The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares

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Summary

1. Prey responses to high predation risk can be morphological or behavioural and ultimately come at the cost of survival, growth, body condition, or reproduction. These sub-lethal predator effects have been shown to be mediated by physiological stress. We tested the hypothesis that elevated glucocorticoid concentrations directly cause a decline in reproduction in individual free-ranging female snowshoe hares, \emph{Lepus americanus}. We measured the cortisol concentration from each dam (using a faecal analysis enzyme immunoassay) and her reproductive output (litter size, offspring birth mass, offspring right hind foot (RHF) length) 30 h after birth.

2. In a natural monitoring study, we monitored hares during the first and second litter from the population peak (2006) to the second year of the decline (2008). We found that faecal cortisol metabolite (FCM) concentration in dams decreased 52% from the first to the second litter. From the first to the second litter, litter size increased 122%, offspring body mass increased 130%, and offspring RHF length increased 112%. Dam FCM concentrations were inversely related to litter size ($r^2 = 0.19$), to offspring birth mass ($r^2 = 0.32$), and to offspring RHF length ($r^2 = 0.64$).

3. In an experimental manipulation, we assigned wild-caught, pregnant hares to a control and a stressed group and held them in pens. Hares in the stressed group were exposed to a dog 1–2 min every other day before parturition to simulate high predation risk. At parturition, unsuccessful-stressed dams (those that failed to give birth to live young) and stressed dams had 837% and 214% respectively, higher FCM concentrations than control dams. Of those females that gave birth, litter size was similar between control and stressed dams. However, offspring from stressed dams were 37% lighter and 16% smaller than offspring from control dams. Increasing FCM concentration in dams caused the decline of offspring body mass ($r^2 = 0.57$) and RHF ($r^2 = 0.52$).

4. This is the first study in a free-ranging population of mammals to show that elevated, predator-induced, glucocorticoid concentrations in individual dams caused a decline in their reproductive output measured both by number and quality of offspring. Thus, we provide evidence that any stressor, not just predation, which increases glucocorticoid concentrations will result in a decrease in reproductive output.

Key-words: 10-year snowshoe hare cycle, glucocorticoid metabolites, maternal effects, natural populations, predation risk

Introduction

Predation is a central organizing agent shaping population and community processes (Krebs \textit{et al.} 2001a; Schmitz 2008). Traditionally, ecologists have focused on the direct effects of predation – the killing of prey (Paine 1966; Taylor 1984; Krebs \textit{et al.} 1995). However, predators also have significant indirect effects on prey populations (see reviews by Lima 1998; Creel & Christianson 2008) and these effects can be as great as their direct effects (Schmitz, Beckerman & O’Brien 1997; Nelson, Matthews & Rosenheim 2004; Preisser, Bolnick & Benard 2005; Fangle, Peacor & Johannsson 2007). Prey responses to the high risk of predation can be morphological such as changes in secondary sexual characteristics and anti-predator defences (Tollrian & Harvell 1999; Day & Young 2004; Vamosi & Schluter 2004) or behavioural such as...
changes in preferred habitats, in vigilance, and in foraging (Hik 1995; Lima & Bednekoff 1999; Childress & Lung 2003; Armitage 2004; Creel et al. 2005; Winnie & Creel 2007). These responses ultimately come at the cost of survival, growth, body condition, or reproduction (Hik 1995; Boonstra et al. 1998; Krebs et al. 2001a; Olaf & Halle 2004; Bian, Wu & Liu 2005; Hodges, Stefan & Gillis 1999).

The indirect effects of predators act through physiological processes. One of the most conserved processes in vertebrates is the ‘stress response’, defined here as the set of neural and endocrine responses that help restore homeostasis ( Sapolsky 1987). Central to the stress response is the activation of the hypothalamic-pituitary-adrenal (HPA) axis and subsequent secretion of glucocorticoids (GC), lasting several minutes to hours (Sapolsky 1992; Wingfield & Romero 2001). A stressor may be any environmental perturbation that disrupts homeostasis, such as harsh weather, habitat changes, anthropogenic disturbances, decreased food availability, and predation attempts (Sapolsky 1987). The presence of short-term elevated GC concentrations facilitates escape from life-threatening situations (Wingfield et al. 1998). However, chronic activation of the HPA axis may trade off future reproduction for present survival (Boonstra & Singleton 1993; Boonstra et al. 1998; Sapolsky, Romero & Munck 2000; Romero & Wikelski 2001; Wingfield & Romero 2001).

The decline in reproduction not only has individual fitness consequences but may also have long-term population consequences (Wingfield & Sapolsky 2003). Although many studies have shown that elevated GC concentration can have negative effects on reproduction, these have been conducted on laboratory animals (e.g. Ferin 1999; Lesage et al. 2001; Hayward & Wingfield 2004; Romero 2004; Eriksen et al. 2006; Götz, Wolf & Stefanski 2008). Studies on free-ranging animals that suggest elevated GC concentrations have negative effects on reproduction often use GC or reproductive proxies without measuring GC concentration or reproduction directly (Bian et al. 2005; Saino et al. 2005; Charbonnel et al. 2008; Lidgard et al. 2008), or they correlate an increase in GC with a decline in reproduction on a population wide level without showing a direct causal link at the individual level (Boonstra et al. 1998; Hackländer, Möstl & Arnold 2003; Lancot et al. 2003; Young et al. 2006; but see Cyr & Romero 2007). Here we carry out a field study on snowshoe hares to examine the causal link between changes in GC concentrations and predator-induced stress.

Snowshoe hares (*Lepus americanus*) are an ideal species to study the effects of GCs on reproduction. Snowshoe hares undergo a regular cyclic fluctuation, with 8–10 years between peak densities (Keith 1963; Krebs et al. 1986). As hare populations increase, so do that of their predators, but with a lag of 1–2 years. During the hare population decline, predators are the direct cause of up to 83% of hare deaths (Boutin et al. 1986; Krebs et al. 1995). Hare reproduction also cycles, with maximum rates occurring during the early increase phase (when predator numbers are lowest), but then progressively declining to a nadir during the decline (when predator numbers are at their peak) (Cary & Keith 1979; O’Donoghue & Krebs 1992; O’Donoghue et al. 1997; Stefan & Krebs 2001). Predators could be the indirect cause of this decline, with the inhibition of the gonadal axis being mediated by the stress of high predation risk through the activation of the HPA axis. Boonstra et al. (1998) showed that plasma cortisol concentrations (the major GC in snowshoe hares) fluctuated with the risk of predation, such that hares experiencing a greater risk of predation had higher plasma cortisol. They proposed that chronic stress, as measured by elevated cortisol concentrations, caused the marked deterioration of reproduction during the decline phase.

Here we test the hypothesis that elevated GC concentrations cause a decline in reproduction in free-ranging hares in two ways. First, in a natural monitoring study, we measured cortisol concentrations and reproduction 30 h after birth in natural populations of free-ranging snowshoe hares from 2006 to 2008. We estimated both the hare and the predator density during this time to determine when the population peak and the maximum risk of predation would occur. Second, in an experimental manipulation, we increased the risk of predation during the last two-thirds of gestation in a sample of wild-caught snowshoe hares held in pens and measured cortisol concentrations and reproduction 30 h after birth. Cortisol concentrations were measured non-invasively using a faecal analysis enzyme immunoassay (EIA). Reproduction was measured as litter size, offspring birth mass and RHF length.

In the natural monitoring study, we predicted that as the risk of predation increased faecal cortisol metabolite (FCM) concentrations in dams would increase. In the experimental manipulation, we predicted that FCM concentrations would be higher in the stressed group compared with the control group. In both studies, we expected that an increase in FCM concentration in dams would cause a decrease in their litter size, offspring birth mass and offspring RHF length.

Materials and methods

**SNOWSHOE HARE BIOLOGY**

Snowshoe hares are synchronous, seasonal breeders with mating occurring immediately post-partum. This results in two to four distinct litter groups, depending on the phase of the population cycle (four litters during the early increase phase and these progressively decline to a nadir of only two litters during the decline phase; Stefan & Krebs 2001). Breeding begins in late April with the first litter born near the end of May, and each subsequent litter borne approximately 36–39 days later (Cary & Keith 1979; Stefan & Krebs 2001). Early litters are weaned at 24–28 days of age, but the last litter of the year may be nursed for up to 40 days (O’Donoghue & Bergman 1992). The young are born precocious and remain together for the first 3–5 days, after which they separate and only come together once a night to nurse (O’Donoghue & Bergman 1992). Snowshoe hares do not have nests or burrows (Severaid 1942; Graf & Sinclair 1987) and are crepuscular, making it nearly impossible to monitor reproduction in the wild.

**ANIMAL TRAPPING**

Our research was approved by the University of British Columbia Animal Care Committee in accordance with the guidelines of the
Canadian Council for Animal Care. Female 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 Co., Tomahawk, WI, USA). The traps were set at 22:00 h and checked at 06:00 h and 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 the appearance of its metabolites in the faeces is between 8–12 h (Sheriff et al. 2009). Therefore, the cortisol metabolites in the faeces represent non-observer induced measures of stress.

Upon capture, each hare was weighed with a Pesola spring scale (± 0.1 g), its right hind foot (RHF) length measured as an index of body size, an ear-tag was placed in its right ear (No. 3 Monel tags, National Band and Tag Co., Newport, KY, USA), and its sexual condition assessed (see Krebs et al. 1986 for details). Pregnancy was determined by body mass, by the colour of the lactational tissue, and by palpating the abdomen (O’Donoghue & Krebs 1992; Stefan & Krebs 2001). Pregnant females were transferred to an outdoor enclosure constructed at the Arctic Institute Base for use in either the natural monitoring experiment or the stress manipulation experiment. The enclosure was a 3-m high game fence with a black, heavy-duty, fabric cloth surrounded by an electric bear-proof fence to protect the hares from mammalian predators such as lynx, coyotes and grizzly bears. The ceiling of the entire enclosure was completely secured with chicken wire (2.5 cm) to prevent raptors (great horned owls and goshawks) and corvids (ravens and magpies) access to the hares in the pens. The enclosure was located in an isolated section of forest approximately 1 km from the main site of human activity.

### NATURAL MONITORING

Population densities of both the hares and the predators were measured, initially as part of the Kluane Boreal Forest Ecosystem Project (Krebs, Boutin & Boonstra 2001b) and thereafter as part of a monitoring study; here we present the data from 1994 to 2008 (two complete cycles). Snowshoe hare densities were estimated on two 36-ha grids. Live-traps were pre-baited with alfalfa cubes for 3–5 days before being set. Trapping sessions consisted of 2–3 nights of trapping within a 5-day period. Trapping did not occur on nights that dropped below ~20 °C. Population density was estimated with the program 
C\textsc{apture} (Otis et al. 1978) and the Jolly-Seber full model, as in previous studies (e.g. Krebs et al. 1995). No. 3 Monel tags.

Avian and mammalian predator populations fluctuate in synchrony with the hare cycle (Doyle & Smith 2001; O’Donoghue et al. 2001; Rohner, Doyle & Smith 2001). An index of the fluctuations in predator populations were obtained by using evidence from lynx and coyote data, as these are reflective of all other predators, including avian predators. We counted lynx and coyote tracks each winter (October through April) along a 25-km transect that traversed our study area, on days after fresh snowfalls while tracks were distinguishable. Track counts for lynx and coyote are highly correlated to their population density in this valley (lynx: \( r^2 = 0.95 \), coyote: \( r^2 = 0.88 \)) and thus give a reliable estimate of changes in predator density (O’Donoghue et al. 1997).

To standardize measurements across years, we monitored females’ cortisol concentration and reproduction during the first and second litters. To estimate reproductive output, we live-trapped hares one-week on either side of the average parturition dates (first litter May 25; second litter June 30; mean parturition dates estimated from O’Donoghue & Krebs 1992 and Stefan & Krebs 2001). Pregnant females \(( n = 30)\) were transferred to the outdoor enclosure and placed in a 60 × 60 × 120 cm chicken wire maternity cage until parturition (for details see O’Donoghue & Krebs 1992). Hares were held in the maternity cages for an average of 3 days and a maximum of 6 days. Hares were fed ad libitum with standard rabbit chow (Unifeed, Okotoks, AB, Canada; Unifeed Ltd Cat. #19-2103, 18% protein, crude fat 2%, crude fibre 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 trapped within the maternity cages and a faecal sample obtained. We recorded litter size and each leveret was sexed, weighed (Pesola spring scales ± 1 g), measured (RHF length – mm), and ear-tagged (No. 1 Monel tags). Families were then released back at the site of capture. Neonates were placed in a litter site created at the base of a willow, under a dead fall, or at the base of a cluster of trees so that the female could easily locate them (Stefan & Krebs 2001). Before releasing the family, all young were held up to the dam and she was released at the litter site after the young. Hares were transported to and from the enclosure in a burlap bag. This procedure did not affect mortality rates of dams as most released hares were re-trapped at a later date. As juveniles disperse at the time when they first enter the traps, it is difficult to assess their survival rate.

### EXPERIMENTAL MANIPULATION

A total of 26 pregnant hares (12 controls and 14 stressed) were live-trapped in the first week of May in 2006 and in 2007. Hares were transferred to the outdoor enclosure (as described above) and placed in individual 4 × 4 m chicken wire pens. Each pen was separated by a burlap covered wall to prevent hares from seeing each other. Control pens were separated from the stress pens by a black, heavy-duty, fabric cloth and a 4-m open corridor. Hares were fed as described above.

A trained dog was used to simulate a mammalian predator in the stress pens. We did this for two reasons. First, lynx and coyotes are responsible for approximately 60% of known hare predation (Krebs et al. 1995) and thus hares should have evolved to be acutely sensitive to a mammalian predator threat. Second, as it was critical that the simulated predator be under tight control, but both visually and olfactory evident, a highly trained dog (as opposed to a raptor) could be more easily handled and directed. The hares in the stress pens were separated from those in the control pens by a heavy black cloth and a 4-m open corridor. The dog was taken into each stress pen for 1–2 min every other day for the last 15 days of gestation. During an exposure, hares adjacent to the pen would remain hidden at the far end of their pen (4 m away). To ensure habituation did not occur (Dallman & Bhatnagar 2001), the dog was used at various times throughout the day and the order of exposure was randomized. The dog was trained not to bark or whine and did not physically contact any of the hares. The dog was not introduced once the females gave birth. Control hares had no contact, visually or physically, with the dog. Although they may have smelled it, control hares did not alter their behaviour during stress exposures. The same dog was used throughout the experiment.

Thirty hours after parturition, dams were live-trapped and a faecal sample taken. Reproductive measurements on the neonates were taken as above. Families were kept in the pens for an additional 28 days for a separate experiment. At the end of experimentation, all hares were released back to the site of their capture.

### FAECAL CORTISOL METABOLITE ANALYSIS

We used an enzyme immunoassay (EIA) to measure faecal cortisol metabolite (FCM) concentration, validated specifically for snowshoe
Fig. 1. Snowshoe hare, lynx, and coyote population density in the south-western Yukon, Canada from 1994 to 2008.

Fig. 2. FCM concentrations (means ± SE) of female snowshoe hares from the two studies. (a) The natural monitoring study 30 h after birth in litter 1 and 2 across 3 years. Dams from litter 1 (poled years) had elevated FCM concentrations compared with those from litter 2 (poled years) (P < 0.005). (b) The experimental manipulation at the time of field capture and 30 h after birth. There was no difference in FCM concentrations at capture (P > 0.05); however at birth, control dams had reduced FCM concentrations compared with stressed successful dams and with stressed unsuccessful dams (P < 0.0001).

Results

Natural Monitoring

Population density

The snowshoe hare population reached a peak of 0.92 hares ha⁻¹ in 2006 and declined to 0.79 and 0.35 hares ha⁻¹ in 2007 and 2008, respectively (Fig. 1). This population peak was considerably lower than the previous peak in 1998 of 1.98 hares ha⁻¹. The track index of mammalian predator populations (lynx and coyotes) was highest in 2007 with approximately 56 predator tracks counted per track night over a 100-km transect, compared with a previous peak of 117 tracks in 1999 (Fig. 1). The track index before the peak, 2006, and just after it, 2008, was approximately 31 and 35 predator tracks counted per track night over a 100-km transect, respectively. Thus, predators peaked 1 year after the hare peak.

Faecal cortisol metabolite concentration

To test for differences in FCM concentrations, we ran a two-way ANOVA (litter group × year). We found an effect of litter group (F₁,24 = 11.70, P < 0.005) but no effect of year (F₂,24 = 1.42, P > 0.05) or interaction between litter group and year (F₂,24 = 2.36, P > 0.05; Fig. 2a). Faecal cortisol metabolite concentrations in dams decreased 52% from the first litter (509.35 ± 107.22 ng g⁻¹ kg⁻¹, n = 15) to the second

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All data are expressed as means ± 1 SE, unless otherwise stated. ANOVAs and ANOVAs were performed using the software package STATISTICA 6. The assumption of normality was tested with Shapiro-Wilk test and the assumption of homogeneity of variances was tested with Levene's test. If these assumptions were not met, the appropriate adjustment was made (log-transformation of data or Greenhouse-Geisser adjustment; Quinn & Keough 2003). Comparisons of the means were considered significant if P < 0.05.
dams (effect of litter group as a continuous covariate. For litter size, we found an ancova way to test for differences in reproductive measures, we ran a two-way ANOVA (litter group × year) and included FCM concentration as a continuous covariate. For litter size, we found an effect of litter group \((F_{1,23} = 8.24, P < 0.01)\) and FCM concentration \((F_{1,23} = 4.47, P < 0.05)\), but no effect of year \((F_{2,23} = 3.37, P > 0.05)\) or interaction between year and litter group \((F_{2,23} = 1.62, P > 0.05)\). Litter size was 19% smaller in the first litter \((3.87 ± 0.22 \text{ young})\) compared with the second litter \((4.73 ± 0.18 \text{ young})\) and litter size was negatively correlated to FCM concentrations in dams (Fig. 3a).

For offspring body mass, we found an effect of litter group \((F_{1,23} = 13.55, P < 0.005)\) and FCM concentration \((F_{1,23} = 4.47, P < 0.05)\), but no effect of year \((F_{2,23} = 0.93, P > 0.05)\) or interaction between year and litter group \((F_{2,23} = 0.56, P > 0.05)\). Offspring had a 24% lower body mass in the first litter \((52.26 ± 298 \text{ g})\) compared with the second litter \((68.03 ± 2.80 \text{ g})\) and offspring body mass was negatively correlated to FCM concentrations in dams (Fig. 4a).

For offspring RHFW length, we found an effect of litter group \((F_{1,15} = 10.94, P < 0.005)\), year \((F_{1,15} = 8.38, P < 0.05)\), and FCM concentration \((F_{1,15} = 18.69, P < 0.001)\), but no interaction between litter group and year \((F_{2,15} = 0.014, P > 0.05)\). Offspring were 11% smaller in the first litter \((33.67 ± 1.22 \text{ mm})\) than in the second litter \((37.61 ± 0.83 \text{ mm})\), and 10% smaller in 2007 \((34.06 ± 1.04 \text{ mm})\) than in 2008 \((37.56 ± 1.14 \text{ mm})\). We did not measure RHFW length in 2006. Offspring RHFW length was negatively correlated to FCM concentrations in dams (Fig. 5a).

**EXPERIMENTAL MANIPULATION**

In the experimental manipulation, 11 out of 12 control hares and 9 out of 14 stressed hares gave birth to viable young. To test for differences in birth rate, we ran a Pearson chi-squared test and found that control hares had a significantly higher birth rate than stressed hares \((χ^2 = 4.91, P < 0.05)\). As the remaining five hares in the stressed group gave birth to preterm or stillborn young, they were considered unsuccessful, and included in the analysis of FCM concentration as a separate group referred to as unsuccessful stressed. They were not included in the reproductive analysis since they did not
give birth to viable young. A single control hare did not give birth and she was not included in any of the analyses.

**Faecal cortisol metabolite concentration**

To test for differences in FCW concentration at the time of capture and 30 h after birth, we ran an ANOVA. We found, at the time of capture, there was no difference in FCW concentration between control and stressed dams, and unsuccessful stressed dams ($F_{2,22} = 0.91, P > 0.05$; Fig. 2b). At the time of birth, FCW concentrations were significantly different in the three groups ($F_{2,22} = 19.20, P < 0.0001$; Fig. 2b). Control dams had FCW concentrations 54% and 89% lower than stressed dams (Tukey’s HSD post-hoc $P < 0.05$) and unsuccessful stressed dams (Tukey’s HSD post-hoc $P < 0.0005$), respectively. Stressed dams had FCW concentrations 75% lower than unsuccessful stressed dams (Tukey’s HSD post-hoc $P < 0.005$).

**Reproduction**

To test for differences in reproductive measures we ran a one-way ANCOVA, including FCW concentration as a continuous covariate. For litter size we found no effect of treatment ($F_{1,17} = 0.06, P > 0.05$) or FCW concentration ($F_{1,17} = 1.70, P > 0.05$). Control dams had a similar litter size (3.64 ± 0.24 young) compared with stressed dams (3.22 ± 0.28 young; Fig. 3b).

For offspring body mass we found an effect of treatment ($F_{1,17} = 11.68, P < 0.005$) and FCW concentration ($F_{1,17} = 13.96, P < 0.005$). Control dams gave birth to offspring with a 58% greater body mass ($57.23 ± 2.69$ g) compared with stressed dams ($36.16 ± 3.35$ g). Increasing FCW concentrations in dams were associated with a reduced offspring body mass (Fig. 4b).

For RHF length, we found an effect of treatment ($F_{1,9} = 10.59, P < 0.01$) and FCW concentration ($F_{1,9} = 5.29, P < 0.05$). Control dams gave birth to offspring with an 18% greater RHF length ($36.08 ± 0.86$ mm) compared with stressed dams ($30.57 ± 0.74$ mm). Increasing FCW concentrations in dams were associated with a reduced offspring RHF length (Fig. 5b).

**Discussion**

The snowshoe hare population in our study area peaked in 2006 and the predators peaked in 2007 (Fig. 1). In the natural monitoring study, we found that female FCW concentrations were higher at parturition of their first litter compared with that at their second (Fig. 2a). However, we did not find differences in female FCW concentrations between years. In the experimental manipulation study, we found that unsuccessful stressed dams (those that did not give birth to viable young) had the highest FCW concentrations followed by stressed dams and then control dams (Fig. 2b). In both studies, dams with higher FCW concentrations had lower reproductive fitness in terms of the number and quality of young (Fig. 3–5).

**Faecal cortisol metabolite concentrations in dams**

Many factors have been shown to affect stress levels in wild animals including density and social status, parasitism, food, and the risk of predation (Boonstra et al. 1998; Creel 2001; Chapman, Saj & Snaith 2007). It has long been recognized that high population densities could disrupt spacing behaviour and increase agonistic interactions and competition leading to an increase in stress and ultimately a decline in reproduction (Christian 1980). More recently, it has been shown that not only density but social status can influence GC concentrations and disrupt breeding (Creel 2001; Young et al. 2006). However, there is little evidence of this in hares. Boonstra et al. (1998) found that hares were less stressed living in experimentally fed populations whose densities were 4 to 13 times those of controls. Although, hares have been shown to display dominance hierarchies in pens and at feeding areas in the wild (Graf 1985), they are not territorial and have broadly overlapping home ranges (Boutin 1984). Furthermore, our results show that hares in 2006 (peak population) were not different from hares in 2008 (second year of the decline; Fig. 1 and 2a).
Parasites can also be important in shaping animal communities and have been shown to influence GC concentrations in mammals (Chapman et al. 2007). In snowshoe hares, Keith, Keith & Cary (1986) studied parasitism for many years in Alberta and concluded that the many parasites of hares were not a direct cause of mortality. Experimental work with anthelminthics in field populations of hares had no measurable impact on survival or reproduction (Sovell & Holmes 1996), or produced effects only in combination with predation and food (Murray, Cary & Keith 1997). Thus, parasitism may affect some hare populations but is likely not a direct factor affecting GC concentrations.

Food and predation are two of the greatest factors affecting animal populations and these have been shown to have interactive synergistic effects (Krebs et al. 1995, 2001a). In snowshoe hares, the change in FCM concentrations between litters (Fig. 2a) could be a result of first, an increase in food availability during the growing season, and second, a decrease in predation risk. The first litter occurs during the late winter–early spring when the winter snowpack is in the process of melting and before the flush of new vegetation, while the second litter occurs during late spring–early summer when new vegetative growth is nearing its peak (Sinclair, Krebs & Smith 1982). This change in quality and quantity of food could explain the differences in FCM concentrations.

Reduced food intake has been found to cause an increase in cortisol levels in mammals and birds (Harris et al. 1994; Kitaysky et al. 1999; Ortiz, Wade & Ortiz 2001). Second, the difference between the litter groups could be due to the decrease in the risk of predation from the first litter to the second. Boutin et al. (1986) found that predation rates decreased from winter to summer. Thus, the risk of predation should also decrease at this time. Since food quantity and quality also increase from the first litter to the second litter, an increase in food availability may also allow hares to forage in a less risk-prone manner (Hik 1995; Murray 2002). Likely, the effects of the increase in food availability and decrease in predation are not mutually exclusive and a combination of the two could explain the decline in FCM concentration from the first to the second litter.

We found that FCM concentrations were similar in the hare peak (2006) and decline (2007 and 2008; Fig. 2a), but predicted that as the risk of predation increased FCM concentrations in hares would also increase. 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. Our experimental results showed that females that gave birth to non-viable young, (either stillborn or aborted), had high FCM concentrations compared with females that gave birth (Fig. 2b). Furthermore, the number of females that gave birth to non-viable young increased during the decline phase (Stefan & Krebs 2001). Had we assessed FCM concentrations from all females within the population, not just the successful ones, we may have seen yearly differences.

In our experimental manipulation, stressed hares had elevated FCM concentrations compared with control hares (FCM concentrations were within the range found in the natural monitoring experiment; Fig. 2b). The extreme sensitivity of hares to predation risk is shown by the lack of habituation to repeated dog exposures. Within treatments, there was also considerable individual variation in FCM concentration (Fig. 2b). This suggests that individuals naturally differed in their physiological response and ability to cope with stressors. This is consistent with the results found by Pride (2005) for ringtailed lemurs, Lemur catta, and by Cabezas et al. (2007) for European wild rabbits, Oryctolagus cuniculus.

Together the natural monitoring and experimental manipulation studies argue that snowshoe hares are highly sensitive to changes in the risk of predation and that slight differences lead to measurable differences in FCM concentrations. Boonstra et al. (1998) found that plasma cortisol levels were higher during the decline phase of the hare cycle compared with the low phase and that this was due to the risk of predation that hares experience. We are currently investigating long-term, population level, seasonal and yearly changes in both plasma cortisol and FCM concentrations in response to the changes in the risk of predation during the snowshoe hare population cycle.

**REPRODUCTION**

In both the natural monitoring and experimental manipulation studies, we found that higher FCM concentrations in hares were associated with a decline in reproductive indices (Figs 3–5) and extremely high FCM concentrations were associated with non-viable births, either abortions or stillborn litters (Fig. 2b). Chronic exposure to elevated GC concentrations can have deleterious physiological consequences to reproduction by decreasing the amount of gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH) produced in the body (Ferin 1999; Owen, Andrews & Matthews 2005). Davis & Meyer (1973) found that in snowshoe hares, seasonal variation in gonadotropins paralleled seasonal changes in reproduction, and that there was a sharp decline in gonadotropin levels which coincided with a sharp decline in reproductive rates. We suggest that predator-induced changes in GC concentrations may be responsible for the changes seen in gonadotropin levels and ultimately the changes in reproduction.

However, the question remains as to why snowshoe hares would decrease their reproductive output when their chance of survival also decreases. There are three possible answers that we will discuss here. The first is that GC concentration affects survival and reproduction in a classic trade-off scenario. The second is that GC concentrations match offspring quality with the dam’s ability for maternal investment. The third is that GC concentrations help regulate maternal programming of the offspring.

A classic trade-off of reproduction for survival is easily understood in predator–prey relationships. As the risk of predation increases, prey species alter their behaviour to increase survival (Hik 1995; Lima 1998) and these changes
are modulated by the short-term release of GCs (Sapolsky et al. 2000; Wingfield & Kitaysky 2002). Although the magnitude of the stress response and a high concentration of GCs can negatively affect survival (Pride 2005; Blas et al. 2007), recent work has shown that at moderate levels chronic exposure to GCs can increase survival (Cote et al. 2006). Cabezas et al. (2007) found that in a free-ranging population of European wild-rabbits, long-term exposure to moderately elevated GC concentrations increased survival after the stressor was removed. Snowshoe hares are a good candidate for increasing GC concentrations in order to survive at the cost of reproduction. Hik (1995) found that snowshoe hares alter their behaviour in response to increasing predation risk, and we know that the stress response of hares is highly sensitive to changes in the risk of predation (shown above; Boonstra et al. 1998). However, the trade-off hypothesis is an unlikely evolutionary driving force in snowshoe hares. Hares have both a limited breeding period and a very poor survival rate. Snowshoe hares breed only during the summer months from May to August, and over 70% of the current year’s breeding population is made up of hares born the previous year (Krebs et al. 2001a). Furthermore, during the decline phase, adult survival rates actually decrease and can drop as low as 65% over 30 days (Krebs et al. 2001a). Since the gestation length for snowshoe hares is 35–37 days (Cary & Keith 1979), there is only a 42% chance of living long enough to give birth to a second litter and only a 23% chance of living long enough to give birth to a third litter. Thus, it would benefit snowshoe hares to maximize reproduction at the cost of an already low chance of survival (see Wingfield & Sapolsky 2003 for review).

Love & Williams (2008) recently suggested that maternally derived GCs could act as an adaptive mechanism linking maternal quality to offspring quality. The GC-induced matching of offspring phenotype could reduce the investment in current reproduction for low-quality mothers resulting in fitness gains through increased survival and future fecundity. Although we did not test this directly, we do not believe that it is occurring in the snowshoe hare. As mentioned above, hare survival rate is extremely low and thus mothers should maximize investment in their current reproduction. Furthermore, Hodges et al. (1999) found that body condition does not directly affect reproduction in snowshoe hares.

The adaptive advantage of maternal programming is easy to conceptualize. If a pregnant female is living in an environment where the risk of predation is high, it is beneficial that she transmit anti-predator behaviours to her offspring. It is logical that this signal is transmitted through the HPA axis, as it is not only responsible for the stress response but it is also associated with certain anti-predator behaviours such as fearfulness, anxiety, and vigilance (Meaney 2001; Seckl 2004). An increase in prenatal GC concentrations has also been shown to influence offspring dispersal and survival (Silverin 1997; Meylan et al. 2002; Meylan & Clobert 2005). Many studies have shown that the HPA axis is highly susceptible to permanent programming during early life development (e.g. Francis & Meaney 1999; Matthews 2002; Seckl 2004; Owen et al. 2005) and prenatally stressed offspring have been shown to have lower glucocorticoid and mineralocorticoid receptor expression in the hippocampus leading to elevated GC concentrations (Wright & Seckl 2001; Wingfield & Seckl 2001) as well as higher reactivity of the HPA axis to stressors (Hayward & Wingfield 2004). For snowshoe hares, maternal programming of offspring through elevated GC concentrations could be highly adaptive. Hares give birth to precocial young without a protective nest or burrow and do not stay with them after birth, returning only briefly each night to nurse (Keith & Windberg 1978). O’Donoghue (1994) showed that the proximate cause of mortality for offspring was predation and that 70% of juvenile mortality occurred within the first 5 days after birth, and 51% of litters had no survivors after 14 days. Thus, young are highly vulnerable to predation. As the risk of predation increases, the elevated GC concentrations of the dam could prenatally program the HPA axis of the offspring such that they are born with a greater stress response, increased vigilance and anxiety-like behaviours, and increased dispersal rates. Thus, elevated GC concentrations during times of high predation risk may cause a decline in reproductive output, but may ultimately increase fitness by promoting juvenile survival.

Here we have shown that increases in GC concentrations in individual dams are associated with a decline in reproduction. For snowshoe hares, these increased GC concentrations are likely explained by both food and predation; however, this is not the case for all species. GC concentrations can also be influenced by density and social status, parasitism, weather, and human activity among other things (Christian 1980; Wasser et al. 1997; Creel 2001; Romero & Wikelski 2001; Chapman et al. 2007). Since we found that elevated GC concentrations, not simply predation, are associated with a decline in reproduction, the type of stressor is essentially irrelevant. Our study has broad implications to all physiologically stressful situations.

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