Journal of Comparative Physiology B (2025) 195:359–377
https://doi.org/10.1007/s00360-025-01617-0

ORIGINAL PAPER

# Seasonal indices of nutrition and stress in a northern population of snowshoe hares

Claire Montgomerie[1] · Marianne Lian[2,3] · Greg Breed[1,4] · Mandy Keogh[5] · Knut Kielland[1,4]

Received: 30 March 2024 / Accepted: 7 April 2025 / Published online: 7 May 2025


## Abstract

Cyclic changes in snowshoe hare (*Lepus americanus*) fecundity have been attributed to changes in winter forage availability and predation pressure. Disentangling how nutrition and predation pressure affect snowshoe hare physiology is complex. As an herbivore of the northern boreal forests, snowshoe hares cope with extreme seasonal changes in diet, ambient temperature, and energy demands. We examined seasonal variation in the body condition index, blood biomarkers indicative of nutritional status, and fecal cortisol metabolite concentrations, in snowshoe hares across five ecologically distinct times of year in relation to adult survival rates. Snowshoe hares sampled from a high-density population in northern Alaska during 2018 showed decreases in survival and in plasma concentrations of total protein (TP), blood urea nitrogen (BUN), hematocrit (Hct), Chloride (Cl) and glucose during March and October. Increased survival and concentrations of Cl, TP, BUN, Hct, sodium (Na) and glucose were observed during August. Decreases in mass and survival from August to October suggest limited forage. Increases in TP, BUN, Hct and glucose in December suggest higher metabolic turnover. Fecal cortisol concentrations were not significantly associated with seasonal nutritional condition. A two-fold increase in mean cortisol was observed during August, potentially associated with energetically costly processes such as increased movement and reproduction. This work provides seasonal observations of snowshoe hare plasma biochemical values (N = 164) indicative of nutritional status, and supports the idea of using a collective biomarker approach to advance our understanding of how seasonality may play a role in snowshoe hare physiology.

**Keywords** Snowshoe hare (*Lepus americanus*) · Nutritional status · Glucocorticoid · Predation · Winter browse · Seasonal variation

## Introduction

The life history of snowshoe hares (*Lepus americanus*) in the boreal forests is dependent, in large part, on coping with seasonal metabolic challenges. Characteristic of high latitude environments, low winter temperatures, extended months of snow, and a short growing season make northern boreal forests an energetically demanding place to live (Anderson and Jetz 2005). Unlike other herbivorous small mammals inhabiting northern latitudes, snowshoe hares do not use the subnivean zone or undertake hibernation/torpor strategies to cope with the cold (Lavergne et al. 2019). Furthermore, they do not cache food and maintain minimal fat stores year-round (Whittaker and Thomas 1983). The shoulder seasons of spring and autumn are critical periods. In the spring, as hares become reproductively active and undergo pelage color change, winter browse still dominates their diet (Majchrzak et al. 2022). Although summer brings

Communicated by Gerhard Heldmaier.

✉ Claire Montgomerie
ckmontgomerie@alaska.edu

[1] Department of Biology and Wildlife, University of Alaska Fairbanks, 2090 Koyukuk Drive, Fairbanks, AK 99775, USA

[2] Department of Forestry and Wildlife Management, Inland Norway University of Applied Sciences, 2480 Koppang, Norway

[3] Department of Veterinary Medicine, University of Alaska Fairbanks, 2141 Koyukuk Drive, Fairbanks, AK 99775, USA

[4] Institute of Arctic Biology, University of Alaska Fairbanks, 2140 Koyukuk Dr., Fairbanks, AK 99775, USA

[5] Alaska Department of Fish and Game, Division of Wildlife Conservation, Fairbanks, AK 99701, USA

better forage, the energetically costly activities of reproduction and juvenile growth, are condensed into a few short months (Anderson and Jetz 2005; Sheriff et al. 2009a, b, c). The onset of autumn represents a drastic decline in dietary protein availability coinciding with increased energetic requirements needed to grow a new winter coat (Sheriff et al. 2009c).These seasonal challenges may contribute to lower body condition and increased mortality rates (Feierabend and Kielland 2015).

Snowshoe hares are well known for their cyclic population dynamics whereby both reproductive rates and survival rates fluctuate significantly over the course of 8–12 year cycles (Krebs et al. 1986; Kielland et al. 2010). Two primary factors are acknowledged as driving variation in the reproductive rates of snowshoe hares: (1) reduction in winter-food availability (Keith 1983), and (2) the development of predator-induced chronic stress (Boonstra et al. 1998). There is further evidence to support the idea that these two factors interact (Krebs et al. 1995, 2013; Majchrzak et al. 2022; Sheriff et al. 2011b; Shiratsuru et al. 2021) imposing synergistic physiological challenges to snowshoe hare homeostasis (Mcarthur et al. 2014).

Snowshoe hare cortisol concentrations have been examined at length, in a variety of studies, and across many cyclic phases (Boonstra et al. 1998; Boudreau et al. 2019; Krebs et al. 1995, 2013; Lavergne et al. 2021; Sheriff et al. 2009a, b, 2011a, b). During years of high predator density, cortisol concentrations in snowshoe hare have been documented to increase; these increases attributed to the idea that hares may develop chronic stress associated with predator avoidance behavior, in turn impacting reproductive rates (Boonstra et al. 1998). Existing literature on snowshoe hare cortisol levels, however, is almost entirely comprised of studies conducted in southwestern Yukon, Canada, near Kluane Lake. There is a lack of literature examining patterns of snowshoe cortisol levels elsewhere across the North America Boreal region.

Furthermore, there are gaps in the literature related to our understanding of how snowshoe hare nutritional status, may impact cortisol production. It is well recognized that following years of high hare abundance, available browse is reduced in both quality and quantity (Bryant et al. 1985; Majchrzak et al. 2022; Sinclair et al 1988). Plant secondary metabolites (PSM) or anti-herbivory defense compounds, known to impart toxicity or reduce nitrogen digestibility in herbivores, may become harder to avoid after years of intensified browsing (Bryant et al. 1985; Mcarthur et al. 2014). Sinclair et al. (1988), and more recently, Majchrzak et al. (2022) have demonstrated that food shortages occur during hare cylces in Kluane, and that food supplimentation trials during late increase phases are correlated with increases in hare density (Sinclair et al. 1988), production of larger leverts, a two-fold increase in over-winter survial (Majchrzak et al. 2022) and decreases in foraging time (Majchrzak et al. 2022; Shiratsuru et al. 2021). These findings suggest that reduced food availabilty may require hares to spend more time foraging to maintain energenic requirements at the cost of increasing predation risk. This further suggests that the predator-induced chronic stress theory may be tightly linked with an underlying source of nutritional stress.

Nutritional stress has been documented to trigger the hypothalamic–pituitary–adrenal axis (HPA) across many taxa (Parker 2003). Moshkin et al. (2003) found that corticosterone concentrations were significantly elevated in association with seasonal food limitation of water voles (*Arvicola amphibius*) and great gerbils (*Rhombomys opimus*). Many of the studies that have investigated predator-induced stress in snowshoe hares have simultaneously monitored aspects of immunology and condition (Boonstra et al. 1998; Boudreau et al. 2019; Lavergne et al. 2021; Majchrzak et al. 2022; Sheriff et al. 2011b). However, most blood biomarkers investigated have been intented to primarly assess stress physiology. Kluane studies, Boonstra et al. (1998), Boudreau et al. (2019), Lavergne et al. (2021) and Sheriff et al. (2011b) brought wild-caught hares into a lab setting and examined plasma levels of glucose and hematocrit, representative of baseline condition before administering hormone tests. There are additional markers of nutritional status that, when measured simutanioulsy with stress levels and survival, may add to our understanding of the relatiohsip between these factors. These investigations could help differientiate if increased snowshoe hares cortisol levels during years of high predator densities are associated only with vigilance-induced stress, or if nutritional stress due to lower food quality, also plays an important role (Hodges and Sinclair 2005). Specifically, assessment of nutritional blood biomarkers related to metabolism, energy availability, and body condition may better our understanding of how seasonal changes in diet affect the physiological state of snowshoe hares. Concentrations of blood urea nitrogen (BUN), total protein (TP), and glucose can be measured as indicators of metabolism and energy availability. BUN is an indicator of nitrogen turnover (Conrado 2020; Kerr 2002) while TP (comprised of albumen and globulin proteins) is synthesized in part by dietary amino-acids and low levels may help identify malnutrition (Conrado 2020; Kerr 2002). Low levels of glucose can also be a sign of poor dietary intake (Conrado 2020; Kerr 2002). Increases in blood glucose levels have been associated with years of high snowshoe hare predation (Boonstra et al. 1998), and predator exposure (Boudreau et al. 2019), and is proposed as a possible explanation for overwinter body mass loss (Boonstra et al. 1998). Hematocrit (Hct), and creatinine can be measured as indices of snowshoe hare body condition, but should be paired with other markers for interpreation purposes (Siegel and Walton 2020). Hct is a measure of the proportion of red blood cells in blood. Low levels have

been used as an integrative index of poor body condition via erythrocyte depletion, mineral loss, or an increase of white blood cells. Boonstra et al. (1998) reported snowshoe hare Hct values to be lower during decline years (when hare fecundity drops), than during low years of a cycle (when hare fecundity starts to increase). Boudreau et al. (2019) found no association between Hct levels and predator exposure in snowshoe hares but did observe annual variation. Creatinine is released into the blood stream in fixed concentrations proportional to muscle mass (Kerr 2002; Siegel and Walton 2020). Seasonal changes in its concentrations may indicate changes in muscle mass. Measuring the ratio of urea nitrogen:creatinine has been used to monitor nutritional deprivation in related lagomorphs such as cottontail rabbits (*Sylvilagus transitionalis*) (Villafuerte et al. 1997). Concentrations of ionized calcium (iCa) and electrolytes Sodium (Na), Chloride (Cl) and Potassium (K) can help address associations between PSM and hare nutrition. Most PSM's are biotransformed to organic acids in the gut of snowshoe hares. These organic acids may cause a reduction of Ca absorption and loss of Na, K and other minerals (Bernays et al. 1989). Buffering and excretion of these acid loads is necessary to avoid detrimental effects of acidosis such as bone calcium loss (Foley et al. 1995). However, unlike other herbivores, lagomorphs may be unable to augment urinary ammonium to excrete acid loads, and instead bicarbonate buffered acids may be accompanied by Na or K to be excreted (Foley et al. 1995). The body condition index (BCI) expressed as the ratio of mass to hindfoot length, is routinely used to evaluate the body condition of snowshoe hares (Boudreau et al. 2019; Lavergne et al. 2021). However, assessement of this metric alone, does not address the specific physiological processes that may be contribtuing to changes in body condition (Hodges et al. 1999). Furthermore, it excludes assessment of growing juvenile hares (small structural size) and potentially pregnant female hares (heavy mass) (Green 2001). Hodges et al. (1999) found that BCI of snowshoe hare exhibits cyclic fluctuations, though no link between BCI and reproductive output was found. These findings demonstrate the importance of simutaniously measuring multiple indices of body condition (Boudreau et al. 2019), both morphological and physiological. Food intake is a primary determinant of an individual's body condition, however interpretation of how diet relates to body condition is complex (Murray 2002). Integration of measuring multiple parameters of nutrition at different levels of biological organization and time representations, may provide a more comprehensive understanding of how the role of diet interplays with other physological factors (Resano-Mayor et al. 2016).

Due to the pronounced variation in seasonal metabolic challenges that snowshoe hares endure, exploring nutrition, stress and survival across a seasonal temporal scale can elucidate patterns related to these factors that may get overlooked when investigation is done at one discrete time period. Glucocorticoid production is responsible for regulating metabolism and behavioral changes needed to meet the energy requirements of routine life history events such as social hierarchy, breeding, and provisioning of young (Garamszegi et al. 2018; Romero 2002). Glucocorticoids increase during energetically demanding activities, and many studies have documented increases specifically during the breeding seasons of mammals and birds (Garamszegi et al. 2018; Czerwinska et al. 2013; Romero 2002; Howland et al. 1985). Examining seasonal glucocorticoid concentrations in combination with seasonal nutritional status, may therefore help interpret changes in glucocorticoid production (Garamszegi et al. 2018). Furthermore, survival rates and BCI have been documented to vary seasonally and be positively correlated (Feierabend and Kielland 2015). Shoulder seasons of spring and autumn show highest mortality rates and lowest body condition, indicating possible stress (Feierabend and Kielland 2015). Quantifying a comphensive look at seasonal nutritional status paired with cortisol levels, may elucidate why such patterns exist. Molt is another seasonal energetically demanding activity that hares undertake during shoulder seasons (Zimova et al. 2016). As climate warming is associated with shortened periods of snow-cover, hares are susceptible to seasonal camouflage mismatch (Mills et al. 2013). Although molt has been linked primarily to photoperiod, wide individual variation in molt phenology exists within populations (Mills et al. 2013). Monitoring seasonal physiological condition of hares could advance our understanding of the causes of molt variation (Zimova et al. 2014).

There is great value in expanding upon, and comparing results with, existing literature related to investigation of how food and fear affect snowshoe hare physiology and ultimately, cyclicity. Lavergne et al. (2021) replicated prevously investigated work monitioring hare stress physiology through an entire hare cycle, yet did not observe the predicted increases in chronic stress metrics that Boonstra et al. (1998) and Sheriff et al. (2011b) found during peak and decline years. Furthermore, variation in herbivory, plant compositon and ecology, and meteorology between southwestern Yukon and interior Alaska have been attributed to observed differences related to the importance of food availability to snowshoe hare cyclicity (Sinclair et al. 1988). Consequently, gaining a comprehensive understanding of seasonal snowshoe hare physiology across broader regions of North America's boreal forests is needed.

In an effort to increase our understanding of how body condition, cortisol production, and survival interplay across seasons, and in relation to pelage molt, we quantified markers of nutrition: (1) blood biomarkers BUN, HCT, Na, iCa, Cl, K, glucose and creatinine and total protein; (2) body condition index (BCI), fecal cortisol metabolite concentrations (FCM), survival, and timing of molt, in wild-caught

snowshoe hares between March of 2018 and April of 2019 in northern Alaska. This work may be the most northern study of snowshoe hares in North America. Additionally, unlike previous work investigating snowshoe hare blood biomarkers of condition, individuals sampled in this study were not transported out of the field setting before blood draw (Boonstra et al. (1998), Boudreau et al. (2019), Lavergne et al. (2021) and Sheriff et al. (2011b). The study was conducted during the late increase phase of a snowshoe hare population cycle, during which predators were increasing (Kielland et al. 2019), and available winter forage was noticeably reduced (Olnes et al. 2018). We sampled hares during five distinct times of the year corresponding to ecologically significant periods pertaining to breeding, molt, changes in the quantity and quality of forage, and increased mortality.

We hypothesized that blood biomarkers and BCI would show pronounced seasonal fluctuations in step with availability of forage. Additionally, we predicted that during times of decreased nutritional status, hares would exhibit elevated FCM concentrations. Moreover, we predicted that survival rates would decrease in association with reduced nutritional status. Lastly, we predicted that snowshoe hares exhibiting indices of better body condition would molt sooner than individuals with reduced body condition. Predictions are presented in Table 1.

## Methods and materials

### Study area

We conducted our study on a population of snowshoe hares inhabiting the northernmost limit of Alaska's boreal forest in the Brooks Range. Located over 400 miles further north than the Kluane Lake region, these mountains separate the final pockets of boreal forest from the treeless arctic coastal plain. Interspersed throughout the range, river drainages and low-lying landscapes hold sizable, discrete patches of boreal flora and fauna. Snowshoe hares were captured in one such area along the Dietrich River (67.859N, 149.826W). This riparian zone is composed primarily of willow (*Salix* spp.) and alder (*Alnus* spp.), as well as sparse balsam poplar (*Populus balsamifera*), and white spruce (*Picea glauca*).

### Population estimates

Pellet count density estimates have been conducted in the study area since 2007. Seven sites each containing 20 pellet plot arrays, are sampled annually using the methods of Krebs

**Table 1** Predicted seasonal outcomes of snowshoe hare: (1) blood biomarkers, (2) body condition index, (3) cortisol metabolite, (4) proportion of molt, and (5) survival, during a late increase year of snowshoe hare cycle in northern Alaska

| Metric | Sample description | Indicator of | Predicted seasonal outcome |
|---|---|---|---|
| Blood urea nitrogen | Measured from blood sample | Nutrition | Decrease during March, October and December sampling periods |
| Hematocrit | | Nutrition | |
| Sodium | | Nutrition | |
| iCa | | Nutrition | |
| Cl | | Nutrition | |
| K | | Nutrition | |
| Glocose | | Nutrition | |
| Creatinine | | Nutrition | |
| Total protein | | Nutrition | |
| Body Condition Index | Ratio of mass to right hindfoot length | Overall condition | |
| 11-Oxoetiocholanolone | Measured as a fecal metabolite of cortisol | Stress | Increase during March and October sampling |
| Proportion of molt | Photo indexed | Molt timing | Individuals who molt first will shower high concentrations of nutrition/condition markers |
| Survival | Monitored VHF collared individuals | Predation and condition | Lowest in spring and fall |

et al. (1987). Estimates made in 2018 indicated hares were in late increase phase of the population cycle, with an average of 4 hares/hectare.

## Live-capture

Blood and fecal samples from 173 snowshoe hares were used for analysis (Table 2). One hundred and thirty-one individuals were sampled, of which, 27 were sampled during two or more seasonal periods.

Hares were captured in #3 Havahart livetraps (model 1085, Lititz, Pennsylvania) baited with alfalfa and carrots. In an effort to minimize the effect of ingested bait on blood parameters measurements, bait was equally distributed to traps. Thirty traps were placed ~ 50 m apart just inside a brush-line parallel to the Dietrich River. The ground below each trap was cleared of all hare fecal pellets each time the trap was opened. This insured that collected fecal pellets were from the trapped individual. A piece of tarpaper was placed on the top of each trap for weather protection. Traps were set each day between 16:00–17:00 and checked between 7:00–8:00 the following morning. Due to high catch rate (summer stage primarily) and logistical constraints (very limited daylight and difficulties sampling in winter conditions), the longest time elapse between a trap being set and checked was 17 h. However, most hares were sampled within 14 h of trap opening. Number of consecutive trap nights was between 2 and 4 days for each season stages.

## Sampling timing

We captured hares in 2018 across five sampling periods corresponding with ecologically significant life history events and environmental conditions:

1. Late Winter (March)

Snowshoe hares are in a pre-breeding behavioral state. Prior to onset of breeding (late April), males become reproductively capable (Whittaker and Thomas 1983; Aldous 1937) and show signs of territorial/courtship displays. Snow depth remains substantial and hares still subsist on a winter diet (Kieth 1983).

2. Early Spring (May)

Males exhibit breeding behavior. Females may be in the early stages of pregnancy and both sexes are in the process of pelage molt (Aldous 1937). Snowmelt in this arctic region exposes new growth but hares still persist primarily on winter browse (Kieth 1983). During this stage, extent of molt was assessed.

3. Late Summer (August)

Females may have had up to three litters in the preceding months and may lactate until end of August (Aldous 1937). Hares persist primarily on summer browse of forbs and grasses (Miller et al. 2024). We also monitored lynx (*Lynx canadensis*) and great-horned owl (*Bubo virginianus*) reproduction in the study area during the summer of 2018 and both species of predators exhibited high reproductive rates indicative of abundant prey (snowshoe hare) availability.

4. Early Winter (October)

With the onset of winter, hares are in the process of another pelage molt. Temperatures are dropping well below freezing at night and some snow is typically present. Hare diet during this period has largely transitioned to winter browse (Bryant 2003).

5. Winter (December)

Temperatures of below − 30 °C are frequent. Hare winter browse consists of twigs of deciduous and conifer trees (Bryant 2003). Despite ingesting nearly 10% of their body mass per day in the winter, low food quality may incur up to 25% reduction in body mass of adult hares over the course of the long winter (Kielland et al. 2010). Hares can no longer be differentiated as an adult or juvenile.

**Table 2** The number and age of snowshoe hares sampled at each of the five sampling periods

| Sample timing | Females (count) | Males (count) |
|---|---|---|
| March | 14 | 20 |
| May | 18 | 19 |
| August | 21 (6 juvenile, 15 adult) | 18 (8 juvenile, 10 adult) |
| October | 16 (3 juvenile, 13 adult) | 21 (9 juvenile, 12 adult) |
| December | 9 | 17 |

## Sample collection

After capture, a pillowcase was wrapped around the door opening of each trap and hares were funneled into and restrained in the pillowcase. Each hare was fitted with a uniquely number steel Monel ear tag (National Band and Tag Co., Newport, Kentucky), weighed (PESOLA 42500 Medio-Line Metric Spring Scale, 2500 g), sexed and length of right hind-foot measured. During Late Summer and Early Winter sampling periods hares were also aged (adult, juvenile), distinguished by weight and pelage coloration—young of the year did not have any apparent white pelage. Adults retained white hare around feet. A

blood sample (0.75–1.0 mL) was collected from the left lateral saphenous vein of individuals using a heparinized (heparin sodium injection, US Pharmacopeia 1,000 US Pharmacopeia units/mL) 1-mL slip-tip syringe (tuberculin slip tip syringe, BD Medical, Franklin Lakes, New Jersey, USA) and a 28-gauge needle (Parasuraman et al. 2010). Blood samples were immediately transferred into plasma tubes (1.3 lithium heparin, polypropylene, micro-tube, NS, Henery Schein, 100/pk) and kept on ice for no more than 6 h prior to analysis. Blood samples used for analysis were never frozen (seven blood samples were discarded because they froze prior to analysis due to low (− 28 °C) field temperatures in December). All capture, handling and sample collection protocols were approved by the University of Alaska Fairbanks Institutional Animal Care and Use Committee (Protocol # 1124642-6). A fecal sample was collected from each individual by picking up pellets from under each cage. During sampling periods where the temperature was below freezing, we avoided collecting pellets with visible urine frozen onto them (Teskey-Gerstl et al. 2000; Sheriff et al. 2011a). Fecal samples were placed in whirl packs and immediately transferred to a field cryogenic shipper (− 180 °C) for up to 6 days before being transferred to a − 80 °C freezer until analysis.

## Collaring hares

Very high frequency (VHF) collars (Advanced Telemetry Systems, Isanti, Minnesota), programmed to deliver a mortality signal after 6 consecutive hours of no movement, were deployed on 16 hares in March to monitor survival rates throughout the study. Collars weighed 26 g and were only fitted on adult hares ≥ 1000 g (< 3% body weight). We fitted additional hares with collars during the May and August sampling periods in an attempt to maintain ~ 10 collared individuals "on-air" throughout this study. A total of 38 adult individuals were monitored. An effort was made to keep an equal number of males and females collared; however, our sample usually included more males than females. Mortality checks were done every 1–3 months between March 2018 and April 2019 using a directional Yagi antenna and handheld receiver (model R1000; Communications Specialists Inc., Orange, California).

## Coat color index

During periods of molt (sampling periods May and October), each hare was photographed in the trap before handling. Photographs were used to index individual coat color as an assessment of the extent the molt (proportion of white coloration present). As hares molt different body regions at different speeds (Ferreira et al. 2017), four regions (head, back, rump, and chest/underside) were first indexed separately, then totaled. Hare were categorized as having a Low, Medium or High amount of white pelage (Mills et al. 2013).

## Blood biomarker investigation

Seven blood biomarkers were identified to investigate seasonal changes in snowshoe hare metabolism and energy availability. Within 6 h of collection, concentrations of blood urea nitrogen (BUN), hematocrit (HCT), sodium (Na), ionized calcium (iCa), chloride (Cl), potassium (K), glucose and creatinine were measured in whole blood samples using a hand-held VETSCAN® i-STAT Analyzer (Abbot Point of Care Inc., Princeton, NJ, USA, *CHEM 8 + cartridge*). Next, whole blood samples were centrifuged at 2000*g* for 5 min. Plasma was transferred to 0.5 mL cryovial tubes. Any plasma samples showing signs of hemolysis (N = 9) were discarded and removed from analysis. Red cells and plasma samples were stored in a field cryogenic shipper (− 180 °C) for up to 6 days before being transferred to a − 80 °C freezer until laboratory analysis. Total protein (TP) was measured with a clinical refractometer (Melillo 2007).

## Extraction and assay of fecal cortisol metabolite

The compound 11-Oxoetiocholanolone is a metabolite of cortisol found in feces of ungulates and other herbivores (Teskey-Gerstl et al. 2000). Sheriff et al. (2009a) validated the use of this metabolite to quantify fecal cortisol in snowshoe hares using a group-specific enzyme immunoassay (EIA) established by Palme and Möstl (1996). Validation of this EIA included a test of dexamethasone suppression and adrenocorticotropic hormone stimulation of adrenal cortisol (Sheriff et al. 2009a). We used a commercially available 11-Oxoetiocholanolone ELISA Kit (Cayman Chemicals, Ann Arbor MI, Item No. 501420) to quantify fecal cortisol metabolites (FCM) in snowshoe hares.

Procedures described in Sheriff et al. (2009a) were followed to store, prepare and extract FCM from snowshoe hares. Fecal pellets from each individual were dried for 14–18 h using a Lyophilizer (LABCONCO Freeze Dry system/FreeZone 4.5, Missouri USA). Dry fecal pellets showing residue of urine, appearing as a white fuzz on pellets (Teskey-Gerstl et al. 2000; Sheriff et al. 2011a) were discarded to reduce urine cortisol metabolite signatures on results of fecal concentrations. Individual's pellets (8–14 pellets) were then homogenized using a coffee grinder (KRUPS F203, China). We removed visible plant material using forceps and only the fine fecal material was kept for analysis. To extract FCM, 2.5 ml of 80% methanol was added to 0.15 ± 0.025 g fecal dust and vortexed for 30 min at 6,000 rpm (HAAKE BUCHLER VORTEX 4322000, Saddle Brook NJ). Samples were then centrifuged for 15 min at

2500*g* (Beckman Coulter Microcentrifuge 20R, California USA). Methanol (~ 300 µl) was transferred to a new 1.5 mL polypropylene cryovial, flash frozen (10 min, − 20 °C), then centrifuged a second time (15 min at 2500*g*). Next, 40 µL of methanol was removed, dried under forced air and reconstituted in 400 µL of assay buffer (1:10). All samples were run in triplicate per manufacturer instructions and each assay included the full standard curve, two controls, non-specific binding wells, total activity wells, maximum binding, and 'zero' (blank) wells. Inter-assay and intra-assay coefficients of variation were 16% and 11%, respectively.

We validated the 11-Oxoetiocholanolone ELISA Kit for use with snowshoe hare fecal samples using standard methods including recovery of added mass, parallelism and dilution linearity ("Appendix 1"). Briefly, pools of methanol extracts for male and female snowshoe hare feces were made from multiple samples and were serially diluted in assay buffer to determine linearity and degree of parallelism to the standard curve. We plotted the results as the percentage bound versus the log of the relative dose, and slopes were inspected for linearity and parallelism. Assay accuracy was assessed by spiking standards with an equal volume of pools, as determined for 50% binding in the parallelism assays. Results were plotted as the observed versus expected standard dose and assessed for linearity, slope, and y-intercept.

## Caveats

Due to high catch rate (summer stage primarily) and logistical constraints (very limited daylight and difficulties sampling in winter conditions), the longest time elapsed between when traps were set and individuals were sampled, was up to 17 h. The gut passage time for fecal steroids in snowshoe hares is 8–12 h (Sheriff et al. 2009a). Therefore, FCM concentrations measured in the study, may have been influenced by capture related stress. Similarly, this confinement duration may have also impacted blood biomarker results. Traps were baited with alfalfa and carrots. Consumption of these foods may have also affected blood biomarker concentrations (Siegel and Walton 2020, Kalita et al. 2001). However, bait and trap confinement variables were held as constant as possible over all five sampling periods. Because the primary objective of this study was to examine seasonal changes in these markers, our results and discussion focus on identifying changes in values rather than absolute values. Nonetheless, we do compare concentrations and values observed in this study to results obtained in other snowshoe hare and lagomorph studies, and our findings were similar to those reported elsewhere ("Appendix 3"). When investigating physiology of wild caught animals, it is nearly impossible to avoid some level of capture and/or handling effect unless animals are immediately euthanized (Boonstra et al. 1998) or sampling is done at a biological level reflecting long-term physiology (i.e., bone marrow (Lavergne et al. 2021), feather, hair or nail sampling (Ataallahi et al. 2022). To our knowledge, this is the first study that attempts to take blood samples from wild caught snowshoe hares without first moving individuals to a lab setting, or euthanizing them in the field.

## Data analysis

Analysis was performed in R version 3.4.3. (R Core Team 2017). Blood biomarker concentrations, FCM concentrations and BCI scores (herein collectively referred to as condition markers) were first tested for normality and equal variance using Shapiro-Wilks test and Levene's test, respectively. Glucose and FCM were log-transformed to achieve normality. Linear mixed-effects models (package *lmer*) were then used to investigate seasonal variation in condition markers. Twenty-seven snowshoe hares were sampled across at least two sampling periods, therefore *individual ID* was classified as a random effect. In addition to the fixed effect of *Season*, we investigated the interaction with *Season* and *Sex,* and—for August and October data only (when *Age* data was collected)—the interaction of *Season* X *Age,* and the interaction *Season* (*Sex* X *Age*).

For each condition marker (seven blood biomarkers, BCI and FCM concentrations), four models were created. The first included the random effect *individual ID* and *Season.* Model two included *individual ID* and the interaction *Season*

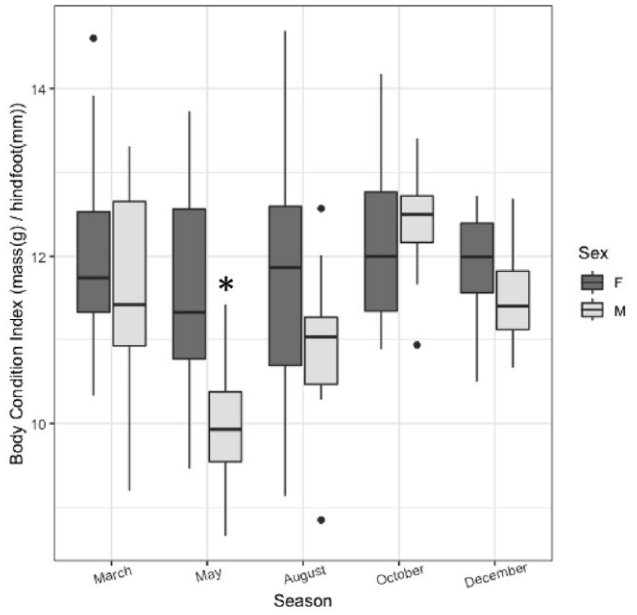


**Fig. 1** Seasonal body condition index scores of male and female snowshoe hares. Results from ANOVA (type III) indicate that male hares were in significantly poorer condition than female hares ($p < 0.001$) in May 2018 (denoted with *)

X *Sex*. The first two models were compared using (anova). Likelihood ratio results ($X^2$, df, *p* value) are presented in Fig. 1 under the heading *Likelihood ratio test*. Models three and four were used to investigate the effect of, *Season* X *Age* and the interaction *Season (Sex* X *Age)*, using only data from the August and October sampling periods (when juveniles could be distinguished). Model threer included *individual ID* and the interaction *Season* X *Age,* and model four included *individual ID* and the interaction *Season* (*Age* X *Sex*). Model comparison and reporting were done as outlined above for models one and two.. Linear mixed-effect models were used over ANOVA models for this analysis due to their robust ability to cope with unbalanced samples sizes.

Analysis of Variance (ANOVA Type III) was used to examine the relationship between individual coat color (amount of white pelage present) and condition markers. Analyses were performed separately for May and October; the two months during which molt was quantified. Chi-squared tests were used to examine the relationship between *coat color* and predictor variables *Sex* and *Age (*October only*)*. ANOVA (Type III) was also used to test for significant differences in BCI between female and male hares in late spring (May).

Survival rates were estimated using Kaplan–Meier method (Kaplan and Meier 1958) adapted for staggered entry (Pollock et al. 1989) in R using the *Survival* package. Survival rates were standardized (30 days) and estimated for each interval between mortality checks (done approximately every 1–3 months), then further grouped into seasonal categories (1) March–June 2018 (2) June–Sept 2018 (3) Sept–Dec 2018 (4) Dec–Jan (5) Jan–March 2019 (6) March–April 2019. Cox Proportional-Hazard for mixed-effects models (*Coxme* and *Survival* package) were used to investigate associations between survival time and condition markers.

## Results

### Body condition index

BCI showed significant seasonal variation ($X^2 = 33.19$, $df = 4$, $p < 0.001$, Table 3). BCI was lowest in May, increased between August and October, and then declined into December. BCI showed significant variation between *Sex* in May, during which male snowshoe hares were in significantly lower body condition than females ($F = 17.97$, $df = 1$, $p < 0.001$, Fig. 1).

### Blood biomarkers

Blood biomarker values varied significantly across seasons: BUN ($X^2 = 13.28$, $df = 4$, $p = 0.01$), TP ($X^2 = 29.63$, $df = 4$, $p < 0.001$), Hct ($X^2 = 39.96$, $df = 4$, $p < 0.001$), Na ($X^2 = 75.99$, $df = 4$, $p < 0.001$), Cl ($X^2 = 18.13$, $df = 4$, $p < 0.001$), glucose ($X^2 = 19.38$, $df = 4$, $p < 0.001$), iCa ($X^2 = 70.13$, $df = 4$, $p < 0.001$) and K ($X^2 = 78.14$, $df = 4$, $p < 0.001$) (Table 3, "Appendix 2"). Creatinine did not vary seasonally ($X^2 = 8.00$, $df = 4$, $p = 0.09$, Table 3).

Across all blood biomarkers except Na and Cl, fixed effects *Sex* and *Age* did not account for additional variance. (Table 3). In August, however, juvenile hares exhibited significantly lower Na ($F = 17.21$, $df = 1$, $p < 0.001$) and Cl ($F = 8.92$, $df = 1$, $p = 0.01$).

In an effort to depict an integrated assessment of seasonal body condition, each blood biomarker is presented relative to BCI for comparison in Fig. 2a–i). BUN, TP, Hct, Cl, Na and glucose all decreased from March to May then increased from May to August (Fig. 2a–f). BUN, TP, Hct, Cl and glucose then decreased between August and October (Fig. 2a–d). BUN, TP, Hct, and glucose increased between October and December (Fig. 2a–c, f). Ionized calcium and K did not follow similar seasonal patterns. In August, iCa decreased then increased through October and into December (Fig. 2g). K was highest in March and December (Fig. 2h).

### Fecal cortisol metabolite

Mean FCM showed significant seasonal variation ($X^2 = 32.54$, $df = 4$, $p < 0.001$, Table 3). FCM varied by less than 35% across all seasons other than in August, when FCM increased more than 100% (Fig. 2i) Interestingly, during the coldest month of December, mean FCM concentrations were lowest.

### Timing of molt

In May, female hares had progressed significantly further in their molt than males ($X^2 = 13.19$, $df = 2$, $p < 0.001$). Males not only retained a higher proportion of white pelage but also exhibited lower BCI scores at this time ($F = 4.69$, $df = 1$, $p = 0.04$). Concentrations of blood biomarkers glucose, Cl, and K were significantly associated with timing of molt (Table 4). Individuals who had molted more of their winter coat (who were browner in color), had significantly lower concentrations of Cl ($F = 5.67$, $df = 1$, $p = 0.02$) and K ($F = 4.67$, $df = 1$, $p = 0.04$) and significantly higher concentrations of glucose ($F = 11.84$, $df = 1$, $p = 0.01$) than individuals who had progressed less in their molting. By contrast, FCM concentrations did not show significant associations with spring timing of molt ($F = 0.06$, $df = 1$, $p = 0.8$) (Table 4).

In October, molt stage did not differ between sex or age. However, individuals further along in molt had significantly higher concentrations of Na ($F = 3.57$, $df = 2$, $p = 0.04$) and lower concentrations of K ($F = 4.00$, $df = 2$, $p = 0.03$). As we observed in spring, FCM concentrations did not

**Table 3** Likelihood ratio test and linear mixed effect model comparison results

| Biomarker | Likelihood ratio test (seasonal significance) | | | Model selection (1) Full dataset (2) Age dataset | | |
|---|---|---|---|---|---|---|
| | $X^2$ | *df* | *p* value | Models | | *df* |
| TP | 29.63 | 4 | < 0.001 | 1 | Season | 7 |
| | | | | | Season*sex | 12 |
| | | | | 2 | Season*age | 6 |
| | | | | | Season*age*sex | 10 |
| Cortisol | 32.54 | 4 | < 0.001 | 1 | Season | 7 |
| | | | | | Season*sex | 12 |
| | | | | 2 | Season*age | 6 |
| | | | | | Season *age*sex | 10 |
| BUN | 13.28 | 4 | 0.01 | 1 | Season | 7 |
| | | | | | Season*Sex | 12 |
| | | | | 2 | Season*age | 6 |
| | | | | | Season*age*sex | 10 |
| Glucose | 19.38 | 4 | < 0.001 | 1 | Season | 7 |
| | | | | | Season*sex | 12 |
| | | | | 2 | Season*age | 6 |
| | | | | | Season*age*sex | 10 |
| Hct | 39.96 | 4 | < 0.001 | 1 | Season | 7 |
| | | | | | Season*Sex | 12 |
| | | | | 2 | Season*age | 6 |
| | | | | | Season*age*sex | 10 |
| iCa | 70.13 | 4 | < 0.001 | 1 | Season | 7 |
| | | | | | Season*sex | 12 |
| | | | | 2 | Season*age | 6 |
| | | | | | Season*age*sex | 10 |
| K | 78.14 | 4 | < 0.001 | 1 | Season | 7 |
| | | | | | Season*sex | 12 |
| | | | | 2 | Season*age | 6 |
| | | | | | Season*age*sex | 10 |
| BCI | 33.19 | 4 | < 0.001 | 1 | Season | 7 |
| | | | | | Season*sex | 12 |
| | | | | 2 | No juveniles | NA |
| Cl | 18.13 | 4 | < 0.001 | 1 | Season | 7 |
| | | | | | Season*sex | 12 |
| | | | | 2 | Season*age | 6 |
| | | | | | Season*age*sex | 10 |
| Na | 75.987 | 4 | < 0.001 | 1 | Season | 7 |
| | | | | | Season*Sex | 12 |
| | | | | 2 | Season*age | 6 |
| | | | | | Season *age*sex | 10 |
| Creatinine | 8.00 | 4 | 0.09 | | No seasonal variation | |

Likelihood ratio results depict significance of condition marker seasonal variation. Concentrations of creatinine did not show significant seasonal variation. Results from mixed-effect model selection (anova) depict predictor variables (season, sex and age) for each condition marker. Two data sets were analyzed for each blood biomarker and cortisol metabolite: (1) Entire data set (2) Data only from seasons containing both Adult and Juvenile age classes. Body Condition Index (BCI) scores were not evaluated for juveniles

show significant associations with timing of autumn molt ($F = 0.96$, $df = 2$, $p = 0.40$) (Table 4).

## Timing of mortality

Mortalities within two days of capture were excluded from analysis (one individual). Nine other individuals were excluded due to loss of signal likely due to transmitter failure. Six collared hares remained at the end of the study. The average life span of adult hares after being collared was approximately 150 days ± 0.03 (Fig. 3). Survival rates did not vary significantly between males and females ($X^2 = 0.31$, $df = 1$, $p = 0.58$). In 2018, 30-day survival rates were lowest between March–June (0.67 ± 0.15) and highest between June

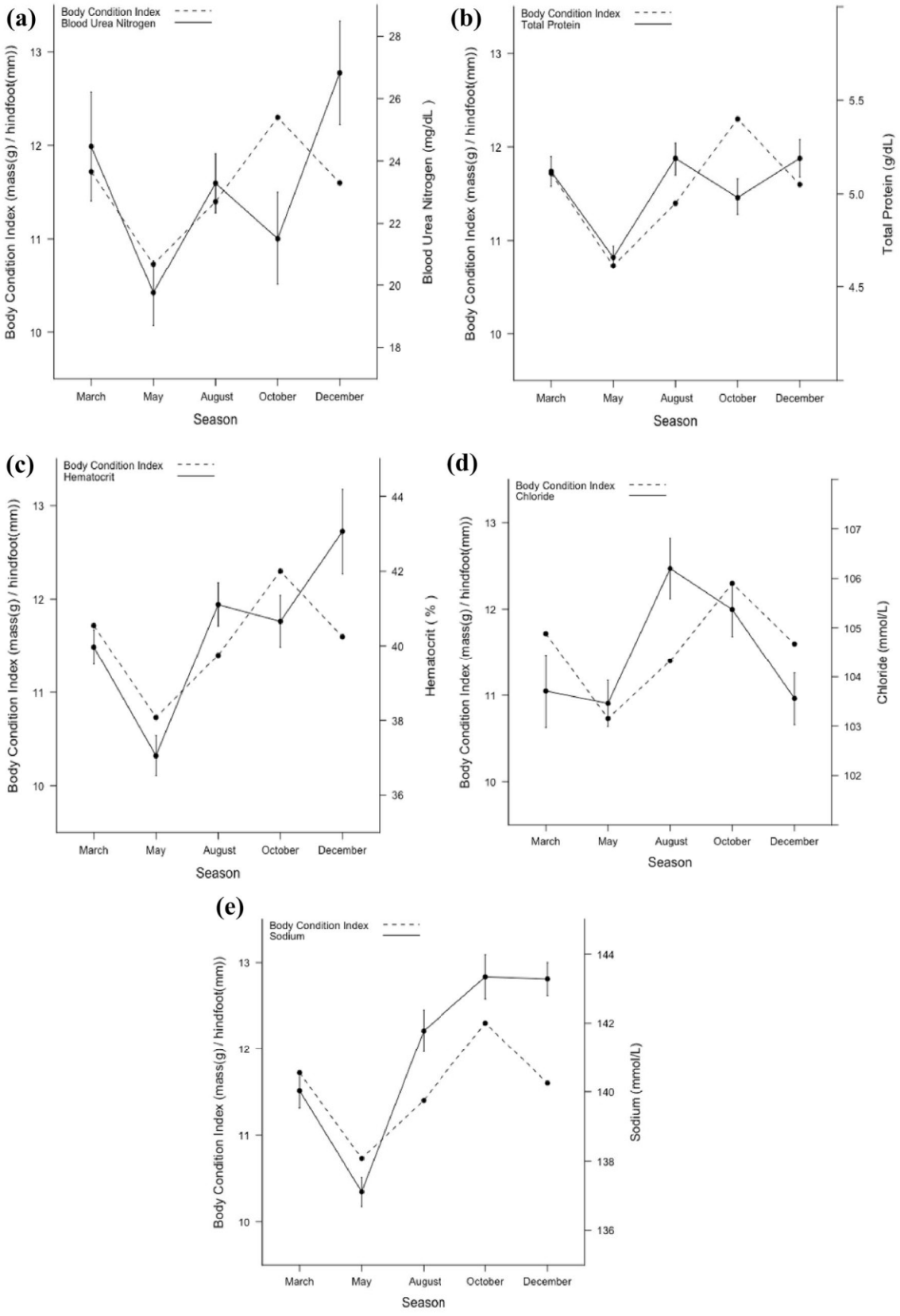


**Fig. 2** **a–i** Integrated assessment of snowshoe hare body condition. Seasonal mean values of different blood biomarkers (solid line) overlaid on seasonal mean values of Body Condition Index scores (BCI, dashed line) of snowshoe hare sampled from a population in northern Alaska in 2018. Graphics depict an integrated assessment of body condition as a function of BCI relative to each biomarker parameter

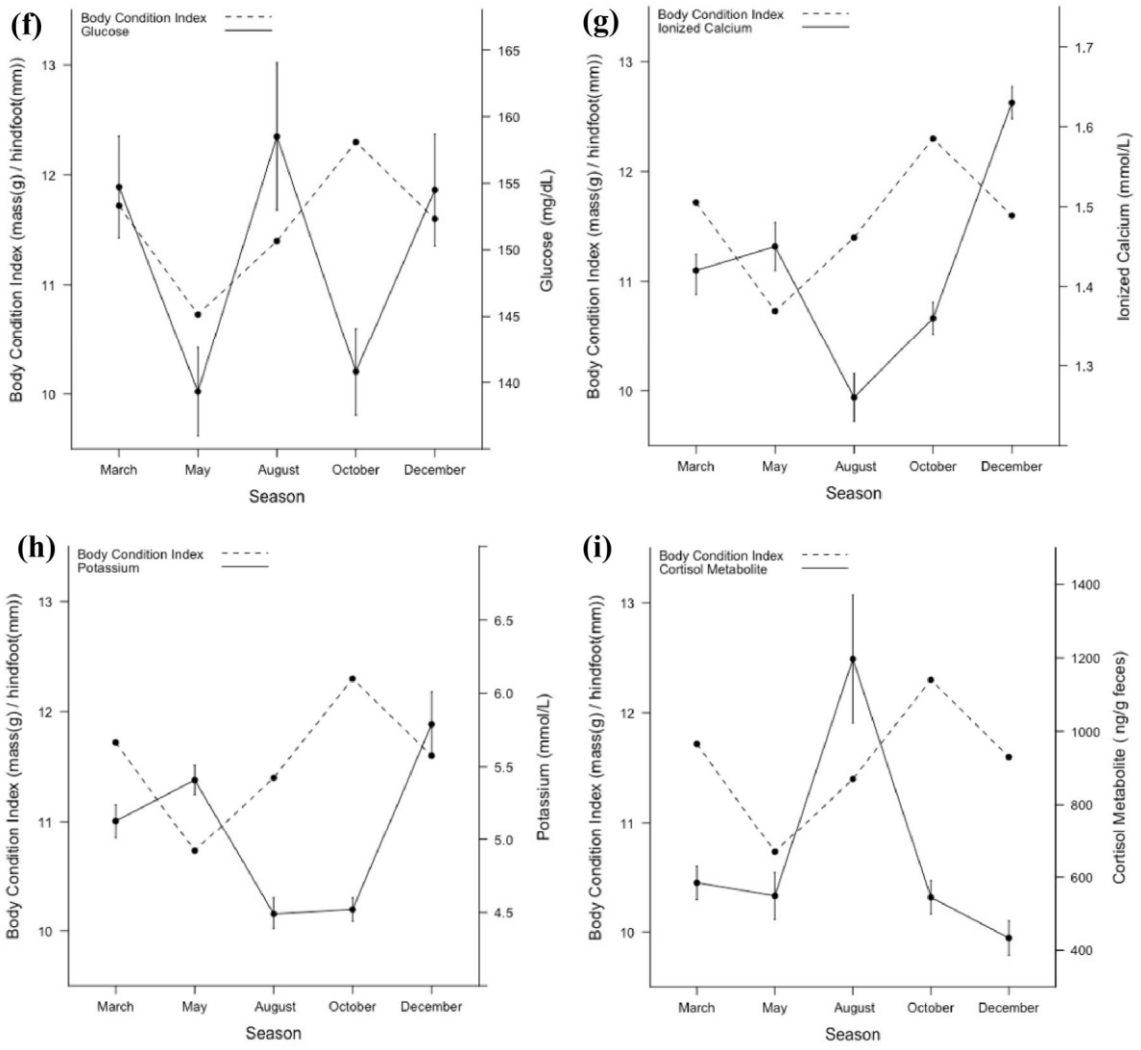


**Fig. 2** (continued)

and September (0.91 ± 0.08) (Fig. 4). Between September of 2018–April of 2019, survival rates decreased by ~ 30% (from 0.91 ± 0.08 to 0.60 ± 0.13; Fig. 4). Individual biomarker values did not show significant association with timing of mortality (Table 5).

## Discussion

Blood biomarkers (BUN, TP, Hct, glucose) and BCI scores concurrently suggest that the physiological condition of snowshoe hares declined in the spring and early winter of 2018, during a late increase phase of the population cycle. Snowshoe hares experienced the most pronounced decrease in body condition between March and May, coincident with lowest 30-day survival rates. These patterns in survival and physiological state are likely associated with reduced forage quality and quantity and increased energetic demands of spring reproduction. In May, male hares exhibited significantly lower BCI scores than females. Although concentrations of blood biomarkers did not mirror this pattern, low male BCI may reflect the role male hares play in reproduction; the lengthy pre-breeding behavior (i.e., courting) which males initiate early in the spring, may take a significant toll on body reserves and therefore body mass. Alternatively, BCI scores of females may have been higher because they were pregnant, however May sampling took place the first 4 days of the month, timed purposely to minimize impacts of pregnancy, as female hares typically become pregnant the end of April–early May (Aldous 1937). Male hares lagged significantly behind females in their spring molt, maintaining their white winter pelage later into spring. Pelage molt progression could be associated with energy availability, parturition timing, or both. It may be that male hares initiate molt later due to reduced body condition, or alternatively, this variation between sexes could be a strategy selected for

**Table 4** Associations between snowshoe condition marker values and timing of molt

| Biomarker | Season | Df | F value | *p* value |
|---|---|---|---|---|
| TP | Spring | 1 | 0.70 | 0.41 |
| | Fall | 2 | 1.50 | 0.24 |
| BUN | Spring | 1 | 2.19 | 0.15 |
| | Fall | 2 | 0.24 | 0.80 |
| Hct | Spring | 1 | 0.02 | 0.87 |
| | Fall | 2 | 2.36 | 0.11 |
| Glucose | Spring | 1 | 11.84 | 0.01* |
| | Fall | 2 | 1.95 | 0.16 |
| Cort | Spring | 1 | 0.06 | 0.81 |
| | Fall | 2 | 0.96 | 0.40 |
| BCI | Spring | 1 | 4.69 | 0.04* |
| | Fall | 2 | 2.92 | 0.06 |
| Na | Spring | 1 | 1.61 | 0.21 |
| | Fall | 2 | 3.57 | 0.04* |
| Cl | Spring | 1 | 5.67 | 0.02* |
| | Fall | 2 | 1.82 | 0.18 |
| K | Spring | 1 | 4.67 | 0.04* |
| | Fall | 2 | 4.00 | 0.03* |
| iCa | Spring | 1 | 1.90 | 0.18 |
| | Fall | 2 | 0.33 | 0.72 |
| Creatinine | Spring | 1 | 0.15 | 0.7 |
| | Fall | 2 | 0.52 | 0.60 |

ANOVA (type III) results testing differences between concentrations of each biomarker and snowshoe hare molt stage (*Low, Medium, High* proportions of pelage that was white). At the spring sampling, no hares were quantified as having a *Low* proportions of white pelage; therefore, comparison was only between *Medium and High* factors. Concentrations of biomarkers Glucose, BCI, Na, Cl and K showed statistically significant differences between molt stage

*Statistical significance

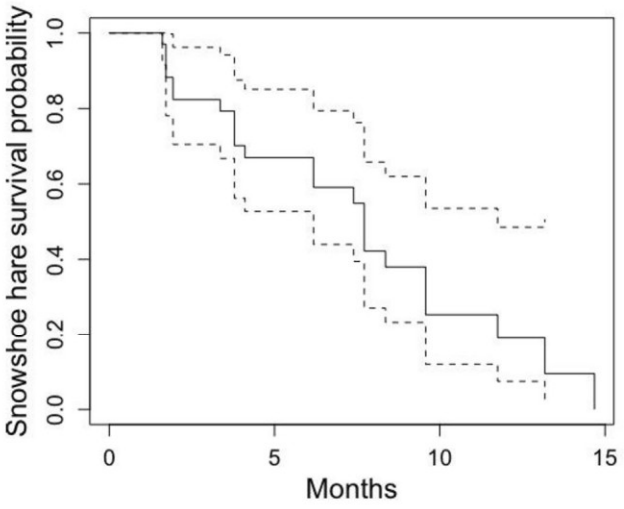


**Fig. 3** Estimated survival curve using the Kaplan–Meier method, for snowshoe hares collared with VHF transmitters and monitored between March 2018 and April 2019

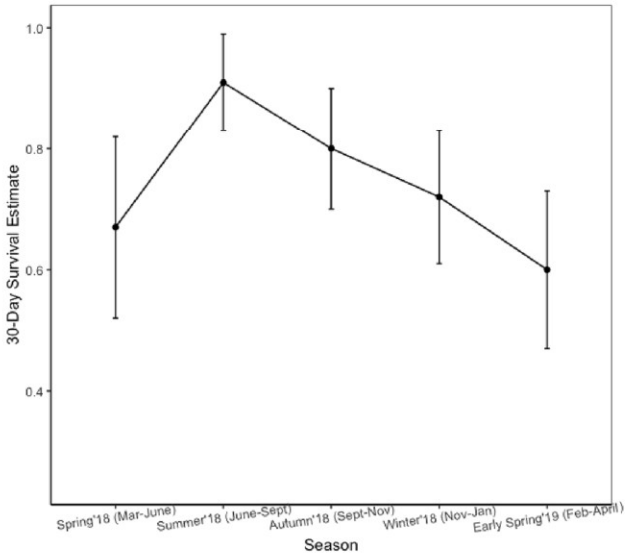


**Fig. 4** Standardized seasonal survival rates of snowshoe hares. Hares were collared between March 2018 and April 2019. Error bars denote survival estimate standard error

by female hares to maintain cryptic coloration prior to their first litter, typically born in late May (Sheriff et al. 2009b). Because we only qunatifed variation in molt between sex at the start of molt, it is unclear if the observered difference in molt timing between females and males represented only a variation in start time, or if rate of molt also varied between sexes (i.e., maybe male hares started later but molted at a faster rate throught spring).

FCM concentrations did not appear elevated during spring months. Mean FCM varied minimally between March and May (from 584 to 549 ng/g). However, snowshoe hares exhibited a strong increase in FCM (more than doubling in concentration) in August (1197 ng/g), even though concurrently sampled blood biomarkers and BCI scores indicated that snowshoe hares had significantly improved nutritional condition. An increase in BCI, concentrations of BUN, TP, Hct, Cl, Na, and glucose between May and August suggest that hares recovered to a better nutritional state, likely due to higher quality green summer forage available during this period. The pronounced doubling of FCM in August suggests that nutritional condition was uncoupled from fecal cortisol concentrations. Increased FCM concentrations may have instead been associated with a myriad of other factors, including: (1) reproductive effort (Romero 2002; Bartoš 1990), (2) increased movement during summer months, (Balm 1999), or (3) changes in diet and FCM excretion concentrations (Goymann 2012). The survival rates of adult snowshoe

**Table 5** Associations between snowshoe hare condition marker values and survival rates

| Biomarker | Coef | exp(coef) | se(coef) | Z | *p* value |
|---|---|---|---|---|---|
| TP | 0.09 | 1.09 | 0.42 | 0.22 | 0.83 |
| Cortisol metabolite | 8.6e−06 | 1.0 | 0.00 | 0.03 | 0.98 |
| Glucose | 0.00 | 1.00 | 0.01 | 0.04 | 0.97 |
| Hct | −0.01 | 0.10 | 0.05 | −0.06 | 0.95 |
| iCa | 0.16 | 1.17 | 1.15 | 0.14 | 0.89 |
| K | 0.05 | 1.05 | 0.25 | 0.20 | 0.84 |
| BCI | −0.09 | 0.91 | 0.22 | −0.43 | 0.67 |
| Cl | −0.01 | 0.99 | 0.05 | −0.20 | 0.84 |
| Na | −0.02 | 0.98 | 0.05 | −0.45 | 0.65 |
| Creatinine | 1.08 | 2.96 | 2.31 | 0.47 | 0.64 |

Mixed effect Cox Proportional-Hazard model results test for associations between concentrations of each biomarker and individual survival time. No statistically significant associations were observed

hare do not corroborate predation related stress. Although our survival monitoring periods varied slightly from our sampling periods, the general trend showed an increase in survival between Spring (March–June) and Summer (June–September). Although we did not assess survival of juvenile hares, the pronounced decrease in the ratio of juveniles to adults over the winter observed elsewhere in Alaska strongly suggest that juveniles bear the brunt of predation (Kielland et al. 2010). However, mean FCM concentrations of adults (1209 ng/g feces) and juvenile hares (1175 ng/g feces) varied minimally in August.

Observed decreases in snowshoe hare blood biomarker concentrations (BUN, TP, Hct, glucose) from August to October suggest hares experienced a decrease in nutritional condition. Seasonal timing of this shift may be associated with the transition of diets from green forage back to winter browse, decreasing ambient temperatures, and energy demands associated with growing a winter coat. Unlike blood biomarkers, BCI continued to increase from August to October. However, by December, BCI had dropped significantly and were similar to March.

Blood biomarkers (BUN, TP, Hct, iCa and glucose) increased in December. We suspect these increases are attributed to energetic demands of winter thermoregulation, which typically cause metabolic rates of small mammals to increase in high latitude environments (Anderson and Jetz 2005). The combined observations of high BUN, glucose and TP concentrations paired with decreased mean BCI, is consistent with this hypothesis and suggests hares may have been experiencing high metabolic turnover, without adequate diet to replenish reserves. Ambient temperatures were below − 30 °C during winter sampling. The effect of sitting in a trap at such extreme temperatures may alone influence metabolic rates. Recent studies in Interior Alaska suggest that in winter hares may double their metabolic rate as air temperature drops from a few degrees below freezing to − 40 °C (Kielland in prep.). Discrepancies between blood biomarkers and BCI exemplify the value of integrating evaluation of parameters of nutrition at different levels of biological organization and time representation to gain a more comprehensive understanding of seasonal changes in condition (Resano-Mayor et al. 2016).

Survival rates decreased steadily from summer to autumn and into the following spring, as observed elsewhere in Alaska (Feierabend and Kielland 2015). Because juveniles were indistinguishable from adults after fall molt, hares collared in December may have been young-of-the year, which could have influenced subsequent estimates of survival rates. At the period of lowest survival, between March–April 2019, survival rate had decreased 7% from survival estimate of March–April 2018 (however this difference falls within the confidence intervals for the survival estimates of these two time periods). This could be indicative of increased predator density during 2018, a greater reduction in available winter forage or a combined effect of these two factors.

Similar to spring sampling, no association between nutritional status and cortisol production was found in fall or winter. As hare nutritional condition decreased from summer to winter, FCM concentrations also showed a significant drop between August and October (from 1197 to 545 ng/g) and were within ~ 5% of concentrations observed in May. Despite the continuing reduction of available food and decreasing temperatures, concentrations of FCM were lowest in mid-winter (433 ng/g), decreasing ~ 25% between October and December. These findings could be attributed to reduced movement rates during winter months, as observed in previous studies in interior Alaska (Feierabend and Kielland 2014; Balm 1999). Tamian et al. (2023) found that, following periods of inclement weather, Columbian ground squirrels (*Urocitellus columbianus*) had lower FCM levels, suggestive of hypo-responsiveness of the HPA axis related to a general hypometabolic response aimed to conserve energy during inclement weather.

A major question left unanswered is whether these patterns are likely to change at different points in the snowshoe hare population cycle. It appeared that during 2018 in northern Alaska, hares were able to physiologically recover from presumed low nutritional status and increased cortisol levels, expressed by the variation from one season to the next (Table 6). FCM concentrations obsereved in this study were similar to observations previously made by: (1) Sheriff et al. (2009b), who observed ranges from 263.92 to 476.62 ng/g, representing concentrations from wild-caught female snowshoe hares 30 h after parturition (fed in captivity) during a cyclic peak and early decline (years 2006–2008) and, (2) Sheriff et al. (2011b), who observed mean ranges of ~ 400 ng/g to ~ 800 ng/g from wild-caught hares during late winters between peak and decline years (2006–2009).

**Table 6** Seasonal mean concentration ± 1SE and range of each biomarker sampled from snowshoe hares in northern Alaska in 2018

| Biomarker | Early spring (March) | Late spring (May) | Late summer (August) | Autumn (October) | Winter (December) | Range |
|---|---|---|---|---|---|---|
| Total protein (g/dl) | 5.12 ± 0.08 | 4.66 ± 0.06 | 5.19 ± 0.09 | 4.98 ± 0.10 | 5.19 ± 0.10 | 4.00–6.40 |
| Cortisol metabolite (ng/g feces) | 584.33 ± 45.43 | 548.74 ± 64.63 | 1197.09 ± 174.80 | 544.79 ± 45.79 | 433.47 ± 46.95 | 90.91–4517.66 |
| Blood urea nitrogen (mg/dL) | 24.47 ± 1.73 | 19.76 ± 1.05 | 23.29 ± 0.95 | 21.51 ± 1.48 | 26.83 ± 1.66 | 3–46 |
| Sodium (mmol/L) | 140.03 ± 0.50 | 137.11 ± 0.42 | 141.77 ± 0.60 | 143.34 ± 0.64 | 143.28 ± 0.48 | 132–152 |
| Ionized calcium (mmol/L) | 1.42 ± 0.03 | 1.45 ± 0.025 | 1.26 ± 0.029 | 1.36 ± 0.02 | 1.63 ± 0.02 | 0.86–1.76 |
| Chloride (mmol/L) | 103.71 ± 0.73 | 103.46 ± 0.50 | 106.20 ± 0.61 | 105.37 ± 0.55 | 103.56 ± 0.53 | 94–116 |
| Glucose (mg/dL) | 154.73 ± 3.81 | 139.30 ± 3.31 | 158.51 ± 5.55 | 140.80 ± 3.26 | 154.50 ± 4.20 | 98–277 |
| Creatinine (mg/dL) | 0.41 ± 0.02 | 0.39 ± 0.02 | 0.35 ± 0.02 | 0.39 ± 0.01 | 0.38 ± 0.03 | 0.20–0.60 |
| Potassium (mmol/L) | 5.13 ± 0.11 | 5.41 ± 0.10 | 4.49 ± 0.10 | 4.52 ± 0.08 | 5.79 ± 0.22 | 3.20–6.90 |
| Hematocrit (%) | 39.97 ± 0.46 | 37.05 ± 0.53 | 41.11 ± 0.58 | 40.66 ± 0.69 | 43.06 ± 1.13 | 28–56 |

Our observed ranges were higher than ranges observed by: (1) Boudreau (2019), who observed ranges of ~ 60 ng/g to ~ 190 ng/g from wild-caught hares during late increase—peak years (2015–2016), and (2) Lavergne et al. (2021), who observed ranges of ~ 80 ng/g to ~ 140 ng/g from wild-caught hares across a snowshoe hare cycle (2014–2020).

This research provides the first evaluation of wild-caught snowshoe hare nutrition condition markers, paired with mean concentrations of FCM, across multiple seasons during a high-density year in Alaska. We can only speculate about the magnitude that these parameters take during different points across the complete population cycle. The intra-annual variation in these indices of nutritional condition may be similar to inter-annual variation due to roughly similar patterns of population fluctuations, food availability, and predation pressure.

Hematology and serum biomarker reference range values are published for many other species of the Leporidae family, however lagomorphs are often confined to enclosures and provided supplemental food in most studies. In comparison to biomarker range values observed for cottontail rabbits (*Sylvilagus floridanus*, Jacobson et al. 1978) and European Brown hares (*Lepus europaeus*, Marco et al. 2003), overall concentrations (mean across all periods of sampling) of snowshoe hare TP, Hct, Na, iCa, and K observed in our study were ~ 20–30% lower. Glucose values were also ~ 55% lower, while BUN levels were ~ 30% higher than European hares and approximately equal to BUN levels of cottontails.

Investigating snowshoe hare physiology is pertinent to understanding the apparent cyclic variation in fecundity (Stefan et al. 2001). We can estimate predator density, track mortality, monitor movement, and quantify available browse, but without relating these findings to underlying physiology, it is challenging to explain how these factors mechanistically affect fitness. Results from this study lend insight to our understanding of how snowshoe hare body condition relates to concentrations of biomarkers representative of both cortisol production and nutritional status. Moreover, our observations bolster interpretation of how seasonal variation in energetically demanding activities, as well as survival rates, are associated with nutrition and cortisol markers in a wild population of snowshoe hares. Lastly, comprehensive assessment of hare body condition (BCI, blood biomarkers indicative of nutrition and FCM) helps provide a better basis for comparison across regions. It has been noted in the literature that plant responses to hare browsing, such as regrowth, nutrient concentrations and PSM accumulation, may vary by browsing pressure and region (Reichardt et al. 1984; Bryant et al. 1985; Krebs et al. 1986; Bryant 2003). Other system differences such as herbivore competition (moose), weather conditions (reoccurring harsh winters/snow depth) and plant and predator species composition, may also vary. This makes it difficult to form broad generalizations about the effects of food availability and predation on cyclic trends across snowshoe hare populations at northern latitudes. A comprehensive, integrative assessment of the physiological status of snowshoe hares provides an opportunity to better tease apart the potential ecological factors that drive variation in nutritional status, stress, reproduction and survival, which ultimately drive the snowshoe hare population cycle across the boreal forests of North America.

## Appendix 1: Validation of ELISA Kit

See Fig. 5.

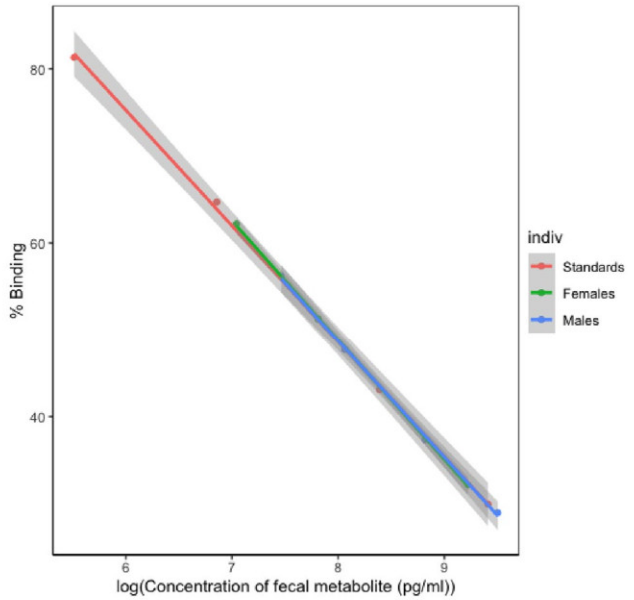


**Fig. 5** Validation of 11-Oxoetiocholanolone ELISA kit. Demonstrates that pooled male and female sample dilution response curves are parallel to the standard concentration response curve

## Appendix 2: Parameter estimates from model comparisons (anova). The letter *J* denotes juvenile hares and the letter *M* denotes male hares

| Biomarker | Parameters of selected model | | | |
|---|---|---|---|---|
| | Variable | Estimate | Std. Error | t value |
| TP | Intercept | 0.71 | 0.01 | 97.43 |
| | Spring | −0.04 | 0.01 | −4.52 |
| | Summer | 0.00 | 0.01 | 0.19 |
| | Fall | −0.01 | 0.01 | −1.39 |
| | Winter | 0.00 | 0.01 | 0.53 |
| Cortisol | Intercept | 2.72 | 0.05 | 58.11 |
| | Spring | −0.08 | 0.06 | −1.27 |
| | Summer | 0.23 | 0.06 | 3.57 |
| | Fall | −0.03 | 0.07 | −0.47 |
| | Winter | −0.13 | 0.08 | −1.71 |
| BUN | Intercept | 24.42 | 1.34 | 18.27 |
| | Spring | −4.66 | 1.83 | −2.55 |
| | Summer | −1.31 | 1.88 | −0.70 |
| | Fall | −2.78 | 1.87 | −1.49 |
| | Winter | 2.62 | 2.26 | 1.16 |
| Glucose | Intercept | 2.18 | 0.01 | 188.93 |
| | Spring | −0.05 | 0.02 | −3.05 |
| | Summer | 0.01 | 0.02 | 0.38 |
| | Fall | −0.04 | 0.02 | −2.61 |
| | Winter | −0.00 | 0.02 | −0.09 |
| Hct | Intercept | 39.95 | 0.61 | 65.48 |
| | Spring | −2.96 | 0.84 | −3.54 |
| | Summer | 1.13 | 0.85 | 1.32 |
| | Fall | 0.70 | 0.85 | 0.81 |
| | Winter | 3.10 | 1.03 | 3.00 |
| iCa | Intercept | 1.42 | 0.03 | 56.33 |
| | Spring | 0.03 | 0.03 | 0.92 |
| | Summer | −0.16 | 0.04 | −4.47 |
| | Fall | −0.06 | 0.04 | −1.61 |
| | Winter | 0.21 | 0.04 | 5.02 |
| K | Intercept | 5.15 | 0.10 | 51.14 |
| | Spring | 0.29 | 0.13 | 2.18 |
| | Summer | −0.65 | 0.14 | −4.78 |
| | Fall | −0.62 | 0.14 | −4.50 |
| | Winter | 0.68 | 0.19 | 3.59 |
| BCI | Intercept | 11.95 | 0.28 | 43.11 |
| | Spring | −0.59 | 0.36 | −1.61 |
| | Summer | −0.29 | 0.38 | −0.77 |
| | Fall | 0.21 | 0.39 | 0.54 |
| | Winter | −0.30 | 0.49 | −0.62 |
| | Intercept M | −0.37 | 0.36 | −1.02 |
| | Spring M | −1.30 | 0.34 | −3.82 |
| | Summer M | −0.61 | 0.41 | −1.48 |
| | Fall M | 0.14 | 0.41 | 0.34 |
| | Winter M | −0.28 | 0.51 | −0.55 |
| Cl | Intercept | 103.59 | 0.58 | 178.05 |
| | Spring | −0.17 | 0.79 | −0.22 |
| | Summer | 2.57 | 0.81 | 3.18 |
| | Fall | 1.72 | 0.81 | 2.13 |
| | Winter | −0.20 | 0.98 | −0.20 |
| | Intercept | 107.22 | 0.67 | 159.59 |
| | Fall | −1.77 | 0.88 | −2.01 |
| | Summer J | −3.33 | 1.16 | −2.88 |
| | Fall J | −0.65 | 1.18 | −0.55 |
| Na | Intercept | 139.91 | 0.53 | 263.35 |
| | Spring | −2.97 | 0.72 | −4.12 |
| | Summer | 1.85 | 0.74 | 2.51 |
| | Fall | 3.41 | 0.74 | 4.62 |
| | Winter | 2.96 | 0.89 | 3.31 |
| | Intercept | 142.82 | 0.67 | 212.92 |
| | Fall | 0.80 | 0.84 | 0.96 |
| | Summer J | −3.86 | 1.16 | −3.32 |
| | Fall J | −1.72 | 1.19 | −1.44 |

## Appendix 3: Comparison of the use of blood biomarkers to assess snowshoe hare condition

Published use of snowshoe hare blood glucose and hematocrit to evaluate condition

| Citation and study location | Study design details leading up to blood draw | Concentrations observed | Cycle phase sampled and trends observed |
|---|---|---|---|
| Boonstra et al. (1998), Kluane | Wild trapped individuals from control plots and experimental plots (food, food + Pred enclosure) transferred to a lab and habituated for three hours before blood draw<br>* Hares were in traps for 6–16 h. No information was provided about travel time from study site to lab | Average plasma glucose concentrations from baseline bleed<br>Control plot: 115 ± 10.10 mg/dL<br>Experimental plot: 126.87 ± 5.04 mg/dL<br>Average Hct from baseline bleed<br>Control plot 40–46%<br>Experimental plot 48%<br>Also compared Hct from this study to shot hares 36.4 ± 1.4% | Samples obtained during 2 years of a decline (1991–1992) and 1 year of a low phase (1994). Glucose levels were 50% higher in the first year of the decline than in the low<br>Control hare glucose levels were significantly higher during decline years than low. Levels of experimental hares were similar in all years<br>Control hare Hct values were consistently lower than experimental hares, showed increases through 1994 when became simialr with values from experimental hares<br>Experimental hare Hct values improved from 1991 to 1992 only |

Published use of snowshoe hare blood glucose and hematocrit to evaluate condition

| Citation and study location | Study design details leading up to blood draw | Concentrations observed | Cycle phase sampled and trends observed |
|---|---|---|---|
| Boudreau et al. (2019), Kluane | Wild trapped individuals transferred to a lab and acclimated for two hours before blood draw<br>* Hares were in traps for up to 8 h. No information was provided about travel time from study site to lab. Reported glucose and Hct ranges are combined averages of control and risk-augmented hares, *estimated* from Fig. 2c, d, respectively. This study did not provide a table depicting results | Average plasma glucose concentrations from baseline bleed ~ 105–125 mg/dL<br>Average Hct from baseline bleed ~ 39–45% | Increase-peak phase (2015–2016)<br>Hares exposed to risk exposure had ~ 10% higher concentrations of glucose than control hares<br>Glucose levels increased from 2015 to 2016<br>Hct levels unaffected by risk exposure. Declined in 2016 |
| Lavergne et al. (2021), Kluane | Wild trapped individuals transferred to a lab and acclimated for two hours before blood draw<br>Hares were in traps for < 8 h. No information was provided about travel time from study site to lab. Reported Glucose values are *estimated* from Fig. 4c | Average plasma glucose concentrations from baseline bleed 80–105 mg/d<br>Average Hct from baseline bleed 38–46% | Entire population cycle (2014–2020)<br>Glucose concentrations were low and similar from last year of the increase phase through to the end of the decline, then increased in the low (2020)<br>Lowest Hct values at peak and first decline year |

Published use of snowshoe hare blood glucose and hematocrit to evaluate condition

| Citation and study location | Study design details leading up to blood draw | Concentrations observed | Cycle phase sampled and trends observed |
|---|---|---|---|
| Sheriff et al. (2011a, b), Kluane | Wild trapped hares transferred to a lab. Hares were in traps for < 8 h. No information was provided about travel time from study site to lab. Reported Glucose and Hct values are *estimated* from Figs. 4c and 6a, respectively | Average plasma glucose concentrations from baseline bleed ~ 110–140 mg/dL<br>Average Hct from baseline bleed ~ 36–49% | Two winter seasons of the decline phase: 2006/2007 and 2007/2008<br>Higher levels of glucose observed in first decline year than in the second<br>"Hares averaged 8% higher glucose levels in the winter of 2006/2007 than in the winter of 2007/2008 and 18% higher glucose levels in late than early winter (Fig. 5c)"<br>Hct values were higher in 2007/2008 than in 2006/2007 and in early winter than in late winter |
| This study, Alaska | Average high and low plasma concentrations from wild trapped individuals (baited with alfalfa carrot). Hares were in traps for up to 17 h and bled in the field immediately upon removal from the trap | Seasonal low and high glucose concentrations 139.30 ± 3.31 (May) 158.51 ± 5.55 (August)<br>Seasonal low and high Hct 39.97 ± 0.46(March) 43.06 ± 1.13(December) | Late increase phase (2018)<br>Glucose concentrations were highest in August and lowest in May<br>Hct values were highest in December and lowest in March |

**Acknowledgements** Support for this project was provided by scholarships to C. Montgomerie from the University of Alaska Fairbanks (UAF) Institute of Arctic Biology, the Alaska Trappers Association, and the Calvin J. Lensink Graduate Fellowship, as well as from funding from the Bonanza Creek Long Term Ecological Research program to K. Kielland (DEB1636476). We thank D. DiFolco for assistance in the field and Gates of the Arctic National Park Service for logistical support. We are grateful to the UAF Department of Veterinary Medicine for providing critical instrument support, and to the Marine Ecotoxicology and Trophic Assessment Laboratory (METAL) for providing the lab space.

**Author contributions** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Claire Montgomerie. The first draft of the manuscript was written by Claire Montgomerie and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. All authors consent that all data and materials as well as software application or custom code support their published claims and comply with field standards. All authors whose names appear on the submission: (1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; (2) drafted the work or revised it critically for important intellectual content; (3) approved the version to be published; and (4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Funding** Support for this project was provided by scholarships to Claire Montgomerie from the University of Alaska Fairbanks (UAF) Institute of Arctic Biology, the Alaska Trappers Association, and the Calvin J. Lensink Graduate Fellowship, as well as from funding from the Bonanza Creek Long Term Ecological Research program to K. Kielland (DEB1636476). All authors certify that they have no affiliations with, or involvement in, any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript. The authors have no competing interests to declare that are relevant to the content of this article.

**Data availability** All data will be made available on request by contacting the first author.

## Declarations

**Ethical approval** This study did not include human participants, their data or biological material, retrospective studies, case studies, or Cell lines. Therefore, ethical approval related to these factors is not applicable. This study involved animals, their data and biological material. All capture, handling and sample collection protocols were approved by the University of Alaska Fairbanks Institutional Animal Care and Use Committee (Protocol # 1124642-6).

## References

Aldous CM (1937) Notes on the life history of the snowshoe hare. J Mammal 18(1):46–57. https://doi.org/10.2307/1374307

Anderson KJ, Jetz W (2005) The broad-scale ecology of energy expenditure of endotherms. Ecol Lett 8:310–318. https://doi.org/10.1111/j.1461-0248.2005.00723

Ataallahi M, Nejad JG, Park KH (2022) Selection of appropriate biomatrices for studies of chronic stress in animals: a review. J Anim Sci Technol 64(4):621–639. https://doi.org/10.5187/jast.2022.e38

Balm PHM (1999) Stress physiology in animals. CRC Press LCC

Bartoš L (1990) Social status and antler development in red deer. In: Bubenik GA, Bubenik AB (eds) Horns, pronghorns, and antlers. Springer New York, New York, pp 442–459. https://doi.org/10.1007/978-1-4613-8966-8_17

Bernays E, Driver C, Bilgener M (1989) Herbivores and plant tannins. Adv Ecol Res 19:263–330. https://doi.org/10.1016/S0065-2504(08)60160-9

Boonstra R, Hik D, Singleton GR, Tinnikov A (1998) The impact of predator-induced stress on the snowshoe hare cylce. Ecol Monogr 79:371–394. https://doi.org/10.1890/0012-9615(1998)068[0371:TIOPIS]2.0.CO;2

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

Bryant JP (2003) Winter browsing on alaska feltleaf willow twigs improves leaf nutritional value for snowshoe hares in summer. Oikos 102(1):25–32. https://doi.org/10.1034/j.1600-0706.2003.12443.x

Bryant JP, Wieland GD, Clausen T, Kuropat P (1985) Interactions of snowshoe hare and feltleaf willow in Alaska 1. Ecol 10(66):1564–1573. https://doi.org/10.2307/1938018

Conrado FO (2020) Hamatology of lagomorphs. Schalm's veterinary hematolgoy, seventh addition. https://doi.org/10.1002/9781119500537.ch117

Czerwinska J, Chojnowska K, Kaminska B et al (2013) Sex-differences and seasonal changes in plasma glucocorticoid concentrations in European beaver (Castor fiber). Reprod Biol 13:34–35

Feierabend D, Kielland K (2014) Movements, activity patterns, and habitat use of snowshoe hares (*Lepus americanus*) in interior Alaska. J Mammal 95:525–533. https://doi.org/10.1644/13-MAMM-A-199

Feierabend D, Kielland K (2015) Seasonal effects of habitat on sources and rates of snowshoe hare predation in Alaskan boreal forests. PLoS ONE 10:e0143543. https://doi.org/10.1371/journal.pone.0143543

Ferreira MS, Alves PC, Callahan CM, Marques JP, Mills LS, Good JM, Melo-Ferreira J (2017) The transcriptional landscape of seasonal coat colour moult in the snowshoe hare. Mol Ecol 26(16):4173–4185. https://doi.org/10.1111/mec.14177

Foley WJ, Mclean S, Cork SJ (1995) Consequences of biotransformation of plant secondary metabolites on acid-base metabolism in mammals—a final common pathway?. J Chem Ecol 21:205–222. https://doi.org/10.1007/BF02033457

Garamszegi SZ, Goymann LZ, Donald WW et al (2018) Do seasonal glucocorticoid changes depend on reproductive investment? A comparative approach in birds. Integr Comp Biol 58:739–750. https://doi.org/10.1093/icb/icy022

Green AJ (2001) Mass/length residuals: measures of body condition or generators of spurious results? Ecology 82:1473–1483. https://doi.org/10.1890/0012-9658(2001)082[1473:MLRMOB]2.0.CO;2

Goymann W (2012) On the use of non-invasive hormone research in uncontrolled, natural environments: the problem with sex, diet, metabolic rate and the individual. Methods Ecol Evol 3:757–765. https://doi.org/10.1111/j.2041-210X.2012.00203.x

Hodges KE, Sinclair AR (2005) Browse site selection by snowshoe hares: effects of food supply and predation risk. Can J Zool 83:280–292. https://doi.org/10.1139/z05-015

Hodges KE, Stefan CI, Gillis EA (1999) Does body condition affect fecundity in a cyclic population of snowshoe hares? Can J Zool 77(1):1–6. https://doi.org/10.1139/z98-188

Howland B, Sanford LM, Palmer WM (1985) Changes in serum levels of LH, FSH, prolactin, testosterone, and cortisol associated with season and mating in male pygmy goats. J Androl 6:89–96. https://doi.org/10.1002/j.1939-4640.1985.tb00822.x

Jacobson HA, Kirkpatrick RL, Burkhart HE et al (1978) Hematological comparison of shot and live trapped cottontail rabbits. Source J Wildl Dis Wildl Dis Assoc J Wildl Dis 14:82–88. https://doi.org/10.7589/0090-3558-14.1.82

Kalita P, Bhuyan R, Chakravorty P, Sarma S (2001) Influence of dietary protein and energy levels on certain bio-chemical constituents of blood in rabbits. Indian Vet J 78(10):893–895. https://doi.org/10.5555/20013172581

Kaplan EL, Meier P (1958) Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481. https://doi.org/10.1080/01621459.1958.10501452

Keith LB (1983) Role of food in hare population cycles. Oikos 40(3):385–395. https://doi.org/10.2307/3544311

Kerr MG (2002) Veterinary laboratory medicine: clinical biochemistry and haematology. Blackwell Science

Kielland K, Olson K, Euskirchen E (2010) Demography of snowshoe hares in relation to regional climate variability during a 10-year population cycle in interior Alaska. This article is one of a selection of papers from The Dynamics of Change in Alaska's Boreal Forests: Resilience and Vulnerability. Can J for Res 40:1265–1272. https://doi.org/10.1139/X10-053

Kielland K, DiFolco D, Montgomerie C (2019) Dining dangerously: geophagy by snowshoe hares. Ecology 100(3):1–3

Krebs CJ, Gilbert BS, Boutin S et al (1986) Population biology of snowshoe hares. I. Demography of food-supplemented populations in the Southern Yukon, 1976–84. J Anim Ecol 55:963

Krebs CJ, Boutin S, Boonstra R et al (1995) Impact of food and predation on the snowshoe hare cycle. Source Sci New Ser Sci Genes Dev Proc Natl Acad Sci USA Mol Cell Biol Genes Dev Biochem J 269:1112–1115. https://doi.org/10.1126/science.269.5227.1112

Krebs CJ, Kielland K, Bryant J et al (2013) Synchrony in the snowshoe hare (*Lepus americanus*) cycle in northwestern North America, 1970–2012. Can J Zool 91:562–572

Lavergne SG, Smith K, Kenney A et al (2019) Physiology and behavior of juvenile snowshoe hares at the start of the 10-year cycle. Anim Behav 157:141–152. https://doi.org/10.1016/j.anbehav.2019.09.003

Lavergne SG, Krebs CJ, Kenney AJ, Boutin S, Murray D, Palme R, Boonstra R (2021) The impact of variable predation risk on stress in snowshoe hares over the cycle in North America's boreal forest: adjusting to change. Oecologia 197(1):71–88. https://doi.org/10.1007/s00442-021-05019-1

Majchrzak YN, Peers MJL, Studd EK, Menzies AK, Walker PD, Shiratsuru S, McCaw LK, Boonstra R, Humphries M, Jung TS, Kenney AJ, Krebs CJ, Murray DL, Boutin S (2022) Balancing food acquisition and predation risk drives demographic changes in snowshoe hare population cycles. Ecol Lett 25(4):981–991. https://doi.org/10.1111/ele.13975

Marco I, Cuenca R, Pastor J et al (2003) Hematology and serum chemistry values of the European brown hare. Vet Clin Pathol 32:195–198. https://doi.org/10.1111/j.1939-165X.2003.tb00335.x

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

Melillo A (2007) Rabbit clinical pathology. J Exotic Pet Med 16(3):135–145. https://doi.org/10.1053/j.jepm.2007.06.002

Miller HA, Gobin J, Boudreau MR, Horne LG, Scholl LE, Seguin JL et al (2024) Cyclic dynamics drive summer movement ecology of snowshoe hares (*Lepus americanus*). Front Collect. https://doi.org/10.3389/fevo.2024.1419245

Mills LS, Zimova M, Oyler J et al (2013) Camouflage mismatch in seasonal coat color due to decreased snow duration. Proc Natl Acad Sci USA 110:7360–7365. https://doi.org/10.1073/pnas.1222724110

Moshkin MP, Gerlinskaya LA, Zavjalov EL et al (2003) Stress and nutrition in the wild. Recent Adv Anim Nutr Aust 14:11–22

Murray DL (2002) Differential body condition and vulnerability to predation in snowshoe hares. J Anim Ecol 71:614–625. https://doi.org/10.1046/j.1365-2656.2002.00632.x

Olnes J, Kielland K, Genet H, Juday GP, Ruess RW (2018) Functional responses of white spruce to snowshoe hare herbivory at the

treeline. PLoS ONE 13(6):e0198453. https://doi.org/10.1371/journal.pone.0198453
Palme R, Möstl E (1996) Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Int J Mammal Biol 62:192–197. https://doi.org/10.1023/a:1014095618125
Parasuraman S, Raveendran R, Kesavan R (2010) Blood sample collection in small laboratory animals. J Pharmacol Pharmacother 1(2):87–93. https://doi.org/10.4103/0976-500X.72350
Parker KL (2003) Advances in the nutritional ecology of cervids at different scales. Écoscience 10(4):395–411. https://doi.org/10.1080/11956860.2003.11682788
Pollock KH, Winterstein SR, Bunck CM, Curtis PD (1989) Survival analysis in telemetry studies: the staggered entry design. J Wildl Manag 53(1):7–15. https://doi.org/10.2307/3801296
R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
Reichardt PB, Bryant JP, Clausen TP, Wieland GD (1984) Defense of winter-dormant Alaska paper birch against snowshoe hares. Oecologia 65:58–69. https://doi.org/10.1007/BF00384463
Resano-Mayor J, Hernández-Matías A, Real J et al (2016) The influence of diet on nestling body condition of an apex predator: a multi-biomarker approach. J Comp Physiol B 186:343–362. https://doi.org/10.1007/s00360-016-0967-3
Romero ML (2002) Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen Comp Endocrinol 128:1–24. https://doi.org/10.1016/S0016-6480(02)00064-3
Sheriff MJ, Bosson CO, Krebs CJ, Boonstra R (2009a) 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 (2009b) The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. J Anim Ecol 78:1249–1258. https://doi.org/10.1111/j.1365-2656.2009.01552.x
Sheriff MJ, Speakman JR, Kuchel L, Boutin S, Humphries MM (2009c) The cold shoulder: Free-ranging snowshoe hares maintain a low cost of living in cold climates. Can J Zool 87(10):956–964. https://doi.org/10.1139/Z09-087
Sheriff MJ, Dantzer B, Delehanty B et al (2011a) Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia 166:869–887. https://doi.org/10.1007/s00442-011-1943-y
Sheriff MJ, Krebs CJ, Boonstra R (2011b) 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
Shiratsuru S, Majchrzak YN, Peers MJL, Studd EK, Menzies AK, Derbyshire R, Humphries MM, Krebs CJ, Murray DL, Boutin S (2021) Food availability and long-term predation risk interactively affect antipredator response. Ecology 102(9):e03456. https://doi.org/10.1002/ecy.3456
Siegel A, Walton RM (2020) Hematology and biochemistry of small mammals. Ferrets Rabbits Rodents. https://doi.org/10.1016/B978-0-323-48435-0.00039-3
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(3):787–806. https://doi.org/10.2307/5093
Stefan CI, Krebs CJ, Krebs S (2001) Reproductive changes in a cyclic population of snowshoe hares. Can J Zool 79:2101–2108. https://doi.org/10.1139/z01-177
Tamian A, Edwards PD, Neuhaus P, Boonstra R, Neuhaus AR, Emmanuel P, Pardonnet S, Palme R, Filippi D, Dobson FS, Saraux C, Viblanc VA (2023) Weathering the storm: Decreased activity and glucocorticoid levels in response to inclement weather in breeding Columbian ground squirrels. Hormones and Behavior. https://doi.org/10.1016/j.yhbeh.2023.105426
Teskey-Gerstl A, Bamberg E, Steineck T, Palme R (2000) Excretion of corticosteroids in urine and faeces of hares (*Lepus europaeus*). J Comp Physiol B Biochem Syst Environ Physiol 170:163–168. https://doi.org/10.1007/s003600050271
Villafuerte R, Litvaitis JA, Smith DF (1997) Physiological responses by lagomorphs to resource limitations imposed by habitat fragmentation: implications for condition-sensitive predation. Can J Zool 75:148–151. https://doi.org/10.1139/z97-019
Whittaker ME, Thomas VG (1983) Seasonal levels of fat and protein reserves of snowshoe hares in Ontario. Can J Zool 61(6):1339–1345. https://doi.org/10.1139/z83-180
Zimova M, Mills LS, Lukacs PM, Mitchell MS (2014) Snowshoe hares display limited phenotypic plasticity to mismatch in seasonal camouflage. Proc R Soc B Biol Sci 281:20140029. https://doi.org/10.1098/rspb.2014.0029
Zimova M, Mills LS, Nowak JJ (2016) High fitness costs of climate change-induced camouflage mismatch. Ecol Lett 19:299–307. https://doi.org/10.1111/ele.12568