



Does maternal stress influence winter survival of offspring in root voles *Microtus oeconomus*? A field experiment

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Maternal stress can have long-term adverse consequences on immunocompetence and disease risk of offspring, and winter survival is a crucial demographic parameter in the life-history of an individual that can substantially affect northern rodent population dynamics. An understanding of the effects of maternal stress on winter survival of offspring may help identify mechanisms driving population fluctuations of northern small mammals. Thus, we assessed the effects of maternal stress, resulting from high population densities, on winter survival of first generation (F_1) and second generation (F_2) in root voles *Microtus oeconomus*. Replicate high- and low-density enclosed parental populations were established, from which we obtained F_1 generation that were used to establish new enclosed, equal-density populations. The adults of the high-density parental populations had higher corticosterone levels, an indication of physiological stress, than did those of the low-density parental populations. Over-winter survival of the F_1 generation voles from the low-density parental populations was greater than that of those from the high-density parental populations. Over-winter survival of F_2 generation voles did not differ between the two treatments. Our results suggest that maternal stress affected over-winter survival of first generations but not second generations. Reduced immunocompetence, resulting from high population density stresses, transferred to offspring may be a factor in annual (winter) population declines. Because the effect is transitory, i.e. immunocompetence of F_2 voles is not affected, reduced immunocompetence resulting from high density stresses would not contribute to lengthy periods of low population densities that are characteristic of multi-annual population fluctuations.

Physiological factors, including the influence of chronic stress, on fitness and life-history traits, such as survival, are important considerations in understanding the dynamics of small mammal populations (Reeder and Kramer 2005, Chown and Storey 2006). A growing body of evidence has demonstrated that maternal stress during gestation impairs both humoral and cellular immune function of offspring, thus increasing susceptibility to diseases (Llorente et al. 2002, Patin et al. 2002, Tuchscherer et al. 2002, Götz and Stefanski 2007). There is a close link between immunocompetence and survival (Mihok et al. 1985, Lochmiller 1996, Sinclair and Lochmiller 2000).

Individual survival is a key life history trait, with major demographic consequences for population fluctuations (Korpimäki et al. 2004, Ozgul et al. 2004). Winter survival has been shown to play a crucial role in population dynamics of small mammals in northern regions (Hansson and Henttonen 1988, Stenseth 1999). Because the number of individuals present at the beginning of the spring breeding period is dependent on over-winter survival (Solonen 2006), winter is an obvious bottleneck for northern small mammal

populations. Low ambient temperature and decreased food availability are the main determinants of winter survival in small mammals. Nelson et al. (1995) proposed that small mammals display enhanced immune-system functions in winter to cope with the challenge of winter conditions. However, the immune function of animals often appears to be depressed in winter (Nelson et al. 1995). Nelson and Demas (1997) hypothesized that winter stresses, such as low temperature and insufficient food, may counteract the short-day enhancement of immune function. As a result, the stress of winter conditions may result in low immune function. One may expect, therefore, that prenatally stressed individuals, when experiencing harsh winters, may exhaust their already diminished immunocompetence, leading to low over-winter survival.

In the study of small mammal population dynamics, it is important to know what initiates population declines and the factors involved in driving populations to extreme lows (Korslund and Steen 2005). Hence, an understanding of the effects of maternal stress on winter survival of offspring may help identify mechanisms driving population fluctuations of northern small mammals.

In this paper we examine the effects of maternal stress on winter survival of offspring of root voles *Microtus oeconomus*, which inhabit the Qinghai-Tibet plateau of the China. Previous results from the same study have shown that prenatally stressed F_1 voles from high density populations had lower immunocompetence, as compared to those from low density populations (Wu et al. 2008). In another study Christian and Lemunyan (1958) showed that crowding affected weight gain and survival, not only of the first generation (F_1) progeny, but also of the second generation (F_2) progeny. Hence, we hypothesized that maternal stress, induced by high density populations, would affect winter survival of F_1 root voles via effects of maternal stress on offspring immunocompetence, and in turn decreased over-winter survival of F_2 voles via the transmission of the effects to offspring of the F_2 generation. To test the hypothesis, we established parental populations of different densities, and quantified plasma corticosterone concentration of the founders to determine if high density induced stress responses in the parents. We then established two, each, replicate populations, whose founders were the F_1 individuals of the original high and low density parental populations, and compared the difference in winter survival of F_1 and F_2 generations. We predicted that F_1 offspring originating from high-density populations would have lower winter survival rates than those originating from low-density populations and that over-winter survival of F_2 voles, whose mothers (F_1) were from high-density parental populations, would have lesser survival than those from low density parental populations.

Methods

Study area

The experiment was conducted at the Haibei Alpine Meadow Ecosystem Research Station, Chinese Academy of Sciences, Menyuan County, approximately 155 km north of Xining, the capital of Qinghai province, China (37°37'N, 101°12'E). Mean annual temperature at the site is -1.6°C and the mean annual precipitation is 562 mm. Mean elevation in the valley bottom is 3200 m.

The study site was located in a winter-grazed *Elymus nutans* meadow, the natural habitat of root voles. The predominant plants were *E. nutans*, *Poa* sp., *Thalictrum alpinum* and *Kobresia humilis*; there was dense plant cover (> 40 cm) above the surface. The soil was loose, moist and fertile.

Establishing parental populations

We established parental populations in four 0.15 ha (30 × 50 m) enclosures in mid-April 2005. The enclosures were constructed of galvanized steel panels extending 0.5 m below and 1.5 m above ground. Each enclosure was larger than the largest home range of root voles (Sun et al. 1982). The sides were high enough to enclose study populations and prevent entry of mammalian predators, such as weasels (*Mustela altaica*, *M. eversmanni*). Avifauna predators had free access to the pens. Above-ground biomass of vegetation did not

differ among the four enclosures (as measured from ten 25 × 25 cm clip-quadrats, randomly located in each enclosure; $F_{3,36} = 0.17$, $p = 0.18$).

The founders of parental populations were captured as juveniles in September 2004 in general study area. The voles were taken to laboratory and maintained in (45 × 30 × 20 cm) cages, two same sex individuals per cage, until released in the experimental study pens. In April 2005, we selected mature and disease-free adults, individually marked by toe clips (≤ 1 toe per foot) as founders of the experimental populations. Prior to establishing the experimental populations, we trapped all enclosures for two weeks to remove resident small mammals. We adopted a systematic design (Hurlbert 1984) to establish two separate, one high-density and one low-density parental populations. High-density populations were established by placing 30 adults of each sex in each enclosure; low-density populations were established by placing 6 adults of each sex in each pen. This created densities of 400 voles ha^{-1} and 80 voles ha^{-1} , representing high- and moderate-densities, respectively, for this species in this study area (Jiang et al. 1991). Founders were allowed one week to become accustomed to the enclosures before trapping began. The body mass of the founders did not differ between enclosures within each population density ($F_{2,68} = 1.12$, $p = 0.33$ for males, $F_{2,68} = 1.18$, $p = 0.31$ for females), or between different parental populations ($F_{1,142} = 0.037$, $p = 0.85$).

Parental populations were trapped, using standard capture-mark-recapture, from late April 2005 to late Jun 2005. We set 60 laboratory-made wooden live-traps (Nie and Liu 2005), baited with carrots, in a 5 × 9 grid (45 traps) in each enclosure. Three-day trapping sessions were conducted at weekly intervals. The traps were set from 06:00 – 12:00 and from 14:30 – 20:30; they were locked closed 12:00 – 14:30 and 20:30 – 06:00, to avoid mortality from high and low temperatures at midday and night. We checked the traps 4 – 5 times a day within a trapping session. The traps were left in place and locked closed between trapping sessions. Each trap was covered with a wooden sheet to reduce exposure to precipitation and temperature extremes. For all captures we recorded trap location, individual identification, body mass, sex, and reproductive status (males, testes abdominal or scrotal; females, closed or perforated vagina, palpable embryos, enlarged teats barren of hair).

Nests of pregnant females were located by the ultraviolet reflective pigment tracking method (Lemen and Freeman 1985, Nie and Liu 2005). We placed 5 – 7 live-traps near the entrance to the nest and in the adjacent surface runways to capture newborn offspring. The newborn (F_1 generation) were 20 – 30 days of age when captured; root voles are weaned at 20 days of age (Jianghui et al. 2005). The F_1 voles were placed in cages (up to 10 individuals per cage) and taken to the Haibei Alpine Meadow Ecosystem Research Station. The F_1 voles were kept in cages (two individuals of the same sex per cage) for 2 – 4 weeks until we started the experimental F_1 populations. We did not control photoperiod and temperature, which varied with the ambient conditions, of the holding room. Offspring were fed pieces of carrots for two weeks, until they were able to eat granulated rabbit chow and had

learned to drink from water bottles. To avoid unnecessary stress induced by laboratory feeding, we did not disturb them except for providing food and water, and clearing cages. All procedures conformed to ASM guidelines for live capture, handling, care of mammals (Animal Care and Use Committee 1998).

Establishing offspring populations

In the end of June 2005, we live-trapped to remove all the parental populations from each enclosure. We then established four F_1 populations by releasing enclosure-born F_1 generation voles into their original enclosures. Two populations consisted of F_1 generation offspring from high-density parental populations (HDOP), the other two offspring populations, F_1 generation voles from low density parental populations (LDOP). All four offspring populations were started with 20 individuals. Owing to few F_1 males, one of replicates of LDOP consisted of eight males and 12 females, the other 10, each, males and females. One of the replicates of HDOP consisted of nine males and 11 females, the other of 10, each, males females. F_1 voles used to establish offspring populations were 40 – 60 days of age, the approximate age at which root voles become reproductively active (Jianghui et al. 2005). The voles were allowed one week to become accustomed to the enclosures before trapping began. The enclosure populations were trapped monthly from mid-July 2005 to mid-April 2006, using standard capture–mark–recapture. Trapping protocol was the same as that used for the parental populations.

Hormone assays

The founders of the original parental populations were live-trapped and transferred in cloth bags to the laboratory, which had a natural photoperiod and ambient temperatures. The founders were allowed to be accustomed to the laboratory for 1 d before measuring corticosterone levels. Nine founders from low-density populations (one male and four females from one population, and two, each males and females from the other) and twenty-four founders from high-density populations (seven males and six females from one and five males and six females from the other) were used to assay hormone levels. Each individual was decapitated at 09:00 – 10:00 in the morning and trunk blood collected within 0.5 min. The blood was centrifuged at 4000 rpm for 15 min. The separated plasma was frozen and stored at -30°C until analysis. Plasma corticosterone concentrations were determined, in duplicate, using commercially available corticosterone ELISA kit. The assay required 125 μl of serum. The sensitivities of the assays were 0.7 nmol l^{-1} for corticosterone. All procedures conformed to guidelines of the American Society of Mammalogists (Animal Care and Use Committee 1998).

Survival modeling and data analyses

We estimated apparent survival (called ‘survival’ in the following) and recapture probability (‘recapture’) of F_1 and F_2

voles using the standard open population Cormack–Jolly–Seber model (Lebreton et al. 1992), implemented in program MARK (White and Burnham 1999). For F_1 voles, the data comprised the capture history of 80 voles (37 males and 43 females), for 10 trapping sessions from July 2005 to April 2006, with an intersession interval of 30 days. For F_2 voles, the data comprised the capture history of 162 voles (77 males and 85 females), for nine trapping sessions from August 2005 to April 2006, with the same time intersession interval for the as F_1 voles.

First, we assessed the goodness of fit (GOF) of the global model to the data, as the CMR models used assume that: 1) every marked animal in the population immediately after time (i) has the same probability of surviving to time ($i + 1$), and 2) every marked animal present in the population at time (i) has the same probability of recapture (p_i). As our focus was to examine whether parental maternal stress (F_1 generation) or grandparental maternal stress (F_2 generation), from density effects, affected over-winter survival of progeny, entire data set using GOF test was split by parental density for F_1 voles and grandparental density for F_2 voles. We carried out the GOF test on the global models, $\Phi_{pd^*t} p_{pd^*t}$ (with both survival and recapture dependent on parental density [denoted as ‘pd’] and time [t]) for F_1 voles. Because all F_2 voles firstly captured in enclosures were juvenile, they were under adult stage when re-captured one month later. While individuals of different developmental stages may differ in the probability of surviving to the next age or stage, thus we constrained an age model as globe model according to Cooch and White (2006) for F_2 voles: $\Phi_{gpd^*a^*t} p_{gpd^*a^*t}$ (with both survival and recapture dependent on grandparental density [‘gpd’], age [‘a’] and time). In this model structure, for a given time interval, the model had two developmental stages of individuals: juveniles (individuals to be firstly captured), and adults (individuals to be re-captured). We call this group ‘age effect’, which was nested in each grandparental group (two level-high and low densities). GOF test was assessed using RELEASE (Burnham et al. 1987), in MARK. The GOF tests were insignificant for both the F_1 voles (test 2 and 3, RELEASE: $\chi^2 = 24.31$, $DF = 20$, $p = 0.23$) and for F_2 voles ($\chi^2 = 24.89$, $DF = 25$, $p = 0.47$), suggesting that both model fits were acceptable. We then used bootstrap-based GOF test to estimated \hat{c} -hat value (1.27 for F_1 and 1.12 for F_2), and then we adjusted \hat{c} -hat to 1.27 and 1.12 in the globe models for F_1 and F_2 voles, respectively. Model notation is based on Lebreton et al. (1992), with subscripts denoting the parameters included within the model. The main effects are denoted by a plus sign (+); specific interactions are denoted by a dot (.); models including all the combinations of additive and interaction effects are denoted by an asterisk (*). The subscripts for model parameters used are denoted in Table 1.

Next, we carried out model selection based on the both global models as recommended by Lebreton et al. (1992). Variations in recapture were modeled before constraining variations in survival, to increase the power of detecting variation in survival. Parsimonious recapture models were selected on the basis of QAICc through the elimination of non-significant variation (Anderson et al. 2000). QAICc is

Table 1. Best model structures of modeling recapture for F_1 and F_2 generations of root voles *Microtus oeconomus*. In each generation, the model with the lowest QAICc is reported first and all models within 3 of the lowest QAICc are included. The model structure for survival was kept constant ($pd \times t$) and ($gpd \times a \times t$) for F_1 and F_2 voles while model structure of recapture were investigated. The most parsimonious models are shown in bold. The starting model for the F_1 generation had an QAICc within 2 of the best model but fewer parameters. Effect of parental and grandparental density is abbreviated pd and gpd, respectively; time effect is t; age effect is a. The main effects are symbolized by a plus sign (+); specific interactions are symbolized by a dot (.); models including all the combinations of additive and interaction effects are represented by an asterisk (*).

Generation	Model no.	Model	Number of parameters	QDeviance	QAICc
F_1 voles	1	p_t	21	130.45	535.37
	2	p_{pd+t}	22	128.17	535.38
	3	$p_{pd+t+pd.t}$	28	124.98	546.36
F_2 voles	4	p_{gpd+t}	21	287.83	1214.78
	5	$p_{gpd+t+gpd \times a.t}$	24	283.84	1217.23
	6	$p_{gpd+a+t+gpd.a+gpd.a.t}$	25	282.77	1218.32

Quasi-AIC where the AICc values are corrected for overdispersion (\hat{c}), and AICc is Akaike's information criterion corrected for small samples (Hurvich and Tsai 1989). Models with differences in QAICc of < 2 were considered similar in their ability to describe the data (Burnham and Anderson 1992). According to the principal of parsimony, if two alternative models had indistinguishable QAICc values ($\Delta AICc < 2$), the model with fewer parameters was selected. Survival was modeled similarly using the best recapture model.

Finally, we tested hypothesis that the parental and grandparental density influenced winter survival of F_1 and F_2 voles by comparing a more parsimonious model containing the density factor, e.g. parental or grandparental density, with neighboring ones without this factor using QAICc.

We used the minimum number known alive (MNKA) method to estimate sizes of parental and offspring populations, as well as numbers of F_1 or F_2 voles in different offspring populations. Mark-recapture sampling trials of known populations in the enclosures showed that MNKA is the best estimate of true population size compared to other estimators (Chambers et al. 1999). The data were examined for homogeneity and normality. Data failing to meet these assumptions were natural log transformed prior to analysis. We used fixed-factor repeated measures one-way analyses of variance (ANOVAs) to test the difference in different population sizes and in numbers of F_1 or F_2 voles between different offspring populations. Owing to insignificant variations in corticosterone level between two replicate enclosures within a high-density parental population ($F_{1,20} = 1.42$, $p = 0.25$; because of insufficient sample sizes, we did not test the difference between two replicates within a low-density population), we pooled data from two replicate and analyzed the effects of parental population density on corticosterone of their founders by using two-way ANOVAs. To explore the effects of body mass on winter survival rates, we analyzed frequency distributions of founder initial body mass between different offspring populations by using likelihood rate (LRT) of χ^2 -test. We also used nested ANOVA to test for difference in founder initial body mass between two replicates within an offspring population and between

different offspring populations. Analyses were performed in SPSS (SPSS Inc., ver. 10.0).

Results

Parental population sizes and corticosterone concentration of founders

The number of founders in the parental populations declined synchronously from the 1st to the 9th week. The differences in density between the LDOP and HDOP remained four-fold ($F_{1,2} = 325.18$, $p = 0.003$; Fig. 1). The mean number of founders at end of the experiment was 5.50 ± 0.50 voles/enclosure in low density populations and 21.00 ± 1.00 voles/enclosure in high-density populations.

Blood corticosterone concentration of the founders differed between the two parental populations ($F_{1,29} = 5.33$, $p = 0.028$; Fig. 2). Founders from the high density populations had higher corticosterone levels than did those from the low density populations. Corticosterone level of males was significantly lower than that of females ($F_{1,29} = 4.42$, $p = 0.044$), but there was no interaction between sex and density ($F_{1,29} = 0.48$, $p = 0.495$).

Founder initial body mass distributions in offspring populations

The frequency distributions of body mass of the initial founders did not differ, either between the two replicates within each offspring population (Fig. 3, LRT $\chi^2 = 3.63$, $DF = 3$, $p = 0.30$, and LRT $\chi^2 = 5.89$, $DF = 4$, $p = 0.21$ for males and females in LDOP, respectively; LRT $\chi^2 = 0.69$, $DF = 4$, $p = 0.95$, and LRT $\chi^2 = 3.09$, $DF = 4$, $p = 0.54$ for males and females in HDOP, respectively), or between different offspring populations (LRT $\chi^2 = 3.15$, $DF = 4$, $p = 0.53$ for males, and LRT $\chi^2 = 5.43$, $DF = 4$, $p = 0.25$ for females). In addition, initial body mass of the founder offspring populations did not differ, either between replicates within each population density (Fig. 3; $F_{2,32} = 1.18$, $p = 0.32$ and $F_{2,35} = 1.13$, $p = 0.34$, for males and females,

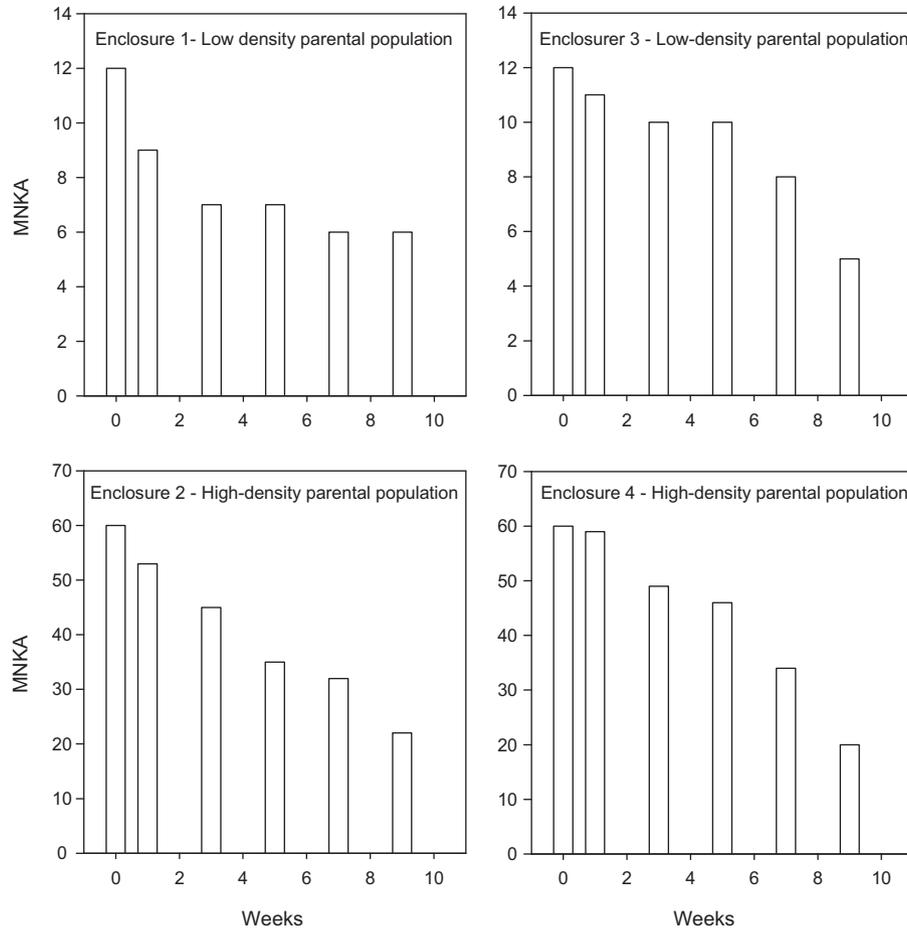


Figure 1. The minimum number known to be alive (MNKA) per 0.15 ha for founders of low- and high-density of parental populations in root voles *Microtus oeconomus*. At 0 week bar indicated number of founders to be introduced to enclosures prior to experiment. For the entire experiment, repeated-measures ANOVA showed significant difference between low- and high-density parental populations ($F_{1,2} = 347.78$, $p = 0.003$).

respectively), or between offspring populations (Fig. 3; $F_{1,32} = 0.48$, $p = 0.49$ and $F_{1,35} = 2.61$, $p = 0.12$, for males and females, respectively).

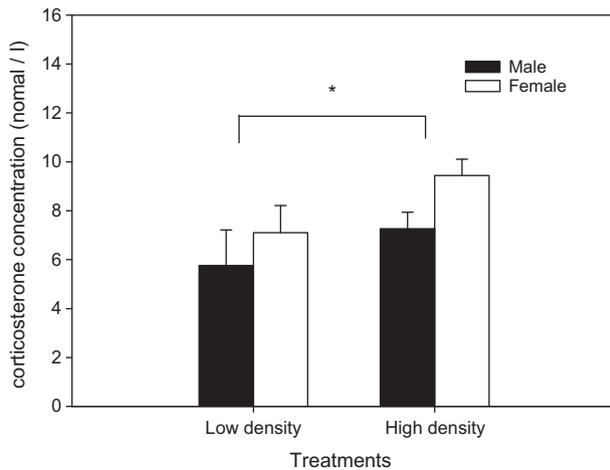


Figure 2. The corticosterone concentration of founders from low- and high-density of parental populations in root voles *Microtus oeconomus*. The data between two replicates within a density population were pooled. Statistically significant effect of parental density on corticosterone level of founders are indicated by * = $p < 0.05$.

Impact of parental population density on survival of offspring

Table 1 presents the best models for recaptures of F_1 and F_2 voles. Model 1, with the lowest QAICc (QAICc = 535.37) and the least parameters, included temporal variation alone and was therefore selected. For F_2 voles, recapture rates were best modeled by including effects of grandparental density and time (model 4). The average recapture rate during the experiment was 58.37% for F_1 voles, and that was 69.73% and 63.11% for F_2 voles for LDOP and HDOP, respectively (estimates derived from model 1 and model 4 in Table 1).

Table 2 presents the best models for survival of F_1 and F_2 voles. The best model structure for describing the survival rate of F_1 voles was $\Phi_{pd + \tau}$ (model 2), which described the survival rate, had effects of parental density and time. Difference in QAICc between model 2 and model 3 was 4.16, indicating that parental density significantly affected winter survival of F_1 voles. Figure 4 showed that survival estimates for nine time intervals (based on the model $\Phi_{pd + \tau} P_t$). Overall survival of F_1 voles was lower in HDOP than LDOP voles. Thirty-day survival of HDOP F_1 voles averaged 8.73% lower than that of LDOP F_1 voles. In addition, survival of F_1 generations in HDOP

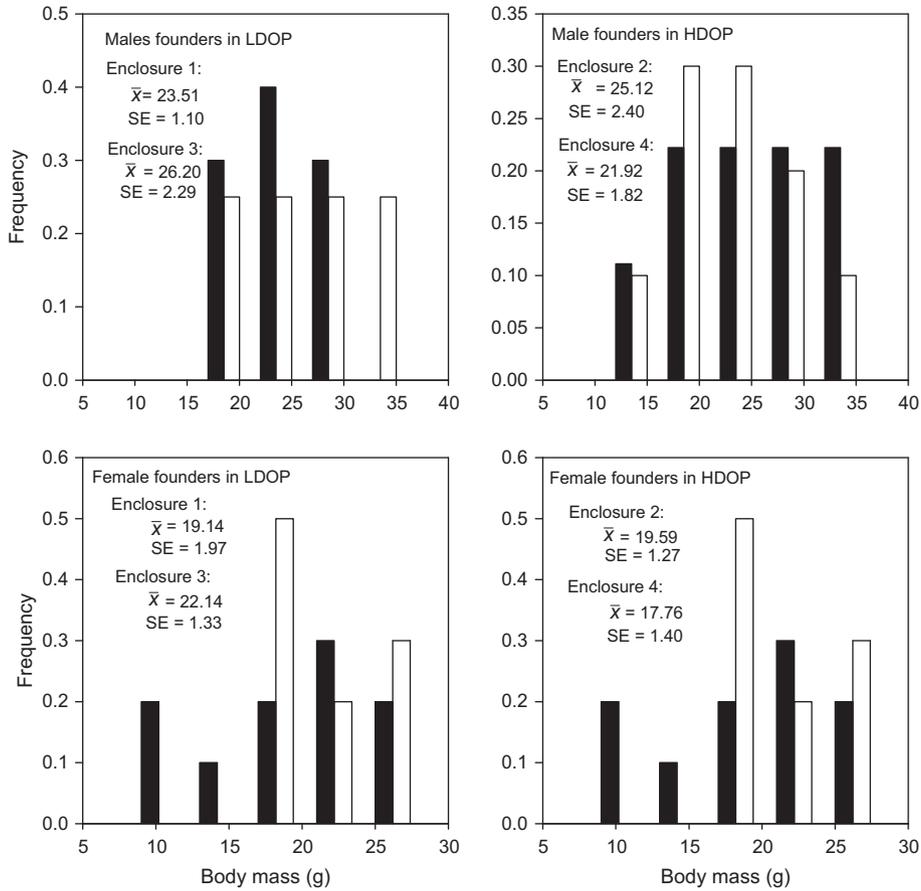


Figure 3. Frequency distributions of founder initial body mass in different offspring populations in root voles *Microtus oeconomus*. The dark and white bar indicated two replicates in each offspring population. LDOP = offspring population which was established by the F_1 founders originating from low-density parental population; HDOP = offspring population which was established by the F_1 founders originating from high-density parental population. The initial body mass distributions were not significantly different either between replicates within a offspring population (LRT $\chi^2 = 3.63$, DF = 3, $p = 0.30$, and LRT $\chi^2 = 5.89$, DF = 4, $p = 0.21$ for males and females in LDOP, respectively; LRT $\chi^2 = 0.69$, DF = 4, $p = 0.95$, and LRT $\chi^2 = 3.09$, DF = 4, $p = 0.54$ for males and females in HDOP, respectively), or between offspring populations (LRT $\chi^2 = 3.15$, DF = 4, $p = 0.53$ for males, and LRT $\chi^2 = 5.43$, DF = 4, $p = 0.25$ for females).

and LDOP displayed two nadirs, October and February–March (Fig. 4). Moreover, the lowest survival of both F_1 populations was in February–March; survival of F_1 voles

in HDOP was 29.60% lower than that in LDOP. In the second nadir, October, survival of F_1 voles in HDOP was 15.64% lower than that in LDOP.

Table 2. Best model structures of modeling survival for F_1 and F_2 generations for root voles *Microtus oeconomus*. In each generation, the model with the lowest QAICc is reported first and all models within 3 of the lowest QAICc are included. The model structure for recapture was kept as the best model from Table 1 (except for the global model for which $p_{pd} \times t$ and $p_{gpd} \times a \times t$ was used for F_1 and F_2 voles, respectively). The parsimonious models are in bold. Abbreviations and subscript meanings are as the same in Table 1

	Model no.	Model	Number of parameters	QDeviance	QAICc
F_1 generation					
Global models					
	1	$\Phi_{pd \cdot t}$	28	124.98	546.36
General models					
	2	$\Phi_{pd + t}$	16	134.95	528.57
	3	Φ_t	15	141.32	532.73
	4	$\Phi_{pd + t + pd \cdot t}$	21	130.45	535.37
F_2 generation					
Global models					
	5	$\Phi_{gpd \cdot a \cdot t}$	30	274.71	1221.17
General models					
	6	$\Phi_{a + t + a \cdot t}$	14	290.55	1202.69
	7	$\Phi_{a + t + a \cdot t + gpd \cdot a \cdot t}$	15	289.13	1203.36
	8	$\Phi_{a + t}$	14	291.82	1203.96

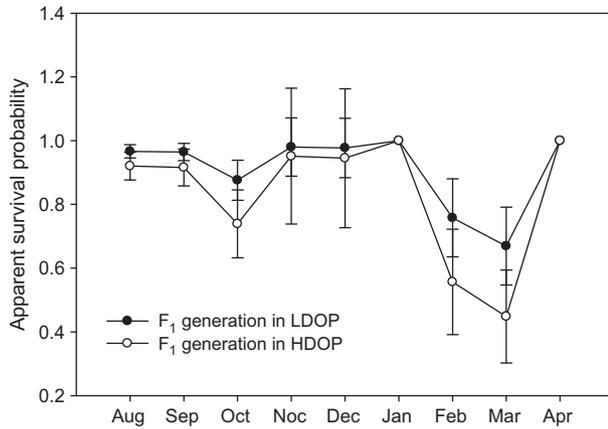


Figure 4. Monthly apparent survival probability \pm SE (model averages) of F_1 voles in different offspring populations of root voles *Microtus oeconomus* during the period from August – April. Abbreviations for offspring populations are as in The survival probabilities were estimated the model $\Phi(d + t) p(t)$.

The best model structure for describing the survival rate of F_2 voles included effects of age and time and an interaction between age and time ($\Phi_{a+t+a,t}$ model 6; Table 2). A model including an factor of grandparental density ($\Phi_{a+t+gpd,a,t}$ model 7; Table 2) did not result in a more parsimonious model (Table 2, model 6 vs model 7, $\Delta AICc = 0.67$), indicating grandparental density did not significantly affect survival rates of F_2 generation.

Impact of parental population density on offspring population size

Population densities of the F_1 generations differed significantly during the period from December to April ($F_{1,1} = 24.12$, $p = 0.039$), with densities in LDOP higher than those in HDOP (Fig. 5A). Densities of the F_1 generations did not differ from July to November. In addition, the numbers of the F_2 generations did not differ during the course of the experiment ($F_{1,1} = 1.49$, $p = 0.347$), or the period from December to April ($F_{1,1} = 1.36$, $p = 0.364$; Fig. 5B). Similarly, the difference in population size between LDOP and HDOP also did not differ over the entire experimental period ($F_{1,1} = 0.42$, $p = 0.583$), including December–April ($F_{1,1} = 0.01$, $p = 0.923$; Fig. 5C). Although parental density significantly reduced number of F_1 voles in HDOP, it did not affect F_2 vole numbers or offspring population sizes. By the end of experiment, the numbers of F_2 generations were more nine times than that of F_1 generations in overall offspring populations.

Discussion

We assessed the effects of maternal stress on winter survival of F_1 and F_2 generation root voles. Replicate high- and low-density enclosed parental populations were established, from which we obtained offspring (F_1 generation) that were used to establish new enclosed, equal-density populations. The adults of the high-density parental populations had higher corticosterone levels, an indication of physiological

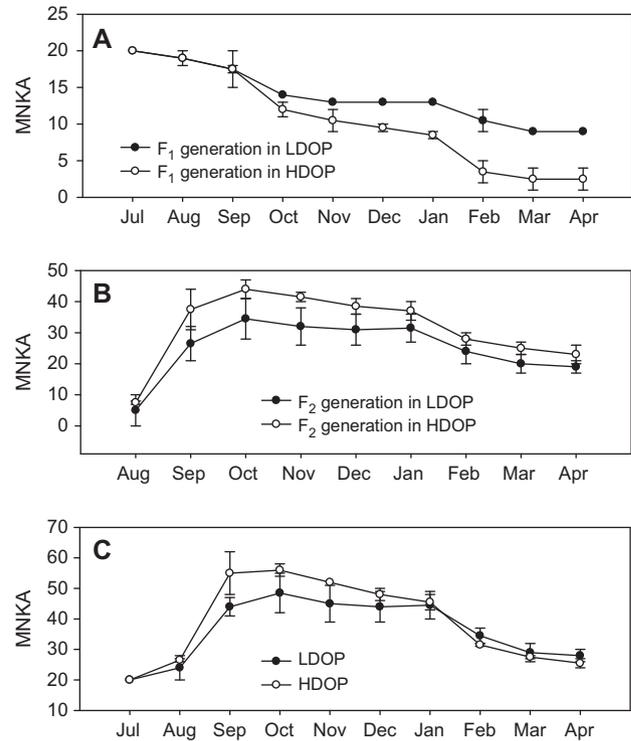


Figure 5. The minimum number known to be alive (MNKA) per 0.15 ha of (A) F_1 and (B) F_2 generations and (C) offspring populations in root voles (*Microtus oeconomus*). Abbreviations for offspring populations are as in Values are means (± 1 SE) for the two replicates within an offspring population. Repeated-measures ANOVA showed statistically significant effect of parental density on F_1 generation from December to April ($F_{1,1} = 24.12$, $p = 0.039$).

stress, than did those of the low-density parental populations. Over-winter survival of the F_1 generation voles from the low-density parental populations was greater than that of those from the high-density parental populations. Over-winter survival of F_2 generation voles did not differ between the two treatments. Furthermore, final population densities (including F_1 and F_2 generation voles) did not differ between treatments. In addition, we found that recapture rate of F_2 voles from high grandparental density was lower than that of those from low grandparental density, which may reflect a reduced explore behavior of F_2 voles from prenatally stressed mothers (F_1 voles). To our knowledge, this is one of first studies that demonstrated an influence of maternal stress on survival of F_1 offspring in a semi-natural setting.

Individuals from some high density populations have been found to display high levels of glucocorticoid concentration (Christian 1980, Boonstra and Boag 1992, Rogovin et al. 2003, Harper and Austad 2004); other populations, however, have not shown such a correlation (Moshkin et al. 2003, Charbonnel et al. 2008). In our study, we recorded higher levels of corticosterone in adults of high-density enclosed of root vole populations than in low density populations. High densities result in increased social strife and instability and parasite load, as well as a decrease in home range size and increased competition for food. The stress responses of individuals evoked by high density is an integrative effect of

these factors and reflects the biological cost of cumulative stress responses (Goymann and Wingfield 2004).

Götz et al. (2007) found that, when offspring were exposed to social confrontations, prenatally stressed male rats had a generally lower number of neutrophils, monocytes, T and NK cells, and lymphocytes than did males that were not prenatally stressed. They also found that non-prenatally stressed offspring, but not prenatally stressed offspring, showed full restoration of all immune cell numbers five days after the social confrontation and a partial recovery of T cell and monocytes numbers within 10 days. Götz and Stefanski (2007) found that prenatally stressed male rats had significantly lower numbers of total leukocytes and lower lymphocyte proliferation after stimulation with pokeweed mitogen, as compared with non-prenatal stressed offspring. In adult mice born to mothers exposed to psychological stress during gestation, macrophage and neutrophil functions were inhibited (Palermo-Neto et al. 2001, Fonseca et al. 2002). In a previous paper, we reported that F_1 voles from the high-density parental populations of our study had lower antibody contents of anti-KLH and greater relative spleen weight, as compared with F_1 voles from the low-density parental populations, indicating maternal stress may suppress immunocompetence of the offspring (Wu et al. 2008).

The effect of maternal stress evoked by high-density populations on immunocompetence of offspring may be responsible for the low over-winter survival of F_1 voles in high density populations. Individuals displaying immunosuppression may be predisposed to morbidity (e.g. sub-clinical infections resulting in enhanced predation risk) and direct mortality (e.g. infectious disease and parasitism). A negative relationship between immune functions and survival has been found in free-living small mammal populations (Mihok et al. 1985, Lochmiller 1996, Sams et al. 1996, Sinclair and Lochmiller 2000). It has been shown that maternal stress may increase susceptibility to infection and disease in later life (Bailey et al. 2004, Kapoor et al. 2006), resulting in decreased survival of offspring (Lordi et al. 2000, Patin et al. 2002, Tuchscherer et al. 2002). Body mass has been suggested to be an important factor influencing on winter survival of northern rodent populations (Aars and Ims 2002, Korslund and Steen 2005). In our study, however, body mass of the founders of the offspring populations did not differ between treatments.

We found that survival of F_1 voles from the high density parental populations was the lowest during the period of February–March, most likely a result of maternal stress and harsh winter conditions. By February and March, the voles had been subjected to winter conditions for approximately four months. The energetically demanding physiological response to low temperatures, combined with a reduced food supply during winter, resulted in a deterioration in immunocompetence (Bian et al. 2008). The deterioration in immunocompetence from the interactive synergistic effects of maternal stress and harsh winter conditions may have resulted in extremely low winter survival of F_1 voles from high density populations.

We also observed that survival of F_1 voles from high density paternal populations reached a second nadir in October. This nadir may be attributed to our experimental design.

Our populations were founded in late June and the founder F_1 voles remained reproductively active into October. October is the beginning of winter in our study area and the time reproduction in root vole populations typically ends. Boonstra (2005) observed that in northern areas, adults often display higher mortality rates at the end of the breeding period, evidence that reproductive effort progressively reduces immunocompetence (Deerenberg et al. 1997, Adamo et al. 2001, Nathalie et al. 2008). As a result, the second nadir in survival of F_1 voles from high density paternal populations may have been a consequence of a combination of maternal stress and reproductive effort on F_1 vole immunosuppression.

Over-winter survival of F_2 voles from low and high density grandparental populations did not differ. The lack of difference in survival was not a result of population density of the experimental populations; all offspring populations were of equal density when they were established. Although over-winter survival of F_1 voles from low density parental populations was higher than that of F_1 voles from high density parental populations, difference in densities of the F_1 voles in the two experimental populations did not occur until December–April. Population density of the F_1 generation voles from the two parental populations did not differ from August to November, the period when F_2 generation voles were developing foetus. Thus, potential maternal stress on the F_2 generation voles was the same, whether from F_1 generation females from low or high density parental populations. We suggest that the variation in over-winter survival of the F_1 generation was not transferred to the F_2 generation, i.e. grandparental population density did not affect over-winter survival of their grand children. Densities did not differ between the experimental populations and thus were not a factor in survival of the F_1 and F_2 generations.

In our study, effects of maternal stress were relatively transitory; we observed effects of maternal stress to affect survival only of the first generation (F_1) offspring, i.e. those whose mothers were exposed to stresses of high population densities. Second generation offspring (F_2 generation) displayed no effects of density stresses on their 'grandmothers', as was transferred to their mothers. Reduced winter survival of voles born during a period of high population density would contribute to a decline in population density during the winter. Survival rates of any peak-born F_1 voles surviving the winter would increase in the spring when the stress of low temperatures no longer adversely affected their immunocompetence and when food availability increased. Because the reduced winter survival of the F_1 generation voles is not transferred to their offspring, which indicated that the suppressed immunocompetence of the F_1 generation voles is not transferred to their offspring, at least not to such an extent that adverse effect at population-level emerged, immunocompetence would not be a factor in survival of young born to these over-winter survivors. Reduced immunocompetence, resulting from high population density stresses, transferred to offspring, thus, may be a factor in annual (winter) population declines. Because the effect is transitory, i.e. immunocompetence of F_2 voles is not affected, reduced immunocompetence resulting from high density stresses would not contribute to lengthy periods of low population densities that are characteristic of multi-annual population fluctuations.

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