

Testing the predictions of energy allocation decisions in the evolution of life-history trade-offs

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Summary

1. Allocating a greater amount of limited resources, such as energy, to current reproduction can reduce the amount of energy available for somatic maintenance and can ultimately impair future breeding success or maternal survival (i.e. cost of reproduction hypothesis). Although there is some support for the cost of reproduction hypothesis in birds, few empirical studies of mammals have demonstrated a trade-off between current and future reproduction. More importantly, most studies testing ultimate costs have neglected to confirm that the proximate costs of reproduction are high.

2. We experimentally manipulated litter size in a wild population of Columbian ground squirrels for 2 years to examine both the proximate energetic and ultimate fitness (i.e. survival and breeding) costs of reproduction. We predicted that females raising augmented litters would have the highest rates of daily energy expenditure and as a result would experience lower survival rates or future fecundity.

3. Females raising augmented litters weaned more pups, had the highest litter masses at weaning, and had field metabolic rates that were almost 1.5 times greater than females raising control or reduced litters. Contrary to our prediction, there were no negative impacts of greater maternal investment and higher energy expenditure on the probability of maternal survival or future reproduction.

4. Pups from augmented litters grew more slowly during the lactation period were smaller at weaning and had a lower probability of survival over-winter. Thus, although females were capable of raising more young than they gave birth to without short-term costs of reduced survival or fecundity, our observations suggest that limitations to litter size are not due to a trade-off in the allocation of energy, but rather due to the reduced survival of offspring from larger litters.

5. Examining the proximate mechanisms hypothesized to underlie life-history trade-offs can be challenging but is critical for a comprehensive understanding of the evolution of life histories.

Key-words: cost of reproduction, doubly-labelled water, fecundity, field metabolic rate, litter size, survival, *Urocitellus columbianus*

Introduction

The allocation of vital resources to various physiological functions drives the evolution of key life history characteristics such as maternal condition, litter size, offspring size and age at first reproduction, to name a few (Fisher 1930; Levins 1968; Van Noordwijk & de Jong 1986). Underlying costs result in negative associations, or trade-offs, among

these traits and prevent selection from simultaneously maximizing all of them. Thus, an organism's life history strategy represents a balance between allocating resources to conflicting needs in a manner that optimizes reproductive performance (Stearns 1989). One of the fundamental trade-offs pervasive in the life-history literature is between current reproduction and future reproduction or survival (Stearns 1989, 1992). The basic premise of this life-history trade-off is that resources invested in the current litter may reduce the resources individual females can allocate to

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self-maintenance; thus, investment can reduce the availability of maternal resources necessary to support future fecundity and survival (cost of reproduction trade-off; Williams 1966a,b; Bell 1980). In polytocous species, the costs of reproduction are expected to be most pronounced for females supporting more young as the relative demand of the litter is greater (Reznick 1985; Sikes & Ylonen 1998; Speakman 2008).

Although there is some support for the cost of reproduction trade-off in birds (Dijkstra *et al.* 1990 and references therein), relatively few studies have shown a decrease in maternal survival or fecundity with increasing litter size in mammals (Festa-Bianchet, Gaillard & Jorgenson 1998; Koivula *et al.* 2003). However, many correlative studies on free-ranging mammals (Murie & Dobson 1987; Millar, Derrickson & Sharpe 1992; Risch, Dobson & Murie 1995) may not have been able to detect ultimate reproductive costs because of confounding individual variation in reproductive capability (Reznick 1985). For this reason, experimental manipulations are preferable. Although brood size manipulations are common in birds, they are infrequently conducted in free-ranging mammals, likely due to the inability to access young or because females are capable of discriminating between their own and fostered young.

Moreover, the limiting resource typically ascribed to life-history trade-offs is energy, but there are few explicit tests that energy allocation decisions are the underpinning causal factors to trade-offs. During reproduction, the energy requirements of the body are elevated above maintenance levels to support behaviours such as mate location, intraspecific competition, copulation and parental care (Trivers 1972). For female mammals, lactation is the most energetically expensive period of reproduction, due in large part to the high metabolic demands of milk production (Ofstedal 1984; Racey & Speakman 1987). Energy intake and/or energy expenditure during the lactation period have been quantified for many female mammals suckling litters of varying sizes, and typically energy intake increases with litter size to a fixed ceiling (Hammond & Diamond 1992; Humphries & Boutin 2000; Johnson, Thomson & Speakman 2001; Speakman 2008). Much emphasis has been placed on identifying intrinsic mechanisms involved in setting this metabolic ceiling (reviewed in Speakman & Król 2005b, 2011), but relatively little is known about the impacts of increasing energetic investment in lactation on a female's future fecundity or survival. Furthermore, most studies on the energetics of reproduction are conducted on captive or domesticated animals. Information on reproductive costs gleaned from these studies cannot be extrapolated to free-ranging animals because reproductive costs will likely be substantially different in the field due to greater activity rates associated with foraging, predator avoidance and interactions with conspecifics, and variation in resource availability, quality and climatic conditions (Stearns & Koella 1986; Nagy 1987; Berteaux 1998).

The objective of this study was to investigate the proximate energetic and ultimate costs of investment in lactation in a free-ranging population of Columbian ground squirrels (Fig. 1). This is a great species for addressing fitness related costs of energy allocation to lactation for several reasons. First, energetic costs to reproduction are expected to be high when there is limited time for the animal to regain depleted energy stores before they are required for use again (Humphries & Boutin 2000). Columbian ground squirrels have a short active season before entry into hibernation, and pups are weaned just a few weeks before hibernation begins (Young 1990; Dobson 1992). This limits the amount of time females have post-weaning to gain somatic fat deposits, which the squirrels depend on for energy during hibernation (Murie & Boag 1984; Young 1990). Thus, if there are energetic costs to reproduction for females, they should be detected in this species. Secondly, unlike many other species of rodents, we are able to obtain survival rates for mothers and juveniles because Columbian ground squirrels exhibit a delayed (i.e. occurs after juveniles' first hibernation) and male-biased dispersal. Annual survival rates of adult females range from 59% to 91% (Murie & Dobson 1987; Festa-Bianchet & King 1991) and average life span is 5 years (Viblanco *et al.* 2010). Finally, previous studies failed to find evidence of reproductive costs when litter size was augmented by one pup (Hare & Murie 1992; Neuhaus 2000). Female metabolic rate was not measured in these studies, so there are no data to confirm that the additional pup was energetically costly to females. After allocating energy to produce one offspring, the energetic cost of lactation for an additional offspring is relatively small, particularly for intermediate litter sizes (Sikes 1998), indicating that females may easily be able to raise a single additional neonate. Therefore, elucidating reproductive costs might be more effective when litter size is augmented by multiple young rather than by one individual.



Fig. 1. An adult female Columbian ground squirrel (photograph by A.L. Skibieli).

We tested for reproductive costs by artificially increasing litter size by two pups and examining effects of litter size manipulation on female energy expenditure, survival to and breeding performance in the following year. If energetic investment in the current litter entails a reproductive cost, we predicted that females raising larger litters would have higher rates of daily energy expenditure and a lower probability of survival to or reduced breeding performance the following year relative to females raising smaller litters.

Materials and methods

A population of Columbian ground squirrels inhabiting Dot Meadow (N 50°38'59.6" W 114°39'40.9", elevation 1565 m) in the Sheep River Provincial Park, Alberta, Canada was studied from 2009 to 2011. This population has been monitored by multiple researchers for more than 10 years, and most squirrels are permanently marked with numbered fingerling eartags. Continuous records of age, based on known births on Dot Meadow, began in 2001. We trapped all squirrels on the meadow at spring emergence from hibernation using Tomahawk live traps (Tomahawk #201 collapsible chipmunk trap, 48 × 15 × 15 cm, Tomahawk, WI, USA) baited with peanut butter. All squirrels were given unique dye (Lady Clairol Hydrience #51 Black Pearl; Proctor and Gamble, Stamford, CT, USA) markings on their backs so individuals could be identified from a distance. Spring emergence mass for all animals was measured to the nearest 5 g using a Pesola spring scale (Pesola Ag, Baar, Switzerland), and zygomatic arch breadth was measured to the nearest 0.1 mm using dial calipers (Swiss Precision Instrument, Garden Grove, CA, USA). Copulation typically occurs underground; therefore, mating dates of all adult females were determined by visual observation of mating behaviours and examination of the degree of vaginal opening and swelling as described by Murie (1995) and Manno *et al.* (2007).

Two to three days prior to the estimated parturition date (24 day average gestation; Murie & Harris 1982), all females that mated were transported to the laboratory at the R. B. Miller field station to give birth. Females were individually housed in polycarbonate microvent rat cages (267 × 483 × 20 mm³; Allentown Caging Equipment Company, Allentown, New Jersey) containing pine chips and strips of newspaper for nesting material. Cages were covered in vented black plastic bags to simulate the burrow environment and to reduce stress. Squirrels were fed lettuce and apples twice daily and horse feed (EQuisine sweet show horse ration, Unifeed, Okotoks, Alberta, Canada; oats, barley, wheat and compressed vegetable material in a molasses mix) *ad libitum*. Cages were checked 3–4 times daily for the presence of pups. On the day of birth, the female and her litter were weighed, pups were sexed, and each pup was marked by removing a small piece of tissue from the outer right and/or left hind toe bud.

Females were paired based on parturition date (within 24 h of each other), and one female of the pair was randomly assigned to one of three treatment groups: litter augmented, litter reduced or control. If the female was assigned to be litter augmented, the paired female's litter was reduced and vice versa. If the female was assigned to be a control, the paired female was also a control. Females were assigned with the stipulation that each female had to retain at least one of her own pups; thus post-manipulation litter size could not be zero. This resulted in some cases ($n = 2$ in 2009, $n = 3$ in 2010), where a female could not be randomly assigned to the litter reduction group because initial litter size at birth was one or two pups. Pups within the litter were randomly chosen either to remain with its siblings or to be transferred to the paired litter. If mass of the fostered pup differed by more than 3 g from the average weight of its new littermates, the pup was reassigned. Litter augmented groups (A) received two additional pups (2009, $n = 9$;

2010, $n = 10$; initial litter size 1–4 pups), litter reduced groups (R) had two pups removed (2009, $n = 9$; 2010, $n = 10$; initial litter size 3–4 pups) and control groups (C-foster) had 1/3 to 1/2 of the litter cross-fostered, so that litter size remained the same as at birth (2009, $n = 14$; 2010, $n = 14$; initial litter size 2–5 pups).

Although Murie, Stevens & Leoppky (1998) found no detrimental effects of cross-fostering on offspring or females, we cross-fostered pups among control litters as an extra precaution to ensure the procedure *per se* did not differentially impact reproductive performance. In some cases, only one female gave birth within a 24-h period and thus, could not be paired with another female for treatment. When this occurred, the female was considered a non-cross-fostered control (C-nonfoster; 2009, $n = 5$; 2010, $n = 6$; initial litter size 1–3 pups). Mothers and pups were then released back to their nest burrows on the meadow 1–2 days following parturition. 60% of females had litter size manipulated in both 2009 and 2010 ($n = 29$); however, all females were randomly assigned to treatment groups without respect to treatment the previous year. Twelve of these females received the same treatment in 2010 as in 2009 (control, $n = 8$; augmented, $n = 2$; reduced, $n = 2$).

Daily energy expenditure (DEE) of females during peak lactation was estimated as field metabolic rate using the doubly-labelled water (DLW) method (Lifson & McClintock 1966; Butler *et al.* 2004). This method has been previously validated by comparison to indirect calorimetry in a range of small rodents. Field metabolic rate measured by DLW during lactation incorporates energy associated with increased foraging rates and milk synthesis but does not include the energy transferred to the young through the milk (Kenagy *et al.* 1990). All females in the population that gave birth in the laboratory were captured on day 21 after parturition (day of parturition = day 0), weighed and injected intraperitoneally with 0.5 mL of sterile DLW (containing approximately 65% ¹⁸O and 35% ²H). After injection, squirrels were held in traps covered with a light cotton pillowcase for 60 min to allow isotopes to equilibrate (Król & Speakman 1999). Following the equilibration period, females were lightly anesthetized by placing them in a 4-L-plastic container with a screw top lid containing a cottonball presoaked in isoflurane, and an initial blood sample was taken. Females were released within 15 min of initial blood collection, recaptured 72 h after initial blood collection, weighed and anesthetized prior to taking a final blood sample. Final blood samples were taken as close as feasible to whole 24-h periods (Speakman & Racey 1988) to estimate isotope elimination rates. Blood samples for determination of background isotopic enrichment were obtained from similarly sized nonreproductive females in the population (Speakman & Racey 1987: method C). Blood samples were obtained by clipping a toenail on the hind-foot to the quick. Whole blood was collected into capillary tubes that were immediately flame-sealed and stored at approximately 5 °C until being shipped to the University of Aberdeen for analysis.

Capillary tubes containing the blood samples were vacuum distilled (Nagy 1983), and water from the resulting distillate was used to produce CO₂ and H₂ (methods in Speakman *et al.* 1990 for CO₂; methods in Speakman & Król 2005a for H₂). The isotope ratios ¹⁸O: ¹⁶O and ²H: ¹H were analysed using gas source isotope ratio mass spectrometry (Isochrom µG and Isoprime 100, respectively, Isoprime Ltd, Cheadle Manchester, UK). Samples were run alongside three laboratory standards for each isotope (calibrated to International standards) to correct delta values to ppm. The three standards were run in increasing order, terminating with the highest enrichment, followed by all the initial samples, then all the final samples and lastly the background samples. The three standards were then rerun in ascending order. For hydrogen analysis, nine, 0.5-µL subsamples of each distillate were sequentially injected, and the data were normally collected from the average of the last five determinations, but this could be reduced if the sequential data indicated that stability had not been reached. In our mass spectrometer, this procedure is generally sufficient to

eliminate any sample-to-sample memory effect. For oxygen analysis of CO₂, we used two independent preparations (by equilibration) and made four injections of 0.5 mL from each sample. We used all eight of these determinations in the calculations, as carry-over is not an issue for CO₂ analysis. All samples were run blind of their biological context. Initial size of the body water pool was estimated using the plateau method (Speakman 1997; Speakman & Król 2005a). CO₂ production was estimated using the single pool equation (equation 7.17) from Speakman (1997) as recommended for this size of animal (Speakman 1993) and converted to daily energy expenditure (kJ day⁻¹) following equation 8.3 from Speakman (1997) assuming a respiratory quotient of 0.85. Given the close association between mother and pups in the burrow, isotope recycling may occur; however, studies using DLW in lactating dogs suggest isotope recycling is not a significant source of error (Scantlebury *et al.* 2000).

Females and pups were captured when pups first emerged from the nest burrows (average 27 days postpartum; Murie 1992). Females and juveniles were weighed and juveniles were given uniquely numbered ear tags and dye-markings. Female change in mass during lactation was calculated as body mass at the time of litter emergence from nest burrows at weaning minus her body mass upon giving birth. Litter size is the number of pups in the litter, and litter mass is the sum of the mass of each individual pup within the litter. Juvenile growth rate during lactation was calculated by subtracting pup mass at birth from pup mass at weaning and dividing by the length of the lactation period. Growth rate and pup birth and weaning masses were averaged within litter (for all statistical tests except for differences between fostered and nonfostered young). Females and juveniles were assumed to have died if they were not seen emerging from burrows the following spring. This can be ascertained because males are the primary dispersers in this species, and juveniles do not typically disperse until the spring following their first hibernation (Murie & Harris 1984).

All statistical analyses were performed in SAS version 9.1.3 for Windows (SAS 2002). Data were examined for normality graphically and statistically by conducting Shapiro–Wilks tests. There were no differences in offspring weaning mass, growth rate or survival to weaning between the two control groups (C-foster and C-nonfoster; weaning mass: $F_{1,42} = 0.20$, $P = 0.66$; growth rate: $F_{1,42} = 0.56$, $P = 0.46$; survival: $\chi^2 = 0.14$, $P = 0.71$). Thus, we combined both cross-fostered and non-cross-fostered control groups into a single control group (C) for all subsequent analyses.

Statistical analyses were conducted in two ways. First, females measured in multiple years were considered independent. For females manipulated in both years, the effect of 2009 treatment on offspring and maternal characteristics measured post-manipulation in 2010 was determined by two-way ANOVAS (PROC GLM; with 2009 treatment and 2010 treatment as independent variables and an interaction term). Whether treatment in 2009 had an effect on whole or partial loss of manipulated litters in 2010 was assessed using logistic regression (PROC LOGISTIC). Treatment in 2009 had no effect on any offspring or maternal characteristics after litter manipulation in 2010 and there was no interaction between treatment in 2009 and treatment in 2010 (Table 1). In addition, treatment in 2009 had no effect on the probability of whole or partial loss of manipulated litters in 2010 (whole litter loss: 2009 treatment, $\chi^2 = 0.13$, d.f. = 2, $P = 0.93$; partial litter loss: 2009 treatment, $\chi^2 = 0.42$, d.f. = 2, $P = 0.81$), suggesting that the two treatments could be considered independent. Differences among treatment groups in litter size, offspring mass, litter mass, offspring growth rates and maternal traits in the year of treatment were then tested using two-way ANOVAS including year and treatment as independent variables. Maternal traits the year after treatment, including litter size and mass at parturition, mating date, spring emergence and parturition masses, were examined using ANOVAS including treatment and year of treatment as independent

Table 1. Effect of treatment in 2009 on offspring and maternal characteristics measured after manipulation in 2010 for females whose litter size was manipulated in both years ($n = 29$)

Traits	d.f.	F-statistic	P-value
Offspring			
Mass at weaning (g)	2, 18	1.77	0.20
Growth rate (g day ⁻¹)	2, 18	2.13	0.15
Maternal			
Litter size after manipulation	2, 24	1.06	0.36
Litter size at weaning	2, 24	1.03	0.37
Mass at weaning (g)	2, 19	0.14	0.87
Female mass change (g)	2, 19	0.08	0.93
DEE (kJ day ⁻¹)	2, 19	0.37	0.69

Two-way ANOVAS included 2009 treatment and 2010 treatment as predictor variables and an interaction term. All interactions NS. Female mass change is change in mass from birth to weaning. Offspring masses and growth rates are averages within litter. Growth rate is mass change from birth to weaning divided by lactation length in days. DEE, daily energy expenditure.

variables. When ANOVAS were significant, Tukey's tests for multiple comparisons were employed. Differences in the probability of litter loss, offspring survival, female survival and breeding among treatment groups were tested with logistic regression including treatment and year as independent variables. Effects of DEE and mass-specific DEE on the probability of maternal survival and subsequent breeding were determined by separate logistic regressions including year as a covariate. All tests with multiple independent variables also included interaction terms.

Secondly, all ANOVAS described above were run using PROC SURVEYREG, and all logistic regressions were run using PROC SURVEYLOGISTIC including female ID as a cluster term. These procedures produce test statistics and robust standard errors that control for dependence among repeated measures. With the exception of the effect of treatment on female spring emergence mass the following year, both statistical methods produced the same results, indicating that the coefficient estimates and standard errors are not substantially affected by the potentially correlated data. Thus, we report only results of tests where multiple treatments of the same individual were considered independent. Data presented as means include \pm SE.

Results

LITTER SIZE, OFFSPRING SIZE AND OFFSPRING SURVIVAL

Litter size at birth ranged from 1 to 5 ($\bar{x} = 2.8 \pm 0.08$); thus, enlarging litters by an additional two pups was on average an increase of about 70%. Initial litter size at birth was significantly different between treatment groups (treatment, $F_{2,73} = 9.70$, $P < 0.001$; year, $F_{1,73} = 0.31$, $P = 0.58$; interaction NS) with females in the reduced treatment group having larger litter sizes at birth (Fig. 2). After the litter manipulation and at weaning, litter size still showed a significant difference among treatment groups (after manipulation: treatment, $F_{2,73} = 128.66$, $P < 0.001$; year, $F_{1,73} = 0.31$, $P = 0.58$; interaction NS; at weaning: treatment, $F_{2,73} = 21.77$, $P < 0.001$; year, $F_{1,73} = 0.11$, $P = 0.74$; interaction NS), but females in the augmented group had the largest litter sizes post-manipulation and at weaning (Fig. 2).

Pup mass at birth did not differ among treatment groups (treatment, $F_{2,73} = 1.67$, $P = 0.20$; year, $F_{1,73} = 0.04$, $P = 0.84$; interaction NS), but pups in reduced and control groups were significantly heavier at weaning than pups raised in augmented litters (treatment, $F_{2,55} = 12.03$, $P < 0.001$; year, $F_{1,55} = 3.07$, $P = 0.09$; interaction NS; Fig. 3). Initial litter mass at birth and litter mass at weaning were significantly different among treatment

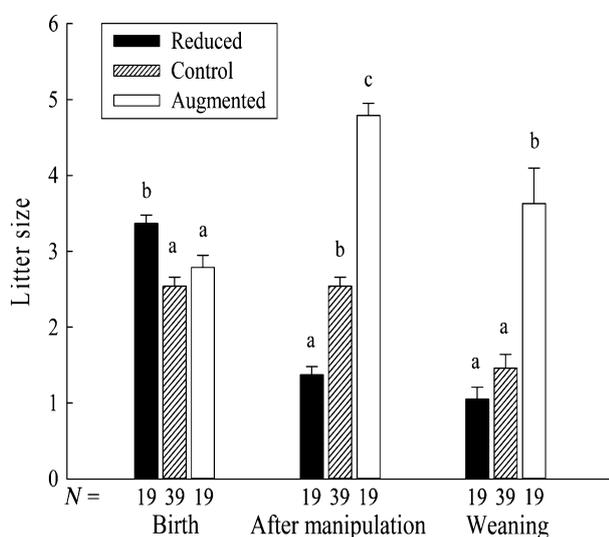


Fig. 2. Comparison of litter size among treatment groups at three different time points: at birth, after litter size manipulation and at weaning. The reduced group had two pups removed after birth, the augmented group had two pups added, and the control group had no change in litter size. Litter size at weaning was coded as 0 for females with complete litter loss. Disparate letters indicate significant differences among treatment groups. Error bars represent standard errors.

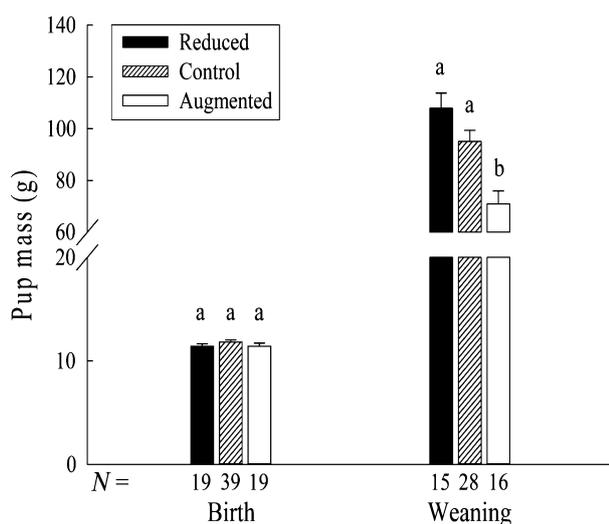


Fig. 3. Comparison of average pup mass at birth and at weaning among treatment groups. The reduced group had two pups removed after birth, the augmented group had two pups added, and the control group had no change in litter size. Disparate letters indicate significant differences among treatment groups. Error bars represent standard errors.

groups (at birth: treatment, $F_{2,73} = 6.52$, $P = 0.003$; year, $F_{1,73} = 0.18$, $P = 0.67$; interaction NS; at weaning: treatment, $F_{2,73} = 13.83$, $P < 0.001$; year, $F_{1,73} = 0.52$, $P = 0.48$; interaction NS). Females in the reduced group had the highest litter masses at birth, whereas females in the augmented group had the highest litter masses at weaning (Fig. 4). Pup growth rate from birth to weaning also differed among treatment groups and pups grew faster in 2010 than in 2009 regardless of treatment (treatment, $F_{2,55} = 10.42$, $P = 0.001$; year, $F_{1,55} = 4.65$, $P = 0.04$; interaction NS). Tukey's tests revealed that rate of pup growth was significantly slower for pups raised in augmented litters than pups in either control or reduced litters (control, $n = 28$, $\bar{x} = 3.10 \pm 0.17$ g day⁻¹; reduced, $n = 15$, $\bar{x} = 3.54 \pm 0.24$ g day⁻¹, augmented, $n = 16$, $\bar{x} = 2.21 \pm 0.19$ g day⁻¹).

Overall 23% of females lost their whole litter during the lactation period. Of the females without whole litter loss, 24% lost at least one pup from birth to weaning. Litter manipulation had no effect on the probability of whole or partial litter loss (whole litter loss: treatment, $\chi^2 = 1.14$, d.f. = 2, $P = 0.57$; year, $\chi^2 = 0.52$, d.f. = 1, $P = 0.47$; interaction NS; partial litter loss: treatment, $\chi^2 = 0.58$, d.f. = 2, $P = 0.75$; year, $\chi^2 = 0.24$, d.f. = 1, $P = 0.62$; interaction NS). Including only pups that survived to weaning, pups raised in augmented litters had an over-winter survival rate of 15%, whereas pups raised in control and reduced litters had survival rates of 46% and 60%, respectively. The probability of juveniles surviving to the next year was dependent on treatment (treatment, $\chi^2 = 17.44$, d.f. = 2, $P < 0.001$; year, $\chi^2 = 1.20$, d.f. = 1, $P = 0.27$; interaction NS). Pups raised in augmented litters (A) had a lower probability of over-winter survival than pups raised in

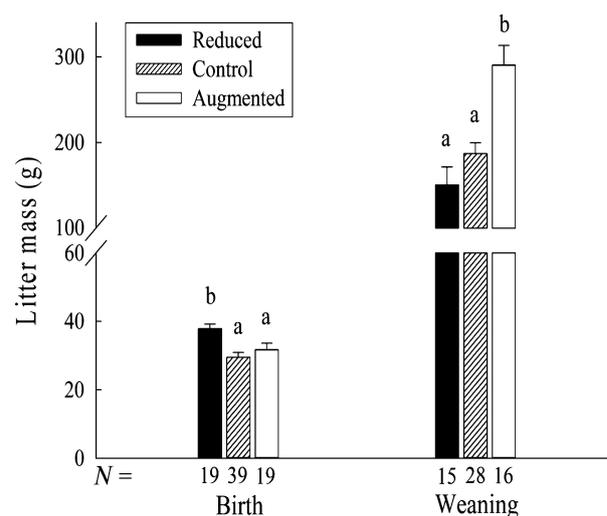


Fig. 4. Differences in litter mass at birth and weaning among treatment groups. The reduced group had two pups removed after birth, the augmented group had two pups added, and the control group had no change in litter size. Disparate letters indicate significant differences among treatment groups. Error bars represent standard errors.

either control (C) or reduced litters (R) (C vs. A: $\chi^2 = 11.29$, d.f. = 1, $P < 0.001$; R vs. A: $\chi^2 = 14.31$, d.f. = 1, $P < 0.001$; R vs. C: $\chi^2 = 1.20$, d.f. = 1, $P = 0.27$).

MATERNAL CHARACTERISTICS

Female age, mass at parturition and parturition date did not differ among treatment groups (Table 2). Females gave birth 5 days earlier on average in 2010 than in 2009 (treatment, $F_{2,73} = 1.24$, $P = 0.30$; year, $F_{1,73} = 18.86$, $P < 0.001$; interaction NS). Of the females that successfully weaned a litter, 53% lost mass from parturition to weaning ($n = 31$), 43% gained mass ($n = 25$) and 3% had no change in mass ($n = 2$). There was no significant relationship between treatment and change in female mass during the lactation period, but females lost more mass in 2010 than in 2009 (treatment, $F_{2,54} = 0.74$, $P = 0.48$; year, $F_{1,54} = 5.15$, $P = 0.03$; interaction NS). Female mass at weaning did not differ among treatment groups but females were heavier at weaning in 2009 than in 2010 regardless of treatment (2009, $\bar{x} = 514.6 \pm 9.3$ g; 2010: $\bar{x} = 485.2 \pm 7.9$ g; treatment, $F_{2,55} = 0.60$, $P = 0.55$; year, $F_{1,55} = 5.86$, $P = 0.02$; interaction NS; Table 2). Females raising augmented litters had the highest DEE and mass-specific DEE (Table 2).

MATERNAL SURVIVAL AND FECUNDITY

Treatment had no effect on the probability of female survival to the next spring ($n = 77$: treatment, $\chi^2 = 0.93$, d.f. = 2, $P = 0.63$; year, $\chi^2 = 0.78$, d.f. = 1, $P = 0.38$; interaction NS) or the probability of breeding the next year ($n = 58$: treatment, $\chi^2 = 0.003$, d.f. = 2, $P = 0.99$; year, $\chi^2 = 0.004$, d.f. = 1, $P = 0.95$; interaction NS). In addition, probability of female survival to the next year was not

related to DEE or mass-specific DEE the year of treatment (DEE, $n = 66$: treatment, $\chi^2 = 2.07$, d.f. = 1, $P = 0.15$; year, $\chi^2 = 1.33$, d.f. = 1, $P = 0.25$; interaction NS; mass-specific DEE, $n = 66$: treatment, $\chi^2 = 1.12$, d.f. = 1, $P = 0.30$; year, $\chi^2 = 1.11$, d.f. = 1, $P = 0.29$; interaction NS). Probability of breeding the next year was not related to DEE or mass-specific DEE the year of treatment (DEE, $n = 51$: treatment, $\chi^2 = 0.24$, d.f. = 1, $P = 0.62$; year, $\chi^2 = 0.003$, d.f. = 1, $P = 0.96$; interaction NS; mass-specific DEE, $n = 51$: treatment, $\chi^2 = 1.00$, d.f. = 1, $P = 0.32$; year, $\chi^2 = 0.003$, d.f. = 1, $P = 0.95$; interaction NS).

Treatment in one year had no effect on a female's mass at parturition, litter size at parturition, litter mass at parturition or date of mating the next year (Table 3). Females gave birth on average 5 days later in 2011 than in 2010, and females were lighter at spring emergence from hibernation in 2011 than in 2010, regardless of treatment. Females raising augmented litters were lighter at spring emergence than females raising their natural litter size but were similar in mass to females raising reduced litters (Table 3). However, when female ID was included in the statistical model as a cluster variable, there were no significant differences in female mass among treatment groups (treatment, $F_{2,39} = 2.10$, $P = 0.14$; year, $F_{1,39} = 11.07$, $P = 0.002$; interaction NS).

Discussion

A fundamental axiom of life-history theory is that individuals must partition limited resources, particularly energy, among competing physiological processes. It is often assumed that allocating energy to reproduction over somatic maintenance will have fitness impacts, but there have been few empirical studies, particularly in mammals,

Table 2. Traits of females raising reduced, control or augmented litters

Trait	Reduced (R)	Control (C)	Augmented (A)	d.f.	F	P	Comparisons		
							C vs. R	C vs. A	R vs. A
Mass at parturition (g)	505.0 ± 9.3 (19)	503.2 ± 7.6 (38)	505.0 ± 10.8 (19)	2, 72	0.02	0.98	NS	NS	NS
Parturition date	141.4 ± 1.3 (19)	143.3 ± 0.9 (39)	141.4 ± 1.3 (19)	2, 73	1.24	0.30	NS	NS	NS
Age (y)	4.4 ± 0.4 (17)	5.0 ± 0.3 (36)	4.1 ± 0.5 (18)	2, 67	1.28	0.28	NS	NS	NS
Mass at weaning (g)	506.0 ± 9.6 (15)	500.4 ± 9.1 (28)	488.8 ± 14.8 (16)	2, 55	0.60	0.55	NS	NS	NS
DEE (kJ day ⁻¹)	715.8 ± 32.5 (16)	777.3 ± 27.5 (33)	955.8 ± 51.9 (17)	2, 62	9.39	<0.001	NS	*	*
Mass-specific DEE (kJ g ⁻¹ day ⁻¹)	1.41 ± 0.1 (16)	1.53 ± 0.1 (33)	1.89 ± 0.4 (17)	2, 62	9.26	<0.001	NS	*	*

Two-way ANOVA including treatment and year as predictor variables and an interaction term. Parturition date and female mass at weaning also differed between years (see Results). All interactions between year and treatment NS. Means ± SE.

*Significant difference between treatment groups according to Tukey's comparison of means. Parturition date is Julian date of litter birth. DEE is daily energy expenditure. Sample size is included in parentheses.

Table 3. Breeding characteristics of females in the year following litter size manipulation

Trait	Reduced (R)	Control (C)	Augmented(A)	d.f.	F	P	Comparisons		
							C vs. R	C vs. A	R vs. A
Litter size at parturition	3.1 ± 0.2 (15)	2.8 ± 0.1 (28)	3.0 ± 0.17 (12)	2, 51	0.65	0.53	NS	NS	NS
Litter mass at parturition (g)	35.7 ± 2.2 (15)	32.8 ± 1.4 (28)	35.8 ± 2.4 (12)	2, 51	0.89	0.42	NS	NS	NS
Mating date [†]	121.9 ± 1.8 (15)	121.4 ± 1.5 (31)	126.1 ± 5.1 (13)	2, 55	1.11	0.34	NS	NS	NS
Female spring emergence mass (g) [†]	386.0 ± 6.6 (15)	406.5 ± 9.3 (31)	363.8 ± 20.4 (13)	2, 55	3.5	0.04	NS	*	NS
Female mass at parturition (g)	503.0 ± 8.1 (15)	513.6 ± 8.4 (28)	503.8 ± 11.3 (12)	2, 51	0.39	0.68	NS	NS	NS

Two-way ANOVA including treatment and year as predictor variables and an interaction term. All interactions between year and treatment NS. Mating date is the Julian date when mating occurred.

*Significant difference between treatment groups according to Tukey's comparison of means.

[†]Denotes significant effect of year on the variable (mating date: year, $F_{1,55} = 5.30$, $P < 0.001$; spring mass: year, $F_{1,55} = 7.47$, $P = 0.008$). Sample size is included in parentheses.

to support this notion. We found that litter enlargement was metabolically costly as females raising augmented litters had significantly higher rates of DEE at peak lactation than females in the control and reduced groups (Table 2). A similar effect of litter size on metabolic rate has been documented in both experimentally manipulated and naturally occurring brood sizes of birds and mammals (Gabrielsen & Mehlum 1987; Kenagy *et al.* 1990; Deerenberg *et al.* 1995; Humphries & Boutin 2000). Furthermore, in our study, augmented females had the lowest litter masses at birth yet the greatest litter masses at weaning (Fig. 4), indicating increased investment in their litters relative to females in the control and reduced groups.

Our experimental design (i.e. excluding females with initial litter sizes of 1 or 2 from assignment to the reduced group) may have resulted in females in poorer condition to be assigned to the augmented or control group. Initial litter size and total litter mass at birth were lower for females in the augmented and control groups compared to the reduced group (Fig. 2 and 4). Because litter size is positively correlated to maternal condition (Dobson, Risch & Murie 1999), giving females with initially small litter sizes more pups to raise would be expected to result in greater energetic costs and/or lower weaning success for these mothers due to their poorer condition. However, female condition (i.e. mass) and weaning success did not differ among treatment groups (Table 2), suggesting that differences in maternal condition and ability to wean a litter are not driving the differences in energetic costs among the treatment groups.

The elevated metabolic rates associated with greater reproductive demand were predicted to result in reduced maternal fecundity or survival. However, we found no relationship between DEE or mass-specific DEE on the probability of over-winter survival or subsequent breeding, indicating a proximate energetic cost to lactation without a short-term trade-off. This is an important finding as prior work has suggested that reproductive effort is costly

in terms of reduced immune function (Deerenberg *et al.* 1997; Drazen, Trasy & Nelson 2003), susceptibility to disease or parasites (Festa-Bianchet 1989; Neuhaus 2003), oxidative stress (Bergeron *et al.* 2011; but see Oldakowski *et al.* 2012; reviewed in Selman *et al.* 2012) and possibly impaired DNA repair mechanisms (Kirkwood & Austad 2000), all of which could increase the probability of mortality. These effects may be a result of reproductive processes causing direct somatic damage independent of energy allocation decisions (Zera & Harshman 2001; Harshman & Zera 2007) and/or may result in a shorter life span or reduced lifetime reproductive success, which would not be detected within the time scale of the current study.

In this study, the only potential negative effect of current reproduction on Columbian ground squirrel mothers we found was on body mass. Females raising augmented litters were lighter at spring emergence from hibernation the year after treatment than females in the control group (Table 3). This trend was also detected in a previous study of Columbian ground squirrels where litter size was enlarged by one pup (Hare & Murie 1992). Interestingly, in the year of treatment, females raising augmented litters had similar body masses at litter weaning to females in control and reduced groups (Tables 2). This suggests that females of augmented litters either lost more body mass or did not gain as much mass between weaning and hibernation or utilized more of their somatic energy reserves during hibernation than control females. However, this did not appear to have long-lasting repercussions as augmented females were able to compensate by gaining mass between emergence the next spring and the start of lactation later that season, as indicated by the similar body masses of females from all three treatment groups at parturition (Table 3).

Contrary to our predictions, litter augmentation and the resulting energetic cost did not result in higher maternal mortality or reduced fecundity the following year, supporting previous experimental and correlative studies that found no evidence of a cost of reproduction in female

Columbian ground squirrels (Murie & Dobson 1987; Hare & Murie 1992; Risch, Dobson & Murie 1995). A short-term ecological cost to reproduction may not be evident for several reasons. First, Tuomi, Hakala & Haukioja (1983) proposed that income breeders could meet elevated reproductive demands by increasing energy intake rather than utilizing somatic stores, thereby circumventing a trade-off between reproduction and survival. Evidence of a cost of reproduction in capital breeders such as bighorn sheep and red deer (Clutton-Brock, Guinness & Albon 1983; Festa-Bianchet, Gaillard & Jorgenson 1998) and no trade-off between current and future reproduction in several income breeders (this study; Murie & Dobson 1987; Hare & Murie 1992; Mappes, Koskela & Ylönen 1995; Risch, Dobson & Murie 1995; Humphries & Boutin 2000) lend support to Tuomi, *et al.*'s (1983) hypothesis. However, effects of current reproductive effort on maternal survival and subsequent fecundity have been detected in other small income breeders (Huber *et al.* 1999; Koivula *et al.* 2003).

Secondly, reproductive costs may only be incurred under stressful environmental conditions, such as poor or highly variable climatic conditions, low food availability or high population density (Boyce & Perrins 1987). Both years of our study were mild in terms of average summer temperature and precipitation rates and were similar to the past three summers (2006–2008: average daytime temperature from May to July = 19.4 °C, average precipitation rate from May to July = 1.7 mm day⁻¹; this study, 2009–2010: average daytime temperature = 17.6 °C, average precipitation rate = 1.4 mm day⁻¹; based on Banff, Alberta, Canada accessed through Environment Canada 2012). However, population density is particularly high in our population of squirrels. The density of our population was higher than the average calculated from other populations of Columbian ground squirrels (our population: 14 lactating females ha⁻¹; average of 11 other populations: 8 lactating females ha⁻¹; Dobson 1990).

Third, any cost of reproduction might be masked by maternal adjustment of litter size post-manipulation. Female Columbian ground squirrels have been shown to adjust reproductive effort to environmental cues by reducing litter size after birth (Murie, Boag & Kivett 1980). However, although litter loss occurred in our population, there were no differences among treatment groups in the probability of whole or partial litter loss. Furthermore, females raising augmented litters weaned more pups than females in control or reduced groups (Fig. 2). These results contrast those of Neuhaus (2000), where female Columbian ground squirrels raising enlarged litters had fewer young survive to weaning.

Pups raised in augmented litters grew at a slower rate, were lighter at weaning and had a lower probability of survival than pups in control and reduced litters (Fig. 3). This corroborates a positive relationship between offspring quality and survival in another population of Columbian ground squirrels (Skibieli, Dobson & Murie 2009). However, these results contrast previous studies of litter

size in Columbian ground squirrels and bank voles (*Clethrionomys glareolus*) that showed no difference in offspring survival after independence between experimentally enlarged and reduced litters (Hare & Murie 1992; Oksanen *et al.* 2001; Koivula *et al.* 2003), but is consistent with reduced survival of red squirrel juveniles (*Tamiasciurus hudsonicus*) from augmented litters (Humphries & Boutin 2000). Our results suggest that in our population of Columbian ground squirrels, like red squirrels, compensatory growth of smaller offspring raised in larger litters does not occur.

In conclusion, although females supporting large litters produced slower growing and smaller young with poorer prospects of survival post-independence, females were not completely able to defray enhanced energetic costs to their offspring as females weaning larger litters invested more in their litters and had elevated rates of energy expenditure relative to females raising smaller litters. Although females raising enlarged litters incurred greater energetic costs during lactation, this did not appear to impact maternal survival or future breeding. Our results suggest that short-term trade-offs in energy allocation are not limiting litter size in this species, but that reduced over-winter survival of offspring from larger litters negates any benefit of weaning a large litter. Overall, our observations suggest that testing the assumptions of underlying causal mechanisms in life-history trade-offs can provide new insights into and further elucidation of the evolution of life-history traits.

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