Ecology, 91(10), 2010, pp. 2983–2994

The ghosts of predators past: population cycles and the role of maternal programming under fluctuating predation risk

Michael J. Sheriff,[1,2,3] Charles J. Krebs,[2] and Rudy Boonstra[1]

[1]*Centre for the Neurobiology of Stress, University of Toronto Scarborough, 1265 Military Trail, Toronto, Ontario M1C 1A4 Canada*
[2]*Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia V6T 1Z4 Canada*

Abstract. Maternal effects may be a major factor influencing the demography of populations. In mammals, the transmission of stress hormones between mother and offspring may play an important role in these effects. Laboratory studies have shown that stressors during pregnancy and lactation result in lifelong programming of the offspring phenotype. However, the relevance of these studies to free-living mammals is unclear. The 10-year snowshoe hare (*Lepus americanus*) cycle is intimately linked to fluctuating predation pressure and predation risk. The enigma of these cycles is the lack of population growth following the decline phase, when the predators have virtually all disappeared and the food supply is ample. We have shown that a predator-induced increase in maternal stress hormone levels resulted in a decline in reproduction. Here we examine population and hormone changes over a four-year period from the increase (2005) to the decline (2008). We report (1) that an index of maternal stress (fecal corticosteroid metabolite [FCM] concentrations) fluctuates in synchrony with predator density during the breeding season; (2) that maternal FCM levels are echoed in their offspring, and this occurs at a population-wide level; and (3) that higher maternal FCM levels at birth are correlated with an increased responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis in their progeny. Our results show an intergenerational inheritance of stress hormones in a free-ranging population of mammals. We propose that the lack of recovery of reproductive rates during the early low phase of the hare cycle may be the result of the impacts of intergenerational, maternally inherited stress hormones caused by high predation risk during the decline phase.

Key words: *free-ranging mammal;* Lepus americanus; *low phase;* Lynx canadensis; *maternal effects; maternal programming; nongenetic phenotypic effects; predation risk; snowshoe hare; stress; ten-year cycle; Yukon, Canada.*

Introduction

Adaptations to the direct (e.g., a severe storm) and indirect (e.g., risk of predation) effects of environmental stressors are essential for ensuring individual fitness in natural populations. The environment that an individual's mother experiences may also affect the offspring's fitness. Maternal effects occur when the maternal phenotype affects the phenotype of her offspring in addition to her direct genetic contribution (Räsänen and Kruuk 2007). These nongenetic phenotypic effects can have a profound influence on offspring and can cause a resemblance not just between a mother and her offspring, but also between grandmothers and grandoffspring (Kirkpatrick and Lande 1989). In the biomedical literature, these nongenetic maternal effects are referred to as maternal or developmental programming. A programming effect reflects the influence of a specific environmental factor during the developmental period, before or just after birth, on the organization of target tissues and/or patterns of gene expression that affect function throughout life (Meaney et al. 2007). Mechanisms responsible for maternal programming may vary among organisms, the nature of the stressor (e.g., malnutrition, trauma, disease, psychological stress, and so forth), and the timing and duration of occurrence (prenatal vs. postnatal; acute vs. chronic). In mammals, stress hormones may play a key organizational role in bringing about these lifelong changes (Seckl 2008, Lupien et al. 2009).

In mammals, numerous laboratory studies have shown that stressors acting during pregnancy and lactation can have long-lasting effects on offspring (Meaney et al. 2007, Weinstock 2008, Mastorci et al. 2009). The mechanisms underlying this relationship involve an increase in glucocorticoids (GC) in either the fetus or neonate. Glucocorticoids (corticosterone in mice and rats; cortisol in most other mammals) are produced by the hypothalamic-pituitary-adrenal (HPA) axis in response to a stressor (defined as any environ-

Manuscript received 19 June 2009; revised 28 January 2010; accepted 1 February 2010. Corresponding Editor: F. S. Dobson.

[3] E-mail: michael.sheriff@utoronto.ca

mental disturbance that disrupts homeostasis; Boonstra 2005, Owen et al. 2005). In laboratory animals, an increased level of maternal stress hormones or exposure to stressors has been linked to offspring depression, anxiety-like behaviors, and alterations in HPA function and brain development (Abe et al. 2007, Meaney et al. 2007, Kapoor et al. 2008).

Extrapolating from laboratory studies to natural populations is problematic in that most of the former have subjected pregnant females to highly artificial stressors (e.g., immobilization, immersion in cold water, bright lights; Henry et al. 1994, Kapoor and Matthews 2005, Meaney et al. 2007), whereas in the latter, reproductive females have a long evolutionary history of adaptation to natural stressors (predation, social interactions, disease, or severe weather). Furthermore, in the laboratory, the timing when mothers are exposed to stressors markedly affects their subsequent impact on the offspring (Kapoor and Matthews 2005). In contrast, although some stressors in nature may be very short-lived (e.g., a severe snowfall), others are chronic for the length of the pregnancy and lactation (e.g., high predation risk or high population density). These chronic stressors are not static and can fluctuate (i.e., although prey may be exposed to a high risk of predation, the number of predators and prey will change). As well, in nature, animals are exposed to multiple stressors simultaneously and may have a variety of coping mechanisms that allow them to integrate all of these (Wingfield and Sapolsky 2003). For example, in the arctic, free-ranging male White-crowned Sparrows abandoned their normal nesting behavior in response to a spring snowstorm, but resumed nesting after it had passed. The change in behavior was associated with plasma corticosterone concentrations going from high to low, respectively, over that interval (Wingfield et al. 1983).

Here we examine a free-ranging population of snowshoe hares, *Lepus americanus* (see Plate 1), from the Yukon, Canada, to elucidate the effects of natural stressors during pregnancy on the physiological development of offspring. Snowshoe hares undergo a regular cyclic fluctuation, with 8–10 years between peak densities (Keith 1963, Krebs et al. 1986). A major factor influencing the hare cycle is predation. As hare populations increase, so do those of their predators, but with a lag of 1–2 years. During the population decline, predators are the direct cause of up to 100% of hare deaths (Hodges et al. 2001); of these, lynx and coyote are responsible for 65–75% of predator-caused deaths, with raptors being responsible for the rest. Furthermore, snowshoe hares are highly sensitive to changes in the risk of predation. Boonstra et al. (1998) showed that plasma cortisol concentrations (the major GC in snowshoe hares) fluctuated in the late winter with the risk of predation, such that hares experiencing a greater risk of predation had higher plasma cortisol. However, they did not link these GC changes directly to the time when hares were breeding. In terms of maternal programming, such predation-induced stress must occur during the summer months when reproduction occurs. In addition, Boonstra et al. (1998) did not link the maternal state with that in their offspring.

In this study we tested whether chronically elevated GC concentrations in dams increased the concentration of the stress hormones of their offspring. We also measured the ability of offspring to mobilize energy and their body condition in response to an increase in maternal GC concentrations. First, in a natural monitoring study in the Yukon we measured fecal cortisol metabolite (FCM) concentrations during the breeding season in dams (within one week after parturition) and in juveniles at weaning (postnatal day [pnd] 28) and after weaning (pnd 60, 90, and 120) to investigate the long-term programming of prenatal GC exposure. Because hares have up to four synchronous litter groups during the breeding season, we assessed whether litter groups differed both within a year and among years to examine the effects of seasonal changes in vegetation growth (Seccombe-Hett and Turkington 2008) and the effects of among-year differences in predation risk (O'Donoghue and Boutin 1995). This study took place from 2005 (increase) to 2008 (decline). Second, in a captive study we measured FCM concentrations in dams at birth and measured their offspring's response to a hormonal challenge at weaning. In the juveniles we obtained measures of the stress response (plasma free-cortisol and maximum corticosteroid-binding capacity, MCBC [a measure of corticosteroid-binding globulin capacity]), of energy mobilization (glucose and free fatty acids), of condition (packed red blood cell volume), and of immunity (white blood cell counts). This study took place in 2006 (peak) and 2007 (decline).

Methods

Natural monitoring study

Population densities of both the hares and lynx were measured as part of a monitoring study (Krebs et al. 2001*b*); densities from 2002 to 2008 are shown here. Snowshoe hare densities were estimated with the program CAPTURE (Otis et al. 1978) and the Jolly-Seber full model from trapping records on two 36-ha grids (Krebs et al. 1986). Lynx tracks were counted along a 25-km transect that traversed our study area, on days after fresh snowfalls while tracks were distinguishable. Lynx densities were estimated from the regression $y = 0.355 + 0.288x$, where x equals track counts (Hone et al. 2007). An index of snowshoe hare overwinter survival was estimated as the proportion of hares present in spring compared to those present in autumn (i.e., density in spring/density in the previous autumn).

Physiological stress measurements occurred over four summers from 2005 to 2008. Snowshoe hares were live-trapped in the Shakwak Trench east of Kluane Lake, Yukon Territory (61° N, 138° W) using Tomahawk live-traps (Tomahawk Live Trap, Tomahawk, Wisconsin,

USA). The traps were set at 22:00 hours and checked at 06:00 hours; thus hares could only be in the traps for a maximum of 8 h. This is relevant, as the lag between the production of cortisol in the body and its appearance in the feces as metabolites is between 8 and 12 h (Sheriff et al. 2009*a*). Therefore, the cortisol metabolites in the feces represent a measure of stress hormones without any stress induced by observer presence or trapping.

We monitored FCM concentration in females one week after the mean parturition date during the first ($n = 32$), second ($n = 32$), and third ($n = 20$) litter of the breeding season (May–August). FCM concentrations were monitored in juvenile hares from the first ($n = 36$) and second ($n = 35$) litter [and third ($n = 5$) litter in 2006] within three days of weaning, which is postnatal day (pnd) 28. Because the relatedness of dams and juveniles is unknown, we compared a pooled sample of the average dam FCM concentrations to the average juvenile FCM concentrations from each litter group in each year.

To examine the long-term effects of prenatal GC exposure, we compared first- and second-litter juveniles in 2006 and 2007 in August (pnd 60 and pnd 28 for first [$n = 25$] and second [$n = 22$] litter, respectively), when juveniles are still growing, and in October (pnd 120 and pnd 90 for first [$n = 13$] and second [$n = 11$] litters, respectively), when juveniles are adult size.

Upon capture, each hare was weighed with a Pesola spring scale (±10 g), its right hind foot (RHF) length was measured as an index of body size, an ear tag was placed in its right ear (No. 3 Monel tags, National Band and Tag, Newport, Kentucky, USA), and its sexual condition assessed (see Krebs et al. 1986). A fecal sample was also collected from below the trap. Lactation was determined by the color of the lactation tissue and matting of the hair around the nipples. Juvenile age was determined by body mass. O'Donoghue and Krebs (1992) found that juveniles weigh 397–492 g and 905–1000 g within 3 days of pnd 28 and pnd 60, respectively. At pnd 90 and 120, juvenile hares are the same size as adults and indistinguishable from them. Thus, we only collected feces from juveniles of known age.

Captive study

In total, nine pregnant hares were transferred to an outdoor enclosure constructed at the Arctic Institute Base, Kluane Lake (southwestern Yukon, Canada) and were placed in individual 4 × 4 m chicken wire (2.5 cm) pens (for details, see Sheriff et al. 2009*b*). Hares were held in pens for an average of 20 days before parturition and for 28 days thereafter. Hares were fed ad libitum with standard rabbit chow (Unifeed, Okotoks, Alberta, Canada; Unifeed, Cat. #19-2103: protein 18%, crude fat 2%, crude fiber 18%) and apples, supplemented daily with natural browse (small branches with leaves and bark from *Salix* spp.), and water ad libitum. Thirty hours after parturition, dams were live-trapped and a fecal sample was taken. Twenty-eight days after parturition, dams and juveniles were live-trapped within the enclosure. Dams were released at the site of their original field capture. Juveniles were subject to a hormone challenge at the Arctic Institute Base before being released to the original field site of capture of their dams.

Fecal cortisol metabolite analysis

We used an enzyme immunoassay (EIA) to measure fecal cortisol metabolite (FCM) concentrations, validated specifically for snowshoe hares. Samples were stored at −80°C within 1 h of collection at the Arctic Institute Base. Samples were kept on ice during transport to the University of Toronto (they were still frozen upon arrival) and stored at −80°C until analyzed.

Fecal samples were freeze-dried using a lyophilizer (LabConco, Kansas City, Missouri, USA) for 14–18 h to control for fiber and water content (Wasser et al. 1993), and were homogenized with a coffee grinder. We then extracted 0.300 g (±0.05 g) of the ground feces with 5 mL 80% methanol (volume/volume) for 30 min at 15 000 rpm (SI units 220.80 rad/s) on a multi-vortexer. After centrifugation (15 min at 2500 *g* [24 526 m/s^2]) an aliquot of the supernatant was diluted (1:10) with assay buffer and frozen at −80°C until analysis. Fecal cortisol metabolite concentrations were measured following the methods outlined by Sheriff et al. (2009*a*) using the 11-oxoaetiocholanolone-EIA developed by Palme and Möstl (1997). This EIA had intra- and inter-assay coefficients of variation of 6.3% and 10.3%, respectively.

Hormone challenge

Female juvenile hares were captured, transferred to a burlap bag, and taken to a dimly lit laboratory and allowed to settle down and habituate for 1–2 h prior to the challenge. Each hare was bled three times (0.3 mL per bleed) from an ear artery using 28-gauge needles (0.36 × 13 mm) into heparinized 0.5-mL syringes (Lo-Dose U-100 insulin syringes; Becton Dickinson, Franklin Lakes, New Jersey, USA). The first blood sample (basal bleed) was immediately followed by an injection of 0.4 mg/kg of dexamethasone sodium phosphate (Sabex, Boucherville, Quebec, Canada) into an ear vein. The second bleed (Dex bleed) occurred 2 h later, followed immediately by an intramuscular injection in the thigh of 40 μg/kg of synthetic adrenocorticotropic hormone (ACTH; Synacthen Depot, CIBA, Mississauga, Ontario, Canada). The final bleed (P60 bleed) occurred 1 h later.

Measurement of glucose concentrations using a FreeStyle glucometer (Abbott Diabetes Care, Alameda, California, USA) and preparation of blood smears were completed within 5 min and measurement of packed red-blood-cell volume, PCV (measured in duplicate after a 9-min centrifugation at 13 460 *g* (132 000 m/s^2) on an IEC Micro-Hematocrit Centrifuge, Model MB, Thermo IEC, Needham Heights, Massachusetts, USA) and

staining of slides (using a modified Wright stain technique called Diff-Quick [Dade International, Miami, Florida, USA]) were completed within 45 min of blood collection. Blood smears and PCV were conducted on the first bleed only and glucose concentrations were measured after every bleed. The remaining blood was centrifuged at 8800 *g* (86 300 m/s^2) for 10 min in an Eppendorf Micro Centrifuge 5413 (Eppendorf, Hamburg, Germany). The separated plasma was then frozen at −80°C at the Arctic Institute Base and at the University of Toronto, until analysis for plasma cortisol, MCBC (maximum corticosteroid-binding capacity), and free fatty acids (FFA).

Total plasma cortisol was measured in duplicate using a radioimmunoassay (Clinical Assays GammaCoat Cortisol ^{125}I RIA Kit, DiaSorin, Stillwater, Minnesota, USA) with inter- and intra-assay coefficients of variation of 12.4% and 2.4%. MCBC levels were measured in duplicate using a radioimmunoassay described by Boonstra and Singleton (1993), with intra- and inter-assay coefficients of variation of 2.6% and 4.9%. Free cortisol concentrations were calculated using the procedures and binding coefficients outlined in Boonstra et al. (1998).

Free fatty acids were measured in duplicate using an in vitro enzymatic colorimetric method to assay quantitative determination of non-esterified fatty acids (HR Series NEFA-HR [2], Wako Diagnostics, Richmond, Virginia, USA). This assay had intra- and inter-assay coefficients of variation of 5.2% and 9.9%.

White blood cell counts from stained slides were carried out at the University of Toronto. Differential white blood cell counts were based on counts of 100 leucocytes.

Statistical analysis

All data are expressed as means ± SE, unless otherwise stated. ANOVAs, general linear models, mixed-effects models, and Newman-Keuls post hoc analyses were performed using the software package STATISTICA 6 (StatSoft 2002). The assumption of normality was tested with Shapiro-Wilks test and the assumption of homogeneity of variances was tested with Levene's test. If these assumptions were not met, data were log-transformed and the assumptions retested. Comparisons of the means were considered significant if $P < 0.05$.

Results

The snowshoe hare population increased from a spring low of 2 hares/km^2 (95% CI: 2–4 hares/km^2) in 2002 to a spring peak of 92 (95% CI: 85–113) hares/km^2 in 2006 and then declined over the next two years to 35 (95% CI: 34–43) hares/km^2 in the spring of 2008 (Fig. 1). During the increase phase, overwinter survival must have been extremely high, with spring density estimates of 81% for 2002/2003, 149% for 2003/2004, 102% for 2004/2005, and 105% for 2005/2006 of those in autumn (Table 1, Fig. 1). The biologically impossible increases in estimates from autumn to spring were probably due to the decreased food availability in the late winter/spring, which lead to an increase in movements and trappability of spring hares relative to autumn hares. However, overwinter survival must have been greatly reduced during the decline, with spring density estimates being only 68% (2006/2007) and 56% (2007/2008) of those in autumn (Table 1, Fig. 1). Across this cycle, the lynx population increased from a low of 1 lynx/100 km^2 (95% CI: 1–2 lynx/100 km^2) in 2002 to a peak of 12 (95% CI: 9–15) lynx/100 km^2 in 2007, and then declined to 5 (95% CI: 3–7) lynx/100 km^2 in 2008 (Fig. 1).

Natural monitoring study

Dam FCM concentrations in free-ranging hares from the first litter varied significantly from 2005 to 2008 (ANOVA: $F_{3,28} = 5.56$, $P = 0.004$; Fig. 1), being higher in 2007 (750.21 ± 65.56 ng/g) than in 2005 (398.49 ± 39.43 ng/g; $P = 0.004$), 2006 (455.60 ± 63.48 ng/g; $P = 0.01$), and 2008 (514.15 ± 51.20 ng/g; $P = 0.02$). To examine how our index of maternal stress (FCM) varied both within and among years, we carried out a mixed-effects model with year and litter group as fixed effects and animal ID as a random variable (this accounted for individuals recaptured in different sessions) comparing 2006, 2007, and 2008 (the only years for which we had data from all three litter groups). We found a year effect ($F_{2,40} = 4.74$, $P = 0.01$), a litter group effect ($F_{2,56} = 19.82$, $P < 0.0001$), and no interaction effect ($F_{4,8} = 1.18$, $P = 0.39$; Fig. 1). Dams in 2007 (515.07 ± 50.51 ng/g) had significantly greater FCM concentrations than those in 2006 (352.22 ± 33.18 ng/g; $P < 0.01$) and in 2008 (430.31 ± 41.41 ng/g; $P < 0.05$). Dams had significantly greater FCM levels at the first litter (560.68 ± 40.80 ng/g) than at the second (290.92 ± 28.42 $P < 0.0001$) and third (424.45 ± 39.03 ng/g; $P < 0.05$). Dams at the second litter had FCM levels that were lower than at the third litter ($P < 0.001$). Thus, dam FCM concentrations fluctuated in synchrony with predator numbers (Fig. 1), increasing from 2005 and 2006 to reach a maximum in 2007, and then declining in 2008. Within a year, dam FCM concentrations declined from the first to the second litter and then increased again at the third litter.

To examine how juvenile FCM levels varied both within and among years (first three litter groups) we carried out a mixed-effects model with year and litter group as fixed effects. We found a year effect ($F_{3,68} = 13.99$, $P < 0.0001$), a litter group effect ($F_{2,68} = 9.33$, $P = 0.0003$), and no interaction effect ($F_{2,68} = 1.62$, $P = 0.21$; Fig. 1). Juvenile FCM concentrations were significantly higher in 2007 (510.74 ± 37.41 ng/g) than in 2006 (285.27 ± 34.85 ng/g; $P = 0.0002$) and in 2008 (288.92 ± 26.40 ng/g; $P = 0.0002$), but were similar in 2007 and 2005, and in 2005, 2006, and 2008. Juveniles born in the first litter (440.10 ± 32.65 ng/g) had significantly higher FCM concentrations than did those born in the second

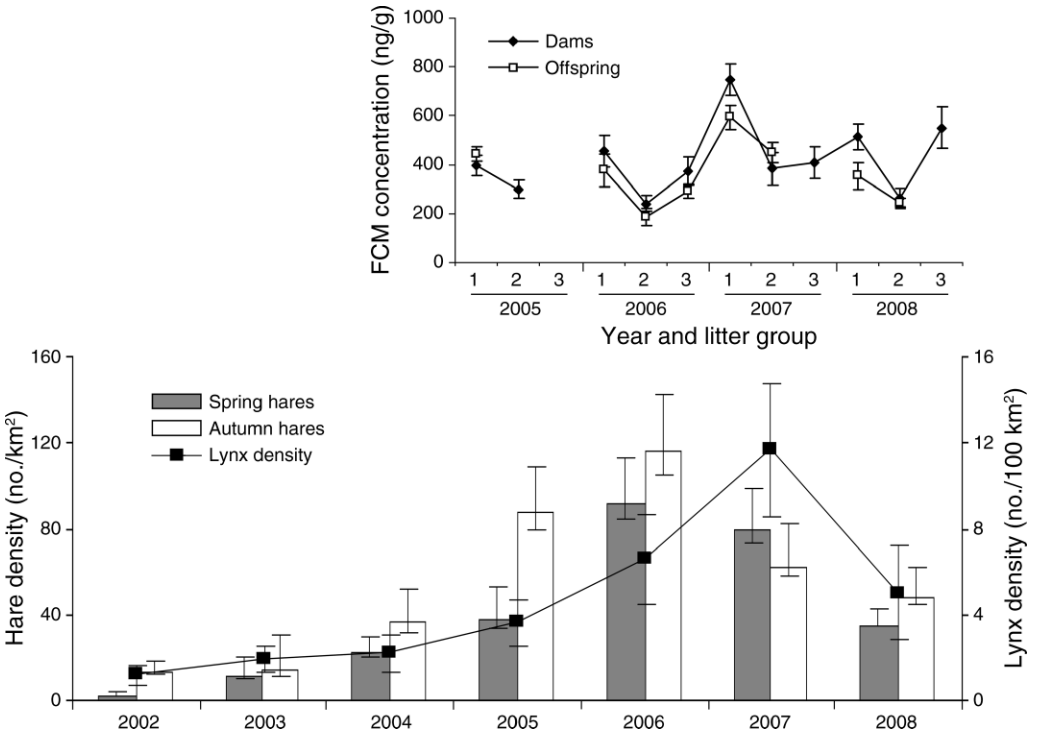

FIG. 1. Fecal cortisol metabolite (FCM) concentration (mean ± SE) in free-ranging adult dams and juvenile snowshoe hares (*Lepus americanus*) during the breeding season in 2005 ($n = 4$, 5, 0 dams caught during the first, second, third litter, respectively; $n = 6$ juveniles caught in the first litter), in 2006 ($n = 10$, 11, 8 adults; $n = 11$, 11, 5 juveniles caught in the first, second, and third litter, respectively), in 2007 ($n = 8$, 8, 8 adults; $n = 10$, 11, 0 juveniles), and in 2008 (10, 8, 4 dams; $n = 9$, 13, 0 juveniles). Juveniles were live-trapped within one week of weaning. FCM is an index of maternal stress; we tested whether chronically elevated glucocorticoids (GC) in dams increased the concentration of the stress hormones of their offspring. The lower graph shows snowshoe hare and lynx population estimates (means with 95% confidence limits) in the southwestern Yukon, Canada, from 2002 to 2008. Lynx densities were estimated during the winter (i.e., from the autumn of one year to the spring of the following year). The snowshoe hare cycle proceeds from a low phase in 2002, increases in 2004 and 2005, reaches a peak in 2006, and then declines in 2008 and 2009, with lynx numbers tracking this change with a lag of one year. Changes in dam FCM levels closely parallel changes in lynx densities, and within a litter group, FCM changes in juveniles echo the FCM changes in the dams.

(290.84 ± 25.98 ng/g; $P = 0.0003$) and the third (292.08 ± 27.26; $P = 0.04$) litter. Although juveniles born in the second and third litter had similar FCM concentrations ($P = 1.00$), this is probably due to the fact that only in 2006 were we able to sample juveniles born in the third litter. Therefore we carried out a separate one-way ANOVA testing for litter differences in 2006 and found significant differences ($F_{2,24} = 4.84$, $P = 0.02$; Fig. 1). Juveniles from the first litter (379.20 ± 67.85 ng/g) had significantly greater FCM levels than those from the second litter (188.25 ± 34.43 ng/g; $P = 0.05$), but levels similar to those from the third litter (292.07 ± 27.26; $P = 0.66$). Juveniles from the second litter had FCM levels that were lower ($P = 0.05$) than those from the third litter. Thus, juvenile FCM concentrations were similar in 2005 and 2006, increased from 2006 to 2007, and declined in 2008. Within a year, FCM concentrations declined from the first litter to the second and increased again in the third. These changes mirrored those found in the dams.

To assess the relationship between dam and juvenile FCM concentration from the same year and litter group, we carried out a general least-squares regression including the first, second, and third litter groups from 2005 to 2008. Dam FCM concentration just after birth was a good predictor of juvenile FCM concentration at weaning ($r^2 = 0.73$, $P = 0.007$; Fig. 2).

To determine whether the differences we observed among years and litter groups in juveniles at weaning

TABLE 1. Yearly and seasonal changes in snowshoe hare (*Lepus americanus*) density from autumn of 2002 to spring of 2008.

Year	Hare density (no./km²)		Change (%)
	Autumn	Spring	
2002/2003	14 (13–18)	11 (10–20)	81%
2003/2004	15 (12–31)	22 (20–30)	149%
2004/2005	37 (32–52)	38 (34–53)	102%
2005/2006	88 (60–110)	92 (85–114)	105%
2006/2007	117 (105–143)	79 (74–100)	68%
2007/2008	63 (59–83)	35 (35–43)	56%

Notes: Values are means with 95% confidence limits in parentheses. The percentage change is the estimate of the spring density compared to that in autumn.

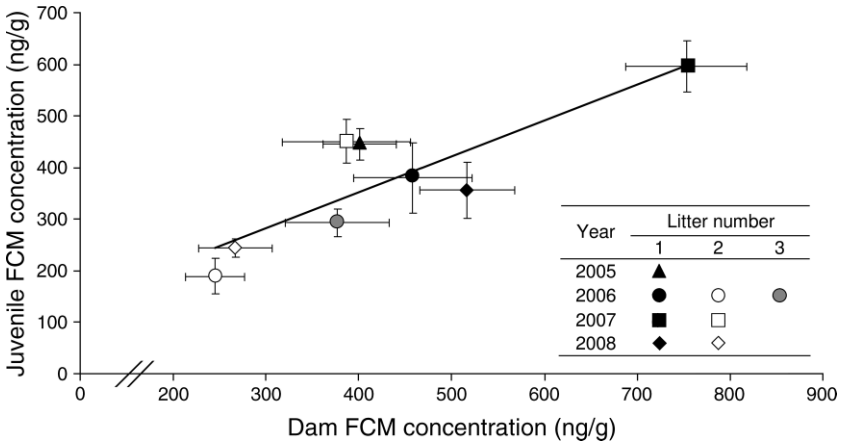

FIG. 2. Fecal cortisol metabolite (FCM) concentration (mean ± SE) in free-ranging snowshoe hare dams and juveniles. Each point is the average from a different litter in 2005, 2006, 2007, and 2008 (from the increase to the decline in the hare population). The line gives the general least-squares regression ($r^2 = 0.73$, $P = 0.007$).

resulted in long-term changes of their FCM levels at older ages, we carried out a two-way ANOVA (litter group × trap date) in 2006 and 2007 to compare FCM concentrations between the first and second litters in August (pnd 60 and 28 for first and second litters, respectively), when the juveniles are still growing, and in October (pnd 120 and 90 for first and second litters, respectively), when juveniles have reached adult size (no individuals were recaptured between sessions, so animal identification did not need to be included in the model). We found a litter effect (for 2006, $F_{1,33} = 8.29$, $P = 0.007$; for 2007, $F_{1,30} = 6.21$, P 0.02), a season effect (for 2006, $F_{1,33} = 5.17$, $P = 0.03$; for 2007, $F_{1,30} = 5.01$, $P = 0.03$), and no interaction effect (for 2006, $F_{1,33} = 0.00$, $P = 1.00$; for 2007, $F_{1,30} = 0.39$, $P = 0.54$; Fig. 3). Thus, first-litter juveniles maintained greater FCM concentrations than second-litter juveniles, irrespective of their age.

Captive study

To test whether the stress hormones of dams (as reflected by their FCM concentrations) were correlated with those of their female offspring, we subjected juvenile hares to a hormone challenge at the time of weaning (pnd 28). We compared dams to their female offspring, as this is the important sex with respect to maternal programming.

As dam FCM concentrations increased, offspring plasma cortisol decreased at the basal bleed ($r^2 = 0.67$, $F_{1,7} = 14.25$, $P = 0.007$; Fig. 4a), increased at the dexamethasone (Dex) bleed ($r^2 = 0.44$, $F_{1,7} = 5.46$, $P = 0.05$; Fig. 4b), and increased at the 60-minute (P60) bleed following adrenocorticotropic hormone (ACTH) administration ($r^2 = 0.60$, $F_{1,7} = 10.41$, $P = 0.01$; Fig. 4c). To determine how dam FCM concentration affected the responsiveness of the juvenile's HPA axis, we subtracted

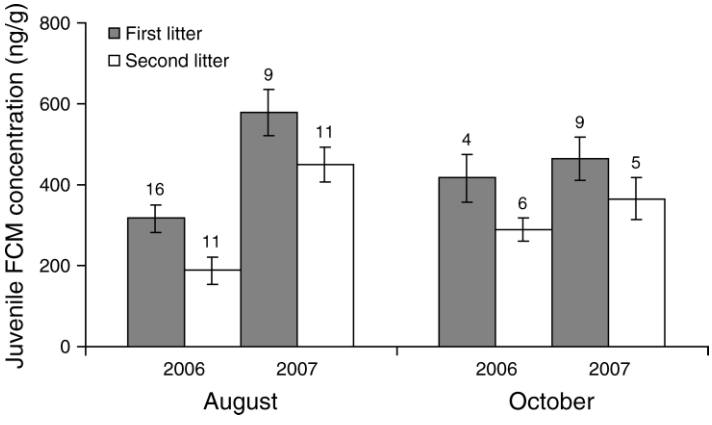

FIG. 3. Fecal cortisol metabolite (FCM) concentration (mean ± SE) of free-ranging juvenile snowshoe hares born in the first and second litter, in August and October of 2006 and 2007. For both 2006 and 2007, first-litter juveniles are postnatal day (pnd) 60 in August and pnd 120 in October; second-litter juveniles are pnd 28 in August and pnd 90 in October. Each pair of first and second litters differs significantly ($P < 0.05$).

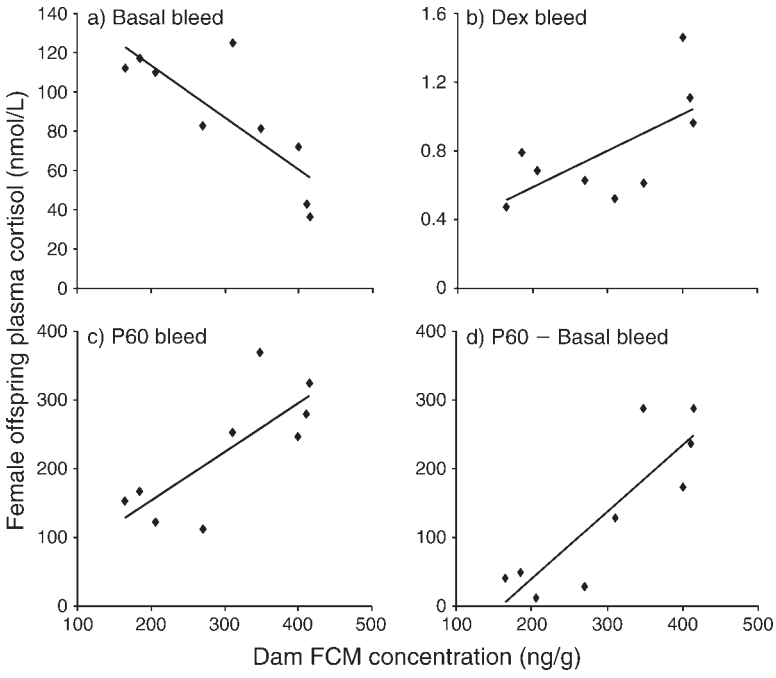

FIG. 4. Fecal cortisol metabolite (FCM) concentrations in dams and the plasma cortisol concentrations of their female offspring in response to a standardized hormone challenge: (a) at basal bleed; (b) 2 hours after a dexamethasone injection (Dex bleed); and (c) 1 hour after an adrenocorticotropic hormone injection (P60 bleed). (d) The change in cortisol levels from the basal plasma concentration to the P60 bleed.

the basal cortisol levels from the 60-minute cortisol levels. We found that as FCM concentration of dams increased, offspring cortisol responsiveness also increased ($r^2 = 0.76$, $F_{1,7} = 20.53$, $P = 0.003$; Fig. 4d). Thus, young became increasingly dexamethasone resistant and ACTH responsive as their mother's FCM concentrations increased.

Dam FCM concentration did not affect the MCBC, free fatty acid (FFA), or glucose levels of their offspring at any time during the challenge: (1) at the basal bleed (for MCBC, $F_{1,7} = 0.25$, $P = 0.63$; for FFA, $F_{1,7} = 2.36$, $P = 0.17$; for glucose, $F_{1,7} = 0.11$, $P = 0.74$); (2) at the Dex bleed (for MCBC, $F_{1,7} = 0.35$, $P = 0.57$; for FFA, $F_{1,7} = 2.21$, $P = 0.18$; for glucose, $F_{1,7} = 0.23$, $P = 0.65$); and (3) at the P60 bleed (for MCBC, $F_{1,7} = 0.61$, $P = 0.46$; for FFA, $F_{1,7} = 0.16$, $P = 0.70$; for glucose, $F_{1,7} = 1.13$, $P = 0.32$).

Dam FCM concentrations significantly affected aspects of their offsprings' hematology (Fig. 5). As dam FCM concentrations increased, the packed red blood cell volume (PCV) of their offspring decreased ($r^2 = 0.47$, $F_{1,7} = 6.12$, $P = 0.04$; Fig. 5a), the neutrophil to lymphocyte (N:L) ratio increased ($r^2 = 0.67$, $F_{1,7} = 13.92$, $P = 0.007$; Fig. 5b), and the eosinophil counts decreased ($r^2 = 0.48$, $F_{1,7} = 6.33$, $P = 0.04$; Fig. 5c). Dam FCM concentrations did not affect monocyte counts ($r^2 = 0.25$, $F_{1,7} = 2.39$, $P = 0.17$; Fig. 5d). Thus, young had worsening indices of condition (PCV) and of immunity as their mothers' FCM concentrations increased.

DISCUSSION

The 10-year snowshoe hare cycle and its attendant cycles in lynx and other furbearers have been fundamental to the development of ecological theory for more than half a century (Stenseth et al. 1999). Empirical studies have been used to examine both this theory and fundamental concepts such as "the balance of nature," predator–prey fluctuations, food web dynamics, and community organization (Elton and Nicholson 1942, Keith 1963, Pimm 1981, Krebs et al. 1986, Boonstra et al. 1998, Stenseth et al. 1998, Sinclair et al. 2000). However, critical elements of the pattern of these cycles have eluded our understanding. During the low phase, virtually all of the predators have died and the vegetation has recovered, yet the hare population remains low for 3–5 years (Krebs et al. 2001*a*). Reproductive rates remain low at this time (Cary and Keith 1979, Stefan and Krebs 2001). In a related paper, we report that a predator-induced increase in maternal FCM levels resulted in a decline in reproduction (Sheriff et al. 2009*b*). An increase in dam FCM concentrations of 250 ng/g resulted in one less baby per litter and the babies born were 24% lighter and 11% smaller. From captive studies on cyclic populations of both snowshoe hares and voles, we know that mothers with high reproductive fitness have daughters with high reproductive fitness; conversely, mothers with low reproductive fitness have daughters with low reproductive fitness. Thus, intrinsic differences between mothers are perpet-

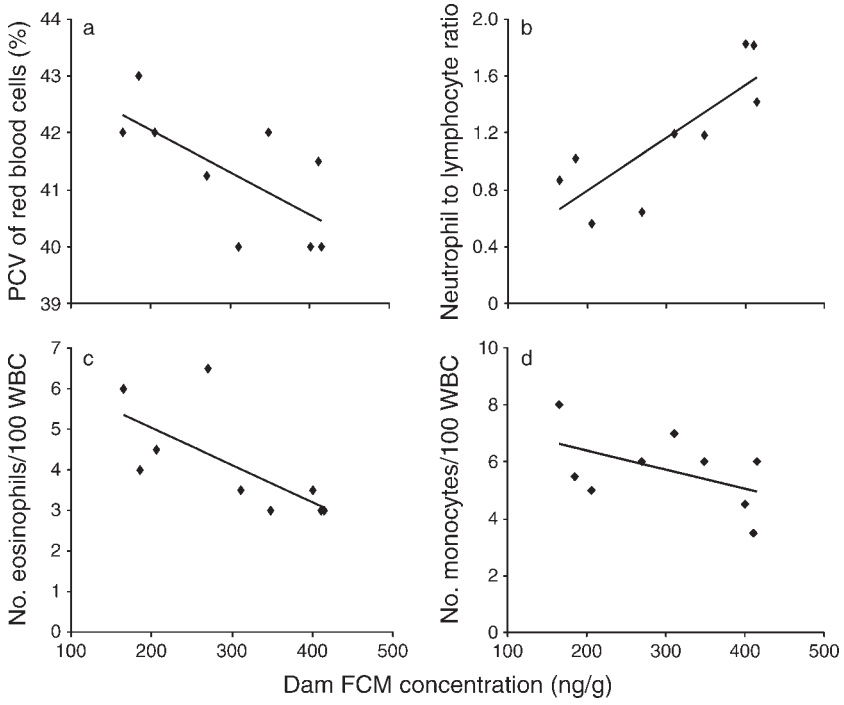

FIG. 5. Fecal cortisol metabolite (FCM) concentrations in dams and the hematology of their female offspring: (a) packed cell volume (PCV) of red blood cells (%); (b) ratio of neutrophils to lymphocytes in 100 white blood cells (WBC); (c) number of eosinophils per 100 WBC; (d) number of monocytes per 100 WBC.

uated in their offspring (Mihok and Boonstra 1992, Sinclair et al. 2003). Our study took place during the increase (2005), peak (2006), and decline phases (2007–2008) of the hare cycle. We elucidate the following connections between the predator-induced increase in stress hormones experienced by the mothers during the population decline and its consequences for their offspring. First, a maternal index of stress hormones (FCM concentrations) fluctuated in synchrony with predator density during the breeding season. Second, this stress hormone index is directly echoed in their offspring, with entire litter groups reflecting the pattern of their mothers at the time the young are born. Third, a mother's FCM levels affect the HPA stress axis of her progeny, with higher maternal FCM levels resulting in increased dexamethasone resistance and a heightened responsiveness to ACTH in progeny. We suggest that maternal programming, linked to the experience of high stress hormones caused by high predation risk, may explain the poor recovery of reproductive rates even after the predator numbers have declined markedly. This maternal programming caused by stress hormones may be a major factor contributing to the low phase of the snowshoe hare cycle.

Maternal stress

Food and predation are two of the greatest factors affecting animal populations and they have interactive and synergistic affects (Krebs et al. 1995, Zanette et al. 2003). In our natural monitoring study, FCM concentrations of dams fluctuated among and within years (Table 1, Fig. 1). Among years, FCM concentrations were highest in 2007 when the number of predators was greatest. We have also found that an experimental increase in the risk of predation resulted in dams with higher FCM concentrations (Sheriff et al. 2009*b*). Boonstra et al. (1998) found that, outside the breeding season, snowshoe hares had a greater plasma cortisol concentration and stress response and a poorer body condition during the decline than during the low phase of the cycle. An increase in the number of predators has also been shown to increase GC levels in other free-ranging mammals and birds (Silverin 1997, Hik et al. 2001, Scheuerlein et al. 2001; but see Creel et al. 2009).

We also expected FCM levels in the second year of the decline (2008) to be higher than in the increase phase (2005); however, we found that levels, although elevated, were statistically similar. Part of the explanation may be that the females in our study were a high-quality subset of females from the hare population, all giving birth within one week of the estimated parturition date. Sheriff et al. (2009*b*) found that females that gave birth to nonviable young (either stillborn or aborted), had high FCM concentrations compared with females that gave birth to viable young. Because the number of females that give birth to nonviable young increases

PLATE 1. A snowshoe hare foraging on a winter morning (February 2007) in Yukon, Canada. Photo credit: M. J. Sheriff.

during the decline phase (Stefan and Krebs 2001), had we assessed FCM concentrations from all females within the population, not just the successful ones, we may have seen greater FCM levels persisting throughout the decline phase.

Within a year, we found that dam FCM concentrations were highest in the first litter, declined in the second, and increased again in the third (Fig. 1). The combined effects of changing risk of predation and food between the first and second litter could explain the changes in FCM concentrations. Boutin et al. (1986) found that predation rates decreased from winter to summer, and thus the risk of predation should also decrease at this time. Furthermore, the first litter occurs during the late winter–early spring when the winter snowpack is in the process of melting and prior to the flush of new vegetation, whereas the second litter occurs during late spring–early summer when new vegetative growth is nearing its peak (Sinclair et al. 1982). Reduced food intake has been found to cause an increase in GC levels in mammals and birds (Harris et al. 1994, Kitaysky et al. 1999, Ortiz et al. 2001). The increase in food availability from the first to the second litter may also allow hares to forage in a less risk-prone manner (Hik 1995, Murray 2002).

The increase in maternal FCM concentrations between the second and the third litter (Fig. 1) could be a result of allostatic load. Allostatic load is the cumulative wear and tear on an animal coping with multiple stressors over its lifetime, such as the risk of predation, the extreme temperatures and food scarcity of winter, and pregnancy (McEwen and Wingfield 2003, Romero et al. 2009). As the allostatic load increases, animals become less able to respond normally and require a greater stress response to counteract the stressor. For snowshoe hares during the decline phase, the high risk of predation during the winter, followed by two litters, would greatly increase their allostatic load. This may be one explanation as to why hares during the increase phase (when the risk of predation is low) may have up to four litters in a summer, whereas those during the decline may have only two litters. Thus, changes in maternal FCM concentrations may be affected primarily by changes in the number of predators, with food augmenting these effects. As allostatic load increases, hares begin to show an even greater stress response to the increase in the risk of predation.

In our captive study, maternal FCM concentrations were within the levels found in the natural monitoring study. Our captive females also showed considerable variation in FCM concentrations, consistent with what we found in the free-ranging females. This suggests that individuals naturally differ in their physiological response and ability to cope with stressors, and this difference was maintained in captivity. This has also been found by Pride (2005) for ring-tailed lemurs *Lemur*

catta, and by Cabezas et al. (2007) for European wild rabbits *Oryctolagus cuniculus*. Thus, our captive results might reflect a wider phenomenon.

Developmental impacts of maternal stress

In our natural monitoring study, dams with increased FCM concentration produced offspring with increased FCM concentrations, and this was maintained into adulthood (Figs. 1–3). Fecal cortisol metabolite levels are an integration of blood cortisol levels over the previous 8–12 hours (Sheriff et al. 2009*a*) and reflect both basal and stress-induced cortisol exposure experienced during this time. In our experimental study, we were able to separate these two factors and found that dams with increased FCM concentration produced offspring with decreased basal plasma cortisol levels and an increased responsiveness of the HPA axis (Fig. 4).

The decrease in basal cortisol and the increased responsiveness seen in experimental juveniles born to mothers with elevated FCM concentrations could be due to an increase in mineralocorticoid receptors (MR) and a decrease in glucocorticoid receptors (GR) in the brain. Densities of these two hippocampal receptors are critical in regulation and feedback of the HPA axis, with MRs regulating basal glucocorticoid levels and GRs regulating glucocorticoid levels in response to stressors (Owen et al. 2005). For pregnant guinea pigs treated with a synthetic glucocorticoid, offspring exhibited increased hippocampal MR expression and reduced basal plasma cortisol concentrations (Liu et al. 2001). Furthermore, exposure of pregnant guinea pigs to a strobe light at gestation day 50 resulted in offspring with reduced GR expression and an elevated activity of the HPA axis (Kapoor et al. 2008). Rat offspring born to mothers that were restrained during the last week of gestation exhibited reduced GR densities (Henry et al. 1994, Maccari et al. 1995). These differences in receptor levels persist into adulthood (Liu et al. 1997, Francis et al. 1999), and we also found that differences in FCM concentrations at weaning persisted into adulthood (Fig. 3). For snowshoe hares, the differences in MR and GR receptor levels would allow them to mount an appropriate response to predation, yet allow them to have low cortisol exposure at times when the risk of predation is low. This would alleviate the negative effects of high cortisol exposure such as a decrease in reproduction and survival. Thus, during the low phase, reproductive output could begin to recover and population density would increase.

Dam FCM concentrations also affected juvenile hematology (Fig. 5). Juvenile hares born to dams with elevated FCM concentration had a lower PCV (Fig. 5a). PCV is an indicator of body condition, with lower values indicating a poorer body condition (Hellgren et al. 1993, Boonstra et al. 1998). Previously, we found that snowshoe hares with predator-induced increases in cortisol levels gave birth to lighter and smaller babies compared with controls (Sheriff et al. 2009*b*). This has also been found for rats and male guinea pigs (Lesage et al. 2001, Emack et al. 2008). Juvenile hares born to dams with elevated FCM concentration also had a higher neutrophil to lymphocyte (N:L) ratio (Fig. 5b). High N:L ratios generally indicate high GC levels (Davis et al. 2008; but see Boonstra et al. 1998). However, this is not the complete picture, as an infection may also create a rise in neutrophil counts sufficient to substantially increase the N:L ratio. In order to disassociate stress from an infection, both eosinophil and monocyte ratios must also be measured, because an infection or parasite will also result in an increase in these WBC types (Davis et al. 2008). We found that an increase in dam FCM concentration resulted in a decrease in eosinophil counts and no change in monocyte counts in juveniles (Fig. 5c). Thus an increase in maternal FCM levels resulted in a shift in leukocyte profile indicative of high GC levels. Eosinophils are important in fighting parasitic infections, and reduced numbers may also result in a decreased immunity (Bullock and Rosendahl 1984). Thus, an increase in prenatal GC exposure results in not only an increase in offspring stress hormones, but also a decrease in body condition and immunity.

The increase in stress hormones in the offspring born to mothers with high FCM concentrations may trade off the decrease in reproduction by increasing the antipredator behavior of the offspring (Emack et al. 2008), thus increasing survival. However, during the low phase when predation risk is low, the same mechanism would lower fitness because the risk of predation is much lower. In order for this mechanism to be adaptive, the gain in fitness during the decrease phase should at least compensate for the loss of fitness in the early low phase. Future work must be done to quantitatively assess different tactics to determine the adaptive component of maternally programmed antipredatory behavior (Lambin et al. 1995).

Acknowledgments

We thank A.T. Sheriff, K. Pieczora, S. Mitford, and E. Hofer for their contributions to this project. The Natural Sciences and Engineering Research Council of Canada, the Department of Indian and Northern Affairs, the EJLB Foundation, and the Arctic Institute of North America all supported this research. We thank Andrew Williams and the Arctic Institute of North America, University of Calgary, for providing facilities at Kluane Lake. The University of British Columbia Animal Care Committee approved all procedures in accordance with the guidelines of the Canadian Council for Animal Care.

Literature Cited

Abe, H., N. Hidaka, C. Kawagoe, K. Odagiri, Y. Watanabe, T. Ikeda, Y. Ishizuka, H. Hashiguchi, R. Takeda, T. Nishimori, and Y. Ishida. 2007. Prenatal psychological stress causes higher emotionality, depression-like behavior, and elevated activity in the hypothalamo-pituitary-adrenal axis. Neuroscience Research 59:145–151.

Boonstra, R. 2005. Equipped for life: the adaptive role of the stress axis in male mammals. Journal of Mammalogy 86:236–247.

Boonstra, R., D. Hik, G. R. Singleton, and A. Tinnikov. 1998. The impact of predator-induced stress on the snowshoe hare cycle. Ecological Monographs 79:371–394.

Boonstra, R., and G. R. Singleton. 1993. Population declines in the snowshoe hare and the role of stress. General and Comparative Endocrinology 91:126–143.

Boutin, S., C. J. Krebs, A. R. E. Sinclair, and J. N. M. Smith. 1986. Proximate causes of losses in a snowshoe hare population. Canadian Journal of Zoology 64:606–610.

Bullock, B. L., and P. P. Rosendahl. 1984. Pathophysiology. Adaptation and alterations in function. Little, Brown, Boston, Massachusetts, USA.

Cabezas, S., J. Blas, T. A. Marchant, and S. Moreno. 2007. Physiological stress levels predict survival probabilities in wild rabbits. Hormones and Behavior 51:313–320.

Cary, J. R., and L. B. Keith. 1979. Reproductive change in the 10-year cycle of snowshoe hares. Canadian Journal of Zoology 57:375–390.

Creel, S., J. Winnie, Jr., and D. Christianson. 2009. Glucocorticoid stress hormones and the effect of predation risk on elk reproduction. Proceedings of the National Academy of Sciences USA 106:12388–12393.

Davis, A. K., D. L. Maney, and J. C. Maerz. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Functional Ecology 22:760–772.

Elton, C., and M. Nicholson. 1942. The 10-year cycle in numbers of the lynx in Canada. Journal of Animal Ecology 11:215–244.

Emack, J., A. Kostaki, C.-D. Walker, and S. G. Matthews. 2008. Chronic maternal stress affects growth, behavior and hypothalamo-pituitary-adrenal function in juvenile offspring. Hormones and Behavior 54:514–520.

Francis, D., J. Diorio, D. Liu, and M. J. Meaney. 1999. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. Science 286:1155–1158.

Harris, S. B., M. W. Gunion, M. J. Rosenthal, and R. L. Walford. 1994. Serum glucose, glucose tolerance, corticosterone and free fatty acids during aging in energy restricted mice. Mechanisms of Ageing and Development 73: 209–221.

Hellgren, E. C., L. L. Rogers, and U. S. Seal. 1993. Serum chemistry and hematology of black bears: physiological indices of habitat quality or seasonal patterns. Journal of Mammalogy 74:304–315.

Henry, C., M. Kabbaj, H. Simon, M. LeMoal, and S. Maccari. 1994. Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. Journal of Neuroendocrinology 6:341–345.

Hik, D. S. 1995. Does risk of predation influence population dynamics? Evidence from the cyclic decline of snowshoe hares. Wildlife Research 22:115–129.

Hik, D. S., C. J. McColl, and R. Boonstra. 2001. Why are arctic ground squirrels more stressed in the boreal forest than in alpine meadows? Ecoscience 8:275–288.

Hodges, K. E., C. J. Krebs, D. S. Hik, E. A. Gillis, and C. E. Doyle. 2001. Snowshoe hare dynamics. Pages 141–178 *in* C. J. Krebs, S. Boutin, and R. Boonstra, editors. Ecosystem dynamics of the boreal forest. The Kluane Project. Oxford University Press, New York, New York, USA.

Hone, J., C. J. Krebs, M. O'Donoghue, and S. Boutin. 2007. Evaluation of predator numerical responses. Wildlife Research 34:335–341.

Kapoor, A., J. Leen, and S. G. Matthews. 2008. Molecular regulation of the hypothalamic-pituitary-adrenal axis in adult male guinea pigs after prenatal stress at different stages of gestation. Journal of Physiology 586:4317–4326.

Kapoor, A., and S. G. Matthews. 2005. Short periods of prenatal stress affect growth, behavior and hypothalamo-pituitary-adrenal axis activity in male guinea pig offspring. Journal of Physiology 566:967–977.

Keith, L. B. 1963. Wildlife's ten-year cycle. University of Wisconsin Press, Madison, Wisconsin, USA.

Kirkpatrick, M., and R. Lande. 1989. The evolution of maternal characters. Evolution 43:485–503.

Kitaysky, A. S., J. F. Piatt, J. C. Wingfield, and M. Romano. 1999. The adrenocortical stress-response of black-legged kittiwake chicks in relation to dietary restrictions. Journal of Comparative Physiology B 169:303–310.

Krebs, C. J., R. Boonstra, S. Boutin, and A. R. E. Sinclair. 2001*a*. What drives the 10-year cycle of snowshoe hares? BioScience 51:25–35.

Krebs, C. J., S. Boutin, and R. Boonstra. 2001*b*. Ecosystem dynamics of the boreal forest. The Kluane Project. Oxford University Press, New York, New York, USA.

Krebs, C. J., S. Boutin, R. Boonstra, A. R. E. Sinclair, J. N. M. Smith, M. R. T. Dale, K. Martin, and R. Turkington. 1995. Impact of food and predation on the snowshoe hare cycle. Science 269:1112–1115.

Krebs, C. J., B. S. Gilbert, S. Boutin, A. R. E. Sinclair, and J. N. M. Smith. 1986. Population biology of snowshoe hares. I. Demography of food-supplemented populations in southern Yukon, 1976–1984. Journal of Animal Ecology 55: 963–982.

Lambin, X., R. A. Ims, H. Steen, and N. G. Yoccoz. 1995. Vole cycles. Trends in Ecology and Evolution 10:204.

Lesage, J., B. Blondeau, M. Grino, B. Bréant, and J. P. Dupouy. 2001. Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids and intrauterine growth retardation and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. Endocrinology 142:1692–1702.

Liu, D., J. Diorio, B. Tannenbaum, C. Caldji, D. Francis, A. Freedman, S. Sharma, D. Pearson, P. M. Plotsky, and M. J. Meaney. 1997. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 277:1659–1662.

Liu, L., A. Li, and S. G. Matthews. 2001. Maternal glucocorticoid treatment programs HPA regulation in adult offspring: sex-specific effects. American Journal of Physiology: Endocrinology and Metabolism 280:E729–E739.

Lupien, S. J., B. S. McEwen, M. R. Gunnar, and C. Heim. 2009. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nature Reviews Neuroscience 10: 434–445.

Maccari, S., P. V. Piazza, M. Kabbaj, A. Barbazanges, H. Simon, and M. Lemoal. 1995. Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. Journal of Neuroscience 15:110–116.

Mastorci, F., V. Massimo, O. Viltart, M. Manghi, G. Graiani, F. Quaini, P. Meerlo, E. Nalivaiko, S. Maccari, and A. Sgoifo. 2009. Long-term effects of prenatal stress: Changes in adult cardiovascular regulation and sensitivity to stress. Neuroscience and Biobehavioral Reviews 33:191–203.

McEwen, B. S., and J. C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. Hormones and Behavior 43:2–15.

Meaney, M., M. Szyf, and J. R. Seckl. 2007. Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. Trends in Molecular Medicine 13:269–277.

Mihok, S., and R. Boonstra. 1992. Breeding performance in captive meadow voles (*Microtus pennsylvanicus*) from decline- and increase-phase populations. Canadian Journal of Zoology 70:1561–1566.

Murray, D. L. 2002. Differential body condition and vulnerability to predation in snowshoe hares. Journal of Animal Ecology 71:614–625.

O'Donoghue, M., and S. Boutin. 1995. Does reproductive synchrony affect juvenile survival rates of northern mammals? Oikos 74:115–121.

O'Donoghue, M., and C. J. Krebs. 1992. Effects of supplemental food on snowshoe hare reproduction and juvenile

growth at a cyclic population peak. Journal of Animal Ecology 61:631–641.
Ortiz, R. M., C. E. Wade, and C. L. Ortiz. 2001. Effects of prolonged fasting on plasma cortisol and TH in postweaned northern elephant seal pups. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology 280:R790–R795.
Otis, D. L., K. P. Burnham, G. C. White, and D. R. Anderson. 1978. Statistical inferences from capture data on closed animal populations. Wildlife Monographs 62:3–135.
Owen, D., M. H. Andrews, and S. G. Matthews. 2005. Maternal adversity, glucocorticoids and programming of neuroendocrine function and behavior. Neuroscience and Biobehavorial Reviews 29:209–226.
Palme, R., and E. Möstl. 1997. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. International Journal of Mammalian Biology 62(Supplement II):192–197.
Pimm, S. L. 1981. The balance of nature. Ecological issues in the conservation of species and communities. University of Chicago Press, Chicago, Illinois, USA.
Pride, R. E. 2005. High faecal glucocorticoid levels predict mortality in ring-tailed lemurs (*Lemur catta*). Biology Letters 1:60–63.
Räsänen, K., and L. E. B. Kruuk. 2007. Maternal effects and evolution at ecological time-scales. Functional Ecology 21: 408–421.
Romero, L. M., M. J. Dickens, and N. E. Cyr. 2009. The reactive scope model: A new model integrating homeostasis, allostasis, and stress. Hormones and Behavior 55:375–389.
Scheuerlein, A., T. J. Van't Hof, and E. Gwinner. 2001. Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). Proceedings of the Royal Society B 268:1575–1582.
Seccombe-Hett, P., and R. Turkington. 2008. Summer diet selection of snowshoe hares: a test of nutritional hypotheses. Oikos 117:1874–1884.
Seckl, J. R. 2008. Glucocorticoids, developmental 'programming' and the risk of affective dysfunction. Progress in Brain Research 167:17–34.
Sheriff, M. J., C. O. Bosson, C. J. Krebs, and R. Boonstra. 2009*a*. A non-invasive technique for analyzing fecal cortisol metabolites in snowshoe hares (*Lepus americanus*). Journal of Comparative Physiology B 179:305–313.
Sheriff, M. J., C. J. Krebs, and R. Boonstra. 2009*b*. The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. Journal of Animal Ecology 78:1249–1258.
Silverin, B. 1997. The stress response and autumn dispersal behavior in willow tits. Animal Behavior 53:451–459.
Sinclair, A. R. E., D. Chitty, C. I. Stefan, and C. J. Krebs. 2003. Mammal population cycles: evidence for intrinsic differences during snowshoe hare cycles. Canadian Journal of Zoology 81:216–220.
Sinclair, A. R. E., C. J. Krebs, J. M. Fryxell, R. Turkington, S. Boutin, R. Boonstra, P. Seccombe-Hett, P. Lundberg, and L. Oksanen. 2000. Testing hypotheses of trophic level interactions: a boreal forest ecosystem. Oikos 89:313–328.
Sinclair, A. R. E., C. J. Krebs, and J. N. M. Smith. 1982. Diet quality and food limitation in herbivores: the case of the snowshoe hare. Canadian Journal of Zoology 60:889–897.
StatSoft. 2002. STATISTICA version 6. StatSoft, Tulsa, Oklahoma, USA.
Stefan, C. I., and C. J. Krebs. 2001. Reproductive changes in a cyclic population of snowshoe hares. Canadian Journal of Zoology 79:2101–2108.
Stenseth, N. C., K.-S. Chan, H. Tong, R. Boonstra, S. Boutin, C. J. Krebs, E. Post, M. O'Donoghue, N. G. Yoccoz, M. C. Forchhammer, and J. W. Hurrell. 1999. Common dynamic structure of Canada lynx populations within three climatic regions. Science 285:1071–1073.
Stenseth, N. C., W. Falck, K.-S. Chan, O. N. Bjornstad, M. O'Donoghue, H. Tong, R. Boonstra, S. Boutin, C. J. Krebs, and N. G. Yoccoz. 1998. From patterns to processes: phase and density dependencies in the Canadian lynx cycle. Proceedings of the National Academy of Sciences USA 95: 15430–15435.
Wasser, S. K., R. Thomas, P. P. Nair, C. Guidry, J. Southers, J. Lucas, D. E. Wildt, and S. L. Monfort. 1993. Effects of dietary fiber on fecal steroid measurements in baboons (*Papio cynocephalus cynocephalus*). Journal of Reproduction and Fertility 97:569–574.
Weinstock, M. 2008. The long-term behavioural consequences of prenatal stress. Neuroscience and Biobehavioral Reviews 32:1073–1086.
Wingfield, J. C., M. C. Moore, and D. S. Farner. 1983. Endocrine responses to inclement weather in naturally breeding populations of White-crowned Sparrows. Auk 100:56–62.
Wingfield, J. C., and R. M. Sapolsky. 2003. Reproduction and resistance to stress: when and how. Journal of Neuroendocrinology 15:711–724.
Zanette, L., J. N. M. Smith, H. Van Oort, and M. Clinchy. 2003. Synergistic effects of food and predators on annual reproductive success in song sparrows. Proceedings of the Royal Society B 270:799–803.