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PHYSIOLOGICAL ECOLOGY – ORIGINAL RESEARCH

The impact of variable predation risk on stress in snowshoe hares over the cycle in North America's boreal forest: adjusting to change

Sophia G. Lavergne[1] · Charles J. Krebs[2] · Alice J. Kenney[2] · Stan Boutin[3] · Dennis Murray[4] · Rupert Palme[5] · Rudy Boonstra[1]

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Abstract

The boreal forest is one of the world's ecosystems most affected by global climate warming. The snowshoe hare, its predators, and their population dynamics dominate the mammalian component of the North American boreal forest. Our past research has shown the 9–11-year hare cycle to be predator driven, both directly as virtually all hares that die are killed by their predators, and indirectly through sublethal risk effects on hare stress physiology, behavior, and reproduction. We replicated this research over the entire cycle by measuring changes in predation risk expected to drive changes in chronic stress. We examined changes in hare condition and stress axis function using a hormonal challenge protocol in the late winter of 7 years—spanning all phases of the cycle from the increase through to the low (2014–2020). We simultaneously monitored changes in hare abundance as well as those of their primary predators, lynx and coyotes. Despite observing the expected changes in hare–predator numbers over the cycle, we did not see the predicted changes in chronic stress metrics in the peak and decline phases. Thus, the comprehensive physiological signature indicative of chronic predator-induced stress seen from our previous work was not present in this current cycle. We postulate that hares may now be increasingly showing behavior-mediated rather than stress-mediated responses to their predators. We present evidence that increases in primary productivity have affected boreal community structure and function. We speculate that climate change has caused this major shift in the indirect effects of predation on hares.

Keywords Chronic stress · Climate change · HPA axis · Population cycles · Non-consumptive predator effects

Introduction

Many vertebrate herbivore populations are regulated top-down by their predators (Krebs et al. 1999; Sinclair and Krebs 2002; Estes et al. 2011). Two factors are potentially at play. Either direct predation mortality acts alone or together with predator–prey interactions that result in fear-induced, non-consumptive stress effects. In the former, though predators may affect the behavior of their prey, reducing their survival and reproduction (they forage in safe habitats and avoid risky ones), there are no significant stress effects (e.g. Creel et al. 2009; Metrione et al. 2020; Pritchard et al. 2020; Spong et al. 2020; reviewed in Say-Sallaz et al. 2019). This has been called the *predator-sensitive foraging hypothesis* (Hik 1995; Sinclair and Arcese 1995). In the latter, predators affect both the behavior of their prey and stress them, affecting key aspects of their physiology and thereby reducing reproduction (e.g. Yin et al. 2017; Bonnot et al. 2018; Dulude-de Broin et al. 2020; reviewed in: Preisser et al.

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✉ Rudy Boonstra
rudy.boonstra@utoronto.ca

[1] Department of Biological Sciences, University of Toronto Scarborough, Toronto, ON, Canada

[2] Department of Zoology, University of British Columbia, Vancouver, BC, Canada

[3] Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada

[4] Department of Biology, Trent University, Peterborough, ON, Canada

[5] Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria

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2005; Peckarsky et al. 2008; Clinchy et al. 2013). This has been called the *predator-induced stress hypothesis* (Boonstra et al. 1998a; Clinchy et al. 2013). These stress effects can act at the level of the individual, the local population, or the entire species over its geographic range. Here we examine the snowshoe hare, which fluctuates in a 9–11 cycle over virtually its entire range (the boreal forest—29% [626 million ha] of North America, Brandt et al. 2013) and where the evidence to date supports the second hypothesis that predators act both directly via mortality and indirectly via the predator-induced stress to drive population changes.

The hypothalamic–pituitary–adrenal (HPA) axis is critical to dealing with stress in mammals. It is a key part of the neuroendocrine system through which life-history decisions—to reproduce, grow, or put energy into storage—are implemented (Ricklefs and Wikelski 2002; Wingfield and Romero 2001). Stressors lead to the rapid release of glucocorticoids (GCs) from the adrenals and these activate a cascade of endocrine signals to mount the appropriate physiological and behavioral responses and then to re-establish homeostasis (Smith and Vale 2006). GCs influence the expression of approximately 10% of the genome, and their targets include genes that control metabolism, growth, repair, reproduction, and the management of resource allocation (Le et al. 2005; Sacta et al. 2016). Temporary suppression of non-essential functions (growth, reproduction, digestion, inflammatory and immune responses) to mobilize energy for flight or flight responses are normal and necessary trade-offs to maximize short-term survival (Monaghue and Spencer 2014). Stressors can be acute, with homeostasis being re-established rapidly, or chronic, leading to a major shift in physiological function. Chronic HPA activation in response to severe long-term stressors (e.g. high predation risk, food scarcity, social conflict, extreme weather), and ongoing suppression of those "non-essential" functions can take a toll on individual health and condition, constrain reproduction and fitness, and have cascading effects on population demography if all individuals are similarly affected (Boonstra 2013; Zanette et al. 2014).

Snowshoe hares (*Lepus americanus*) are the keystone herbivore species whose predictable population cycles dominate terrestrial community dynamics in North America's boreal forest (Keith 1990; Krebs et al. 2018a, b). Winter climate is the key determinant of the dominant understory vegetation in these forests (tall shrubs) and their counterparts in northwestern Eurasia (dwarf shrubs), structuring the contrasting herbivore–predator food web interactions in them (Boonstra et al. 2016). The hare cycles have been a constant feature in the North American forests for at least 300 years (Elton and Nicholson 1942). Over the course of a cycle, hare densities can fluctuate up to 40-fold. The prevailing evidence since the late-1990s is that predation is the primary driver of the cycle both via top-down consumptive effects on mortality and through stress-mediated non-consumptive effects on reproduction (Boonstra et al. 1998a; Krebs et al. 2001a; Krebs et al. 1995, 2018a, b). As the hare population increases, so do their predators (Canada lynx—*Lynx canadensis,* coyotes—*Canis latrans*, great-horned owls—*Bubo virginianus*, and northern goshawks—*Accipiter gentilis*), lagging behind by 1–2 years. Hares must simultaneously cope with these 4 predator species who have markedly different hunting strategies (ambush, cursorial, avian night, avian day, respectively; Doyle and Smith 1994; Rohner and Krebs 1996; O'Donoghue et al. 1998). During the decline and early low phases of the hare cycle, direct predation causes ~ 100% of hare deaths and annual adult survival can drop to 0.5% (Boutin et al. 1986; Hodges et al. 2001). Simultaneously, from the peak into the decline phase, reproductive output per female declines to about a third of what it is when predator density is low (late low and increase phases; Cary and Keith 1979; Stefan and Krebs 2001). The low phase remains the enigma of the cycle (Boonstra et al. 1998b) as the hare population fails to recover even though predators have declined markedly and there is an abundance of forage (Smith et al. 1988; Krebs et al. 2001b).

In the southern Yukon the drivers of these cycles have now been studied over 5 cycles using both detailed monitoring of hares and their predators and experimentation of the entire ecosystem (Krebs et al. 2001a; Krebs et al. 2018a, b). A detailed examination of the hare stress axis functioning started in the 2nd cycle (over 3 winters during decline and low phases—Boonstra et al.1998a), and was repeated in the 4th cycle (over 2 winters during decline—Sheriff et al. 2011a). All condition and physiological measures from these studies (changes in stress axis reactivity, energy mobilization, indices of condition and immunity) were in agreement: hares during the decline were chronically stressed, those in the low were not. Other explanations—lack of winter food, disease, and social interactions at high density during the decline—were rejected (Boonstra et al. 1998a; Sheriff et al. 2011a; Krebs et al. 2018a, b).The weight of evidence also indicated that this predator-induced fear had long-term consequences (Sinclair et al. 2003; Sheriff et al. 2015; MacLeod et al. 2018). The lag in population recovery after the decline phase (*i.e.* the 2–5 year low phase) was hypothesized to be mediated by phenotypically inherited maternal effects (Boonstra et al. 1998b). Lavergne et al. (2014) found changes in the expression of genes from the peak to the decline phase whose functions were consistent with the physiological changes associated with fluctuating predation risk. A plausible hypothesis is that these changes are linked to epigenetic changes in expression of key regulatory genes during the peak and decline, especially those affecting the stress axis (Bossdorf et al. 2008; Ho and Burggren 2010). There was a major knowledge gap from our previous work in that we did not understand how the hare stress

axis functioned during the increase and peak phase of the cycle. Our goal was to investigate stress axis function over all phases of the hare population cycle.

There is a critical need in science to replicate research to be confident that findings of the past are robust and repeatable and to avoid confirmation bias (Fidler et al. 2017; Baker 2016). However, repeatability assumes that all the relevant conditions remain the same. For laboratory research this is relatively easy to do, with only the investigator and the lab changing. For field research this may prove much more difficult, even when the investigator and the site remain the same, especially given the rapid anthropogenic impacts on habitat and climate. On the land north of 60°N (boreal and Arctic regions) temperatures have increased at double the global mean rate, with the boreal forest projected to be 4–5 °C warmer by 2100 (Price et al. 2013). Our study extended previous research at a time when the impacts of climate warming were becoming increasingly evident (see Andreassen et al. 2021 for a review of the impact of climate warming on global small mammal cycles and outbreaks).

We assessed snowshoe hare stress physiology and individual condition in late winter throughout all phases of the cycle (increase, peak, decline, and low) and simultaneously monitored changes in hare, lynx, and coyote densities. We assessed hares in February when the cumulative effects of needing to both search for food and avoid predators under severe cold should be the most taxing, but prior to the onset of reproduction and its potentially confounding hormonal effects. Hares were subjected a hormone challenge protocol to systematically probe hare HPA axis function, as well as measurements of hematological and body indices of condition and immunity.

To increase scientific understanding and certainty, we recognized the need to use multiple approaches to assess the costs of predation risk over the cycle (Munafò and Smith 2018). We had 7 objectives in our study. We deal with the first 3 in this paper and the last 4 in subsequent papers (the latter research is ongoing). All of these are set within the context of the climate change we have observed at our research site over the last 50 years. First, we wanted to obtain an integrated picture of hare stress physiology across their entire population cycle (all 4 phases). Second, we wanted to assess if intensifying predator-induced stress from the peak and decline correlated with hormonal and physiological profiles and lack of population recovery in the low phase. Third, we wanted to assess if the pattern of stress dynamics across the hare decline and low were consistent with past findings. Fourth, we are simultaneously assessing epigenetic changes in key brain regions involved in HPA axis regulation occur over the cycle (c.f. Edwards et al. 2021). Fifth, we are examining the stress effects on dendritic plasticity in the prefrontal cortex, the hippocampus, and the amygdala over the hare cycle. Laboratory studies have documented that stress can affect both cognition and behavior through changes in dendritic connectedness in these brain regions (Wellman et al. 2020). Sixth, we are examining if stress-induced changes in the microbiome occur and how these track and give insight into the cycle (c.f. Stothart et al. 2016). Seventh, we are assessing if the pre- and postnatal effects of predation risk affect offspring behavior, physiology, and gene expression (Lavergne 2018; McCaw 2020).

We made the following predictions with respect to the first 3 objectives: the effect of chronic stress, assuming that the current cycle was a replicate of past ones and that evidence of chronic stress would only appear during the peak and decline (Table 1; for extensive physiological references on which these predictions were made, see Boonstra et al. 1998a; Sheriff et al. 2011a,b; Breuner et al. 2013): first, that free cortisol levels would be higher; second, that corticosteroid binding globulin (CBG) levels (the protein that binds cortisol strongly in blood, preventing it from being free and biologically active and is inhibited by stress) would be lower; third, that blood glucose levels would be higher (increased liver gluconeogenesis would move energy substrates to the liver for rapid mobilization); fourth, that indices of condition—adult body mass, bone marrow fat, and hematocrit (% packed red-blood cell volume)—would be lower; and fifth, that measures of immunity would change (neutrophils higher; lymphocytes and eosinophils lower).

Materials and methods

Study area

All research and monitoring activities took place in the Shakwak Trench east of Kluane Lake in the southwestern Yukon Territory (61° 03' N, 138° 41' W). Here the boreal forest is dominated by white spruce (*Picea glauca*) with a shrub understory of willow (primarily *Salix glauca*),

Table 1 Predicted pattern of predator-induced chronic stress in snowshoe hares its associated effects on physiology, condition, and immunity across the snowshoe hare cycle

Variable	Cycle phase predictions			
	Increase	Peak	Decline	Low
Chronic stress	Low	High	Highest	Low
Baseline HPA axis activity	Low	High	Highest	Low
CBG binding capacity	High	Low	Lowest	High
Dexamethasone resistance	Low	High	Highest	Low
Adrenal capacity	Low	High	Highest	Low
Energy mobilization	Low	High	Highest	Low
Body condition	High	Lower	Lowest	High
Immunity	High	Lower	Lowest	High

bog birch (*Betula glandulosa*), and soapberry (*Shepherdia canadensis*). All trapping and study procedures were approved by Yukon Territorial Government Scientists and Explorers Act Licenses and Wildlife Permits, as well as the University of Toronto Animal Care Committee in accordance with the guidelines of the Canadian Council for Animal Care.

Predator–prey population monitoring

Snowshoe hare population densities were estimated via mark–recapture trapping sessions on two 36-hectare monitoring grids (Silver and Sulphur) in the spring (March/April) and fall (September/October) of every year. Each grid had 86 Tomahawk traps (No. 106; Tomahawk Live Trap, Tomahawk, WI, USA) along four trap lines, for an effective grid size of ~ 60 ha. Following a 3–5-day period of pre-baiting, hares were live-trapped and tagged during 2–3 night sessions (see Hodges et al. 2001 for full monitoring details and protocols). Absolute population density estimates for each session were then calculated from the maximum likelihood spatial estimator in Efford's DENSITY 4.4 program (Efford et al. 2009). Upon capture, each hare was weighed with a Pesola spring scale (± 10 g), their right-hind foot (RHF) length was measured as an index of skeletal size, their sex and reproductive status were assessed, and they were given an identifying ear tag. We used the finite rate of annual change in snowshoe hare density (spring to spring) to distinguish between the phases of the cycle (Keith 1990). Specifically, decline and increase phases were characterized by rates of change < 0.44 and > 1.89, respectively. All years between the decline and increase phases were defined as the low phase, and years between increase and the next decline were defined as the peak phase (used similarly by Sheriff et al. 2015 and Oli et al. 2020).

We estimated the abundance of lynx and coyotes in the valley each winter by track counts along 22 km transect. Tracks per 24 h were calculated by counting tracks crossing the transect 24–48 (but up to 72) hours following fresh snowfall events from October–April every year (see O'Donoghue et al. 1997 for more details). These track counts were correlated with density estimates obtained by telemetry of collared individuals and extensive snowtracking. We used the relationship in O'Donoghue et al. (submitted June 2021) to convert tracks per 24 h per km into density estimates for lynx and coyote.

Kluane winter conditions

Long-term temperature data (1970–2020) were obtained from the Environment Canada Meteorological Station in Haines Junction, Yukon, located approximately 50 km SE of our study site. Since 2004, we also have detailed measurements of temperature and snow depth on our study area. Overwinter temperatures were recorded every 4 h (1 Nov to 28 Feb) using duplicate iButton data loggers (Maxim Integrated, San Jose, CA, USA) on both control hare monitoring grids. iButtons positioned above the snow pack (80 cm above ground level) were used to assess winter severity for snowshoe hares given that they do not burrow and thus spend the majority of their time exposed to ambient temperatures. These data were used to calculate an index of winter severity as the number of degree-days below − 10 °C (e.g. if the average temperature on a day was − 23 °C, that would mean 13 degree-days below − 10 °C and these were summed over the 4-month period from 1 Nov to 28 Feb). The lower critical temperature for snowshoe hares in winter is − 10 °C (Sheriff et al. 2009a). Snow depth was measured every 5 km during predator track transects (1 Nov to 28 Feb) and used to calculate the annual average snow depth (mean of all stakes averaged from all transects, 1–3 transects per month) and to determine the maximum snow depth recorded in each year.

Experimental animals

Winter live trapping

We assessed snowshoe hare stress physiology and condition between 14 and 26 February from 2014 to 2020. Hares were captured in the boreal forest surrounding a 30 km stretch of the Alaska Highway. Live traps (No. 106; Tomahawk Live Trap, Tomahawk, WI, USA) were deployed at forested sites along old roads, cutlines, and paths that radiated off from the highway as well as 50–75 m off the highway where hare tracks were evident. These sites were chosen to permit us to get our research vehicle off of the highway to avoid collisions with snowplows that kept the highways clear of snow. Traps were locked open and prebaited once with a handful of rabbit chow one week prior to trapping. Traps (25–50 set per night, depending on hare density) were set near midnight (2200–0100 h) and checked before daybreak (0600–0800 h) to ensure that hares were in the traps < 8 h. A sample of feces (not contaminated by urine) were collected underneath the traps. Thus, the cortisol metabolite concentrations in feces (see Sect. 2.5) represented baseline levels unaffected by capture and handling (Sheriff et al. 2009b). Traps were not set if temperatures dropped below − 20 °C. Upon capture, hares were transferred to burlap bags to limit visual stimuli and minimize stress during handling. Each hare was transported to the Kluane Lake Research Station for hormone challenges and condition assessment where it was weighed (Pesola scale ± 10 g), sexed, sexual condition assessed, and right hind foot measured (RHF, mm). At all times, hares remained in their burlap bags and between blood sampling

were placed on straw bedding in open individual containers at ambient temperatures of ~ 5 °C to avoid overheating.

Hormonal challenges

We used hormonal challenges to get an integrated picture of the hares' recent past while overriding their immediate physiological response to the effects of capture and handling. The hormone challenge is a well-established protocol for the assessment of chronic stress in mammals (Boonstra et al. 1998a; Sheriff et al 2011b) and it involves two steps: a dexamethasone (Dex) suppression test followed by an adrenocorticotropic hormone (ACTH) stimulation test. Dex is a synthetic glucocorticoid used to assess the brain's sensitivity to circulating corticosteroid concentrations and inhibit further ACTH release via negative feedback. ACTH stimulates the adrenals directly and tests their capacity to release cortisol. The protocol consists of 5 sequential bleeds from an ear artery. The first (*Base* sample) was collected after a 2 h acclimation period, and is an integrative measure of both HPA axis activation and inhibition in response to capture and handling. This was immediately followed by venous ear injection of dexamethasone sodium phosphate (0.4 mg/kg; Vétoquinol, Quebec, Canada). The second blood sample (*Dex response* bleed) was taken two hours later and followed with an intramuscular injection of synthetic ACTH to the thigh (4 IU; Cortrosyn, Amphastar Pharmaceuticals Inc. USA). The adrenal response to ACTH was assessed using the next three samples—taken 30, 60, and 120 min post-injection. A small portion of the *Base* bleed was used to obtain indices of condition (hematocrit and leukocyte profiles). Circulating glucose concentrations were quantified for each blood sample using a FreeStyle glucometer (Abbott Laboratories). FreeStyle control solutions were used to ensure consistency in readings across years. The remaining whole blood was centrifuged for 10 min (5500 g) for plasma hormone analysis.

Indices of condition, immunity, reproduction, and age

Hematocrit (percent packed red blood cell volume) was measured in duplicate using 75 µl capillary tubes that were centrifuged for 8 min (13,460 g). Two blood smears were made and stained with Diff-Quick (Dade International Inc., Florida, USA). Leukocyte profiles were determined as the proportion (out of 100 cells) of neutrophils, lymphocytes, monocytes, and eosinophils. These values were used to determine neutrophil:lymphocyte (*N:L*) ratios for each hare. Stress-induced neutrophilia can be dissociated from disease effects by also examining the proportions of monocytes and eosinophils, which would increase in the case of infection.

After completion of the hormone challenge, hares were put under inhalational anesthesia with isoflurane, euthanized (University of Toronto SOP 12.11.4), and autopsied. A limited number of hares were released at their original site of capture in 2014 ($n = 3$) and 2016 ($n = 1$) as they were being monitored in another study. Body condition was estimated as the percentage of bone marrow fat (BMF). Bone marrow was dissected from the right tibia bone, weighed, oven-dried (70 °C for 24 h), and then reweighed to calculate the amount of fat (% of fresh weight; Brooks et al. 1977). We used the paired testes mass in males as an index of reproductive status, with heavier testes indicative of greater reproductive investment or breeding onset, and used the dry mass (80 °C for 5 days) of the heaviest crystalline eye lens as a relative estimate of age (Cary and Keith 1979; Keith et al. 1968).

Hormone analyses

Fecal cortisol metabolites

Fecal samples were stored at − 20 °C in the Yukon and transported to the University of Toronto for analysis. The samples were freeze-dried in a FreeZone 4.5L lyophilizer (LabConco, MO, USA) for 14–18 h, then manually homogenized in liquid nitrogen using a mortar and pestle. Dried ground feces (60 mg ± 5 mg) were extracted in 1 ml of 80% methanol for 30 min (1500 rpm) on a Vibrax orbital shaker (IKA-Werke GmbH & Co Germany). Following a 15 min centrifugation (2500 g), 100 µl of the extract supernatant was diluted [1:10] in assay buffer. FCMs were quantified for all samples in duplicate using an 11-oxoaetiocholanolone enzyme immunoassay validated specifically for snowshoe hares (Sheriff et al. 2009b). This assay had intra- and inter-assay coefficients of variation of 6% and 8%, respectively.

Plasma cortisol concentrations

For each hormone challenge sample we quantified total plasma cortisol concentrations in duplicate (10 µl) using a commercially available Cortisol ^{125}I radioimmunoassay kit (MP Biomedicals, NY, USA). We added 60 µl of [1:3] ammonium hydroxide (NH_4OH) to each reaction to saponify triglycerides and minimize any binding interference. This assay had intra- and inter-assay coefficients of variation of 2% and 6%. The maximum corticosteroid-binding capacity (MCBC) of CBG was measured for each sample in duplicate using an H^3 protocol (Boonstra and Singleton 1993) that had intra- and inter-assay coefficients of variation of 4% and 6%. Total cortisol and MCBC values were then used to determine the concentration of free (unbound) cortisol at each time point using the calculations and binding coefficients detailed in Boonstra et al. 1998a). Though chronic stress causes CBG to start declining in many mammals after ~ 24 h (Breuner et al. 2013), lagomorphs are unique in that MCBC increases rapidly after the ACTH injection (Boonstra et al.

1998a). This is caused by an ACTH-induced increase in free fatty acids which temporarily increases binding properties of albumin (Boonstra and Tinnikov 1998), causing a temporary increase in binding capacity. Thus, here we report absolute MCBC value from the initial Base bleed only and focus on the changes in free cortisol as they are the biologically relevant metric—only the unbound cortisol portion is available for tissue uptake—and free cortisol values incorporate total cortisol and MCBC dynamics for each individual hare.

Statistical analysis

Data are expressed throughout as means ± 1 SE. Data were tested for assumptions of normality and homogeneity using a Shapiro–Wilk test and Levene's equality of variances test, respectively. Logarithmic transformations were applied to achieve normality where necessary, and datasets that could not be normalized but met the assumptions of homogeneity and had large enough samples sizes ($n > 30$) were treated as normal (Krebs 2014). Preliminary analyses found significant within-phase (i.e. inter-annual) variation for both the increase and decline phases of the cycle, precluding the use of "phase" as our factor of interest. General Linear Models (ANOVA) were used to test the main effects and interaction effects of year and sex (SPSS v.25, IBM, Armonk, NY, USA) A Bonferroni multiple testing correction was applied in post hoc comparisons. We estimated effect sizes using partial eta-squared (η^2_p) values (moderate effect > 0.06, large effect > 0.14; Cohen 1988) and report these values for all results where $p < 0.1$.

Results

Boreal forest dynamics

Long-term climatic record

Over the last 50 years, both summer and winter temperatures have become progressively warmer in the southwestern Yukon (Fig. 1). There is an upward trend for both seasons overall, with winter temperatures increasing at twice the rate of summer temperatures. On average, winter temperatures increased 0.75 °C per decade ($-165.51 + (0.075 \times \text{year})$, $n = 51$ $R^2 = 0.14$, CV = 19.0%; slope $P = 0.007$, CL 0.022–0.129) and summer temperatures increased 0.29 °C per decade ($-46.12 + (0.029 \times \text{year})$, $n = 50$, $R^2 = 0.17$, CV = 8.0%; slope $P = 0.003$, 95% CL 0.012–0.047). Thus, over these 50 years average winter temperatures increased 21% (3.75 °C) and summer temperatures increased 13% (1.45 °C).

Winter conditions across the study

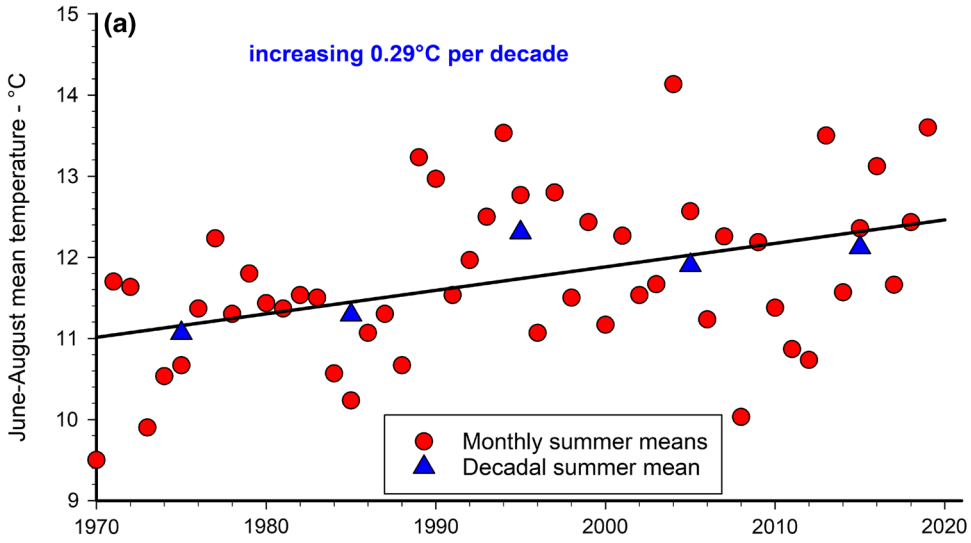
Average winter temperatures (1 Nov to 28 Feb) fluctuated from − 11.8 to − 15.5 °C over the 7 years of our study (Min: − 38.9 °C; Max: 6.1 °C). Hares experienced the most severe overwinter temperatures in 2016–17, 2017–18, and 2019–20 (Appendix S1 Supplementary material). Those years had 74–80% more degree-days below − 10 °C than in 2015–16, the mildest winter for hares in terms of thermoregulatory demand. Average snow depth ranged from 31.6 to 55.1 cm across years (mean = 37.0 ± 2.5 cm), with the two coldest winters (2016–17 and 2017–18) also having the highest average and highest maximum snow depths (Appendix S1 Supplementary material).

Predator–prey dynamics

The most recent hare population peak prior to this cycle occurred in 2006. Following the decline and low phases, hare numbers began to recover over the summer of 2013. Our study began the following winter and spanned all four phases of the cycle (*increase* 2013–14 to 2015–16; *peak* 2016–17; *decline* 2017–18 to 2018–19; *low* 2019–20; Fig. 2). Spring density (numbers/ha; 95% CI) increased 36.6-fold from the end of the low phase (2012; 0.03; 0.03–0.08) to their population peak in 2017 (1.22; 0.93–1.60), and then declined for 2 years, reaching a low in spring 2020 (0.13; 0.07–0.25).

Predator populations (lynx and coyote) more than doubled over the same period, reaching a maximum combined density of 33.7 predators/100 km^2 in the winter of 2016–17. Predator density remained high into the first year of hare decline (2017–18), then fell to levels roughly half of maximum in the second year of the decline (19.6/100 km^2) and into the low (17.3/100 km^2). Coyote numbers peaked during the middle of the increase phase and then declined across the rest of the cycle. Thus the key mammalian predator on hares from the peak to the low were lynx.

Snowshoe hares experienced peak rates of annual population growth (132%) mid-way through the increase phase (from spring 2014 to 2015), and the least overwinter population loss the following year (− 12.2%, 2015–16; Fig. 2). Putative predation risk—measured as the ratio of predators (lynx and coyotes) to hares in spring (Sheriff et al. 2011a)—was lowest as the hare population peaked in 2016 and 2017, then increased steadily across the decline and low phases to a maximum of 0.013 predators/hare in spring 2020. This period of increased risk also saw the most dramatic rate of overwinter hare population loss in our study period, an 81% decline in hare density over the 2018–2019 winter and a 58% decline over the 2019–2020 winter (Appendix S2 Supplementary material).



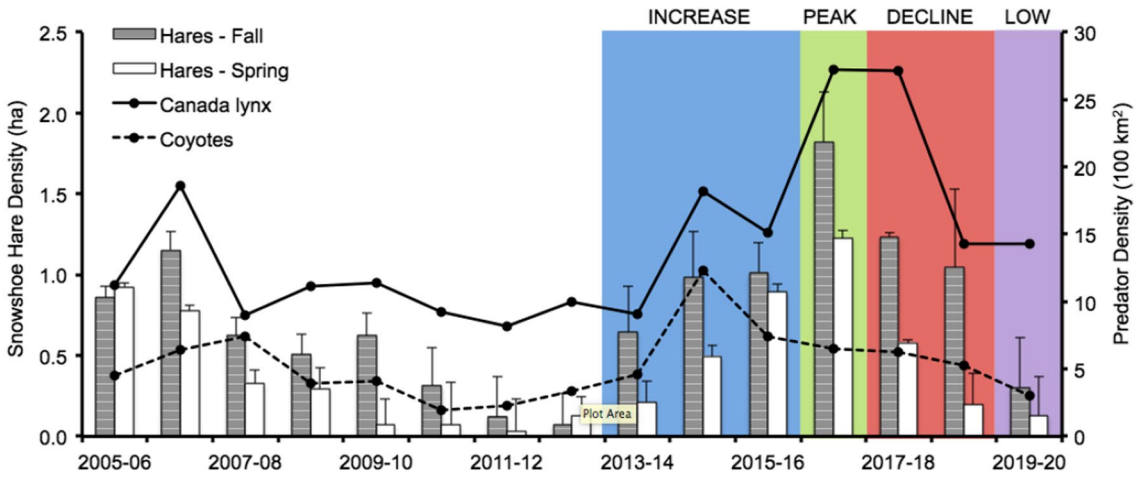


Fig. 1 Changes in temperature from 1970 to 2020 in Haines Junction, Yukon. **a** The summer temperatures represent monthly means across the average growing season (1 Jun to 31 Aug) and **b** the winter temperatures represent annual averages across the coldest winter period (1 Nov to 28 Feb)

Fig. 2 Density estimates for snowshoe hares and their predators (lynx and coyote) in the Kluane Lake region, Yukon (2005–2020). Hare density estimates from fall (September/October) and spring (March/April) mark–recapture trapping sessions. Estimates with 95% confidence levels are shown and colored boxes corresponding to cycle phases indicate the study period. Predator density estimates are from overwinter (November–March) snow tracking

Winter hares—morphometrics, age, and reproductive status

We examined the stress physiology, condition, energetics, and immunology of 92 hares over the 7 years of our study. February hare body mass varied by year ($F_{6,72} = 3.68$, $p < 0.01$, $\eta^2_p = 0.24$), even when accounting for the variation explained by our index of skeletal size (right hind foot length; $F_{1,72} = 16.64$, $p < 0.01$, $\eta^2_p = 0.19$) and our index of age (dry weight of the heaviest crystalline eye lens; $F_{1,72} = 19.83$, $p < 0.01$, $\eta^2_p = 0.22$). Body mass differed between sexes ($F_{1,72} = 11.70$, $p < 0.01$, $\eta^2_p = 0.14$), and there was no interaction effect between year and sex ($F_{6,78} = 1.75$, $p = 0.12$; Table 2). On average, females were 5% heavier than males across all years. Bonferroni post hoc comparisons conducted for each sex indicated that males were 21% heavier in 2016 than in 2020 ($P < 0.01$) and females were 17% heavier in 2016 than in 2015 ($P = 0.04$). Differences in mass do not appear to be caused by variation in population age structure across years, as neither right-hind foot (RHF) length (skeletal size), nor the dry mass of the heaviest eye lens (our index of age) differed by year (RHF: $F_{6,78} = 1.37$, $P = 0.24$; Age: $F_{6,74} = 1.17$, $P = 0.33$), sex (RHF: $F_{1,78} = 0.03$, $P = 0.87$; Age: $F_{1,74} = 1.48$, $P = 0.23$), or their interaction (RHF: $F_{6,78} = 2.02$, $P = 0.07$; Age: $F_{6,74} = 0.61$, $P = 0.72$; Table 2). In contrast, male testes mass, our index of reproductive onset, varied significantly among years ($F_{6,39} = 3.97$, $P < 0.01$, $\eta^2_p = 0.38$), with males in the early increase (2014) having testes that were at least twice as heavy in late February than in all other years examined ($p < 0.01$). Males in the first year of the decline (2018) had the lowest paired testes mass of all years, weighing 3.9 × less than those of males in the first year of the increase (2014) (Table 2). Only 2 males in the entire study were in reproductive condition (scrotal), and both were seen during the increase (2014 $n = 1$, 2015 $n = 1$). Taken together, these data suggest that we sampled a similar cross-section of the hare population in each year, and that there is evidence of variation in condition over the cycle. Though certain aspects are consistent with chronic stress predictions for individual parts of the cycle (e.g. highest body mass during the mildest winter—2015–16, lowest testes mass in the first year of decline), we saw comparable values for these metrics among other years of the cycle. Thus there was not a direct phase-based link between the numerical changes in predator–prey densities and the impacts on hare body condition in this cycle.

Hare stress physiology

Baseline cortisol concentrations

We predicted that FCMs would be higher in the peak and the decline. However, we found no significant differences in mean fecal cortisol metabolite (FCM) concentrations as a function of year ($F_{6,78} = 1.07$, $P = 0.39$), sex ($F_{1,78} = 0.99$, $P = 0.32$), or their interaction ($F_{6,78} = 1.16$, $P = 0.34$; Fig. 3). Thus, this integrated measure of baseline cortisol levels was similar from the increase to the low, contrary to our prediction.

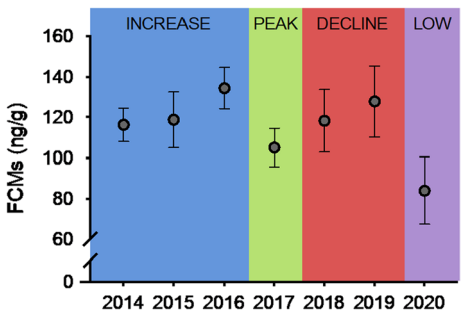


Fig. 3 Concentrations of fecal cortisol metabolites (FCMs, means ± 1 SE) in snowshoe hare feces collected at capture. These values are an estimate of baseline levels of circulating free cortisol (unaffected by the stress of capture and handling). These hares were subsequently given hormonal challenges

Table 2 Sample size, body mass, right hind foot length, dry crystalline eye lens mass, and paired testes mass (means ± 1 SE) of adult snowshoe hares sampled in February given hormone challenges (2014–2020)

Phase	Year	Sample size		Body mass (g)		Right hind foot (mm)		Lens weight (mg)		Testes weight (g)
		Male	Female	Male	Female	Male	Female	Male	Female	Male
Increase	2014	8	6	1384 (34)	1547 (60)	136 (1)	138 (1)	179 (10)	196 (12)	6.6 (0.5)
Increase	2015	9	6	1459 (33)	1418 (36)	141 (3)	139 (3)	175 (7)	168 (7)	3.1 (0.4)
Increase	2016	11	6	1523 (26)	1665 (44)	138 (1)	141 (2)	182 (10)	178 (11)	2.7 (0.5)
Peak	2017	7	7	1467 (43)	1454 (87)	140 (2)	134 (3)	177 (11)	182 (8)	3.0 (0.6)
Decline	2018	5	7	1430 (75)	1547 (28)	137 (2)	140 (1)	197 (11)	194 (15)	1.7 (0.1)
Decline	2019	5	6	1377 (37)	1475 (43)	142 (3)	138 (1)	165 (12)	178 (13)	2.7 (0.4)
Low	2020	3	6	1255 (61)	1458 (52)	134 (3)	136 (2)	157 (4)	189 (16)	2.4 (0.7)

Initial response to capture and handling (base bleed)

We found that maximum corticosteroid binding capacity (MCBC) in plasma varied among years ($F_{6,77}=4.46$, $P<0.01$, $\eta^2_p=0.26$), but not by sex ($F_{1,77}=0.35$, $P=0.56$) or their interaction ($F_{6,77}=1.23$, $P=0.30$; Fig. 4a). Hares had higher MCBC in 2018, the first year of decline, than in 2016 ($p=0.03$) or 2020 ($P<0.01$), and lower MCBC in 2020 than in 2015 ($P<0.01$) or 2019 ($P=0.04$). In contrast, free cortisol concentrations did not vary as a function of year ($F_{6,78}=1.69$, $P=0.14$), sex ($F_{1,78}=0.01$, $P=0.92$), or their interaction ($F_{6,78}=0.59$, $p=74$; Fig. 4b). Glucose concentrations varied by year ($F_{6,73}=4.82$, $P<0.01$, $\eta^2_p=0.28$), but not by sex ($F_{1,73}=0.24$, $P=0.62$) or their interaction ($F_{6,73}=0.41$, $P=0.87$; Fig. 4c). Post hoc analysis indicated significant variation across the increase phase, with higher glucose levels at *Base* in 2014 and 2015 than in 2016 ($p<0.01$). In general, levels were low and similar from the last year of the increase through to the end of the decline and then increased in the low (2020). Thus, neither MCBC nor free cortisol concentrations showed signs of chronic stress effects at the peak and decline, and in fact MCBC showed exactly the opposite pattern, with the highest levels of CBG binding capacity in decline phase animals. Similarly, there was no evidence of stress-induced variation in energy mobilization during periods of high predation risk. What is also striking with the MCBC and glucose data is how little variation was seen among individuals within a year, indicating that all hares were remarkably similar in this aspect of their physiology.

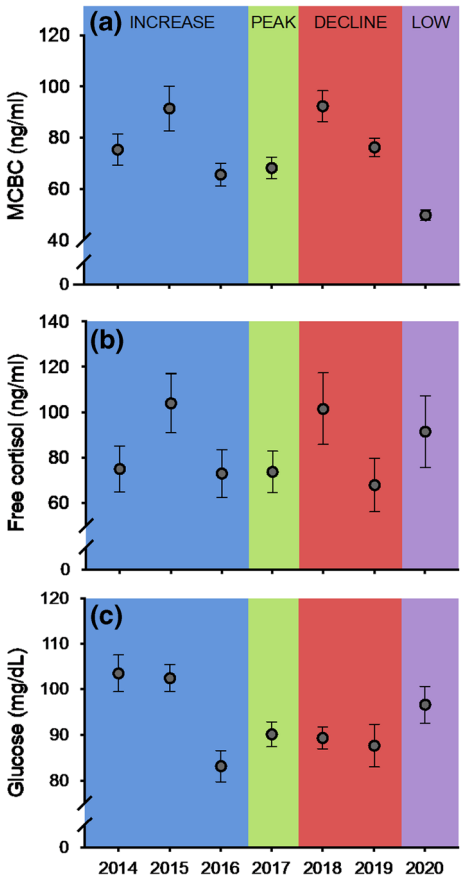


Fig. 4 Measures of the stress response and energy mobilization in snowshoe hares at the initial *Base* bleed throughout the cycle (means ± 1 SE). Three measures were quantified in response to capture and handling: **a** maximum corticosteroid binding capacity (MCBC), **b** free cortisol in plasma, and **c** blood glucose

HPA axis inhibition: response to dexamethasone

Nearly all hares ($n=88$) showed evidence of robust HPA axis negative feedback responses across the cycle, with free cortisol declining to very low concentrations (< 5 ng/ml) following the administration of Dex. After omitting the 4 aberrant responses to Dex from the analysis (see below), we found that free cortisol concentrations varied by year ($F_{6,74}=7.19$, $P<0.01$, $\eta^2_p=0.37$), but not by sex ($F_{1,74}=0.90$, $P=0.35$) or their interaction ($F_{6,74}=1.24$, $P=0.30$). Broadly, hares in the both decline phase years (2018, 2019) had lower free cortisol concentrations than those in the increase and peak phases (2014, $P=0.00$–0.03; 2016, $P<0.01$; 2017, $P<0.01$). Animals in the low phase (2020) also had lower free cortisol levels than those in the last increase year (2016) ($P=0.01$; Appendix S3 Supplementary material).

We found evidence of impaired negative feedback in four individuals—all female. Three were from the decline phase (2018 $n=2$; 2019 $n=1$), and one from the increase phase (2016). Their free cortisol concentrations were extreme outliers (28.36—87.22 ng/mL) relative to the rest of the population (2.47 ± 0.22 ng/mL) and the 2019 female appeared to be fully GC-resistant: with free cortisol concentrations increasing by 30% in response to the Dex injection.

Thus, there were no evidence of HPA axis inhibition (dexamethasone resistance) indicative of predator-induced chronic stress during the peak and decline, contrary to our predictions. We found that negative HPA axis feedback functioned robustly throughout the cycle in virtually all (96%) hares.

Adrenal capacity: response to ACTH stimulation

The magnitude of free cortisol response to ACTH stimulation was quantified using area under the curve (AUC) (Delehanty and Boonstra 2011). AUC was measured from the free cortisol concentration at the DEX bleed (used as a baseline) through to the P120 bleed (i.e. *Dex* to *P120;* Fig. 5a). It provided an integrated measure of the hares' sensitivity and capacity to produce cortisol. A greater AUC would be indicative of a chronically stressed hare. The 4 females with aberrant Dex responses (see above) were excluded

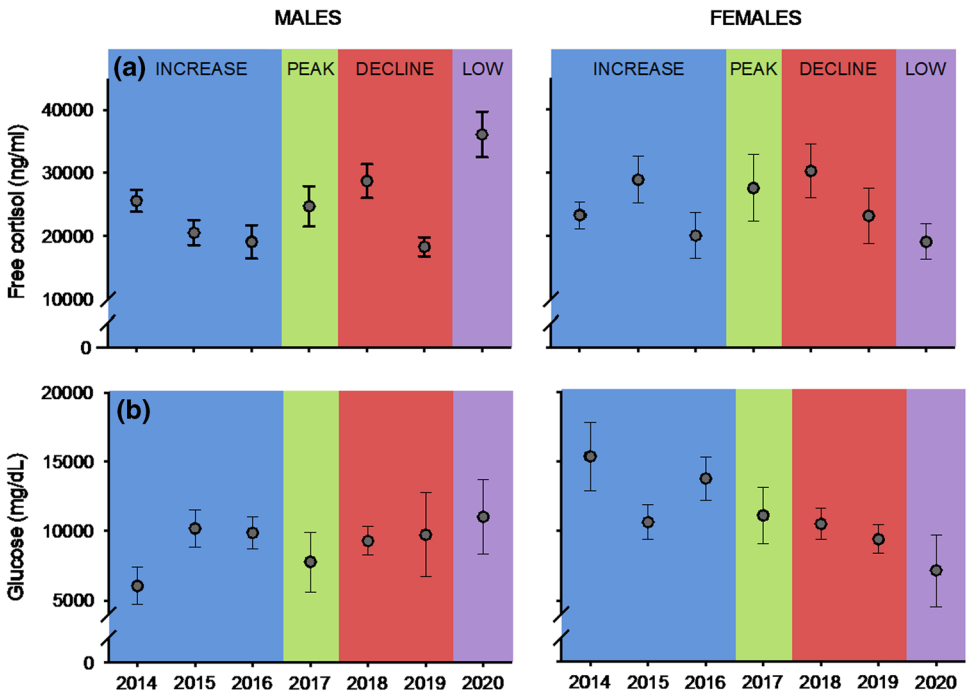


Fig. 5 Snowshoe hare hormone challenge responses over the cycle (means ± 1 SE): **a** adrenal capacity was quantified as the magnitude of free cortisol response to ACTH stimulation (*Area under the curve, Dex* to *P120*); **b** energy mobilization was quantified as the magnitude of glucose response to Dex and ACTH injections (*Area under the curve*, *Base* to *P120)*

from the analysis. Changes in the amount of free cortisol showed a year effect ($F_{6,74}=2.24$, $P=0.05$, $\eta^2_p=0.15$), no sex effect ($F_{1,73}=0.05$, $P=0.83$), and a marginal interaction effect ($F_{6,74}=2.09$, $P=0.07$, $\eta^2_p=0.15$). Post hoc comparisons indicated higher free cortisol responses in males from 2020 than 2016 ($P=0.03$), but no variation among years in females. These results do not support our prediction of higher free cortisol responses during the peak and decline phases. We found similar levels of stress reactivity across the cycle, and an increase in stress reactivity only in low phase males.

Energy mobilization

As both Dex and ACTH have been shown to mobilize glucose from hepatic glycogen stores (Dallman et al 1989), glucose response magnitude was quantified as the area under the curve (AUC) from *Base* to *P120*, corrected to starting concentration (AUC—*Base* value × 240). A greater AUC would be indicative of a chronically stressed hare. There was no year effect ($F_{6,78}=0.57$, $P=0.76$), but a sex effect ($F_{1,78}=4.10$, $P=0.05$, $\eta^2_p=0.05$) and an interaction effect ($F_{6,78}=2.41$, $P=0.04$, $\eta^2_p=0.16$) (Fig. 5b). Females consistently mobilized more energy than males (4–153%) in response to the hormone challenge across the increase, peak, and first year of the decline. However, this pattern reversed in 2019 and 2020 when males' responses were 4% and 30% greater than that in females', respectively. Overall, male glucose responses were broadly similar across the entire cycle, with the exception of the first year of the increase when values were low. In contrast, female glucose responses started high and progressively declined across the cycle. Thus, in both sexes our findings are in sharp contrast to our chronic stress predictions: we expected energy mobilization to be low during the increase phase and then progressively increase in tandem with increasing predation risk into the peak and decline.

Indices of condition and immunity

Body condition

We found that hematocrit (% packed red blood cell volume) varied by year ($F_{6,78}=5.45$, $P<0.01$, $\eta^2_p=0.30$), but not by sex ($F_{1,78}=0.34$, $p=0.56$) or their interaction ($F_{6,78}=0.20$, $P=0.98$; Fig. 6a). Average hematocrit ranged from 38 to 46% across the study period, with the lowest values occurring in the peak (2017) and the first year of the decline (2018). Post hoc analyses indicated that hematocrit levels in the second year of the decline (2019) were greater than those in increase (2014, 2015), peak (2017), and first decline year (2018) (all $P<0.01$). Variation within a year was slight (narrow SE's), indicating that all hares were similar in this measure of condition. Thus, consistent with our chronic predator-induced stress prediction, the hematocrit measure of condition was lower during the peak and early decline, but contrary to it, late decline hares were in the best condition.

Our second index of condition was the percentage of bone marrow fat (BMF). Keith et al. (1984) found that BMF values < 29.5% were indicative hares that likely had likely

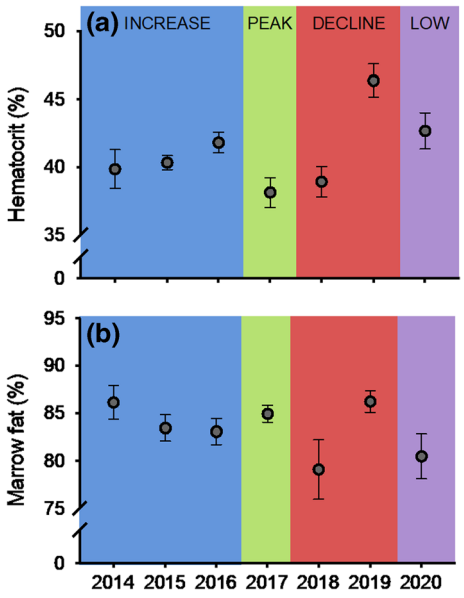


Fig. 6 Indices of snowshoe hare condition in winter over the cycle (means ± 1 SE) **a** hematocrit is an estimate of mean packed red blood cell volume (%) and **b** tibia bone marrow fat (%) is an estimate of the amount of whole body fat

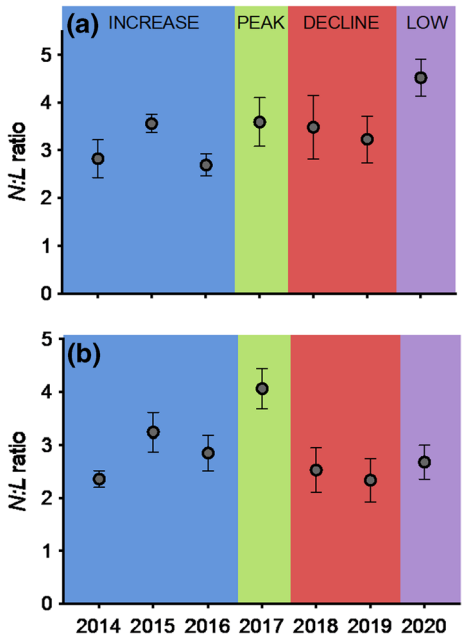


Fig. 7 Neutrophil:Lymphocyte (*N:L*) ratios (means ± 1 SE) calculated from blood smear differential white blood cell counts were used as an index of immunity in **a** male and **b** female snowshoe hares over the cycle

died of starvation. BMF varied among years ($F_{6,73}=2.23$, $P=0.05$, $\eta^2_p=0.16$), but not by sex ($F_{1,73}=0.23$, $P=0.64$) or their interaction ($F_{6,73}=1.19$, $P=0.32$; Fig. 6b). Average BMF ranged from 79 to 86% across the cycle, with the lowest values occurring in the first year of the decline (2018) and the low (2020)—however, none of the post hoc comparisons among years were significant. Thus, contrary to our chronic stress predictions, the BMF condition of hares were extremely good throughout the cycle, respective of changes in predation risk.

Immunity

We used the neutrophil:lymphocyte (*N:L*) ratios as our index of immunity, given that an increase in this ratio can arise from an individual being under chronic stress or experiencing an acute infection. The *N:L* ratio varied by year ($F_{6,78}=3.46$, $P=0.004$, $\eta^2_p=0.19$), but not bysex ($F_{1,78}=2.44$, $P=0.12$, $\eta^2_p=0.08$) and marginally by their interaction ($F_{6,78}=2.17$, $P=0.054$; Fig. 7). We, therefore, analyzed only by year ($F_{6,85}=3.18$, $P=0.007$, $\eta^2_p=0.19$). N/L ratios were significantly higher in the peak (2017), than in 2 of the increase years (2014, 2016) and the last decline year (2019).

Eosinophil levels are an index of parasitism. Eosinophil levels varied by year ($F_{6,78}=3.62$, $P=0.02$, $\eta^2_p=0.19$), but not by sex ($F_{1,78}=0.32$,) or their interaction ($F_{6,78}=0.45$). We therefore analyzed only by year ($F_{6,85}=2.09$, $P=0.01$, $\eta^2_p=0.19$). Overall levels were low over the 7 years, with levels in the peak (2017) being lower than in the last decline year (2019) and the low (2020); the first year of the decline (2018) was also lower than the second (Appendix S4 Supplementary material).

Monocyte levels are associated with defence against infections and bacteria, and chronically high corticosteroid levels can lead to a reduction in monocytes; however, high or low levels show species specificity and thus are harder to interpret (Jain 1993). Monocyte levels varied by year ($F_{6,78}=13.7$, $P=0.001$, $\eta^2_p=0.19$), but not by sex ($F_{1,78}=0.11$,) or their interaction ($F_{6,78}=0.44$). We therefore analyzed only by year ($F_{6,85}=14.81$, $P=0.0001$, $\eta^2_p=0.19$). Both the last year of the increase (2016) and the peak (2017) had lower levels than the other increase years, the decline years, and the low year (Appendix S4 Supplementary material). In summary, we found no evidence of chronic stress effects on immunity (i.e. increased *N:L* ratios), no marked increases in eosinophils during the decline, and no reduction in monocytes that was restricted only to the decline phase, the late increase and the peak had lower levels.

Discussion

We examined the functioning of the snowshoe hare stress axis across all phases of the most recent population cycle (from 2014 to 2020) in the southwestern Yukon. The broad demography of the cycle—from increase to decline—has

remained similar to past cycles. The unknown is how and if the low phase, which currently under way, will play out. We predicted that changes in predation risk would drive changes in chronic stress in hares, being low in the increase, higher in the peak, highest in the decline, and low again in the low. However, in contrast to the evidence from two previous cycles where all metrics indicated that decline phase hares were chronically stressed (Boonstra et al. 1998a; Sheriff et al. 2011a), we found no evidence of chronic stress as hare numbers peaked, and no cohesive, unified chronic stress phenotype as they declined. Table 3 lays out our predictions and summarizes our findings. Because the various measures are complex, we discuss each in turn, compare them to past results, explore the possible reasons for this dramatic shift in physiological function, and finally consider what this means for the future of the 10-year cycle.

Changes in stress axis function

We obtained 5 key measures of stress axis function in this cycle, and most of these departed from the predictions of predator-induced chronic stress effects seen in two previous cycles. The first was fecal cortisol metabolite (FCM) levels at capture, our integrated measure of baseline HPA axis activity over the hares' recent past (Sheriff et al. 2011b; Palme 2019). We found no change in FCMs across the hare cycle (Fig. 3). In contrast, Sheriff et al. (2011a) found that winter hare FCMs fluctuated in synchrony with predator density across the peak, decline, and low phases, and that FCMs in the first year of that decline were 40% higher than in peak or low years. Studies in other species have also found that heightened predation risk increases FCMs (Monclús et al. 2009; Hammerschlag et al. 2017; Fauteux et al. 2018; c.f. St Juliana et al. 2017). The second index was the changes in maximum steroid binding capacity (MCBC), a measure of corticosteroid binding globulin—the key protein that binds cortisol strongly in blood. All evidence indicates that chronically high stress levels depress liver production of CBG (Breuner et al. 2013). When that happens, it leads to an increase in free (unbound) glucocorticoids, further intensifying the impact of chronic stress. We did not see a reduction in MCBC during the decline phase (Fig. 4a), one of the hallmark signatures of hare chronic stress. In fact, hares in the first decline year (2018) had the highest MCBC at the base bleed of all years studied and values that were similar to those seen in the second year of the increase phase. In contrast, the two previous cycles examined found that MCBC values were lower during the first year of the decline than the second year of the decline (Boonstra et al. 1998a; Sheriff et al. 2011a) or the low phase (Boonstra et al. 1998a). The third index was the lack of variation in free cortisol concentrations at *Base* (response to capture and handling) across all 7 years (Fig. 4b). In contrast, Boonstra et al. (1998a) found higher free cortisol levels at *Base* in the 2 decline years than in the low year, and Sheriff et al. (2011a) saw higher values in the first than in the second decline year. The fourth index was the HPA axis' negative feedback mechanism that continued to function well in virtually all hares, irrespective of phase. Similarly, in the two previously investigated cycles, negative feedback also continued to function well irrespective of phase or year. Thus, the negative feedback mechanism from the adrenals to the HPA persists, irrespective of phase of the cycle and predation risk. The fifth index was our measure of adrenal capacity and overall stress reactivity. The magnitude of free cortisol responses to ACTH stimulation differed among years (Fig. 5a), but our *post-hoc* comparisons found that the only difference was that males in 2016 (increase) were lower than in 2020 (low). Hence, the predicted phase differences in stress reactivity were not seen. The previous two hare studies did not use the integrative area-under-the-curve (AUC) measure as we did here. However, both found marked increases in free cortisol in the

Table 3 Predicted impacts of chronic stress on the physiological targets examined during the peak and decline phases of the hare cycle, a summary of the year and sex effects seen over the entire cycle, and their support for the predator-induced chronic stress hypothesis of population regulation in snowshoe hares

Target	Peak & Decline Phase Predictions	Outcome	Chronic Stress Hypothesis
Baseline HPA axis activity	↑ *FCMs*	No year effects	Reject
CBG binding capacity	↓ *MCBC*	Greatest during the early decline	Reject
Dexamethasone resistance	↑ *Free cortisol*	Decline, Low < Increase, Peak*	Partial Support*
Adrenal capacity *(Capture)*	↑ *Free cortisol*	No year effects	Reject
Adrenal capacity *(ACTH)*	↑ *Free cortisol*	Low > late Increase (Males only)	Reject
Energy mobilization *(Capture)*	↑ *Glucose*	Early Increase > late Increase	Reject
Energy mobilization *(Challenge)*	↑ *Glucose*	Males < Females	Reject
Body condition	↓ *Winter weight*	Late Increase > All years; Males < Females	Reject
Body condition	↓ *Marrow fat*	Lowest during early Decline, Low	Support
Body condition	↓ *Hematocrit*	Late Decline > early Increase, Peak, early Decline	Reject
Immunity	↑ *N:L ratio*	Highest in Peak (Females); Males > Females	Partial Support

** Excludes 4 females from the increase (n = 1) and decline (n = 3) with evidence of impaired HPA axis negative feedback*

first year of the decline relative to either the second year or the low year. In summary, the functioning of the hare stress axis over this cycle differed markedly from that in the previous two cycles studied: *hares showed no evidence of chronic stress during the decline phase.*

Changes in energy mobilization

Chronic stress is known to increase hepatic production and storage of glucose as glycogen (Fujiwara et al. 1996) by enhancing the liver's capacity for gluconeogenesis (Miller and Tyrell 1995). This glycogen can then be rapidly mobilized by a severe stressor, such as a predator attack. We saw no evidence of change in the ability to mobilize glucose over this cycle, either in response to capture and initial handling (*Base* bleed; Fig. 4c) or in response to our hormonal challenge (Fig. 5b). In contrast, Boonstra et al. (1998a) found that at *Base*, hares had > 50% higher glucose levels in the first year of the decline than in the low. In response to DEX and ACTH, they found that hares in their first decline year mobilized much more glucose than those from the low; the second decline year hares were intermediate. Sheriff et al. (2011a) also found higher levels in first decline year than in the second. What is not captured by these statistical differences is the magnitude of the absolute differences among the studies. If we look only at the first decline year from each of these cycles, the baseline levels were approximately ~ 56% (50 mg/dl) higher in the first two cycles (i.e. 1991 ~ 145 mg/dl, 2007 ~ 138 mg/dl; Figs. 4d, 5c therein, respectively) than in this cycle (2018 ~ 89 mg/dl; Fig. 4c). Thus, hares in this current cycle showed no evidence of chronic stress effects on energy mobilization and glucose levels were much lower overall throughout all years of our study. Hence, we conclude that stress-induced gluconeogenesis was not occurring in the present cycle.

Changes in condition and immunity

We had two measures of hare condition over the cycle—hematocrit and bone marrow fat. Hematocrit levels are an integrative index of body condition and predicted to decline under chronic stress. Stress decreases erythropoiesis (Leung and Gidari 1981; Northrop-Clewes 2008) and lower hematocrit levels have been tied to increased predation risk (Hik et al. 2001; Clinchy et al. 2004) and to poorer nutritional and health status (Hellgren et al. 1993; Lochmiller et al. 1986; Moreno et al. 1998). We expected hematocrit levels to be high during the increase, low during the peak and decline, and intermediate during the low. However, we found that levels were low across the increase (39.9–41.8%) and low (42.7%) phases, lowest in the peak and first year of the decline (< 39%), and markedly higher in the second year of the decline (46.4%; Fig. 6a). Hares in the two previous cycles (Boonstra et al.1998a; Sheriff et al. 2011a) had hematocrit levels that were low in the first year of the decline (~ 40%) and higher by the second year of the decline (~ 45–46%). For those cycles, we had concluded that the latter levels were the norm and that elevated predation risk in the early decline drove them down. However, this cannot explain the observed low values in the increase phase of the present cycle, suggesting that condition was either poor over all these years (unlikely based on bone marrow fat), or that for this cycle, these lower values are the new norm.

Bone marrow fat (BMF) was our second index of condition. In cottontail rabbits *(Sylvilagus floridanus)*, BMF is a sensitive physical index to both recent (within 3 weeks) and relatively subtle changes (25% reduction) in nutritional intake (Warren and Kirkpatrick 1978). In ungulates, BMF is the last fat source to be mobilized before starvation (Mech and Delgiudice 1985). It is strongly correlated to whole body fat levels in snowshoe hares and is thus a good reflection of condition (Wirsing et al. 2002). In our study, BMF levels were high, ranging from 79.1% (first decline year) to 86.1% (second decline year; Fig. 6b). Of the two previous cycles that examined hare physiology, only Boonstra et al. (1998a) measured BMF. Their values were much lower (68.6% in the first decline year, 51.4% in the second decline year, and 56.6% in the low). Autopsy work from that same cycle by Hodges et al. (2006) echoed those results ($n = 570$ of which 499 came from the peak and decline winters), with BMF levels of hares killed by predators (51.0%) or humans (58.4%) being markedly higher than those dying of presumed starvation (29.5%). Thus, in contrast to the earlier work in the Yukon and in Alberta (Keith et al. 1984), our results indicate that throughout the present cycle, hares were in extremely good condition.

Our measure of immunity was a relatively crude leukogram. Chronic stress is predicted to depress immunity, resulting in an overall decline in white blood cell numbers (WBCs; Kelley 1985; Dhabhar 2002; Martin 2009), an increase in neutrophils, and a decrease in lymphocytes (i.e. increased *N:L* ratios; Davis et al. 2008). Field conditions in our study did not permit us to quantify absolute levels of WBCs. Though we saw a sex-specific effect on immunity, with females having a higher *N:L* ratios at the peak, there was no indication of stress-induced neutrophilia during the decline phase in either sex (Fig. 7a,b). In contrast, Boonstra et al. (1998a) found that total WBCs were significantly lower during the decline but no change in *N:L* ratios, whereas Sheriff et al. (2011a) found higher neutrophils during the first year of the decline than the second. Chronic stress was predicted to increase parasitic infections and this should be reflected in higher eosinophil levels in the decline and possibly the peak phases. Indeed, Keith et al. (1986) found higher levels of parasitic worms in declining hare population in Alberta. Consistent with this, Boonstra

et al. (1998a, b) found higher eosinophil levels in the decline than the low year and Sheriff et al (2011a, b) found higher eosinophil levels in the second than the first decline year. In contrast, we found levels lower in the peak and first decline year than the low year (Appendix S4 Supplementary material). Chronic stress decreases monocyte levels, cells that are indicative of infections and the results of both Boonstra et al. (1998a, b) and Sheriff et al. (2011a, b) are consistent with this. In contrast, we found lower levels during the last increase year and the peak year, but not during the decline. Thus, our blood cell measures were not indicative of chronic stress during the decline phase.

Evidence for a changing boreal forest

Our detailed physiological evidence from this cycle leads us to conclude that chronic predator-induced stress played no causal role in driving it. What happened? Was our focus on stress and its confirming evidence in the two previous cycles leading us in the wrong direction while some other factor was simultaneously playing a defining role? We think not. The mountain of work on stress in laboratory rodents, humans, and wild populations over the last century indicates that the stress axis is central to coping with acute and chronic changes in all vertebrates and that our focus was not misplaced (Sapolsky et al. 2000; Boonstra 2013). The alternative is that the environment has changed so that the relative influences of environmental stressors (predators and winter food) on snowshoe hares have shifted. Climate change could be the agent of that shift. Though the climate hypothesis is difficult to test directly as the causal mechanism driving this change in hare stress physiology over the cycle, there are a series of lines of evidence consistent with it.

Long-term warming trends in the Yukon have resulted in temperatures that are now 21% warmer in summer and 13% warmer in winter than they were 50 years ago (1970–2020; Fig. 1). Four lines of evidence from our area indicate that this warming is having marked ecosystem impacts. First, a decade-long spruce bark beetle (*Dendroctonus rufipennis*) outbreak ravaged large swaths of standing forest in the Yukon and Alaska (1994–2005; Berg et al. 2006) and likely occurred because of both warmer winter temperatures and drier summer conditions. The mountain pine bark beetles are having major impact on pine species throughout western Canada and the US for similar reasons (Bentz et al. 2010). Second, there has been a major increase in the standing biomass or "shrubification" of the Kluane boreal forest understory (> 100% increase in dwarf birch, and > 50% increase in grey willow; Grabowski 2015; Boonstra et al. 2017). These two shrubs are the hares' primary food in winter. Third, the increase in primary productivity has had cascading impacts on a number of small mammal species (Krebs et al. 2019). Arctic ground squirrels (*Urocitellus parryii)* went from approximately 17% of the Yukon's boreal herbivore biomass in the 1980s and 1990s, to less than 2% by 2015 (Boonstra et al. 2018). They are now functionally extinct from the forest valley meadows (Werner et al. 2016) due to their inability to detect predators (squirrels are line-of-sight herbivores) when the vegetation grows too high. Over the same time period, the density of red-backed voles (*Myodes rutilus*) and *Microtus* voles have also increased dramatically (Krebs et al. 2019). Fourth, there has been a shift in the boreal predator landscape. The increased density of small mammals led to the appearance of marten (*Martes americana)*, a major vole predator (Fig. 6, Boonstra et al. 2018). Marten are also capable of killing juvenile hares. Higher rodent densities can support a greater abundance of coyotes, another of the hare's major predators (Krebs et al. 2019). Moreover, a climatic trend toward shallower snow conditions in the Kluane region over the last two decades benefits the coyote (a mesopredator), by increasing their hunting success of hares (Peers et al. 2020). Snowshoe hares are particularly vulnerable to coyotes when snow depths are < 35 cm. The decline in coyotes densities from 2017 to 2020 (Fig. 2) is likely related to deeper snow levels. In contrast, warming-induced boreal shrubification has likely had the opposite impact on avian hare predators. An increase in the amount of understory cover should decrease hare susceptibility to predation by the great-horned owls and northern goshawks (Rohner and Krebs 1996) that previously accounted for up to 25% of hare kills (Hodges et al. 2001). Increased cover should also reduce the burden of predator-sensitive foraging—that is, the necessity of trading off resource quantity and quality for safety when predator risk is high (Hik 1995). All of these changes likely play a role in the physiological changes we have seen and the attenuation (or disappearance) of chronic stress effects in snowshoe hares. Finally, evidence from others indicates that the boreal forest tree chemistry is changing in response to increases in both temperature and CO_2 levels (Berini et al. 2018; Holopainen et al. 2018). Thus, the hares' summer and winter foods are likely also being affected, which may in turn affect their nutrition and population dynamics.

Are these negative results informative?

Our results are paradoxical and do not support the *predator-induced chronic stress hypothesis* for hare population regulation in this most recent cycle. Hares during the peak and decline phases were largely similar to those during the increase. However, neither do our results support the alternative—the *predator-sensitive foraging hypothesis* (Hik 1995). It predicts that condition would decline because of poor nutrition during the peak and decline (i.e. hares would forage in safe, but poor quality habitats), without a necessary increase in concurrent chronic stress. We found very high levels of BMF, our index of body condition, across all

phases of the cycle. Though Hik (1995) found evidence of hares foraging in safer habitats during the decline phase in the 2nd cycle, Hodges and Sinclair (2003) found that lasting predator effects on hares during the low phase were not mediated by a nutritional mechanism, with hares being able to maintain their nutritional intake irrespective of predator pressure. Hodges (1999) also found that hares moved more, not less, under risk of predation. Though we found some evidence of heightened energy mobilization and poor body condition in hares during the decline phase of this cycle, these effects were not dramatic and quickly ameliorated by the second year of the decline and into the low. Interestingly, a concurrent study during this same cycle demonstrated that experimental provisioning of individual wild female hares with high quality rabbit chow overwinter had a protective effect against overwinter mass loss and resulted in better body condition (hematocrit) from the increase to the first year of the decline, with both control and fed animals showing similar and elevated hematocrit values by the second year of the decline (Majchrzak 2020). Similar results were found with our whole population feeding experiment in the first decline winter and then in the whole population/predator exclusion in the second decline winter in the 2nd cycle (Boonstra et al. 1998a, b). Nonetheless, though winter food limitation (birch and willow shrubs) may have exacerbated sublethal predator effects at the peak in past cycles (Sinclair et al. 1988; Smith et al. 1988; Krebs et al. 2001b), virtually all the metrics detailed herein argue against food limitation now. Thus, hares may increasingly be showing behavior-mediated rather than stress-mediated prey responses (McArthur et al. 2014; Creel 2018; Majchrzak 2020). This explanation finds support in the results of an experimental manipulation of summertime predation risk during the increase phase of this most recent hare cycle. Boudreau et al. (2019) were able to elicit the anticipated effects of chronic stress (depressed CBG binding capacity, heightened cortisol responses) in snowshoe hares that were subjected to unavoidable, simulated predator encounters in the form of chases with a trained dog. Thus, though the capacity for predator-induced chronic stress in hares remained, the current environmental conditions (food, cover) appear sufficient to have buffered hares from the negative consequences of increasing risk, precluding the appearance of chronic stress signatures seen in previous cycles. This suggests the existence of a potential "threshold of susceptibility" to predator-induced chronic stress, mediated by the interacting influences of winter temperatures and snow depth, resource availability, foraging activity, and body condition.

What critically remains to be seen is whether the lack of decline-phase chronic stress effects in hares will have affected the hares' phased-based patterns of variable reproductive output, as well as the duration of the low phase currently underway. Other questions that need to be addressed are: whether organizational effects in the brain and on body physiology are occurring independent of impacts on HPA axis function, and whether changes in offspring fitness via maternal nutritional or predator-induced programming during the decline phase explain the lag in population recovery during the low. Thus, are the cyclic patterns we have studied for almost 50 years starting to disappear or changing? We see no evidence of this yet. Nevertheless, given that hares are no longer being chronically stressed during the decline, we predict that the low phase will disappear or be severely truncated, and the length of the cycle will shift from 9–11 years to 7–8 years. Thus predator-induced stress will cease to be a necessary and sufficient cause for the entire hare cycle. We also predict that the intense herbivory of the key winter shrubs documented during previous cycles in the peak and early decline phases will no longer be there. From the standpoint of the North American boreal forest, it will be critical to investigate the changes in the quantity and quality of the key vegetation components, the demographic, reproductive, physiological, and fitness changes in hares, hare–predator interactions, and the climate that underpins this ecosystem. Climate is the central bottom-up driver for the difference in boreal forest ecosystem organization in northwestern Eurasia from that in western North America (ericaceous dwarf shrubs and 3–4 year vole–mustelid cycles versus tall deciduous shrubs and 9–11 year snowshoe hare–lynx cycles, respectively, Boonstra et al. 2016). A speculative prediction is that climate change will increasingly drive the latter to resemble the former, but this will depend on the speed of change in temperature, moisture, and fire.

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References

Andreassen HP, Sundell J, Ecke F et al (2021) Population cycles and outbreaks of small rodents: ten essential questions we still need to solve. Oecologia 195:601–622. https://doi.org/10.1007/s00442-020-04810-w

Baker M (2016) Reproducibility crisis? Nature 533:353–366

Bentz BJ, Régnière J, Fettig CJ et al (2010) Climate change and bark beetles of the western United States and Canada: direct and indirect effects. Bioscience 60:602–613

Berg EE, Henry JD, Fastie CL et al (2006) Spruce beetle outbreaks on the Kenai Peninsula, Alaska, and Kluane National Park and Reserve, Yukon Territory: relationship to summer temperatures and regional differences in disturbance regimes. For Ecol Manag 227:219–232. https://doi.org/10.1016/j.foreco.2006.02.038

Berini JL, Brockman SA, Hegeman AD et al (2018) Combinations of abiotic factors differentially alter production of plant secondary metabolites in five woody plant species in the boreal-temperate transition zone. Front Plant Sci 9:1257. https://doi.org/10.3389/fpls.2018.01257

Bonnot NC, Bergvall UA, Jarnemo A, Kjellander P (2018) Who's afraid of the big bad wolf? Variation in the stress response among personalities and populations in a large wild herbivore. Oecologia 188:85–95

Boonstra R (2013) Reality as the leading cause of stress: Rethinking the impact of chronic stress in nature. Funct Ecol 27:11–23. https://doi.org/10.1111/1365-2435.12008

Boonstra R, Singleton GR (1993) Population declines in the snowshoe hare and the role of stress. Gen Comp Endocrinol 91:126–143. https://doi.org/10.1006/gcen.1993.1113

Boonstra R, Tinnikov AA (1998) Increased corticosteroid binding capacity of plasma albumin but not of corticosteroid-binding globulin caused by ACTH-induced changes in free fatty acid concentrations in snowshoe hares and rabbits. J Endocrinol 156:205–212. https://doi.org/10.1677/joe.0.156020

Boonstra R, Hik DS, Singleton GR, Tinnikov A (1998a) The impact of predator-induced stress on the snowshoe hare cycle. Ecol Monogr 79:371–394

Boonstra R, Krebs CJ, Stenseth NC (1998b) Population cycles in small mammals: the problem of explaining the low phase. Ecology 79:1479–1488

Boonstra R, Andreassen HP, Boutin S et al (2016) Why do the boreal forest ecosystems of northwestern Europe differ from those of western North America? Bioscience 66:722–734

Boonstra R, Krebs CJ, Cowcill K (2017) Responses of key understory plants in the boreal forests of western North America to natural versus anthropogenic nitrogen levels. For Ecol Manage 401:45–54. https://doi.org/10.1016/j.foreco.2017.06.065

Boonstra R, Boutin S, Jung TS et al (2018) Impact of rewilding, species introductions and climate change on the structure and function of the Yukon boreal forest ecosystem. Integr Zool 13:123–138. https://doi.org/10.1111/1749-4877.12288

Bossdorf O, Richards CL, Pigliucci M (2008) Epigenetics for ecologists. Ecol Lett 11:106–115

Boudreau MR, Seguin JL, Boonstra R et al (2019) Experimental increase in predation risk causes a cascading stress response in free-ranging snowshoe hares. Oecologia 191:311–323. https://doi.org/10.1007/s00442-019-04500-2

Boutin S, Krebs CJ, Sinclair ARE, Smith JNM (1986) Proximate causes of losses in a snowshoe hare population. Can J Zool 64:606–610

Brandt J, Flannigan P, Maynard MD et al (2013) An introduction to Canada's boreal zone: Ecosystem processes, health, sustainability, and environmental issues. Environ Rev 21:207–226. https://doi.org/10.1139/er-2013-0040

Breuner CW, Delehanty B, Boonstra R (2013) Evaluating stress in natural populations of vertebrates: total CORT is not good enough. Funct Ecol 27:24–36. https://doi.org/10.1111/1365-2435.12016

Brooks PM, Hanks J, Ludbrook JV (1977) Bone marrow as an index of condition in African ungulates. S Afri J Wildl Res 7:61–66

Cary JR, Keith LB (1979) Reproductive change in the 10-year cycle of snowshoe hares. Can J Zool 57:375–390. https://doi.org/10.1139/z79-044

Clinchy M, Zanette L, Boonstra R et al (2004) Balancing food and predator pressure induces chronic stress in songbirds. Proc Roy Soc B 271:2473–2479. https://doi.org/10.1098/rspb.2004.2913

Clinchy M, Sheriff MJ, Zanette LY (2013) Predator-induced stress and the ecology of fear. Funct Ecol 27:56–65. https://doi.org/10.1111/1365-2435.12007

Cohen J (1988) Statistical power analysis for the behavioral sciences. Routledge Academic, New York

Creel S (2018) The control of risk hypothesis: reactive vs. proactive antipredator responses and stress-mediated vs. food-mediated costs of response. Ecol Lett 21:947–956. https://doi.org/10.1111/ele.12975

Creel S, Winnie JA, Christianson D (2009) Glucocorticoid stress hormones and the effect of predation risk on elk reproduction. PNAS 106:12388–12393. https://doi.org/10.1073/pnas.0902235106

Dallman MF, Levin N, Cascio CS et al (1989) Pharmacological evidence that the inhibition of diurnal adrenocorticotropin secretion by corticosteroids is mediated via type i corticosterone-preferring receptors. Endocrinology 124:2844–2850. https://doi.org/10.1210/endo-124-6-2844

Davis AK, Maney DL, Maerz JC (2008) The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol 22:760–772. https://doi.org/10.1111/j.1365-2435.2008.01467.x

Delehanty B, Boonstra R (2011) Coping with intense reproductive aggression in male Arctic ground squirrels: the stress axis and its signature tell divergent stories. Physiol Biochem Zool 84:417–428

Dhabhar FS (2002) Stress-induced augmentation of immune function—the role of stress hormones, leukocyte trafficking, and cytokines. Brain Behav Immun 16:785–798. https://doi.org/10.1016/S0889-1591(02)00036-3

Doyle FI, Smith JNM (1994) Population responses of northern goshawks to the 10 year cycle in numbers of snowshoe hares. Stud Avian Biol 16:122–129

Dulude-de Broin F, Hamel S, Mastromonaco GF, Côté SD (2020) Predation risk and mountain goat reproduction: evidence for stress-induced breeding suppression in a wild ungulate. Funct Ecol 34:1003–1014. https://doi.org/10.1111/1365-2435.13514

Edwards PD, Frenette-Ling C, Palme R, Boonstra R (2021) A mechanism for population self-regulation: social density suppresses GnRH expression and reduces reproductivity in voles. J Anim Ecol 90:784–795. https://doi.org/10.1111/1365-2656.13430

Efford MG, Borchers DL, Byrom AE (2009) Density estimation by spatially explicit capture-recapture: likelihood-based methods. In: Thomson DL, Cooch EG, Conroy MJ (eds) Modeling demographic processes in marked populations. Springer, New York, pp 255–269

Elton C, Nicholson M (1942) The ten-year cycle in numbers of the lynx in Canada. J Anim Ecol 11:215–244

Estes JA, Terborgh J, Brashares S et al (2011) Trophic downgrading of planet earth. Science 333(6040):301–306. https://doi.org/10.1126/science.1205106

Fauteux D, Gauthier G, Berteaux D et al (2018) High Arctic lemmings remain reproductively active under predator-induced elevated stress. Oecologia 187:657–666. https://doi.org/10.1007/s00442-018-4140-4

Fidler F, Chee YE, Wintle BC et al (2017) Metaresearch for evaluating reproducibility in ecology and evolution. Bioscience 67:282–289
Fujiwara T, Cherrington AD, Neal DN, McGuinness OP (1996) Role of cortisol in the metabolic response to stress hormone infusion in the conscious dog. Metabolism 45:571–578. https://doi.org/10.1016/S0026-0495(96)90026-8
Grabowski M (2015) Interspecific boreal shrub growth response to climate, fertilization and herbivory. Master thesis, Department of Zoology, University of British Columbia, Vancouver, Canada
Hammerschlag N, Meyer M, Searkarmela SM et al (2017) Physiological stress responses to natural variation in predation risk: evidence from white sharks and seals. Ecology 98:3199–3210
Hellgren EC, Rogers LL, Seal US (1993) Serum chemistry and hematology of black bears: physiological indices of habitat quality or seasonal patterns? J Mamm 74:304–315. https://doi.org/10.2307/1382385
Hik DS (1995) Does risk of predation influence population dynamics? Evidence from cyclic decline of snowshoe hares. Wildl Res 22:115–129. https://doi.org/10.1071/WR9950115
Hik DS, McColl CJ, Boonstra R (2001) Why are Arctic ground squirrels more stressed in the boreal forest than in alpine meadows? Ecoscience 8:275–288
Ho DH, Burggren WW (2010) Epigenetics and transgenerational transfer: a physiological perspective. J Exp Biol 213:3–16. https://doi.org/10.1242/jeb.019752
Hodges KE (1999) Proximate factors affecting snowshoe hare movements during a cyclic population low phase. Ecoscience 6:487–496
Hodges KE, Sinclair ARE (2003) Does predation risk cause snowshoe hares to modify their diets? Can J Zool 81:1973–1985
Hodges KE, Krebs CJ, Hik DS et al (2001) Snowshoe hare demography. In: Krebs CJ, Boutin S, Boonstra R (eds) Ecosystem dynamics of the boreal forest: the Kluane Project. Oxford University Press, NY, pp 141–178
Hodges KE, Boonstra R, Krebs CJ (2006) Overwinter mass loss of snowshoe hares in the Yukon: Starvation, stress, adaptation or artefact? J Anim Ecol 75:1–13. https://doi.org/10.1111/j.1365-2656.2005.01018.x
Holopainen JK, Virjamo V, Ghimire RP et al (2018) Climate change effects on secondary compounds of forest trees in the northern hemisphere. Front Plant Sci 9:1445. https://doi.org/10.3389/fpls.2018.01445
Jain NC (1993) Interpretation of leukocyte parameters. In: Jain NC (ed) Essentials of veterinary hematology. Blackwell, Philadelphia, PA, pp 295–306
Keith LB (1990) Dynamics of snowshoe hare populations. Current Mamm 2:119–195
Keith LB, Meslow EC, Rongstad OJ (1968) Techniques for snowshoe hare population studies. J Wildl Manage 32:801–812. https://doi.org/10.2307/3799556
Keith LB, Cary JR, Rongstad OJ, Brittingham MC (1984) Demography and ecology of a declining snowshoe hare population. Wildl Monogr 90:1–43
Keith IM, Keith LB, Cary JR (1986) Parasitism in a declining population of snowshoe hares. J Wildl Dis 22:349–363
Kelley KW (1985) Immunological consequences of changing environmental stimuli. In: Moberg GP (ed) Animal stress. Amer Physiol Soc, Baltimore, pp 193–223
Krebs CJ (2014) Ecological Methodology, 2nd edn. Pearson, New York
Krebs CJ, Boutin S, Boonstra R et al (1995) Impact of food and predation on the snowshoe hare cycle. Science 269(5227):1112–1115. https://doi.org/10.1126/science.269.5227.1112
Krebs CJ, Sinclair ARE, Boonstra R et al (1999) Community dynamics of vertebrate herbivores—how can we untangle the web? In: Olff H, Brown VK, Drent RH (eds) Herbivores: Between plants and predators Blackwell Science Ltd. UK, London, pp 447–473
Krebs CJ, Boutin S, Boonstra R (eds) (2001a) Ecosystem dynamics of the boreal forest: the Kluane Project. Oxford University Press, New York
Krebs CJ, Dale MRT, Nams VO et al (2001b) Shrubs. In: Krebs CJ, Boutin S, Boonstra R (eds) Ecosystem dynamics of the boreal forest: the Kluane Project. Oxford University Press, NY, pp 92–115
Krebs CJ, Boonstra R, Boutin S (2018a) Using experimentation to understand the 10-year snowshoe hare cycle in the boreal forest of North America. J Anim Ecol 87:87–100. https://doi.org/10.1111/1365-2656.12720
Krebs CJ, Boonstra R, Kenney AJ, Gilbert BS (2018b) Hares and small rodent cycles: a 45-year perspective on predator-prey dynamics in the Yukon boreal forest. Aust Zool 39:724–732. https://doi.org/10.7882/AZ.2018.012
Krebs CJ, Boonstra R, Gilbert BS et al (2019) Impact of climate change on the small mammal community of the Yukon boreal forest. Integr Zool 14:528–541. https://doi.org/10.1111/1749-4877.12397
Lavergne SG (2018) Neurobiology of predation risk and the inheritance of stress in snowshoe hares *(Lepus americanus)* PhD. Thesis, University of Toronto, Toronto, Canada
Lavergne SG, McGowan PO, Krebs CJ, Boonstra R (2014) Impact of high predation risk on genome-wide hippocampal gene expression in snowshoe hares. Oecologia 176:613–624
Le PP, Friedman JR, Schug J et al (2005) Glucocorticoid receptor-dependent gene regulatory networks. PLoS Genet 1:159–170. https://doi.org/10.1371/journal.pgen.0010016
Leung P, Gidari AS (1981) Glucocorticoids inhibit erythroid colony formation by murine fetal liver erythroid progenitor cells in vitro. Endocrinology 108:1787–1794. https://doi.org/10.1210/endo-108-5-1787
Lochmiller RL, Hellgren EC, Varner LW, Grant WE (1986) Serum and urine biochemical indicators of nutritional status in adult female collared peccaries, *Tayassu tajacu* (Tayassuidae). Comp Biochem Physiol Part A Physiol 83:477–488
MacLeod KJ, Krebs CJ, Boonstra R, Sheriff MJ (2018) Fear and lethality in snowshoe hares: the deadly effects of non-consumptive predation risk. Oikos 127:375–380. https://doi.org/10.1111/oik.04890
Majchrzak YN (2020) The role of food limitation in the snowshoe hare *(Lepus americanus)* population cycle. PhD Thesis, University of Alberta, Edmonton, Canada
Martin LB (2009) Stress and immunity in wild vertebrates: timing is everything. Gen Comp Endocrinol 163:70–76. https://doi.org/10.1016/j.ygcen.2009.03.008
McArthur C, Banks PB, Boonstra R, Forbey JS (2014) The dilemma of foraging herbivores: dealing with food and fear. Oecologia 176:677–689. https://doi.org/10.1007/s00442-014-3076-6
McCaw LK (2020) Cyclical variation in predation risk: impacts on snowshoe hare *(Lepus americanus)* offspring through maternal programming. MSc. thesis, University of Toronto, Toronto, Canada
Mech LD, Delgiudice GD (1985) Limitations of the marrow-fat technique as an indicator of body condition. Wildl Soc Bull 13:204–206
Metrione LC, Hunter D, Penfold LM (2020) Seasonal changes in fecal glucocorticoid metabolite concentrations in bison *(Bison bison)* living with or without wolves *(Canis lupus)*. J Wildl Dis 56:175–178
Miller WL, Tyrell JB (1995) The adrenal cortex. In: Felig P, Baxter JD, Frohman LA (eds) Endocrinology and metabolism, 3rd edn. McGraw-Hill, New York, pp 555–711
Monaghue P, Spencer KA (2014) Stress and life history. Current Biol 24:R408–R412

Monclús R, Palomares F, Tablado Z et al (2009) Testing the threat-sensitive predator avoidance hypothesis: Physiological responses and predator pressure in wild rabbits. Oecologia 158:615–623. https://doi.org/10.1007/s00442-008-1201-0

Moreno J, De León A, Fargallo JA, Moreno E (1998) Breeding time, health and immune response in the chinstrap penguin *Pygoscelis antarctica*. Oecologia 115:312–319. https://doi.org/10.1007/s004420050522

Munafò MR, Smith GD (2018) Robust research needs many lines of evidence. Nature 553(7689):399–401. https://doi.org/10.1038/d41586-018-01023-3

Northrop-Clewes CA (2008) Interpreting indicators of iron status during an acute phase response—lessons from malaria and human immunodeficiency virus. Ann Clin Biochem 45:18–32. https://doi.org/10.1258/acb.2007.007167

O'Donoghue M, Boutin S, Krebs CJ, Hofer EJ (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, Boutin S, Krebs CJ, Murray DL, Hofer EJ (1998) Behavioural responses of coyotes and lynx to the snowshoe hare cycle. Oikos 82:169–183

Oli MK, Krebs CJ, Kenney AJ, Boonstra R, Boutin S, Hines JE (2020) Demography of snowshoe hare population cycles. Ecology 101:1–15. https://doi.org/10.1002/ecy.2969

Palme R (2019) Non-invasive measurement of glucocorticoids: advances and problems. Physiol Behav 199:229–243. https://doi.org/10.1016/j.physbeh.2018.11.021

Peckarsky BL, Abrams PA, Bolnick DI et al (2008) Revisiting the classics: considering nonconsumptive effects in textbook examples of predator–prey interactions. Ecology 89:2416–2425

Peers MJL, Majchrzak YN, Menzies AK et al (2020) Climate change increases predation risk for a keystone species. Nat Clim Change 10:1149–1153. https://doi.org/10.1038/s41558-020-00908-4

Preisser EL, Bolnick DI, Benard MF (2005) Scared to death? The effects of intimidation and consumption in predator–prey interactions. Ecology 86:501–509. https://doi.org/10.1890/04-0719

Price DT, Alfaro RI, Brown KJ et al (2013) Anticipating the consequences of climate change for Canada's boreal forest ecosystems. Environ Rev 21:322–365. https://doi.org/10.1139/er-2013-0042

Pritchard CE, Palme R, Langkilde T (2020) Glucocorticoid and triiodothyronine concentrations do not correlate with behavior in vicuñas *(Vicugna vicugna)*. Gen Comp Endocrinol 286:113299

Ricklefs R, Wikelski M (2002) The physiology/life-history nexus. Trends Ecol Evol 17:462–468

Rohner C, Krebs CJ (1996) Owl predation on snowshoe hares: consequences of antipredator behaviour. Oecologia 108:303–310

Sacta MA, Chinenov Y, Rogatsky I (2016) Glucocorticoid signaling: an update from a genomic perspective. Annu Rev Physiol 78:155–180

Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 21:55–89

Say-Sallaz E, Chamaillé-Jammes S, Fritz H, Valeix M (2019) Non-consumptive effects of predation in large terrestrial mammals: mapping our knowledge and revealing the tip of the iceberg. Biol Cons 235:36–52. https://doi.org/10.1016/j.biocon.2019.03.044

Sheriff MJ, Speakman J, Kuchel JR et al (2009a) The cold shoulder: free-ranging snowshoe hares maintain a low cost of living in cold climates. Can J Zool 87:956–964. https://doi.org/10.1139/Z09-087

Sheriff MJ, Bosson CO, Krebs CJ, Boonstra R (2009b) A non-invasive technique for analyzing fecal cortisol metabolites in snowshoe hares *(Lepus americanus)*. J Comp Physiol B: Biochem Syst Environ Physiol 179:305–313. https://doi.org/10.1007/s00360-008-0314-4

Sheriff MJ, Krebs CJ, Boonstra R (2011a) 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 MJ, Dantzer B, Delehanty B et al (2011b) Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia 166:869–887. https://doi.org/10.1007/s00442-011-1943-y

Sheriff MJ, McMahon EK, Krebs CJ, Boonstra R (2015) Predator-induced maternal stress and population demography in snowshoe hares: The more severe the risk, the longer the generational effect. J Zool 296:305–310. https://doi.org/10.1111/jzo.12249

Sinclair ARE, Arcese P (1995) Population consequences of predation-sensitive foraging: the Serengeti wildebeest. Ecology 76:882–891. https://doi.org/10.2307/1939353

Sinclair ARE, Krebs CJ (2002) Complex numerical responses to top-down and bottom-up processes in vertebrate populations. Phil Trans Roy Soc B Biol Sci 357(1425):1221–1231. https://doi.org/10.1098/rstb.2002.1123

Sinclair ARE, Krebs CJ, Smith JNM, Boutin S (1988) Population biology of snowshoe hares. III. Nutrition, plant secondary compounds and food limitation. J Anim Ecol 57:787–806

Sinclair ARE, Chitty D, Stefan CI, Krebs CJ (2003) Mammal population cycles: evidence for intrinsic differences during snowshoe hare cycles. Can J Zool 81:216–220. https://doi.org/10.1139/z03-006

Smith SM, Vale WW (2006) The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialogues Clin Neurosci 8:383–395

Smith JNM, Krebs CJ, Sinclair ARE, Boonstra R (1988) Population biology of snowshoe hares. II. Interactions with winter food plants. J Anim Ecol 57:269–286. https://doi.org/10.2307/4778

Spong G, Gould NP, Sahlén E et al (2020) Large-scale spatial variation of chronic stress signals in moose. PLoS ONE 15:e0225990. https://doi.org/10.1371/journal.pone.0225990

St. Juliana JR, Kotler BP, Wielebnowski N, Cox JG (2017) Stress as an adaptation I: stress hormones are correlated with optimal foraging behaviour of gerbils under the risk of predation. Evol Ecol Res 18:571–585

Stefan CI, Krebs CJ (2001) Reproductive changes in a cyclic population of snowshoe hares. Can J Zool 79:2101–2108. https://doi.org/10.1139/cjz-79-11-2101

Stothart MR, Bobbie CB, Schulte-Hostedde AI et al (2016) Stress and the microbiome: linking glucocorticoids to bacterial community dynamics in wild red squirrels. Biol Lett 12:20150875. https://doi.org/10.1098/rsbl.2015.0875

Warren RJ, Kirkpatrick RL (1978) Indices of nutritional status in cottontail rabbits fed controlled diets. J Wildl Manage 42:154–158

Wellman CL, Bollinger JL, Moench KM (2020) Effects of stress on the structure and function of the medial prefrontal cortex: insights from animal models. Int Rev Neurobiol 150:129–153

Werner JR, Gillis EA, Boonstra R, Krebs CJ (2016) You can hide but you can't run: apparent competition, predator responses and the decline of Arctic ground squirrels in boreal forests of the southwest Yukon. PeerJ 4:e2303. https://doi.org/10.7717/peerj.2303

Wingfield JC, Romero M (2001) Adrenocortical responses to stress and their modulation in free-living vertebrates. In: McEwen BS (ed) Handbook of Physiology, Section 7: the endocrine system. coping with the environment: neural and endocrine mechanisms. Oxford Univ Press, NY, pp 211–236

Wirsing AJ, Steury TD, Murray DL (2002) Relationship between body condition and vulnerability to predation in red squirrels and snowshoe hares. J Mamm 83:707–715

Yin B, Yang S, Shang G, Wei W (2017) Effects of predation risk on behavior, hormone levels, and reproductive success of plateau pikas. Ecosphere 8:e01643. https://doi.org/10.1002/ecs2.1643

Zanette LY, Clinchy M, Suraci JP (2014) Diagnosing predation risk effects on demography: can measuring physiology provide the means? Oecologia 176:637–651