

Experimental manipulation of breeding density and litter size: effects on reproductive success in the bank vole

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Summary

1. Reproductive success of individual females may be determined by density-dependent effects, especially in species where territory provides the resources for a reproducing female and territory size is inversely density-dependent.

2. We manipulated simultaneously the reproductive effort (litter size manipulation: ± 0 and $+ 2$ pups) and breeding density (low and high) of nursing female bank voles *Clethrionomys glareolus* in outdoor enclosures. We studied whether the reproductive success (number and quality of offspring) of individual females is density-dependent, and whether females can compensate for increased reproductive effort when not limited by saturated breeding density.

3. The females nursing their young in the low density weaned significantly more offspring than females in the high density, independent of litter manipulation.

4. Litter enlargements did not increase the number of weanlings per female, but offspring from enlarged litters had lower weight than control litters.

5. In the reduced density females increased the size of their home range, but litter manipulation had no significant effect on spacing behaviour of females. Increased home range size did not result in heavier weanlings.

6. Mother's failure to successfully wean any offspring was more common in the high density treatment, whereas litter manipulation or mother's weight did not affect weaning success.

7. We conclude that reproductive success of bank vole females is negatively density-dependent in terms of number, but not in the quality of weanlings.

8. The nursing effort of females (i.e. the ability to provide enough food for pups) seems not to be limited by density-dependent factors.

Key-words: density dependence; intraspecific competition; mammals; reproductive costs, reproductive effort.

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Introduction

Reproductive success (i.e. number and quality of offspring produced) of a female is determined by trade-offs between different life-history traits, constraints and several ecological factors. Of the ecological determinants, intraspecific competition and density-dependent effects on reproduction have received much attention, particularly in birds (e.g. Perrins 1965; Lundberg *et al.* 1981; Nilsson 1987; Cooch *et al.* 1989) and also to some extent in mammals (e.g. Clutton-

Brock, Guinness & Albon 1982; Morris 1989; Wauters & Lens 1995). In general, breeding success of females seems to be negatively correlated with density and competition between individuals. This has further been experimentally studied both in birds (e.g. Alatalo & Lundberg 1984; Török & Tóth 1988) and mammals (Ostfeld, Canham & Pugh 1993; Ostfeld & Canham 1995). However, in mammals studies have concentrated on reproductive success at the population level (mean number of recruits to the population) and density-dependent effects on reproductive success of individual females have not been experimentally studied.

Increasing density may influence the average reproductive output in a population by affecting the repro-

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ductive success of all females (Lack 1954) or by forcing more females to breed in poor-quality breeding sites where reproductive success is lower (Andrewartha & Birch 1954). Density-dependence in brood size in heterogeneous environments has been observed both in birds (Dhondt, Kempenaers & Adriansen 1992) and in small mammals (Morris 1992). However, density-dependent changes in reproductive success may also occur regardless of environmental heterogeneity. In small mammals increasing the density of reproducing females have been found to reduce the size of territories (e.g. Mazurkiewicz 1971; Viitala 1977; T. Mappes & E. Koskela, unpublished data). Reduced territory size may consequently decrease the amount of food resources and/or the number of secure nest sites within each territory. Territory quality may affect reproduction of individual females by proximately constraining their reproductive effort (e.g. females have limited amount of food) or, alternatively, by adaptive adjustment of their reproductive effort according to the quality of territory (individual optimization hypothesis, Perrins & Moss 1975; Morris 1985; Pettifor, Perrins & McCleery 1988). Until now most of the manipulations of breeding density of female birds (e.g. Tompa 1967; Alatalo & Lundberg 1984; Virolainen 1984; Török & Tóth 1988) have been conducted by offering nest-boxes in different densities before females have started laying eggs. This study design lacks the ability of randomizing the females into different treatments. Furthermore, to be able to control for the possible adjustment of brood size to density, the density manipulation should be performed after females have given birth. Territorially breeding species in which the territory provides all resources for a breeding female, offer a good possibility to study density-dependence of reproductive success by manipulating density of breeding females. This is not possible in many birds, in which foraging trips outside of the territory are frequent and the estimation of territory quality is difficult. In contrast, in many territorially breeding small mammals, home range provides all resources for a breeding female and density manipulations can be carried out at every stage of the reproductive cycle.

In our study species, the bank vole *Clethrionomys glareolus* Schreber, breeding females are territorial (e.g. Bujalska 1973; Koskela, Mappes & Ylönen 1997) and home range sizes of nursing females have been found to be negatively correlated with density (T. Mappes and E. Koskela, unpublished data). We recently studied reproductive effort and reproductive costs in a litter size manipulation experiment in the bank vole (Mappes, Koskela & Ylönen 1995). In that study, bank vole females did not seem to compensate increased nursing costs with increased parental effort. In other words, they did not trade their own condition against the quality of offspring or enlarged the size of their home range during nursing. We proposed two different explanations for this (Mappes *et al.* 1995):

1. females attempted to maximize their survival probability during the present breeding event or to the next breeding attempt, which may maximize their lifetime reproductive success (Williams 1966); or
2. density-dependent factors in saturated breeding density limited the current nursing effort (i.e. the ability to provide sufficient food for pups).

We used a novel experimental approach to study the effects of breeding density and litter size on reproductive success in female bank voles. In particular, we studied the density-dependent effects on reproductive success in female small mammals during nursing, at the time when the energy needs are greatest (Kaczmariski 1966; Gittleman & Thompson 1988). By manipulating the litter size and breeding density simultaneously, we created a situation where females had the possibility to compensate for increased nursing costs by enlarging their territories to obtain sufficient amounts of resources for the current breeding attempt. Because, especially in mammals, the prenatal environment (e.g. intra-uterine position) and mother's quality may have significant influence on behaviour and life history strategies of individuals (reviewed in vom Saal 1981 and Clark & Galef 1995), cross-fosterings were performed to randomize the influence of prenatal maternal effects on the performance of pups.

Materials and methods

STUDY SITE AND ANIMALS

The study was conducted at Konnevesi, central Finland (62°37'N, 26°20'E) in 0.25 ha outdoor enclosures. Because of a limited number of enclosures in use, four separate runs of the experiment were carried out: the first in May–June, the second in June–July, the third in July–August and the fourth in August–September (Table 1). For monitoring the individual voles, 25 multiple-capture live traps were distributed in each enclosure in a 5 × 5 array with 10 m between the trap stations. For a detailed description of the habitat and design of the enclosures see Koskela *et al.* (1997). All female voles used in the study were wild-caught and had over-wintered, except for females in the second run which were young-of-the-year and originated from a laboratory colony. The normal range of litter size for bank vole females in our study area is from 1 to 10 (usually 4–8, T. Mappes & E. Koskela, unpublished data). The males were wild-caught and showed scrotal testes as a sign of their maturity.

STUDY DESIGN

At the start of the experiment six (individually marked) females were randomly assigned to each enclosure and released simultaneously in the middle of each enclosure. This density is comparable to the highest breeding densities observed in earlier studies

Table 1. Number of successful (weaned at least one pup) and unsuccessful females in different treatments

Replicate	1 (5 enclosures)				2 (3 enclosures)				3 (4 enclosures)				4 (2 enclosures)			
	High		Low		High		Low		High		Low		High		Low	
Litter manipulation	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
Successful females	4	3	3	2	2	0	2	2	0	2	2	2	2	1	1	1
Unsuccessful females	2	3	0	1	1	3	0	0	3	1	1	1	1	2	0	0
Total	6	6	3	3	3	3	2	2	3	3	3	3	3	3	1	1

C = control litters, E = enlarged litters. The number of enclosures in use in different replicates is given in the brackets.

carried out in the same enclosures (Ylönen, Kojola & Viitala 1988; T. Mappes & E. Koskela, unpublished data). There were no differences in the size of females (body weight and width of head) in different enclosures at the beginning of the study runs (one-way ANOVA, $P > 0.9$ for both variables). After a 7-day habituation period three mature, randomly chosen males were introduced into each enclosure. The spacing dynamics of individuals were monitored two times during the experiment: (i) when females were in the late pregnancy of their first litters; and (ii) after litter size and density manipulations when females were nursing their young. During trapping periods traps were checked 10 times, twice a day (morning and evening) for 5 days. At each capture, vole identity, sex, trap location, weight and reproductive status were recorded.

After the first trapping period (late pregnancy), 17 days after releasing males, all females were removed from enclosures and housed in standard breeding cages in the laboratory until they gave birth. The breeding of females within enclosures were in close synchrony: all gave birth within 1–4 days. Mothers were inspected twice a day for parturition. Right after pups were found, they were counted, weighed and their sex was determined (by the length of the anogenital distance). Litters were manipulated and cross-fosterings performed within 2 days from the birth. In cross-fosterings all pups in all the litters were changed (no littermates in the same litter) and when possible, the sex ratio of litters was not changed. In our previous experiment, the growth or survival of pups did not differ between the female's own pups and foreign pups (Mappes *et al.* 1995). Nursing density was manipulated in two treatments: reduced, where two randomly selected females from the same enclosure were released to their original enclosure with pups, and control, where the density of females was not changed from the original six. In litter size manipulations we assigned litters of each original size randomly to two treatment groups: enlarged litters, 'E', where two pups were added, and control litters, 'C', where the original litter size was not changed. The pups for enlarged litters originated from mothers that were not released back to the enclosures (low density treatment). So, as

a result of the manipulations we had high density enclosures with six females in each, three nursing control and three enlarged litters, and low density enclosures with two females in each, one female nursing a control and one an enlarged litter. The original litter size did not differ among the density treatments or litter manipulation groups (three-way ANOVA, run: $F_{3,41} = 2.51$, $P = 0.072$; density: $F_{1,41} = 1.54$, $P = 0.222$; litter: $F_{1,41} = 0.37$, $P = 0.544$; density*litter: $F_{1,41} = 0.02$, $P = 0.879$). After performing manipulations within the enclosures, females and their litters (pups individually marked) were returned in breeding cages to enclosures and placed in the activity centre which should lie very near to their nests (Mironov 1990). Cages were left open so the mothers could carry pups back to the nests. This method has operated well in our other studies (Mappes *et al.* 1995; Koskela, Jonsson, Hartikainen & Mappes 1998).

When offspring were 30 days old, they were captured and taken to the laboratory where they were individually weighed. Before the possible birth of second litters, females were removed from enclosures to the laboratory to determine the characteristics of subsequent breeding (litter size, birth weight of pups, post-partum weight of mothers). During these two last trapping sessions trapping was continued until all individuals were caught (in the case of mothers) or new individuals were not found (weanlings).

Home range sizes and activity centres were estimated for individuals separately for two trapping periods. Home ranges were calculated using 90% mononuclear probability polygon centred on arithmetic mean (90% MPP; Kenward 1987). Home range size was not correlated with the number of captures (before manipulations: $r_s = 0.195$, $n = 43$, $P > 0.2$, after manipulations $r_s = -0.025$, $n = 47$, $P > 0.9$). The activity centre was calculated as the arithmetic mean point of each individual's capture co-ordinates.

DATA ANALYSIS

As the four runs of the experiment were carried out in different seasons it is necessary to take into account possible effects of changing environment on the results. Our experiment did not aim to study seasonal

effects, as for that the sample sizes would have been too low. However, in the analyses of variance study run was included in the models as a separate factor. In other analysis, the effect of run was studied first and if found significant ($P < 0.05$) it was included in the analysis. Within each run, the possible effect of enclosure on dependent variables (home range size, litter size, birth weight, weight at 30 days) was studied using either one-way ANOVA or Kruskal–Wallis one-way ANOVA (depending on whether the assumptions of parametric test were met). We did not find any significant effect of enclosure on any trait (P for all > 0.1) and, hence, the enclosure was not used as a separate factor in the following statistical analyses. A total of 141 weanlings were caught of which 112 were weighed at birth and 139 at 30 days old. In the analyses of offspring weight (at birth and at 30 days) between treatments the mean values of litters of foster mothers were used. Possible factors behind total losses of litters (weaning success) were studied using logistic regression and log-linear models. For correlation analyses Spearman rank correlations were used. All the tests are two-tailed. The statistical analyses were performed by using SPSS for Windows (SPSS Inc. 1992).

Results

NUMBER AND SIZE OF WEANLINGS AND WEANING SUCCESS

After litter size manipulations in the laboratory, the number of offspring differed significantly between litter manipulation groups, but not between density treatments (Fig. 1, Table 2). However, the number of young weaned per female was greater in the low density treatment, but there was no significant difference between litter manipulation groups or interaction between litter and density treatments (Fig. 1, Table

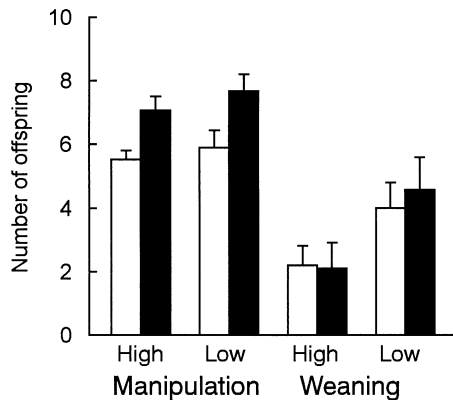


Fig. 1. Number of offspring per female after manipulation and at weaning in different treatments. High = high density treatment; Low = low density treatment; control litters = white bars; enlarged litters = black bars. Bars show the mean \pm SE. For statistics see Table 2.

Table 2. Number of offspring after manipulation and at 30 days of age (weaning) in relation to manipulation groups. Run = study period; density = high/low density; litter = control/enlarged litter. Three-way ANOVA used

	d.f.	MS	F	P
After manipulation				
Run	3, 41	5.35	2.51	0.072
Density	1, 41	3.28	1.54	0.222
Litter	1, 41	33.80	15.84	<0.001
Litter \times density	1, 41	0.05	0.02	0.879
At weaning				
Run	3, 41	0.77	0.10	0.962
Density	1, 41	53.21	6.59	0.014
Litter	1, 41	0.50	0.06	0.805
Litter \times density	1, 41	1.33	0.17	0.687

2). If the analysis is performed only with females that weaned at least one young (see below), the size of litters at weaning were not significantly different between the litter manipulation groups regardless of nursing density (control density: C litters 4.1 ± 0.5 , E litters 5.2 ± 1.1 , reduced density: C litters 4.5 ± 0.7 , E litters 5.9 ± 0.7 , three-way ANOVA, run: $F_{3,22} = 0.74$, $P = 0.538$; density: $F_{1,22} = 0.17$, $P = 0.686$; litter manipulation: $F_{1,22} = 1.68$, $P = 0.208$; density*litter: $F_{1,22} = 0.08$, $P = 0.775$).

Nineteen out of 48 females released in the enclosures with their litters failed to wean successfully any young to the age of 30 days (Table 1). Factors affecting weaning success were studied using a logit-model with weaning success as a dependent variable, and density and litter manipulations as explanatory factors (Table 3). All the models which included density-factor fitted to the data significantly ($P > 0.05$). In further analyses, density of nursing females explained significantly the weaning success of mothers ($G = 6.889$, d.f. = 1, $P = 0.009$), so that breeding failures were more common in high as compared to low density. The effects of litter manipulation or the interaction term between the two treatments were not significant (litter: $G = 0.908$, d.f. = 1, $P = 0.341$, interaction: $G = 0.036$, d.f. = 1, $P = 0.849$). Weaning success was not affected by the number of pups the mother was nursing after manipulation (logistic regression,

Table 3. The logit models of weaning success of females in relation to density manipulation (density) and litter manipulation (litter)

Model	G	d.f.	P
(1) Density + litter + density \times litter	0.000	0	1.000
(2) Density* litter	0.036	1	0.849
(3) Density	0.944	2	0.624
(4) Litter	6.925	2	0.031
(5) Constant	7.712	3	0.052

$G = 1.58$, $n = 48$, d.f. = 1, $P = 0.691$) or mother's post-partum weight (logistic regression, $G = 0.18$, $n = 44$, d.f. = 1, $P = 0.671$). Neither did the number of disappeared offspring correlate with mother's initial litter size (pooled data from both density manipulation groups; for C litters: $r_s = 0.162$, $n = 24$, $P = 0.450$, for E litters, $r_s = 0.110$, $n = 24$, $P = 0.610$).

At manipulation, the initial mean weight of pups did not differ among the treatments (three-way ANOVA, run: $F_{3,36} = 0.35$, $P = 0.791$, density: $F_{1,36} = 0.17$, $P = 0.681$, litter: $F_{1,36} = 0.60$, $P = 0.443$, density*litter: $F_{1,36} = 0.17$, $P = 0.686$). At 30 days of age, the weight of offspring tended to be lower in enlarged litters compared with the control group, but the effect of density was not significant (three-way ANOVA, run: $F_{3,22} = 0.63$, $P = 0.606$, litter: $F_{1,22} = 3.45$, $P = 0.077$, density: $F_{1,22} = 0.00$, $P = 0.968$, litter*density: $F_{1,22} = 0.00$, $P = 0.963$, Fig. 2.). However, if we control for variation in birth weight by introducing it to the model as a covariate, the effect of litter manipulation is significant (run: $F_{3,16} = 3.04$, $P = 0.059$, litter: $F_{1,16} = 4.76$, $P = 0.044$, density: $F_{1,16} = 1.12$, $P = 0.305$, covariate: $F_{1,16} = 5.15$, $P = 0.037$). Female home range size did not correlate significantly with the weight of weanlings ($r_s = -0.056$, $n = 28$, $P = 0.777$).

SPACING BEHAVIOUR AND LITTER SIZE

Before manipulations the home range size of females did not differ between treatments (three-way ANOVA, run: $F_{3,37} = 3.18$, $P = 0.035$, density: $F_{1,37} = 0.53$, $P = 0.472$, litter: $F_{1,37} = 0.03$, $P = 0.854$). Females enlarged the size of their home range in response to reduced density, but litter size manipulation had no effect on home range size (run: $F_{3,40} = 3.32$, $P = 0.029$, density: $F_{1,40} = 11.36$, $P = 0.002$, litter: $F_{1,40} = 0.69$, $P = 0.410$, density*litter: $F_{1,40} = 0.04$, $P = 0.843$, Fig. 3). The body weight and head width of females at the start of the experiment correlated significantly with their initial litter size (weight:

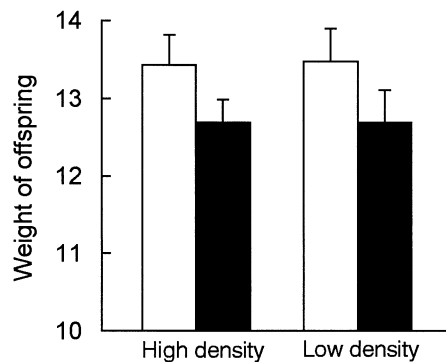


Fig. 2. Weight of 30-day-old offspring (in grams) in different treatments. Control litters = white bars; enlarged litters = black bars. Bars show the mean \pm SE.

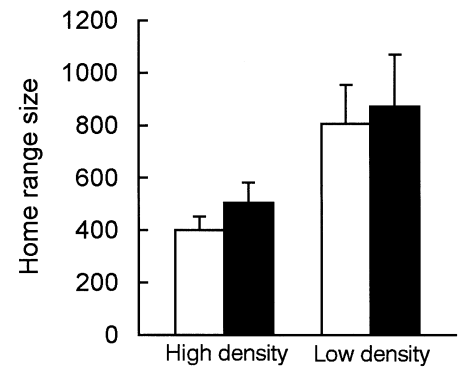


Fig. 3. Home range size of females (90% mononuclear probability polygon, in m^2) in different treatments after manipulation. Control litters = white bars; enlarged litters = black bars. Bars show the mean \pm SE.

$r_s = 0.492$, $n = 47$, $P < 0.001$, head: $r_s = 0.534$, $n = 47$, $P < 0.001$). Home range size of females before manipulations did not correlate with initial litter size ($r_s = 0.010$, $n = 43$, $P = 0.951$).

REPRODUCTIVE COSTS

Two females died after experimental manipulations, one in the enclosures and one in the laboratory before the birth of second litter. Both these females were from control density, one from a C litter and the other from an E litter. None of the eight females in the last run of the study had a subsequent litter, most likely because the breeding season was almost over. These females have not been included in the following analyses of subsequent breeding. Descriptive statistics for characteristics of subsequent breeding in different treatments are given in Table 4.

Subsequent breeding of mothers was studied by using logit-models with reproduction as the dependent variable. Litter and density manipulation groups, and the weaning success of mothers (did mothers successfully wean any pups to trappable age) were used as explanatory factors in the model. According to low Z-values ($|z| < 0.5$) of parameter estimates from the saturated model, the weaning success-factor was excluded from further models. This was supported by the fact that, if analysed separately, the success-factor did not affect the probability of subsequent breeding ($\chi^2 = 0.17$, $P = 0.676$). All the models (including the constant effect) with litter and density manipulation as explanatory factors fitted the data significantly. In further analyses neither the effect of density manipulation ($\chi^2 = 1.709$, d.f. = 1, $P = 0.191$) nor litter manipulation ($\chi^2 = 1.565$, d.f. = 1, $P = 0.211$) on probability of second breeding were significant. The size of subsequent litters, mean birth weight of pups, or female weight change during the experiment (post-partum weight after subsequent litters minus initial weight) did not seem to differ between manipulation

Table 4. Descriptive statistics about breeding parameters following the manipulations. Values represent mean \pm SE except for the first variable. For statistics see Table 5

Density manipulation	High		Low	
	Control	Enlarged	Control	Enlarged
Proportion of mothers producing second litters (<i>n</i>)	73.3 (15)	46.7 (15)	77.8 (9)	77.8 (9)
Litter size	5.7 \pm 0.8	7.4 \pm 0.2	7.4 \pm 0.8	7.4 \pm 0.3
Mean weight of pup at birth (g)	1.9 \pm 0.1	1.7 \pm 0.1	1.7 \pm 0.1	1.7 \pm 0.1
Female weight change (g)	5.9 \pm 1.3	3.9 \pm 1.2	4.9 \pm 0.6	4.7 \pm 0.9

groups and were not affected by success in the previous breeding attempt (Table 5).

Discussion

We used a novel approach to study the effects of breeding density and litter size on reproductive success of bank vole females. We further studied whether density-dependent factors, such as home range size and/or number of neighbouring females, limit the nursing effort (i.e. the ability to provide sufficient food for pups) of females. According to the results, density had clear effects on the reproductive success of females: mothers nursing their young in the low density weaned significantly more offspring than mothers in the high density. Litter enlargements did not increase the number of weanlings per female, but offspring from enlarged litters suffered from lower mass as compared to offspring from control litters. This was true independently of density treatment: even though the mothers increased the size of their home ranges when unlimited by saturated breeding density, it did not result in better quality (i.e. heavier) weanlings. This indicates that density-dependent factors

were not limiting the nursing effort of bank vole mothers.

DENSITY-DEPENDENT EFFECTS ON REPRODUCTIVE SUCCESS

Earlier breeding density manipulations studying the reproductive success of individual females have been conducted in birds, particularly in the pied (*Ficedula hypoleuca* Pall.) and collared flycatcher (*F. albicollis* Temm.) (Tomba 1967; Alatalo & Lundberg 1984; Virolainen 1984; Török & Tóth 1988). The general finding from these experiments is that the negative effects of density on breeding success of females are manifested in lower numbers and quality of fledglings, probably due to competition for food during the nestling period. In the present study, the reproductive success of mothers in terms of number of offspring weaned was strongly density-dependent. However, while density did not affect the weight of weanlings, litter enlargements seemed to have a negative effect on weanling weights as found also in other studies (Mappes *et al.* 1995; Koskela 1998; Koskela *et al.* 1998). There was no interaction between litter and density manipulations in the weight of weanlings. Hence, it seems that density-dependent factors do not limit the nursing effort of female bank voles.

Recent results from a food manipulation experiment suggest that the reproductive success of bank vole females is limited by food availability: with supplemental food the home range size of females decreased and the weight of weanlings increased compared to control females (Koskela *et al.* 1998; P. Jonsen, T. Hartikainen, E. Koskela and T. Mappes unpublished data). Why did larger home range in the low density treatment not allow females to produce larger weanlings? In the food manipulation experiment (Koskela *et al.* 1998) supplemental food (rodent chow) was provided *ad libitum* in feeders scattered evenly in the enclosures, and the costs of utilizing these extra resources were most likely low. However, patrolling over a large home range and foraging was not probably efficient enough to affect the quality of weanlings in this study. Consequently, it may be that only in a high quality habitat enlargement of home range size would result in larger size of weanlings.

Table 5. Three-way ANOVAs of litter size, female weight change and mean birth weight of pups from subsequent breeding

Variable	Factor	d.f.	F	P
Litter size	Success	1, 24	0.00	0.976
	Density	1, 24	1.86	0.185
	Litter	1, 24	0.49	0.492
	Density \times litter	1, 24	1.58	0.221
Female weight change	Success	1, 19	0.92	0.350
	Density	1, 19	0.04	0.842
	Litter	1, 19	1.00	0.329
	Density \times litter	1, 19	0.10	0.758
Mean birth weight	Success	1, 21	0.03	0.854
	Density	1, 21	0.38	0.544
	Litter	1, 21	1.77	0.198
	Density \times litter	1, 21	0.99	0.331

Success = weaning success (yes/no), density = high/low density, litter = control/enlarged litter. All other two-way and all three-way interactions were not significant ($P > 0.05$).

The weaning success of females (probability to wean at least one young) was strongly density-dependent, independent of litter size or mother's weight. In the current study, the number (and size) of offspring weaned was determined when the pups were 30 days old and they were probably already independent of their mother. Thus, it is unclear whether most of the litter losses took place at very early stages of lactation, or whether post-weaning mortality was important determinant of the pup survival. Infanticide (killing of foreign pups by conspecifics) has been observed in many small mammal species including the bank vole (Ylönen, Koskela & Mappes 1997) and it might be one cause of unsuccessful breeding. Infanticide occurs soon after birth and usually all the pups in the nest are lost. When density increases, pups may be more exposed to infanticide because the probability that an infanticidal individual encounters the nest is greater, as suggested in Mallory & Brooks (1978) (but see Boonstra 1980). Furthermore, the detrimental effect of adults, particularly adult females, on juvenile survival has been observed in many small mammal species, indicating that pup loss may also happen later in life (e.g. Boonstra 1978; Rodd & Boonstra 1988; and references therein, but see Ostfeld & Canham 1995). In this study, the greater mean number of weanlings per mother in the low density treatment did not result in lower weaning weight as compared to high density treatment. Also, because the litter sizes did not differ at weaning between density treatments (if only successful mothers were included), this suggest that most of the offspring mortality occurred at early age. However, we conclude that firm conclusions of the mechanisms causing lowered reproductive success in higher density can not be drawn from the current data. It is possible that both infanticide and post-weaning mortality are more frequent in high as compared to low density.

REPRODUCTIVE COSTS

Mappes *et al.* (1995) proposed two different explanations for why mothers do not trade off their condition against the quality of pups. First, females can increase their survival probability during the present breeding event and/or to the next breeding attempt, which may maximize their lifetime reproductive success (Williams 1966). Secondly, intraspecific competition in saturated breeding density may limit the current breeding effort. The results from the present experiment do not support the latter hypothesis as there was no difference in the weight of offspring between the density treatments and emphasize the importance to study reproductive costs as determinants of optimal reproductive effort in small mammals. However, earlier experimental studies in mammals have not found evidence of reproductive costs in terms of reduced fecundity or condition of mothers (Hare & Murie 1992; Mappes *et al.* 1995). Although

our main aim in the present study was not to examine reproductive costs, the data gave us an opportunity to cautiously investigate the possible joint effects of density and litter manipulations on subsequent performance of females. We did not find any significant results showing differential survival or fecundity between females from different treatments. However, the used experimental design (brood enlargement) does not necessarily 'force' females to increase their effort (e.g. Smith *et al.* 1988; Moreno *et al.* 1995; Tolonen & Korpimäki 1996) and therefore may fail to measure costs which do exist. Furthermore, considerable sample sizes are required for reliable statistical analyses of the probability of subsequent breeding (e.g. Graves 1991; Roff 1992). Pooling the data from our earlier study (Mappes *et al.* 1995) and from the control (high) density of the present study, gives the proportions of females producing subsequent litters: 72% ($n = 25$) in the control litters and 52% ($n = 27$) in enlarged litters ($\chi^2 = 2.23$, d.f. = 1, $P = 0.136$, $\alpha = 0.05$, Effect size $w = 0.2$, Power of test = 0.30, from tables in Cohen 1988). With the present sample size, the power of test would be 0.80 (the desired power value proposed by Cohen 1988) only if the effect size would be 0.4, twice as high as it is now. In other words, with our sample size we would have an 80% probability of rejecting the null hypothesis only if the probability not to reproduce in experimental females would be twice as high as found.

TRADE-OFF BETWEEN NUMBER AND QUALITY OF OFFSPRING

Is the quality of smaller individuals lower than that of the larger ones in terms of future survival and reproductive success? Size is usually considered to be positively correlated with fitness of an individual: larger individuals often enjoy a competitive advantage in reproduction or have faster growth rate and/or better survival (Roff 1992). However, there are surprisingly few data on subsequent performance (e.g. growth, survival, reproductive success) of different sized offspring in small mammals. Furthermore, the studies conducted (e.g. Fleming & Rauscher 1978; Myers & Master 1983; Kaufman & Kaufman 1987; Derrickson 1988; Solomon 1991, 1994) have not controlled for maternal effects arising from mother's quality or litter *per se*. Nevertheless, in general, the findings seem to support the view that large size in small mammals is beneficial. For example, Mappes *et al.* (1995) showed that the probability of *Clethrionomys glareolus* females starting to breed during the summer of their birth increases with the body weight at weaning. Furthermore, in prairie voles *Microtus ochrogaster* Wagner higher weaning weight enhances future survival and breeding success (Solomon 1991, 1994). In the present study, the weight of offspring at 30 days (representing weaning weight) was not only affected by the treatment, but also by the birth weight of pups.

This was true even when cross-fosterings were performed to randomize for prenatal maternal effects. Furthermore, in a recent enclosure experiment (T. Mappes & E. Koskela, unpublished data), birth weight of bank vole pups correlated positively with their probability to mature and also with the size of their first litters. This suggests that influence of the prenatal environment on future performance of pups is important. Taken together, there are some indications of advantages of large size at birth and at weaning for future survival and reproductive success in small mammals. Clearly, more data and experimental studies are needed to assess the significance of size for future performance.

Conclusions

Our experiment suggests that reproductive success of bank vole females is negatively density-dependent in terms of number of weanlings. Density-dependent factors do not seem to limit the nursing effort of females as there was no difference in the quality of offspring between the density treatments. The results support earlier findings of the existence of a trade-off between the number and quality of offspring in small mammals, and calls for more detailed investigations about the role of reproductive costs as determinants of optimal reproductive effort in small mammals.

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