8 a.m.–8 p.m.) as a fixed factor. To control for the playing order of control and owl sounds, we originally included "order" as a fixed factor. However, this caused strong multicollinearity between variables and we omitted this variable from the model. Instead, we tested the effect of playing order separately for stress levels and activity and found no significant effect. To test the effects of our treatments on body mass, we used temperature treatment, time (start and end of the experiment) and sex as fixed factors, and ID and chamber as random factors. Food consumption was tested with temperature, sound treatment, and sex as fixed factors, and again, ID and chamber as random factors. Daily food consumption was calculated by dividing the amount of food eaten by the number of days between measurements. All interactions between fixed factors were tested. Results for the interactions relevant for the final model are reported in the "Results" section (Table 2). For model selection, we used the Akaike information criterion (AIC), selecting the model with the lowest AIC value for the analyses. Also, graphical residual analysis was used to validate the model. To test for a correlation between stress level and total activity, and activity and food consumption, we used Spearman's correlation.

Results

Stress

Physiological stress levels measured as corticosteroid metabolites were significantly higher in males than in females throughout the

Table 2 Summary of the results for the final models for all fixed factors. *Time in "Activity" refers to the time of the day (night vs. day), and time in "Body mass" to the course of the experiment (start vs end)

	Stress level, both sexes			Stress level, males			Stress level, females		
	df	F value	p value	df	F value	p value	df	F value	p value
Temperature	1, 64	0.807	0.3725	1, 34	0.398	0.532	1, 30	0.3436	0.562
Sound	2, 115	1.526	0.222	2, 62	0.0278	0.973	2, 53	5.052	0.010
Sex	1, 64	28.565	<0.001	x	x	x	x	x	x
Temperature: Sound	2, 115	5.181	0.007	2, 62	6.889	0.002	2, 53	2.114	0.131
Temperature: Sex	1, 64	0.041	0.841	x	x	x	x	x	x
Sound: Sex	2, 115	1.691	0.189	x	x	x	x	x	x
	Activity			Food consumption			Body mass		
	df	χ^2	p value	df	F value	p value	df	F value	p value
Temperature	1	1.709	0.191	1, 64	1.232	0.271	1, 67	0.067	0.796
Sound	2	13.306	0.001	2, 126	43.372	<0.001	x	x	x
Sex	1	1.246	0.264	1, 64	3.834	0.055	1, 67	59.646	<0.001
Time*	1	67.967	<0.001	x	x	x	1, 56	31.888	<0.001
Temperature: Sound	2	7.670	0.022	2, 126	4.547	0.012	x	x	x
Temperature: Sex	x	x	x	1, 64	2.496	0.119	x	x	x
Sound: Sex	x	x	x	2, 126	0.738	0.480	x	x	x
Temperature: Time*	1	18.595	<0.001	x	x	x	x	x	x
Sound: Time*	2	8.531	0.014	x	x	x	x	x	x

experiment (Table 2, Fig. 1). When the sexes were analyzed separately, stress levels in females were significantly affected by the sound treatment but not by the temperature treatment (Table 2, Fig. 1). In a pairwise comparison, the stress levels in the control were significantly higher than in NS ($T_{55.37} = 3.090$, $p = 0.009$), and the difference between NS and owl was nearly significant ($T_{55.91} = -2.384$, $p = 0.052$, Fig. 1). However, there was no significant difference between control and owl ($T_{54.51} = 0.674$, $p = 0.779$). In males, there were no significant differences between any treatments (Table 2). However, there

Fig. 1 LS-mean (with SE) of corticosterone metabolite levels indicating stress in different treatments for females and males. *ST* stable temperature treatment, *UT* unstable temperature treatment, *NS* no sound, *Control* control sound, and *Owl* predator sound (*To enable accurate comparison between sexes the metabolite levels are adjusted)

was a significant interaction between temperature treatment and sound treatment (Table 2) but in pairwise comparisons all differences were not significant (all comparisons $p > 0.080$).

Activity

Total activity was not affected by the temperature treatments (Table 2) but there was a significant interaction between temperature treatment and time of the day, and in a pairwise comparison, the daytime activity in ST treatment was significantly lower than in UT treatments ($Z = -3.032$, $p = 0.013$). No significant difference in nighttime activity between temperature treatments was observed (ST vs UT, $Z = 0.498$, $p = 0.956$). Sound treatments had a significant effect on activity, and there was a significant interaction between sound and temperature treatments (Table 2, Fig. 2). In a pairwise comparison, we observed different trends in the response to the sound treatments between temperature groups (Fig. 2). In UT treatment, individuals reacted to both control and owl sounds by reducing their activity, but only reactions to owl sound were significant (NS vs control; $Z = 2.682$, $p = 0.079$, NS vs Owl; $Z = 4.404$, $p < 0.001$). There was no significant difference between control and owl sound (control vs owl, $Z = 1.555$, $p = 0.628$). In ST treatments, no similar response was observed (NS vs control; $Z = -0.042$, $Z = 1.000$, NS vs owl; $Z = 0.879$, $p = 0.952$, control vs owl; $Z = 0.877$, $p = 0.952$). There were no significant differences between sexes (Table 2), and we
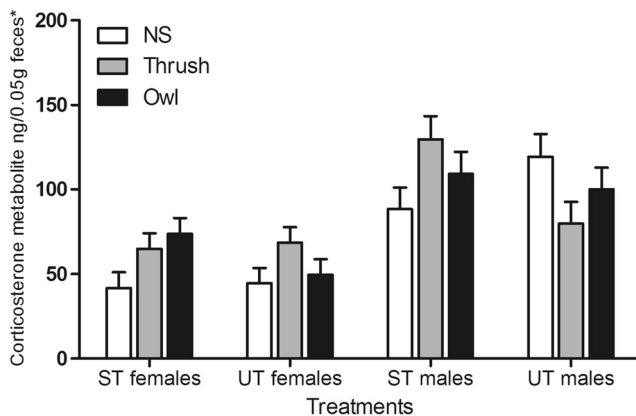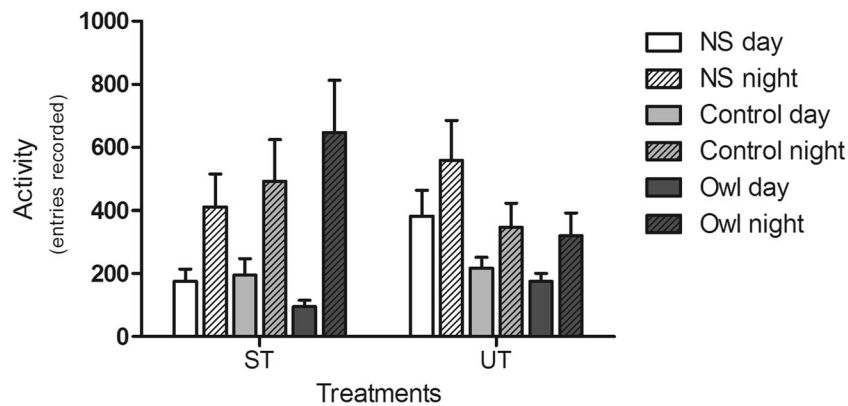
Fig. 2 LS-mean (with SE) activity during day and night in different treatments. Activity on x-axis represents the frequency of recorded entries through the sensor hole in the experimental cages during 24 h (see "Experimental cages" section). *ST* stable temperature treatment, *UT* unstable temperature treatment, *NS* no sound, *Control* control sound, and *Owl* predator sound

observed no correlation between stress level and total activity (rho = 0.116, p = 0.120).

Body mass and food consumption

Males were heavier than females throughout the experiment (Table 2). Time had an effect on body mass, as the mean body mass was significantly lower at the beginning of the experiment than in the end (Table 2). There were no significant differences between temperature groups (Table 2).

Food consumption was significantly affected by the sound treatments but not by temperature treatments (Table 2). Food consumption was significantly lower in NS than control and owl (NS vs control: $Z = -7.613$, $p < 0.001$, NS vs owl $Z = -8.441$, $p < 0.001$). There was no difference in food consumption between control and owl treatments ($Z = -0.823$, $p = 0.689$). Males tended to eat more than females but the difference was not significant (Table 2). There was no correlation between activity and food consumption (rho = 0.136, $p = 0.218$).

Discussion

Winter in boreal and polar regions is the most challenging season for most animal species. Thermoregulation is a physiological constraint and a stressor to animals experiencing temperatures far below their so-called lower critical temperature (LCT) (Huey and Bennett 1990; Marchand 1996; Moshkin et al. 2002). LCT is the ambient temperature where an animal needs to adjust its behavior and physiological functions (e.g., increased/reduced mobility, energy intake, shivering) in order to maintain homeostasis (Marchand 1996). LCT is often season and acclimatization dependent (McDevitt and Speakman 1994; Marchand 1996). Exposure to cold increases the metabolic rate in non-hibernating small mammals (Wang et al.

1999; Cichon et al. 2002) and it decreases immunocompetence (Cichon et al. 2002). We hypothesized that fluctuating cool temperatures (1–12 °C) could be even more challenging for small animals than a cool but stable temperature (6 °C) due to the constant adjustment in thermoregulation it requires. However, contrary to expectations, we did not find significantly elevated stress levels in the unstable temperature treatment. This result could indicate that voles acclimatized to cool temperature (see "Experimental animals" section) are relatively adaptable to changes in temperature, at least in the case of unrestricted food availability. Further, we observed a clear plasticity in their circadian activity patterns, which likely plays an important role in their successful adaptation to different temperature conditions. In bank voles, the circadian activity rhythm is polyphasic (Ylönen 1988; Halle 2000) with several activity bouts separated by resting periods. The majority of these activity bouts occur between dusk and dawn, while daytime activity is often lower (Ylönen 1988). However, as predicted, animals in unstable temperature treatments increased their daytime activity compared to individuals under stable temperature. Total activity did not differ between temperature treatments. Thus, our results suggest that bank voles are able to adjust their circadian activity patterns according to their environment without changes in their physiological stress levels, at least for a short time period.

Introducing the sound treatments caused significantly elevated corticosterone metabolite levels in females, but did not differ between the experienced temperature treatments. This implies that due to the behavioral adjustments, e.g., shift in their circadian activity pattern, the fluctuating temperature combined with predation risk did not induce higher stress levels compared to the stable temperature treatments. The inability to differentiate neutral sounds from predator sounds (also, the thrush song caused significantly elevated corticosterone metabolite levels) may be explained by the laboratory origin of the voles. In males, there were no significant

differences in stress levels between any treatments. However, corticosterone levels as indicators of stress in males may be perhaps somewhat more complicated compared to females, as testosterone has been shown to strongly impact glucocorticoids and fear. It is generally accepted that elevations of glucocorticoid levels reduce testosterone level (Chichinadze and Chichinadze 2008; Bronson 2009), but there is also strong evidence that animals with high testosterone level have lower stress hormone levels and are less fearful to novel stimuli (Cornwell-Jones and Kovanic 1981; Boissy and Bouissou 1994; Place and Kenagy 2000; Schradin 2008; Kohlhause et al. 2011). These results could explain some of the variance in male stress levels observed in our experiment.

Despite the lack of response on stress levels, the temperature treatments seemed to affect the behavioral response of bank voles when encountering a threatening sound. Voles exposed to unstable temperature conditions significantly reduced their activity in response to the sound treatment, whereas voles exposed to the stable temperature regime tended to increase their activity, though not significantly. This may indicate that abiotic environmental conditions affect antipredator behavior in bank voles. However, we should point out that the voles within a temperature group showed no statistically significant difference in activity when comparing their responses between the thrush and owl sounds. That is why the term "anti-predator" behavior should be interpreted cautiously. Nevertheless, we consider using the term in this context justified, as based on our results it seems that both of these sounds were interpreted as a threat.

In prey animals, like voles, anti-predator behavior is often divided into two main responses: fleeing and freezing. Based on the trend observed in our experiment, it seems that the freezing response was more pronounced under unstable temperatures. It is known that anti-predator behavior can differ according to predator type (terrestrial or avian), distance to predator and behavior of the predator (immobile or approaching etc.) or whether the threat is direct or indirect (Ydenberg and Dill 1986; Eilam et al. 1999; Edut and Eilam 2004; Stankowich and Blumstein 2005; Blumstein 2006; Cooper et al. 2012). Also, the geography of a habitat (open or covered) makes a difference (Jacob and Brown 2000). There are also indications that the reproductive stage and life history of an animal can affect anti-predator behavior (Borowski 2002; Brown and Shine 2004; Trebatická et al. 2010). Our results suggest that abiotic environmental conditions, such as climatic or weather conditions, can affect the chosen anti-predator strategy. Banks et al. (2002) showed that, due to the innate fear response, unfamiliar habitat increases immobility in *Microtus* voles. We suggest that an unstable abiotic environment could act as an unpredictability factor comparable to the unfamiliar habitat in the study of Banks et al. (2002).

Laboratory experiments often provide only a coarse indication and idea of the ecological interactions occurring under natural conditions. As anti-predator behavior is a summary of multiple factors, extrapolating our results to natural conditions as such is not possible. However, when speculating the observed responses, one could argue that the shift in the circadian activity pattern as well as reduced activity under predation risk observed in individuals experiencing unstable temperatures may be considered beneficial response for the animal under the conditions of the climate change scenario. Owls hunt mainly based on their vision and hearing, thus, prey living in a habitat with little shelter, e.g., no snow cover, would probably survive better by reducing activity. Shifts in activity patterns to avoid predation or other disturbance are observed in many other species as well. Many desert rodents decrease their above-ground activity during the full moon (Lockard and Owings 1974; Kaufman and Kaufman 1982; Kotler et al. 1991; Daly et al. 1992), whereas some larger mammals such as deer, boars, and bobcats have changed their activity patterns in response to human recreation and hunting seasons (George and Crooks 2006; Ohashi et al. 2013). However, under long-term predation risk, the reduced activity could lead to energy deficiency if the food availability is scarce, as often is the case during winter. In our experiment, there was no difference in food consumption nor body mass between temperature groups. This is probably due to the abundance and vicinity of the food source. The consumption was higher under control and owl treatments compared to NS but the increase was, however, more likely time dependent rather than a consequence of the sound treatments. In a previous experiment conducted in the same climate chambers at the same time of the year (Sipari et al. 2014), an identical pattern in food consumption was observed without stressors other than temperature regimes.

In the wild, reduced mobility may not only lead to energy deficiency but would eventually also increase the accumulation of scent cues (Banks et al. 2000) and likely increase the risk of mammalian scent-based predators. The shift in their circadian rhythm towards more diurnal activity is an adaptive response against nocturnal predators, as well as against cold nights, but could again increase the likelihood of being preyed upon by day-active predators. Nevertheless, these results provide an interesting aspect to the study of anti-predator behavior by suggesting that abiotic environmental factors might affect the anti-predator strategy in certain species.

Conclusion

Our results suggest that changes in winter conditions caused by the climate change may alter the circadian activity pattern and possibly, even the anti-predator behavior of bank voles. However, the adaptability and behavioral plasticity in bank

voles may diminish the risk of pathological accumulation of stress effects in cases of multiple simultaneous stressors. On the other hand, not all stressors are equal. In addition to the suggested increase in winter time predation pressure and temperature-related stress in small mammals, food availability may become compromised. Thus, in future studies, it would be interesting to test how food limitation combined with different temperature or predation stressors would affect the behavioral and physiological responses, and potentially winter survival in bank voles.

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