THE DETERMINANTS OF OPTIMAL LITTER SIZE IN FREE-RANGING RED SQUIRRELS

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Abstract. Food availability, energetic ceilings, and life-history trade-offs have been proposed as potential determinants of offspring number in many animals. We investigated the role of these factors in determining litter size in a free-ranging population of red squirrels (*Tamiasciurus hudsonicus*). Long-term observational data were used to assess the influence of food availability, while experimental manipulations of litter size permitted evaluation of the importance of energetic ceilings and life-history trade-offs. Among unmanipulated litters, juvenile growth rate and survival, but not litter size, were significantly related to annual food supply. Experimental increases in offspring number were successfully sustained in a high- and a low-food year, but in both years increases in litter sizes were associated with pronounced declines in juvenile growth rates. However, the reduced size of offspring in augmented litters did not fully compensate for the increase in offspring number, so that the total litter mass supported by augmented females was much higher than that of control females. During late lactation, augmented females were characterized by increased daily energy expenditure, but not by significant changes in time budgets, relative to control females. Increases in litter size did not appear to reduce maternal survival, but were associated with declines in offspring survival. Together, these results indicate that food availability and energetic ceilings do not limit litter size in red squirrels directly, but that trade-offs between offspring number and offspring survival may eliminate any advantage of weaning larger-than-normal litters.

Key words: boreal forest (Yukon, Canada); energy budgets; field metabolic rate; lactation; life-history trade-offs; limits to reproductive performance; litter-size manipulations; offspring number, optimal; red squirrels; reproductive effort, mammals; reproductive energetics; Sciuridae; Tamiasciurus hudsonicus.

INTRODUCTION

Individual breeders should produce the number and size of offspring each breeding season that maximizes their lifetime reproductive success (Lack 1947, 1954, Williams 1966, Charnov and Krebs 1974). Although the determinants of optimal offspring number have been well studied in free-ranging birds (see reviews by Martin [1987], Lindén and Møller [1989], Godfray et al. [1991], and Monaghan and Nager [1997]), relatively little is known about the role of these factors in shaping the reproductive patterns of free-ranging mammals.

Food availability, energetic ceilings, and life-history trade-offs are all thought to be potential determinants of optimal offspring number. In situations where food availability limits offspring production (Lack 1947, 1954, Martin 1987, Wiehn and Korpimäki 1997), the energy transferred from a lactating female to her offspring is limited by her ability to locate and ingest sufficient amounts of food prior to (capital breeders) or during (income breeders; Drent and Daan 1980) lac-

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tation. Boutin's (1990) review of food-supplementation studies indicates that in a majority of the mammal species studied, increased food availability resulted in increased litter size, offspring growth, and offspring survival. In situations where offspring production is limited by energetic ceilings (Drent and Daan 1980, Peterson et al. 1990, Hammond and Diamond 1997), the energy transferred from a lactating female to her offspring is limited by her capacity to digest and assimilate acquired food, or to synthesize the assimilated resources into milk. The existence of energetic ceilings in small-mammal reproduction is frequently speculated on (e.g., Drent and Daan 1980, Peterson et al. 1990, Thompson 1992, Hammond and Diamond 1997) and has been demonstrated in laboratory studies (Hammond and Diamond 1992, Hammond et al. 1994, Koteja 1996), but their importance under natural conditions is poorly understood (Karasov 1986, Weiner 1992, Koteja 1996, Hammond and Diamond 1997). Finally, life-history trade-offs (i.e., a cost of reproduction or an offspring number vs. offspring quality trade-off) may result in selection for levels of offspring production below limits imposed by food availability or energetic ceilings (Lack 1947, 1954, Williams 1966, Roff 1992, Stearns 1992). Negative correlations between natural litter size and subsequent maternal or offspring survival have been demonstrated in only a few mammal species,

TABLE 1. Our predictions, used to evaluate the potential determinants of optimal offspring number in a population of red squirrels (near Kluane Lake, Yukon, Canada).

Factor	Predicted response to litter-size manipulations	Predicted inter-annual relationship between food availability and offspring production
Food limitation	not sustained	positive
Energetic ceilings	not sustained	no relationship
Life-history trade-offs	sustained, with subsequent decline in maternal or offspring survival/reproduction	no relationship, or positive (if food availability affects the life-history costs of increased offspring production)

most frequently in larger mammals under food-stressed or high-density conditions (Waser and Jones 1991, Millar et al. 1992, Festa-Bianchet et al. 1998, and references therein).

Because natural variation in offspring number can be confounded with individual variation in reproductive potential, studies that document the energy and life-history consequences of manipulated litter size provide a stronger test of the determinants of offspring number than do correlative studies (van Noordwijk and deJong 1986, Roff 1992, Stearns 1992). Litter-size manipulations have been conducted on two species of mammals, and results to date indicate that, unlike many bird species (Monaghan and Nager 1997), small mammals may produce the maximum number of offspring permitted by food availability or energetic ceilings. In Hare and Murie's (1992) study of free-ranging columbian ground squirrels (*Spermophilus columbianus*), only 4 of the 22 enlarged litters were successfully weaned. Similarly, in an outdoor enclosure study, Mappes et al. (1995) found that experimental increases in bank vole (*Clethrionomys glareolus*) litter sizes did not result in more offspring surviving past weaning, unless supplemental food was provided (Koskela et al. 1998).

In the present study we investigated the determinants of optimal litter size in a population of red squirrels (*Tamiasciurus hudsonicus*) near the northern limit of the species' large geographic range, where the predominance of a single, mast-seeding conifer species causes pronounced annual variation in food supply. Because females in this population rear their litters in easily accessed tree nests and readily accept foster neonates, it was possible to manipulate litter sizes in two years with widely varying food supply. We documented the energy, behavioral, and life-history consequences of these manipulations, and combined these results with long-term data on food supply and offspring production in the study site, to evaluate the role of food availability, energetic ceilings, and life-history trade-offs in red squirrel reproduction (Table 1).

METHODS

Red squirrels were studied on a 130-ha site near Kluane Lake, Yukon, Canada (61° N, 138° W), from 1989 to 1997. Detailed descriptions of red squirrel ecology and energetics are provided by Smith (1968), Pauls (1978, 1981), and Obbard (1987). The study site is dominated by white spruce (*Picea glauca*) with a sparse understory of willow (*Salix* spp.). The staple food source of this populations consists of seeds from white spruce cones, but spruce buds, mushrooms, invertebrates, and small vertebrates are also eaten. Red squirrels maintain year-round territories, and within their territory, maintain one or more middens of hoarded spruce cones (Gurnell 1984). Because larder hoards can greatly exceed, and may be used in place of, on-body energy stores (e.g., fat), variation in body mass and body composition may be less important in this species than in larger or non-hoarding mammals.

Adult female red squirrels in the Kluane Lake population usually give birth to a single litter in early spring (late March to early May). Gestation is ~35 d long (Obbard 1987), and weaning occurs ~65 d following parturition (Humphries and Boutin 1996; S. Boutin, *unpublished data*). Juveniles are first observed outside the nest at ~40 d of age (Humphries and Boutin 1996; S. Boutin, *unpublished data*), approximately 2 wk prior to weaning. Because mortality of juveniles following emergence is high (Larsen and Boutin 1994, Stuart-Smith and Boutin 1995a) and emergent juveniles begin foraging independently once outside of the nest, we documented litter size and juvenile growth rates, as well as maternal behavior and energy expenditure, prior to juvenile emergence.

Food supply and natural variation in litter size, juvenile growth, and juvenile survival

White spruce cone productivity was measured in early August of each year from 1989 to 1997. The numerical index presented represents the average number of cones present on one side of the top third of 160 trees distributed across the study site. The reproductive activity of females was monitored using methodology described in Boutin and Larsen (1993) and Humphries and Boutin (1996). Briefly, all individuals present in the study site are marked with numbered eartags and unique combinations of colored wire threaded through the eartags. Nests of lactating females were located with radio telemetry, and litter size, mass, and sex ratio was documented shortly after parturition and again prior to juvenile emergence. During the second nest visit, juveniles were given numbered eartags to allow identification following emergence. Litter sizes reported are

those recorded during all first nest entries that occurred 1–10 d following parturition.

Growth rates were calculated for only those juveniles whose mass was measured 0–10 d and 18–36 d following parturition. A longer interval that included measurements of juvenile size just prior to emergence would have been preferable, but after ~35 d juveniles are capable of exiting the nest and climbing up the nest tree, and thus are difficult to count and weigh. Growth of juvenile red squirrels during this period approximates linearity (S. Boutin, *unpublished data*). Offspring survival in different years was compared based on both the frequency of complete litter failures (i.e., the death of all offspring in a litter) and partial litter reductions.

We evaluated the effects of food availability on reproductive output by first establishing whether litter size, juvenile growth rate, and juvenile survival differed significantly between years. We then evaluated whether the year-to-year changes in these reproductive measures were related to the corresponding changes in cone supply.

Litter-size manipulations

As described in Humphries and Boutin (1996), we created donor litters ($n = 8$ squirrels in 1994, $n = 7$ squirrels in 1995) by removing two juveniles, and augmented litters by adding two juveniles ($n = 8$ squirrels in 1994, $n = 17$ squirrels in 1995), shortly after the first nest visit. More than two offspring were removed from the nests of some females in 1995 (to facilitate creation of additional augmented litters) but these litters were not studied further. In 1994 two juveniles were cross-fostered between control females ($n = 8$ squirrels) to mimic the fostering associated with the augmented treatment. Because there was no evidence of an effect of the fostering technique per se (Humphries and Boutin 1996), we did not cross-foster juveniles between females in 1995 and, in both years, we included the large sample of unmanipulated females that were studied on the same site (see preceding section) as control individuals in some analyses. In 1994 all augmented, donor, and cross-foster control females had original litter sizes of three offspring except for two cross-foster control females and four donor females who had four offspring. Litter sizes of the larger sample of unmanipulated control females ranged from 1 to 4 offspring (mean = 3.02 offspring, $n = 96$ mothers). In 1995 the original litter sizes of augmented females were 2 offspring ($n = 2$ mothers), 3 offspring ($n = 5$ mothers), 4 offspring ($n = 8$ mothers), and 5 offspring ($n = 2$ mothers) and litter sizes of donor females were 3 offspring ($n = 4$ mothers) and 4 offspring ($n = 3$ mothers). Litter sizes of unmanipulated control females ranged from 1 to 5 offspring (mean = 3.17 offspring, $n = 79$ mothers).

During manipulations, juveniles were weighed, sexed, and those transferred between nests were marked with small, temporary eartags. Nests were reentered ~15 d later to determine the success of the manipulation, and to weigh and permanently ear-tag the juveniles.

Behavioral and energetic responses to litter-size manipulations

Because we were interested in studying the effect of offspring number on reproductive effort, we excluded females from behavioral and energy analyses if, after the period of manipulation, their litter sizes were naturally reduced by >1 juvenile. Because no litter reductions occurred prior to emergence in 1994, all 24 females were included in the study. In 1995, of the 17 augmented litters created, only seven females maintained their manipulated litter size (original litter sizes of 3 offspring, $n = 2$ mothers; 4 offspring, $n = 4$ mothers; and 5 offspring, $n = 1$ mother), and an additional two lost only one juvenile (original litter sizes of 3 and 4 offspring). Thus, nine augmented females were deemed eligible for study in 1995. Exclusion of unsuccessful females is potentially problematic, because the subset of females that are studied are those best able to sustain the experimental treatment, rather than a random sample of the population (Hare and Murie 1992). However, because in this study the proportion of females excluded from the augmented treatment (47%) was lower than the proportion excluded from the control treatment (58%), the differences reported between augmented and control females in 1995 may be slightly conservative.

Behavior was recorded with instantaneous sampling at 30-sec intervals during 10-min focal sessions (Altmann 1974). Methodology and behavior classifications were similar to those presented in Stuart-Smith and Boutin (1995b), except we discriminated between feeding (handling and ingestion of food) and searching (slow locomotion with head oriented towards the substrate), and recorded details about the foods consumed, including the rate with which spruce cones were processed. Focal sessions were distributed systematically between 34 and 45 d after parturition, between 0600 and 2300, with intervals of at least 100 min between focal sessions on the same individual. In 1994 we recorded the behavior of eight augmented, eight control, and eight donor females during 30 focal sessions per female. In 1995 we recorded the behavior of four augmented and six control females, during 20 focal sessions. The number of focal sessions per female was reduced in 1995 because preliminary analysis of 1994 data indicated that within-female variance increased only slightly if time budgets were calculated from 20 focal sessions rather than 30 focal sessions.

Behavior during a focal session was summarized by calculating the proportion of each behavior category recorded over the 20 sampling points (10-min session, behavior recorded at 30-sec intervals). For comparisons between treatments and years, proportions re-

corded during each focal session were averaged for each female and arcsine transformed. The average cone-processing rate was calculated for each female, based on all observations of uninterrupted foraging bouts (feeding or searching) when two or more cones were processed.

Daily energy expenditure was measured with the doubly labeled water technique (Nagy 1988), generally following the methodology described in Kenagy et al. (1990). Because the doubly labeled water technique measures respiratory energy expenditure, measurements made on lactating females incorporate the energy expended for the acquisition of additional food resource and the synthesis of milk, but not the energy that is transferred directly to the young through milk (Kenagy 1987, Kenagy et al. 1989, 1990). The energy expenditure of augmented, donor, and control females was measured in 1994 only, for a 2-d or 3-d interval between day 39 and 43 of lactation. Females were injected intramuscularly with doses of ~2.8 mL ^{18}O (95 atom %) and 25.9 MBq of tritium per kilogram body mass. Following a 1-h equilibration period, blood samples were collected with heparinized microhematocrit tubes from a clipped toenail. Females were recaptured 48 ± 1.5 h ($n = 9$ females) or 72 ± 1.5 h ($n = 3$ females) later, and a second blood sample was taken. Blood samples were refrigerated until ^{18}O and tritium concentrations were measured at the Laboratory of Structural Biology and Molecular Medicine, University of California at Los Angeles (California, USA), using methodology described by Nagy (1983). CO_2 production was estimated using the single-isotope pool equation (Lifson and McClintock 1966), and converted to energy expenditure based on 21.7 J/mL CO_2 (Nagy 1983). Measures of energy expenditure during late lactation were successfully obtained from 11 females (4 augmented, 5 donor, and 2 control animals).

In addition to analyzing the effect of treatment on daily energy expenditure, we investigated if expenditure was related to female behavior or ambient temperature during the measurement period. For behavioral analysis, based on focal sessions conducted between initial and final blood samples (mean = 6.7 focal sessions per female, range: 3–10 focal sessions), we evaluated whether the proportion of time spent out of the nest or a behavioral activity index were significant covariates of any observed relationship between litter-size treatment and energy expenditure. The activity index was calculated in the following manner:

$$\text{activity} = n\left(\frac{t + f}{r}\right)$$

where n, t, f, r are the proportions of time spent out of the nest, traveling, foraging, and resting, respectively. One donor female was excluded from this analysis because we conducted only two focal sessions on her during the sampling interval. Focal sessions were never

conducted within 2 h of blood sampling. To examine the effects of ambient temperature on daily energy expenditure, we calculated the average ambient temperature during each female's sampling interval. Average temperatures were calculated from maximum and minimum temperatures recorded during daylight hours in shaded forest habitat, 0.5–3 km away from the territories of study animals. Average ambient temperatures were then subtracted from the lower critical temperature of red squirrels during spring (18°C; Pauls 1981) to obtain an index of thermoregulatory requirements during the sampling period. The effect of this thermal index on expenditure was then tested as a covariate of any observed relationship between litter-size treatments and energy expenditure.

Post-emergent survival and reproduction of offspring and mothers

We monitored the long-term survival of females and litters that were manipulated in 1994. Due to the very low dispersal distances and high observability of both juvenile and adult red squirrels, disappearance from the study site is almost always indicative of mortality (Larsen and Boutin 1994, Stuart-Smith and Boutin 1995a). However, in 1995 several study females occupied territories on the periphery of the long-term study site, and therefore the possibility of dispersal outside the study site prevented us from accurately monitoring survival. The survival and future reproduction of 1994 study animals (mother and offspring) from emergence in 1994 until spring 1999 was documented using the methodology described above (see *Food supply and ...*) and elsewhere (Boutin and Larsen 1993, Humphries and Boutin 1996). Twenty of 24 females that were studied in 1994 were 1 or 2 yr old, while the remaining 4 females were 4 ($n = 2$ females), 5, and 6 yr old. Although female age and parity did not differ significantly between treatments, the effects of litter-size manipulations on future survival and reproductive output of mothers were evaluated with and without inclusion of this older group of females.

Statistical analyses

When assumptions of normality could be met, results were analyzed with parametric tests, including one-way and two-way ANOVA, ANCOVA, and least-squares linear regression (see *Results*). All proportional data were arcsine transformed prior to application of parametric techniques. When small sample sizes and/or skewed distributions resulted in violations of normality assumptions, nonparametric tests were used (Wilcoxon-Mann-Whitney, Kruskal Wallis, and *G* tests; see *Results*). All tests of significance are two-tailed and the significance level, α, was set at 0.05. Unless otherwise indicated values are presented in the text as mean ± SE.
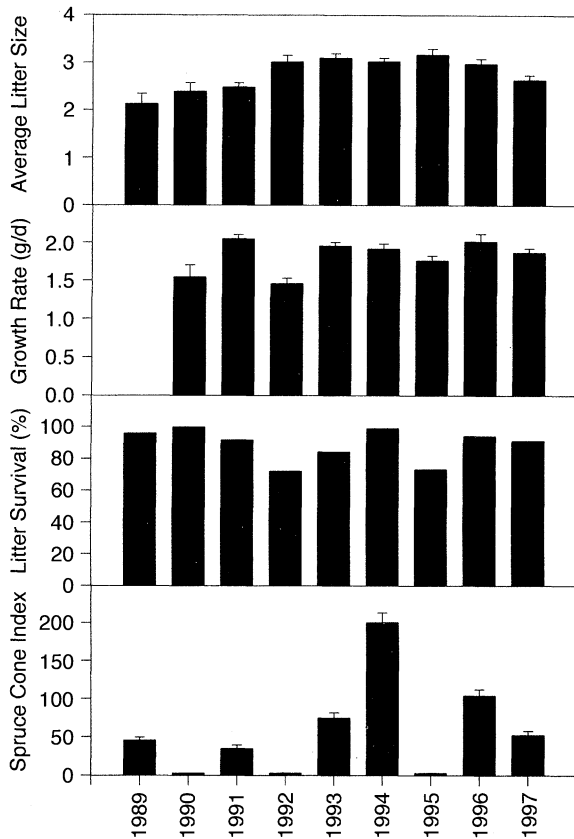
FIG. 1. Long-term observational data on the relationship between spruce cone production and litter size, juvenile growth rate, and juvenile survival in red squirrels. Because availability of food in the spring is determined by cone production from the previous autumn, values for the spruce cone index are presented in the year after they were measured. Data are means and 1 SE where appropriate.

RESULTS

Natural food supply, litter size, juvenile growth and survival

The annual production of white spruce cones on the study site varied markedly between 1988 and 1997 (Fig. 1). Among the 583 red squirrel litters that were studied between 1989 and 1997, litter size at parturition varied between 1 and 7 offspring, and averaged 2.95 offspring. Litter sizes differed significantly between years ($F_{8, 574} = 5.8$, $P < 0.001$), with the smallest average litter size occurring in 1989 (2.13 ± 0.22 offspring) and the largest in 1995 (3.17 ± 0.13 offspring; Fig. 1). The change in average litter size from one year to the next was not significantly related to the corresponding change in cone supply ($F = 0.58$, $n = 8$ yr, $r^2 = 0.09$, $P = 0.48$).

Average annual growth rates of nursing juveniles also differed significantly between years ($F_{7, 274} = 6.6$, $P < 0.001$), but year-to-year changes in average growth rates were not linearly related to the corresponding

change in cone supply ($F = 1.48$, $n = 7$ yr, $r^2 = 0.23$, $P = 0.28$). However, juvenile growth appeared to be affected by cone production in a threshold manner (Fig. 1). In the three springs preceded by cone crop failures, growth rates were lower (1.46, 1.54, and 1.76 g/d) than during all other springs (1.87–2.05 g/d; Wilcoxon-Mann-Whitney $U = 0.0$, $n = 8$ yr, $P = 0.03$), but in years when cone crops were produced, growth rates did not appear to vary according to the size of the cone crop (Fig. 1).

The proportion of litters from which at least one juvenile survived to tagging (18–36 d of age) varied from 70–75% in 1992 and 1995 to above 95% in 1990 and 1994, differing significantly between years (Fig. 1; $G = 75.9$, df = 8, $P < 0.001$). Annual change in survival was significantly related to the corresponding change in cone supply ($F = 14.75$, $n = 8$ yr, $r^2 = 0.71$, $P = 0.009$). Mortality of some but not all juveniles in a litter occurred infrequently (6.8% of 311 litters, all years combined), and did not differ significantly between years ($G = 5.07$, df = 6, $P = 0.55$).

Litter-size manipulations

Ecological context.—Litter-size manipulations in 1994 coincided with very high food availability, due to the abundant white spruce cone crop of autumn 1993 (200.2 cones/tree; 9-yr average: 64.3 cones/tree). In contrast, manipulations in 1995 coincided with very low food availability, due to a cone crop failure in autumn 1994 (1.2 cones/tree). However, because the autumn 1995 cone crop was large (103.9 cones/tree), and the seed energy content of ripening cones on spruce trees increases from spring to late summer (Schopmeyer 1974, Edwards 1980), food availability increased somewhat as the 1995 reproductive season advanced. In both years average litter sizes were larger than the 9-yr average of 2.95 offspring, with averages of 3.17 offspring in 1995 and 3.02 offspring in 1994 representing the largest and third-largest litter sizes recorded during the 9-yr study period. Average spring body mass of reproductive females in the study site did not differ between the two years (245 ± 2 g in 1994; 241 ± 3 g in 1995; $t = 0.95$, df = 147, $P = 0.34$), but body composition, and likely more importantly, external food hoards, may have differed markedly.

Pre-emergent offspring growth and survival.—Juvenile growth rates were 22% higher in 1994 than in 1995 ($P = 0.001$), and 33% higher among control litters than augmented litters ($P = 0.009$; Table 2). Growth rates of control and augmented litters differed by a similar amount in 1994 (0.53 g/d) and 1995 (0.41 g/d). Both of these differences exceeded the greatest inter-year variation in growth rates observed among control litters (0.34 g/d). Juvenile survival was also much lower in the low-food year of 1995 than in the high-food year of 1994. However juvenile survival did not differ between treatments in either year (Wilcoxon-Mann-Whitney U tests, all $P > 0.3$; Table 2).

TABLE 2. Year and treatment effects on offspring growth and survival and on maternal mass and behavior, in a population of red squirrels near Kluane Lake (Yukon, Canada).

Year	Cone supply	Litter treatment	Offspring growth (g/d, mean ± 1 SE)	Offspring survival to 25 d (%)	Offspring survival to emergence (%)	Litter mass at day 40 (g)†	Female mass at day 35–45 (g; mean ± 1 SE)	Female behavior (% of time; mean ± 1 SE)		
								In nest	Foraging	Resting
1994	high	augmented	1.53 ± 0.12	100	95	356	261 ± 9	15 ± 2	28 ± 4	23 ± 5
		control	2.06 ± 0.07	98	80	219	255 ± 5	18 ± 7	25 ± 4	24 ± 6
1995	low	augmented	1.29 ± 0.10	71	31	308	266 ± 9	27 ± 8	45 ± 2	3 ± 2
		control	1.70 ± 0.10	64	40	193	270 ± 11	35 ± 7	42 ± 5	5 ± 2

Notes: Differences between years and treatments for juvenile growth, maternal mass, and maternal behavior were analyzed using two-way ANOVAs. Juvenile growth rate differed between augmented and control litters ($n = 30$ litters, $P < 0.009$) and between 1994 and 1995 ($P < 0.001$). Maternal mass did not differ between treatments ($n = 28$ females, $P = 0.87$) or years ($P = 0.26$). Time allocated to any of the three behavioral categories did not differ between treatments ($P > 0.3$ in all cases) but in each case did differ between years ($n = 30$ females, $P < 0.01$ in all cases).

† Litter mass estimates apply only to females whose litter size was not reduced by more than one offspring prior to tagging (see *Methods*), and are based on growth rates and offspring number prior to emergence.

Maternal behavior.—Experimental increases in offspring number did not lead to significant changes in time budgets, but the decline in cone availability between 1994 and 1995 was associated with pronounced differences in the proportion of time females allocated to being in the nest, foraging, and resting (Table 2). Average time spent in the nest and time spent foraging increased between 1994 and 1995 by 94% and 65%, respectively, while time spent resting (while outside the nest) declined by 83%. Regressions between time allocated to individual behaviors and parturition date revealed no significant relationships, whether treatments and years were analyzed collectively or separately.

In accordance with the abundant cone crop in August 1993 and the cone crop failure in August 1994, females in 1994 were commonly observed processing hoarded cones (52% of 3129 instantaneous observations of feeding by 24 females) and never observed processing new cones. Females in 1995 rarely processed hoarded cones (4% of 1946 observations of 14 females) and commonly processed new cones (89% of 1946 observations). Hoarded cones in 1994 and new cones in 1995 were processed at a similar rate during uninterrupted bouts of foraging (0.96 ± 0.05 hoarded cones/min vs. 1.11 ± 0.12 new cones/min; $t = 1.05$, df $= 26$, $P = 0.31$).

Because the rate of cone processing declined significantly with date in 1995 ($r^2 = 0.32$, $n = 21$ females, $P = 0.007$) but not in 1994 ($r^2 = 0.19$, $n = 13$ females, $P = 0.13$), we used date as a covariate when testing for treatment effects on cone processing rate in 1995 but not in 1994. In 1994, the processing rate of augmented and control females was 1.0 ± 0.06 and 0.88 ± 0.04 cones/min, respectively ($t = 1.59$, df $= 11$, $P = 0.14$). Differences were more pronounced in 1995, when augmented females processed cones at more than 1.5 times the rate of control females (least-square means; 1.5 ± 0.1 vs. 0.9 ± 0.1 cones/min; ANCOVA treatment term, $F = 5.4$, df $= 1$, $P = 0.0003$).

We also calculated the proportion of time spent feeding relative to time spent foraging (feeding plus searching for food) for each bout of cone foraging, to determine if differences in cone processing rate were due to increases in search or handling times. Proportions were averaged for each female who was observed processing cones for at least 10 cumulative minutes. Feed/forage ratios were generally high (range 0.67–0.90), but were lower and more variable in 1994 (0.79 ± 0.016) than in 1995 (0.88 ± 0.007; $F_{1,16} = 10.80$, $P = 0.0004$). There was, however, no treatment effect on feed/forage ratio ($F_{1,16} = 0.14$, $P = 0.71$).

Maternal energy expenditure.—In 1994 the average daily energy expenditure of females between day 39 and day 43 of lactation was 448 ± 17 kJ/d ($n = 11$ females). The expenditure of augmented females (492 ± 10 kJ/d, $n = 4$ females) was about 8% greater than control females (457 ± 31 kJ/d, $n = 2$ females) and 21% greater than donor females (408 ± 26 kJ/d, $n = 5$ females). Mass-specific rates of daily energy expenditure averaged 1.73 kJ·g^{-1}·d^{-1} (± 0.07, $n = 11$ females) and differed significantly among the three treatments (Fig. 2a; $F_{2,10} = 6.18$, $P = 0.02$). The amount of time females spent out of the nest during the sampling interval was not a significant covariate of the energy expenditure–treatment relationship ($F_{1,9} = 1.758$, $P = 0.23$) and neither was the activity index ($F_{1,9} = 0.804$, $P = 0.40$). However, the thermal index (lower critical temperature minus average ambient temperature) was a significant covariate of the expenditure–treatment relationship (Fig. 2b; $F_{1,7} = 21.86$, $P = 0.003$). Inclusion of the thermal index as a covariate also increased the significance of the treatment effect on energy expenditure ($F_{2,7} = 22.43$, $P = 0.001$). The expenditure–temperature relationship is not confounded by date, as average daily temperature was not correlated with date
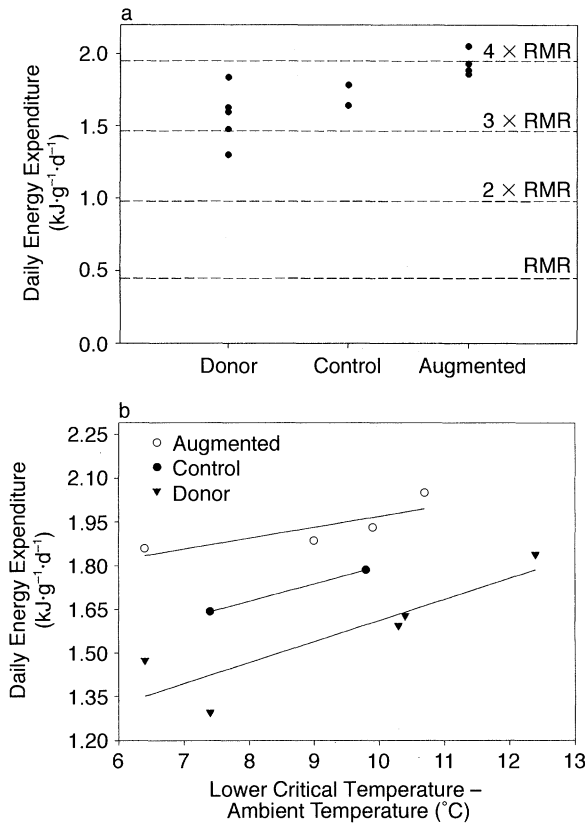
FIG. 2. Effects of the three litter-size treatments on maternal energy expenditure. (a) Treatment effects on daily energy expenditure from day 40 to day 43 of lactation. Dashed lines indicate multiples of the red squirrels resting metabolic rates (RMRs), based on values reported by Pauls (1981). (b) Effect of treatment and temperature index on energy expenditure. The temperature index reflects the average of maximum and minimum daylight temperatures recorded during each female's sampling interval, subtracted from the lower critical temperature of red squirrels in spring (18°C; Pauls 1981).

during the 17-d period when energy expenditure of all females was measured ($r = -0.18$, $n = 17$ days, $P = 0.5$).

Post-emergent offspring survival.—In 1994 the proportion of augmented juveniles that survived past 75 d of age was much lower ($10 \pm 4\%$) than both control ($58 \pm 15\%$; Wilcoxon-Mann-Whitney $U = 12.0$, $n = 16$ litters, $P = 0.03$), and donor juveniles (56 ± 18 %). However, in this year, overwinter survival of juveniles was very low with only 3 of 73 juveniles surviving until spring 1995. Two of these juveniles were from donor litters, and one was from an augmented litter.

From the perspective of the mother, both augmented and donor treatments reduced reproductive output. By 75 d after parturition, control females tended to have more surviving offspring than donor females (Wilcoxon-Mann-Whitney $U = 45$, $n = 16$ females, $P = 0.15$), and had significantly more than augmented females ($U = 14$, $n = 16$ females, $P = 0.04$; Fig. 3).

Maternal survival and future reproductive output.— Small sample sizes limit our ability to evaluate the consequences of litter-size manipulations on maternal survival and future reproductive output, but the available data provide no support for the cost-of-reproduction hypothesis. One year following the manipulations, five of eight females from each treatment remained alive. The total number of years that each female survived following manipulations ranged from zero to four years, but did not differ between treatments ($n = 24$ females, Kruskal-Wallis test $H = 0.04$, $P = 0.98$). Considering only those study females that were one or two years old in the year that manipulations were conducted also provides no indication of a treatment effect on survival ($n = 20$ females, Kruskal-Wallis test $H = 0.33$, $P = 0.85$).

Our litter-manipulation treatment also appeared to have no effect on the future reproductive output of mothers. All surviving study females produced a litter in spring 1995. Average parturition dates (Julian date) of donor, control, and augmented females were very similar: 155 ± 4 d ($n = 5$ females), 153 ± 3 d ($n = 5$ females), and 153 ± 5 d ($n = 4$ females), respectively. Because litter reductions and subsequent second litters were common in 1995, the size of first litters at birth could not be measured for all females, and was a relatively poor indicator of their reproductive output in 1995. Instead, we compared the reproductive output of females in 1995 based on the total number of offspring they successfully raised to emergence, whether those offspring originated from a first or second litter. Observed differences in reproductive output were not significant ($H = 3.6$, $P = 0.17$) and in the opposite direction than that predicted by the cost of reproduction hypothesis. Surviving augmented females from 1994 ($n = 4$ squirrels) successfully raised 1.5 ± 0.7 offspring
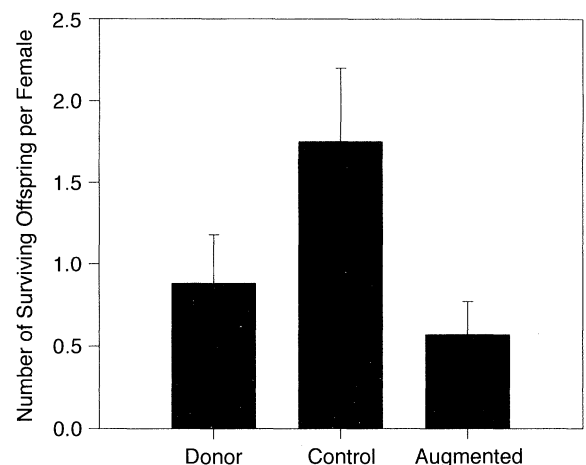

FIG. 3. Effects of the three litter treatments on the number of surviving offspring per female squirrel at 75 d post-partum. Each bar represents an average (and 1 SE) based on the eight mothers in each treatment.

to emergence in 1995, compared to 0.2 ± 0.2 offspring raised by control females (n = 5 squirrels), and 1.0 ± 0.5 offspring raised by donor females (n = 5 squirrels). Total future reproductive output, measured as the total number of offspring surviving to emergence in all years following manipulation, ranged from zero to four offspring and also did not differ between treatments, whether only 1- and 2-yr-old females (n = 20 females, Kruskal-Wallis Test H = 0.17, P = 0.92) or all females (n = 24 females, Kruskal-Wallis Test H = 0.21, P = 0.90) were included in the analysis.

DISCUSSION

Food availability

In both years that manipulations were conducted, augmented females (females whose litters had been augmented by the addition of two juveniles) sustained experimental increases in offspring production equivalent to ~50% of their body mass. Clearly, females were generally less successful sustaining reproductive demands in the low-food year of 1995 than in the high-food year of 1994, but even under low-food conditions, augmented females fared no worse than control or donor females. The ability of augmented females to sustain these increases in both years despite pronounced differences in cone production is inconsistent with food availability imposing a direct limit on offspring production. However, the long-term data suggest that both offspring growth and offspring survival are related to spruce cone abundance. Taken together, these results are consistent with food availability affecting offspring production indirectly by moderating the life-history consequences of increases in production. Otherwise, given that females are capable of supporting experimental increases in offspring production during low-food years, offspring growth and food supply would not be expected to be related.

Food availability is generally considered to be a key determinant of reproductive success (Martin 1987, Boutin 1990), and the effects of food availability on red squirrel reproduction have been reported previously (Boutin and Larsen 1993, Wauters and Lens 1995, but see Larsen et al. 1997). Although Gittleman and Thompson (1988) proposed that behavioral responses may be among the most important of the energetic compensations made by reproducing individuals, we observed no differences in the time budgets of augmented and control females. The increase in time spent foraging from 1994 to 1995 may have permitted females to partially compensate for the decline in food availability between the two years, but it is less clear why the decrease in time spent resting from 1994 to 1995 coincided with a nearly equivalent increase in time spent in the nest.

Energetic ceilings

In contrast to predictions based on the hypothesis that energetic ceilings limit offspring production, fe-

male red squirrels in this Yukon (Canada) population were capable of supporting reproductive demands much higher than those associated with natural litter sizes. The capacity of female red squirrels to support additional offspring requirements contrasts with results from the previous studies that have manipulated mammal litter sizes in the field (Hare and Murie 1992, Mappes et al. 1995, Koskela et al. 1998) and with physiological literature that has speculated a direct, limiting role for energetic ceilings in mammalian reproduction (e.g., Kenagy et al. 1990, Thompson 1992, Weiner 1992, Koteja 1996).

Measures of red squirrel FMR (field metabolic rate) during late lactation in 1994 establish that experimental increases in offspring production were associated with increased levels of energy expenditure. This increase appears to have been fueled by a combination of increased energy intake (since augmented and control females spent a similar proportion of time foraging but augmented females processed cones at a faster rate while foraging), and depletion of body energy reserves (Humphries and Boutin 1996). This apparent simultaneous increase in energy intake, energy expenditure, and offspring growth during peak periods of lactation provides further evidence against an important role of energetic ceilings in red squirrel reproduction, since it implies that lactating females are not subject to either central or peripheral limitations (Koteja 1996).

The mean mass-specific rate of energy expenditure by control females in this study (1.71 $kJ \cdot g^{-1} \cdot d^{-1}$), was 18% higher than rates measured for similar-sized, free-ranging ground squirrels (*Spermophilus saturatus*) during the same stage of lactation (1.45 $kJ \cdot g^{-1} \cdot d^{-1}$; Kenagy et al. 1990). However values for the two species were similar in terms of multiples of resting metabolic rate (RMR) (control red squirrels this study, 3.5 RMR using RMR measurements of Pauls [1981]; *S. saturatus*, 3.4 RMR, Kenagy et al. 1990). The augmented female that was exposed to the lowest ambient temperatures had a rate of energy expenditure that was 4.2 RMR. This latter value approximates the 4 RMR level that Drent and Daan (1980) originally postulated as an upper limit to sustained metabolic scope in a wide array of organisms. More recent reviews indicate that although most measurements of sustained metabolic scope in vertebrates do not exceed about 4.0 RMR, this level is surpassed in several species, and values approximating 7.0 RMR have been documented (Peterson et al. 1990, Hammond and Diamond 1997).

The apparent effect of ambient temperature on energy expenditure during late lactation suggests that thermoregulatory costs may be an important component of the energy budgets of reproducing females. We did not study the heat exchange of lactating red squirrels in any detail, but variation in mean ambient temperature by 9°C (3–12°C) was associated with a 15% difference in daily expenditure during late lactation. This difference exceeded the average change in ex-

penditure associated with the litter-size manipulation treatment (augmented females +11%, donor females −11%). Inter-annual variation in the timing of reproduction and infrequent incidences of multiple litters per year in this population result in parturition dates ranging from late March to late July (S. Boutin, *unpublished data*). During this interval, monthly mean temperatures vary by 10°C and daily mean temperatures can vary by more than 25°C (Atmospheric Environment Service, Environment Canada, Whitehorse, Yukon). With this degree of variation in ambient temperature, reproductive timing should have large effects on the energy expenditure of lactating females and presumably their ability to sustain reproductive energy demands.

Life-history trade-offs

The survival and future reproduction of red squirrel mothers appeared to be unaffected by our litter manipulation treatments, and thus results to date are inconsistent with a cost of reproduction occurring in red squirrels. Tuomi et al. (1983) proposed that a cost of reproduction was unlikely to occur in organisms that sustain the energy demands of reproduction by increasing energy intake and not draining somatic energy reserves. Since red squirrels and other small mammals rely mainly on increased energy intake to support reproductive demands (Millar 1987), an absence of a cost of reproduction might be expected. However, because reliance on hoarded food is, in several ways, similar to reliance on an internal energy reserve, food-hoarding animals may also experience a direct relationship between their current use of energy and its future availability. Extending Tuomi et al.'s (1983) ideas to more widely applicable criteria, the prevalence of a cost of reproduction should be positively associated with (1) the degree to which the energy source used to support reproductive demands can be, and is, depleted, (2) the limitations (e.g., time, food availability) and costs (e.g., predation) associated with replenishing the energy source, and (3) the brevity of the interval between when the source is depleted and when it is again required to support future energy requirements. At this time, it is unclear which of these factors may be responsible for the absence of a cost of reproduction in red squirrels.

The reduced growth rate and survival of experimentally enlarged litters was consistent with the existence of a trade-off between offspring number and offspring fitness. This trade-off is usually expressed in terms of an offspring number vs. offspring size trade-off (Roff 1992, Stearns 1992), with the implicit assumption that offspring size is an important determinant of offspring fitness. An inverse relationship between litter size and offspring growth rate or size has been reported frequently in the laboratory (e.g., König et al. 1988, Hammond and Diamond 1992, Genoud and Perrin 1994, Rogowitz and McClure 1995) but less commonly in the field (e.g., Wauters et al. 1993, Koskela et al. 1998). Although the reduced body size of juveniles arising

from augmented litters provides a plausible explanation for why augmented juveniles had lower survival over the summer of 1994, other studies on North American red squirrels have found no relationship between juvenile body size and survival (Larsen and Boutin 1994; S. Boutin, *unpublished data*). Alternatively, augmented juveniles may have had lower survival because sibling competition for vacant territories makes juveniles from large litters less likely to acquire a territory. This seems plausible in this species since few juveniles are successful in acquiring a territory, and those that are tend to take over the natal territory from their mother or settle on vacant territories adjacent to the natal territory (Boutin et al. 1993, Price and Boutin 1993, Larsen and Boutin 1994).

Further research is required to confirm that an offspring number vs. survival trade-off normally occurs in red squirrel reproduction, and to establish why this trade-off exists. In the two other systems where mammal litter sizes have been manipulated in the field (Hare and Murie 1992, Koskela et al. 1998), increases in offspring number also appear to reduce offspring survival, but it is unclear when (i.e., prior to or shortly after emergence) or why juveniles from augmented litters suffer disproportionate mortality. Although litter-size manipulations do not evaluate the genetic basis of life-history trade-offs (Reznick 1992) or incorporate the prepartum costs and adjustments that may be associated with natural increases in litter size (Gittleman and Thompson 1988, Jameson 1998), they remain one of the most useful approaches for evaluating what limits the reproductive success of mammals in their natural environment. Field studies that combine litter-size manipulations with careful documentation of offspring growth and survival prior to emergence, and offspring dispersal and survival after emergence, will help to address the general question of why female mammals do not produce larger litters.

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