

**This is an electronic reprint of the original article.
This reprint *may differ* from the original in pagination and typographic detail.**

Author(s): Sipari, Saana; Haapakoski, Marko; Klemme, Ines; Palme, Rupert; Sundell, Janne; Ylönen, Hannu

Title: Population sex-ratio affecting behavior and physiology of overwintering bank voles (*Myodes glareolus*)

Year: 2016

Version:

Please cite the original version:

Sipari, S., Haapakoski, M., Klemme, I., Palme, R., Sundell, J., & Ylönen, H. (2016). Population sex-ratio affecting behavior and physiology of overwintering bank voles (*Myodes glareolus*). *Physiology and Behavior*, 159, 45-51.
<https://doi.org/10.1016/j.physbeh.2016.03.008>

All material supplied via JYX is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of the repository collections is not permitted, except that material may be duplicated by you for your research use or educational purposes in electronic or print form. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone who is not an authorised user.

Accepted Manuscript

Population sex-ratio affecting behavior and physiology of overwintering bank voles (*Myodes glareolus*)

Saana Sipari, Marko Haapakoski, Ines Klemme, Rupert Palme, Janne Sundell, Hannu Ylönen

PII: S0031-9384(16)30095-6
DOI: doi: [10.1016/j.physbeh.2016.03.008](https://doi.org/10.1016/j.physbeh.2016.03.008)
Reference: PHB 11245

To appear in: *Physiology & Behavior*

Received date: 1 December 2015
Revised date: 23 February 2016
Accepted date: 9 March 2016



Please cite this article as: Sipari Saana, Haapakoski Marko, Klemme Ines, Palme Rupert, Sundell Janne, Ylönen Hannu, Population sex-ratio affecting behavior and physiology of overwintering bank voles (*Myodes glareolus*), *Physiology & Behavior* (2016), doi: [10.1016/j.physbeh.2016.03.008](https://doi.org/10.1016/j.physbeh.2016.03.008)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Population sex-ratio affecting behavior and physiology of overwintering bank voles (*Myodes glareolus*)

Saana Sipari^a, Marko Haapakoski^a, Ines Klemme^a, Rupert Palme^c, Janne Sundell^b and Hannu Ylönen^a

^a*Department of Biological and Environmental Science, Konnevesi Research Station, University of Jyväskylä, P.O.Box 35, FI-40014 Jyväskylä, Finland*

^b*Lammi Biological Station, University of Helsinki, Pääjärventie 320, 16900 Lammi, Finland*

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

Correspondence: Saana Sipari, tel: +358407633324, e-mail: saana.m.sipari@jyu.fi,

saana.m.sipari@gmail.com

ABSTRACT

Many boreal rodents are territorial during the breeding season but during winter become social and aggregate for more energy efficient thermoregulation. Communal winter nesting and social interactions are considered to play an important role for the winter survival of these species, yet the topic is relatively little explored. Females are suggested to be the initiators of winter aggregations and sometimes reported to survive better than males. This could be due to the higher social tolerance observed in overwintering females than males. Hormonal status could also affect winter behavior and survival. For instance, chronic stress can have a negative effect on survival, whereas high gonadal hormone levels, such as testosterone, often induce aggressive behavior. To test if the winter survival of females in a boreal rodent is better than that of males, and to assess the role of females in the winter aggregations, we generated bank vole (*Myodes glareolus*) populations of three different sex ratios (male-biased, female-biased and even density) under semi-natural conditions. We monitored survival, spatial behavior and hormonal status (stress and testosterone) during two winter months. We observed no significant differences in survival between the sexes or among populations with differing sex-ratios. The degree of movement area overlap was used as an indicator of social tolerance and potential communal nesting. Individuals in male biased populations showed a tendency to be solitary, whereas in female biased populations there was an indication of winter aggregation. Females living in male-biased populations had higher stress levels than the females from the other populations. The female-biased sex-ratio induced winter breeding and elevated testosterone levels in males. Thus, our results suggest that the sex-ratio of the overwintering population can lead to divergent overwintering strategies in bank voles.

Key words: Overwintering, winter aggregation, social interactions, population sex-ratio, hormonal status, survival

1. INTRODUCTION

Overwintering strategies of different organisms in boreal and arctic regions can be divided roughly into three categories; migration, hibernation and resistance. Migration is most common among birds, whereas reptiles and amphibians, as well as some mammals rely on hibernation. However, surprisingly many animals remain active throughout the winter, despite the challenging environmental conditions. For instance, northern cervids, like moose, deer and caribou are very well equipped against cold with their large body size, thick winter pelage and fat reserves. But not all winter-active animals are large and armed with similar traits. In fact, many homeothermal winter-active animals are small mammals like rodents and shrews. Unlike large animals, their winter survival is directly and strongly dependent on snow cover. Under the snow pack these small animals exploit the subnivean space formed between the ground, withered vegetation and the snow for moving and foraging, but most importantly for protection against cold and predators. However, besides the importance of the physical protection provided by snow cover, also physiological and behavioral overwintering adaptations are required. Particularly, the hypothalamic-pituitary-adrenal axis (HPA axis) is essential in equipping mammals to endure the harsh conditions of northern winter (Boonstra 2004, Boonstra *et al.* 2014). It regulates several substantial body processes such as energy metabolism, reproduction, growth, immune system and stress reactions (Boonstra 2004, Campbell and Reece 2005). The function of the endocrine system during winter varies amongst species but in small winter-active rodents decreasing day length and temperature are known to induce changes e.g., in fur thickness and metabolism. Also, many small mammals reduce their body mass to lower energy needs, and often all reproductive functions cease in

order to allocate energy for survival (Hyvärinen 1984, Feist 1984, Hansson 1990, Marchand 1996, Merritt and Zegers 2002, Aars and Ims 2002). In addition to these physiological changes, the HPA axis is considered to be responsible for changes in winter time behavior as well (Boonstra *et al.* 2014). Some species that are solitary and territorial during the breeding season are known to become more social during winter, and to construct communal nests (Webster and Brooks 1981, West and Dublin 1984, Ylönen and Viitala 1985, Ylönen and Viitala 1991, Merritt and Zegers 2002). This is often related to the seasonal decrease in gonadal steroid levels, e.g. testosterone in males (Bronson 1985, Boonstra *et al.* 2014).

The increased social tolerance and the social interactions in the overwintering population are considered to play an important role in the winter survival of many small rodents. The main benefit gained from aggregation seems to be social thermoregulation, as cold is one of the main stressors during winter. Huddling keeps the nest temperature higher, which is more energy efficient compared to individual nesting (Sealander 1952, Vickery and Millar 1984). In Taiga voles (*Microtus xanthognathus*), foraging bouts are timed between individuals in a way that the nest is never left empty to prevent nest temperatures from dropping (Wolff and Lidicker Jr 1981). However, in case of scarce food resources the communal nesting creates an inevitable trade-off between energy gain and energy consumption (West and Dublin 1984). Further, the degree of communal nesting seems to vary between species, years, habitats and winter characteristics (Viitala 1984, West and Dublin 1984) comprising incomplete understanding of the actual determinants of successful overwintering. Due to the nature of their overwintering environment, i.e. under the snow pack, the role of social interactions and behavior on winter survival in small winter-active ground-dwelling mammals is relatively little explored.

In order to define the impact and importance of behavior and social dynamics on overwintering survival in a rodent species, we performed a field enclosure experiment at the

onset of winter. As a model species we used the bank vole (*Myodes glareolus*), one of the most common winter-active boreal rodent in Eurasia. Bank voles are short lived, granivorous-omnivorous small rodents. During the breeding season, from April-May to September, they are territorial. Female bank voles, like all *Myodes* females, defend their exclusive breeding territories, whereas the home ranges of males are larger and overlap with each other and with several females' territories (Bujalska 1973). However, it appears that during winter their social tolerance increases, and similar to many *Microtus* species they exploit communal winter nests (Ylönen and Viitala 1985, Ylönen and Viitala 1991). Interestingly, females are reported to survive better over winter than males (Ylönen and Viitala 1985, Klemme *et al.* 2008). Their smaller body size, and thus, lower absolute energetic need is assumed to be a major factor behind this. In addition, it has been suggested by Ylönen *et al.* (1995) that females with their better "social skills" form the core of winter aggregations and that this could play an important role in the overwintering success of the whole overwintering population. Based on an experiment on odor preference, also Ferkin & Seamon (1987) suggested that overwintering groups of meadow voles (*Microtus pennsylvanicus*) might be female biased. It was shown that non-breeding meadow vole females preferred the odor of female conspecifics, whereas males showed no preference and displayed more antagonistic acts against other males than females did against female conspecifics. Thus, male-male aggressive interactions may preclude males from joining the same winter aggregation with each other. A tendency for aggressive behavior could be reflected in higher hormonal levels related to competitive ability and aggression, like testosterone, as well as high stress levels if there are many competing males present. Being isolated from communal nests would also mean higher energetic costs in terms of thermoregulation and thus, possibly increased stress levels caused by the cold. This could decrease the survival possibility of solitary overwintering individuals. However, maintaining

testosterone levels high enough for breeding during winter could be beneficial, because winter breeding occurs occasionally in many small boreal rodent species (Hansson 1984; Jannett 1984; Kaikusalo & Tast 1984). As the winter mortality of small rodents is relatively high, trading off survival for reproduction may increase fitness.

To test the hypothesis of better winter survival of females, assess their role as initiators of winter aggregations and investigate the impact of population structure and hormonal status on winter time behavior and survival, we generated three different population types in semi-natural outdoor enclosures in late October: Female-biased population type (F), male-biased population type (M) and even-sex ratio population type (E). Spatial and temporal variation in the sex-ratio is relatively common in natural animal populations and can be caused by several factors depending on the species (Werken and Charnov 1978). For example, mammalian predators such as weasels tend to kill more female than male voles, whereas males are more susceptible to avian predation (Norrdahl and Korpimäki 1998). Thus, the relative abundance of different predator species could locally lead to biased population sex-ratios.

To estimate social dynamics, we monitored space use of the voles and the degree of movement area overlap as an indicator of social tolerance and decreased level of aggression between individuals which could imply communal nesting. We also observed how the hormonal status of individuals affects their behavior and survival, and further, whether the population type (F, M or E) affects hormonal status. To do this we monitored corticosterone (stress) and testosterone levels in the form of fecal steroid metabolites. Stress was monitored in both sexes, but testosterone only in males.

We hypothesized that voles in female biased populations would survive best and express the highest social tolerance and the lowest stress levels. Male biased populations were expected to survive worst and have the highest stress levels due to high encounter probability and low tolerance between males. Also, if females are the ones maintaining the

communal winter nests it is likely that the total number of communal nests or aggregations would be lower in male biased populations due to the shortage of females. Bank voles are suggested to aggregate in groups of 2-5 individuals, of which the majority are females and perhaps 1 to 2 males (Ylönen and Viitala 1991). This could mean that some of the males are left out of the aggregations. If these surplus males attempt to join these winter nests, it could disturb the nesting and increase stress level also in females. Further, males with high levels of testosterone were hypothesized to express low survival. Testosterone is known to be immunosuppressive, and maintaining high testosterone level is energy consuming (Mills *et al.* 2009, Boonstra *et al.* 2014). Further, high testosterone would likely maintain social intolerance and aggression and probably lead to isolation from communal nests. This would expose the individual to cold stress.

Briefly, the aims of this experiment were to verify the supposed better winter survival of females compared to males, and to test how the population sex-ratio affects winter survival, behavior and physiology in bank voles.

2. MATERIAL AND METHODS

2.1 *Experimental animals*

All animals used in this study (72 males and 72 females) were born in the laboratory during April-July 2012 at Konnevesi research station. One month before the actual experiment commenced, all voles were transferred to a greenhouse for acclimatizing to outdoor temperatures and light rhythms. All test animals were adults at this point in time. The greenhouse was not heated and the temperature in the greenhouse was similar to outdoors. Voles were housed separately in standard mouse cages (43×26×15 cm) with wood shavings

and hay as bedding. Standard mice pellets (Labfor R36, Lantmännen) and water were provided *ad libitum*.

2.2 Experimental design

The experiment was carried out in 12 large outdoor enclosures (0.25ha each) from late October to mid December 2012. The enclosures were made of galvanized steel sheet 125cm in height, with the fence reaching 0.5 m under the ground and 0.75 m above ground. The fence prevents voles from escaping and small mammalian predators from entering the study area, but it does not prevent avian predation. Voles were divided into three population types; female biased populations (F), male biased populations (M) and even sex-ratio populations (E). Each population type was replicated in four enclosures with 12 animals in each. In sex biased populations, the ratio of the sexes was 8: 4, and in even population (6: 6). Populations were distributed randomly among the 12 enclosures. As some of the experimental animals were of different ages (born April-July) individuals were evenly distributed among all populations so that no age group was more represented than the other. Before releasing the voles into enclosures all individuals were weighed. To identify the animals we used small metal ear tags with individual numbering (Hasco Metal Tags Style # 1005-1, Hasco Tag Company, Dayton, Kentucky, U.S.A). One eartag was attached to the right ear lobe of the animal by using applicator tool (Hasco applicator Style # 1005-1S, Hasco Tag Company, Dayton, Kentucky, U.S.A). No anaesthetic or antiseptic was used. However, the puncture heals quickly and we have not observed any adverse effects of this marking method.

We also collected fecal samples for the evaluation of corticosterone metabolite levels. In males, fecal testosterone metabolites were also measured (see *Fecal sampling and analysis*). At the beginning of the experiment there were no differences in corticosterone metabolite levels between groups (males: $F_{2,48} = 0.325$, $p = 0.724$; females: $F_{2,46} = 1.455$, $p =$

0.244. Females have a naturally significantly lower basal level, ergo, separate analysis), nor in testosterone metabolite levels ($F_{2,53} = 0.356$, $p = 0.703$).

During the experiment voles were monitored by multiple live trappings. In each enclosure we had 25 multiple-capture live traps (Ugglan special®, Grahnb AB, Hillerstorp, Sweden) distributed evenly in 5x5 grids. All traps were covered with trap chimneys (40 x 40 x 50 cm) made of metal sheet, which allows trapping during the snowy season. In total we performed three trapping sessions; two for monitoring the survival and space use in different populations, and one for fecal sample collection. Sunflower seeds were used as bait. The first session was carried out during early November, one week after releasing the animals into the enclosures to monitor the survival and space use. Trapping was conducted three times per day (early morning, afternoon, and late evening) with ten trap checks in total. The second trapping was conducted for fecal sample collection in late November (see *Fecal sampling and analysis*). The third and last trapping session was performed in mid-December again to monitor the space use and survival, similar to the first session. This trapping session followed the same protocol as the first one, but this time we also weighed the captured individuals with Pesola spring scales. For weighing the animals in the field, we placed the animal into a small (open) plastic bag and the clip-on of the spring scale was then attached to the bag.

Permanent snow came in the beginning of December, and during the last trapping session the average snow depth was 25-30 cm.

2.3 *Fecal sampling and analyses*

To collect the fecal samples in laboratory conditions, before the animals were released to the enclosures, each vole was temporarily moved to an empty (no beddings) mouse cage (43×26×15 cm). After the vole defecated, it was returned to its home cage. This usually happened fast, and took a maximum of one hour. After this, feces were collected in empty

Eppendorf-tubes (1.5 ml, one tube per individual) from the sampling cage using tweezers. Samples were stored at -20 °C. Fecal samples were collected from voles released into 9 enclosures out of 12 in total (54 males and 54 females).

One month after the release, we performed a trapping session for fecal sample collection from the field. All traps used for this session were new and unused. Samples were collected from the floor of the traps with tweezers to plastic Eppendorf tubes and the trap was wiped clean with paper towels after each sampling. The feces were usually very dry and did not stain the trap. Also, fecal samples are not highly sensitive to contamination. With larger animals, samples are often collected directly from the ground so this method is well suited for field conditions as ultimate sterility is not required. However, samples soaked in urine were not used as it can distort the results (Sipari, Ylönen, Palme, unpublished results). Samples were stored at -20 C. When two or more voles were inside the same trap simultaneously we did not collect the samples. This happened twice. The traps were checked every three hours, three times per day, and one day per enclosure. In total, samples were collected from nine enclosures, three enclosures for each population type (see above). This took four days. No overnight trapping was done in order to keep the time the voles spend captured in the traps as low as possible to avoid possible stress caused by the captivity. Trapping data were not used for evaluating space use and survival.

After the experiment, the remaining voles were left to overwinter in the enclosures and afterwards were used for other experiments.

All fecal samples were analyzed using 5 α -pregnane-3 β ,11 β ,21-triol-20-one enzyme immunoassay (EIA), with methods described in Touma et al. 2003. This specific EIA has been validated and proven suitable for measuring fecal corticosterone metabolites in bank voles (Sipari, Ylönen, Palme, unpublished results). In that validation experiment it was shown that males excrete on average 70 % of corticosterone metabolites via feces, whereas

females excrete only around 50 %. Due to that significant difference we adjusted the measured corticosterone metabolite levels to the estimated value of 100% for both sexes for meaningful comparisons in our statistical analyses and in the illustrations for this paper. To measure fecal testosterone metabolites in males we used EIA for 17 β -hydroxyandrogen, first described by Palme & Möstl (1994), and validated for bank voles by Sipari, Ylönen and Palme (Unpublished results). All laboratory analyses were performed in the University of Veterinary Medicine, in Vienna, Austria.

All procedures conducted during the experiment were approved by the Finnish State Committee for Animal Experimentation (Licence Code: ESLH-2008-05258/Ym-23).

2.4 Statistical analyses

Statistical analyses were performed using R 3.0.3 and IBM SPSS Statistics 20. Survival was analysed using generalized linear mixed model (GLMM) with binomial distribution, and for analysing space use, corticosterone metabolite levels, testosterone metabolite levels and body mass we used linear mixed model (Gaussian distribution) fitted with restricted maximum likelihood (REML).

To test if survival was affected by time (first and third trapping session), sex and population type (F, M, E) were set as fixed factors, and individual nested within enclosure as a random factor. We also tested the time points separately but that did not change the final result. The space use data were first analysed using Ranges 6 program (Anatrack Ltd. Wareham, UK), using 100% convex polygon for calculating movement areas. For statistical testing, time, sex and population type were used as fixed factors and individual nested within enclosure as a random factor. For better fit the data were log-transformed. To test the effect of time on the degree of movement area overlap, we set time as a fixed factor and individual nested within enclosure as a random factor. We also tested if there was a difference in the

tendency to overlap female or male area between population types between the sexes. We tested sexes separately against different overlapping types (e.g. males overlapping females, males overlapping males etc.) We set time and population type as fixed factors and individual nested within enclosure as a random factor. First, for testing corticosterone metabolite concentrations, we set sex, population type and time as fixed factors and individual nested within enclosure as a random factor. Additionally, for a more meaningful comparison of the population types we analysed the sexes separately and used only data collected from the field, as that was our main interest. For this test we used population type as a fixed factor and individual nested within enclosure as a random factor. The data were log-transformed. To test the possible effect of stress on survival we compared the corticosterone metabolite concentrations measured in the field (late November) between individuals that were still alive in December and individuals who had died by December. Survival status (dead or alive) was set as a fixed factor and individual nested within enclosure as a random factor. Sexes were tested separately. Testosterone metabolite levels were tested first with time and population type as fixed factors and individual nested within enclosure as a random factor. For more meaningful comparison between population types we then used only the data collected from the field. For better fit the data were log-transformed. To test if there was a difference in testosterone metabolite levels measured in field (late November) between individuals alive and dead by December we used survival status as a fixed factor and individual nested within enclosure as a random factor. Body mass was tested with time, sex and population type as fixed factors and individual and enclosure as random factors. To test if the body mass of the individuals at the beginning of the experiment would reflect survival probability we compared the starting body masses of individuals still alive in December and individuals who had died by December. Survival status and sex were set as fixed factors and individual nested

within enclosure as a random factor. For model selection we used Akaike information criterion (AIC), the model with the lowest AIC value was selected for the analyses.

3. RESULTS

3.1 Survival

Sex had no significant effect on survival ($F_{1,120} = 1.1942$, $p = 0.277$), nor did the population type ($F_{2,8} = 1.470$, $p = 0.293$). In general, the male biased populations tended to survive worse than even sex-ratio or female biased populations (Fig. 1), but not significantly. Time, on the other hand, had a clear effect, as expected. One week after release the average survival was 66.7%, but it decreased significantly by December, when only 32.3% of the animals were still alive ($F_{1,131} = 49.1298$, $p < 0.001$). No significant interactions were detected.

3.2 Space use in the enclosures

The movement areas inside the enclosures were significantly larger for males than females ($F_{1,72} = 8.982$, $p = 0.003$). In November, the average movement area in males was 156 ± 21 m² and in females 95.5 ± 21.8 m² (values reported with standard errors). By December the areas had decreased significantly in both sexes ($F_{1,33} = 9.772$, $p = 0.004$, in males 77 ± 24.2 m², and in females 28 ± 9.6 m²). There were no significant differences between populations ($F_{2,8} = 2.153$, $p = 0.179$).

The degree of movement area overlap decreased significantly from November till December ($F_{1,57} = 19.094$, $p < 0.001$). We also tested if there was a difference in the tendency to overlap female or male areas between population types and sexes. The population type significantly affected the ratio of males overlapping male areas ($F_{2,8} = 10.527$, $p = 0.006$), as

males from male biased population seemed to avoid overlapping with other males (Fig. 2). By December the overlapping percent in male biased populations was 0% (male-male overlap, pairwise comparison: E vs M; $Z = 4.763$, $p < 0.001$, F vs M; $Z = 5.738$, $p < 0.001$, E vs F; $Z = -0.591$, $p = 0.992$). However, there were no significant differences in the tendency of males to overlap female areas between population types ($F_{2,7} = 2.421$, $p = 0.159$). The population type did not affect the overlapping tendency or direction in females (females overlapping males: $F_{2,7} = 2.100$, $p = 0.193$, females overlapping females: $F_{2,8} = 1.724$, $p = 0.239$).

3.3 Fecal steroid metabolites

Sex had a significant effect on stress levels measured as fecal corticosterone metabolite concentrations ($F_{1,24} = 43.029$, $p < 0.001$). However, this was likely due to the naturally lower basal corticosterone level in females (Sipari, Ylönen, Palme, unpublished results) rather than a difference in the stress experienced. After spending 1 month in the enclosures the stress levels had increased significantly ($F_{1,24} = 6.183$, $p = 0.020$). There were no significant interactions. For a more meaningful comparison between populations we tested the sexes separately and compared only the stress levels measured from the field. The population type significantly affected stress levels in females ($F_{2,6} = 5.248$, $p = 0.048$) but not in males ($F_{2,5} = 1.067$, $p = 0.411$, Fig. 3). In females, pairwise comparison showed that the M populations had significantly higher stress levels than the F populations ($Z = -3.186$, $p = 0.004$), but the other comparisons were not significant. Stress levels during November did not affect the survival observed in December (males: $F_{1,13} = 0.942$, $p = 0.349$; females: $F_{1,7} = 2.812$, $p = 0.138$).

By the end of November, testosterone metabolite levels had decreased significantly ($F_{1,18} = 10.119$, $p = 0.005$), except for female biased populations, where the testosterone metabolite levels actually increased, though not significantly (Fig. 4). The population type

had a significant effect on testosterone metabolite levels ($F_{2,19} = 9.099$, $p = 0.002$). In pairwise comparisons the levels were significantly higher in female biased than male biased or even sex-ratio populations (E vs. F; $Z = -3.241$, $p = 0.003$, F vs. M; $Z = 4.199$, $p < 0.001$). The E and the M population had no significant difference ($Z = 0.806$, $p = 0.699$). Testosterone metabolite levels measured in the field in November affected the survival observed in December ($F_{1,13} = 6.000$, $p = 0.029$). Individuals who were still alive by December had significantly lower testosterone metabolite levels than those who had died by then ($Z = -2.449$, $p = 0.014$).

3.4 *Body mass*

There were no significant differences in the body mass between different populations throughout the experiment ($F_{2,8} = 0.406$, $p = 0.679$). Males were significantly heavier than females ($F_{1,117} = 4.803$, $p = 0.030$). The body mass of the animals decreased significantly after the release ($F_{1,28} = 16.892$, $p < 0.001$). Body mass in the beginning of the experiment had a significant effect on the survival observed in December ($F_{11,115} = 4.460$, $p = 0.037$). In pairwise comparisons females that survived better were heavier at the beginning of the experiment than those that had died by December ($Z = 3.017$, $p = 0.014$). In males there was no significant difference ($Z = -0.368$, $p = 0.983$).

4. DISCUSSION

Our results suggest that the sex-ratio of the overwintering population may affect the behavior and overwintering strategies of bank voles. In our experiment we were not able to verify earlier findings of higher winter survival in females compared to males. However, our results

suggest that females may play an important role in creating and maintaining the winter aggregations and communal winter nests, as the degree of overlapping movement areas indicating winter aggregation was higher in female biased and even sex-ratio population types than male biased populations. Also, the sex-ratio of the population significantly affected the hormonal status of the individuals. Voles living in male biased populations tended to have higher stress levels than individuals from other population types, whereas in female biased populations the males showed significantly higher testosterone levels compared to other populations. Differences in stress levels did not result in different survival. Individuals in male biased populations tended to have a lower survivability than in other population types but the difference was not significant. However, the overlapping of the movement areas between individuals indicating social interactions, and perhaps a tendency for communal nesting, was 0 % in male biased populations in December even though the total sizes of the movement areas within a gender did not differ between population types. This suggests divergent social dynamics compared to even sex-ratio and female biased populations. Our interpretation is that the elevated stress levels and the lack of overlapping areas in male biased populations indicate a solitary overwintering strategy and possibly antagonistic social dynamics between individuals.

Aggression is often related to testosterone in males (Bronson 1989), and thus it was surprising to notice that the fecal testosterone metabolite concentrations were actually lowest in males from male biased populations. The difference was significant between female biased population type but not with the even sex-ratio population type. This suggests that the winter time aggressiveness cannot be solely testosterone induced. There are similar observations in some other rodents, such as hamsters, showing that in seasonal environments the short days reduce testosterone levels but induce aggressive behavior in males (Jasnow *et al.* 2000, Jasnow *et al.* 2002). In prairie voles (*Microtus ochrogaster*) castration does not reduce

aggressive behavior in males, and it seems that anterior hypothalamic vasopressin, rather than testosterone, might be involved in the regulation of aggression in this species (Demas *et al.* 1999, Gobrogge *et al.* 2007, Gobrogge *et al.* 2009). In red squirrels (*Tamiasciurus hudsonicus*) aggressiveness during the non-breeding season has been suggested to be related to the high concentrations of dehydroepiandrosterone (DHEA), an androgen precursor (Boonstra *et al.* 2008). DHEA is also linked to aggression in non-breeding songbirds (Soma *et al.* 2008). Density and territorial conflicts appear to increase DHEA levels, and it is noticed to be higher in stressed animals (Boonstra *et al.* 2008). This could explain the assumed antagonistic behavior occurring even with low testosterone levels in males from the male biased populations in our experiment. Winter time aggression in red squirrels was explained by the attempt to defend their food stores as food is often highly limited during winter (Dantzer *et al.* 2012). This could also be the case with the voles in the male biased populations. Males are larger than females and thus their absolute energy needs are higher. In populations with high male density and restricted food resources, staying solitary may overcome the benefits of communal nesting (West & Dublin 1984). It has been reported that the food distribution inside a given area does not affect the overwintering strategy in bank voles (Ylönen and Viitala 1991), but perhaps total food availability could. The higher stress levels observed in male biased populations could be derived from territorial conflicts as well as cold stress due to the possible solitary nesting.

Female biased sex ratio did not increase the survival probability, at least not during early winter. However, compared to male biased populations the degree of males' movement area overlap with other males was significantly higher, suggesting more amicable social interactions. Also the tendency of females to overlap the areas of other females and males was higher, but not significantly. Further, the higher female density in the population resulted in significantly higher fecal testosterone metabolite concentrations in males compared to

males from other population types. During the acclimatization period in the green house, before the actual experiment and division into different populations, males had already lowered their testosterone production towards the overwintering levels. In the enclosures, by the end of November, males in male biased populations and even sex-ratio populations had decreased their testosterone levels even further, but males in female biased populations showed quite the opposite trend. On average, males in female biased populations had testosterone levels high enough for reproduction in late November. During the last trapping session in December, we observed one female visibly pregnant and several receptive females (vaginal opening used as an indication) in female biased populations.

The presumed social aggregation and possible communal nesting combined with winter breeding in female biased populations differs quite drastically from the overwintering strategy observed in male biased populations. The ultimate reason for this, however, is not clear. Perhaps the benefits of communal nesting combined with the lower food consumption of females provide circumstances favorable enough for restoring breeding condition and reproducing during winter (Ylönen and Viitala 1985). In general, high testosterone level in males had a negative effect on survival. However, the observed high testosterone levels of males in female biased populations did not result in lower survival at the population level compared to other population types, at least not during the early winter. Furthermore, there were no observable differences in female space use between populations, which would indicate territoriality in female biased populations due to the restored breeding activity. Neither the elevated testosterone levels seemed to cause observable (in terms of movement area overlaps) aggressive behavior in males, which supports the idea of testosterone not being responsible for winter aggression in bank voles.

Winter breeding is known to occur occasionally in many vole species (Jannett 1984, Kaikusalo and Tast 1984, Ylönen and Viitala 1985) but the factors enabling or causing this

phenomenon are not always clear. High food abundance strongly correlates with the probability of winter breeding, but it does not explain all of the observed occasions (Jannett 1984). Population density is considered to be an important factor, as winter breeding is often reported to occur at low or increasing population densities (Jannett 1984). Our results, however, suggest that instead of population density *per se*, the female density and the low male-male competition in the population could play a key role inducing winter breeding.

In the populations with even sex-ratio, the degree of overlapping movement areas was quite similar to female biased population, suggesting that social aggregation occurred also in even sex-ratio populations. However, the observed movement areas of females were so small by December that interpreting the results of females' movement area overlap should be done cautiously. The hormonal responses in even sex-ratio populations showed no prominent trends compared to the other population types. Perhaps in populations with a balanced female-male density the number of females is high enough to enable a certain degree of communal nesting, but not necessarily favorable for inducing winter breeding.

Conclusion

It is intriguing how the gender distribution of an overwintering population can lead to such divergent overwintering strategies in bank voles, while living in almost identical habitats and experiencing the same environmental conditions. To determine whether one of the strategies is better than the other in terms of higher survival or direct fitness gain would require more extensive long-term experiments. Based on the trends observed in our experiment it seems possible that the solitary overwintering strategy presumably exploited in male-biased populations could eventually result in lowest survival, though we could not verify this during this short-term early winter experiment. On the other hand, in the case of a rapid decrease in food abundance the communal nesting could turn out to be an unfavorable strategy.

Particularly, the energetically demanding winter breeding could easily result in high mortality in the population in case of suddenly deteriorated environmental conditions. Nevertheless, our results show evident behavioral plasticity, as well as opportunism in the overwintering strategies in bank voles under northern winter conditions.

Acknowledgement

We would like thank Professor Jack Millar and Professor Anders Angerbjörn for commenting on earlier versions of the manuscript, John Loehr for checking the language and Konnevesi research station staff; Jyrki Raatikainen, Risto Latvanen, Janne Koskinen and Helinä Nisu for taking such a good care of our animals. This research was financed by the Academy of Finland and Biological Interactions Graduate School.

References

- Aars J. & Ims R.A. 2002. Intrinsic and climatic determinants of population demography: the winter dynamics of tundra voles. *Ecology* 83: 3449-3456.
- Boonstra R., Dantzer B., Delehanty B., Fletcher Q.E. & Sheriff M.J. 2014. Equipped for Life in the Boreal Forest: The Role of the Stress Axis in Mammals. *Arctic* 67: 82-97.
- Boonstra R., Lane J.E., Boutin S., Bradley A., Desantis L., Newman A.E. & Soma K.K. 2008. Plasma DHEA levels in wild, territorial red squirrels: seasonal variation and effect of ACTH. *Gen Comp Endocrinol* 158: 61-67.
- Boonstra R. 2004. Coping with changing northern environments: the role of the stress axis in birds and mammals. *Integr Comp Biol* 44: 95-108.
- Bronson F.H. 1989. *Mammalian reproductive biology*. University of Chicago Press.
- Bronson F.H. 1985. Mammalian reproduction: An ecological perspective. *Biology of Reproduction* 32: 1-26.
- Bujalska G. 1973. The role of spacing behaviour among females in the regulation of reproduction in the bank vole. *Journal Reprod Fert* 19: 465-474.
- Campbell N. & Reece J. 2005. *Biology*. 7th. Benjamin Cummings, San Francisco.
- Dantzer B., Boutin S., Humphries M.M. & McAdam A.G. 2012. Behavioral responses of territorial red squirrels to natural and experimental variation in population density. *Behav Ecol Sociobiol* 66: 865-878.
- Demas G.E., Moffatt C.A., Drazen D.L. & Nelson R.J. 1999. Castration does not inhibit aggressive behavior in adult male prairie voles (*Microtus ochrogaster*). *Physiol Behav* 66: 59-62.
- Feist D.D. 1984. Metabolic and thermogenic adjustments in winter acclimatization of subarctic Alaskan red-backed voles. In: Merritt J.F. (ed.), *Winter Ecology of Small Mammals*, Carnegie Museum of Natural History, Spec. Pub 10, Pittsburgh, pp. 131-137.
- Gobrogge K.L., Liu Y., Jia X. & Wang Z. 2007. Anterior hypothalamic neural activation and neurochemical associations with aggression in pair-bonded male prairie voles. *J Comp Neurol* 502: 1109-1122.
- Gobrogge K.L., Liu Y., Young L.J. & Wang Z. 2009. Anterior hypothalamic vasopressin regulates pair-bonding and drug-induced aggression in a monogamous rodent. *Proc Natl Acad Sci U S A* 106: 19144-19149.
- Hansson L. 1990. Ultimate factors in the winter weight depression of small mammals. *Mammalia* 54: 397-404.

- Hyvärinen H. 1984. Wintering strategy of voles and shrews in Finland. In: Merritt J., F. (ed.), *Winter Ecology of Small Mammals*, Carnegie Museum of Natural History, Spec. Pub 10, Pittsburgh, pp. 139-148.
- Jannett F.J., Jr. 1984. Reproduction of the montane vole, *Microtus montanus*, in subnivean populations. In: Merritt J., F. (ed.), *Winter Ecology of Small Mammals*, Carnegie Museum of Natural History, Spec. Pub 10, Pittsburgh, pp. 215-224.
- Jasnow A.M., Huhman K.L., Bartness T.J. & Demas G.E. 2002. Short Days and Exogenous Melatonin Increase Aggression of Male Syrian Hamsters (*Mesocricetus auratus*). *Horm Behav* 42: 13-20.
- Jasnow A.M., Huhman K.L., Bartness T.J. & Demas G.E. 2000. Short-Day Increases in Aggression Are Inversely Related to Circulating Testosterone Concentrations in Male Siberian Hamsters (*Phodopus sungorus*). *Horm Behav* 38: 102-110.
- Kaikusalo A. & Tast J. 1984. Winter breeding of microtine rodents at Kilpisjärvi, Finnish Lapland. In: Merritt J., F. (ed.), *Winter Ecology of Small Mammals*, Carnegie Museum of Natural History, Spec. Pub 10, Pittsburgh, pp. 243-252.
- Klemme I., Ylonen H. & Eccard J.A. 2008. Long-term fitness benefits of polyandry in a small mammal, the bank vole *Clethrionomys glareolus*. *Proc Biol Sci* 275: 1095-1100.
- Marchand P.J. 1996. *Life in the cold: an introduction to winter ecology*. University Press of New England.
- Merritt J.F. & Zegers D.A. 2002. Maximizing survivorship in cold: thermogenic profiles of non-hibernating mammals. *Acta Theriol* 47: 221-234.
- Mills S.C., Grapputo A., Jokinen I., Koskela E., Mappes T., Oksanen T.A. & Poikonen T. 2009. Testosterone-Mediated Effects on Fitness-Related Phenotypic Traits and Fitness. *Am Nat* 173: 475-487.
- Norrdahl K. & Korpimäki E. 1998. Does mobility or sex of voles affect risk of predation by mammalian predators? *Ecology* 79: 226-232.
- Palme R. & Möstl E. 1994. Biotin-streptavidin enzyme immunoassay for the determination of oestrogens and androgens in boar faeces. *Advances of Steroid Analysis '93" (Ed. S. Görög), Akadémiai Kiadó, Budapest* : 111-117.
- Sealander J.A., Jr. 1952. The Relationship of Nest Protection and Huddling to Survival of *Peromyscus* at Low Temperature. *Ecology* 33: 63-71.
- Soma K.K., Scotti M.L., Newman A.E., Charlier T.D. & Demas G.E. 2008. Novel mechanisms for neuroendocrine regulation of aggression. *Front Neuroendocrinol* 29: 476-489.
- Touma C., Sachser N., Möstl E. & Palme R. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *Gen Comp Endocrinol* 130: 267-278.

- Vickery W.L. & Millar J.S. 1984. The energetics of huddling by endotherms. *Oikos* : 88-93.
- Viitala J. 1984. Stability of overwintering populations of *Clethrionomys* and *Microtus* at Kilpisjärvi, Finnish Lapland. In: Merritt J., F. (ed.), *Winter Ecology of Small Mammals*, Carnegie Museum of Natural History, Spec. Pub 10, Pittsburgh, pp. 109-112.
- Webster A.B. & Brooks R.J. 1981. Social behavior of *Microtus pennsylvanicus* in relation to seasonal changes in demography. *J Mammal* : 738-751.
- Werken J.,H & Charnov E.,L. 1978. Facultative sex ratios and population dynamics. *Nature* 272: 349-350.
- West S.D. & Dublin H.T. 1984. Behavioral strategies of small mammals under winter conditions: Solitary or social?. In: Merritt J., F. (ed.), *Winter Ecology of Small Mammals*, Carnegie Museum of Natural History, Spec. Pub 10, Pittsburgh, pp. 293-299.
- Wolff J. & Lidicker Jr W.Z. 1981. Communal winter nesting and food sharing in taiga voles. *Behav Ecol Sociobiol* 9: 237-240.
- Ylönen H. & Viitala J. 1985. Social organization of an enclosed winter population of the bank vole *Clethrionomys glareolus*. *Ann Zool Fenn* 22: 353-358.
- Ylönen H. & Viitala J. 1991. Social overwintering and food distribution in the bank vole *Clethrionomys glareolus*. *Ecography* 14: 131-137.

FIGURE 1 Survival rates of different population types during the experiment (LS-means \pm SE). (F = female biased population type, M = male biased population type, E = even sex-ratio population type). No significant differences ($P < 0.05$) between population types were observed.

FIGURE 2 The sizes of the movement areas and the degree of overlapping areas in males (a) and in females (b) during November and December in different population types. Significant differences ($P < 0.05$) in movement areas (Area, left y- axis) were observed between time (November $>$ December) and sexes (males $>$ females), but not between population types. Time had a significant effect on the overlap ratio (November $>$ December right, y-axis), and the population type affected the ratio of males overlapping male areas (M $<$ E and M $<$ F). (MM = males overlapping males, MF = males overlapping females, FM = females overlapping males, FF = females overlapping females).

FIGURE 3 Fecal corticosterone metabolite levels (LS-means with SE) indicating stress in different population types in females and males. In the laboratory (Lab) all animals were housed individually. Field measurements were done at the end of November. Significant differences ($P < 0.05$) in CM levels were found between the sexes (males $>$ females) and between laboratory and field conditions (Lab $<$ Field). The only significant difference between population types was observed in females under field conditions (F $<$ M). (F = female biased population type,

M = male biased population type, E = even sex-ratio population type, * to enable accurate comparison between sexes the metabolite levels are adjusted. See Material and methods, 2.3).

FIGURE 4 Fecal testosterone metabolite (TM) levels (LS-means with SE) in different population types in males. In the laboratory (Lab) all animals were housed individually. Field measurements were done at the end of November. Significant differences ($P < 0.05$) were found between laboratory and field conditions (Lab > Field) and between population types under field conditions (F > M and F > E). (F = female biased population type, M = male biased population type, E = even sex-ratio population type).

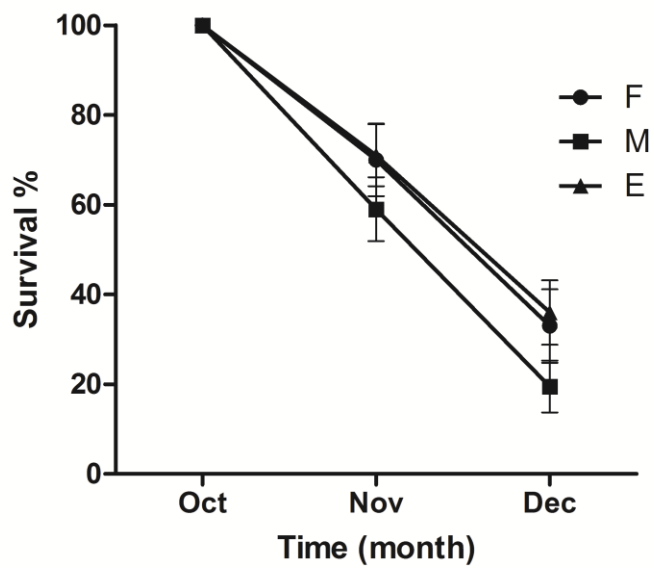


Fig. 1

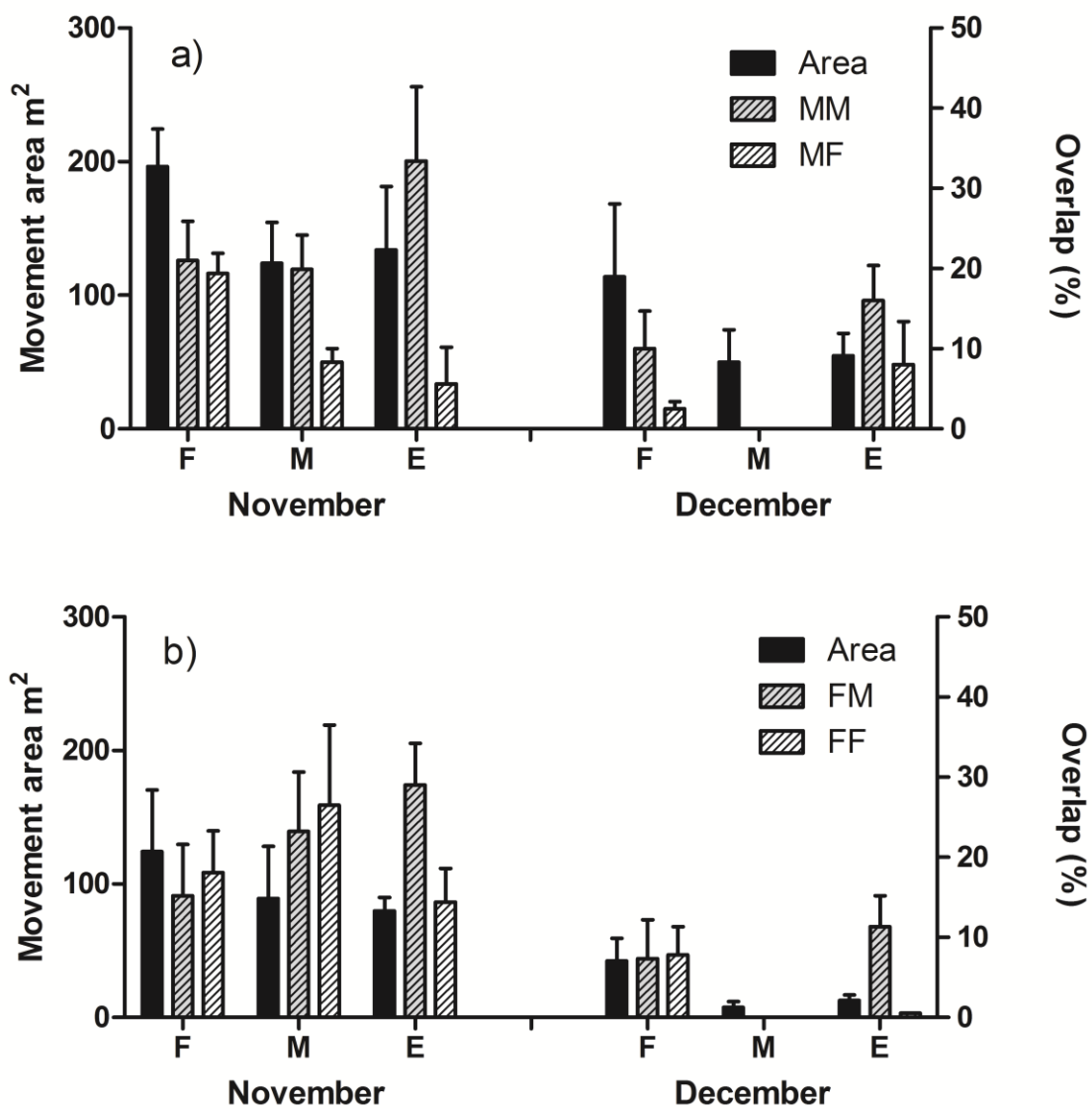


Fig. 2

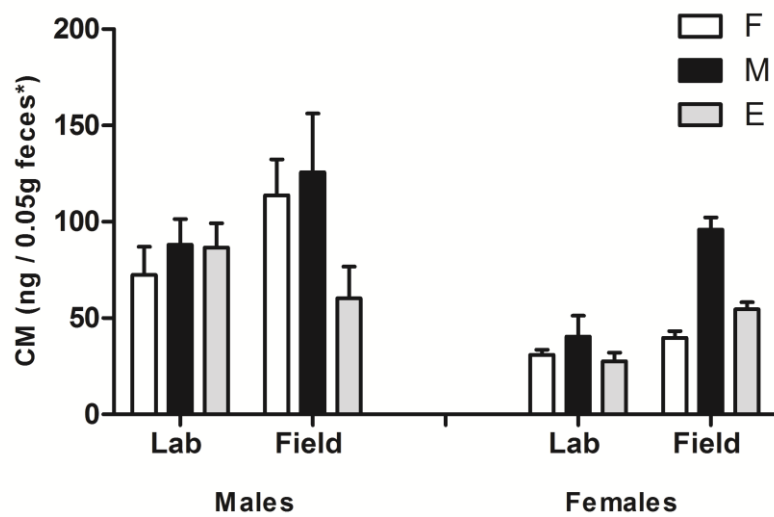


Fig. 3

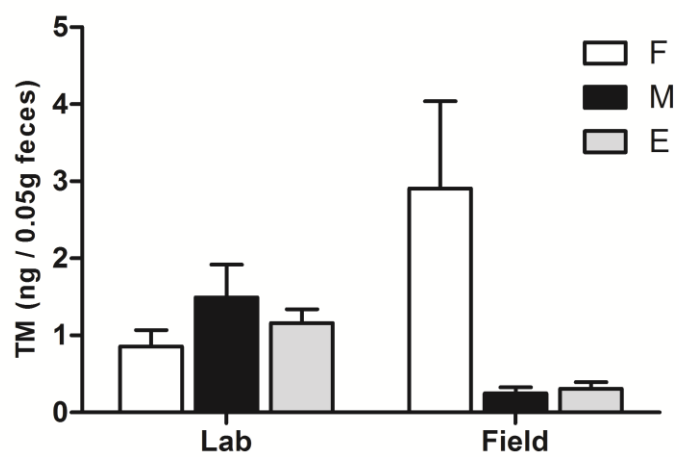


Fig. 4

ACCEPTED MANUSCRIPT

Highlights

- Female biased population sex-ratio induces social overwintering and winter breeding
- Male biased sex-ratio can lead to solitary overwintering
- Male biased sex-ratio induces elevated stress levels in overwintering females

ACCEPTED MANUSCRIPT