



Physiology and behaviour of juvenile snowshoe hares at the start of the 10-year cycle

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Snowshoe hares, *Lepus americanus*, are the dominant prey species in the boreal forest and they undergo regular, predator-driven population cycles every 8–11 years. Previous research has found that hare stress physiology is highly sensitive to both within-year (seasonal/litter group) and across-year (cycle phase) variation in predation risk and that maternal–offspring stress profiles are tightly correlated. Thus, both pre- and postnatal environments may interact to shape offspring physiological phenotype. If changes in the function and reactivity of the maternal stress axis are associated with variation in offspring behaviours that increase survival during periods of high predation risk, then the maternal effects on offspring physiology seen previously could represent a mechanistic route of adaptive maternal programming. To examine the relationship between physiology and behaviour in snowshoe hares, we monitored free-ranging adult and juvenile stress hormone levels in the first and second litters of the breeding season and assessed open field exploratory behaviour in weaning-age and independent juveniles in the southwestern Yukon (Canada) during 2013–2015, when predator density was low but increasing. Thus, our study spanned the late low phase (2013) and the early increase phase (2014–2015) of the hare population cycle. We found that increased concentrations of faecal cortisol metabolites (FCMs) were associated with aspects of risk avoidance in weaning-age hares. Juveniles with higher stress hormone levels spent more time under cover and were less active during open field trials, highlighting a potential mechanistic route to allow individuals to sensitively cope with a changing environment. Although average FCM levels of breeding females and juveniles were not correlated with one another and litter-based differences in physiology and behaviour were not present during these low-risk phases of the cycle, the association between stress hormone levels and behaviour sets the stage for adaptive maternal effects on offspring behaviour and survival as the cycle progresses and predation risk intensifies.

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The parental experience of stressful environments prior to conception and during gestation can have profound developmental effects on offspring phenotype (Welberg & Seckl, 2001). In addition to postnatal stressors and environmental influences, exposure to maternal glucocorticoids in utero can programme offspring responses to stress (McGowan & Matthews, 2018; Thayer, Wilson, Kim, & Jaeggi, 2018), influencing their behaviour and coping

styles, including the ability to deal with environmental challenges and threats (Langenhof & Komdeur, 2018; Reddon, 2012; Weinstock, 2008). Developmental plasticity in behaviours that influence offspring survival is most likely to occur in environments where the type or intensity of a stressor varies over time (Mateo, 2014; Sheriff et al., 2017). In general, prenatal stress effects are expected to increase reactivity of the hypothalamic–pituitary–adrenal (HPA) axis (Clarke, Wittwer, Abbott, & Schneider, 1994; Schneider, Moore, Kraemer, Roberts, & DeJesus, 2002; St-Cyr & McGowan, 2015), decrease locomotion and increase vigilance and anxiety-like behaviour in mammals (Dickerson, Lally, Gunnell, Birkle, & Salm, 2005; Fride & Weinstock, 1988; Ward, Johnson, Salm, & Birkle, 2000; Wilcoxon & Redei, 2007). While these behaviours can be

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adaptive and increase survival in risky environments through reduced exposure and conspicuousness to predators, they may also impose fitness costs by limiting the time allocated to resource acquisition or mating opportunities. The relative influence of prenatal versus postnatal influences on offspring phenotype and the resulting impact on offspring fitness (net benefit versus cost) largely depend on the degree of similarity between the maternal environment and the environment that offspring encounter (Love, McGowan, & Sheriff, 2013; Sheriff & Love, 2013).

REPRODUCTION AND EARLY LIFE SURVIVAL

The 8–11-year population cycle of the snowshoe hare, *Lepus americanus*, dominates terrestrial community dynamics throughout Canada and Alaska's boreal forest (Krebs et al., 1995, 2018). Hare predators such as lynx, *Lynx canadensis*, coyotes, *Canis latrans*, and great-horned owls, *Bubo virginianus*, regulate hare demography via both direct mortality and nonconsumptive effects on reproduction (Boonstra, Hik, Singleton, & Tinnikov, 1998; Hodges, Krebs, Hik, Gillis, & Doyle, 2001). Total breeding season output varies from 6.9 young/female (over 2 litters) when predator density is high (the decline phase) to 18.9 young/female (over 3–4 litters) when predator density is low (the late low/early increase phase; Hodges et al., 2001; Stefan & Krebs, 2001). The changes in reproductive output across the cycle are the product of variation in both the number of litters (pregnancy rate) and litter size (Hodges et al., 2001). Snowshoe hares breed synchronously, have postpartum oestrus and have a gestation period of 36–39 days (Cary & Keith, 1979; Keith, Rongstad, & Meslow, 1966; Severaid, 1942). They produce precocial young that are born fully furred and ambulatory, and maternal care appears largely limited to a single nightly nursing bout (Graf & Sinclair, 1987; O'Donoghue & Bergman, 1992) until leverets are weaned at approximately 24–28 days of age (Rongstad & Tester, 1971).

This early life period is a precarious time. Littermates stay together for approximately 3–5 days after birth and during that time they survive or die together (O'Donoghue, 1994; O'Donoghue & Bergman, 1992). Nearly 70% of juvenile mortality occurs in the first 5 days after birth, primarily due to a specialist class of juvenile predators – red squirrels, *Tamiascurus hudsonicus*, and Arctic ground squirrels, *Urocitellus parryii* – which are the leading cause of mortality in very young hares (O'Donoghue, 1994). Squirrel predation accounts for the particularly poor survival probabilities of second-litter offspring, whose birth coincides with the timing of increased juvenile red and ground squirrel independence. All juvenile hares are extremely vulnerable to predation in early life, with markedly low 30-day survival probabilities across all litters (litter 1 = 0.46; litter 2 = 0.15; litter 3 = 0.43; O'Donoghue, 1994). While the influence of litter size and morphology on juvenile snowshoe hare survival has been investigated (O'Donoghue, 1994), the impact of individual variation in other aspects of offspring phenotype (whether shaped by prenatal or postnatal conditions, or both) that may influence early life fitness has not.

Physiological Sensitivity to Predation Risk

Monitoring of snowshoe hare stress physiology in the southwestern Yukon has revealed an acute sensitivity to predators (Boonstra et al., 1998; Sheriff, Krebs, & Boonstra, 2011). In reproductive adults, circulating levels of glucocorticoids are highest during the population decline phase, when predator numbers reach a maximum, and early in the breeding season, when both hare:lynx ratios and food quantity and quality are at their lowest. The first litter is born in late May when the weather is unpredictable, snow cover is likely and summer vegetation has yet to grow.

The second litter is born in late June, when there is abundant fresh vegetation and the relative risk of predation tends to be lower owing to dilution of individual risk from first-litter recruits. These differences produce offspring that are born to adults with markedly different stress profiles across the breeding season (i.e. from first to second and later litters). During the most recent hare population cycle, wild-caught breeding adults had faecal cortisol metabolite (FCM) concentrations (a noninvasive index of stress; Palme, 2019) that were 101% (males) and 62% (females) higher after the birth of the first litter than after birth of the second litter (Sheriff et al., 2011). Sheriff, Krebs, and Boonstra (2010a) also found that average FCM concentrations in free-ranging juvenile hares were tightly correlated with the average FCM concentrations of breeding dams in the population both across years and between litters, and that higher maternal stress hormone levels at birth were associated with increased reactivity of the stress axis of offspring at weaning. Critically, differences in faecal stress profiles of juveniles in first and second litters were consistent over time and maintained into the autumn, when both groups had reached adult size (3–4 months old) and were sharing the same postnatal environment (Sheriff et al., 2010a). Together these findings highlight the potential for lasting perinatal stress effects (parental, in utero and early postnatal influences) on offspring physiology in this species. If adult hormone concentrations accurately reflect fluctuations in predation risk and if variation in maternal stress levels during gestation are consistently 'inherited' by the offspring, then maternal physiological programming could translate to adaptive downstream offspring behaviours during periods of high environmental risk. We explore this relationship in snowshoe hares given the potential for individual variation in personality and behaviour (e.g. predator avoidance, risk aversion, boldness) to have pronounced fitness consequences in natural populations and influence large-scale ecological and evolutionary processes (Dingemanse & Réale, 2005; Wolf & Weissing, 2012).

We had two objectives. First, we wanted to assess population levels of FCMs – and specifically the relationship between breeding female and juvenile stress profiles – during the low and increase phases of the hare population, when the overall risk of predation is relatively low but increasing as the lynx population also starts to recover. Second, we examined the association between maternal/individual offspring stress hormone levels and ecologically relevant and potentially adaptive early life behaviour. This behaviour has not yet been examined in snowshoe hares, but is probably critical to juvenile survival and ultimately to population growth. We hypothesized that increased reactivity of the juvenile HPA axis in response to increasing predation risk (as a function of postnatal environment or via stress-mediated maternal programming of offspring physiology) would be associated with an adaptive, risk-averse juvenile behavioural phenotype. We tested this hypothesis by monitoring FCM levels in free-ranging reproductive adult and juvenile snowshoe hares, and conducted standardized open field tests (OFT) of behaviour on juveniles at two developmental time points: near weaning (~1 month old) and when fully independent (~2 months old). The open field test is a commonly used behavioural test for the assessment of general emotionality, boldness and exploratory behaviour (Réale, Reader, Sol, McDougall, & Dingemanse, 2007; Suarez & Gallup, 1981). It has been used to measure the behaviour of a variety of small mammals in natural and seminatural contexts, including red squirrels (Boon, Réale, & Boutin, 2007; Taylor, Boutin, Humphries, & McAdam, 2014), chipmunks (*Tamias striatus*; Careau et al., 2015), voles (*Microtus arvalis*; Eccard & Herde, 2013) and alpine marmots, *Marmota marmota* (Ferrari et al., 2013). Open field behaviour has been particularly well studied in rabbits (the sister-group to hares in the leporids family) in relation to reproductive state (García-Dalmán & González-

Mariscal, 2012; González-Mariscal, Albonetti, Cuamatzi, & Beyer, 1997), production (Kowalska, Bielanski, & Pietras, 2008), early development (Rödel, Bautista, Roder, Gilbert, & Hudson, 2017) and novel object response (Hoffman, Hernández Decasa, Beyer Ruiz, & González-Mariscal, 2010). We compared offspring born in the first and second litters of the breeding season, as we anticipated marked variation in parental stress levels (and thus maternal programming of offspring traits) due to differential food availability and relative predation risk between litters (Sheriff et al., 2011). This approach allowed us to test the following predictions.

(1) Faecal cortisol metabolite (FCM) concentrations in the breeding population fluctuate in synchrony with predation risk both among years (2013 = 2014 < 2015) and within breeding seasons (litter 1 > 2).

(2) Average breeding dam FCM levels are correlated with the average FCM levels of juveniles born to each litter group.

(3) Increased predator avoidance behaviours (decreased exploratory activity, more time spent under cover and heightened behavioural responses to predatory cues) are associated with higher juvenile FCM concentrations.

METHODS

Population Demography

Snowshoe hare abundance has been continuously monitored in the Shakwak Trench east of Kluane Lake, Yukon Territory (61°N, 138°W) for over 40 years (Krebs et al., 2001, 2018). Spring hare density was estimated in March/April 2013–2015 during 2–3 night mark–recapture trapping sessions on 36 ha grids (following methods detailed in Hodges et al., 2001). Absolute density estimates for each session were calculated from the maximum likelihood spatial estimator in Efford's Density v.4.4 program (Efford, Borchers, & Byrom, 2009). We estimated the annual abundance of lynx, the hares' main specialist predator, from a 25 km track transect that traverses our study area. Track counts were taken following fresh snowfall events during October–April in each year, an approach that yields a reliable index of predator density in this valley (O'Donoghue, Boutin, Krebs, & Hofer, 1997).

Breeding Population Stress Physiology

To establish stress hormone levels in the adult population, we live-trapped hares for 1 week on either side of mean parturition dates for first litters (24 May–7 June) and second litters (24 June–7 July) across three breeding seasons in the Kluane Lake region (2013–2015). Mean parturition dates were estimated from reproductive monitoring in previous hare population cycles (Stefan & Krebs, 2001; M. Sheriff, personal communication) and personal observations of weight change and reproductive status in females (palpation of developing embryos, mammary tissue development, onset of lactation; Keith, Meslow, & Rongstad, 1968; O'Donoghue & Krebs, 1992). We collected faecal samples from scrotal males and postpartum females during overnight trapping sessions (set 2200–0100 hours, checked starting at 0600 hours). Faecal cortisol metabolites (FCMs) are an easily measured, noninvasive index of stress and can provide an integrated assessment of glucocorticoid levels that is unaffected by the stress of trapping and handling if collected within 8 h of capture in this species (Sheriff, Bosson, Krebs, & Boonstra, 2009). Only clean faecal pellets uncontaminated by urine were selected and collected into 7 ml polypropylene tubes. Upon return to the research station (<4 h from time of collection in all cases) samples were stored in a –20 °C freezer and later transported to the University of Toronto (Ontario, Canada) on

dry ice. FCM concentrations were quantified for all samples in duplicate using an 11-oxoetiocholanolone enzyme immunoassay (EIA) developed by Palme and Möstl (1997) and validated specifically for snowshoe hares (Sheriff et al., 2009). Samples were freeze-dried for 14–18 h (FreeZone 4.5L Lyophilizer, LabConco, MO, U.S.A.), manually homogenized in liquid nitrogen, extracted for 30 min at 15 000 rpm on a multitube vortexer (60 mg of dried faeces in 1 ml of 80% methanol), then diluted (1:10) with assay buffer for analysis. EIAs had intra- and interassay coefficients of variation of 5.8% and 5.7%, respectively.

Juvenile Stress Physiology and Behaviour

We monitored stress hormone levels and behaviour of wild-caught juveniles in the first litter (L1) and the second litter (L2) at two developmental ages: leverets that were still nursing or recently weaned and juveniles that were fully independent. Given that hares show linear growth in the first 3 months of life, we estimated individual age from body weight at capture (Keith et al., 1968; O'Donoghue & Krebs, 1992). Mean parturition dates were similar across the study period, so we included animals in the 'Leveret' age group if they weighed 300–530 g during targeted capture sessions for each litter (L1: 13–20 June; L2: 17–26 July) and in the 'Independent' age group if they weighed 780–1030 g (L1: 17–26 July; L2: 16–22 August). Thus, Leverets were estimated to be 19–33 days old and Independents were estimated to be 49–65 days old. Traps were baited with an apple slice and rabbit chow. Upon capture, individuals were transferred to a burlap bag for handling and basic measurements were taken just prior to each juvenile's behavioural trial. We weighed individuals with a Pesola spring scale, measured the length of the right hindfoot (RHF) as an index of body size, assigned sex based on external anatomy and gave each an identifying eartag (Monel No. 3 tags, National Band and Tag, Newport, KY, U.S.A.). Faeces were collected from below each trap, and samples were handled as above to quantify faecal cortisol metabolite concentrations. As free-ranging adult females and juveniles were of unknown relatedness, group means were used to evaluate the relationship between population-level 'maternal' and juvenile stress hormone levels for each litter (Sheriff et al., 2010a).

We assessed juvenile behaviour during a 10 min modified open field test (OFT). We built a portable arena so that juveniles could be tested on-site immediately after checking traps (0600 hours onward). The arena (0.9 × 1.8 m and 1.2 m high) had corrugated plastic walls (Kowalska et al., 2008), a 25 cm chicken wire ledge covered in freshly collected natural browse along the inside of each wall and was set on natural forest floor (Fig. 1). A fresh piece of apple was placed in the centre of the arena for each trial. Thus, the juvenile had to decide whether to risk exposure in the open centre of the arena for access to a highly desired nutritional reward or to remain under cover and minimize the risk of detection by potential predators (Lockie, McAuley, Rawlinson, Guiney, & Andrews, 2017). Juveniles were first transferred from their handling bag to a corrugated plastic 'start box' that was then gently positioned inside the testing arena (see Fig. 1 inset). This box was cleaned with mild detergent and water between each trial, and the same experimental apparatus was used in all years. After a 2 min acclimation period, the start box door was lifted open by a researcher sitting a few metres away. Care was taken to ensure that the researcher was always positioned downwind of the testing arena and that their shadow was not visible to the individual within. The hare was then able to explore the arena for 10 min, at which point the experimenter would 'fly' a northern goshawk, *Accipiter gentilis*, silhouette (30 × 60 cm) over the arena to assess the juvenile's response to an ecologically relevant predator cue (Mueller & Parker, 1980; Yilmaz & Meister, 2013). All trials were recorded with an overhead digital



Figure 1. Portable open field test arena for assessment of wild juvenile snowshoe hare behaviour. The door of the start box was opened remotely and the hare was free to explore open and covered portions of the arena (see inset) during the 10 min trial.

video camera (GoPro Hero3, San Mateo, CA, U.S.A.) for later analysis. At the end of the test, juveniles were returned to their handling bag and released at their site of capture. We have no reason to believe the trials affected juvenile survival, as several of the individuals tested were recaptured throughout the summer.

Ethical Note

All experimental procedures were performed by trained individuals and were approved by the University of Toronto Animal Care Committee and the Yukon Territorial Government (WRP95, WRP120 and WRP141) in accordance with the guidelines of the Canadian Council for Animal Care. Standard operating procedures for live-trapping, capture and handling were followed to minimize stress and disturbance to experimental animals. Hares were handled gently and quietly at all times and were held in fitted breathable burlap bags during handling to obscure visual distractions, keep them calm and prevent injury. Hares and hare traps were kept in the shade and provided with cover and apple for hydration.

Analysis of Open Field Behaviour

Video files were analysed with JWatcher v.1.0 (Blumstein, Daniel, & Evans, 2016) and Tracker v.4.94 (Brown, 2017) open source behavioural software. We used continuous coding in JWatcher to determine the latency to emerge from the start box and to track the hare's position in the arena for the duration of the trial (start box, in the open, or under cover; see Fig. 1 inset). Because 'postemergence' trial length differed for all hares based on how quickly they left the start box, we used the first 3 min post-emergence to standardize measures of exploratory behaviour in the arena. For this portion of the trial, we calculated the proportion of time spent under cover (under ledge or back into start box) and assessed activity using the number of position transitions between the open and covered portions of the arena (frontlimbs and hindlimbs) as well as the distance travelled (m). The latter was calculated by marking coordinate positions of a reliable body landmark, the base of the rump, every 30 frames (1 s of video) to obtain the path length in Tracker. In two cases, individuals entered the arena when there were fewer than 3 min remaining in the test (101 s, 106 s). These were included in the analysis of the proportion of time spent under cover postemergence but excluded from analyses of distance travelled and position transition. Juvenile

responses to the hawk silhouette were qualitatively categorized as 'None' (showed no visible reaction), 'Mild' (flinched, froze or slowly moved to cover), or 'Severe' (ran for cover or tried to escape from arena) by a single observer, blind to the test subject's litter group and year.

Statistical Analyses

Data were tested for normality and homogeneity of variance assumptions using Shapiro–Wilk and Levene's tests, respectively. Logarithmic transformations were applied to achieve normality where necessary, and general linear model ANOVAs and calculations of effect size (partial Eta squared) were performed using SPSS v.25 statistical software (IBM, Armonk, NY, U.S.A.). All adults and juveniles were sampled only once across the study period, so all data were treated as independent samples. There was a significant interaction between year and sex in the breeding population's faecal cortisol metabolite (FCM) concentrations ($F_{2,129} = 5.48$, $P < 0.01$), so adult males and females were analysed separately, using two-way ANOVAs (year, litter) and Tukey's HSD post hoc analysis. For analyses of juvenile physiology and behaviour, we first tested the effect of sex or sex interactions (with year, litter, or age) on our variables of interest. None were statistically significant, so we pooled data sets from both sexes and analysed the data using three-way (year, litter, age class) ANOVAs. Categorical data were compared between groups using chi-square contingency tables with Fisher's exact test of significance, and correlations between variables using Pearson's correlation coefficient (r). Data were considered significant at $P < 0.05$, and effect sizes (partial η^2 : moderate effect > 0.06 , large effect > 0.14 ; Cohen, 1998) are reported for all tests where $P < 0.1$. Unless otherwise indicated, data presented are means \pm SE.

RESULTS

Hare and Lynx Population Dynamics

Our experiment started in the late low phase of the snowshoe hare cycle (2013) and continued for two increase phase years (2014 and 2015; Fig. 2). The spring density of hares (mean per km², 95% CI) approximately doubled in each year, from 12.5 (10.0–15.0) in 2013 to 21.2 (12.5–40.5) in 2014 and 49.1 (33.3–72.9) in 2015. Overwinter lynx density estimates were constant in the first 2 years (18.7 ± 3.6 mean tracks per track night/100 km (2012–2013) and 16.5 ± 3.8 (2013–2014)), but increased 132% (to 38.3 ± 5.9) in the winter of 2014–2015.

Stress Physiology

To obtain faecal samples from adult animals, live-trapping sessions targeted breeding hares following the birth of first and second litters. Samples were collected from 80 adult males in 2013, 2014 and 2015 (L1: $N = 8, 20, 17$; L2: $N = 4, 18, 13$) and from 61 adult females (L1: $N = 3, 10, 16$; L2: $N = 7, 12, 13$). Due to the low number of juvenile captures in 2013, we limited analyses of physiology and behaviour to 2014 and 2015 in leverets (L1: $N = 6, 5$; L2: $N = 5, 7$) and independents (L1: $N = 6, 4$; L2: $N = 7, 13$). Adults of both sexes showed variation in FCM concentrations among years (males: $F_{2,74} = 5.44$, $P < 0.01$, $\eta^2 = 0.13$; females: $F_{2,55} = 3.67$, $P = 0.03$, $\eta^2 = 0.12$), but there were no significant differences as a function of litter group (males: $F_{1,74} = 2.17$, $P = 0.15$; females: $F_{1,55} = 1.55$, $P = 0.22$) or year * litter interactions (males: $F_{2,74} = 1.20$, $P = 0.31$; females: $F_{2,55} = 0.15$, $P = 0.86$). Post hoc comparisons indicated that mean male FCM concentrations were similar in 2013 and 2014 but increased significantly from 2014 to 2015 ($P < 0.01$), whereas mean

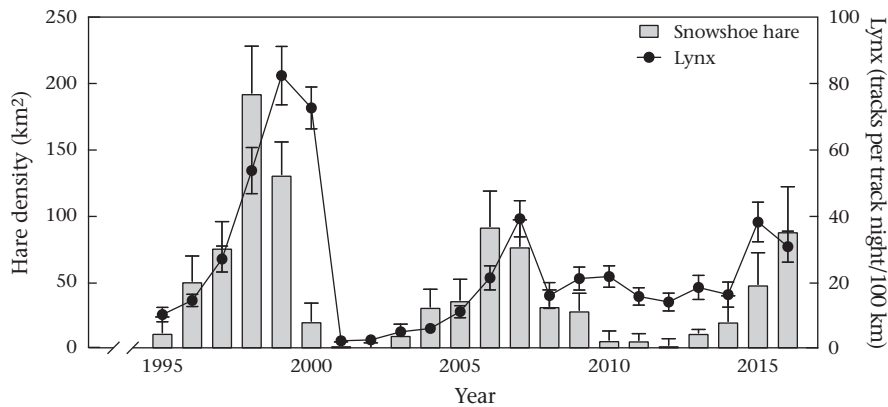


Figure 2. Changes in spring snowshoe hare density (mean hares/km², 95% CI) and overwinter track counts for lynx (mean tracks per track night/100 km \pm 1 SE) in the boreal forest of the southwestern Yukon during 1995–2016. Our study spanned the late low and increase phases of the hare population cycle (2013–2015).

female FCM concentrations increased significantly from 2013 to 2014 ($P = 0.01$) but were similar in 2014 and 2015 (Fig. 3). Thus, there appears to be no consistent pattern in breeding adult stress hormone levels as a function of increasing predator density across years, and seasonal variation in FCM (i.e. between litter groups) was negligible in both sexes.

Similarly, there was no effect of litter group ($F_{1,43} = 1.27$, $P = 0.27$) or year ($F_{1,43} = 0.20$, $P = 0.66$) on juvenile FCM concentrations. Leverets had significantly higher FCM concentrations than Independents ($F_{1,43} = 11.48$, $P < 0.01$, $\eta^2 = 0.21$). There was also a significant interaction of age * litter ($F_{1,43} = 5.81$, $P = 0.02$, $\eta^2 = 0.12$) but nonsignificant interactions of age * year ($F_{1,43} = 3.13$, $P = 0.08$, $\eta^2 = 0.07$) and year * litter ($F_{1,43} = 0.60$, $P = 0.44$; Fig. 4).

Mean FCM concentrations in breeding dams following the birth of each litter group were not correlated with mean FCMs in juveniles at either age (Leverets: $r_2 = -0.03$, $P = 0.49$; Independents: $r_2 = 0.45$, $P = 0.28$; Fig. 4). We also examined the correlation between breeding dam FCMs and Leveret FCMs during our study years relative to similar data from the most recent population cycle (Sheriff et al., 2010a). Although the relationship was relatively weak during the low and increase phases of the current hare cycle, the combined dataset showed a very strong overall relationship between breeding dam and leveret FCM across all phases ($r_{10} = 0.91$, $P < 0.01$; Fig. 5).

Juvenile Behaviour

Emergence from Start Box

Although there were no significant differences in the proportion of juveniles that left the start box as a function of either year

(2014 = 0.88, 2015 = 0.69; chi-square test: $\chi^2_1 = 2.58$, $P = 0.19$), litter (litter 1 = 0.90, litter 2 = 0.69; $\chi^2_1 = 3.42$, $P = 0.10$), or age class (Leveret = 0.87, Independent = 0.70; $\chi^2_1 = 2.14$, $P = 0.19$), a greater proportion of juveniles left the start box and emerged significantly sooner in 2014 than in 2015 ($F_{1,32} = 5.64$, $P = 0.02$, $\eta^2 = 0.15$; Fig. 6a). The latency to emerge did not differ as a function of litter ($F_{1,32} = 1.97$, $P = 0.17$), age class ($F_{1,32} = 1.90$, $P = 0.18$), or interaction effects (year * litter $F_{1,32} = 0.41$, $P = 0.53$; year * age $F_{1,32} = 0.04$, $P = 0.85$; litter * age $F_{1,32} = 1.86$, $P = 0.18$). These results suggest that juveniles were more hesitant to leave the relative safety of the start box in 2015.

Exploratory Behaviour

Of all factors investigated, age class had the most pronounced effect, and we found differences as a function of age on all three measures of exploratory behaviour. Leverets spent more time under cover than Independents ($F_{1,32} = 17.00$, $P < 0.01$, $\eta^2 = 0.35$; Fig. 6b). Independents showed greater levels of activity – travelling greater relative distances ($F_{1,30} = 3.59$, $P = 0.07$, $\eta^2 = 0.10$; Fig. 6c) and displaying a greater number of position transitions ($F_{1,30} = 6.47$, $P = 0.02$, $\eta^2 = 0.19$; Fig. 6d) in the first 3 min post-emergence. While there was a significant effect of year on the time spent under cover, with juveniles spending a greater proportion of their trials under cover in 2014 than in 2015 ($F_{1,32} = 8.92$, $P = 0.01$, $\eta^2 = 0.22$), neither measure of activity varied between years (distance: $F_{1,30} = 0.02$, $P = 0.88$; position: $F_{1,30} = 0.10$, $P = 0.75$). There were no significant differences between litters 1 and 2 (time under cover: $F_{1,30} = 0.61$, $P = 0.44$; distance: $F_{1,30} = 0.00$, $P = 0.95$; position: $F_{1,30} = 0.56$, $P = 0.46$) and no significant interaction effects for any of the behaviours examined.

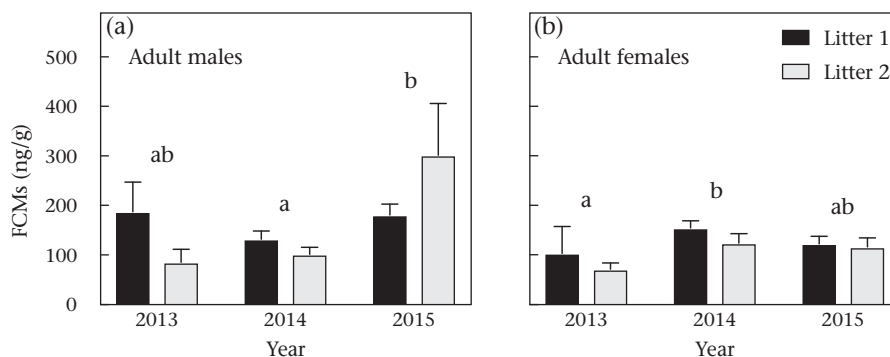


Figure 3. Mean faecal cortisol metabolite (FCM) concentrations (\pm 1 SE) in free-ranging adult (a) male and (b) female snowshoe hares following the birth of the first and second litters of the breeding season (2013–2015). Significant post hoc differences are denoted by different letters above the bars.

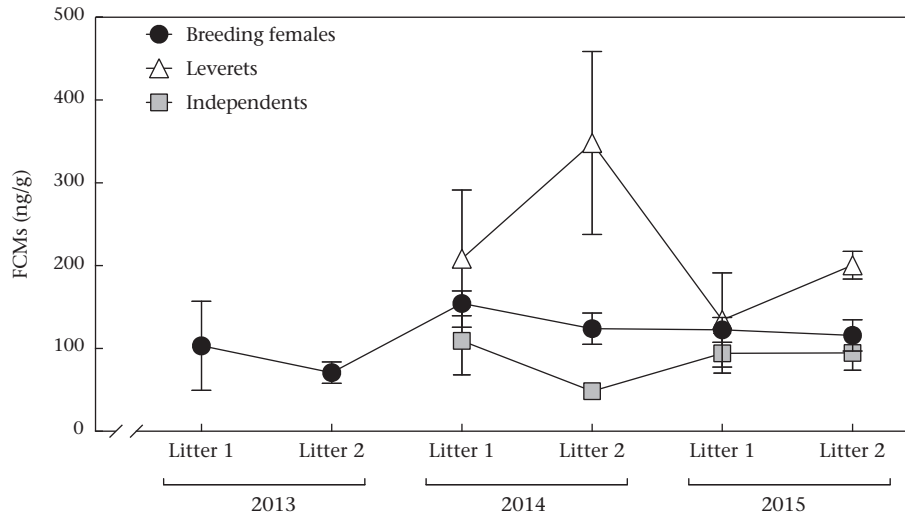


Figure 4. Mean faecal cortisol metabolite (FCM) concentrations (± 1 SE) in breeding females following the birth of first and second litters, and in first- and second-litter juveniles at ~ 1 month (Leverets) and at ~ 2 months of age (Independents). The snowshoe hare population was in the late low/early increase phase in 2013 and in the increase phase in 2014 and 2015.

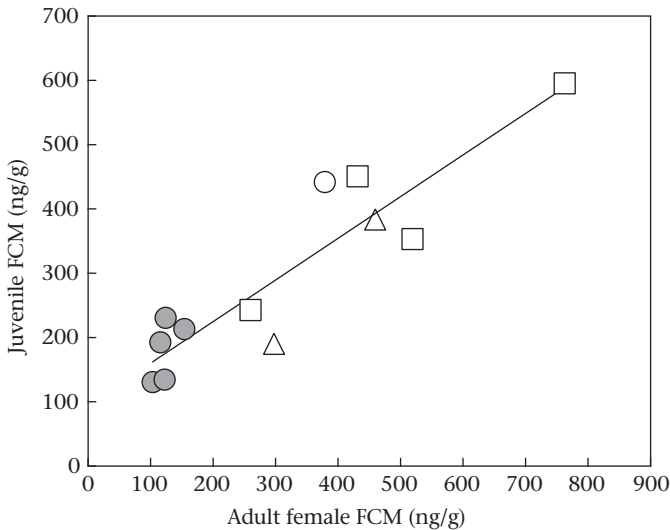


Figure 5. Mean faecal cortisol metabolite (FCM) concentrations in first- and second-litter offspring born during increase (circles), peak (triangles) and decline (squares) phases of the snowshoe hare cycle. Open symbols are data collected during 2005–2008 by Sheriff et al. (2010a), and shaded circles are data collected during 2013–2015). Identical trapping, sample preparation and analysis methods were used in both studies. Since the relatedness of wild individuals in the population was unknown, average dam and juvenile FCM concentrations were calculated for each litter group. Faecal samples were collected from live-trapped juveniles at approximate weaning age (25–31 days old based on body weight) and postpartum adult females within 1 week of mean parturition dates for first and second litters. Adapted from Figure 2 in Sheriff et al. (2010a).

Influence of Individual and Dam FCM Concentrations

Given the significant differences in FCM concentrations between age classes, we assessed the relationship between FCMs and behaviour in Leverets and Independents separately. Individual Leveret FCM concentrations were correlated with the proportion of time spent under cover during the first 3 min postemergence (Pearson correlation: $r_{18} = 0.54$, $P < 0.01$) and inversely correlated to our two measures of activity during the same period: the number of position transitions between the different zones ($r_{16} = -0.44$, $P = 0.03$) and the distance travelled in the open field

arena ($r_{16} = -0.46$, $P = 0.03$; Fig. 7). These patterns were no longer present in Independents (under cover: $r_{18} = 0.13$, $P = 0.30$; position transitions: $r_{18} = -0.13$, $P = 0.30$; distance travelled: $r_{18} = 0.02$, $P = 0.46$; Fig. 7). Thus, there appears to be a consistent relationship between individual FCMs and risk avoidance behaviour (seeking cover and decreased activity levels) in the earliest stage of life.

To examine population-level trends, we also examined correlations between average concentrations of breeding dam FCMs following the births of first and second litters in 2014 and 2015 with average measures of behaviour for juveniles born to each of those litter groups ($N = 4$). Mean dam FCM levels were positively correlated with the proportion of time spent under cover in both Leverets ($r_2 = 0.32$, $P = 0.34$) and Independents ($r_2 = 0.90$, $P = 0.06$), although the relationship only neared significance in the latter. In contrast, average dam FCM levels had less consistent relationships to average measures of juvenile activity, with opposite effects in each age class (Leverets: position transitions: $r_2 = 0.62$, $P = 0.19$; distance travelled: $r_2 = 0.88$, $P = 0.06$; Independents: position transitions: $r_2 = -0.51$, $P = 0.25$; distance travelled: $r_2 = -0.37$, $P = 0.32$).

Predator Cue Response

The hawk silhouette served as a salient risk cue, eliciting an immediate behavioural response (mild or severe) from nearly all juveniles tested ($N = 38$, 93%). Although nonresponders were spread across groups (Fig. 8), a significantly higher proportion of juveniles showed a severe response in first litters than in second litters (litter 1 = 0.84, litter 2 = 0.50; chi-square test: $\chi^2_1 = 5.31$, $P = 0.05$), and there was a nonsignificant tendency for a greater proportion of severe responses in the older age class (Leveret = 0.55, Independent = 0.76; $\chi^2_1 = 2.05$, $P = 0.20$). We found no difference in the proportion of juveniles showing severe behavioural responses to the predator cue as a function of year (2014 = 0.67, 2015 = 0.65; $\chi^2_1 = 0.01$, $P = 1.0$).

DISCUSSION

This study represents the first standardized test of snowshoe hare behaviour. We hypothesized that interindividual differences in activity and exploration are linked to variation in stress physiology, and thus sensitive to environmental stressors experienced in early

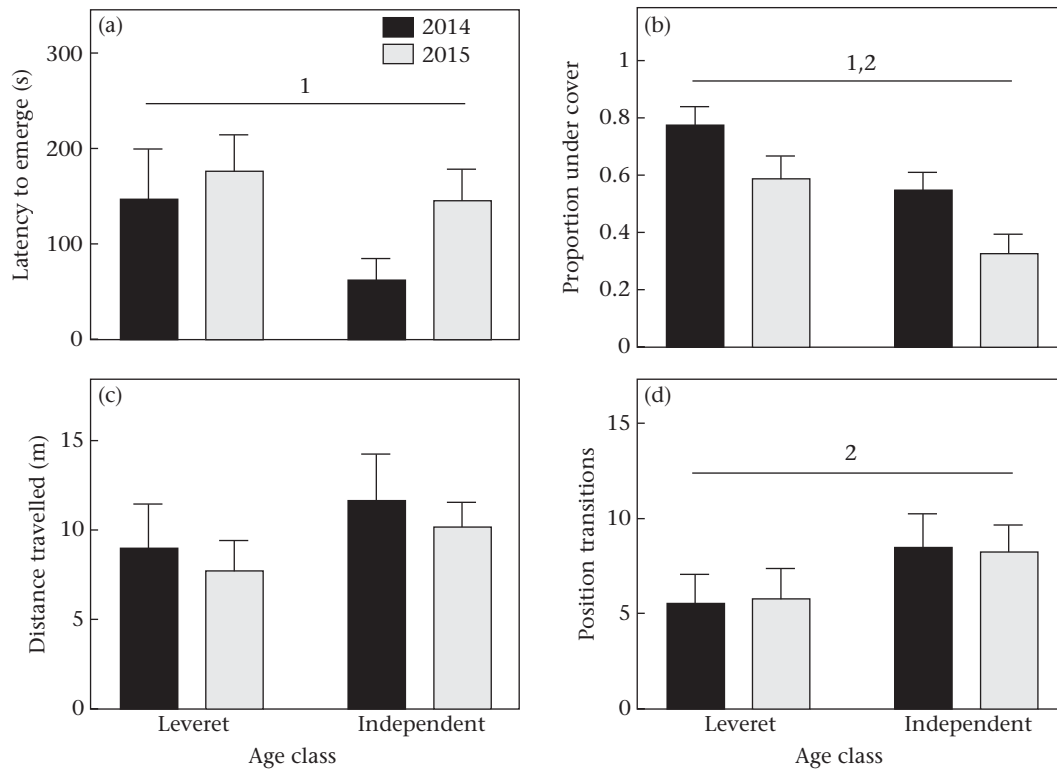


Figure 6. Exploratory behaviours of Leveret and Independent hares during open field tests. We measured (a) the latency to emerge from the start box, (b) the proportion of time spent under cover, (c) the relative distance travelled (m) and (d) the number of position transitions in the arena. Behaviours (b–d) were quantified for the first 3 min postemergence from the start box. All variables were tested for significant effects of litter, year (1) and age (2).

life. Programming of behavioural phenotypes that better allow individuals to cope with predation risk in early life (a critical and particularly vulnerable life stage) could have major implications on juvenile recruitment, which in turn affects population-level cyclical dynamics. We found that higher faecal cortisol metabolite (FCM) concentrations were associated with behavioural components of predator avoidance and a ‘risk-averse’ juvenile phenotype in early life. Leverets (within 1 week of weaning) increased the amount of time spent under cover and decreased their exploratory activity as a function of increasing cortisol concentrations (Fig. 7). The relationship between individual FCM levels and behaviour weakened by the time hares reached full independence (~2 months of age), but the average proportion of time spent under cover in this age class was strongly correlated with average FCM concentrations in the breeding dam population. These findings provide support for a link between HPA axis activity and juvenile behaviour in early life that could become an important mediator of juvenile survival, particularly as maternal effects and early postnatal conditions on offspring physiology converge and intensify under increasing predator pressure during the peak and decline phases of the snowshoe hare cycle.

Low and Increase Phase Stress Physiology

Contrary to our expectations, we found no consistent patterns of variation in average stress hormone levels during the low and early increase phases of the snowshoe hare cycle. In Prediction 1, we anticipated that FCM levels in the population would closely track annual variation in lynx density and show consistent differences between litters (Sheriff et al., 2011). Although breeding adults did show variation in FCMs across years, these effects were sex specific, with only breeding male FCMs fluctuating in synchrony with

predator density (Fig. 3a). Average juvenile FCMs did not vary between years, and neither adults nor juveniles showed significant differences in stress profiles between the first and second litters of the breeding season. Neither did our data bear out Prediction 2, failing to replicate the tight relationship between average dam and average juvenile FCM concentrations in the population seen previously (Sheriff et al., 2010a). However, when we compared the two most recent hare cycles directly (Fig. 5), it was evident that average stress hormone levels during our study were low relative to the late increase, peak and decline phase levels of the cycle studied by Sheriff et al. (2010a). Predator-induced maternal stress can have variable impacts depending on the frequency or intensity of exposure, and may need to reach a certain threshold or become chronic (as is seen during the peak and decline phases of the cycle) for convergent developmental programming effects on offspring HPA function to occur at a population-wide scale (McGowan & Matthews, 2018; Roff, 1996; Vitousek et al., 2018). Similarly, it would appear that the cumulative overwinter stress and nutritional effects that drive litter-based differences in breeding population stress levels and body condition (Sheriff et al., 2010a; Sinclair, Krebs, & Smith, 1982) are reduced when overall predator density is low, resulting in broadly similar average parental stress levels during the first and second litters at this stage of the cycle (Fig. 3). Comparison of our results with those of Sheriff et al. (2010a) suggests that, during the increase phase of the cycle, individual parental experience and postnatal offspring experience (rather than average predator density) are the primary drivers of variation in stress physiology, and thus no strong correlations are seen between average adult and juvenile FCMs in the population. Later in the cycle, risk intensifies, leading to strongly convergent stress effects across the population and increased synchrony in parent–offspring programming effects.

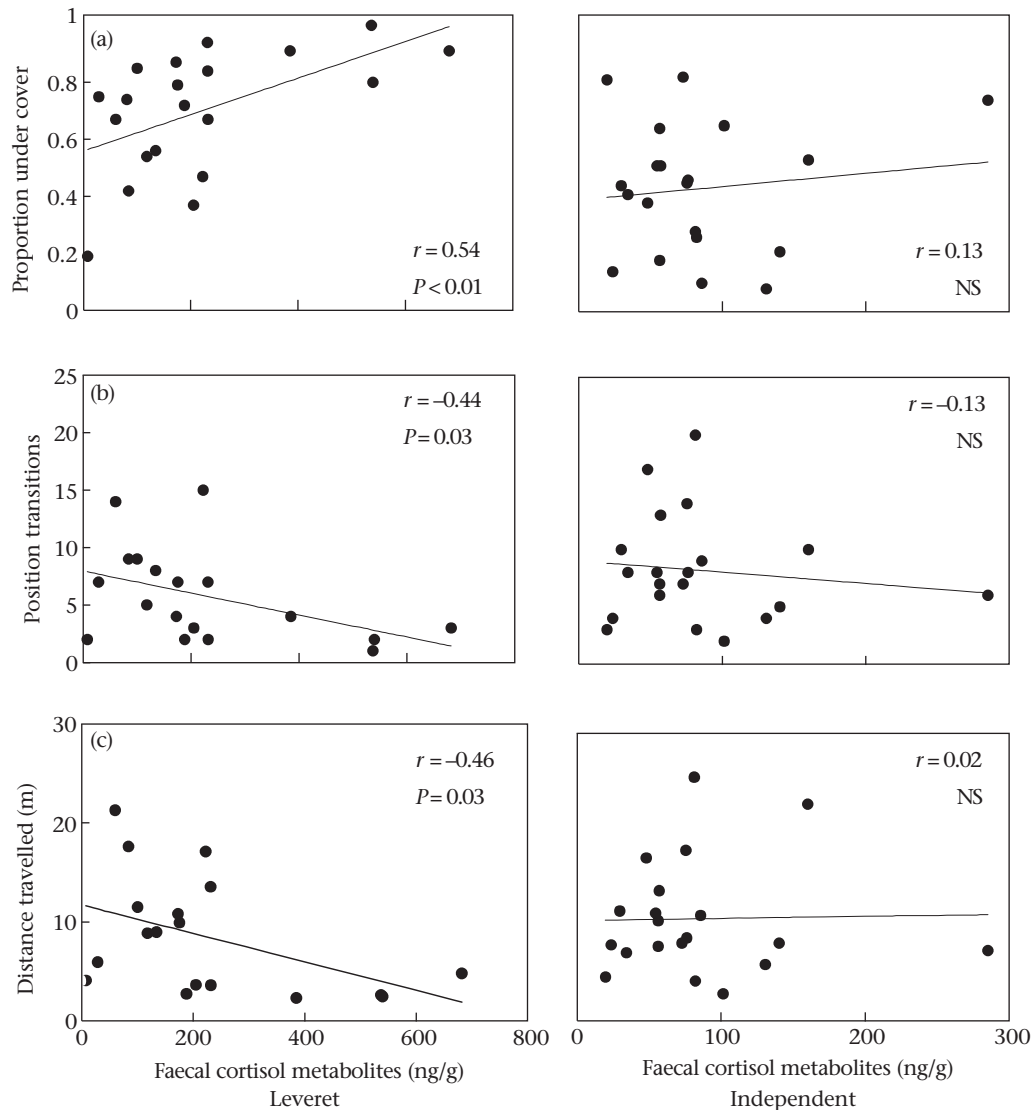


Figure 7. Correlations between faecal cortisol metabolite concentrations and open field exploratory behaviour in Leveret and Independent juvenile snowshoe hares. Three behaviours were assessed during the first 3 min postemergence: (a) the proportion of time spent under cover, (b) the number of position transitions among zones of the arena and (c) the relative distance travelled (m) during the trial (see text for details).

Interpreting Open Field Behaviour

We provide evidence for a significant relationship between FCM concentrations and risk-averse behaviours in weaning-age juveniles (Prediction 3). Measures of exploration and activity during standardized personality tests, or the tendency to investigate a novel or unfamiliar environment, are associated with boldness and risk-taking behaviour in variety of species (e.g. Dammhahn & Almeling, 2012; Mikics, Barsy, Barsvári, & Haller, 2005; van Oers, Klunder, & Drent, 2005). Natural monitoring of wild snowshoe hares suggests that variation in behaviour can have important repercussions on survival. Hares have been shown to adopt a predator-sensitive foraging strategy under periods of high predation risk (Hik et al., 1995; Wolff, 1980), increasing their use of closed forest habitats that are safer but of poorer food quality (Hik, 1994). A study of radiocollared hares by Rohner and Krebs (1996) found that owls kill hares more frequently in open habitat than in closed forest, and preferentially kill younger (but not necessarily the smallest) juveniles. They argue that variation in antipredator behaviour is the most likely explanation for differential mortality in

that age class. Our findings thus highlight a potential and directly translatable fitness consequence for the individual differences in time spent under cover during juvenile open field trials, if the behaviours seen therein are consistent between experimental and natural contexts. While a single behavioural trial allows for a comparative approach across groups, establishing a true measure of individual temperament or personality would require repeated capture and testing of the same individuals over time or across situations (Gosling, 2001; Réale et al., 2007; Sih, Bell, & Johnson, 2004). This approach is particularly challenging in wild species, and so our understanding of how behaviour quantified in the laboratory or under artificial scenarios translates to natural and ecologically relevant contexts is limited (Arvidsson, Adriaensen, van Dongen, De Stobbeleere, & Matthysen, 2017; Niemelä & Dingemanse, 2014). Nevertheless, there is evidence for consistent individual variation in behaviour across captive and wild contexts (e.g. Cole & Quinn, 2014; Fisher, James, Rodríguez-Muñoz, & Tregenza, 2015; Herborn et al., 2010; Yuen, Pillay, Heinrichs, & Schradin, 2016), and a meta-analysis on the repeatability of behaviour by Bell, Hankinson, and Laskowski (2009) provides

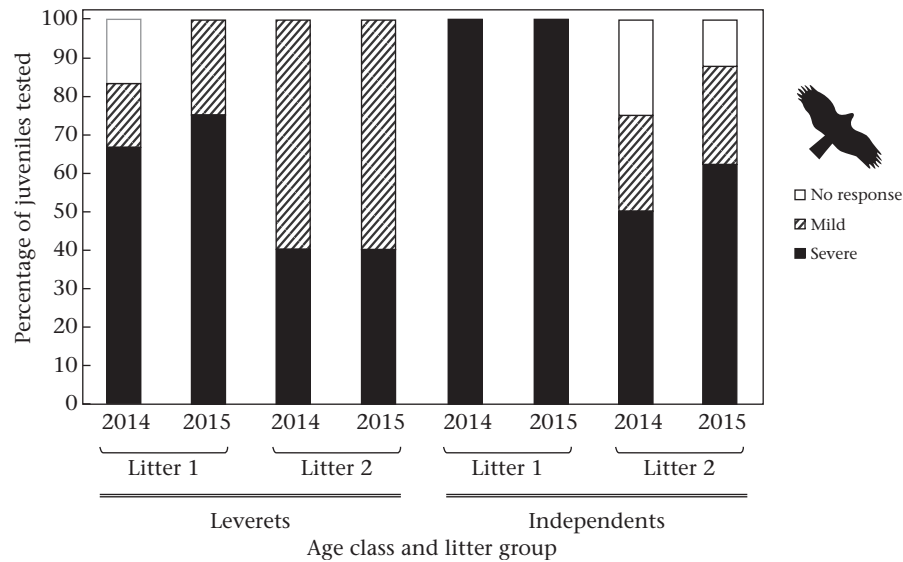


Figure 8. Juvenile hare responses to a predator cue (hawk silhouette) during open field behavioural tests. Responses were classified as ‘None’ when no behavioural response occurred, as ‘Mild’ when flinching or a slow movement to cover occurred and as ‘Severe’ when darting for cover or attempts to escape from the testing arena occurred.

strong support for consistent individual differences in behaviour across taxa, with higher repeatability in wild populations than in captive populations.

Influence of Year, Litter and Age on Juvenile Behaviour

Given the lack of strong differences in stress profiles across the breeding season, it is perhaps unsurprising that we found limited evidence for strong litter-based differences in juvenile behaviour. A notable exception was the proportion of juveniles showing severe behavioural responses to the hawk silhouette, which was significantly greater in first-litter offspring than in second-litter offspring (Fig. 8). Although our study design allowed for the comparison of behaviour between age-matched litters, there was inherent temporal variation in when each litter reached testable age throughout the summer. While it is unlikely that aspects of the environment that could influence the detection of overhead visual cues varied significantly across testing sessions (density of overhead canopy cover similar June–August), differences in response to the predator cue between litters may be attributable to experience and learning from differential postnatal exposure to risk and potential stressors. It is possible that first-litter offspring that survived to weaning and independence were a subset of all first-litter offspring. The survivors showed heightened behavioural responses to predator cues (bolting, escape, etc.), and those that failed to do so early in the breeding season (when predator:prey ratios were at their highest) had already been eliminated from the population. Alternatively, given that second-litter snowshoe hare births coincide with the timing of increased independence of red squirrel and ground squirrel juveniles (O’Donoghue, 1994), their early life experiences of risk or predator interactions may have overwhelmingly been with small mammal predators, resulting in weaker responses to alternative predators, such as birds of prey. That a similar trend for more severe responses to the hawk was seen in Independent-age hares relative to Leverets (Fig. 8) also suggests that there may be some degree of learning involved in the detection and response to predators. This result might also reflect alternative strategies based on physical ability, with older, more mature hares having increased escape ability. However O’Donoghue and Bergman (1992) found that although very young hares freeze when approached, juveniles

more than 10 days old tend to flee from danger. Nevertheless, the almost ubiquitous perception/behavioural response to the predator cue across ages and groups (whether mild or severe) may also indicate a degree of innate response to predator cues that is independent of personal experience, given that failure to do so would result in death and thus be heavily selected against (Tinbergen, 1953).

On the whole, the most pronounced group differences in open field exploratory behaviour were those seen between age classes, with Independents being more active (travelling greater distances and changing positions among the different zones of the arena more frequently) and spending less time under cover than Leverets (Fig. 6). While this result is in keeping with the general positive relationship expected between the degree of physical maturation and activity (Rödel et al., 2017), the older juveniles were also characterized by lower mean FCM concentrations than Leverets (Fig. 4). This is consistent with a bolder, more exploratory behavioural profile in novel environments. The pattern of year effects on behaviour was less consistent. Juveniles that emerged from the start box took significantly longer to do so in 2015 than in 2014 (Fig. 6a). It has been suggested that latency and activity levels are behavioural components of prey conspicuousness to potential predators and are thus meaningful measures of antipredator behaviour (Eccard & Herde, 2013). This cycle’s first year of significant increase in lynx density in the valley was 2015 (Fig. 2), and increased behavioural ‘caution’ might be a response to the increase in environmental risk if juveniles are able to perceive that the environment has changed, either via direct encounters or through sensitivity to predator cues (scent, vocalizations, conspecific alarm cues, etc.) in the environment (Lima & Dill, 1990; Weissburg, Smeed, & Ferner, 2014). However, a contradictory pattern is seen when considering the amount of time spent under cover between years: juveniles that took longer to emerge in 2015 also spent more time out in the open thereafter than did juveniles in 2014 (Fig. 6b). The low overall stress levels during our study period meant that group-based comparisons of behaviour were more challenging to interpret, and additional drivers of variation may need to be considered during these phases, such as weather or hyperlocal predation risk (i.e. resident bird of prey nest).

Caveats

There are a few caveats to consider in the interpretation of these findings. The first is that we aged and categorized hares based on a linear growth curve for wild hares (O'Donoghue & Krebs, 1992), but major differences in body condition between individuals could have a skewing effect. We controlled for this by confirming that overall distributions for weight and right hindfoot length (index of skeletal size) were not significantly different between litters or years. Given that hares do not use nests or burrows, finding newborn litters in the wild is extremely difficult (Graf & Sinclair, 1987; Severaid, 1942). Thus, capture and testing of wild individuals of known birth date could potentially be accomplished via a maternity cage technique (O'Donoghue & Krebs, 1992; Stefan & Krebs, 2001) to tag newborn litters and attempt recapture later in life. This approach could provide valuable life history information on focal individuals, as early life parameters such as litter size, birth mass and growth rate can all influence juvenile behaviour (Dimitsantos, Escorihuela, Fuentes, Armario, & Nadal, 2007; Guenther, 2018; Rödel et al., 2017). Although we do not expect those life history variables to have major impacts at the population level relative to the effects of predation risk during the population peak and decline (which should be sizable and affect individuals similarly), they could have a relatively greater influence on experimental outcomes during periods of low predator abundance and risk. Although methodologically challenging, particularly at low hare densities, the use of maternity cages would allow us to examine related mother–offspring dyads, instead of the rather coarse population-level correlations used here. This approach would provide a much finer-scale investigation of the impacts of variation in maternal glucocorticoids during the perinatal period on offspring physiology and behaviour and greater power to detect adaptive programming effects therein. Furthermore, knowledge of maternal characteristics and reproductive histories that can affect offspring condition (e.g. age: Monclús, Tiulim, & Blumstein, 2011; lactating only versus lactating and pregnant: González-Mariscal, Gallegos, Sierra-Ramírez, & Garza Flores, 2009) could also help disentangle interlitter variation and pre- and postnatal maternal influences on juvenile physiology and behaviour.

The second caveat is that we have related juvenile behaviour in response to stressors (novel environment, predator cue) with a single physiological index that reflects basal precapture levels of circulating cortisol concentrations (Sheriff, Krebs, & Boonstra, 2010b). A meta-analysis of prenatal stress effects on the developing HPA axis in vertebrates found that the most pronounced effects were on glucocorticoid recovery (e.g. impaired negative feedback of the HPA axis) rather than programming of either basal levels or peak stress responses (Thayer et al., 2018). Thus, it may be that our analysis did not encompass the most salient physiological predictors of behaviour. Nevertheless, cautious (low exploration) behavioural phenotypes have been associated with both higher basal and stress responses of the HPA axis in rats (Koolhaas, de Boer, Coppens, & Buwalda, 2010), and higher FCM concentrations have been associated with higher levels of vigilance in response to a novel object in wild Richardson's ground squirrels, *Urocitellus richardsonii* (Clary et al., 2014), and greater mobilization of cortisol in response to an adrenal stimulation challenge in adult snowshoe hares (Sheriff et al., 2010b). Thus, in addition to the ethical and methodological advantages of noninvasive sampling that is unbiased by the stress of capture and handling, quantification of faecal cortisol metabolite levels appears to provide a meaningful profile of stress physiology in relation to behaviour. Future work could include collection of peripheral blood (Schöpfer, Palme, Ruf, & Huber, 2012; Stein & Bell, 2014) or saliva (Nemeth, Pschernig, Wallner, & Millesi, 2016; Shin & Shin, 2016) following handling

and behavioural testing to capture a glucocorticoid index that also incorporates both stress reactivity and recovery (negative feedback) of the HPA axis following a challenge.

Conclusions

Our objectives were to assess the relationship between an index of stress physiology (FCMs) and behaviour in juvenile snowshoe hares and between dam and juvenile FCMs from the late low to the early increase phases of the hare cycle (as predator density also increases). We aimed to determine whether the glucocorticoid-mediated maternal effects on offspring physiology seen previously (Sheriff et al., 2010a) were associated with behaviours that could increase the probability of survival in high predation environments, and thus represent a mechanistic route of adaptive maternal programming (Mousseau & Fox, 1998; Sheriff & Love, 2013). While overall levels and the correlation between breeding dam and juvenile FCMs were low during our study, we found that stress hormone levels were correlated with levels of activity and hiding behaviour in weaning-age snowshoe hares. Coupled with earlier evidence of hare sensitivity to fluctuating predator density, as well as the maternal 'programming' of offspring stress reactivity, the relationships between heightened juvenile FCM levels and cautious behaviour (decreased activity, more time under cover) seen here suggest that highly stressed mothers would be likely to produce 'predator-sensitive' offspring that are predisposed to explore their environment in a risk-averse manner. Our findings lay the groundwork for stronger maternal programming effects on offspring physiology as predation intensifies during peak and decline phases of the hare cycle, which could decrease offspring susceptibility to predatory encounters when risk is high. Connecting behavioural phenotypes to direct fitness outcomes will be key in testing this adaptive maternal programming hypothesis, and future work should examine individual juvenile behaviour in the peak and decline phases of the cycle with concurrent monitoring of survival. As the relative impacts of pre- and postnatal influences on offspring appear variable across the hare cycle, further investigation of these interacting effects at multiple phenotypic levels of integration as the cycle progresses is warranted.

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References

- Arvidsson, L. K., Adriaensen, F., van Dongen, S., De Stobbeleere, N., & Matthysen, E. (2017). Exploration behaviour in a different light: Testing cross-context consistency of a common personality trait. *Animal Behaviour*, 123, 151–158. <https://doi.org/10.1016/j.anbehav.2016.09.005>.
- Bell, A. M., Hankinson, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: A meta-analysis. *Animal Behaviour*, 77, 771–783. <https://doi.org/10.1016/j.anbehav.2008.12.022>.
- Blumstein, D. T., Daniel, J. C., & Evans, C. S. (2016). *JWatcher (Version 1.0)*. <http://www.jwatcher.ucla.edu>.
- Boon, A. K., Réale, D., & Boutin, S. (2007). The interaction between personality, offspring fitness and food abundance in North American red squirrels. *Ecology Letters*, 10, 1094–1104. <https://doi.org/10.1111/j.1461-0248.2007.01106.x>.

- Boonstra, R., Hik, D., Singleton, G. R., & Tinnikov, A. (1998). The impact of predator-induced stress on the snowshoe hare cycle. *Ecological Monographs*, 79, 371–394. <https://doi.org/10.2307/2657244>.
- Brown, D. (2017). *Tracker* (Version 4.94). <https://physlets.org/tracker/>.
- Careau, V., Montiglio, P. O., Garant, D., Pelletier, F., Speakman, J. R., Humphries, M. M., et al. (2015). Energy expenditure and personality in wild chipmunks. *Behavioral Ecology and Sociobiology*, 69, 653–661. <https://doi.org/10.1007/s00265-015-1876-2>.
- Cary, J. R., & Keith, L. B. (1979). Reproductive change in the 10-year cycle of snowshoe hares. *Canadian Journal of Zoology*, 57, 375–390. <https://doi.org/10.1139/z79-044>.
- Clarke, A. S., Wittwer, D. J., Abbott, D. H., & Schneider, M. L. (1994). Long-term effects of prenatal stress on HPA axis activity in juvenile rhesus monkeys. *Developmental Psychobiology*, 27, 257–269. <https://doi.org/10.1002/dev.420270502>.
- Clary, D., Skyner, L. J., Ryan, C. P., Gardiner, L. E., Anderson, W. G., & Hare, F. J. (2014). Shyness–boldness, but not exploration, predicts glucocorticoid stress response in Richardson's ground squirrels (*Urocitellus richardsonii*). *Ethology*, 120, 1–9. <https://doi.org/10.1111/eth.12283>.
- Cohen, J. (1998). *Statistical power analysis for the behavioural science* (2nd ed.). Hillsdale, NJ: L. Erlbaum.
- Cole, E. F., & Quinn, J. L. (2014). Shy birds play it safe: Personality in captivity predicts risk responsiveness during reproduction in the wild. *Biology Letters*, 10, 20140178. <https://doi.org/10.1098/rsbl.2014.0178>.
- Dammhahn, M., & Almeling, L. (2012). Is risk taking during foraging a personality trait? A field test for cross-context consistency in boldness. *Animal Behaviour*, 84, 1131–1139. <https://doi.org/10.1016/j.anbehav.2012.08.014>.
- Dickerson, P. A., Lally, B. E., Gunnell, E., Birkle, D. L., & Salm, A. K. (2005). Early emergence of increased fearful behaviour in prenatally stressed rats. *Physiology & Behavior*, 86, 586–593. <https://doi.org/10.1016/j.physbeh.2005.08.025>.
- Dimitsantos, E., Escorihuela, R. M., Fuentes, S., Armario, A., & Nadal, R. (2007). Litter size affects emotionality in adult male rats. *Physiology & Behavior*, 92, 708–716. <https://doi.org/10.1016/j.physbeh.2007.05.066>.
- Dingemans, N. J., & Réale, D. (2005). Natural selection and animal personality. *Behaviour*, 142, 1165–1190. <https://doi.org/10.1163/156853905774539445>.
- Eccard, J. A., & Herde, A. (2013). Seasonal variation in the behaviour of a short-lived rodent. *BMC Ecology*, 13, 43. <https://doi.org/10.1186/1472-6785-13-43>.
- Efford, M. G., Borchers, D. L., & Byrom, A. E. (2009). Density estimation by spatially explicit capture–recapture: Likelihood-based methods. In D. L. Thomson, E. G. Cooch, & M. J. Conroy (Eds.), *Modeling demographic processes in marked populations* (pp. 255–269). New York, NY: Springer.
- Ferrari, C., Pasquarotta, C., Carere, C., Cavallone, E., von Hardenberg, A., & Réale, D. (2013). Testing for the presence of coping styles in a wild mammal. *Animal Behaviour*, 85, 1385–1396. <https://doi.org/10.1016/j.anbehav.2013.03.030>.
- Fisher, D. N., James, A., Rodríguez-Muñoz, R., & Tregenza, T. (2015). Behaviour in captivity predicts some aspects of natural behaviour, but not others, in a wild cricket population. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20150708. <https://doi.org/10.1098/rspb.2015.0708>.
- Fride, E., & Weinstock, M. (1988). Prenatal stress increases anxiety related behaviour and alters cerebral lateralization of dopamine activity. *Life Sciences*, 42, 1059–1065. [https://doi.org/10.1016/0024-3205\(88\)90561-9](https://doi.org/10.1016/0024-3205(88)90561-9).
- García-Dalman, C., & González-Mariscal, G. (2012). Major role of suckling stimulation for inhibition of estrous behaviors in lactating rabbits: Acute and chronic effects. *Hormones and Behavior*, 61, 108–113. <https://doi.org/10.1016/j.yhbeh.2011.11.003>.
- González-Mariscal, G., Albonetti, M. E., Cuamatzi, E., & Beyer, C. (1997). Transitory inhibition of scent marking by copulation in male and female rabbits. *Animal Behaviour*, 53, 323–333. <https://doi.org/10.1006/anbe.1996.0327>.
- González-Mariscal, G., Gallegos, J. A., Sierra-Ramírez, A., & Garza Flores, J. (2009). Impact of concurrent pregnancy and lactation on maternal nest-building, estradiol and progesterone concentrations in rabbits. *World Rabbit Science*, 17, 145–152. <https://doi.org/10.4995/wrs.2009.654>.
- Gosling, S. D. (2001). From mice to men: What can we learn about personality from animal research? *Psychological Bulletin*, 127, 45–86. <https://doi.org/10.1037/0033-2909.127.1.45>.
- Graf, R. P., & Sinclair, A. R. E. (1987). Parental care and adult aggression toward juvenile snowshoe hares. *Arctic*, 40, 175–178. <https://doi.org/10.14430/arctic1763>.
- Guenther, A. (2018). Life-history trade-offs: Are they linked to personality in a precocial mammal (*Cavia aperea*)? *Biology Letters*, 14, 20180086. <https://doi.org/10.1098/rsbl.2018.0086>.
- Herborn, K. A., Macleod, R., Miles, W. T. S., Schofield, A. N. B., Alexander, L., & Arnold, K. E. (2010). Personality in captivity reflects personality in the wild. *Animal Behaviour*, 79, 835–843. <https://doi.org/10.1016/j.anbehav.2009.12.026>.
- Hik, D. S. (1994). *Predation risk and the snowshoe hare cycle* (Ph.D. thesis). Vancouver, BC: University of British Columbia.
- Hik, D. S. (1995). Does risk of predation influence population dynamics? Evidence from the cyclic decline of snowshoe hares. *Journal of Wildlife Research*, 22, 115–129. <https://doi.org/10.1071/WR9950115>.
- Hodges, K. E., Krebs, C. J., Hik, D. S., Gillis, E. A., & Doyle, C. E. (2001). Snowshoe hare dynamics. In C. J. Krebs, S. Boutin, & R. Boonstra (Eds.), *Ecosystem dynamics of the boreal forest. The Klane Project* (pp. 141–178). New York, NY: Oxford University Press.
- Hoffman, K. L., Hernández Decasa, D. M., Beyer Ruiz, M. E., & González-Mariscal, G. (2010). Scent marking by the male domestic rabbit (*Oryctolagus cuniculus*) is stimulated by an object's novelty and its specific visual or tactile characteristics. *Behavioural Brain Research*, 207, 360–367. <https://doi.org/10.1016/j.bbr.2009.10.021>.
- Keith, L. B., Meslow, E. C., & Rongstad, O. J. (1968). Techniques for snowshoe hare population studies. *Journal of Wildlife Management*, 32, 801–812. <https://doi.org/10.2307/3799556>.
- Keith, L. B., Rongstad, O. J., & Meslow, E. C. (1966). Regional differences in reproductive traits of the snowshoe hare. *Canadian Journal of Zoology*, 44, 953–961.
- Koolhaas, J. M., de Boer, S. F., Coppens, C. M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology*, 31, 307–321. <https://doi.org/10.1016/j.yfrne.2010.04.001>.
- Kowalska, D., Bielanski, P., & Pietras, M. (2008). Suitability of behavioural tests for determining the ways rabbits function in the environment and their relationship with some productive traits. In *Proceedings of the 9th World Rabbit Congress, 10–13 June 2008, Verona, Italy* (pp. 1195–1199). Valencia, Spain: World Rabbit Science Association. <http://www.world-rabbit-science.com/WRSA-Proceedings/Co>.
- Krebs, C. J., Boonstra, R., & Boutin, S. (2018). Using experimentation to understand the 10-year snowshoe hare cycle in the boreal forest of North America. *Journal of Animal Ecology*, 87, 87–100. <https://doi.org/10.1111/1365-2656.12720>.
- Krebs, C. J., Boutin, S., & Boonstra, R. (2001). *Ecosystem dynamics of the boreal forest. The Klane project*. New York, NY: Oxford University Press.
- Krebs, C. J., Boutin, S., Boonstra, R., Sinclair, A. R. E., Smith, J. N. M., Dale, M. R. T., et al. (1995). Impact of food and predation on the snowshoe hare cycle. *Science*, 269, 1112–1115. <https://doi.org/10.1126/science.269.5227.1112>.
- Langenhof, M. R., & Komdeur, J. (2018). Why and how the early-life environment affects development of coping behaviours. *Behavioral Ecology and Sociobiology*, 72, 34. <https://doi.org/10.1007/s00265-018-2452-3>.
- Lima, S. L., & Dill, L. M. (1990). Behavioural decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology*, 68, 619–640. <https://doi.org/10.1139/z90-092>.
- Lockie, S. H., McAuley, C. V., Rawlinson, S., Guiney, N., & Andrews, Z. B. (2017). Food seeking in a risky environment: A method for evaluating risk and reward value in food seeking and consumption in mice. *Frontiers in Neuroscience*, 11, 24. <https://doi.org/10.3389/fnins.2017.00024>.
- Love, O. P., McGowan, P. O., & Sheriff, M. J. (2013). Maternal adversity and ecological stressors in natural populations: The role of stress axis programming in individuals, with implications for populations and communities. *Functional Ecology*, 27, 81–92. <https://doi.org/10.1111/j.1365-2435.2012.02040.x>.
- Mateo, J. M. (2014). Development, maternal effects, and behavioural plasticity. *Integrative and Comparative Biology*, 54, 841–849. <https://doi.org/10.1093/icb/ucu044>.
- McGowan, P. O., & Matthews, S. G. (2018). Prenatal stress, glucocorticoids, and developmental programming of the stress response. *Endocrinology*, 159, 69–82. <https://doi.org/10.1210/en.2017-00896>.
- Mikics, E., Barsy, B., Barsvári, B., & Haller, J. (2005). Behavioural specificity of non-genomic glucocorticoid effects in rats: Effects on risk assessment in the elevated plus-maze and the open-field. *Hormones and Behavior*, 48, 152–162. <https://doi.org/10.1016/j.yhbeh.2005.02.002>.
- Monclús, R., Tuilim, J., & Blumstein, D. T. (2011). Older mothers follow conservative strategies under predator pressure: The adaptive role of maternal glucocorticoids in yellow-bellied marmots. *Hormones and Behavior*, 60, 660–665.
- Mousseau, T. A., & Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends in Ecology & Evolution*, 13, 403–407. [https://doi.org/10.1016/S0169-5347\(98\)01472-4](https://doi.org/10.1016/S0169-5347(98)01472-4).
- Mueller, H. C., & Parker, P. G. (1980). Naïve ducklings show different cardiac response to hawk than to goose models. *Behaviour*, 74, 101–113. <https://doi.org/10.1163/156853980X00339>.
- Nemeth, M., Pschernig, E., Wallner, B., & Millesi, E. (2016). Non-invasive cortisol measurements as indicators of physiological stress responses in Guinea pigs. *PeerJ*, 4, 1590. <https://doi.org/10.7717/peerj.1590>.
- Niemelä, P. T., & Dingemans, N. J. (2014). Artificial environments and the study of 'adaptive' personalities. *Trends in Ecology & Evolution*, 29, 245–247. <https://doi.org/10.1016/j.tree.2014.02.007>.
- O'Donoghue, M. O. (1994). Early survival of juvenile snowshoe hares. *Ecology*, 75, 1582–1592. <https://doi.org/10.2307/1939619>.
- O'Donoghue, M. O., & Bergman, C. M. (1992). Early movements and dispersal of juvenile snowshoe hares. *Canadian Journal of Zoology*, 70, 1787–1791. <https://doi.org/10.1139/z92-246>.
- O'Donoghue, M. O., Boutin, S., Krebs, C. J., & Hofer, E. J. (1997). Numerical responses of coyotes and lynx to the snowshoe hare cycle. *Oikos*, 80, 150–162. <https://doi.org/10.2307/3546526>.
- O'Donoghue, M. O., & Krebs, C. J. (1992). Effects of supplemental food on snowshoe hare reproduction and juvenile growth at a cyclic population peak. *Journal of Animal Ecology*, 61, 631–641. <https://doi.org/10.2307/5618>.
- Palme, R. (2019). Non-invasive measurement of glucocorticoids: Advances and problems. *Physiology & Behavior*, 199, 229–243. <https://doi.org/10.1016/j.physbeh.2018.11.021>.
- Palme, R., & Möstl, E. (1997). Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. *Zeitschrift für Säugetierkunde*, 62(Suppl. II), 192–197.
- Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemans, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, 82, 291–318. <https://doi.org/10.1111/j.1469-185X.2007.00010.x>.

- Reddon, A. R. (2012). Parental effects on animal personality. *Behavioral Ecology*, *23*, 242–245. <https://doi.org/10.1093/beheco/arr210>.
- Rödel, H. G., Bautista, A., Roder, M., Gilbert, C., & Hudson, R. (2017). Early development and the emergence of individual differences in behaviour among littermates of wild rabbit pups. *Physiology & Behavior*, *173*, 101–109. <https://doi.org/10.1016/j.physbeh.2017.01.044>.
- Roff, D. A. (1996). The evolution of threshold traits in animals. *Quarterly Review of Biology*, *71*, 3–35.
- Rohner, C., & Krebs, C. J. (1996). Owl predation on snowshoe hares: Consequences of antipredator behaviour. *Oecologia*, *108*, 303–310. <https://doi.org/10.1007/BF00334655>.
- Rongstad, O. J., & Tester, J. R. (1971). Behaviour and maternal relations of young snowshoe hares. *Journal of Wildlife Management*, *35*, 338–346. <https://doi.org/10.2307/3799610>.
- Schneider, M. L., Moore, C. F., Kraemer, G. W., Roberts, A. D., & DeJesus, O. T. (2002). The impact of prenatal stress, fetal alcohol exposure, or both on development: Perspectives from a primate model. *Psychoneuroendocrinology*, *27*, 285–298. [https://doi.org/10.1016/S0306-4530\(01\)00050-6](https://doi.org/10.1016/S0306-4530(01)00050-6).
- Schöpper, H., Palme, R., Ruf, T., & Huber, S. (2012). Effects of prenatal stress on hypothalamic–pituitary–adrenal (HPA) axis function over two generations of guinea pigs (*Cavia aperea f. porcellus*). *General and Comparative Endocrinology*, *176*, 18–27. <https://doi.org/10.1016/j.ygcen.2011.12.010>.
- Severaid, J. H. (1942). *The snowshoe hare, its life history and artificial propagation*. Augusta, ME: Maine Department of Inland Fisheries and Game.
- Sheriff, M. J., Bell, A., Boonstra, R., Dantzer, B., Lavergne, S. G., McGhee, K. E., et al. (2017). Integrating ecological and evolutionary context in the study of maternal stress. *Integrative and Comparative Biology*, *57*, 437–449. <https://doi.org/10.1093/icb/ixc105>.
- Sheriff, M. J., Bosson, C. O., Krebs, C. J., & Boonstra, R. (2009). A non-invasive technique for analyzing fecal cortisol metabolites in snowshoe hares (*Lepus americanus*). *Journal of Comparative Physiology B*, *179*, 305–313. <https://doi.org/10.1007/s00360-008-0314-4>.
- Sheriff, M. J., Krebs, C. J., & Boonstra, R. (2010a). The ghosts of predators past: Population cycles and the role of maternal programming under fluctuating predation risk. *Ecology*, *91*, 2983–2994. <https://doi.org/10.1890/09-1108.1>.
- Sheriff, M. J., Krebs, C. J., & Boonstra, R. (2010b). Assessing stress in animal populations: Do fecal and plasma glucocorticoids tell the same story? *General and Comparative Endocrinology*, *166*, 614–619. <https://doi.org/10.1016/j.ygcen.2009.12.017>.
- Sheriff, M. J., Krebs, C. J., & Boonstra, R. (2011). From process to pattern: How fluctuating predation risk impacts the stress axis of snowshoe hares during the 10-year cycle. *Oecologia*, *166*, 593–605. <https://doi.org/10.1007/s00442-011-1907-2>.
- Sheriff, M. J., & Love, O. P. (2013). Determining the adaptive potential of maternal stress. *Ecology Letters*, *16*, 271–280. <https://doi.org/10.1111/ele.12042>.
- Shin, Y. J., & Shin, N. S. (2016). Evaluation of effects of olfactory and auditory stimulation on separation anxiety by salivary cortisol measurement in dogs. *Journal of Veterinary Science*, *17*, 153–158. <https://doi.org/10.4142/jvs.2016.17.2.153>.
- Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioural syndromes: An ecological and evolutionary overview. *Trends in Ecology & Evolution*, *19*, 372–378. <https://doi.org/10.1016/j.tree.2004.04.009>.
- Sinclair, A. R. E., Krebs, C. J., & Smith, J. N. M. (1982). Diet quality and food limitation in herbivores: The case of the snowshoe hare. *Canadian Journal of Zoology*, *60*, 889–897. <https://doi.org/10.1139/z82-121>.
- St-Cyr, S., & McGowan, P. O. (2015). Programming of stress-related behaviour and epigenetic neural gene regulation in mice offspring through maternal exposure to predator odor. *Frontiers in Behavioral Neuroscience*, *9*, 145. <https://doi.org/10.3389/fnbeh.2015.00145>.
- Stefan, C. L., & Krebs, C. J. (2001). Reproductive changes in a cyclic population of snowshoe hares. *Canadian Journal of Zoology*, *79*, 2101–2108. <https://doi.org/10.1139/cjz-79-11-2101>.
- Stein, L. R., & Bell, A. (2014). Paternal programming in sticklebacks. *Animal Behaviour*, *95*, 165–171. <https://doi.org/10.1016/j.anbehav.2014.07.010>.
- Suarez, S. D., & Gallup, G. G. (1981). Predatory overtones of open-field testing in chickens. *Animal Learning & Behavior*, *9*, 153–163. <https://doi.org/10.3758/BF03197812>.
- Taylor, R. W., Boutin, S., Humphries, M. M., & McAdam, A. G. (2014). Selection on female behaviour fluctuates with offspring environment. *Journal of Evolutionary Biology*, *27*, 2308–2321. <https://doi.org/10.1111/jeb.12495>.
- Thayer, Z. M., Wilson, M. A., Kim, A. W., & Jaeggi, A. V. (2018). Impact of prenatal stress on offspring glucocorticoid levels: A phylogenetic meta-analysis across 14 vertebrate species. *Scientific Reports*, *8*, 4942. <https://doi.org/10.1038/s41598-018-23169-w>.
- Tinbergen, N. (1953). *Social behaviour in animals*. New York, NY: J. Wiley.
- van Oers, K., Klunder, M., & Drent, P. J. (2005). Context dependence of personalities: Risk-taking behaviour in a social and a nonsocial situation. *Behavioral Ecology*, *16*, 716–723. <https://doi.org/10.1093/beheco/ari045>.
- Vitousek, M. N., Taff, C. C., Ardia, D. R., Stedman, J. M., Zimmer, C., Salzman, T. C., et al. (2018). The lingering impact of stress: Brief acute glucocorticoid exposure has sustained, dose-dependent effects on reproduction. *Proceedings of the Royal Society B: Biological Sciences*, *285*, 20180722. <https://doi.org/10.1098/rspb.2018.0722>.
- Ward, H. E., Johnson, E. A., Salm, A. K., & Birkle, D. L. (2000). Effects of prenatal stress on defensive withdrawal behaviour and corticotropin releasing factor systems in rat brain. *Physiology & Behavior*, *70*, 359–366. [https://doi.org/10.1016/S0031-9384\(00\)00270-5](https://doi.org/10.1016/S0031-9384(00)00270-5).
- Weinstock, M. (2008). The long-term behavioural consequences of prenatal stress. *Neuroscience & Biobehavioral Reviews*, *32*, 1073–1086. <https://doi.org/10.1016/j.neubiorev.2008.03.002>.
- Weissburg, M., Smee, D. L., & Ferner, M. C. (2014). The sensory ecology of nonconsumptive predator effects. *American Naturalist*, *184*, 141–157. <https://doi.org/10.1086/676644>.
- Welberg, L. A. M., & Seckl, J. R. (2001). Prenatal stress, glucocorticoids and the programming of the brain. *Journal of Neuroendocrinology*, *13*, 113–128. <https://doi.org/10.1111/j.1365-2826.2001.00601.x>.
- Wilcoxon, J. S., & Redei, E. E. (2007). Maternal glucocorticoid deficit affects hypothalamic–pituitary–adrenal function and behaviour of rat offspring. *Hormones and Behavior*, *51*, 321–327. <https://doi.org/10.1016/j.yhbeh.2006.11.006>.
- Wolff, J. O. (1980). The role of habitat patchiness in the population dynamics of snowshoe hares. *Ecological Monographs*, *50*, 111–130. <https://doi.org/10.2307/2937249>.
- Wolf, M., & Weissing, F. J. (2012). Personalities: Consequences for ecology and evolution. *Trends in Ecology & Evolution*, *27*, 452–461. <https://doi.org/10.1016/j.tree.2012.05.001>.
- Yilmaz, M., & Meister, M. (2013). Rapid innate defensive responses of mice to looming visual stimuli. *Current Biology*, *23*, 2011–2015. <https://doi.org/10.1016/j.cub.2013.08.015>.
- Yuen, C. H., Pillay, N., Heinrichs, M., & Schradin, C. (2016). Personality traits are consistent when measured in the field and in the laboratory in African striped mice (*Rhabdomys pumilio*). *Behavioral Ecology and Sociobiology*, *70*, 1235–1246. <https://doi.org/10.1007/s00265-016-2131-1>.