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Reproductive Energetics and Life-history Trade-offs in Female Red Squirrels

by

Murray M. Humphries ©

A thesis submitted to the faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Department of Biological Sciences

Edmonton, Alberta

Spring 1996

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Reproductive Energetics and Life-history Trade-offs in Female Red Squirrels** submitted by Murray M. Humphries in partial fulfillment of the requirements for the degree of Master of Science.

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Date: Jan. 25, 1996

DEDICATION

This thesis is dedicated to Bob and Ruby Mitchell
for showing me that hard work in the
field leads to good things.

ABSTRACT

The influence of reproductive effort limitations and life-history trade-offs on optimal offspring number was investigated in female red squirrels (*Tamiasciurus hudsonicus*). I manipulated offspring number of females shortly after parturition, measured indices of their resulting reproductive effort, and monitored the future survival and fecundity of study females and their offspring. Females successfully sustained experimental increases in offspring number through a combination of a 25% reduction in juvenile growth, a 15% increase in foraging efficiency, a 10% increase in energy expenditure, and an increased reliance on stored energy. These responses did not lead to reductions in the future survival or fecundity of females with increased demands. However, increases in offspring number did lead to reduced offspring survival, suggesting that a trade-off between offspring number and fitness is a more important determinant of optimal offspring number than is energy limitation during lactation or the cost of reproduction.

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Chapter One. Introduction

Identification of selective forces that influence offspring number is complex. Lack (1947; 1954) initiated the modern-day study of the evolution of offspring number by proposing that natural selection should favor the clutch size that on average produces the greatest number of offspring that survive to reproductive age. Faced with mounting evidence that average clutch size in many bird populations was smaller than that predicted by Lack, evolutionary ecologists refined Lack's hypothesis (eg. Williams 1966b). The interpretation of the fitness contribution of a given clutch size was expanded to include consideration of the effect clutch size had on the future survival and fecundity of parents, and the fecundity of offspring. If, in addition to reducing offspring survival, increases in clutch size reduced offspring fecundity, or parental survival or fecundity, then clutch sizes smaller than those predicted by Lack would be favoured by natural selection. In the thirty years since Williams' presentation of these ideas (Williams 1966a, 1966b), the negative effects of offspring number on offspring fitness, and offspring number on parental survival and future fecundity, have come to be referred to as life-history trade-offs (Stearns 1992), and have become pervasive in explanations of the evolution of offspring number (Godfray, Partridge, & Harvey 1991; Stearns 1992; Roff 1992).

Despite their widespread application in explanations of the evolution of offspring number, life-history trade-offs remain poorly understood and controversial. The proposed trade-off between offspring number and the survival and fecundity of parents, commonly referred to as the cost of reproduction, is especially controversial due to inconsistent empirical results (Reznick 1985; Lindén & Møller 1989; Lessells 1991) and debate over appropriate methods for identification of the trade-off (Partridge & Harvey 1985; Reznick 1985, 1992; Stearns 1989). Despite inconclusive theoretical and empirical support for the cost of reproduction, and valid alternative explanations for the discrepancies between observed clutch sizes and Lack's clutch (see Morris 1992; Risch, Dobson, & Murie 1995), the trade-off between current offspring number and future survival and fecundity is still regarded as one of the most important determinants of offspring number (Lessells 1991; Stearns 1989, 1992). The persistence of support for the cost of reproduction hypothesis likely results from the following three factors: 1) general acceptance that a true cost of reproduction is expressed in the genotype, as well as the phenotype, of a parent (Reznick 1985, 1991), has made convincing confirmation or refutation of the cost of reproduction hypothesis very difficult. 2) The principle of allocation, the notion that when resource availability is limited, resources can be invested into a behaviour only at the expense of investment into other behaviours (Levins 1968, Sibly & Calow 1986), offers a mechanistic and intuitive basis for the cost of reproduction and, given the considerable resource requirements of reproduction, makes reduction in future survival and fecundity seem to be an almost unavoidable result of increases in offspring number. 3) Despite general opinion to the contrary, the majority of experimental studies of the cost of reproduction, have confirmed the presence of the trade-off (17 of 20 studies reviewed by Reznick 1985; 12 of 19 studies reviewed by Stearns 1992).

Investigations of the genetic basis of life-history trade-offs (eg. Lande 1982; de Jong 1990) have simultaneously diversified and polarized life-history research. Genetic life-history studies are concerned with how selection affects gene frequencies. If there is a trade-off between two traits, then the loci affecting allocation of resources will affect both traits; a genetic increase in allocation to one trait will necessarily be associated with a genetic decrease in allocation to the other (Reznick 1985). If the traits are related in this fashion, they are said to be linked by negative genetic correlation or antagonistic pleiotropy. Characters that have been subject to simultaneous selection (eg. reproductive output and longevity) are expected to develop negative correlations because natural selection will favor alleles that affect both traits positively, until it is no longer possible to affect one trait positively without having deleterious effects on the other trait (Reznick 1985). Researchers investigating the genetic basis of life-history trade-offs measure genetic correlations between relatives, or conduct selection experiments, to determine if genetic increases in one trait are associated with genetic decreases in the associated trait (Reznick 1985).

Investigators working at the genotypic level have argued that studies that do not investigate the genetic basis of life-history traits fail to validly test for the presence of a trade-off (Reznick 1985, 1992). However, researchers working at the phenotypic level have argued that phenotypic studies can identify trade-offs as validly as genetic methods, but with much less effort, and that genetic methods are 1) suitable for only a very limited range of organisms and 2) offer little insight into the role of trade-offs in the life history patterns observed among free-ranging organisms (Partridge 1992; see also Reznick 1992). Both perspectives are mostly, but not totally, correct (Lessells 1991). Studies at the genotypic and phenotypic levels clearly offer different insights into life history evolution, and therefore, have developed into two different, but equally valid, avenues of investigation of life-history trade-offs.

A gradual, but significant shift in phenotypic studies of the evolution of offspring number, has been away from Lack and Williams' focus on the determinants of the optimal mean litter size of a population or species, toward consideration of the optimal litter size for each individual comprising the population. Early proponents of this approach (Perrins & Moss 1975; Drent & Daan 1980) suggested that "*differences between parents and among the same parents from year to year thus become the vital points demanding explanation rather than annoying uncertainties clouding the major issues of species patterns*" (Drent & Daan 1980, p. 225) and proposed that these differences result from variation in the parent's ability to raise offspring due to individual variation in body condition, experience, food resources or territory quality (Perrins & Moss 1975). Implicit in this approach, now referred to as the individual optimization hypothesis, is a fundamental shift in the way variation of offspring number within populations is viewed. In contrast to Lack and Williams' predictions that the number of offspring surviving from different clutch sizes should be normally distributed around an optimal population litter size, the individual optimization hypothesis predicts survival to be independent of offspring number; individuals producing an above average number of offspring will have an above average ability to raise those offspring.

The apparent validity of the individual optimization hypothesis (Perrins & Moss 1975; Drent & Daan 1980; Hogstedt 1980; Pettifor, Perrins, McCleery 1988; Waser & Jones 1991; Pettifor 1993a, 1993b; Risch et al. 1995) is one of the factors that has made investigation of the cost of reproduction difficult at the phenotypic level. Many early tests of the cost of reproduction hypothesis were correlative; the future survival and fecundity of individuals producing different numbers of offspring were compared to determine if current reproduction negatively influenced future reproduction (eg. Kluyver 1963; Lack 1966; Wootton & Evans 1976; Clutton-Brock, Guinness & Albon 1983). van Noordwijk & de Jong (1986) illustrated why, given that breeders are individually optimizing, studies of this nature may identify positive correlations between offspring number and future reproduction, even if a cost of reproduction exists. Presumably the cost of reproduction occurs because resource availability is limited, and therefore individuals invest in current reproduction at the expense of investment in future survival or fecundity. van Noordwijk and de Jong (1986, p.141) suggest that this situation is analogous to an economic situation faced by humans. "*If the budget is fixed, people spending more on housing should spend less on cars. In fact, the amount of expendable income is variable and in many situations positive correlations are observed between the per-family expenses on housing and on cars. There is little problem in identifying rich and poor families on this basis...Where biologists have observed positive correlations between life-history traits, they have also identified individuals that perform well or poorly*". As an empirical example of this phenomenon, song sparrows (*Melospiza melodia*) that acquire territories in better habitat, subsequently experience both higher reproductive success and survival (Smith 1981; Hochachka, Smith, & Arcese 1989).

Because adaptive variation in clutch size can undermine the ability of correlative studies to identify life-history trade-offs, experimental manipulation of life-history traits is widely regarded as the best method for identifying phenotypic trade-offs (Reznick 1985, Partridge & Harvey 1988, Roff 1992, Stearns 1992). The extensive literature on clutch size manipulations in birds (reviewed by Lindén & Møller 1989) is the best known research of this type, but similar techniques have been applied to invertebrates (reviewed by Godfray, Partridge, & Harvey 1991), and one species of mammal (Hare & Murie 1992). Reznick (1985) and Lessells (1991) distinguished between using indirect and direct manipulations of life-history traits to test for the presence of trade-offs. For example, in studies designed to test for the cost of reproduction, investigators normally manipulate offspring number following egg-laying (parturition in mammals) and monitor subsequent survival and fecundity of parents whose clutches were or were not manipulated. Reznick (1985) classified this type of manipulation as direct, since current offspring number, which forms part of the cost of reproduction trade-off, is altered directly. The cost of reproduction was tested in the Antarctic petrel (*Thalassoica antartica*), by placing lead loads on the leg of one parent during the chick provisionment period (Saether, Andersen, & Pedersen 1993). This is considered an indirect manipulation, as would an experimental design that manipulated offspring number by supplementary feeding (eg. Kent 1981). Direct manipulations are considered to be more suitable tests for life-history trade-offs (Reznick 1985, Lessells 1991). Indirect manipulations can be problematic if either the manipulation does not have the intended affect on the desired trait (eg. the lead load did not result in higher reproductive effort of the petrel parents), or if the manipulation itself is responsible for an accompanying change in other life-history traits (eg. supplemental feeding increases offspring number and adult survival).

Although manipulative studies are better tests of phenotypic life-history trade-offs than are correlative studies, and direct manipulations are preferable over indirect manipulations, experimental designs incorporating direct manipulations do not provide fool-proof tests of life-history trade-offs. Lessells (1991) correctly states that even manipulations of offspring number are not truly direct manipulations. Reproductive effort is the life-history trait most directly involved in the proposed cost of reproduction trade-off, and therefore is the trait that should be manipulated to test the hypothesis. Increases in offspring number will necessarily increase reproductive effort only if offspring number or growth rate is not reduced following the manipulation. In some bird species, manipulation of clutch size did not result in increases in provisionment rate (Korpimaki 1988; Smith et al. 1988). A second shortcoming of direct manipulations is that they usually do not incorporate the full costs that a breeder would experience due to an increase in offspring number (Lessells 1991). For example, clutch size manipulations in birds do not include the costs of synthesizing, laying, and incubating additional eggs. This is often considered a minor omission because the energy costs of offspring care generally increase from conception to near offspring independence (Clutton-Brock 1991), but this may be an over-simplified interpretation of the energy demands faced by breeders. Presumably, the costs of each reproductive stage will not be determined by its absolute energy requirements, but by its energy requirements relative to energy availability during the stage. A final problem with either indirect or direct manipulations of life-history traits occurs because study organisms have the ability to modify the experimental treatment. According to the individual optimization hypothesis, breeders adjust offspring number according to their ability to raise them. It is naive to believe that this adjustment process is completely circumvented by manipulating offspring number. If all parents do not raise the experimentally increased number of offspring, the subset of study animals in the augmented group consists of the individuals most able to sustain increases in reproductive demands, rather than a random sample of the population (Hare and Murie 1992). Even if all breeders raise the assigned number of offspring adjustment of growth rates, offspring dependence periods (eg. premature fledgling), or even future fecundity (see Lessells 1991), could still confound the experimental treatment.

The current study was designed to test for the presence of life-history trade-offs in female red squirrels, as well as to gain insight into the proximate strategies used by females squirrels to sustain the energy demands of lactation. To identify trade-offs between offspring number and 1) offspring survival and fecundity and 2) parental survival and future fecundity, I manipulated the litter sizes of females by two offspring, and monitored the future survival and fecundity (females only) of mothers and offspring for at least one year following the manipulations. In order to ensure that the manipulations of offspring number had the desired effect on reproductive effort, I quantified the time budgets, energy expenditure and mass changes of mothers, and the growth and survival rates of their offspring, prior to weaning. These data also provided insight into the energetic compensations made by females following an unpredicted increase in reproductive demands. Were increases in energy demands sustained, and if they were, did females accommodate the increased demands by increasing energy intake, reducing expenditure, or by increased reliance on energy stores?

In chapter II, I describe the effects of experimental variation in offspring number and natural variation in food supply on female reproductive effort. This chapter includes findings pertaining to juvenile growth and survival prior to weaning, and female behaviour and energy expenditure during the latter stages of lactation. Because of extreme natural variation in spruce cone abundance during the two years of the study, I was also able to incorporate investigation of the effects of food availability on female reproductive effort. Chapter III consists of data from 1994 on the effect of reproductive demands on the dynamics of maternal energy stores during lactation. The findings in this chapter can be regarded as an extension of the reproductive effort data in chapter II, but also as a demonstration of the adjustments breeders can make to avoid or minimize the life-history trade-offs tested for in chapter IV. In chapter IV, I compare the future survival and fecundity of females assigned to different litter size treatments in 1994 to determine if there is evidence for a cost of reproduction in red squirrels. The survival of juveniles from litters assigned to the three treatments in 1994 are also compared to determine if increases in offspring number lead to a reduction in offspring fitness.

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Chapter Two. Offspring size and compromise: an experimental study of reproductive effort in the red squirrel.

Optimal offspring number theory proposes that an individual should produce the number of offspring each breeding season that will maximize its lifetime reproductive success (Lack 1947, 1954; Williams 1966; Charnov & Krebs 1974). Thus, offspring number is thought to be affected by proximate limitations, such as energy or time availability during reproduction (Drent & Daan 1980), and by trade-offs between offspring number and quality (Lack 1947, 1954; Stearns 1992) and current and future reproduction (i.e. the cost of reproduction; Williams 1966; Stearns 1992). In most cases these limitations and trade-offs are linked. For example, a trade-off between offspring number and quality may result from limitations faced by the parent while provisioning offspring (Drent & Daan 1980) or from regulation of parental reproductive effort because of selective pressures stemming from the cost of reproduction (Stearns 1989).

The proposed basis of most proximate limitations and life-history trade-offs is the limited availability of energy. Indeed, the most commonly used operational definition of reproductive effort, the proportion of available energy allocated to reproduction, confines reproductive effort to energy distribution alone. This makes the measurement of energy expenditure critical in studies of reproductive effort, and the doubly-labeled water technique has made this possible in field studies (e.g. Kenagy et al. 1990; Moreno et al. 1995). However, breeders may face other limitations in addition to, or in place of energy limitations, such as specific nutritional requirements (e.g. proteins; Bolton, Houston, & Monaghan 1992) or time. In addition, the basis of reproductive costs and trade-offs that are imposed by predation or intra-specific competition, may be due to behavioural, not energetic, responses to increased demands. Therefore, it is important to incorporate several measures of costs in studies of reproductive effort (Gittleman & Thompson 1988).

Manipulation of clutch size has proven to be a useful technique for investigating the determinants of optimal offspring number in birds and invertebrates (Godfray 1987; Godfray, Partridge & Harvey 1991; Linden & Moller 1989). Because natural variation in offspring number tends to be confounded with other parental or environmental variables (e.g. breeding experience, access to food resources), correlations between natural offspring number and life-history traits are often misleading (Hogstedt 1981; Reznick 1985; Stearns 1989). Similarly, correlative studies of the relationship between natural offspring number and reproductive effort offer little insight into the costs an individual would experience due to increases in offspring number. If offspring number is adjusted according to a parent's ability to provision offspring (Drent & Daan 1980), no strong relationship between natural offspring number and indices of reproductive effort should be expected to occur, even if reproductive effort is a direct function of offspring number within an individual.

Mammalian reproduction is characterized by increasing energy demands from conception to the onset of weaning; in most mammals, female energy expenditure is higher during the latter stages of lactation than during any other period of their life-history (Gittleman & Thompson 1988). Nevertheless, reproductive strategies of mammals are diverse. Life-history strategies range from extended iteroparity (e.g. red deer, *Cervus elaphus*, hinds produce offspring every one to two years for up to sixteen years; Clutton-Brock, Albon, & Guinness 1988) to semelparity (e.g. males of several species in the marsupial genus, *Antechinus*, die soon after mating; Cheal, Lee, & Barnett 1976). Strategies of energy procurement for reproduction ranging from complete reliance on stored energy (e.g. females of many species of phocid seals fast from parturition to weaning of their litters; Bonner 1984) to almost complete reliance on increases in ingested energy (e.g. body energy stores provide less than 4% of energy requirements during lactation in golden-mantled ground squirrels, *Spermophilus saturatus*; Kenagy et al. 1990). Although these general patterns are well described for many mammal species, little is known about the roles of proximate limitations and life-history trade-offs in shaping these patterns. Several authors have proposed that reproductive effort during lactation, especially the stages of lactation just prior to the onset of weaning, should be most subject to energy limitation and most likely to lead to life-history trade-offs (Gittleman & Thompson 1988; Kenagy et al. 1990; Sadleir 1984).

Recent studies incorporating manipulations of litter size have begun to test this hypothesis (Hammond & Diamond 1992, 1994; Hare & Murie 1992; Genoud & Perrin 1994).

In the present study, I manipulated the litter size of female red squirrels (*Tamiasciurus hudsonicus*) and measured behavioural and energetic indices of their resulting reproductive effort during lactation. I also measured the food supply of this population by measuring spruce cone and mushroom production (both important food sources for red squirrels; Obbard 1987) in both years of the study. The objectives of this research were to determine if females could sustain increased reproductive demands, and if they could, to gain insight into the energetic compensations that enabled the females to do so. Operating under the general hypothesis that different methods of compensation (e.g. increased energy intake, use of body energy stores, decreased energy expenditure) should contribute differently to life-history trade-offs (Tuomi, Hakala, & Haukioja 1983), I use the results to predict what, if any, types of trade-offs are likely to occur in red squirrel reproduction.

METHODS

Red squirrels were studied on a 130 ha site near Kluane Lake, Yukon, Canada (61°N, 138°W), during spring and summer (March-August) in 1994 and 1995. The study site is dominated by white spruce (*Picea glauca*) with a sparse understory of willow (*Salix sp.*). The staple food source of this population 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 cached spruce cones (Gurnell 1984). Caches are normally developed or supplemented in late summer when spruce cones have matured. Cone crop failures occur periodically (three years in the last nine years in the Yukon; Boutin unpublished data), and in these years squirrels rely on cones still cached from previous years and the other food sources listed above.

Red squirrels are diurnal, but in spring and summer tend to be most active in the morning and evening, spending nights and portions of the afternoon in nests (Pauls 1978). Nests, which are usually grass structures suspended among conifer branches, or less commonly, tree cavities or burrows lined with grass, are occupied by adults and independent juveniles throughout the year and by dependent juveniles and their mothers in spring and summer. When foraging, red squirrels are highly observable regardless of food type. When squirrels are extracting seeds from cones, it is usually possible to count the number of cones processed per unit time, which we did in this study.

Food Supply

Spruce cone and mushroom productivity was measured in August 1993, 1994 and 1995. At 160 grid stakes that were distributed systematically on two 25 ha grids within the study site, the nearest spruce tree with a DBH > 5 cm was marked with flagging tape. In the first week of August of each year, the number of cones visible on the top third of flagged trees was counted. If the total number of cones visible in that portion of the tree exceeded 100, the tree was photographed using a telephoto lens, and cones were counted from the photographs using a magnifying glass. Because cone production did not differ between grids, I present cone abundance in each year as the average number of cones present on each tree for both grids combined. At the same 160 locations, the number of mushrooms present within a 3 m radius of the grid stake was counted in early August 1993, 1994 and 1995. Mushroom abundance is presented as the total number of mushrooms counted in each year.

Litter-size Manipulation

The reproductive activity of females was monitored by observations of weight changes and nipple condition during repeated captures in spring. Shortly before or after parturition, females were fitted with radio collars to aid in location of their nests. Once nests were located , young were counted, sexed, and weighed. Parturition date was estimated from the trapping records of the female as well as the size of the young when I visited the nest (Boutin & Larsen 1993). The number and masses of juveniles in the nest were determined twice; shortly after parturition and approximately 25 days following parturition. Juvenile masses were measured to the nearest gram with 100g spring-slide scales. During the second nest visit, juveniles were given numbered eartags to allow identification following emergence.

We created donor litters by removing two juveniles, and augment litters by adding two juveniles, shortly after the first nest visit. See Chapter III for a more detailed description of manipulations. In 1994, two juveniles were cross-fostered between control females to mimic the fostering associated with the augment treatment. Because there were no effects of relatedness on survival or growth rates among control juveniles in 1994 (see chapter III), we did not cross-foster juveniles between control females in 1995. In 1994, 8 augment, 8 donor, and 8 control (cross-fostered) litters were created. In 1995 only augment and control (unmanipulated) females were studied intensively. Seventeen augment litters were created in 1995.

Because I was interested in studying the effect of offspring number on reproductive effort, I excluded females from further study if, after manipulation, their litter sizes were naturally reduced by more than one juvenile. Because no litter reductions occurred prior to emergence in 1994, all 24 females were included in the study. In 1995, of the 17 augment litters created, only seven females maintained their manipulated litter size, and an additional two lost only one juvenile. Thus, nine augment females were deemed eligible for study in 1995.

To increase the sample sizes pertaining to juvenile survival, I encorporated data on a larger number of 1994 and 1995 control litters, that were not cross-fostered. Because the mothers of these litters were not studied as intensively as cross-fostered control, donor, and augment females, I was less likely to observe the juveniles immediately following emergence. Therefore estimates of survival to emergence of these juveniles will tend to be lower than survival estimates for juveniles from the other three treatments. However, the same census technique and effort was applied to all juveniles for the rest of the summer, so survival estimates other than at emergence should not be biased.

Time Budgets

Behaviour was recorded with instantaneous sampling at 30 second intervals during ten minute focal sessions (Altmann 1974). Females were located with radio telemetry prior to, and when lost during focal sessions. Behaviour was classified as one of ten categories (feeding, searching for food, vigilant, traveling, resting, in nest, interacting with another squirrel, vocalizing, out of site, lost, and other).

In 1994, I recorded the behaviour of augment (n=8), donor (n=8), and control females (n=8) during 30 focal sessions per female, 35-45 days following parturition. In 1995, I recorded the behaviour of augment and control females only, during 40 focal sessions, 15-25 and 35-45 days following parturition (20 focal sessions per interval). Sample sizes, for interval one and two respectively, were eight and six for control females and five and four for augment females. The behaviour of four control females and two augment females were measured in both intervals. In both years, sessions were distributed systematically across the ten day periods, between 0600h and 2300h, with intervals of at least 100 minutes between focal sessions on the same individual.

Behaviour during a focal session was summarized by calculating the proportion of each of the 11 behaviour categories recorded over the 20 sampling points (10 minute session, behaviours recorded at 30 second intervals). When a female was recorded as lost or out of site during a focal session, I ensured that she was not occupying her nest. Therefore, lost and out of site behaviours were included in the denominator of calculated proportions of time spent in nest, but excluded from calculations of proportions for all other behaviours. For comparisons between treatments, years, or sessions (in 1995), proportions recorded during each focal were averaged for each female. Comparisons between years are limited to the behaviour of augment and control females, 35-45 days following parturition (interval 2). All proportions were arcsin transformed prior to statistical analysis.

Profitability of Cone Foraging

When behaviour was recorded as feeding, I recorded details of the food type, and in the case of spruce cones, counted the number of cones processed during the session. The average processing rate was calculated for each female, based on all observations of uninterrupted foraging bouts (feeding or searching) when 2 or more cones were processed. Squirrels extract seeds from cones while the cones are maturing on the tree and after they have matured and have been cached; I refer to these as new cones and cached cones respectively.

Based on the energy content of conifer seeds, the seed yield of cones at different stages of maturation, and the cone processing rate of foraging squirrels, I calculated the profitability of cone foraging in different years and at different times within years. White spruce cones are fertilized in May, become ripe in August, and cones open and seeds begin to disperse in September (Schopmeyer 1974). Between fertilization and maturity (in 1995 squirrels were first observed processing immature cones on June 23), the weight of each seed increases due to the gradual accumulation of carbohydrates, fats, oils, and proteins (Edwards 1980). Weight-specific energy content of seeds is relatively constant during this phase of maturation, but the total seed energy content within a cone is steadily increasing. Maturity of cones, and hence seed weight and number, can be predicted by the accumulation of degree days since fertilization (Mosseler 1992). Based on 1) degree day accumulations in Yukon from May to August 1995, 2) the slope of the relationship between degree days and seed accumulation reported in Mosseler (1992), and 3) the average seed yield of mature cones in mesic sites in Northern Alberta (Alberta Forest Service 1978), I estimated the relationship between seed quantity per cone (seed number • seed weight) and date. Then, based on a constant energy value of white spruce seed from Alaska (6.62 cal mg^{-1}; Brink & Dean 1966) and average cone processing rate of squirrels during focal sessions, I calculated the profitability of cone foraging (cal min^{-1}).

Energy Expenditure

Daily energy expenditure was measured with the doubly labeled water technique (Nagy 1988), generally following the methodology described in Kenagy et al. (1990). Energy expenditure of augment, donor, and control females was measured in 1994 only, for a two or three day interval between day 39 and 43 of lactation. Females were injected intramuscularly with doses of approximately 2.8 ml kg^{-1} ^{18}O (95 atom %) and 0.7 mCi kg^{-1} tritium. Following a one hour equilibration period, blood samples were collected with heparinized microhematocrit tubes from a clipped toe-nail. Females were recaptured 24 ± 1.5 hours or 36 ± 1.5 hours later, and a second blood sample was taken. Blood samples were refrigerated until ^{18}O and tritium concentrations were measured using techniques described in Nagy (1983). Twenty-three females were injected with isotopes, but problems with untrappability (n=3), injectate leakage (n=6), and faulty seals on microhematocrit tubes (n=3) reduced the useable measures of energy expenditure to 11 females (4 augments, 5 donors, and 2 controls).

RESULTS

Cone production varied significantly between years ($F_{2,513}$=44.4, p=0.0001), with high production in August 1993 (150 ± 22 cones/tree) and 1995 (89 ± 16 cones/tree), and very low production in August 1994 (0.9 ± 0.3 cones/tree). Over the last nine years, cone production at this site has averaged 44 ± 16 cones per tree per year (Boutin unpublished data). Thus, due to the abundant cone crop in 1993, cone supply was abundant for red squirrels in spring and summer 1994. However, because of the low production in August 1994 squirrels depleted their cone caches in late summer, autumn, and winter 1994, resulting in low cone supply in late winter and spring 1995. In 1995 conditions improved gradually as the abundant 1995 crop matured on the trees until August, when the cone supply was again abundant. Mushrooms were much less abundant in 1994 (total=18) and 1995 (23) than in 1993 (905) and previous years (Boutin pers. obs.), and thus were not a viable alternate food source during the spring 1995 cone shortage.

Behaviour

In both years, all treatments combined, females spent 24% of the time between 6:00h and 23:00h in the nest. When out of the nest, and in sight of the observer, 50% of time was spent foraging, 20% resting, and the remaining 30% in various other activities including traveling, vocalizing, and vigilance.

There were no significant treatment effects on proportion of time spent in the nest (two-way ANOVA of treatment and year effects, treatment term F=0.93, df=1, p=0.34), but females were in the nest twice as often in 1995 than in 1994 (31 ± 5 % vs. 16 ± 3 %) which was a significant difference (year term F=7.48, df=1, p=0.01). Augment females in 1995 tended to spend less time in the nest as the season progressed (interval one r^2=0.44, n=6, p=0.04; interval two r^2=0.66, n=5, p=0.09). This trend was not present for control females in either interval in 1995 (interval one r^2=0.27, n=8, p=0.19; interval two r^2=0.01, n=6, p=0.86) or for either treatment in 1994 (augment r^2=0.09, n=8, p=0.46; control r^2=0.13, n=8, p=0.37). The increase in time spent in the nest in 1995, relative to 1994, was associated with a decline in time spent resting. Females spent only 4 ± 1 % of time resting in 1995 compared to 24 ± 3 % in 1994 (two-way ANOVA, year term F=16.28, df=1, p<0.001). As with time spent in the nest, there was no significant effect of treatment on time spent resting (treatment term F=0.08, df=1, p=0.78). Regression analysis of the effect of date on time spent resting indicated that there were no significant seasonal trends among either treatment in both years (p>0.25 in all cases).

In addition to the increase in time spent in the nest, females spent significantly more time foraging in 1995 (43 ± 3%) than in 1994 (26 ± 3%; two-way ANOVA year term $F_{1,26}$=16.1, p=0.0005), but again there was no significant difference between augments and controls (two-way ANOVA treatment term $F_{3,26}$=0.76, p=0.39). The proportion of time spent foraging did not vary significantly with date in 1994 or 1995, whether treatments were analyzed collectively or separately (p>0.35 for all regressions).

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 cached cones (52% of 3,129 instantaneous observations of feeding by 24 females) and never observed processing new cones, and females in 1995 rarely processed cached cones (4% of 1,946 observations of 14 females) and commonly processed new cones (89% of 1,946 observation). The rate of cone processing declined significantly with date in 1995 (r^2=0.32, n=21, p=0.007) but not in 1994 (r^2=0.19, n=13, p=0.13).

Because of the date effects on processing rate in 1995, I used date as a covariate when testing for treatment effects on processing rate in 1995 but not in 1994. Analysis of the effect of treatment on processing rate in 1994 indicated that the processing rates of augment (1.0 ± 0.06 cones per minute) and control females (0.88 ± 0.04) were not significantly different (t=1.59, df=11, p=0.14). Differences were more pronounced in 1995, when augment females processed cones at almost double the rate of control females (Fig. 2-1; least square means; 1.5 ± 0.1 vs. 0.9 ± 0.1; ANCOVA treatment term, F=5.4, df=1, p=0.0003).

Combining the seasonal effects on processing rate (negative relationship) and cone energy content (positive relationship) into a single profitability measure revealed a highly significant, positive relationship between date and profitability of cone foraging in 1995 (r^2=0.85, df=16, p=0.0001). Profitability of cone foraging in 1995 increased with season among augment (r^2=0.83, df=5, p=0.01) and control females (r^2=0.95, df=10, p=0.0001; Fig. 2-2), and using this relationship as a covariate, I found that augment females acquired 20% more energy per unit foraging time than control females (178 cal min^{-1} vs. 143 cal min^{-1}; treatment term F=6.3, p=0.025). However, because profitability increased over time to a lesser degree among augment females than control females, the slopes are marginally heterogeneous (interaction term F=10.8, p=0.059), and thus the analysis of covariance is not completely valid. Because the energy content of cached cones did not change with date in 1994, the non-significant, 12% difference in cone processing rate between control and augment females should have resulted in a similar 12% difference in energy intake per unit foraging time.

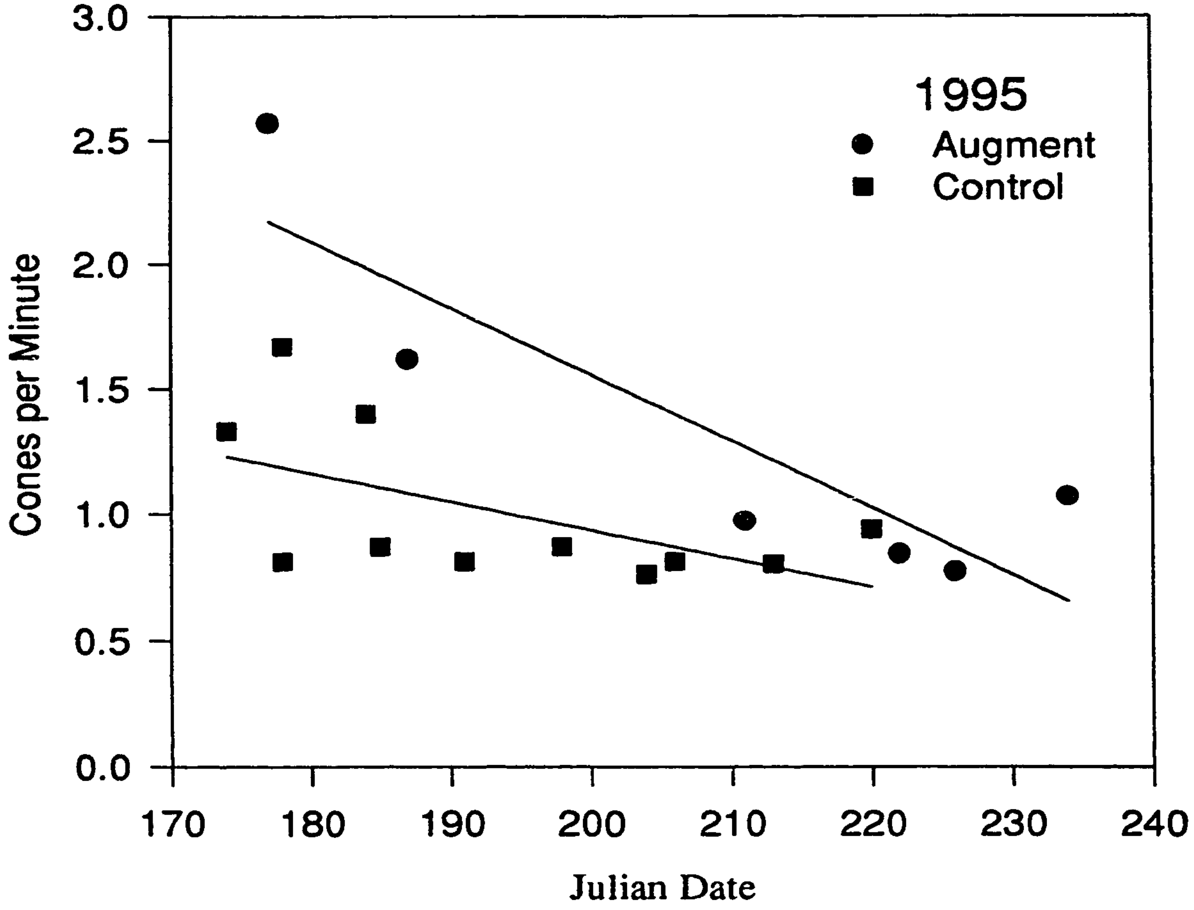


Figure 2-1. Relationship between spruce cone processing rate and date in 1995. Each point represents an average rate for a female during a ten day period. Lines represent least-square regressions.

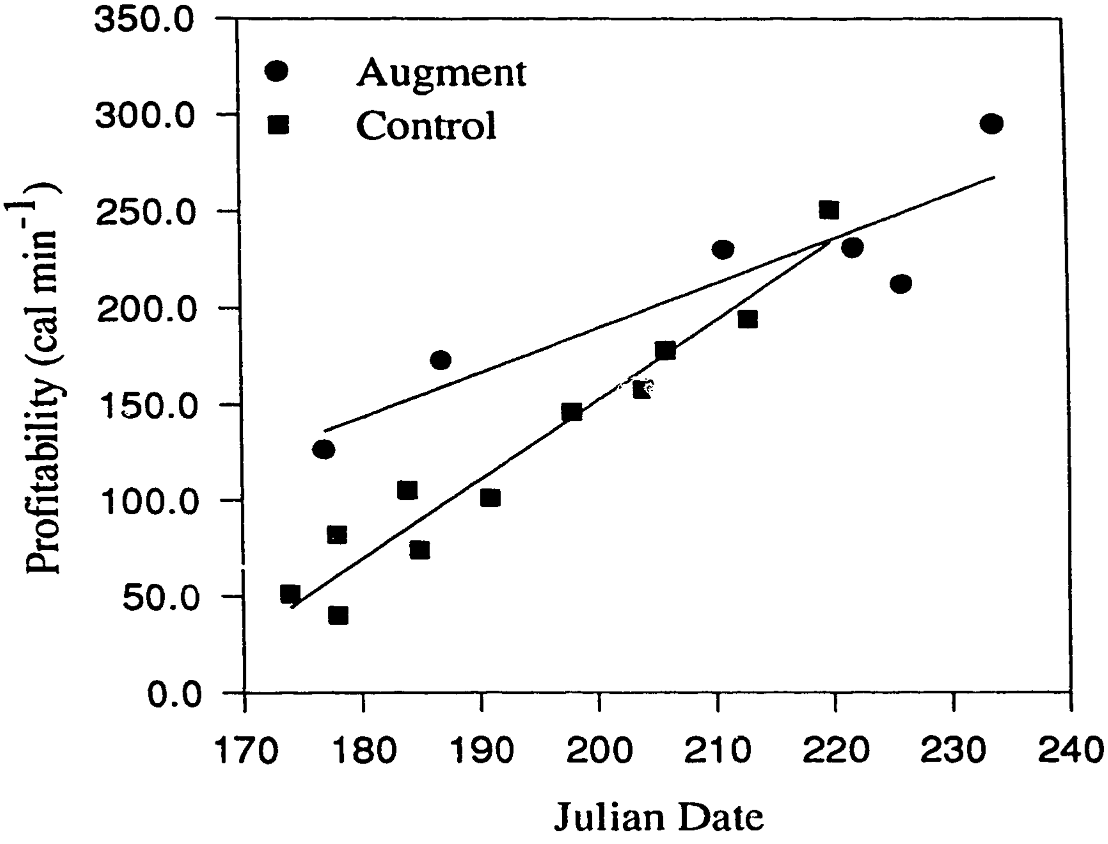


Figure 2-2. Relationship between profitability of cone foraging bouts and date in 1995. Profitability based on processing rates in Fig. 2-1, adjusted according to the seasonal caloric content of spruce cones. Lines represent least-square regressions.

I calculated the ratio of time spent feeding to time spent foraging (feeding and searching) for each bout of cone foraging. Ratios were averaged for each female who was observed processing cones for at least 10 cumulative minutes. Regressing this variable on date, revealed that the feed:forage ratio did not change with date in 1994 (r^2=0.001, n=13, p=0.90) but tended to decline with date in 1995, although the relationship was not significant (r^2=0.39, n=7, p=0.09). Low sample size in 1995 prevented analysis of each treatment separately, but separate analysis in 1994 revealed that there was no relationship among control females (r^2=0.05, n=6, p=0.67), and a marginally significant negative relationship among augment females (Fig. 2-3; r^2=.54, n=7, p=0.06). Augment females in 1994 tended to spend more time searching per unit time foraging as the season progressed.

Comparison of female behaviour in interval one (day 15-25) and two (day 35-45) in 1995 revealed that females spent almost twice as much time in the nest during the earlier interval (55% vs. 31%; paired t-test p=0.02). The decreased time spent in the nest in the second interval was associated with a 50% increase in time spent foraging (interval 1, 30 ± 5%; interval 2, 45 ± 3%; paired t-test p=0.04).

Energy Expenditure

Daily energy expenditure at day 39-43 of lactation was significantly different for the three treatments (Fig. 2-4; Kruskal-Wallis Test, χ=7.6, n=11, p=0.02). The expenditure of augment females (492 ± 9 kJ day^{-1}) was higher and less variable than that of control (457 ± 22 kJ day^{-1}) and donor (408 ± 23 kJ day^{-1}) females. Because the donor DEE measures included females with litter sizes of one and two, I tested the effect of litter size on donor energy expenditure. Although the three donor females with litter sizes of two tended to have higher expenditure than the two females with one offspring (424 ± 29 vs. 383 ± 44 kJ day^{-1}), the difference was not significant (Kruskal-Wallis Test, χ=0.33, n=5, p=0.56).

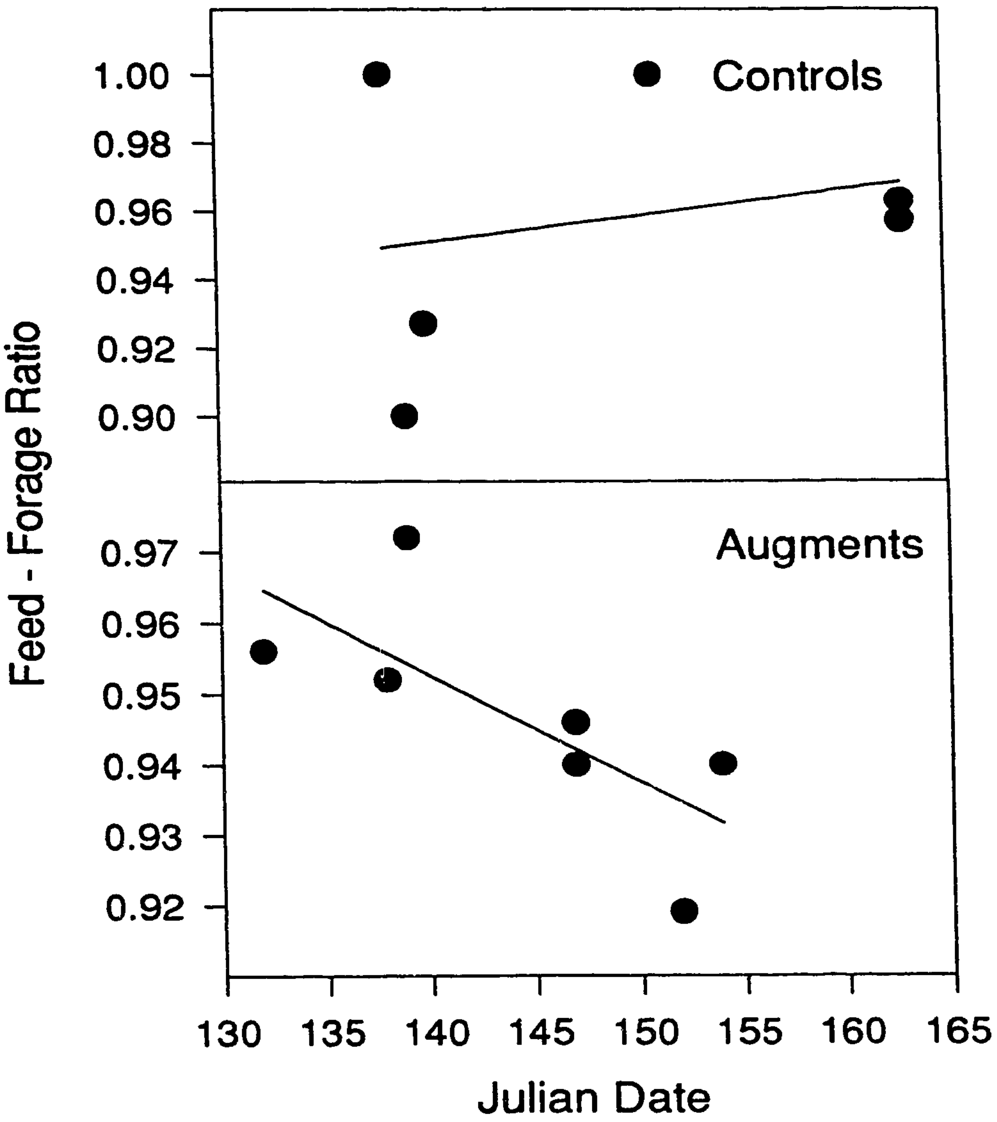


Figure 2-3. Relationship between the ratio of foraging time spent feeding and date in 1994. Feed-forage ratio was calculated by dividing the time spent feeding into time spent foraging (feeding or searching) during an uninterrupted bout.

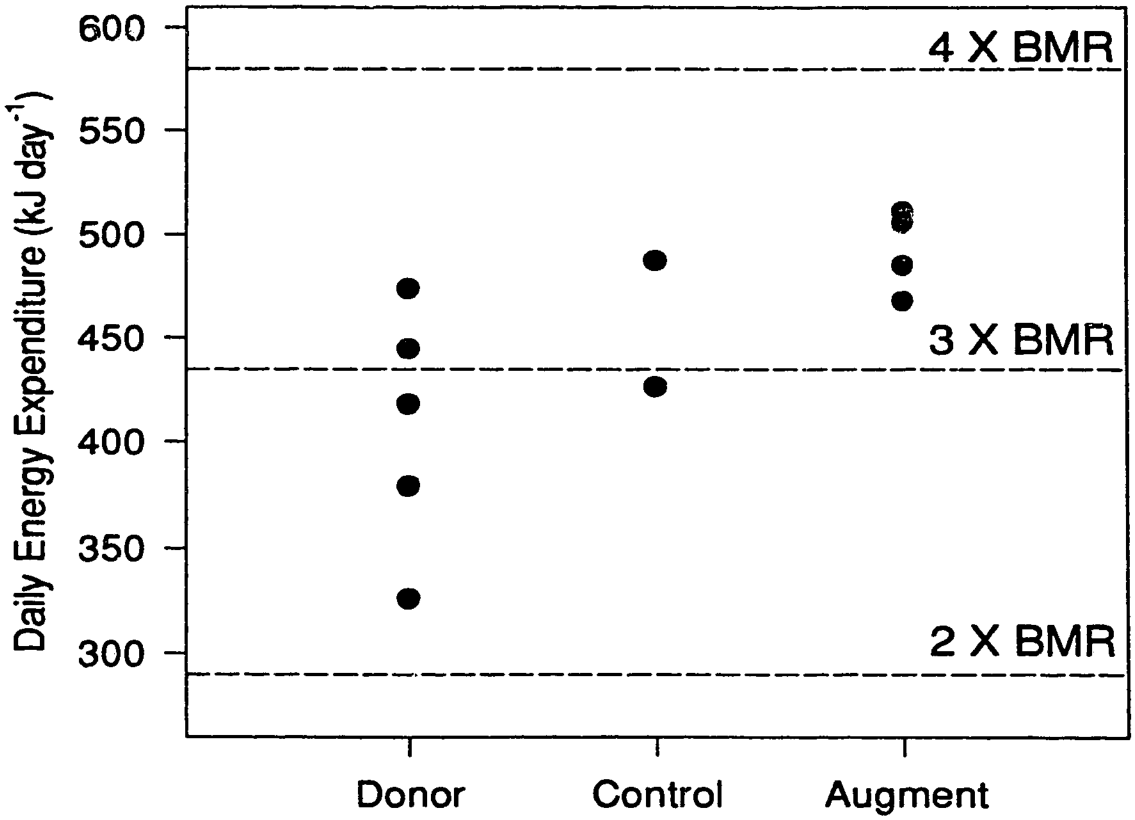


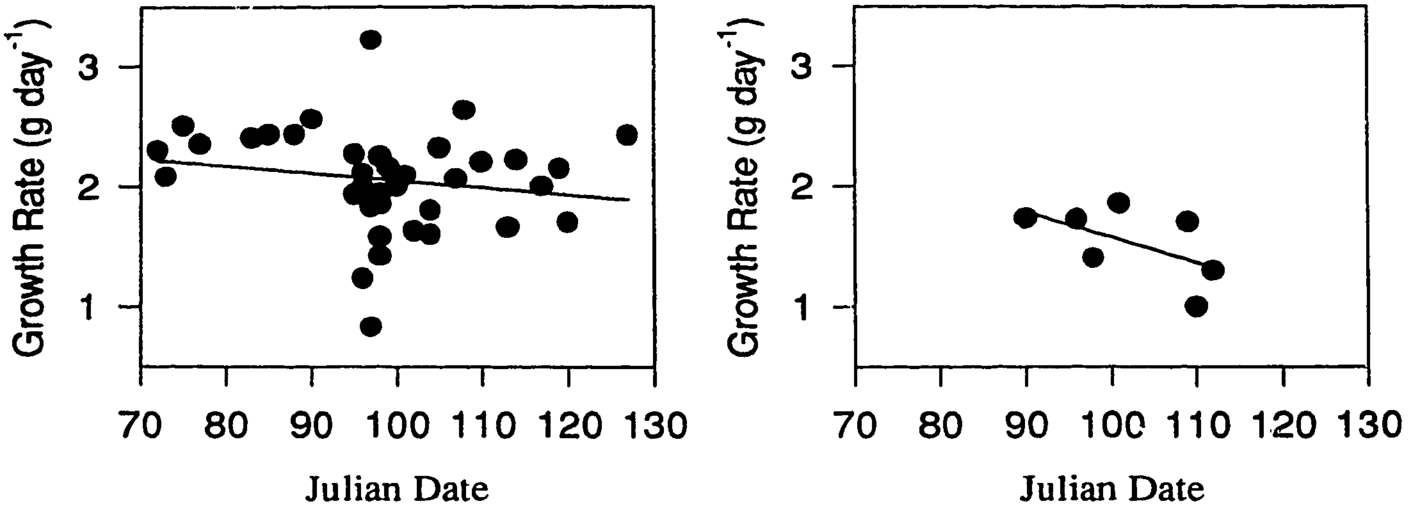
Figure 2-4. Treatment effects on daily energy expenditure from day 40 to day 43 of lactation. Dashed lines indicate multiples of basal metabolic rates, based on values reported by Pauls (1981)

Juvenile Growth and Survival

In 1994, survival of offspring until day 25 was very high for control (96%, n=195 juveniles from 65 litters) and experimental litters (cross-fostered controls 100%, n=26 juveniles from 8 litters, augment 100%, n=40 juveniles from 8 litters; donor 100%, n=12 juveniles from 8 litters). Similarly, survival from birth to emergence (day 40-45) was high for all treatments (control 80%, cross-fostered control 100%, augment 95%, donor 100%). The non-significant difference (G=0.71, df=1, p=0.40) between survival of control and cross-fostered control juveniles likely resulted from more intensive observation of cross-fostered juveniles when they emerged from the nest.

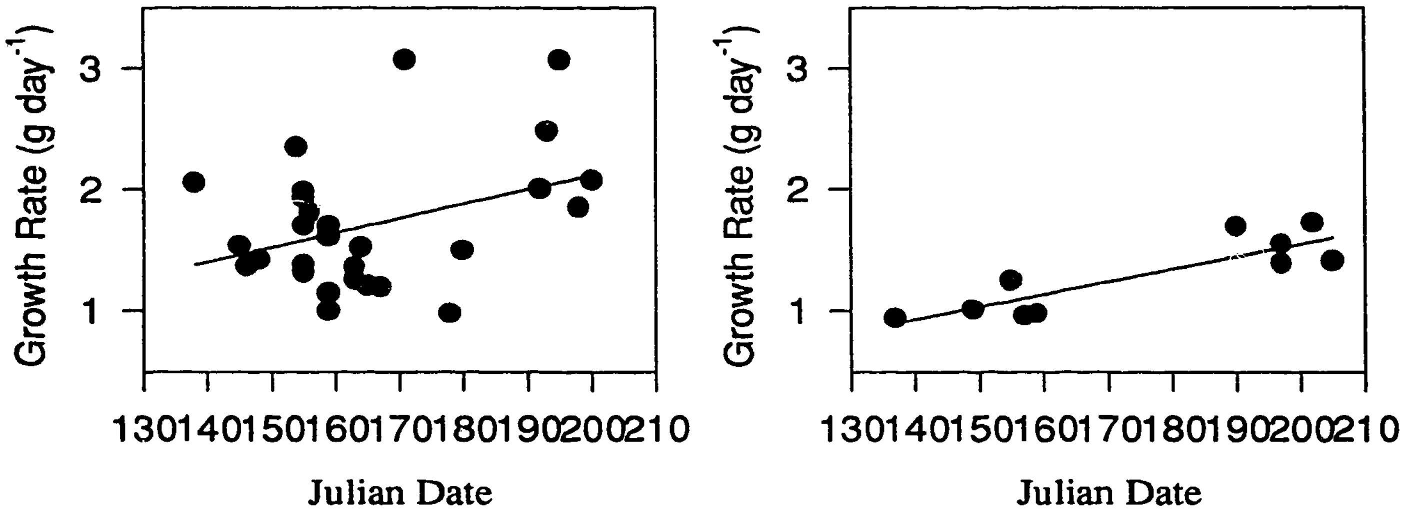
Juvenile survival was lower in 1995. Among 57 control litters, 19 (40%) were characterized by the death of all juveniles before day 25, which was significantly higher than the 2% (n=65) occurrence of complete litter loss in 1994 (G=25.2, df=1, p<0.0001). The incidence of complete litter loss was similar for donor litters (50%, n=10; G=.32, df=1, p=0.57), but significantly lower for augment litters (12%, n=17; G=5.5, df=1, p=0.02). Although the prevalence of complete litter loss was low among augment females, only 41% (n=17) were successful in maintaining their augmented litter sizes until tagging, and only 15% (n=13) did so until weaning.

Juvenile growth rates differed between 1994 (1.90 ± 0.04 g/day) and 1995 (1.56 ± 0.04), and between control (1.86 ± 0.03) and augment (1.40 ± 0.04) treatments. Combined analysis of the year and treatment effects (two-way nested ANOVA, nesting juveniles within females), yielded significant treatment (F=6.9, df=1, p=0.009) and year effects (F=21.8, df=1, p<0.0001) and no significant interaction effect (F=0.01, df=1, p=0.94). Because of the seasonal increase in food supply in 1995, I tested for a relationship between parturition date and juvenile growth rate (average rate calculated for each litter). Growth rate (including control and augment litters) was significantly related to parturition date in both years (1994, r^2=0.12, n=45, p=0.02; 1995, r^2=0.10, n=40, p=0.04), but the relationships were opposite; growth rate decreased over time in 1994 and increased over time in 1995 (Fig. 2-5). Separate analysis of each year-treatment combination showed that the strongest relationship was among augment litters



a) Control females in 1994

b) Augment females in 1994



c) Control females in 1995

d) Augment females in 1995

Figure 2-5, a-d. Treatment and year effects on offspring growth in relation to birth date in 1995 (r^2=0.75, n=10, p=0.001), with the other relationships characterized by low explanatory power, and marginal significance (1994 control r^2=0.10, n=38, p=0.04; 1995 control r^2=0.13, n=30, p=0.05) or, moderate explanatory power, but due to a smaller sample size, non-significance (1994 augment, r^2=0.33, n=7, p=0.18; Fig. 5).

DISCUSSION

During the period when reproductive effort was measured (day 35-45), the total mass of offspring supported by augment females was 137 ± 43 g greater than that supported by control females in 1994, and 115 ± 12 g more than control females in 1995. Thus, in both years the reproductive demands of augment females were manipulated by approximately 50% of maternal body mass. Not all females sustained their natural or experimentally increased reproductive demands, and those females whose manipulated litter size (or original litter size in the case of controls) had decreased by more than one juvenile prior to emergence, were excluded from analysis. Exclusion of females was necessary only in 1995, since no litter sizes were reduced prior to emergence in 1994. 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 & Murie 1992). However, in this study, the proportion of females excluded from augment and control treatments were approximately equal, and so, although the total group of study females may not be representative of the population, the relative comparison of females with and without augmented demands should be valid.

Because of the natural variation in spruce seed abundance experienced by this population during the study period, I was able to investigate the effects of inter-annual (1994 vs. 1995) and intra-annual (early 1995 vs. late 1995) variation in food availability on the ability of females to sustain natural and experimentally increased reproductive demands. Intra-annual variation could also have occurred in 1994 if cone caches were becoming depleted as the reproductive season progressed. The marginally significant regression between the proportion of time spent searching while foraging and date for

1994 augment females suggests that this may have been the case, at least for females with augmented demands. However, this trend did not translate into any significant seasonal trends of juvenile growth or survival in 1994.

In 1995 when cone availability was low, females spent more time foraging and in the nest, and less time resting than females in 1994. Despite the seasonal increase in food supply in the second year of the study, behaviour of females in 1995 generally did not change significantly with date, except for time spent in the nest by augment females, which declined over time. Differences in time spent foraging likely resulted in part from differences between old and new cones in the energy acquired per unit time foraging. Control females in 1994 acquired 40% more calories per minute of cone foraging than females in 1995 (240 cal min^{-1} vs. 143 cal min^{-1}).

In 1995, females sustained the high energy demands of lactation when the availability of their staple food source was low. Females could have maximized the amount of energy they allocated to their offspring by either increasing their energy intake or decreasing their energy expenditure (except for that allocated to their offspring). By spending more time in the nest, with the associated low activity and thermoregulatory costs, and more time foraging, 1995 females should have partially compensated for the reduced food availability. However, the increase in nest use in 1995 was associated with a decline in time spent resting; females substituted time spent resting in 1994 with time spent in the nest in 1995. Therefore, the increased nest use would have resulted in reduced expenditure only if females have lower energy expenditure while in the nest than while resting outside the nest. Most observations of squirrels in both years were made when the ambient temperature was below the lower critical temperature of summer-acclimatized red squirrels (21°C; Pauls 1981). However, postural adjustments, temperature-modulated behaviour, and micro-climate selection allow red squirrels to remain normeothermic at temperatures characteristic of the study site in late spring and summer (Pauls 1978). Since resting outside the nest was likely associated with only a slight thermoregulatory cost, and a probable energy conservation benefit due to reduced disturbance by offspring, it is unlikely that replacement of time spent resting with time spent in the nest by females in 1995 was an energy conservation strategy.

Alternatively, females may have spent more time in the nest in 1995 because of changes in water balance and milk production due to the water content of their food source. The production of more highly concentrated milk by water-restricted females has been demonstrated, and is believed to be a direct, physiological consequence of reduced water intake (Baverstock, Spencer, & Pollard 1976). Although it has not been demonstrated, females with increased water intake will presumably produce more dilute milk. An additional characteristic of conifer seed maturation, not mentioned in the methods section, is an approximate two-fold decline in seed moisture content from the time squirrels began processing immature cones in June until they matured in mid-August (Ching & Ching 1962; Edwards 1980). Thus the water content of spruce seed consumed by females in this study would have been low throughout 1994, and declining from high levels in spring 1995 to near 1994 levels by August 1995. Red squirrels are rarely observed ingesting free water (Boutin per. obs.), so their water requirements are believed to be met by principally preformed and metabolic water (Bartholomew & Cade 1972). The large differences in water content of immature and mature cones, and the predominance of spruce seed in the diet of red squirrels, should have lead to 1995 females producing larger volumes of milk than 1994 females. Although milk production of 1995 females may have been high, the total energy they transferred to their litters was likely lower than that transferred by 1994 females, as suggested by the lower food availability and juvenile growth rates in 1995. If females characterized by higher milk output spend proportionally more time nursing their litters, fluctuation in water intake may be responsible for the observed patterns of nest use. The increased nest use by females in 1995 relative to 1994, and augment females in early 1995 relative to late 1995, is consistent with this hypothesis. In an evolutionary sense, dilute milk is not an unavoidable consequence of high water intake (body water could be eliminated by means other than milk production), and thus is expected to be linked to water intake only if production of dilute milk increases, or does not decrease, the reproductive efficiency of females.

Reductions in food supply and increases in offspring number should both elevate the energetic stress experienced by females during lactation. Therefore, if the general behavioural differences between 1994 and 1995 females (more time spent foraging and in nest, and less time resting in 1995) were due to differences in the energetic stress experienced in the two years, the behaviour of control and augment females could be expected to differ in a similar manner. These differences did not occur; time budgets of augment and control females were remarkably similar in each year. Thus, either the magnitude of the energetic effect of food supply fluctuations was greater than the energetic effect of litter size manipulations, or behavioural differences between 1994 and 1995 were due to specific effects of changes of food supply (water content and profitability of new and old cones), and not general effects of energetic stress.

Although time budgets of augment and control females were similar, their rate of energy acquisition during foraging bouts was different. In both 1994 and 1995 augment females tended to acquire more spruce seed per unit time of cone foraging, although the difference was significant only in 1995. Because search time during foraging bouts was not significantly different between treatments, the increased efficiency appears to be due to decreases in food handling time alone. Despite the elevated energy demands associated with the latter stages of lactation (Gittleman & Thompson 1988), females with natural litter sizes do not appear to maximize their rate of energy intake per unit time foraging.

Although daily energy expenditure around day 40 of lactation varied significantly between treatments, the differences were small. The average expenditure of augment females was only 7% higher than that of control females. In fact, observed increases in energy intake per unit time foraging by augment females (non-significant 12% increase in 1994, significant 20% increase in 1995), should have allowed augment females to sustain their rate of energy expenditure with less foraging time than control females. However, daily energy expenditure is not a complete measure of reproductive effort;

although it incorporates the costs of acquiring and digesting the resources required for lactation and the costs of synthesizing milk, it does not measure the energy transferred directly to the offspring via milk (Kenagy 1987). Additionally, when stored energy is used to support reproductive requirements, the costs of metabolizing that energy will be measured, but of course, the original foraging costs of obtaining that energy will not. Because augment females were mobilizing energy stores at a faster rate than control females during the measurement period (see chapter III), daily energy expenditure measurements will slightly under estimate the differences in their reproductive effort. Mean energy expenditure of control females was 25% higher than that reported for golden-mantled ground squirrels (*Spermophilus saturatus*; mean and range of post-partum body masses is the same as red squirrels) at the same period of lactation. However, in terms of multiples of basal metabolic rate, values for the two species were very similar (red squirrels, 3.2 BMR; *S. saturatus*, 3.4 BMR). These values and those for female red squirrels with augmented demands (3.4 BMR) are less than the 4 BMR level that Drent & Daan (1980) have proposed as a possible upper limit to daily sustained workload in a wide array of organisms.

Experimental increases in litter size were associated with a pronounced reduction in juvenile growth rate, even in the abundant food conditions of 1994. Nevertheless, the 18% decrease in growth rate from 1994 to 1995, and the 40% increase from early 1995 to late 1995 suggests that juvenile growth rate is at least partly determined by food supply. Juvenile survival also appears to be related to food supply, as indicated by the 50% decrease in survival to emergence in 1995, relative to 1994

The 18% increase in time spent foraging by control females in 1995, relative to females in 1994, should have reduced the 40% discrepancy in energy intake due to differences in cone profitability to about 20%. This discrepancy may have been compensated for by either increasing intake from alternate food sources or reducing energy expenditure. But the 18% reduction in juvenile growth rates between 1994 and 1995 (1.90 g day^{-1} vs. 1.56 g day^{-1}), suggests that females did not compensate for this deficit, nor did they respond by committing a greater proportion of available resources to their offspring; instead they appear to have reduced their energy investment in their offspring.

Female red squirrels appear to be characterized by levels of reproductive effort that are below levels imposed by time or physiological constraints. Through increased efficiency during foraging bouts and reduced offspring growth rates, females were able to sustain increases in offspring number without biologically significant changes in time budgets or energy expenditure. Findings reported in chapter III, indicate that by adjusting energy stores early in lactation, females can also sustain increases in offspring number without experiencing declines in body condition.

The variation in food supply between 1994 and 1995 affected females more negatively than did changes in offspring number in either year. Reductions in food supply caused a sharp increase in rates of pre-emergent juvenile mortality, and significant increases in the proportion of female time budgets that were allocated to foraging. Nevertheless, even under conditions of apparent food limitation, females were able to sustain increases in offspring number without altered time budgets. The additional mass of offspring supported by augment females at emergence of their litters (see first paragraph of discussion) can be used as a measure of the margin between the reproductive effort of females with natural litter sizes and a maximum upper limit of reproductive effort. Using this measure, control females in 1995 appear to have been only slightly closer to maximum levels than 1994 females; the additional offspring mass supported by 1994 augment females is only 16% higher than that supported by 1995 augment females. However, this is likely not a fair representation of upper limits of reproductive effort. Given the strong evidence for regulation of reproductive effort by females with natural litter sizes, it is likely that the reproductive effort of augment females does not reflect maximum levels, but simply individually regulated levels at a slightly higher set point.

Why are female red squirrels characterized by a conservative level of reproductive effort? Life-history trade-offs which, by definition, will result in expression of reproductive effort below maximum levels, offer the most parsimonious explanation.

If juvenile size at emergence is an important determinant of survival, the reduction in size of augment juveniles reported in this study should lead to an offspring number vs. fitness trade-off. Correlative data from the same population of red squirrels indicates that juvenile size at emergence has no significant effect on survival to recruitment (Larsen & Boutin *in prep.*). However, the differences in juvenile size that resulted from my offspring number manipulations were considerably larger than the differences that occurred between unmanipulated litters, and therefore may be more likely to lead to survival effects.

Tuomi et al. (1983) proposed that the cost of reproduction is less likely to occur in systems where individuals sustain increased reproductive demands by increasing resource input, and more likely to occur in systems characterized by reliance on body energy stores. Superficially, results from this chapter (and chapter III) suggest that red squirrels belong in the former category. However, increases in resource intake by larder-hoarding red squirrels may be equivalent to declines in body energy stores for non-hoarding animals, as both result in depleted levels of stored energy. If elevated reproductive effort leads to advanced depletion of cone caches, offspring number could be directly linked to survival of female red squirrels.

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Chapter Three. Reproductive demands and mass gains: a paradox in female red squirrels

Reproduction in vertebrates involves periods of high energy demand. Because offspring size and quality are, to a large extent, determined by a parent's ability to maximize the energy transfer from itself to its offspring (Drent & Daan 1980), responses to reproductive energy demands are key determinants of reproductive success. To maximize reproductive success, a breeder must maximize the number and quality of offspring produced, while minimizing the mortality risk associated with extreme declines in body condition. The reproductive success achieved from a given reproductive attempt may be highly dependent on the breeder's ability to meet demands during short energetic bottlenecks, such as the late stages of mammalian lactation (Gittleman & Thompson 1988). Many vertebrates store energy prior to or during the reproductive season, then mobilize these stores during periods of peak reproductive demands (Sadleir 1984; Gittleman & Thompson 1988). This reliance on stored energy (rather than that acquired directly from foraging) increases the total, or proportion of, daily energy expenditure that can be allocated to reproduction.

Because of the allometric relationship of energy demands and storage capacity, small animals are not able to sustain reproductive demands with stored energy (Lindstedt & Boyce 1985) and therefore, relative to larger animals, are typically more reliant on ingested energy during reproduction (Millar 1987). Nevertheless, many species of small birds and mammals are known to use some energy reserves during reproduction (Drent & Daan 1980, Gittleman & Thompson 1988, Millar 1987), and even relatively small energy stores can be significant (Lindstedt & Boyce 1985). In mammals, energy reserves are usually developed prior to conception and during gestation, but not during lactation when energy demands are highest (Gittleman & Thompson 1988).

Increases in body mass during early lactation have been reported for several species of small mammals (Millar 1978; Konig, Riester & Markl 1988; Kenagy, Stevenson & Masman 1989; Sikes 1995). Although it is clear that female reproductive demands increase from conception to late lactation, the occurrence of surplus energy budgets may not be limited to pre-reproductive or early reproductive periods. For example, in highly seasonal environments, seasonal increases in food supply and decreases in thermoregulatory costs may facilitate positive energy budgets during advanced stages of reproduction. Thus energy storage during early lactation, like storage prior to and during gestation, may be a strategy employed by breeders to maximize the transfer of energy to their offspring during late lactation. Because lactational energy storage occurs nearest to the period of peak demands, and thus at a point when the extent of these demands should be most predictable, it may confer the additional benefit of allowing breeders to adjust their stores according to late lactation requirements.

The purpose of this study was to investigate the energy dynamics of breeding red squirrels (*Tamiasciurus hudsonicus*) to gain insight into the proximate strategies breeders use to maximize the transfer of energy from themselves to their offspring. Female reproductive demands were experimentally altered by litter-size manipulations, and their subsequent mass changes were documented. We determined if squirrels were capable of mass increases during early lactation, and if so, whether or not these increases were correlated with demands during late lactation.

METHODS

Red squirrels were studied on a 130 ha site near Kluane Lake, Yukon, Canada (61°N, 138°W) in spring and summer 1994. During the study period, white spruce (*Picea* glauca) seed, the principal food source of this population, was abundant (see chapter II), and the seasonal temperatures were above average (Boutin unpublished data). All squirrels on this site have been marked with numbered ear-tags, and the reproductive success of individual females has been monitored since 1990 (Boutin & Larsen 1993).

The reproductive activity of females was monitored by observations of mass changes and nipple condition during repeated captures beginning in late March of each year. Shortly before or after parturition, females were fitted with radio collars to aid in locating their nests. Once nests were located the young were counted, sexed and weighed. Parturition date was estimated from the trapping records of the female and the size of the young when we visited nests (after Boutin & Larsen 1993).

The number and mass (nearest gram with 100g spring-slide scale) of juveniles in the nest were determined twice; shortly after and approximately 25 days following parturition. During the second nest entry, juveniles were given numbered eartags to allow identification following emergence. After emergence and until late summer, juveniles were live-trapped and weighed as frequently as possible. Post-emergence mass of a juvenile was considered to be the first measured mass following emergence, but before weaning. Female masses were measured repeatedly during pregnancy, lactation, and post-weaning (nearest gram with 500 g spring-slide scale). All means are presented with ± 1 standard deviation (SD), unless otherwise noted.

Litter-size manipulation

Augmented litters were created by adding two juveniles taken from nests of other females present on the study site. Two juveniles were cross-fostered between control females to mimic the fostering associated with the augment treatment. During manipulations, juveniles were weighed, sexed and those transferred between nests were marked with small, temporary ear tags. Nests were re-visited ~15 days later to determine the success of the manipulation, and to weigh and permanently ear-tag the juveniles.

Cross-fostering and litter additions between 7 April and 9 May, 1994 resulted in eight augment and eight control females. All females had original litter sizes of three except for two control females who had four. No females experienced litter reductions prior to juvenile emergence and age or mass at parturition did not differ between augment and control females. Transfers of juveniles were conducted when they were 5-13 days old (mean=8.6); the age difference between foster-siblings was 0-4 days (mean=1.3).

When nests were revisited ~15 days later (for tagging), all litter members (original and fostered juveniles) were still alive. Mass increases of fostered and non-fostered juveniles within control and augment nests did not differ (paired t-test of average growth rates; t=-0.35, p=0.73, df=15). For fifteen of the sixteen litters, all juveniles (i.e. 5 from augment nests and 3 or 4 from control nests) were observed and/or trapped following emergence. The single exception was one augment litter from which only two juveniles were observed following emergence. Data from this female following emergence of her young were excluded from analyses.

The body masses of augment and control females were recorded whenever they were live-trapped, but the majority of our trapping effort concentrated on four discrete periods. These periods, in terms of days since parturition, were 1) day 0-12, 2) day 35-45, the period of juvenile emergence (range=36-51, mean=42), 3) day 60-70, the period when weaning of juveniles was completed (range=57-73 , mean=65), and 4) day 111-144, corresponding to late summer. There was no effect of treatment on the timing of emergence (T-test, t=0.07, p=0.95, df=12) or weaning (T-test, t=-0.70, p=0.51, df=6), relative to date of birth.

Because female masses were measured repeatedly, and during discrete periods, the most appropriate analysis of the treatment effect on mass changes would have been an analysis of variance with repeated measures (Zar 1984). However, because masses of all females were not measured at every period (due to our failure to capture, or due to the death of some females) a number of females had incomplete repeated measures and, as a result, replication was reduced to insufficient levels (eg. n=3 for control females). Therefore, we analysed each interval (eg. parturition to emergence) separately and, for any given interval, included all females whose mass change was measured. This incorporated more replication in the analysis, but due to the lack of independence between intervals, allowed us to statistically test for treatment effects at only one interval.

The total body water of eleven augmented and control females was measured at day 40 of lactation by the tritiated water dilution method (Pace et al. 1947). Because total body water and body fat are inversely correlated (Robbins 1993), determination of total body water provides a measure of the total energy stores possessed by an animal (Torbit et al. 1985). Squirrels were injected intramuscularly with tritiated water at a dosage of about 0.7 mCi/kg of body mass. If leakage of injectate occurred (3 of 11 females), that individual's total body water measurement was omitted from analyses. Following an equilibration period of one hour, blood samples were collected with heparinized microhematocrit tubes from a clipped toe-nail. Blood samples were flame-sealed, then refrigerated until analysis of tritium levels was performed.

RESULTS

Juvenile growth and the magnitude of manipulation

At the point of manipulation, total litter masses of augment females were increased by 63% ± 17% by the addition of two young. Control juveniles gained significantly more mass per day than augment juveniles between manipulation and tagging (2.0 ± 0.49 g/day vs. 1.6 ± 0.35 g/day; nested ANOVA with juveniles nested within females, $F_{1,14}$=35.2, P< 0.0001) and between tagging and post-emergence (2.5 ± 0.43 g/day vs. 2.1 ± 0.43 g/day; nested ANOVA, $F_{1,10}$=16.9, P=0.002). Despite slower growth of their juveniles, augment females continued to support much higher litter masses than control females throughout lactation. By the average day of emergence (day 42), augment females were supporting an additional 137 ± 43 g of offspring, relative to controls (~55% of adult female body mass).

Female mass changes in response to manipulations

Augment females gained 25 ± 13 g between parturition and emergence, significantly more than the 7 ± 8 g increase of control females (Fig. 3-1; T-test, t=-3.1, df=12, p=0.01). Between emergence and weaning, augment and control females lost similar amounts of mass (augments, 14 ± 12 g; controls, 11 ± 12 g). Masses continued to decline between weaning and late summer, especially among augment females (11 ± 8 g vs. 4 ± 8 g decline of controls). The cumulative result of the summer mass changes was a negligible mass change between parturition and late summer for females of both treatments (augments, 0 ± 8 g; controls, -2 ± 3 g). Total body water at day 40 of lactation ranged from 79 to 91%, and did not differ significantly between treatments. However, the extent of mass gains made by control and augment females between parturition and emergence of their litters (day 35-45) was inversely correlated with measures of their total body water at day 40 (Fig. 3-2; r=-0.70, p=0.05, n=8).

Because the mass of each foster juvenile, relative to those already present in the nest, varied by as much as ± 10 g, the percent manipulation (calculated as mass added/original litter mass*100) varied from 46-79% (63 ± 17 %). Thus, percent manipulation can be used as an indicator of the demand imposed on augment females by the litter additions. A regression between the percent manipulation and female mass increase between parturition and emergence was significant (r^2=0.504, p=0.05, n=8) and the slope positive (Fig. 3-3).

Lactational mass gains from 1990-1994

Between 1990 and 1994, unmanipulated females gained, on average, 9.8 ± 15.5 g (n=226) between parturition (day 1-10) and emergence of their young (day 35-45). The amount gained varied significantly between years (ANOVA $F_{4,221}$=3.74, p=0.006), ranging from 5.4 ± 18.9 g (n=38) in 1992 to 15.0 ± 16.0 (n=67) in 1993. The pattern of mass gain and natural litter size among this large sample of females was consistent with the trend observed among manipulated females, as natural litter size and early lactation

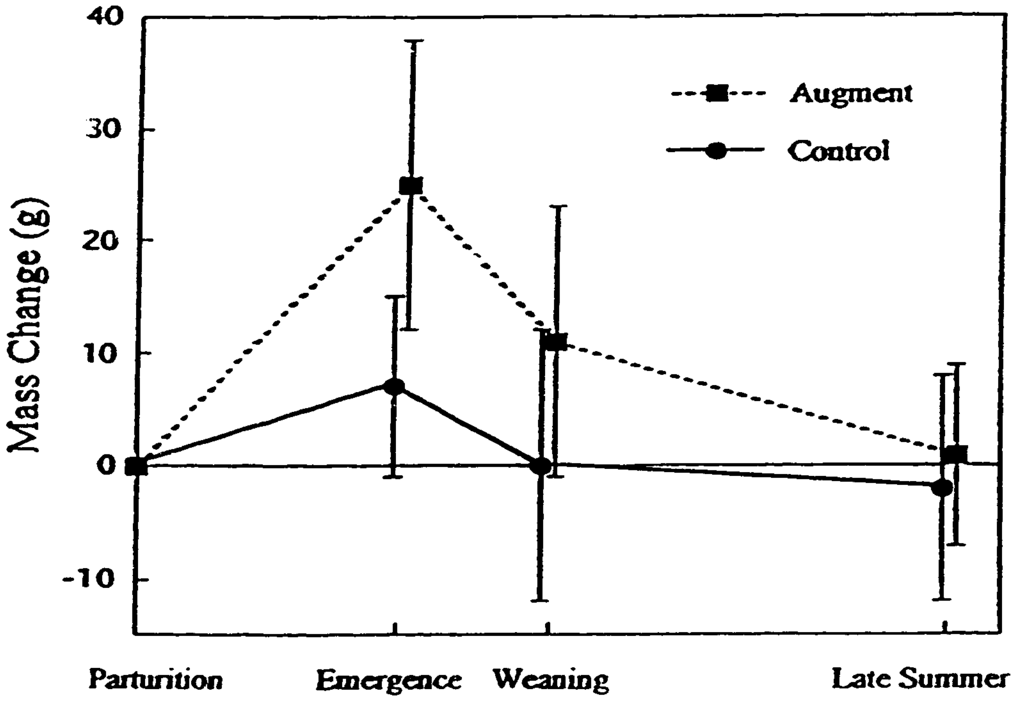

Figure 3-1. Mass changes of females from parturition to late summer. Emergence refers to day 35-45 following parturition when most juveniles emerged, weaning refers to day 60-70 when most juveniles were fully weaned, and late summer refers to a five day period in August (day 111-144). Sample sizes for the three intervals are 7, 7, and 6 for controls, and 8, 7, and 6 for augments. Bars indicate SD.

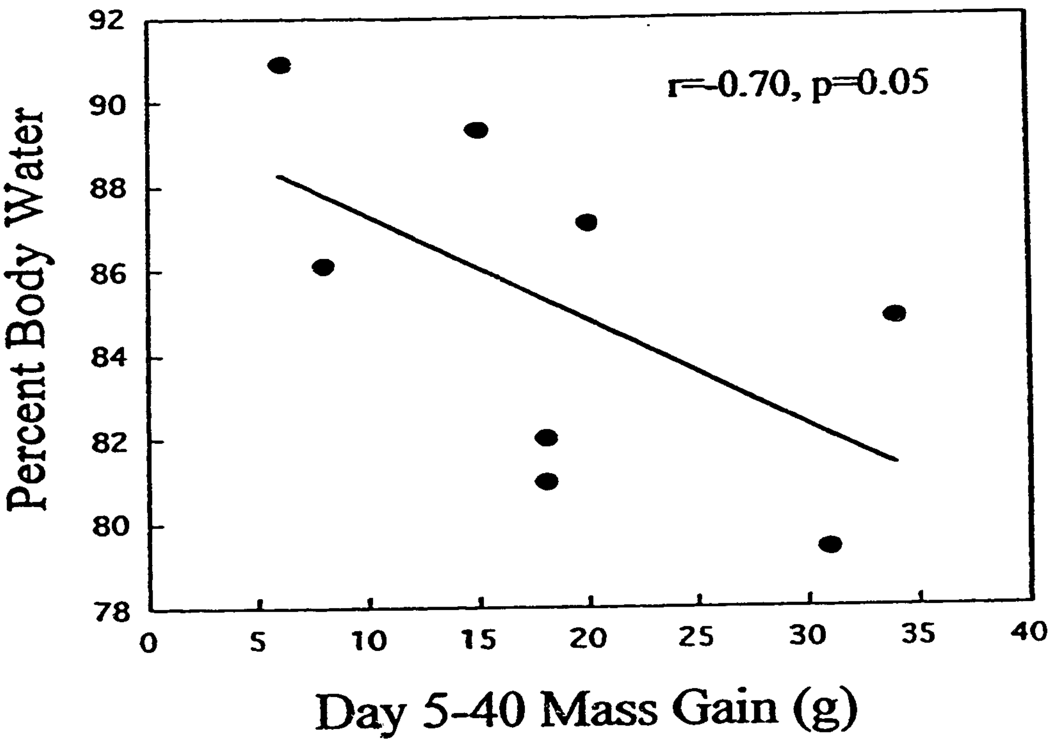

Figure 3-2. Correlation between early lactation mass gain and total body water at day 40, as measured by the tritiated water dilution technique.

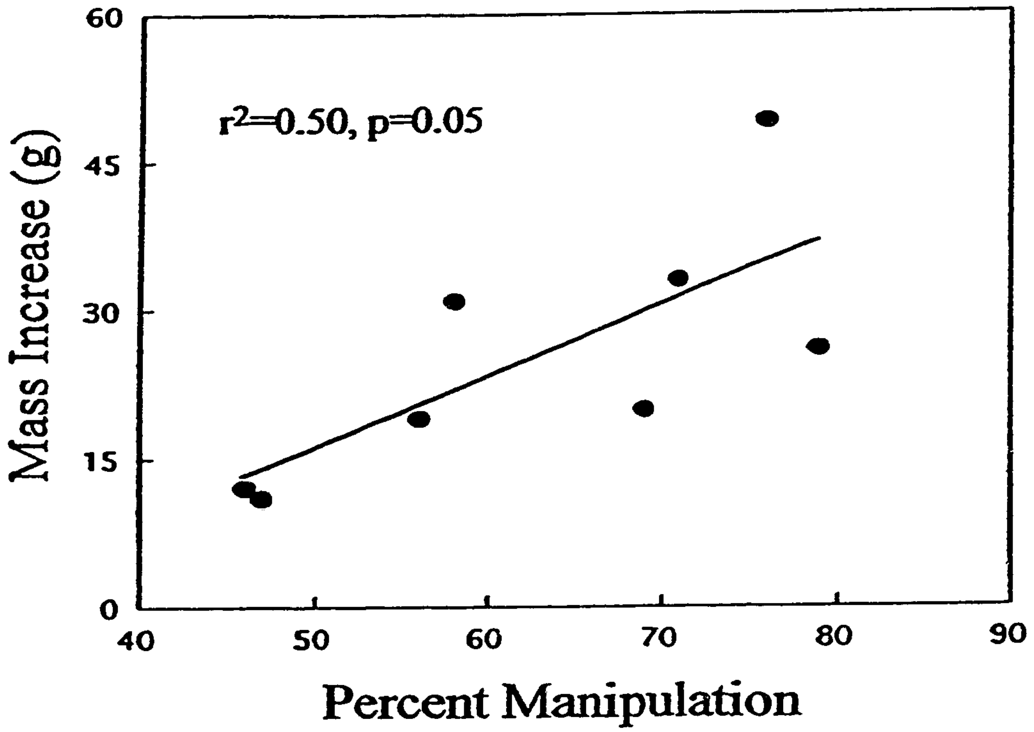

Figure 3-3. Augment mass increases from parturition to juvenile emergence (day 35-45) in response to the extent that their litter masses were manipulated.
Percent manipulation = (total mass of offspring added to litter / original litter mass) * 100.

mass gain were positively related (all years pooled, Fig. 3-4; r^2=0.05, p=0.002, n=201). The regression remains significant when the two litter sizes of five are excluded from the analysis (r^2=0.04, p=0.008, n=199), and marginally significant when litter sizes of five and litter sizes of one are excluded (r^2=0.02, p=0.06, n=192). Although these results are statistically significant, the proportion of variation explained is small.

The extent of the early lactation mass gain by females was significantly related to the survival of their offspring to weaning. Weight gain was a significant covariate of the relationship between litter size and percent survival at emergence (Fig. 3-5; ANCOVA, mass-gain term, $F_{1,213}$=7.2, p=0.008). Although the positive relationship between mass gain and offspring survival was weak among litter sizes of four relative to litters of two and three, the three slopes did not differ from each other significantly (ANCOVA, mass-gain/litter size interaction term, $F_{2,212}$=1.2, p=0.31).

DISCUSSION

Do changes in mass indicate changes in energy stores?

Body mass is commonly used as a measure of the energy stores possessed by an animal. However, body mass is a function of a number of variables in addition to energy stores, including structural size (Dobson 1992), gut contents, body water, and growth or senescence of various tissues (Kenagy & Barnes 1988). Repeated measures of body mass over time has permitted us to use mass change rather than fixed body mass as an index of condition and thus, to exclude the influence of structural size. Additionally, we have used the tritiated water dilution technique (Pace et al. 1947) to determine to what extent measured changes in body mass reflect changes in energy stores.

Given that total body water and body fat are inversely correlated (Robbins 1993), the inverse relationship between total body water at the midpoint of lactation and the extent of early lactation mass gain in red squirrels indicates that these changes in

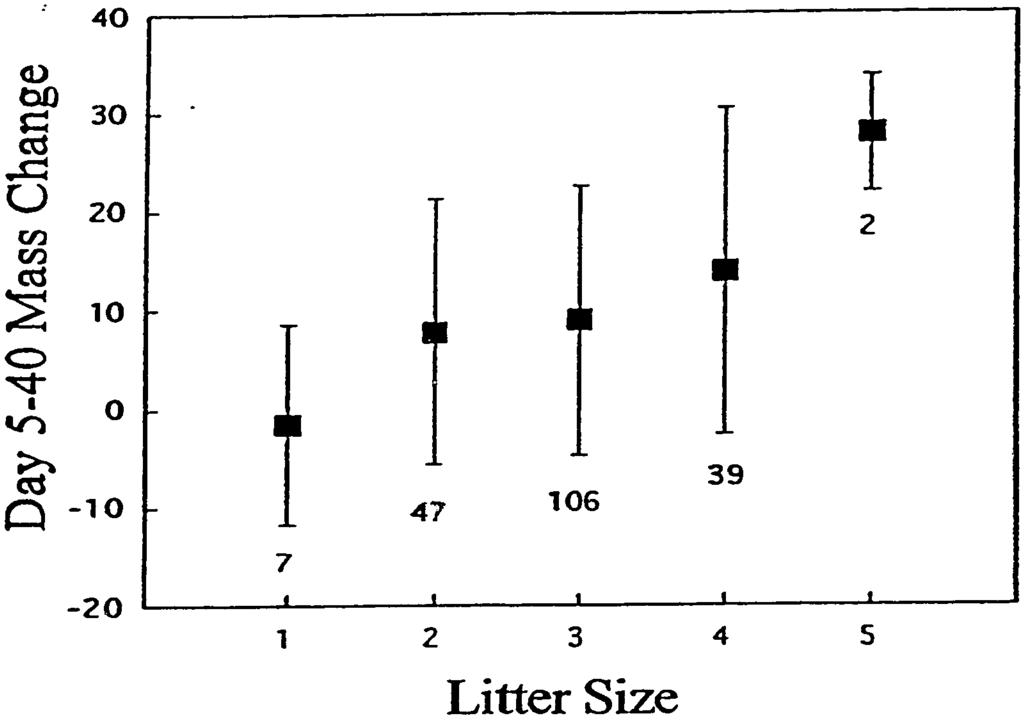

Figure 3-4. The relationship between early lactation mass gain and litter size among control females between 1990 and 1994.

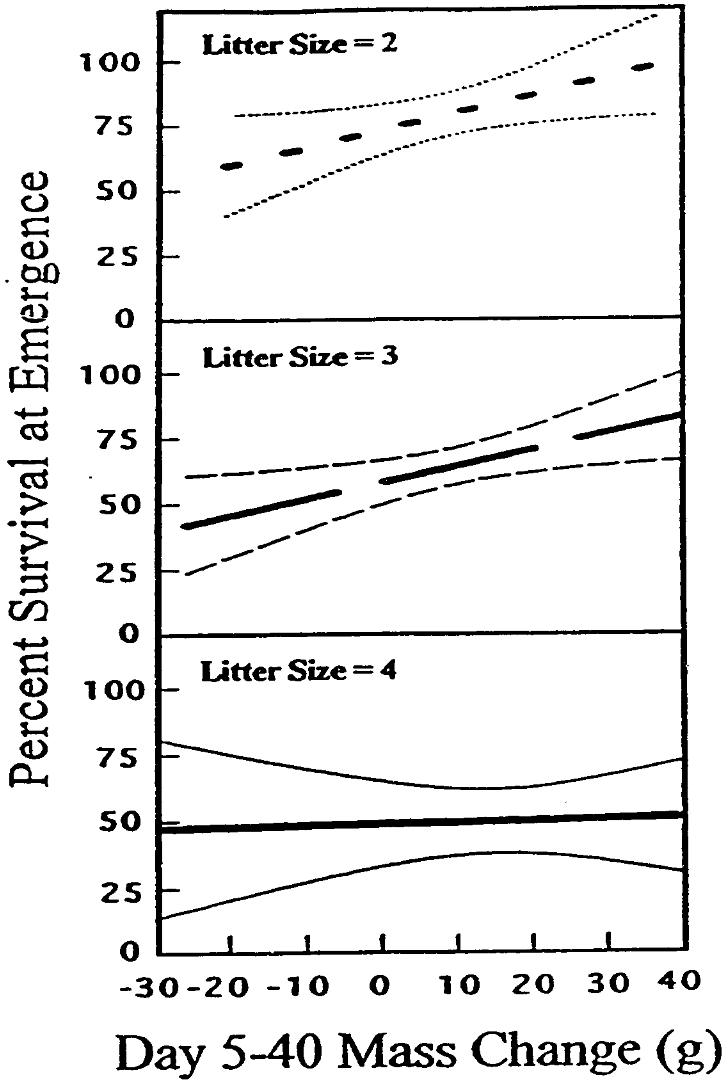

Figure 3-5. The relationship between early lactation mass gain, litter size, and juvenile survival at emergence. Regression line and 95% confidence intervals are indicated for each litter size. Analysis of covariance indicates a significant effect of litter size on juvenile survival ($F_{3,211}$=6.2, p=0.0005) and a significant effect of female mass gain on juvenile survival ($F_{1,213}$=7.2, p=0.008). The effect of mass gain on survival did not differ significantly between litter sizes ($F_{2,212}$=1.2, p=0.31).

mass did reflect changes in fat stores. Furthermore, Becker (1992) found that increases in fat and protein stores accounted for more than 75% of spring mass gains by adult female red squirrels, and in general, that body mass was an excellent predictor of body fat in adult squirrels (Becker 1992).

Energy storage during early lactation

The pattern of mass change in females was indicative of energy storage from parturition to emergence of young, followed by mobilization of stores between emergence and weaning and, to a lesser extent, between weaning and late summer. The same pattern of lactational mass gain followed by mass loss has been reported for several rodent species (Millar 1978; Konig, Riester & Markl 1988; Kenagy, Stevenson & Masman 1989). If more than 75% of the mass gains of lactating red squirrels were due to changes in fat (Becker 1992), energy stores were supplemented on average by at least 6 g in control females (i.e. 75% of 10g mass increase) and 19 g in augment females, or 229 kJ and 724 kJ respectively (Robbins 1993). Although average gains in controls likely accounted for less than one day of energy requirements during the peak of lactation (field metabolic rate of red squirrels during late lactation is 355 ± 40 kJ, Humphries unpublished data), the significant regression between female mass gain and juvenile survival indicates that the variance in gains made by unmanipulated females is nonetheless ecologically significant.

Early lactation mass gain may be a critical process for sustaining the extreme energy demands associated with late lactation. Because energy requirements of lactation increase dramatically from parturition to near weaning (eg. Kenagy et al. 1989), early lactation energy storage and late lactation energy mobilization reduces the maximum daily requirements that a female must sustain, or conversely, increases the requirements that a female *can* sustain, during late lactation. We expect energy deposition during early lactation, as opposed to pre-reproductive or gestational deposition, to be especially important among mammals in highly seasonal environments where 1) the offspring survival benefits associated with early emergence may select for initiation of reproduction before sizable energy stores can be accumulated (Slade, Sauer & Glass 1984, Hickling, Millar & Moses 1991) and 2) energy storage capabilities prior to and during pregnancy may be limited by food scarcity and/or thermoregulatory constraints (Bronson 1989). For red squirrels, thermoregulatory constraints and selection for early juvenile emergence are likely more important than food scarcity in the selection for this pattern of energy storage. Red squirrels maintain a hoard of high energy food throughout the year and females do not appear to be limited by short-term food availability during pregnancy or lactation (Becker 1994, Boutin unpublished data). However, because juvenile red squirrels must acquire a territory and establish an overwinter food hoard between spring emergence and fall (Larsen & Boutin 1994), early emergence likely conveys survival benefits. Additionally, red squirrel thermoregulatory costs and activity are highly temperature dependent throughout the range of ambient temperatures that occur at Kluane during late winter and spring (Irving, Krog & Monson 1955, Pauls 1978), and thus, energy storage capabilities prior to lactation are likely limited by thermoregulatory constraints.

Adjustment of energy stores according to reproductive demands

The adjustment of reproductive timing and offspring number according to current levels of energy stores is well documented in a variety of organisms (Drent & Daan 1980, Bronson 1989). However, our data suggest that causality in this relationship can be reversed; energy stores can be adjusted according to reproductive demands. When stored energy is required to support the demands of later reproductive stages, the ability to adjust levels of stored energy according to "predicted" future requirements may be important. If this adjustment occurs, a paradoxical situation is expected; individuals with the highest reproductive demands will accumulate the greatest amounts of stored energy. Mammalian lactation provides an excellent example where adjustment of this form could operate. Energy demands increase from relatively low levels early in lactation to high levels as weaning is approached, stored energy is commonly used to sustain requirements during late lactation, and demands during early lactation should, in most cases, predict demands during late lactation.

As a result of our litter manipulations, augment females were immediately imposed with a 63 ± 17 % increase of litter mass, and continued to support an additional 33 ± 7 % of daily litter growth until after emergence. The pattern of greater mass gain during early lactation and mass loss during late lactation among augment females (relative to controls), suggests that female red squirrels mobilize available energy stores to support the demands of late lactation and they adjust the level of these stores during early lactation according to demands at that time. The early lactation mass gain of females with natural litter sizes between 1990 and 1994 was consistent with this pattern. Although variance was high, there was a significant, positive relationship between natural litter size and female mass gain.

Three additional studies demonstrate that the adjustment of energy storage according to future reproductive demands may occur in other mammals. Millar (1978), in interpreting mass changes of lactating female *Peromyscus leucopus* Fischer in the laboratory, mentioned two significant trends; females lost mass toward the end of lactation and heavier females tended to support larger litters. A third trend not discussed, but apparent in Table 2 (pp. 1056), concerns mass changes of females with different litter sizes. Average female mass gains from day 1 to day 13 of lactation (weaning occurred at day 22) with natural litter sizes of 2 through 7 were 0.2, 1.2, 1.7, 2.9, 3.5, and 5.1 g respectively, suggesting that females with the highest reproductive demands accumulated the greatest amounts of stored energy. Similarly, captive northern grasshopper mice (*Onychomys leucogaster*) supporting natural litter sizes of 1 through 6 gained, on average, 1.1, 0.4, 3.9, 5.4, 5.5, and 9.2 g respectively, between day 0 and day 16 of lactation (Table 1; Sikes 1995). In a third laboratory study, Kam and Degen (1993) found the average energy retention during the first half of lactation (metabolizable energy intake - [estimated maintenance requirements + energy transfer to young]) was -75, +8, and +63 kJ for female fat sand rats (*Psammomys obesus*) with small, medium, and large litters respectively (Kam & Degen 1993).

However, these results must be interpreted with caution. The positive relationship between mass gain and litter size of *Peromyscus leucopus* and *Onychomys leucogaster* may result from differences in gut contents and not necessarily energy stores, as there was a significant, positive relationship between litter size and food intake in both studies (Millar 1978; Sikes 1995). Additionally, positive relationships between natural litter size and early lactation mass gain may be spurious if extraneous variables such as food supply, breeding experience, or female quality affect both litter size and mass gain. Because of the potential influence of the confounding factors, correlations between litter size and mass gain, and for that matter, correlations between most other life-history traits, must be interpreted with caution (Clutton-Brock 1991). Nevertheless, in our study, the response of augment females to litter-size manipulations indicates, that at least in red squirrels, the relationship between reproductive demands and early lactation mass gain is causal.

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Chapter Four. Life-history trade-offs in red squirrel reproduction

To maximize lifetime reproductive success, individuals must maximize both the number and quality of offspring produced in their lifetime. If resources necessary for production of offspring are limited, increases in one reproductive trait, such as offspring number, will result in declines in associated traits, such as offspring quality (Williams 1966). Past research has demonstrated that individuals can sustain experimental increases in reproductive traits such as offspring number (see reviews by Reznick 1985; Andérs & Møller 1989; Lessells 1991), indicating that in many situations traits are expressed at levels well below those imposed by limits. It is generally assumed that such levels of expression can only be optimal, in terms of maximizing reproductive success, if trade-offs occur between reproductive traits (Stearns 1989). However, alternative explanations, including models of optimizing reproductive success in fluctuating environments (Boyce & Perrins 1987), can explain the expression of conservative life-history traits without the existence of trade-offs (see Morris 1992, Risch, Dobson, & Murie 1995).

Two life-history trade-offs that may affect optimal offspring number are the proposed negative relationships between current and future reproduction, and between offspring number and offspring fitness (Stearns 1989, 1992). The presumed basis of these trade-offs is limitations on the acquisition and allocation of resources, especially energy (Drent & Daan 1980; Gittleman & Thompson 1988). Thus the trade-off between current and future reproduction (ie. the cost of reproduction; Williams 1966; Reznick 1985), should result in selection for a level of resource allocation during each reproductive attempt that will maximize the total resource allocated to offspring during the lifetime of the individual. It follows that these two trade-offs (the cost of reproduction and offspring number vs. fitness) are hierarchical in nature. The cost of reproduction, or in its absence, energetic limitation, determines the amount of resources parents allocate to their current offspring. The amount of resources allocated to each offspring then becomes a function of offspring number which can be increased only at the expense of offspring fitness.

Results from laboratory studies on invertebrates, and field studies on invertebrates and birds indicate that both trade-offs are prevalent in a diverse group of species (Lessells 1991; Godfray, Partridge & Harvey 1991). Although many methods have been used to test for the presence of life-history trade-offs, genetic correlations, selection experiments, and experimental manipulations of life-history traits are now generally accepted as the most valid and useful techniques for investigating trade-offs (Reznick 1985; Stearns 1989, Lessells 1991; but see Reznick 1992). Much of the insight we have into the role of trade-offs in free-ranging, vertebrate populations has come from brood manipulation experiments in birds. About two-thirds of studies of this nature have demonstrated a cost of reproduction trade-off among females (9 of 14 studies reviewed by Lindén & Møller 1989; trade-offs E-I, L, N, P, R in Lindén & Møller's table 1) and a similar proportion have demonstrated a number vs. fitness trade-off among offspring (6 of 10 studies reviewed by Lindén & Møller 1989; Table 1, trade-offs C, K, T, U in Lindén & Møller's table 1).

Less is known about the role of life-history trade-offs in mammalian reproduction. In general, correlative studies have shown that litter size, or other indices of reproductive effort, do not affect maternal or juvenile survival (Murie & Dobson 1987; Michener & Locklear 1990; Millar, Derrickson, & Sharpe 1992; Waser & Jones 1991; but see Clutton-Brock, Guiness, & Albon 1983). In the only experimental study of the cost of reproduction in free-ranging mammals, Hare & Murie (1992) compared the survival and fecundity of four female Columbian ground squirrels (*Spermophilus columbianus*) with augmented litter sizes, six females with unmanipulated litter sizes and fourteen females with reduced litter sizes, and found no evidence for a cost of reproduction. No study to date has experimentally tested for an offspring number vs offspring fitness trade-off in mammals. Experimental increases in litter sizes of greater white-toothed shrews (*Crocidura russula*) reduced offspring size at weaning (Genoud & Perrin 1994), but the subsequent effect on future survival or fecundity of the juveniles was not investigated.

The purpose of this study was to test for the cost of reproduction and offspring number versus fitness trade-offs in a population of free-ranging red squirrels (*Tamiasciurus hudsonicus*). I experimentally increased or reduced the litter sizes of female red squirrels by two offspring, and compared the future survival and fecundity of these females during next 18 months with females whose litters were cross-fostered but not augmented or reduced. To test for the offspring number versus fitness trade-off, the survival of juveniles belonging to litters of the three treatments were compared over the same time period.

Analysis of the reproductive effort of 1994 manipulated females in chapter II and III leads to several predictions pertaining to life-history trade-offs. Because augment females had significantly higher reproductive output at emergence than control females, a cost of reproduction, a trade-off between offspring number and fitness following emergence, or both are predicted to occur; otherwise females should be producing litter sizes equivalent to augment litters. Because augment females sustained their elevated demands without declines in body condition (chapter III) or significant behavioural changes (chapter II), a cost of reproduction is not expected to occur. Finally, because there was a significant treatment effect on juvenile size at emergence, a trade-off between offspring number and fitness is predicted to occur.

METHODS

Red squirrels were studied on a 130 ha site near Kluane Lake, Yukon, Canada (61°N, 138°W), during spring and summer (March-August) in 1994 and 1995. The study site is dominated by white spruce (*Picea glauca*) with a sparse understory of willow (*Salix sp.*). Litter size manipulations were conducted in spring 1994, and the survival and fecundity of females, as well as the survival of their 1994 offspring, was followed until August 1995. As a result of a large spruce cone crop in summer 1993 and a cone crop failure in summer 1994, the food supply of this population was relatively high in spring 1994, but declined to very low levels by spring 1995 (see chapter II).

Litter-size manipulation

Augmented litters were created by adding two offspring taken from donor litters. Two offspring were cross-fostered between control females to mimic the fostering associated with the other two treatments. During manipulations, offspring were weighed, sexed and those transferred between nests were marked with small, temporary ear tags. Nests were re-visited ~15 days later to determine the success of the manipulation, and to weigh and permanently ear-tag the juveniles.

Cross-fostering and litter additions between 7 April and 9 May, 1994 resulted in eight augment, eight donor, and eight control females. All females had original litter sizes of three except for three donor females and two control females who all had four. There were no significant differences in age of females assigned to the three treatments. Transfers of juveniles were conducted when they were 5-13 days old (mean=8.6); the age difference between foster-siblings was 0-4 days (mean=1.3).

When nests were revisited ~15 days later (for tagging), all litter members (original and fostered juveniles) were still alive. Mass increases of fostered and non-fostered juveniles within control and augment nests did not differ (paired t-test of average growth rates; t=-0.35, p=0.73, df=15). For all but one litter, all juveniles (i.e. five from augment nests, three or four from control nests, one or two from donor nests) were observed and/or trapped following emergence. The single exception was one augment litter from which only two juveniles were observed following emergence. This female and her juveniles were excluded from analyses.

Survival and fecundity of females and their offspring

Survival of study individuals was documented through spring and autumn population censuses, as well as continuous monitoring of territory ownership within the study site from April to September in each year. All individuals present on the study site were marked with small numbered ear-tags, and a unique combination of colored wires or disks threaded through ear-tags. Survival of individuals was documented by live-trapping or visual observation. Due to the very low dispersal distances, readily identifiable territory locations, and high observability and trappability of red squirrels, it is possible to census all squirrels present in an area (Price *et al.* 1986; Boutin & Larsen 1993; Larsen & Boutin 1994).

The reproductive activity of females was monitored by observations of mass changes and nipple condition during repeated captures beginning in late March of each year. Shortly before or after parturition, females were fitted with radio collars to aid in locating their nests. Once nests were located the young were counted, sexed and weighed. Parturition date was estimated from the trapping records of the female and the size of the young when the nest was visited (after Boutin & Larsen 1993). The number and mass (nearest gram with a 100g spring scale) of juveniles in the nest were determined twice; shortly after and approximately 25 days following parturition. During the second nest visit, juveniles were given numbered eartags to allow identification following emergence

Data Analyses

Because this experiment was designed to test the consequences of either an increase or decrease in offspring number, augment and donor treatments were compared separately to controls, and not to each other. Treatment effects on female survival were analysed using contingency analysis. To determine if reproductive effort affected future reproduction, I compared the parturition dates, and the number of emerged juveniles of females in 1995 assigned to the three treatments in 1994. The proportion of juveniles surviving until 50 (emergence), 75 (weaning), and 365 days (recruitment) of age was calculated for each litter, and treatment effects on juvenile survival were tested by comparing the 23 resulting proportions.

RESULTS

Female survival and future reproduction

The litter manipulation treatments did not significantly affect female survival (Fig. 4-1). Survival during the first four months following the manipulations was higher among

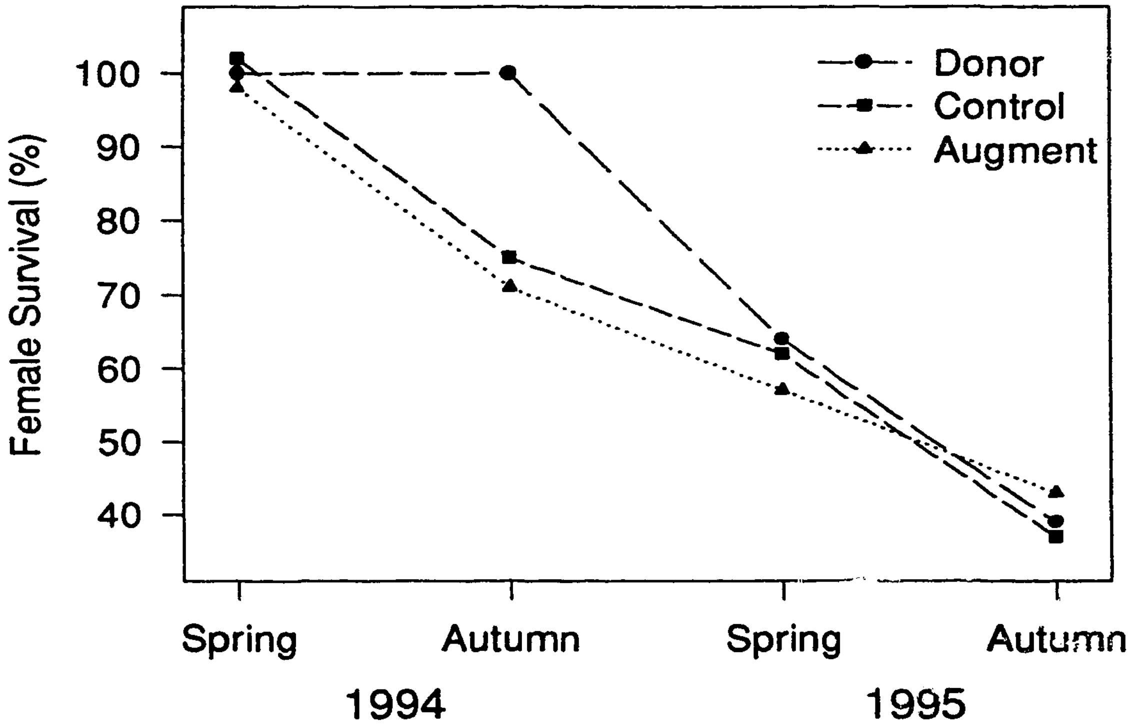

Figure 4-1. Survival of study females from spring 1994 to autumn 1995. Sample sizes are eight donor, eight control, and seven augment females.

donor females than among control and augment females. Small sample sizes makes statistical analysis of the difference between control and donor survival relatively unreliable; parametric G-test analysis indicated a significant difference (G=3.1, df=1, 1-tailed p=0.04) but non-parametric analysis using Fisher's exact test indicated a non-significant difference (1-tailed p=0.23). The validity of applying either a G-test or Fisher's exact test to these data is questionable because of low sample sizes, and non-fixation of row and column totals, respectively (Sokal & Rohlf 1981). Therefore, I will side with the more conservative of the two outcomes, and assume the difference is not significant. Survival among the three treatments was similar in all other periods. Eighteen months after the manipulations, about 40 % of females from each treatment (range 38 to 43 %) were still alive

There was also no treatment effect on future reproductive output of females. Average parturition dates (julian date) of donor, control, and augment females were very similar: 155 ± 4 (n=4), 153 ± 3 (n=4), and 153 ± 5 (n=5), respectively. Because litter reductions and subsequent second litters were common in 1995 (see chapter II), first litter offspring number at birth could not be determined for all females, and was a relatively poor indicator of their reproductive output in 1995. Therefore, I 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 in the opposite direction than that predicted by the cost of reproduction hypothesis. Surviving augment females from 1994 (n=4) successfully raised 1.5 ± 0.7 offspring to emergence in 1995, compared to 0.2 ± 0.2 raised by control females (n=5), and 1.0 ± 1.0 raised by donor females (n=3). However, the differences between augments and controls (Kruskal-Wallis test, χ=3.1, df=1, p=0.08), and controls and donors (χ=0.34, df=1, p=0.56) were not significant.

Juvenile survival

The survival of augment juveniles was consistently lower than that of control and donor juveniles until after their first winter when high mortality rates made meaningful comparisons of survival trends difficult (Fig. 4-2). The survival of augment juveniles to 50 days of age (37 ± 12 %) was significantly lower than the survival of control juveniles (81 ± 6 %; t=3.5, df=13, 1-tailed p=0.002), and the survival of donor juveniles (63 ± 16%) was lower, but not significantly different, than that of control juveniles (t=1.1, df=14, 1-tailed p=0.14). By 75 days of age, the survival of augment juveniles remained significantly lower than control juveniles (11 ± 4 % vs. 58 ± 15%; t for unequal variances=3.0, df=8, 1-tailed p=0.008), but the survival of controls and donors was similar (58 ± 15 % vs. 56 ± 18 %). Over-winter survival of juveniles was very low; only 3 of 73 juveniles survived until spring 1995. Two of these juveniles were from donor litters, and one was from an augment litter.

From the perspective of the female, both augment and donor treatments reduced reproductive output. By 75 days after parturition, control females averaged almost twice as many surviving juveniles as donor females (t=1.6, df=14, 1-tailed p=0.06), and over twice as many as augment females (t=2.3, df=13, 1-tailed p=0.02; Fig. 4-3).

DISCUSSION

The absence of significant treatment effects on female survival or future fecundity and the presence of significant effects on juvenile survival, suggests that, in red squirrels, a trade-off between offspring number and fitness is more important than the cost of reproduction in determination of optimal offspring number. However, small sample sizes in this experiment prohibited the detection of more subtle survival or fecundity effects. The absence of a cost of reproduction in red squirrels is consistent with findings for other small mammals, including experimental (Hare & Murie 1992) and correlative studies (Michener & Locklear 1990; Waser & Jones 1991; Millar, Derrickson, & Sharpe 1992). The only mammals known to experience a trade-off between offspring number and future survival and fecundity are large ungulates (*Cervus elaphus*, Clutton-Brock et

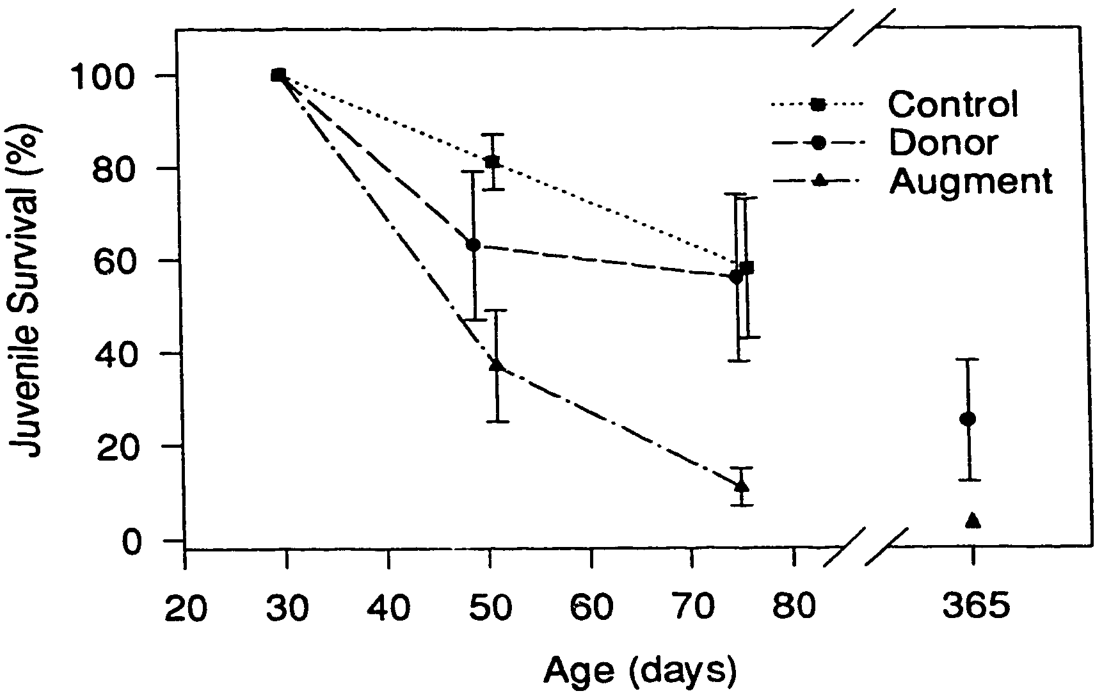

Figure 4-2. Survival of juveniles at 50, 75, and 365 days of age. Indicated percentages are the averages of the percentage of juveniles surviving in each litter (n=8 control and donor litters, n=7 augment litters). Bars represent ± 1 standard error. Seventy-two juveniles (35 augment, 25 control, and 12 donor) were alive at 35 days of age. Only two donor juveniles, and one control juvenile survived to 300 days of age.

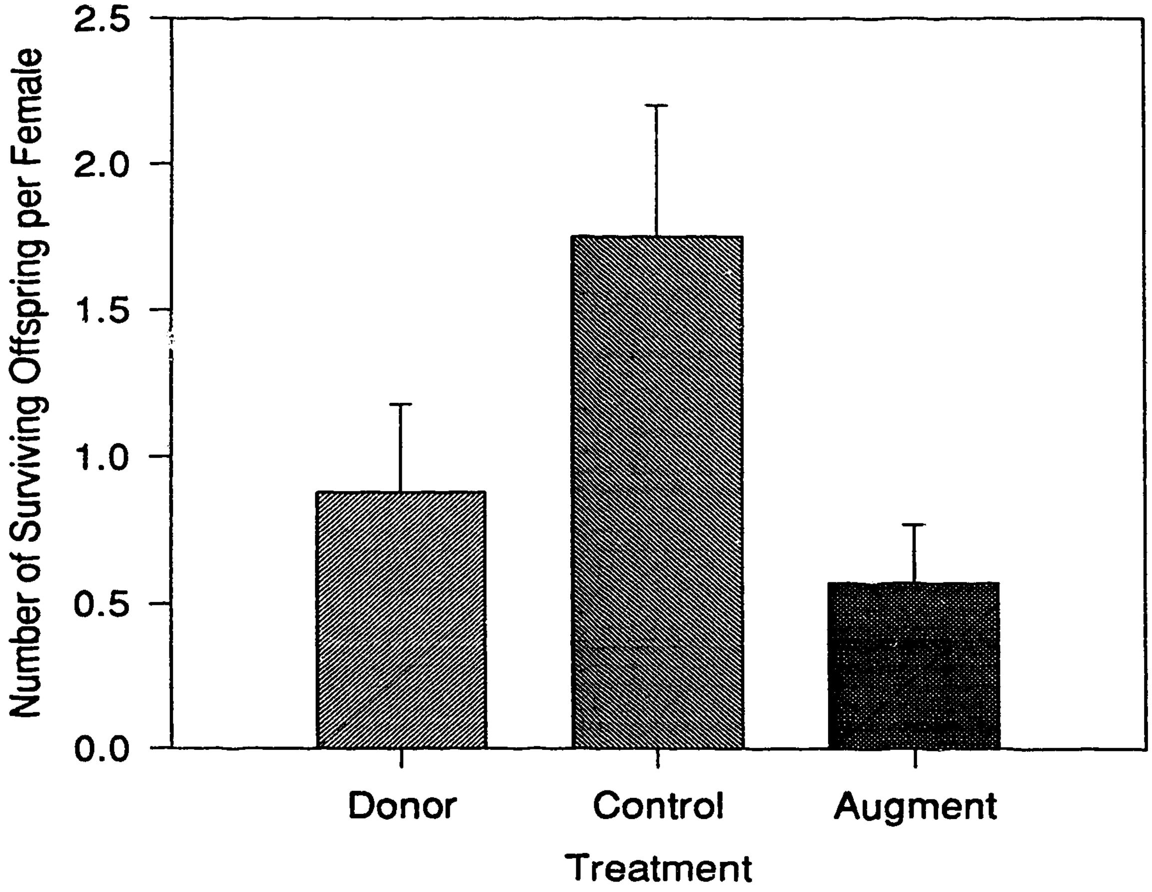

Figure 4-3. Average number of juveniles surviving to 70 days of age. Treatment averages are based on eight control, eight donor, and seven augment females. Bars represent ± 1 SE.

al. 1983; *Ovis canadensis*, Festa-Bianchet 1989). Tuomi, Hakala, & Haukioja (1983) proposed that breeders who use body energy stores to support reproductive demands, rather than increases in energy intake, will be more likely to experience a cost of reproduction. Because large mammals rely on stored energy during reproduction to a much greater extent than small mammals (Gittleman & Thompson 1988; Robbins 1993), indications that the cost of reproduction occurs only in large mammals is consistent with Tuomi et al.'s (1983) hypothesis. However, the support for the presence or absence of the cost of reproduction in mammals remains weak because experimental studies with adequate sample sizes have yet to be conducted.

The lower reproductive output of donors and augments relative to controls, in terms of number of offspring surviving to 75 days of age, suggests that the natural litter size of female red squirrels leads to the greatest number of surviving offspring in each year. Thus, adopting terminology common in literature pertaining to optimal offspring number in birds, female red squirrels could be said to be producing a "Lack litter" (Lack 1947; Drent & Daan 1980; Stearns 1992). This finding implies that deleterious effects of offspring number on offspring survival, eliminate the potential benefits of increased litter sizes.

Many factors, or interactions between factors, could be responsible for the reduced survival of augment juveniles relative to controls. The survival of augment juveniles was already lower than controls by the time litters were weaned (weaning occurred at 63 ± 5 days of age in 1994), and therefore, direct mortality due to inadequate provisioning by augment females could have played a role. Considering that 1) augment juveniles sustained reduced provisioning rates from manipulation to emergence (as indicated by lower growth rates) without experiencing higher mortality rates, 2) emergence and weaning date did not differ among treatments (see Chapter II) and 3) post-emergent and even late pre-emergent juveniles can survive without any nutritional support from their mothers (Boutin unpublished data), this seems an unlikely explanation. However, this does not eliminate the possibility of an indirect provisionment effect. In response to reduced energy acquisition from their mother, augment juveniles may have spent more time out of the nest or dispersed sooner than control juveniles, and therefore experienced higher predation rates. Lower augment survival could have also been due to their smaller body size, which may have 1) reduced their ability to acquire or defend territories from other squirrels, 2) made maintenance of a balanced or surplus energy budget more difficult because of higher thermoregulatory costs, or 3) reduced their ability to sustain periods of low energy intake or high energy expenditure (Robbins 1993).

Finally, the reduced survival of augment juveniles could have been due to increased sibling competition for territory acquisition. Juvenile red squirrels that are successful in acquiring a territory almost always do so on or adjacent to their natal midden (Larsen & Boutin 1994; Price & Boutin 1993), and acquiring a territory is considered necessary for survival (Larsen & Boutin 1994). Therefore, the increased number of emerging offspring likely decreased the probability of individual augment juveniles acquiring a territory. But this effect should have resulted in an equal number of offspring surviving from augment and control litters, rather than fewer surviving augment juveniles, as was observed. The observed difference could be due to competition alone only if elevated competition among augment juveniles resulted in additional mortality risks (eg. predation, declines in body condition) other than the failure to obtain a territory.

Thus, the most parsimonious and plausible explanation for the observed offspring number vs. fitness trade-off, is that the survival of augment juveniles was reduced, relative to controls, due to the energetic or competitive disadvantages associated with their smaller body size. However, this conclusion is obviously speculative. Studies incorporating more replication, and designs capable of differentiating between the various potential causes of the offspring number vs. fitness trade-off, will lead to a better understanding of the role of life-history trade-offs in red squirrel reproduction.

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Chapter Five. Conclusion

In this study, I investigated life-history trade-offs and reproductive energetics of female red squirrels in an attempt to gain insight into the factors that determine optimal offspring number. The study was designed to provide a valid test for life-history trade-offs, but also to gain insight into aspects of general reproductive biology outside of the trade-off paradigm. Red squirrels proved to be very amenable subjects for a study of this design. Due to the accessibility of pre-emergent juveniles, high trapability of adults, and relative ease with which individual survival and female fecundity can be assessed, red squirrels are excellent candidates for further research to evaluate theory relating to mammalian life histories. Nevertheless, red squirrels impose rather restrictive sample size limitations, as do most free-living vertebrates other than birds that occupy nest boxes. The inability of researchers to achieve adequate replication may present the greatest obstruction to the study of the life history strategies of free-ranging mammals.

The response of female red squirrels to manipulations of offspring number demonstrates the considerable ability of breeders to accommodate changes in reproductive demands through adjustments of rates of energy intake, storage, and mobilization. Based on my preliminary trade-off data, these adjustments may allow breeders to avoid the potential survival costs associated with increased offspring number, but may be insufficient to avoid survival effects on their offspring. Thus it appears that females regulate their reproductive effort at the expense of a reduction in the value of their current offspring.

Red squirrel ecology provides some clues as to why females might pursue this strategy. Red squirrels are relatively long-lived (up to 6 years of age), females, on average, have few offspring that survive to reproduce (Larsen & Boutin *in prep.*), abundance of their main food source is characterized by periodic, severe fluctuations, and juvenile over-winter survival is strongly affected by the fluctuations in food availability (Boutin unpublished data). As a result, in the reproductive life span of a female, juvenile survival is likely to fluctuate from relatively low to relatively high levels, independent of maternal care effects. Some threshold level of maternal care will no doubt affect offspring survival, but beyond that threshold, offspring survival may be controlled by variables outside the control of the mother. Selection in environments with these characteristics my result in strong selection for female survival and rather loose selection for offspring care above threshold levels.

As with most studies, more questions exist at the completion of this research then at its inception. Many of the basic life-history questions remain. Why is offspring survival reduced by increases in offspring number? Does the cost of reproduction exert any effect on optimal offspring number in red squirrels? Have females been selected to optimize reproductive success per breeding season or per lifetime? The rather striking regulation ability of females, generates questions about optimizing reproductive success in fluctuating environments. Have cone crop failures affected reproductive strategies of female red squirrels? If cohort effects are important components of reproductive success, do females employ conservative strategies throughout their lifetime, or can they adjust their reproductive strategies to take maximum advantage of positive cohort effects? Although these questions are relatively simple, achieving answers likely will not be. As stated previously, identification of the selection forces that influence offspring number is complex.