VARIATION IN MINERAL LEVELS AND IMMUNE RESPONSES RELATIVE TO ENVIRONMENTAL AND INDIVIDUAL CONDITIONS IN ADULT FEMALE MOOSE IN CENTRAL BRITISH COLUMBIA

by

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Abstract

Environmental change can compromise the health and fitness of individual wildlife, leading to negative consequences for populations. Understanding how environmental change relates to wildlife health and fitness is therefore crucial for informing effective conservation and management strategies. Mineral status and immune function are key components of animal health that are sensitive to changes in habitat, climate, and disturbance regimes, and may therefore serve as useful biomarkers for examining how environmental variation corresponds with health and population resilience in wildlife.

Moose (*Alces alces*) are one species whose health may be affected by environmental change. Over the past two decades, moose populations in central British Columbia (BC) have declined dramatically following a severe mountain pine beetle epidemic and subsequent timber salvage logging, which resulted in a heavily altered landscape. In response to these declines, the Province of BC initiated a long-term research project on adult female moose. This research documented cases of starvation and health-related mortalities, along with suboptimal pregnancy rates, which suggests that bottom-up factors may have contributed to the observed declines. My thesis draws on and supplements information collected as part of the BC Provincial Moose Research Project to investigate associations between bottom-up factors and moose health. Specifically, I examined environmental and individual correlates of essential mineral concentrations and immune responses in female moose to better characterize patterns linking environmental variation and moose health.

First, I examined whether mineral concentrations in the hair of adult female moose were associated with environmental factors in their summer–autumn habitat. I used hair samples collected during winter captures to quantify the concentrations of 15 macro and trace minerals.

Using generalized linear mixed-effects models, I tested whether variation in mineral concentrations may have reflected differences in habitat composition, landscape disturbance, and climatic conditions. I found that precipitation was an important predictor of selenium and zinc concentrations, suggesting that mineral uptake could be influenced by climate-driven effects on vegetation. Moose that spent more time in deciduous forests had greater concentrations of potassium and magnesium, possibly reflecting the nutritional value of these forest stands. Furthermore, moose with access to recent wildfire burns had greater zinc levels, suggesting that fire could enhance forage quality or availability. Collectively, these findings reveal patterns in moose nutritional health in relation to environmental conditions.

Second, I measured concentrations of multiple immune biomarkers in the serum of female moose and investigated how these markers related to individual condition and parasite exposure. Moose with greater fat reserves had higher concentrations of interleukin-12, suggesting that individuals in better condition may be able to allocate more resources toward immune function. Total globulin concentrations were elevated in moose exposed to both micro- and macro-parasites, reflecting immune activation in response to parasitic challenges. I also found correlations between zinc levels and both IL-12 and total globulin, whereas copper concentrations were associated with haptoglobin, indicating a potential role of trace minerals in modulating immune responses. Combined, my results highlight connections between nutrition, immune function, and parasite exposure in moose.

Collectively, my findings offer novel insights into patterns of variation in moose health in relation to environmental conditions. Moreover, my findings provide baseline data on a range of health biomarkers in female moose and highlight the importance of future monitoring to assess the effects of environmental change on wildlife health.

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CHAPTER 1: Introduction

Background

In the face of rapid environmental change, wildlife are increasingly subjected to pressures that may compromise individual fitness and population performance (Acevedo-Whitehouse and Duffus, 2009). Landscape disturbances caused by both natural and human activities, along with climate change, altered predator-prey dynamics, pollution, pathogen spill-over, overexploitation, and the complex interactions among these pressures, have contributed to the declines of many species (Ceballos *et al.*, 2015; Newbold *et al.*, 2015). Understanding the relative importance and mechanisms by which these pressures affect different species is essential for identifying and prioritizing stewardship strategies that promote the long-term sustainability of wildlife populations.

Monitoring biomarkers of health in wildlife provides valuable insight into the physiological impacts of environmental change on wildlife populations. Historically, wildlife health was viewed narrowly as the mere presence or absence of disease (Stephen, 2014). However, in recent decades, the concept of wildlife health has evolved to encompass a dynamic interplay of biological, social, and environmental factors that collectively influence the resilience and sustainability of wildlife populations through effects on individual fitness (Stephen, 2014; Thacker *et al.*, 2019; Wittrock *et al.*, 2019; Aleuy *et al.*, 2023). Health biomarkers are inherently dynamic, varying across time and space, and can reflect both intrinsic biological characteristics (*e.g.*, immunity, nutrient acquisition, stress response, genetics) and extrinsic factors (*e.g.*, climate and weather, habitat features) (Thacker *et al.*, 2019; Aleuy *et al.*, 2023). Assessing biomarkers of wildlife health at both the individual and population level allows for early detection of deviations from baseline health, thereby informing targeted and effective monitoring strategies (Wikelski and Cooke, 2006; Cooke *et al.*, 2013).

Climate and landscape disturbances can affect wildlife health and fitness by altering habitat conditions and resource availability, ultimately shaping population dynamics through complex interactions (Acevedo-Whitehouse and Duffus, 2009). These disturbances can directly influence the quality and quantity of forage within habitats and the energetic balance (*i.e.*, intake versus expenditure) of some wildlife species (Schmidt *et al.*, 2002; van Beest *et al.*, 2012; Oster *et al.*, 2018; van Beest *et al.*, 2023). Understanding the relative influences of climate and landscape disturbances on the nutritional value of habitat is particularly important in large herbivores, as nutrition is a key determinant of reproduction and survival for many species (Parker *et al.*, 2009; Stephenson *et al.*, 2020). Among the various indicators of wildlife health, biomarkers representing mineral status and immune function offer valuable insight into the multiple, interacting environmental factors affecting wild animals through changes in nutritional status and physiological responses. Since mineral status and immune competence are closely linked to fitness traits (Ohmer *et al.*, 2021; Mosbacher *et al.*, 2022; Rioux *et al.*, 2022), including reproduction and survival, measuring these biomarkers in response to environmental variation can help uncover the mechanisms connecting anthropogenic disturbances to individual health, condition, and ultimately population dynamics.

Macro and trace minerals, including essential elements and toxic heavy metals, are important components of an animal's diet that are associated with individual health. Mammals require up to 29 minerals to grow, reproduce, survive, and maintain proper physiological function (Kincaid, 2000; Suttle, 2010; Oster *et al.*, 2024). Macro minerals, such as calcium, magnesium, and potassium, are required in relatively large amounts to facilitate various metabolic and physiological processes, such as maintaining homeostasis, regulating metabolism, ensuring organ function, and promoting optimal growth and development (Kincaid, 2000; Underwood, 2012). In contrast, trace minerals, such as copper, selenium, and zinc, are required in much smaller amounts but are crucial for supporting enzymatic reactions, immune function, and antioxidant defenses (Kincaid, 2000; Underwood, 2012). In free-ranging ungulates, mineral deficiencies or toxicities have been associated with impaired growth (Pollock, 2005), reduced reproductive success (Flynn *et al.*, 1977; Stephenson *et al.*, 2001), and compromised immunity (Flueck, 2012). In some cases, inappropriate levels of minerals have been implicated in population declines (O'Hara *et al.*, 2001; Frank *et al.*, 2004; Murray *et al.*, 2006). Thus, identifying factors associated with mineral concentrations in animal tissues may provide important insights into both population health and broader patterns of nutritional condition.

The bioavailability of minerals to herbivores is likely to change with shifts in vegetation quality and availability caused by environmental change (Ohlson and Staaland, 2001; van Beest *et al.*, 2023), which could have consequences for animal health. Climate and landscape alterations can influence mineral bioavailability by modifying soil chemistry, nutrient cycling, and plants' ability to uptake minerals (Schmidt *et al.*, 2002; Oster *et al.*, 2018; van Beest *et al.*, 2023). Additionally, reductions in overall forage abundance due to environmental change may limit herbivores' capacity to consume sufficient quantities of mineral-rich plants. Understanding the effects of environmental conditions on mineral levels can therefore provide insights into herbivore responses to environmental change, thereby informing management strategies aimed at improving health. For example, identifying areas where herbivores exhibit lower essential mineral levels could help prioritize habitat protection or guide efforts such as mineral supplementation where appropriate.

Traditionally, mineral concentrations in animals have been measured using organ tissues (*e.g.*, liver or kidney) or serum samples (McDowell, 1992; Herdt and Hoff, 2011). In recent years, hair analysis has emerged as a valuable alternative for assessing mineral status in wildlife, particularly in research and monitoring contexts (Cygan-Szczegielniak *et al.*, 2018; Jutha *et al.*, 2022; Mosbacher *et al.*, 2022; Rioux *et al.*, 2022). Hair offers a practical advantage over other tissue types as it can be easily collected during capture, is simple to store, and reflects long-term mineral accumulation, making it particularly suitable for field studies. Additionally, hair mineral concentrations may offer valuable insights into population-level trends, as they are often associated with individual health and demographic outcomes. For example, Rioux *et al.* (2022) observed that higher zinc and sodium concentrations, along with lower cesium and manganese levels, were linked with improved adult survival in caribou (*Rangifer tarandus caribou*). A similar study in muskoxen (*Ovibos moschatus*) revealed that variations in copper, selenium, and molybdenum levels were associated with changes in annual calf recruitment (Mosbacher *et al.*, 2022). Together, these studies highlight the potential of hair as an informative tissue for measuring mineral concentrations and examining their relationships with environmental characteristics in free-ranging wildlife.

Immune function is another physiological process associated with wildlife health and resilience, serving as a primary defense against pathogens and shaping patterns of disease occurrence and outcomes in the host (Schmid-Hempel, 2021). The immune system is complex and multifaceted, encompassing innate and adaptive responses that work together to protect animals from infections, parasites, and other challenges. Hosts differ markedly in their ability to mount immune responses, reflecting differences in genetic background, life-history characteristics, nutritional condition, prior exposure to pathogens, and environmental factors.

Understanding the dynamics of immunity is therefore useful in monitoring population health for conservation (Brock *et al.*, 2012, Ohmer *et al.*, 2021).

Given that immune function is energetically costly and requires considerable nutrients, immune biomarkers can offer insight into fitness-related traits and energetic trade-offs. Animals allocate limited energy among immunity, growth, reproduction, and maintenance, and these investments may shift under nutrient restrictions (Stearns, 1989; Sheldon and Verhulst, 1996). For example, animals in poor body condition may exhibit reduced immune reactivity (Gilot-Fromont *et al.*, 2012). In roe deer (*Capreolus capreolus*), immune phenotypes varied with body condition, where individuals in better condition had higher levels of parameters related to innate immunity (Gilot-Fromont *et al.*, 2012). In addition, direct trade-offs between reproductive effort and immune function have been documented in Soay sheep (*Ovis aries*), where high antibody responsiveness was associated with improved survival during harsh winters but reduced reproductive output (Graham *et al.*, 2010). Certain nutrient restrictions, including imbalances in trace minerals, may also influence immunity independent of energy restriction (Jolles *et al.*, 2015). Trace minerals play a crucial role in immune function by supporting enzymatic processes, antioxidant defenses, and cellular immunity, further linking nutritional status with immunocompetence (Kincaid, 2000; Underwood, 2012). Therefore, environmental change may exacerbate trade-offs in resource allocation by altering immunological, endocrinological, and physiological responses, which in turn could profoundly affect immunity and infectious disease dynamics.

Moose ecology in central British Columbia

A better understanding of moose (*Alces alces*) health can provide valuable insights into patterns associated with environmental change at both individual and population levels. As the largest living members of the deer family (*Cervidae*), moose occur throughout the boreal and temperate forests of North America and Eurasia (Franzmann, 1981). Moose are an iconic species of northern ecosystems that play critical roles in predator-prey systems, nutrient cycling, and forest succession. Moreover, moose are culturally significant to many First Nations and economically important to hunters and guide outfitters.

Although moose populations fluctuate naturally over time, there have been notable declines in parts of North America (Timmermann and Rodgers, 2017). In some regions of British Columbia (BC), Canada, moose populations declined by 50–70% between the early 2000s and 2010 (Kuzyk *et al.*, 2018). This period coincided with a severe mountain pine beetle (*Dendroctonus ponderosae*) outbreak, which caused extensive pine tree mortality across much of the province. Subsequently, large-scale salvage logging, primarily through clearcutting, led to a sharp increase in the Allowable Annual Cut (Parfitt, 2007). Rapid forest disturbance has been hypothesized as a factor contributing to moose declines in the central interior of BC.

In 2012, the Province of British Columbia and partners initiated a long-term research project to understand the effects of natural and anthropogenic disturbances on moose population declines and to identify restoration management options (Kuzyk and Heard, 2014). As part of this initiative, provincial biologists have monitored adult female and calf moose across five study areas representing a range of forest disturbance intensities and ecosystem types to examine patterns in moose distribution, health, and survival. Since the beginning of the project, adult females have been captured annually to maintain approximately 30 active GPS collars per study area, and biological samples have been collected from both live-captured animals and mortality sites. Although predation was the most frequently identified cause of mortality, investigations into adult female deaths revealed a higher-than-expected number of cases linked to health-related factors, along with evidence of suboptimal pregnancy rates across the province (Thacker *et al.*, 2019). Interestingly, an initial assessment of trace mineral concentrations in organ tissues and serum revealed variation across populations, with suboptimal levels of several trace minerals (*e.g.*, iron, cobalt, copper, manganese, selenium, and zinc) in some individuals. Moreover, investigations of adult female moose health suggest that the occurrence and potential impacts of several additional health determinants, such as body fat, stress, and pathogen prevalence, may vary across study areas and years. Together, these findings highlight the need for further research to better understand how moose health relates to environmental variation across different landscapes.

Given the complex interactions among natural and human disturbances and climate factors affecting moose habitat and population dynamics, a deeper understanding of moose health at individual and population levels is essential. Health biomarkers can serve as early indicators of broader ecological change, especially in regions undergoing rapid landscape transformation. Currently, no single factor fully explains the observed differences in overall health among BC moose populations. Although some populations in central BC have shown signs of stabilization in recent years, ongoing monitoring remains critical to determine whether these trends persist and to support effective management interventions. Therefore, ongoing monitoring that expands the range and types of health biomarkers, combined with long-term, multi-level studies, is critical to thoroughly assessing how biomarkers of individual health may relate to and potentially help to predict moose population dynamics in the region.

Thesis objectives and format

My thesis explores how mineral concentrations and immune responses vary in relation to environmental and individual conditions in moose from two of the provincial study areas. It is structured into four chapters. In Chapter 1, “Introduction”, I provided an overview of the importance of assessing wildlife health in the context of environmental change. I also described the historical and ecological context of moose populations in British Columbia (BC), introduce the study system, and outline the landscape change hypothesis originally proposed by Kuzyk and Heard (2014). In Chapter 2, titled “Habitat composition, landscape disturbance, and climatic conditions are associated with hair mineral concentrations in moose”, I examined associations between hair mineral concentrations and environmental conditions using GPS collar data, spatial datasets, and hair samples collected during winter captures. I also established baseline hair mineral concentrations for this demographic. In Chapter 3, titled “Immune biomarkers vary in relation to body fat, trace mineral status, and parasite exposure in moose”, I investigated how aspects of host physiological condition and parasite exposure are associated with variation in immune function by measuring a suite of serum immune biomarkers that represent different components of the immune system. Chapters 2 and 3 are presented in manuscript format for journal publication and use first-person plural to recognize the contributions of my collaborators. In Chapter 4, “Conclusions”, I provide a final summation of the results and synthesize the findings of both chapters. I also address the general limitations of this study and offer recommendations for future research. Overall, this research advances our understanding of adult female moose health in changing environments. My work offers novel insights into potential ways by which moose respond to landscape disturbances and climate change and expands the suite of health biomarkers that can be used for longitudinal monitoring at individual and population levels.

CHAPTER 2: Habitat composition, landscape disturbance, and climatic conditions are associated with hair mineral concentrations in moose

Introduction

The current unprecedented rate of environmental change is imposing stressors on wildlife populations around the globe. Climate change and landscape disturbances shape wildlife population dynamics in part via bottom-up effects on wildlife health and fitness (Acevedo-Whitehouse and Duffus, 2009). Climate, for example, affects forage quantity and quality for herbivores via effects on plant growth rate, species composition, and phenology (Post *et al.*, 2008; Park *et al.*, 2020; Brown *et al.*, 2022). Moreover, anthropogenic and natural landscape disturbances, such as industrial forest harvesting and wildfire, can alter the quality and composition of vegetation through changes in forest structure and successional stages (Kasischke *et al.*, 2006; Rocha-Santos *et al.*, 2016; Whitman *et al.*, 2019). These changes in vegetation affect large herbivores that rely on plant communities for nutrition and whose population growth may be limited by the nutritional quality of their habitat (Parker *et al.*, 2009; Stephenson *et al.*, 2020). Understanding how climate and landscape disturbances relate to the nutritional value of habitat is therefore particularly important in large herbivores.

Mineral status is one nutritional currency that is associated with the health and fitness of large herbivores (Barboza *et al.*, 2009). Essential minerals, including macro minerals (elements required in large amounts) and trace minerals (elements needed in smaller quantities), are required in the diet of all animals to support physiological and biochemical functions such as growth, immunity, reproduction, and survival (Kincaid, 2000; Underwood, 2012). In contrast, non-essential elements, such as toxic heavy metals, have no established biological functions but can interfere with the absorption or activity of essential minerals (Abdulla and Chmielnicka, 1989; Ali and Khan, 2018). Animals may experience adverse health effects when homeostatic mechanisms that regulate levels of specific minerals are disrupted and mineral levels in tissues fall above or below the normal physiological range (Baj *et al.*, 2023). Although the importance of minerals for physiological functioning in domestic livestock is well established, little is known about their role in wild herbivores (Blakley *et al.*, 2000; French *et al.*, 2017; Bondo *et al.*, 2019; Jutha *et al.*, 2022). However, similar effects of mineral imbalances—whether deficiencies or toxicities—are presumed to occur in wild herbivores, and have been linked with poor health, reduced reproductive success, and compromised population performance (Flynn *et al.*, 1977; Flueck, 1994; O'Hara *et al.*, 2001; Flueck *et al.*, 2012; Newby and DeCesare, 2020). Therefore, monitoring of minerals should be a key component of evaluating health trends in individuals and populations of free-ranging herbivores.

Large herbivores derive the bulk of their minerals from forage but may supplement their intake with alternative nutrient-rich sources, such as mineral licks (Spears, 1994; Ayotte *et al.*, 2006). The quality, abundance, and accessibility of minerals in forage vary across time and space (Ohlson and Staaland, 2001; van Beest *et al.*, 2023). Despite this variation, individuals must obtain sufficient quantities of minerals, as demanded and constrained by their physiology. Mineral acquisition is further complicated by changes in mineral bioavailability in forage resulting from climate change and landscape disturbances. These factors can modify mineral bioavailability by altering soil chemistry, nutrient cycling, and the mineral uptake capacity of plants (Schmidt *et al.*, 2002; Oster *et al.*, 2018; van Beest *et al.*, 2023). Moreover, reductions in overall forage abundance caused by environmental change may limit the ability of herbivores to ingest sufficient quantities of mineral-rich plants. Although recent studies have explored associations between minerals and health in free-ranging herbivores (Jutha *et al.*, 2022;

Mosbacher *et al.*, 2022), few have investigated how environmental conditions correspond to variation in mineral concentrations. Exploring these associations could offer insights into herbivore responses to environmental change and reinforce the use of mineral concentrations as biomarkers of health.

Moose (*Alces alces*) in central British Columbia (BC), Canada, are ideal subjects to explore associations between environmental conditions and mineral concentrations. In this region, populations of moose declined by as much as 70% in the early 2000s (Kuzyk *et al.*, 2018). This period coincided with a widespread outbreak of mountain pine beetle (*Dendroctonous ponderosae*) and subsequent salvaging logging of beetle-killed timber, resulting in a heavily altered landscape (Alfaro *et al.*, 2015). The dramatic declines led to a coordinated research project between the Province of BC and partners to understand the effects of landscape disturbances on moose populations. Observations of apparent starvation and health-related mortalities, coupled with suboptimal pregnancy rates, suggest that bottom-up factors may be influencing the viability of populations (Thacker *et al.*, 2019). Minerals play a particularly important role in the health of moose, where deficiencies in essential minerals such as cobalt, copper, iron, and selenium have been identified as contributing to moose mortalities, poor reproductive output, and ultimately poor performance in several populations in North America (Flynn *et al.*, 1977; O'Hara *et al.*, 2001; Frank *et al.*, 2004; Murray *et al.*, 2006). Despite these findings elsewhere, our understanding of mineral levels in moose in central BC remains somewhat limited, partly due to logistical challenges associated with assessing mineral concentrations in free-ranging wildlife.

In animals, mineral concentrations are typically quantified using storage organs (*i.e.*, liver or kidney) since these organs reflect the availability of minerals in the body (McDowell, 1992).

However, analyzing organs in wildlife requires either invasive biopsies or post-mortem sampling. In free-ranging wildlife, organs are often consumed by predators or scavengers before they can be collected as part of mortality investigations. Therefore, in live-captured animals, mineral concentrations are measured most commonly in blood or serum (Herdt and Hoff, 2011). Mineral concentrations in blood and serum, however, reflect only short-term mineral status that fluctuates with metabolic demands and homeostatic controls (Underwood, 2012) and often correlate poorly with those in storage organs (Blakley *et al.*, 2000). As an alternative approach to evaluate mineral concentrations, hair sampling and analysis is increasingly being used to assess mineral concentrations in wildlife research and monitoring (*e.g.*, Jutha *et al.*, 2022; Mosbacher *et al.*, 2022; Rioux *et al.*, 2022; Herrada *et al.*, 2024; Dickinson *et al.*, 2025). Minerals are incorporated into the hair shaft during the period of hair growth from circulating blood that feeds the growing hair follicle (Combs, 1987). Hair growth occurs in a defined time period after which it becomes metabolically inactive; thus, hair mineral concentrations reflect an animal's physiological status at the time of hair growth (Combs, 1987). Previous research has used hair to assess individual health in woodland caribou (*Rangifer tarandus caribou*; Jutha *et al.*, 2022) and as an indicator of demographic rates and broader population trends in woodland caribou (Rioux *et al.*, 2022) and muskoxen (*Ovibos moschatus*; Mosbacher *et al.*, 2022). These studies highlight the potential of using hair as an indicator of mineral status, however, the extent to which hair concentrations reflect whole-body mineral levels remains unclear. Hair may not always provide an accurate representation of an animal's overall mineral status, and factors such as age and sex can also influence mineral concentrations (Combs, 1987). Despite these limitations, hair sampling offers a promising alternative approach for assessing long-term concentrations of select minerals in wildlife, particularly once validated and in situations where conventional monitoring methods are impractical (Jutha *et al.*, 2022).

Here, we studied the associations between environmental conditions, habitat composition, and mineral concentrations in moose sampled from two populations in central British Columbia, from 2020 to 2022. We used hair samples collected from adult female moose at capture in winter to measure a suite of 15 minerals known to be related to health and fitness outcomes. Then, we tested whether indices of habitat composition, landscape disturbance, and/or climatic conditions were correlated with differences in the uptake of minerals into hair. We predicted that environmental conditions that are known to influence the quality or quantity of forage would correspond with differences in mineral concentrations in moose hair. We also documented baseline hair mineral concentrations in female moose and demonstrated the potential of using hair mineral concentrations to reveal patterns in moose responses to environmental variation.

Methods

Study areas

Our study took place in two areas in central British Columbia, Canada (Fig. 2.1). The Bonaparte Plateau (BP) is located on the traditional territory of the Secwépemc First Nation, north of Kamloops, BC. The BP study area encompasses 6,800 km^2 and lies at 51°13′ N latitude and 120°81′ W longitude. The Prince George South (PGS) study area is located on the traditional territories of the Lheidli T'enneh and Saik'uz First Nations, southwest of Prince George, BC. The PGS study area covers an area of 11,000 km^2 and lies at 53°56′ N latitude and 123°63′ W longitude. The climate of both study areas is humid continental, characterized by short and dry, warm summers and long, cold winters. On the BP, the long-term mean annual temperature is

9.5°C, with long-term average winter temperatures around 0.1°C (December to March) and summer temperatures around 20.5°C (June to August) (Environment and Climate Canada, 2024). The BP area receives an average of 215.9 mm of rainfall and 63.1 cm of snowfall annually (Environment and Climate Change Canada, 2024). In PGS, the mean annual temperature is 4.3°C, with winter conditions averaging -5.2°C and summer temperatures near 14.9°C (Environment and Climate Change Canada, 2024). The PGS area receives an average of 432.0 mm of rainfall annually, along with 203.9 cm of snowfall (Environment and Climate Change Canada, 2024).

The BP area is characterized by three dominant Biogeoclimatic Ecosystem Classification Zones, including Interior Douglas-fir (IDF), Sub-Boreal Pine-Spruce (SBPS), and Montane Spruce (MS), whereas PGS is primarily within the Sub-Boreal Spruce (SBS) Biogeoclimatic Ecosystem Classification (BEC) zone (Meidinger and Pojar, 1991). Vegetation in the study areas is diverse, consisting of coniferous and deciduous forests at various seral stages, along with non-forested habitats such as wetlands and lakes. Common coniferous tree species in both study areas include lodgepole pine (*Pinus contorta var. latifolia*), hybrid spruce (*Picea glauca* × *engelmannii*), subalpine fir (*Abies lasiocarpa*), and Douglas-fir (*Pseudotsuga menziesii*), while broadleaf deciduous species include black cottonwood (*Populus balsamifera*), trembling aspen (*Populus tremuloides*), and paper birch (*Betula papyrifera*; Meidinger and Pojar, 1991). Historically, wildfire was the primary disturbance regime in central BC but has largely been replaced by commercial forestry. Both study areas have recently undergone extensive salvage logging in response to large-scale mountain pine beetle outbreaks (Alfaro *et al.*, 2015).

In addition to moose, both study areas support populations of other large cervids, including mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and elk

(*Cervus canadensis*). The primary predator of adult moose is wolves (*Canis lupus*), though black bears (*Ursus americanus*), grizzly bears (*Ursus arctos*), and cougar (*Puma concolor*) are also present. Moose density was estimated to be 254 ± 41/1000 km² in BP during the winter of 2017–2018 and 400 ± 78/1000 km² in PGS during the winter of 2016–2017 (Kuzyk *et al.*, 2018).

Figure 2.1. Locations of Prince George South (top) and the Bonaparte Plateau (bottom), the two study areas for exploring connections between adult female moose (*Alces alces*) hair mineral concentrations and environmental conditions in central British Columbia, Canada, from 2020 to 2022.

Animal capture, radio-collaring, and sample collection

We focused our study on 31 adult female moose (≥1.5 years old, $n_{\mathrm{BP}} = 17$, $n_{\mathrm{PGS}} = 14$) captured in the winters (December–February) of 2020–2022 by the Province of BC as part of a large-scale province-wide study investigating factors affecting moose population change (Kuzyk *et al.*, 2018). For simplicity, we grouped moose captured between the consecutive months of December and February as part of a single study year (*i.e.*, individuals captured between December 2019 and February 2020 are considered part of the 2020 study year). Moose were captured using either aerial net gunning and physical restraint or chemical immobilization by aerial darting. Capture personnel equipped female moose with GPS-telemetry collars (Vectronic Aerospace VERTEX Survey Globalstar or Survey Iridium radio collars, or Advanced Telemetry Systems G2110E radio collars) that were programmed to record one to six fixes per day (Procter *et al.*, 2020). Hair samples were collected from each individual moose between the shoulders using needle-nosed pliers in an area that was dry and visually free of contaminants (Procter *et al.*, 2020). The hair samples were dried and stored in envelopes at room temperature prior to sample analysis (Thacker *et al.*, 2019). Moose were recaptured and sampled in successive years where possible to support longitudinal monitoring of the health status of individual moose (Thacker *et al.*, 2019). The detailed methods used for animal capture, sampling, and monitoring have been previously described (Kuzyk *et al.*, 2018, Procter *et al.*, 2020). All captures were conducted in accordance with the British Columbia *Wildlife Act* under permit CB17-277227. In addition, analysis of data from this project was approved by the Animal Care and Use Committee at the University of Northern British Columbia (ACUC Protocol Number 2021-01).

Analysis of hair mineral concentrations

The sample preparation and analysis of hair were conducted following methods previously described (Jutha *et al.*, 2022; Rakic, 2022; Aguilar *et al.*, 2023). We inspected hair samples under a dissecting microscope and manually removed hair follicles, bulbs, or undercoat if present. Using plastic forceps, we thoroughly washed hair samples three times in 95% ethanol (Greenfield Global Inc.) followed by ultrapure Type 1 reagent-grade water to eliminate all possible surface contamination and external element deposits from environmental exposure (Smith *et al.*, 2007). We transferred washed samples into clean paper envelopes and oven-dried them at 50˚C for 24 hours. Once fully dried, we weighed 70 mg of dried hair and added it to a Teflon vial along with 2 mL of 70% nitric acid (HNO_3 [TraceMetal™ Grade], Fisher Chemical™). The vials were digested using a high-pressure microwave reactor (ETHOS EZ Microwave Digestion System, Milestone, Shelton, CT, USA). The digester temperature was gradually increased from room temperature to a peak of 220°C over one hour, and then gradually cooled to room temperature over one hour. We transferred digested samples to Falcon tubes, diluted them to 4 mL with Type 1 water, and stored them at 5˚C until analysis.

Hair mineral concentrations were measured at the Alberta Centre for Toxicology, University of Calgary. There, digested samples were further diluted 10X with Type 1 water and introduced to the inductively coupled plasma mass spectrometer (ICP-MS, 8800 Triple Quadrupole ICP-MS, Agilent) to analyze a panel of 15- minerals: calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), potassium (K), selenium (Se), sodium (Na), and zinc (Zn), along with three contaminant heavy metals: arsenic (As), cadmium (Cd), and lead (Pb). Instrument calibration verification was conducted before, during, and after sample analyses using certified reference materials (Trace

Elements in Natural Water [NIST1643f]; Multi-Element Standard [SCP Science]; and Environmental Calibration Standard [Agilent]). Within each analysis run, one digested hair sample was randomly selected to be run in duplicate, where a maximum deviation limit of 20% between duplicates was set for the results to be accepted. For samples run in duplicate, the average of the two mineral concentration values was used for analysis. The limit of quantification (LOQ; wet weight, digested sample) for Na was 3.0 mg/L, for Ca, Mg, and K was 1.0 mg/L, for Fe was 0.5 mg/L, for Cr, Cu, Zn, As, and Cd was 0.005 mg/L, for Mn and Se was 0.001 mg/L, and for Co, Mo, and Pb was 0.0001 mg/L. Mineral concentrations detected but falling below the LOQ were included in the analysis; values were omitted in cases where concentrations fell below the limit of detection (LOD). Quality assurance was verified in each batch using certified reference materials (NIST2976 freeze-dried mussel tissue, National Institute of Standards and Technology; and NRC DORM-4 "Fish Protein Certified Reference Material for Trace Metals", National Research Council Canada) as positive controls, along with blank samples as negative controls. We reported mineral concentrations on a µg/g dry hair weight basis.

Environmental variables

We surveyed the literature to identify explanatory variables to test *a-priori* hypotheses about how environmental conditions might predict variation in the hair mineral concentrations of moose. These variables corresponded with hypotheses within three explanatory categories: habitat composition, landscape disturbance, and climatic conditions (Table 2.1). Minerals are incorporated into hair from circulating blood during the period when hair is growing (Combs, 1987), which is assumed to begin in early summer and continue until late fall in moose, with moulting commencing the following early spring (Sokolov and Chernova, 1987). Accordingly, hair samples collected in the winter (December–February) from female moose during captures reflect mineral deposition that occurred during the preceding early summer to late fall. To characterize the environmental conditions and habitat used by moose, we therefore used GPS collar locations collected between June 1 and October 31 in the year preceding hair collection. In instances where moose were captured for the first time and GPS collar data were not available prior to capture (n = 18 hair samples), we assumed site fidelity and used GPS collar locations from the June–October period succeeding the capture event (Van Dyke *et al.*, 1995; Scheideman, 2018; McLaren and Patterson, 2021).

Plant community composition and diversity have strong effects on the diversity of minerals available in forage plants (Ohlson and Staaland, 2001). In central BC, moose consume both coniferous and deciduous trees across seasons (Koetke *et al.*, 2023), which vary in their nutritional properties, including the concentrations of several minerals (*e.g.*, Ca, Cu, K, Mg; Richardson and Friedland, 2016). Therefore, we expected that the vegetation composition of a forest stand would affect the forage species moose consume, and consequently, an individual's mineral status. To assess use of different stand types by individual moose, we calculated the proportion of GPS points for each individual moose in coniferous, mixed, and deciduous forest stands, using the Provincial Vegetation Resource Inventory (VRI; DataBC, 2024). We classified each stand as either coniferous or deciduous if the associated dominant leading species made up ≥70% of the trees in the patch. If the leading species made up <70%, we classified the stand based on the leading species if the second most common species belonged to the same category (*i.e.*, both coniferous or both deciduous). A patch was classified as mixed if the leading tree species comprised <70% of trees in the patch and the second leading tree species was in the alternate category.

Wetlands and riparian habitat contain aquatic plant species that enable moose to meet essential mineral and nutrient requirements (Tischler *et al.*, 2019). For instance, aquatic plants tend to have greater concentrations of Na, and to a lesser degree, greater concentrations of Co, Cu, Fe, and K, compared with terrestrial plants (Ohlson and Staaland, 2001; Staaland and White, 2001). We calculated the proportion of GPS points for each moose that fell within wetlands and riparian habitats using the Freshwater Atlas (FA) (DataBC, 2024). We defined riparian habitat as a 50 m-buffer around FA lakes, rivers, and streams (Koetke *et al.*, 2023).

Both natural and human disturbances shape forest structure and composition. Historically, wildfire has been a dominant disturbance type leading to the development of early seral conditions that increase the quality and quantity of preferred moose food types (*e.g.*, young trees and shrubs; Maier *et al.*, 2005; Lord and Kielland, 2015; Joly *et al.*, 2017).Wildfires also create a pulse of plant-available nutrients in the soil which can be taken up by regenerating vegetation (Kelsall *et al.*, 1977; Simard *et al.*, 2001). Therefore, we used the BC Wildfire Service Historical Fire Perimeters data to calculate the proportion of moose GPS collar points in wildfire burns ≤10 years old (DataBC, 2024). We focused on burns ≤10 years old as they provide high-quality forage for moose in the early seral stages of plant succession (Lord and Kielland, 2015). Additionally, most wildfires in our study areas occurred within the past decade (Mumma *et al.*, 2024), and few moose were located near older burns, limiting our ability to assess moose use of older post-fire habitats. Due to a large number of zeros in the data, we treated fire as a factor, categorizing moose with a proportion of GPS-points > 0.1 as having access to burns and those with < 0.1 as not having access to burns.

Forest harvesting is the primary resource-based land use in central BC. Forest harvesting, like wildfire, promotes the growth of early successional vegetation that serves as forage for moose (Fisher and Wilkinson, 2005). However, the removal of forest canopy alters the conditions (*e.g.*, sunlight, water, soil nutrients) that influence the growth and composition of regenerating vegetation (Hjeljord *et al.*, 1990; Wurtz and Zasada, 2001). These shifts in growing conditions can also affect plant chemical composition, such as the production of plant secondary metabolites, which may reduce the digestibility and bioavailability of key nutrients for herbivores (Roberge, 2023). Therefore, we calculated the average age of forest stands across GPS points using Harvested Areas of BC (Consolidated Cutblocks) data (DataBC, 2024). In instances where stand age data were missing (*e.g.*, non-harvested areas), we used the VRI data to estimate stand age.

Climate change is altering weather patterns, including temperature variability and the amount of precipitation, which can affect plant growth, phenology, and forage quality (Rustad *et al.*, 2001; Post *et al.*, 2008; Park *et al.*, 2020; Brown *et al.*, 2022). These changes in climate also drive the decomposition of soil parent materials and the subsequent release of nutrients that are taken up by plants (Kabata-Pendias, 2010). Thus, we calculated seasonal averages of the mean monthly temperature and total monthly precipitation (June–October) across GPS collar locations for each moose using monthly climate data from ClimateBC version 7.42 (Wang *et al.*, 2016).

Table 2.1. Variables considered to explain patterns of essential mineral concentrations in the hair (*n* = 60) of adult female moose (*Alces alces*) in central British Columbia, Canada, from 2020 to 2022.

Explanatory variable category	**Explanatory variable**	**Description**	**Data Source**
All	Study area	Categorical; PGS or BP	Observation
	Year	Categorical; 2020, 2021, or 2022	Observation
Habitat Composition	Deciduous forest	Proportion of GPS points in deciduous leading forest stands	VRI
	Mixed forest	Proportion of GPS points in mixed forest stands	VRI
	Wetland	Proportion of GPS points within wetlands	Freshwater Atlas
	Riparian	Proportion of GPS points within riparian habitat, defined as a 50 m buffer around lakes, rivers, and streams	Freshwater Atlas
Landscape Disturbance	Wildfire	Factor; 1 = "burns" (proportion of GPS points in wildfire burns ≤10 years old is > 0.1), 0 = "no burns" (proportion of GPS points in wildfire burns ≤10 years old is < 0.1)	Historical Fire Perimeters
	Forest stand age	Average forest age class across GPS points	Consolidated Cutblocks, VRI
Climatic Conditions	Temperature	Average mean monthly temperature across GPS points (°C, Jun–Oct)	ClimateBC
	Precipitation	Average total monthly precipitation across GPS points (mm, Jun–Oct)	ClimateBC

Abbreviations: VRI, Vegetation Resources Inventory

Statistical analysis

We calculated descriptive statistics (*i.e.*, mean, standard deviation, median, and range) for the 15 minerals across study areas, years, and individuals (*i.e.*, including repeated measures). For our environmental analysis, we focused on minerals considered essential for metabolism (Underwood, 2012) and excluded As, Cd, and Pb from further consideration. We also excluded Cr, Co, and Na from further analysis as their concentrations fell below the LOQ in more than 85% of hair samples. We used pair-plots (Pearson $r \geq 0.70$) to test for collinearity between minerals (Zuur *et al.*, 2010). Most minerals were weakly correlated (Pearson $r \leq 0.70$), except for Ca and Mg, which were highly correlated ($r = 0.90$); thus, we removed Ca to avoid measuring the same signal unnecessarily. We used Cleveland dot-plots to evaluate potential outliers for each mineral (Zuur *et al.*, 2010) and removed one extreme outlier from Fe that was nearly 18 times higher than the median value. The cause of this extreme value is unknown; however, it could be due to external contamination from another substance that was not fully removed during the laboratory wash procedure.

We developed a set of 17 candidate models to investigate the variance observed in hair minerals according to habitat composition, landscape disturbance, and climatic conditions (Table 2.2). These models were developed *a-priori* to reflect plausible relationships between minerals and environmental characteristics based on the literature. We applied the same set of models to each mineral individually. Given that mineral values were always positive and typically displayed a positively skewed distribution, we fit generalized linear mixed effects models using a γ distribution and log link (package glmmTMB; Brooks *et al.*, 2017). We included a random effect of individuals to account for heterogeneity and potential non-independence of repeated measures. We included study area and year as fixed effects in all models to explain potential spatial and temporal variation in mineral concentrations not explained by our environmental predictors. We centered temperature and precipitation within each study area and year, which allowed us to assess relative deviations from local climatic conditions. By contrast, including year and study area in our models accounted for broader temporal and geographical differences in climate. We tested for multicollinearity between continuous explanatory variables using variance inflation factors (VIF; Zuur *et al.*, 2010). All variables were weakly correlated (VIF < 3), except for coniferous forest with both broadleaf deciduous forest and mixed forest (VIF > 3), so we removed coniferous forest from our models. We standardized continuous predictor variables by subtracting the mean from the observed values and dividing by the standard deviation.

We compared our *a-priori* models and a null model using Akaike's Information Criterion corrected for small sample sizes (AIC_c) and model weights (Burnham and Anderson, 2002). We considered models with $\Delta AIC_c < 2$ to be equally likely hypotheses (Burnham and Anderson, 2002), provided they did not include uninformative parameters (*i.e.*, those that include one extra parameter without meaningfully improving the model's log-likelihood but are ranked close to more parsimonious models with lower AIC values; Leroux, 2019). We interpreted parameters within models to be influential if their 85% confidence intervals (CIs) did not overlap zero. We used 85% CIs as this confidence level reflects the significance threshold consistent with the decision-making framework of AIC-based model selection (Sutherland *et al.*, 2023). We evaluated model fit of our top models by visualizing scaled residuals simulated from the fitted model to assess uniformity, dispersion, and overall model assumptions using the DHARMa package (Hartig, 2024). All statistical analyses were performed using R (version 4.3.1; R Core Team, 2023).

Table 2.2. *A-priori* mixed effects models used to explain concentrations of minerals in hair (*n* = 60) collected from adult female moose (*Alces alces*) during the winters of 2020–2022 in central British Columbia, Canada. All models included a random intercept for Individual ID to account for repeated measures. Study area and year were included as fixed effects in every model.

Model category	**Independent variables**
Habitat composition	Study area + Year + Deciduous forest + Mixed forest
	Study area + Year + Wetland + Riparian
	Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian
Landscape disturbance	Study area + Year + Wildfire
	Study area + Year + Forest stand age
	Study area + Year + Wildfire + Forest stand age
Climatic conditions	Study area + Year + Temperature + Precipitation
Habitat composition and landscape disturbance	Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire
	Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Forest stand age
	Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age
Habitat composition and climatic conditions	Study area + Year + Deciduous forest + Mixed forest + Temperature + Precipitation
	Study area + Year + Wetland + Riparian + Temperature + Precipitation
	Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Temperature + Precipitation
Landscape disturbance and climatic conditions	Study area + Year + Wildfire + Temperature + Precipitation
	Study area + Year + Forest stand age + Temperature + Precipitation
	Study area + Year + Wildfire + Forest stand age + Temperature + Precipitation
Global model	Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age + Temperature + Precipitation
Null model	Study area + Year

Results

In total, we analyzed mineral concentrations in 60 hair samples ($n_{BP} = 31$, $n_{PGS} = 29$) collected between 2020 and 2022 in the two study areas. These samples corresponded to 31 individuals, of which eight had only one measurement, 17 had two measurements in two years, and six had three measurements in three years. We found detectable concentrations (*i.e.*, above the LOD) of all 15 minerals in hair from female moose (Table 2.3). Concentrations in hair samples fell below the LOQ for the macro minerals K (1.7% samples) and Na (98.3% samples), and for the trace minerals Co (86.7% samples), Cr (91.7% samples), Mo (5% samples), and Se (1.7% samples). For non-essential minerals, the concentrations fell below the LOQ for each of the heavy metals tested: As (88.3% samples), Cd (96.7% samples), and Pb (5% samples). For As, 11.7% of samples ($n = 7$) fell below the LOD.

Table 2.3. Mean, standard deviation (SD), median, and range mineral concentrations (µg/g, dry weight) in hair ($n = 60$) from female moose (*Alces alces*) collected in the winters of 2020–2022 from populations in Bonaparte (BP; $n = 31$) and Prince George South (PGS; $n = 29$).

	Mineral	**Mean ± SD**	**Median**	**Range**	**# <LOQ**
Essential macro minerals	Ca	689.39 ± 280.37	645.56	331.29–2088.80	0
	K*	267.19 ± 215.10	202.10	24.93–964.10	1
	Mg	123.89 ± 41.40	117.65	62.87–307.13	0
	Na*	76.97 ± 23.76	74.05	64.76–257.52	59
Essential trace minerals	Co*	0.01 ± 0.01	0.01	0.01–0.05	52
	Cr*	0.22 ± 0.44	0.12	0.12–2.75	55
	Cu	4.87 ± 0.93	4.66	3.20–8.14	0
	Fe	35.81 ± 52.00	21.11	8.78–368.05	0
	Mn	6.00 ± 4.45	4.75	0.73–17.72	0
	Mo*	0.07 ± 0.04	0.06	0.01–0.22	3
	Se*	0.22 ± 0.15	0.19	0.02–0.76	1
	Zn	80.56 ± 4.56	80.76	70.55–90.12	0
Non-essential heavy metals	As*§	0.12 ± 0.00	0.12	0.11–0.13	53
	Cd*	0.13 ± 0.03	0.12	0.11–0.28	58
	Pb*	0.32 ± 0.60	0.11	0.01–3.91	3

* Includes values below the LOQ, § $n = 53$ because 7 hair samples below the LOD were omitted

Essential macro minerals

In adult female moose, variables related to habitat composition best explained K concentrations in hair (Fig. 2.2A). Our model selection approach identified a single top model with a ΔAIC_c score less than two that carried 59% of the model set weight (Table 2.4). Moose that had a greater proportion of their GPS points in deciduous forest had greater concentrations of hair K (β = 0.32, SE = 0.10, 85% CI = [0.18, 0.46]). Similarly, moose that had a greater proportion of their GPS points in mixed forest had greater concentrations of hair K (β = 0.15, SE = 0.09, 85% CI = [0.02, 0.29]). On average, moose from the PGS study area had lower K concentrations than those in the BP area (β = -0.57, SE = 0.15, 85% CI = [-0.79, -0.36]). Concentrations of K in hair were lower in moose sampled in 2021 (β = -0.42, SE = 0.19, 85% CI = [-0.69, -0.15]) and 2022 (β = -0.49, SE = 0.18, 85% CI = [-0.75, -0.24]) relative to 2020. Although climatic conditions were not among predictors in the top model set, temperature and precipitation were influential in lower-ranked models at least two AIC scores higher than the null model, suggesting that moose exposed to relatively warmer and wetter conditions had higher K concentrations (*e.g.*, 3rd ranked model—temperature: β = 0.39, SE = 0.08, 85% CI = [0.27, 0.50]; precipitation: β = 0.15, SE = 0.08, 85% CI = [0.04, 0.26]; Appendix A Table A.1).

Similarly, habitat composition variables were important predictors of Mg concentrations in female moose (Fig. 2.2B), with one top model having a ΔAIC_c score under two and accounting for 44% of the total model set weight (Table 2.4). Moose who had a greater proportion of their GPS points in deciduous forest had greater concentrations of hair Mg (β = 0.13, SE = 0.04, 85% CI = [0.06, 0.19]). The model also included mixed forest as a parameter, however, the confidence interval for the effect of mixed forest on Mg concentrations encompassed zero (β = -0.02, SE = 0.05, 85% CI = [-0.09, 0.04]). Moose in PGS exhibited lower Mg concentrations in hair than those in the BP area (β = -0.23, SE = 0.08, 85% CI = [-0.35, -0.12]). Concentrations of Mg were lower in 2021 (β = -0.11, SE = 0.07, 85% CI = [-0.21, -0.01]) and marginally lower in 2022 (β = -0.09, SE = 0.06, 85% CI = [-0.19, 0.00]) relative to 2020.

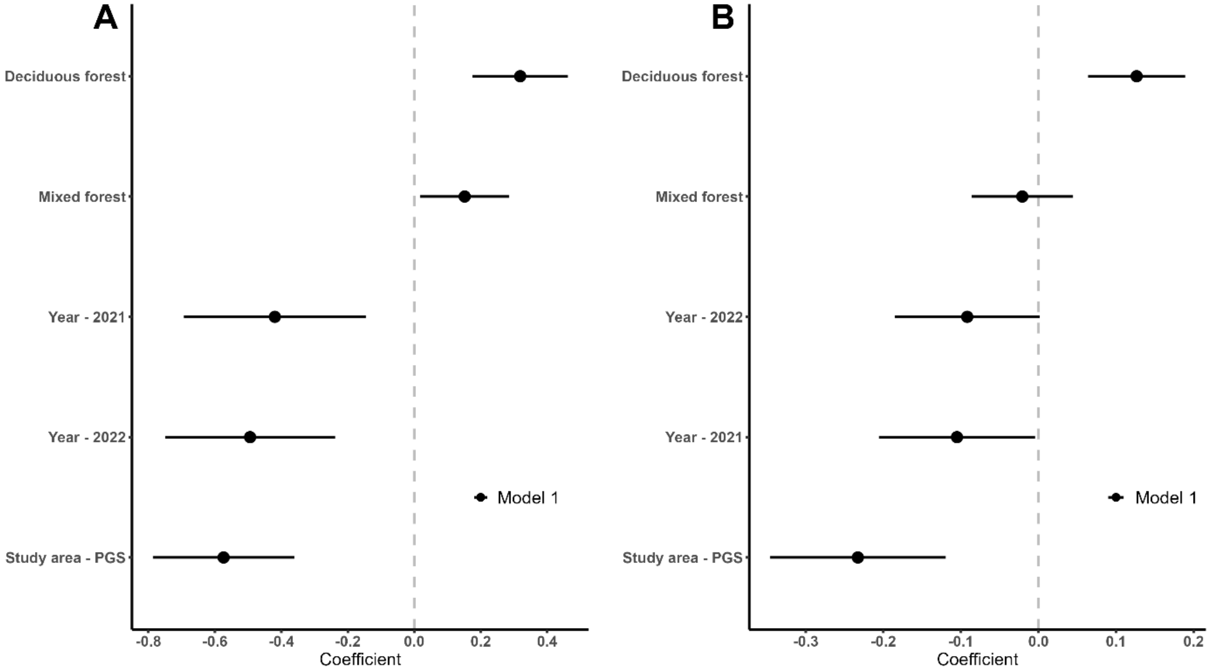

Figure 2.2. Coefficient estimates and 85% confidence intervals (CI) for independent variables in the top-ranked generalized linear mixed effects models (< 2 ΔAIC_c) explaining **(A)** K concentrations and **(B)** Mg concentrations, two macro minerals in female moose (*Alces alces*) hair. Hair samples (*n* = 60) were collected during capture events in the winters of 2020–2022 from two study areas (Prince George South and the Bonaparte Plateau) in central British Columbia, Canada. An independent variable has an influential relationship with the mineral if the CI does not overlap 0. Continuous predictors deciduous forest and mixed forest were standardized to a mean of zero and a standard deviation of one prior to analysis. Coefficients were ordered from most positive to most negative based on standardized estimates.

Table 2.4. Candidate models and model selection statistics used to explain the hair mineral concentrations (n = 60) of adult female moose (*Alces alces*) in two study areas in central British Columbia, Canada, from 2020–2022. Models that were within the top model set (< 2 ΔAIC$_c$) and ranked higher than the null model, as well as null models, are included. The full list of candidate models and model selection statistics can be found in Appendix A. Variables in bold font indicate an influential relationship with the dependent variable (85% CI does not overlap 0). Predictor variables did not explain substantially more variation in the data than the null model for minerals Cu, Fe, Mn, and Mo, and therefore, these results are not presented. All models included a random intercept for Individual ID to account for repeated measures. Study area and year were included as fixed effects in every model.

	Dependent variable	Rank	Model category	Independent variables	AIC$_c$	ΔAIC	AIC$_c$ w_i
Essential macro minerals	K	1	Habitat composition	**Study area** + **Year** + **Deciduous forest** + **Mixed forest**	756.42	0.00	0.59
		14	Null model	**Study area** + **Year**	773.51	17.09	<0.01
	Mg	1	Habitat composition	**Study area** + **Year** + **Deciduous forest** + Mixed forest	587.36	0.00	0.44
		5	Null model	**Study area** + **Year**	592.11	4.76	0.04
Essential trace minerals	Se	1	Climatic conditions	Study area + Year + **Temperature** + **Precipitation**	-138.05	0.00	0.51
		14	Null model	Study area + Year	-110.18	27.87	0.00
	Zn	1	Landscape disturbance and climatic conditions	Study area + **Year** + **Wildfire** + Temperature + **Precipitation**	347.56	0.00	0.37
		2	Climatic conditions	Study area + **Year** + Temperature + **Precipitation**	347.89	0.33	0.31
		6	Null model	Study area + **Year**	352.04	4.48	0.04

Essential trace minerals

Climatic conditions best explained Se concentrations in hair from female moose (AIC_c w_i = 0.51; Table 2.4 and Fig. 2.3A); no other model had a $\Delta\mathrm{AIC}_c$ score less than two. Moose that experienced warmer local temperatures (β = 0.38, SE = 0.07, 85% CI = [0.29, 0.48]) and greater amounts of precipitation (β = 0.28, SE = 0.06, 85% CI = [0.20, 0.36]) had greater concentrations of hair Se. Concentrations of Se in hair were similar between study areas (β = 0.01, SE = 0.14, 85% CI = [-0.20, 0.21]) and years (2021: β = 0.09, SE = 0.10, 85% CI = [-0.05, 0.23]; 2022: β = -0.02, SE = 0.08, 85% CI = [-0.13, 0.10]).

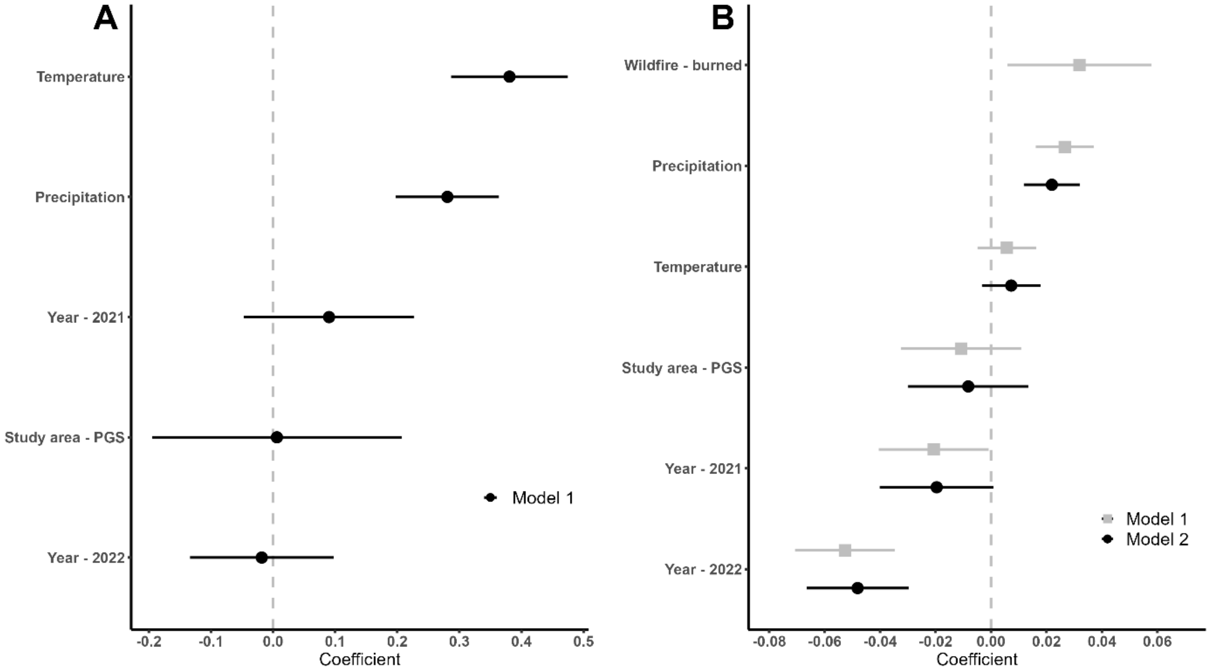

Figure 2.3. Coefficient estimates and 85% confidence intervals (CI) for independent variables in the top-ranked generalized linear mixed effects models (< 2 $\Delta\mathrm{AIC}_c$) explaining **(A)** Se concentrations and **(B)** Zn concentrations, two trace minerals in female moose (*Alces alces*) hair. Hair samples (*n* = 60) were collected during capture events in the winters of 2020–2022 from two study areas (Prince George South and the Bonaparte Plateau) in central British Columbia, Canada. An independent variable has an influential relationship with the mineral if the CI does not overlap 0. Continuous predictors temperature and precipitation were standardized to a mean of zero and a standard deviation of one prior to analysis. Coefficients were ordered from most positive to most negative based on standardized estimates.

Our model selection approach indicated that climatic conditions, or a combination of climatic conditions and landscape disturbance variables, best explained hair Zn concentrations in female moose (Fig. 2.3B). The two models in the top model set, which were the only models with a ΔAIC_c score under two, accounted for a combined weight of 68% (Table 2.4). Individual female moose who used habitats burned by wildfires exhibited higher concentrations of Zn compared to those that did not use burned areas (β = 0.03, SE = 0.02, 85% CI = [0.01, 0.06]). Precipitation was a consistently important predictor of Zn; Zn concentrations increased with greater amounts of precipitation (1st ranked model: β = 0.03, SE = 0.01, 85% CI = [0.02, 0.04]; 2nd ranked model: β = 0.02, SE = 0.01, 85% CI = [0.01, 0.03]). Temperature was positively associated with Zn concentrations, though the confidence interval for the coefficient overlapped zero (1st ranked model: β = 0.01, SE = 0.01, 85% CI = [-0.01, 0.02]; 2nd ranked model: β = 0.01, SE = 0.01, 85% CI = [-0.01, 0.02]). Concentrations of Zn were similar between the two study areas (1st ranked model: β = -0.01, SE = 0.02, 85% CI = [-0.03, 0.01], 2nd ranked model: β = -0.01, SE = 0.02, 85% CI = [-0.03, 0.01]). Moose had marginally lower Zn concentrations in 2021 (1st ranked model: β = -0.02, SE = 0.01, 85% CI = [-0.04, -0.01], 2nd ranked model: β = -0.02, SE = 0.01, 85% CI = [-0.04, 0.00]) and lower Zn concentrations in 2022 (1st ranked model: β = -0.05, SE = 0.01, 85% CI = [-0.07, -0.03], 2nd ranked model: β = -0.05, SE = 0.01, 85% CI = [-0.07, -0.03]) relative to 2020.

For the remaining essential minerals (*i.e.*, Cu, Fe, Mn, and Mo), environmental variables did not explain more variation in the data than the null model (AIC_c w_i = 0.29, 0.39, 0.30, and 0.32 for Cu, Fe, Mn, and Mo, respectively), which included study area and year. Female moose in PGS had lower concentrations of Fe (β = -0.80, SE = 0.18, 85% CI = [-1.05, -0.53]) and Mn (β = -0.60, SE = 0.19, 85% CI = [-0.87, -0.32]) compared to BP; however, Cu and Mo were similar between study areas (Cu: β = 0.03, SE = 0.05, 85% CI = [-0.04, 0.10]; Mo: β = 0.24, SE = 0.17, 85% CI = [-0.01, 0.49]). Concentrations of Mo differed between years, with levels lower in 2021 (β = -0.39, SE = 0.16, 85% CI = [-0.62, -0.15]) and 2022 (β = -0.40, SE = 0.15, 85% CI = [-0.62, -0.18]) compared to 2020. Concentrations of Mn were higher in 2022 than 2020 (β = 0.45, SE = 0.20, 85% CI = [0.16, 0.74]) and similar between 2021 and 2020 (β = -0.02, SE = 0.21, 85% CI = [-0.33, 0.29]). Concentrations of Fe were lower in 2022 compared to 2020 (β = -0.30, SE = 0.13, 85% CI = [-0.49, -0.11]) and similar between 2021 and 2020 (β = 0.01, SE = 0.14, 85% CI = [-0.20, 0.22]). No year-to-year variation was detected in Cu concentrations (2021: β = -0.03, SE = 0.06, 85% CI = [-0.12, 0.05]; 2022: β = -0.04, SE = 0.05, 85% CI = [-0.12, 0.04]).

Discussion

Nutritional condition can be an important indicator of the responses of herbivores to environmental change, with implications for long-term population monitoring and conservation. In this study, we demonstrated that concentrations of essential minerals in hair from adult female moose may be related to climatic conditions, habitat composition, and landscape disturbance. Specifically, we found that greater concentrations of the trace minerals Se and Zn in moose hair were associated with more precipitation, possibly suggesting that mineral uptake is influenced by climate-driven effects on vegetation. Mineral concentrations also varied with habitat composition; moose that spent more time in deciduous forest had greater K and Mg concentrations in their hair, potentially reflecting the nutritional value of deciduous forest. In addition, moose with access to recent wildfire burns had greater Zn levels in their hair, consistent with the idea that post-fire environments may improve forage quality and/or quantity for herbivores. We also successfully documented concentrations of macro minerals, trace minerals, and heavy metals in the hair of female moose from central BC and revealed substantial variation in mineral concentrations between study populations and across years. This resulted in the development of a baseline dataset that will be valuable for long-term health research and monitoring within this demographic. Together, these findings lend support to the utility of hair as a biomonitoring tool and may provide foundational data for assessing the effects of environmental change on mineral nutrition in free-ranging moose.

Our study confirms that at least 15 minerals can be detected in moose hair to some extent, contributing to a growing body of literature examining mineral status in wildlife through hair analysis (Jutha *et al.*, 2022; Mosbacher *et al.*, 2022; Rioux *et al.*, 2022; Herrada *et al.*, 2024; Dickinson *et al.*, 2025). Ten minerals were readily detectable and present above the LOQ in 95% or more of samples (Table 2.3), and these minerals could be prioritized for testing in future validation studies. Although the moose sampled in our study showed no obvious signs of mineral deficiency or toxicity at capture, the average concentrations of several minerals (*i.e.*, Ca, Cu, Fe, Se) in their hair were similar to those reported in free-ranging female moose from an Alaskan population identified as having low or deficient mineral levels, alongside observations of poor calf survival and high adult mortality (O'Hara *et al.*, 2001). Specifically, O'Hara *et al.* (2001) reported mean concentrations of Cu, Ca, Fe, and Se in female moose hair samples as 2.77, 599.7, 38.2, and 0.50 ppm, respectively. However, differences in analytical methods and reporting (*e.g.*, O'Hara *et al.*, 2001 use wet weight, ppm) complicate direct comparisons between our study population and theirs and highlight the need for standardized methods and established mineral reference ranges in hair.

Compared to studies of other cervids using the same analytical methods and reporting protocols, moose in our study had, on average, lower Cu and Zn concentrations and higher Fe and Pb concentrations than those reported in the hair of male Northern Mountain caribou

(*Rangifer tarandus caribou*) from northwestern British Columbia (Jutha *et al.*, 2022). These differences could represent variation in habitat quality or population health; however, mineral concentrations are known to vary by sex and species (Vikøren et al., 2011), as well as by age and dietary preferences, which may also contribute to the observed variation. These findings therefore underscore the need for research that provides a better understanding of mineral requirements in different species and their implications for growth, reproduction, and survival. Moreover, future studies should assess correlations between mineral concentrations in hair and those in storage organs to identify which hair minerals can reliably indicate moose nutritional status. This was not feasible in the present study due to the minimally invasive live sampling methods employed but could be addressed through harvest-based sampling (Jutha *et al.*, 2022). Nevertheless, mineral concentrations in hair have been used as a biomonitoring tool in a range of species, and our results serve as a basis for longitudinal monitoring and future comparisons within this moose demographic.

We observed considerable variation in several of the mineral concentrations between study populations and among years, highlighting the dynamic nature of mineral bioavailability in the environment. A notable pattern was that average monthly precipitation levels during the hair growth period were lowest in 2022 ($\bar{x}$ = 41.2 mm) compared to 2020 ($\bar{x}$ = 61.4 mm) and 2021 ($\bar{x}$ = 73.4 mm), coinciding with the lowest average concentrations of several minerals (*i.e.*, Fe, K, Mo, Zn) recorded in moose hair. We speculate that lower-than-average precipitation, in conjunction with other climate-mediated conditions, may reduce mineral availability by causing vegetation on which moose depend to become scarce or decline in quality (Rustad *et al.*, 2001). We also found that female moose in PGS had lower average concentrations of several minerals (*i.e.*, Fe, K, Mg, Mn) compared to those in the BP area, which probably reflects the relatively large geographical scale of this study. These differences could be due to variation in underlying geology, soil weathering, and atmospheric deposition (Brown *et al.*, 1999). Combined, these findings demonstrate that hair is reflective of spatial and temporal fluctuations in mineral bioavailability.

On a local scale within study areas, we observed that relative deviations in climatic conditions were associated with variation in concentrations of the trace minerals Se and Zn, and to a lesser extent, the macro mineral K, in female moose hair. Specifically, warmer temperatures were associated with higher concentrations of Se and K and more precipitation was associated positively with concentrations of Se, K, and Zn. Although the underlying processes driving these mineral patterns are unknown, temperature and precipitation have pronounced influences on the species composition, quality, quantity, and phenology of forage plants available to herbivores (Rustad *et al.*, 2001; Post *et al.*, 2008; Park *et al.*, 2020; Brown *et al.*, 2022). We suspect that warmer average temperatures during summer and fall in our study may extend the growing season, leading to greater plant biomass available to moose. Increased precipitation can enhance soil moisture, which in turn promotes plant growth and nutrient uptake (Kabata-Pendias, 2010), possibly leading to a greater abundance of high-quality forage available to moose. These findings align with studies on other herbivores, such as white-tailed deer, which showed higher liver Cu and Zn concentrations following increased summer precipitation (Hollingsworth *et al.*, 2021). Irrespective of the mechanism, our results indicate that moose mineral concentrations vary with even small local fluctuations in climatic conditions. As extreme weather events become more frequent in British Columbia due to climate change, mineral status represents one of many nutritional currencies that could be affected, further emphasizing the need for continued research and monitoring in this system.

Habitat composition was associated with variation in the concentrations of macro minerals in female moose hair. Specifically, female moose that spent more time in deciduous forest stands had greater concentrations of the macro minerals K and Mg in their hair. Previous work in woodland caribou has shown that hair mineral content reflects diet composition (Rioux *et al.*, 2022). Therefore, we postulate that this relationship could reflect the nutritional value of a diet consisting of deciduous plant species. Indeed, deciduous shrubs and trees serve as important forage for moose (Koetke *et al.*, 2023; Spitzer *et al.*, 2024; Breithaupt *et al.*, 2025) and are a rich source of many essential minerals (*e.g.*, Co, Cu, K, Mg, Mn, Zn; Ohlson and Staaland, 2001; Staaland and White, 2001). Deciduous plants are particularly valuable in the summer due to their high digestible protein and energy content, which support growth and lactation (Ohlson and Staaland, 2001; Staaland and White, 2001; Spitzer *et al.*, 2024). Furthermore, we found that moose with a higher proportion of GPS points in mixed forest stands had greater K concentrations, which could be due to a higher availability of deciduous plants or the nutritional advantages of diet mixing in diverse plant communities (Wang *et al.*, 2010). Notably, we measured broad-scale relationships between habitat composition and mineral concentrations in hair using the proportion of GPS collar points in each habitat type, which provides insight into habitat use rather than the direct consumption of specific plant species. Future research integrating plant mineral analyses and moose diet composition using feces or isotopic signatures would help clarify these relationships (Rioux *et al.*, 2022; Spitzer *et al.*, 2023). Nonetheless, our findings highlight a potential role of deciduous and mixed forests in supporting moose nutrition.

Our finding that female moose with GPS points in areas burned by wildfire within the last 10 years had higher concentrations of Zn in their hair is consistent with the presumption that fire can be beneficial to the nutritional condition of moose. Considerable research suggests that moose respond positively to early seral conditions following fire, both behaviourally and demographically (Maier *et al.*, 2005; Lord and Kielland, 2015; Joly *et al.*, 2017), likely due to the rapid increase in preferred forage such as deciduous woody browse and herbaceous plants (Landhausser and Wein, 1993). Fire also increases the availability of minerals in the soil for plant uptake (*e.g.*, Cu, Fe, K, Mn, and Zn; Kelsall *et al.*, 1977; Simard *et al.*, 2001), which in turn influences the quantity of minerals herbivores can acquire through forage. Therefore, the increase in hair Zn concentrations could reflect a short-term nutritional gain tied to improved forage quality in the early years following fire. Similar findings have been reported for white-tailed deer, where individuals in recently burned areas (≤ 3 years old) exhibited higher hepatic Zn concentrations compared to those in unburned habitats (Zimmerman *et al.*, 2008). As a potential caveat, a large proportion of moose in our study did not have GPS points in areas recently disturbed by wildfire (n = 46 classified as 'no burns' using a >0.10 threshold). One possible explanation is high site fidelity, as moose may remain in established home ranges that have not recently burned, even if nearby burned areas offer improved habitat conditions. Alternatively, moose might avoid recently burned areas due to increased risks or costs associated with open habitats, such as greater thermal stress and predation risk, especially during warm weather and parturition (Bowyer *et al.*, 1999; Melin *et al.*, 2014). Previous studies suggest that moose show the strongest selection for burned habitats at intermediate successional stages (~11–30 years old; Maier *et al.*, 2005; Joly *et al.*, 2017), likely because these areas provide a balance of high forage availability and sufficient cover. Since most burns in our study areas were less than 10 years old, we could not assess whether intermediate or older burns were associated with variation in mineral concentrations. However, recent work by Mumma *et al.* (2024) in this system suggests that the use of burns by moose will increase as vegetation regrows and structural cover improves. Hence, our findings provide early evidence that younger burns may offer a short-term improvement in forage quality, while highlighting the need for long-term monitoring to determine whether nutritional gains persist or shift as burns mature.

Although mineral deficiencies are more commonly documented in wild ungulate populations, excessive intake of some minerals can also lead to toxicity or adverse health effects. For example, the macro minerals K and Mg are essential for muscle and nerve function, but excessive dietary levels can interfere with rumen health and lead to digestive or metabolic dysfunction (Puls, 1988; McDowell, 1992). Moreover, trace minerals such as Se, though critical for immune function and antioxidant defense, can lead to hair loss, hoof deformities, and reproductive impairment when chronically elevated (Puls, 1988; Flueck *et al.*, 2012; Raisbeck, 2020). Moose must therefore navigate a narrow nutritional window—ensuring they meet mineral requirements without exceeding tolerable limits. This delicate nutritional balancing act is further complicated by interactions among minerals that can affect their absorption and utilization within the body (Underwood, 2012). Although our study identifies environmental correlates of minerals as reflected in hair, we acknowledge that higher hair concentrations do not necessarily equate to better health. Further understanding of the mechanisms moose use to regulate mineral intake to avoid deficiency and excess is essential for fully interpreting these patterns and informing effective management strategies.

Another important consideration to our study is that mineral imbalances in wild ungulates are not always due to insufficient mineral concentrations in forage alone but may also arise from digestive challenges associated with seasonal dietary shifts. When moose switch from highly fibrous winter diets to lush, low-fiber spring vegetation, their ability to absorb or retain minerals may be compromised, even if mineral levels in forage are adequate (Ayotte *et al.*, 2006). Moose often seek mineral licks, especially in spring and summer (Tankersley and Gasaway, 1983; Rea *et al.*, 2013; Huxter *et al.*, 2024), where elevated levels of essential minerals such as Ca, Fe, Mg (Rea *et al.*, 2013), as well as carbonates (Ayotte *et al.*, 2006), are available. Nutritional supplementation from these licks can support rumen function, improve nutrient absorption, and enhance overall body condition (Kreulen, 1985). Moose may also use mineral licks to counteract other aspects of forage quality such as the increasing presence of plant defensive compounds during the summer (Ayotte *et al.*, 2008). Due to a lack of spatial data on mineral lick locations within our study areas, we were unable to evaluate their role directly; however, this represents a valuable direction for future research. Nonetheless, because hair integrates mineral intake over an extended period, we expect that forage remains a major contributor to mineral acquisition in moose, and thus an important influence on hair mineral concentrations, although the relative importance of forage versus other sources such as mineral licks likely varies among individual minerals, individual animals, and forage nutrient concentrations.

In summary, by integrating hair mineral concentrations with measures of habitat composition, landscape disturbance, and climatic conditions, we offer novel insights into patterns of mineral variation in moose in response to environmental change. The tight linkage between mineral status and population stability in herbivores is well documented (O'Hara *et al.*, 2001; Flueck *et al.*, 2012), as many minerals play a crucial role in maintaining health and supporting reproduction and survival (Kincaid, 2000; Underwood, 2012). Therefore, understanding the factors associated with mineral concentrations in moose is necessary for sound population management. Moreover, our findings provide a baseline to support the monitoring of mineral status in hair and underscore the importance of continued monitoring of minerals as a tool for assessing moose responses to environmental change. Minerals are only one of many nutritional currencies essential for the health and fitness of moose, and these results underscore the importance of considering multiple environmental and physiological factors when studying wildlife nutrition. Ultimately, our preliminary study could pave the way for novel research on the causes and consequences of mineral variability in moose and other large herbivores in landscapes subject to environmental change.

CHAPTER 3: Immune biomarkers vary in relation to body fat, trace mineral status, and parasite exposure in moose

Introduction

Wildlife today face widespread changes in habitat and climate that can affect their ability to contend with parasitic infections. Parasites, including microparasites (*e.g.*, viruses, bacteria) and macroparasites (*e.g.*, helminths, ticks), can compromise the health of individuals and persistence of wildlife populations, shaping a range of life-history traits in their hosts (Gulland, 1995). Central to host–parasite dynamics is the immune system, which acts as the primary line of defense against the establishment, replication, and spread of infectious agents (Schmid-Hempel, 2021). Although parasites are ubiquitous in natural systems, hosts differ markedly in their ability to mount effective immune responses. This variation is often influenced by environmental conditions, which can suppress, modify, or enhance immune function through factors such as temperature fluctuations, pollution, and limited food availability (Acevedo-Whitehouse and Duffus, 2009; Jolles *et al.*, 2014; Becker *et al.*, 2019; Ohmer *et al.*, 2021). Under such constraints, individuals may face trade-offs in the allocation of resources to immune defenses versus other physiological demands, such as reproduction (Sheldon and Verhulst, 1996; Lochmiller and Deerenberg, 2000).

Indeed, a central concept in eco-immunology is that immunity is costly to maintain and deploy (Lochmiller and Deerenberg, 2000). Given a finite amount of nutritional and energetic resources, individuals must balance investments across competing physiological demands such as immunity, growth, maintenance, and reproduction (Stearns, 1989; Sheldon and Verhulst, 1996), and these trade-offs have been observed across diverse taxa (*e.g.*, Ilmonen *et al.*, 2000; Ruiz *et al.*, 2011; Hayward *et al.*, 2019; Encel *et al.*, 2023). For example, female Soay sheep

(*Ovis aries*) with higher antibody responsiveness showed improved survival during harsh winters but also exhibited reduced reproductive output (Graham *et al.*, 2010). However, such trade-offs are not universal; they may vary depending on the type of immune response (Albery *et al.*, 2019; Ruoss *et al.*, 2019) and can be influenced by social and ecological contexts (French *et al.*, 2007; Wallace *et al.*, 2023). For instance, when resources are abundant, hosts may afford simultaneous investment in immune defense and other energetically costly traits (van Noordwijk and de Jong, 1986; Becker *et al.*, 2019). Understanding the context-dependent nature of these trade-offs is essential to understanding how fluctuating environmental conditions relate to wildlife health.

Nutritional condition plays a central role in mediating physiological trade-offs and shapes an individual's ability to resist and tolerate infectious agents (Downs and Stewart, 2014; Ohmer *et al.*, 2021). In general, individuals in good nutritional condition are expected to invest in immune responses that balance effective parasite control with minimal self-damage (Downs *et al.*, 2014; Downs and Stewart, 2014), whereas those in poor condition often exhibit reduced immunocompetence and increased susceptibility to disease (Beldomenico and Begon, 2010). Experimental studies have demonstrated that food restriction can suppress multiple components of immunity, such as cellular responses to gastrointestinal parasites and antibody production (Valderrábano *et al.*, 2006; French *et al.*, 2007; Martin *et al.*, 2007; Bourgeon *et al.*, 2010). Yet, in natural populations, these relationships are often more complex. For example, in roe deer (*Capreolus capreolus*), immune responses shift with body condition, but the direction and magnitude of change vary depending on the specific immune biomarker examined (Gilot-Fromont *et al.*, 2012). Therefore, individuals may adjust their investment in immune strategies depending on their nutritional condition. A more nuanced understanding of these dynamics in wild animals is essential for clarifying how nutritional condition relates to immunocompetence under natural ecological pressures.

Limited macronutrient (*e.g.*, energy, protein) and micronutrient (*e.g.*, vitamins, minerals) intake can influence immune responses and underlie trade-offs across physiological systems (French *et al.*, 2007; Brunner *et al.*, 2014; Downs *et al.*, 2018; Bariod *et al.*, 2024). Among micronutrients, trace minerals such as copper (Cu), selenium (Se), and zinc (Zn) play particularly important roles in regulating immune responses and may influence immunity independently of energetic constraints (Jolles *et al.*, 2014). These minerals act as essential cofactors in enzymatic pathways that support immune cell activation, proliferation, and differentiation, and contribute to reactive oxygen species generation, oxidative stress modulation, and regulation of inflammation and immune resolution (Kincaid, 2000; Underwood, 2012; Paul and Dey, 2015). As such, deficiencies or imbalances in trace minerals can impair immune system function, leaving individuals more vulnerable to infection and influencing the trajectory of disease outcomes (Chandra, 1996; Kincaid, 2000). Although these relationships are well-documented in domestic animals (McClure, 2003; McClure, 2008), they remain relatively underexplored in wild populations. Furthermore, the physiological mechanisms underlying these relationships in wildlife are often not fully investigated or understood.

Measuring immunological biomarkers in wildlife enables an understanding of immune function and its connection to physiological condition, fitness, and parasite exposure (Ohmer *et al.*, 2021). The immune system is a complex and multifaceted defense network, and interpreting variation in immune function requires assessing multiple immune indices that capture different components of this system. Cytokines are signaling proteins secreted by various immune cells that regulate immune responses by either enhancing or suppressing activity, and include, for example, interferons (INF), interleukins (IL), interferon gamma-induced protein (IP), and tumor necrosis factors (TNF) (Graham *et al.*, 2007; Zimmerman *et al.*, 2014). Cytokines are multifunctional molecules that can promote pro- or anti-inflammatory responses and modulate specific subsets of immune cells in both innate and adaptive immunity. Acute phase proteins (APP), such as haptoglobin, are synthesized in the liver in response to the production inflammatory cytokines (Libera *et al.*, 2022). Haptoglobin scavenges free hemoglobin, inhibits microbial iron uptake, and reduces oxidative damage (Peck *et al.*, 2016). Production of haptoglobin is typically upregulated during the course of both acute and chronic bacterial or viral infections, reflecting increased erythrocyte turnover linked to inflammatory processes (Gruyse *et al.*, 2005; Peck *et al.*, 2016; Libera *et al.*, 2022). Total globulins, encompassing alpha, beta, and gamma proteins, represent a broad group of serum proteins critical to immune defense, including antibodies, enzymes, and carrier proteins (Alberghina *et al.*, 2011; Couch *et al.*, 2017). Globulins contribute to innate and adaptive immune processes, including humoral responses, while also playing roles in inflammation and molecule transport, making their levels useful indicators of immune activation or ongoing infection. Therefore, measuring concentrations of a panel of cytokines alongside haptoglobin and total globulins provides broad coverage of the immune system, offering complementary insights into multiple aspects of immune function and enabling a more integrated assessment of immune status in wild animals.

Moose (*Alces alces*) in central British Columbia (BC), Canada, are well suited for studying immune variation within a dynamic and changing ecological context. This region has faced extensive pine tree mortality caused by a severe mountain pine beetle (*Dendroctonus ponderosae*) outbreak, which led to large-scale salvage logging operations (Alfaro *et al.*, 2015). Concurrent with these landscape changes, some moose populations experienced steep declines of up to 70% (Kuzyk *et al.*, 2018). Early findings from long-term research and monitoring suggest that bottom-up factors may be contributing to fluctuations in the viability of populations, as indicated by observed starvation and health-related mortalities, coupled with suboptimal pregnancy rates (Thacker *et al.*, 2019). Furthermore, moose face exposure to multiple pathogens reported to cause mortality and reproductive failure in other wild ungulates (das Neves *et al.*, 2010; Thacker *et al.*, 2019). To our knowledge, no previous studies have comprehensively quantified a broad range of immune biomarkers in moose reflecting different components of immunity nor examined patterns of variation in these responses. Measuring immune biomarkers in moose offers the opportunity to assess associations with parasitic infections and physiological condition and ultimately informs conservation efforts aimed at improving wildlife health.

The aim of the present study was to explore how individual physiology and immune challenges are associated with immune function in wild moose. To this end, we analyzed serum samples from adult female moose collected during the winters of 2020–2022 from two study areas in central British Columbia. We quantified the concentrations of haptoglobin, total globulins, and 13 cytokines, which represent the first such cytokine data for moose. We then examined correlations between immune responses and pregnancy status, body fat, key trace minerals, and exposure to micro- and macro-parasites. We expected that immune responses might differ between pregnant and non-pregnant individuals, as pregnancy is an energetically demanding stage. We also expected that individuals with greater body fat could show variation in immune responses, reflecting differences in energetic reserves. Because trace minerals serve as essential cofactors in many immune processes, we anticipated that greater mineral concentrations could be associated with differences in immune biomarker levels. Finally, we predicted that parasite exposure would be associated with increased immune biomarker levels due to immune activation. We sought to provide a foundational understanding of the dynamic immune responses in free-ranging moose and lay groundwork for monitoring health and physiological adaptations in wildlife facing environmental change.

Methods

Ethics statement

The capture and handling of adult female moose was undertaken by the Province of British Columbia as part of a large-scale, provincial-wide moose research project. All procedures adhered to the British Columbia *Wildlife Act* (permit CB17-277227). Data analysis for this project was approved by the Animal Care and Use Committee at the University of Northern British Columbia (ACUC Protocol Number 2021-01).

Study system

We carried out our study on female moose from two populations in central British Columbia, Canada, located in the Bonaparte Plateau (BP) and Prince George South (PGS) regions. These populations have been studied as part of a long-term monitoring effort. The BP (6,800 km^2) is located north of Kamloops, BC (51°13′ N, 120°81′ W), on the traditional territory of the Secwépemc First Nation. The PGS area (11,000 km^2) is situated southwest of Prince George, BC (53°56′ N, 123°63′ W), on the traditional territories of the Lheidli T’enneh and Saik’uz First Nations. Both regions experience a humid continental climate with short, warm summers and long, cold winters. Average annual temperatures are higher in BP (9.5°C) than in PGS (4.3°C), with BP receiving less precipitation overall (216 mm rain, 63 cm snow) compared to PGS (432 mm rain, 204 cm snow; Environment and Climate Change Canada, 2024). The BP landscape spans three Biogeoclimatic Ecosystem Classification (BEC) zones—Interior Douglas-fir (IDF), Sub-Boreal Pine–Spruce (SBPS), and Montane Spruce (MS)—whereas PGS is dominated by the Sub-Boreal Spruce (SBS) zone (Meidinger and Pojar, 1991). Both areas include mixed coniferous and deciduous forests at varying successional stages, alongside lakes and wetlands. Historically shaped by wildfire, these ecosystems are now primarily influenced by industrial forestry and have undergone widespread salvage logging following mountain pine beetle outbreaks (Alfaro *et al.*, 2015). Alongside moose, local wildlife includes mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), elk (*Cervus canadensis*), and predators such as wolves (*Canis lupus*), black bears (*Ursus americanus*), grizzly bears (*U. arctos*), and cougar (*Puma concolor*). Moose densities were estimated at 254 ± 41/1,000 km^2 in BP (2017–2018) and 400 ± 78/1,000 km^2 in PGS (2016–2017; Kuzyk *et al.*, 2018).

Sample collection

To identify how physiological variables and immune challenges may relate to variation in immune biomarker levels, we analyzed serum samples from adult female moose ($\geq$1.5 years old, $n = 31$) captured during early winter (December–February) from 2020 to 2022. For simplicity, individuals captured between December and February were grouped into a single study year (*e.g.*, moose captured between December 2019 and February 2020 were classified as part of the 2020 study year). Comprehensive descriptions of animal capture, sampling, and monitoring have been previously described (Kuzyk *et al.*, 2018, Procter *et al.*, 2020). In brief, adult females were captured using either aerial net-gunning with physical restraint or chemical immobilization via aerial darting from a helicopter. Each moose underwent a comprehensive physical examination and health assessment performed by the attending wildlife veterinarian or an experienced wildlife biologist. Blood samples (20–35 mL) were drawn from the jugular vein using an 18-gauge, 1.5-inch needle, and placed into 6.0 mL royal blue top collection tubes (for trace mineral testing) and 5.0 mL serum separator tubes (gold-top SST; for all other serological testing). Within 12 hours, samples were centrifuged for 15 minutes, and the resulting serum was decanted into cryovials and frozen at −20°C until laboratory analysis. Recaptures were conducted in successive years when possible to facilitate longitudinal monitoring of individual health status (Thacker *et al.*, 2019).

Immune biomarkers

We submitted frozen serum samples to Eve Technologies Corporation, Calgary, Alberta, for the multiplexed quantification of 13 cytokines using Luminex xMAP technology. Due to the absence of cross-reactivity data for moose, we initially submitted three pilot samples for testing using commercial assay kits developed for bovine, ovine, and porcine species. Based on these pilot samples, we selected the porcine panel for analysis of the remaining samples ($n = 57$), as it produced the highest detection rates across individual cytokines and yielded the greatest number of values within the assay's dynamic range. Cytokine concentrations were quantified using Eve Technologies' Porcine Cytokine 13-Plex Discovery Assay® (MilliporeSigma, Burlington, Massachusetts, USA) on the Luminex™ 200 system (Luminex, Austin, Texas, USA), following the manufacturer's protocol. The assay measures fluorescence intensity emitted by fluorescently labeled beads bound to cytokines, and cytokine concentrations are derived by comparing fluorescence signals to a standard curve created from known concentrations. The 13-plex assay measured granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFNγ), interleukin-1 alpha (IL-1α), IL-1β, IL-1 receptor antagonist (IL-1ra), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, and tumor necrosis factor alpha (TNF-α). Each sample was analyzed in duplicate, and the average of the two replicate values was used in the final analysis, reported in pg/mL. Assay sensitivities of the biomarkers range from 5–42 pg/mL.

Serum was also submitted to the Animal Health Laboratory, University of Guelph, for the quantification of globulin (Glb) and haptoglobin (Hp). To calculate globulin concentrations (g/L), total protein and albumin were first measured directly using colorimetric assays on the Roche Cobas 6000 c501 biochemistry analyzer (Roche Diagnostics, Indianapolis, Indiana, USA); globulin was then derived by subtracting albumin from total protein. Haptoglobin (g/L) concentrations were determined using a photometric method on the same analyzer, following protocols based on the methods of Makimura and Suzuki (1982) and Skinner *et al.* (1991). These methods rely on the peroxidase activity of the hemoglobin–haptoglobin complex at low pH, which is measured spectrophotometrically.

Physiological and parasitic variables

Reproduction requires substantial energy in mammals and, as a result, pregnant individuals may have reduced capacity to invest in other costly physiological processes such as immune function. To explore how immunity may relate to pregnancy, we submitted serum samples to the Herd Health Diagnostics Center (Pullman, Washington, USA) for pregnancy testing using a BioPRYN enzyme-linked immunosorbent assay (ELISA). Adult females were classified as pregnant if serum concentrations of pregnancy-specific protein B (PSPB) exceeded 0.21 mg/mL.

Body fat serves as the primary energetic reserve in ungulates, fluctuating seasonally and in response to environmental conditions, making it a key indicator of nutritional status. Moreover, winter body fat in our study system has been shown to reflect whether or not a female successfully raised a calf in the previous year, and therefore provides insight into both recent reproductive investment and current energetic condition (Jefferies, 2024). Therefore, to assess how immunity may vary in relation to energy, we estimated body fat using maximum rump fat thickness (MAXFAT) measured at capture via ultrasonography (FUJIFILM Sonosite M-Turbo®, Toronto, ON, or Ibex® Pro, Loveland, CO), and converted values to ingesta-free body fat percentage (IFBFAT; hereafter, body fat) using the equation: IFBFAT = 5.61 + 2.05 × MAXFAT (Stephenson *et al.*, 1998).

Trace minerals are essential micronutrients that play critical roles in maintaining immune function, supporting antioxidant defenses, and regulating inflammatory responses (Kincaid, 2000; Underwood, 2012; Paul and Dey, 2015). To assess how variation in mineral status may be associated with variation immune responses, we submitted serum samples collected in royal blue top tubes to the Animal Health Laboratory at the University of Guelph. Concentrations of seven minerals were quantified using inductively coupled plasma mass spectrometry (ICP-MS). For this study, we focused on copper (Cu), selenium (Se), and zinc (Zn), as these elements have well-established roles in immune regulation, oxidative stress response, and inflammation in model organisms (Underwood, 2012).

To evaluate immune responses in relation to parasitic challenge, we selected one macroparasite and one microparasite based on their prevalence in our study population. As part of the broader provincial moose health monitoring program, serum samples were screened for exposure to a wide array of infectious agents; however, most pathogens exhibited very low seroprevalence (≤10%) and were excluded from analysis. Full diagnostic protocols and prevalence estimates for all screened pathogens are reported in Thacker *et al.* (2019).

For this study, we retained two parasites with sufficient prevalence to support statistical modeling: winter tick (*Dermacentor albipictus*) infestation and an alphaherpesvirus. Winter tick is a single-host ectoparasite that can cause substantial energetic costs through blood loss, skin damage, and thermoregulatory stress, especially in late winter (Samuel, 2004). Winter tick infestation was assessed at capture at two body sites: the upper shoulder and the rump. At each site, ticks were counted along four parallel 10 cm transect lines spaced 2 cm apart. The total number of ticks across all transects at both sites was summed to provide an overall infestation score for each individual. Due to several zero counts in the data, we treated winter tick infestation as a binary variable, classifying individuals as tick-positive if ticks were observed at either site and tick-negative if no ticks were present.

Alphaherpesviruses are a group of viruses that can establish latent infections and reactivate under stress or immunosuppression. In domestic livestock, bovine herpesvirus-1 (BoHV-1), which causes infectious bovine rhinotracheitis, is a well-known alphaherpesvirus linked to respiratory, reproductive, and systemic illnesses (Muylkens *et al.*, 2007). We assessed seropositivity to an alphaherpesvirus, which has not yet been identified in moose, using an ELISA at the BC Animal Health Centre in Abbotsford, British Columbia. This assay detects antibodies specific to bovine herpesvirus-1. Seropositivity was treated as a binary variable indicating prior exposure or infection.

Statistical analysis

We computed descriptive statistics (*i.e.*, mean, standard deviation, median, and range) for the observed concentrations of 13 cytokines, total globulin, and haptoglobin, across study areas, years, and individuals (*i.e.*, including repeated measures). All cytokines exhibited some out-of-range (OOR) concentrations below the assay's detection limit, although the extent of OOR values varied among biomarkers (see results). Low or undetectable cytokine levels still represent biologically meaningful variation in immune expression; therefore, we assigned concentrations equal to half the lowest observed concentration for each biomarker to OOR samples and retained them in analyses (Balle *et al.*, 2020). Concentrations of cytokines that were considered OOR were, however, excluded from descriptive statistics. In addition to concentrations of biomarkers, we reported descriptive statistics for raw fluorescence intensity values for each cytokine analyte on the full dataset. Fluorescence intensity is a more reliable indicator of sample-to-sample variation, particularly in samples with low concentrations of an analyte, as values do not require defining a limit of detection (Breen *et al.*, 2016). By contrast, concentrations are determined from raw fluorescence values through comparisons with a standard curve and can therefore only be quantified for values that fall within the linear range of the standard curve.

We selected immune biomarkers for further analysis by first examining patterns of covariation using pairwise Spearman's rank correlations. Cytokines GM-CSF, IFN-γ, IL-8, and TNF-α were excluded from pairwise correlations and subsequent analyses due to a high proportion of samples falling below the minimum detectable concentration. The remaining nine cytokines were all highly correlated (all $\rho > 0.93$; see results). Based on these results, we selected four cytokines for further analyses that are known to have distinct, non-redundant biological roles in an attempt to capture meaningful variation in immune function: IL-1β, a pro-inflammatory cytokine important in innate immune activation; IL-10, an anti-inflammatory cytokine that regulates immune suppression and resolution; IL-12, which promotes Th1-type cellular immunity; and IL-4, which supports Th2-type humoral responses (Graham *et al.*, 2007; Zimmerman *et al.*, 2014). Total globulin and haptoglobin were also retained, as they were weakly correlated with cytokines ($\rho \leq 0.20$) and represent different axes of immune function.

We used generalized linear mixed effects models (GLMMs) implemented in the glmmTMB package (Brooks *et al.*, 2017) to identify variables associated with the six selected immune biomarkers. Given that cytokines (*i.e.*, IL-1β, IL-12, IL-10, IL-4) and haptoglobin were restricted to positive values and their distribution is right skewed, we assumed a Gamma distribution of the residuals and used a logarithmic link function. In contrast, globulin concentrations were approximately normally distributed and therefore modeled using a Gaussian distribution with an identity link. Prior to fitting models, we used Cleveland-plots to evaluate outliers for each biomarker (Zuur *et al.*, 2010) and removed two severe outliers from haptoglobin that were approximately 9 and 12 times higher than the median value, respectively. These values (1.59 and 2.08 g/L) were considered outliers as they were substantially higher than the next highest reported haptoglobin level of 0.68 g/L in our dataset and exceeded levels previously documented in another moose population and other cervid species (McDonough *et al.*, 2022; Lamb *et al.*, 2024).

Model selection was conducted using a model dredging approach via the *dredge* function in the MuMIn package (Bartoń, 2023), which fits all possible subsets of a global model and ranks them based on Akaike's Information Criterion corrected for small sample size (AIC_c; Burnham and Anderson, 2002). The global models included the following fixed effects to reflect biologically plausible relationships: pregnancy status, body fat, serum concentrations of Cu, Se, and Zn, winter tick infestation, and alphaherpesvirus serostatus. Study area and year were retained as conditional fixed effects in all models to control for spatial and temporal variation, allowing us to isolate the effects of our variables of interest. Individual ID of female moose was included as a random intercept to account for repeated sampling. We assessed multicollinearity among explanatory variables within our global models using variance inflation factors (VIFs; Zuur *et al.*, 2010) and found all variables to be weakly correlated with VIFs less than two. Continuous predictors were standardized by centering on the mean and scaling by the standard deviation prior to analysis.

We considered models with ΔAICc < 2 to be equally supported (Burnham and Anderson, 2002), provided they did not include uninformative parameters (*i.e.*, those that include one extra parameter without meaningfully improving the model's log-likelihood but are ranked close to more parsimonious models with lower AIC values; Leroux, 2019). If the set of similarly supported models contained the null model (study area and year only), we considered that the most parsimonious model. We interpreted parameters within models to be influential if their 85% confidence intervals (CIs) did not overlap zero. We used 85% CIs as this confidence level reflects the significance threshold consistent with the decision-making framework of AIC-based model selection (Sutherland *et al.*, 2023). We evaluated model fit of our top models by visualizing scaled residuals simulated from the fitted model to assess uniformity, dispersion, and overall model assumptions using the DHARMa package (Hartig, 2024). All statistical analyses were conducted using R (version 4.3.1; R Core Team, 2023). Given the exploratory nature of our analysis and the number of model comparisons conducted, the reported covariate effects should be interpreted as preliminary hypotheses requiring validation with independent data.

Results

We sampled 31 unique adult female moose during winter between 2020 and 2022 in two study areas in central British Columbia: the Bonaparte Plateau (BP; *n* = 17) and Prince George South (PGS; *n* = 14). Of these individuals, eight were sampled once, 17 were sampled twice in two different years, and six were sampled three times in three years, resulting in a total of 60 serum samples in which immune biomarkers were quantified. We detected measurable concentrations of all tested immune biomarkers in moose serum (Table 3.1). Of the 13 cytokines measured using the porcine cytokine multiplex test, the proportion of samples within the assay's sensitivity range varied from 23.3% for GM-CSF to 96.7% for both IL-1β and IL-4. Cytokines

GM-CSF, IFN-γ, IL-8, and TNF- α fell below the minimum detectable concentration limit in 77%, 30%, 57%, and 53% of samples, respectively, and were removed from analyses. All other immune biomarkers were detectable in at least 80% of samples. No samples exceeded the assay's upper sensitivity limit. All immune biomarkers, except total globulins, showed moderate to strong right-skewed distributions, indicating that most individuals had low concentrations and a few had markedly higher levels. Summary statistics for key predictor variables used in our models are presented in Table 3.2.

The nine cytokines retained were strongly and positively correlated, with Spearman's rank coefficients ranging from 0.94 to 0.98 (all *p*-values <0.001; Appendix B Fig. B.1). In contrast, correlations between cytokines and total globulins were weak but generally positive, ranging from $\rho = 0.10$ to 0.20 (all *p*-values > 0.13). Correlations between cytokines and haptoglobin were weak and mostly negative, ranging from $\rho = -0.14$ to –0.04 (all *p*-values >0.28). Total globulins and haptoglobin were effectively uncorrelated ($\rho = 0.03$, *p*-value = 0.65).

Table 3.1. Serum immune biomarker concentrations ($n = 60$) from adult female moose (*Alces alces*) sampled in winter (2020–2022) from two populations in central British Columbia, Canada: the Bonaparte Plateau (BP; $n = 31$) and Prince George South (PGS; $n = 29$). Cytokine concentrations are reported in pg/mL, and globulin and haptoglobin in g/L. Cytokine concentration values that fell below the assay detection range (out of range; OOR) and could not be extrapolated were excluded from descriptive statistics. Fluorescence intensity values, which do not have a defined limit of detection, are reported for the full sample size.

Immune biomarker	**Mean conc. ± SD**	**Median conc.**	**Conc. range**	**OOR (*n*/60)**	**Mean FI**	**Median FI**	**FI range**
GM-CSF	26.07 ± 25.54	17.92	1.85–81.85	46	12.78 ± 4.16	11.50	8.50 – 29.50
IFN-γ	3034.56 ± 5014.00	1340.81	24.84–28771.74	18	84.53 ± 157.92	36.40	13.80 – 1066.00
IL-1α	75.41 ± 141.69	25.42	0.12–705.58	6	264.59 ± 503.95	81.90	9.50 – 2650.00
IL-1β	601.35 ± 1193.03	123.45	1.01–5703.34	2	256.13 ± 469.29	67.15	9.00 – 2419.50
IL-1ra	704.01 ± 1416.55	244.57	4.71–6261.22	10	282.17 ± 507.38	91.15	14.50 – 2619.80
IL-2	557.37 ± 1236.10	142.45	0.57–5744.94	6	259.50 ± 526.49	61.50	11.30 – 2636.30
IL-4	3849.75 ± 10376.39	522.76	2.84–51308.42	2	285.49 ± 554.89	87.75	13.30 – 2860.80
IL-6	252.20 ± 546.26	75.54	0.47–2760.12	12	249.95 ± 481.83	75.40	11.50 – 2690.80
IL-8	67.03 ± 73.05	48.46	4.55–329.72	34	56.37 ± 80.72	27.75	11.50 – 444.30
IL-10	2745.18 ± 6281.27	842.35	4.76–36118.42	10	254.30 ± 519.88	63.75	9.50 – 2896.30
IL-12	363.76 ± 671.91	115.88	0.35–3031.10	11	247.73 ± 470.66	65.90	10.80 – 2620.00
IL-18	3594.05 ± 8397.79	1028.07	4.52–38674.41	9	228.85 ± 462.09	55.75	12.00 – 2332.50
TNFα	34.79 ± 25.51	29.64	6.15–100.81	32	21.84 ± 6.37	20.75	12.00 – 46.80
Globulin	29.63 ± 7.26	28.00	17.00–58.00	0	-	-	-
Haptoglobin	0.25 ± 0.31	0.17	0.12–2.08	0	-	-	-

Abbreviations: conc., concentration, FI, fluorescence intensity, GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon gamma; IL, interleukin; TNFα, tumor necrosis factor alpha

Table 3.2. Summary of physiological and parasitic variables (*n* = 60) used to predict immune biomarker concentrations in female moose (*Alces alces*) serum sampled during the winters of 2020–2022 in two populations: the Bonaparte Plateau (BP; *n* = 31) and Prince George South (PGS; *n* = 29), central British Columbia, Canada. Continuous variables (body fat percentage and serum trace minerals Cu, Se, and Zn) are presented as mean ± standard deviation. Categorical variables (pregnancy status, winter tick presence, and alphaherpesvirus serostatus) are presented as counts with corresponding percentages in parentheses. These values represent averages and proportions calculated across both study areas, all three sampling years, and include repeated measurements from individuals.

Variable	**Type**	**Summary**
Body fat (%)	Continuous	9.00 ± 2.00
Serum Cu (µg/mL)	Continuous	0.40 ± 0.07
Serum Se (µg/mL)	Continuous	0.05 ± 0.02
Serum Zn (µg/mL)	Continuous	0.56 ± 0.08
Pregnancy status — Pregnant	Categorical	42 (70%)
Winter tick — Presence	Categorical	43 (72%)
Alphaherpesvirus — Seropositive	Categorical	45 (75%)

Our model selection approach indicated that body fat, or a combination of body fat and serum Zn concentrations, best predicted IL-12 concentrations in the serum of adult female moose (Fig. 3.1). The two top-ranked models with ΔAIC_c scores less than two accounted for a combined 24% of the cumulative model weight (Table 3.3). Moose with greater amounts of body fat had greater concentrations of IL-12 (1st ranked model: β = 0.69, SE = 0.24, 85% CI = [0.35, 1.03]; 2nd ranked model: β = 0.59, SE = 0.25, 85% CI = [0.23, 0.95]). Additionally, moose with higher serum Zn concentrations also had elevated IL-12 concentrations (1st ranked model: β = 0.76, SE = 0.33, 85% CI = [0.11, 1.40]). Concentrations of IL-12 were similar between the two study areas (1st ranked model: β = 0.73, SE = 0.98, 85% CI = [-0.69, 2.15]; 2nd ranked model: β = 0.74, SE = 0.96, 85% CI = [-0.65, 2.12]). Moose sampled in 2022 had higher IL-12 concentrations compared to those sampled in 2020 in the first ranked model (β = 1.07, SE = 0.55, 85% CI = [0.29, 1.86]); however, the 85% confidence interval overlapped zero in the second ranked model (β = 0.27, SE = 0.48, 85% CI = [−0.42, 0.96]). Moose sampled in 2021 had similar

IL-12 concentrations to those sampled in 2020 in both models (1st ranked model: β = −0.05, SE = 0.54, 85% CI = [−0.83, 0.73]; 2nd ranked model: β = −0.69, SE = 0.54, 85% CI = [−1.46, 0.09]).

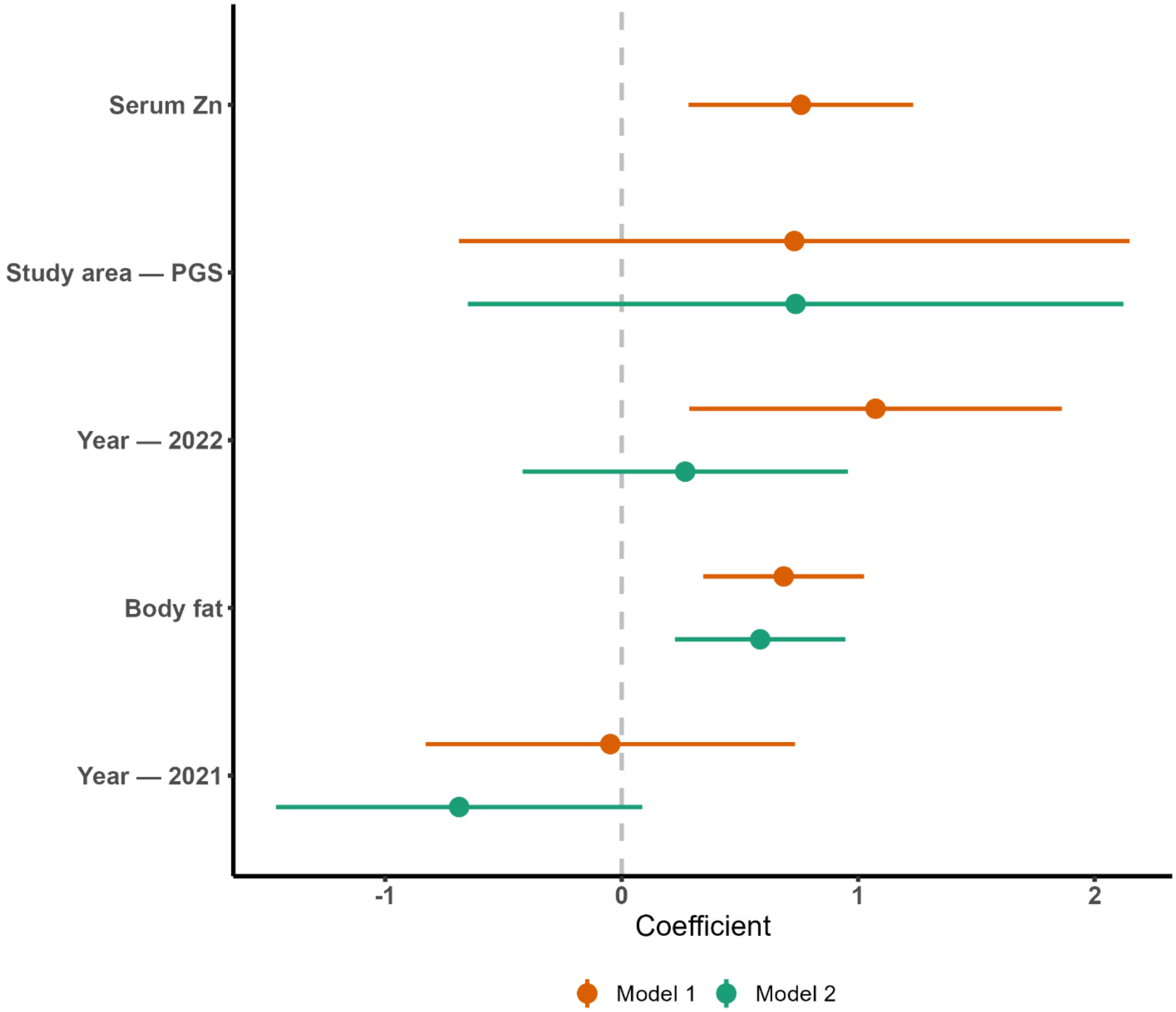

Figure 3.1. Coefficient estimates and 85% confidence intervals (CI) for independent variables in the top-ranked generalized linear mixed effects models (< 2 ΔAICc) explaining interleukin-12 concentrations in female moose (*Alces alces*) serum. Serum samples (*n* = 60) were collected during capture events in the winters of 2020–2022 from two study areas (Prince George South and the Bonaparte Plateau) in central British Columbia, Canada. An independent variable has an influential relationship with the mineral if the CI does not overlap 0. Continuous predictors serum Zn and body fat were standardized to a mean of zero and a standard deviation of one prior to analysis. Coefficients were ordered from most positive to most negative based on standardized estimates.

Table 3.3. Model selection statistics used to predict serum immune biomarker concentrations (*n* = 60 for most biomarkers; haptoglobin: *n* = 58 due to removal of two outliers) in adult female moose (*Alces alces*) in two study areas in central British Columbia, Canada, from 2020–2022. Models that were within the top model set (< 2 ΔAICc) and ranked higher than the null model, as well as null models, are included. Variables in bold font indicate an influential relationship with the dependent variable (85% CI does not overlap 0). Predictor variables did not explain substantially more variation in the data than the null model for immune biomarkers IL-1β, IL-10, and IL-4, and therefore, these results are not presented. All models included a random intercept for Individual ID to account for repeated measures. Study area and year were included as conditional fixed effects in every model.

Dependent variable	**Rank**	**Independent variables**	**AIC_c**	**ΔAIC**	**AIC_c w_i**
IL-12	1	Study area + **Year** + **Body fat** + **Serum Zn**	674.23	0.00	0.17
	2	Study area + Year + **Body fat**	676.15	1.92	0.07
	13	Study area + Year (null)	678.83	4.59	0.02
Total globulins	1	Study area + **Year** + **Winter tick** + **Alphaherpesvirus** + **Serum Zn**	381.12	0.00	0.11
	2	Study area + **Year** + **Winter tick** + **Alphaherpesvirus**	382.11	0.99	0.07
	3	Study area + **Year** + **Winter tick**	382.73	1.61	0.05
	18	Study area + **Year** (null)	385.09	3.97	0.02
Haptoglobin	1	Study area + Year + **Serum Cu**	-172.21	0.00	0.10
	2	Study area + Year + **Serum Cu** + Alphaherpesvirus	-171.05	1.17	0.07
	3	Study area + Year + **Serum Cu** + Serum Cu	-170.87	1.34	0.05
	4	Study area + Year + **Serum Cu** + Winter tick	-170.45	1.76	0.04
	7	Study area + Year (null)	-169.73	2.45	0.03

Abbreviations: IL, interleukin

Among the models evaluated to predict total globulin concentrations, three had ΔAIC_c scores below two and collectively accounted for 23% of the cumulative model weight (Fig. 3.2, Table 3.3). Winter tick presence consistently emerged as an important predictor of total globulins; female moose infested with winter ticks had elevated concentrations of total globulins (1st ranked model: $\beta = 3.31$, SE = 1.41, 85% CI = [1.29, 5.34]; 2nd ranked model: $\beta = 3.20$, SE = 1.44, 85% CI = [1.13, 5.26]; 3rd ranked model: $\beta = 3.27$, SE = 1.43, 85% CI = [1.21, 5.33]). Additionally, total globulin concentrations were greater in moose seropositive for alphaherpesvirus (1st ranked model: $\beta = 3.89$, SE = 1.78, 85% CI = [1.33, 6.46]; 2nd ranked model: $\beta = 3.23$, SE = 1.80, 85% CI = [0.63, 5.82]). Total globulin concentrations decreased with higher serum Zn levels (1st ranked model: $\beta = -1.53$, SE = 0.78, 85% CI = [-2.65, -0.41]). Total globulin concentrations were greater in moose sampled in 2022 (*e.g.*, 1st ranked model: $\beta = 4.88$, SE = 1.52, 85% CI = [2.69, 7.08]) and marginally greater in moose sampled in 2021 (*e.g.*, 2nd ranked model: $\beta = 3.00$, SE = 1.52, 85% CI = [0.82, 5.19]) relative to 2020. Concentrations of total globulins in female moose were similar between study areas (*e.g.*, 1st ranked model: $\beta = 0.33$, SE = 1.95, 85% CI = [-2.47, 3.14]).

Four models predicting haptoglobin concentrations showed ΔAIC_c scores less than two, collectively comprising 25% of the cumulative model weight (Fig. 3.3, Table 3.3). Across all top models, serum Cu was the only consistent and influential predictor of haptoglobin (*e.g.*, 1st ranked model: $\beta = 0.10$, SE = 0.04, 85% CI = [0.04, 0.17]). Although the second through fourth ranked models included additional predictors such as alphaherpesvirus serostatus, serum Se, or winter tick presence, they differed from the top-ranked model by only one parameter each and had higher AIC_c scores. This pattern suggests they likely contain uninformative parameters and that the most parsimonious model is the first ranked model with serum Cu alone. Haptoglobin concentrations were similar between study areas (1st ranked model: β = 0.01, SE = 0.11, 85% CI = [-0.16, 0.16]) and across years (1st ranked model — 2021: β = -0.06, SE = 0.07, 85% CI = [-0.16, 0.05]; 2022: β = 0.02, SE = 0.06, 85% CI = [-0.06, 0.12]).

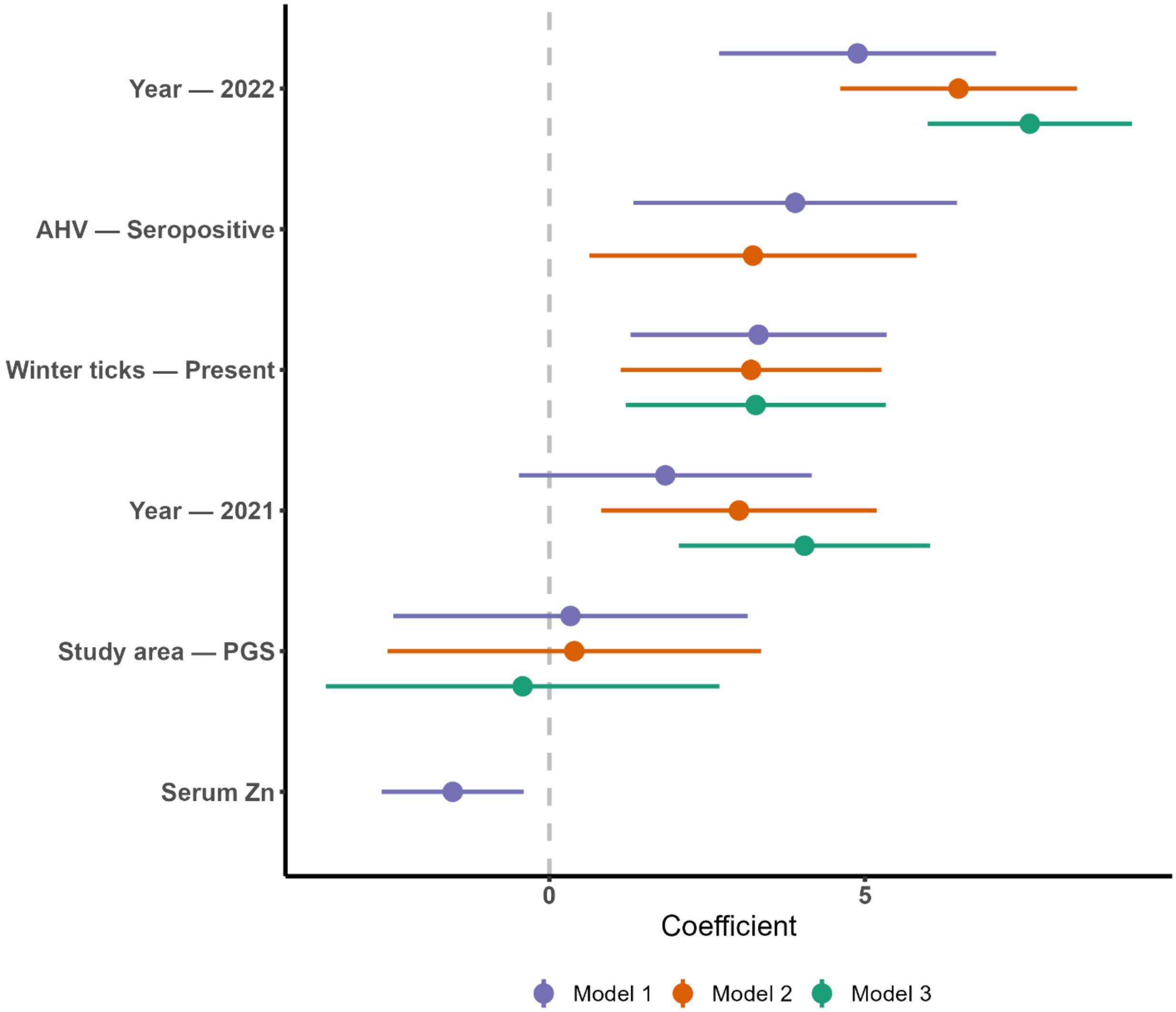

Figure 3.2. Coefficient estimates and 85% confidence intervals (CI) for independent variables in the top-ranked generalized linear mixed effects models (< 2 ΔAICc) explaining total globulin concentrations in female moose (*Alces alces*) serum. Serum samples (*n* = 60) were collected during capture events in the winters of 2020–2022 from two study areas (Prince George South and the Bonaparte Plateau) in central British Columbia, Canada. An independent variable has an influential relationship with the mineral if the CI does not overlap 0. The continuous predictor serum Zn was standardized to a mean of zero and a standard deviation of one prior to analysis. Coefficients were ordered from most positive to most negative based on standardized estimates. AHV = Alphaherpesvirus.

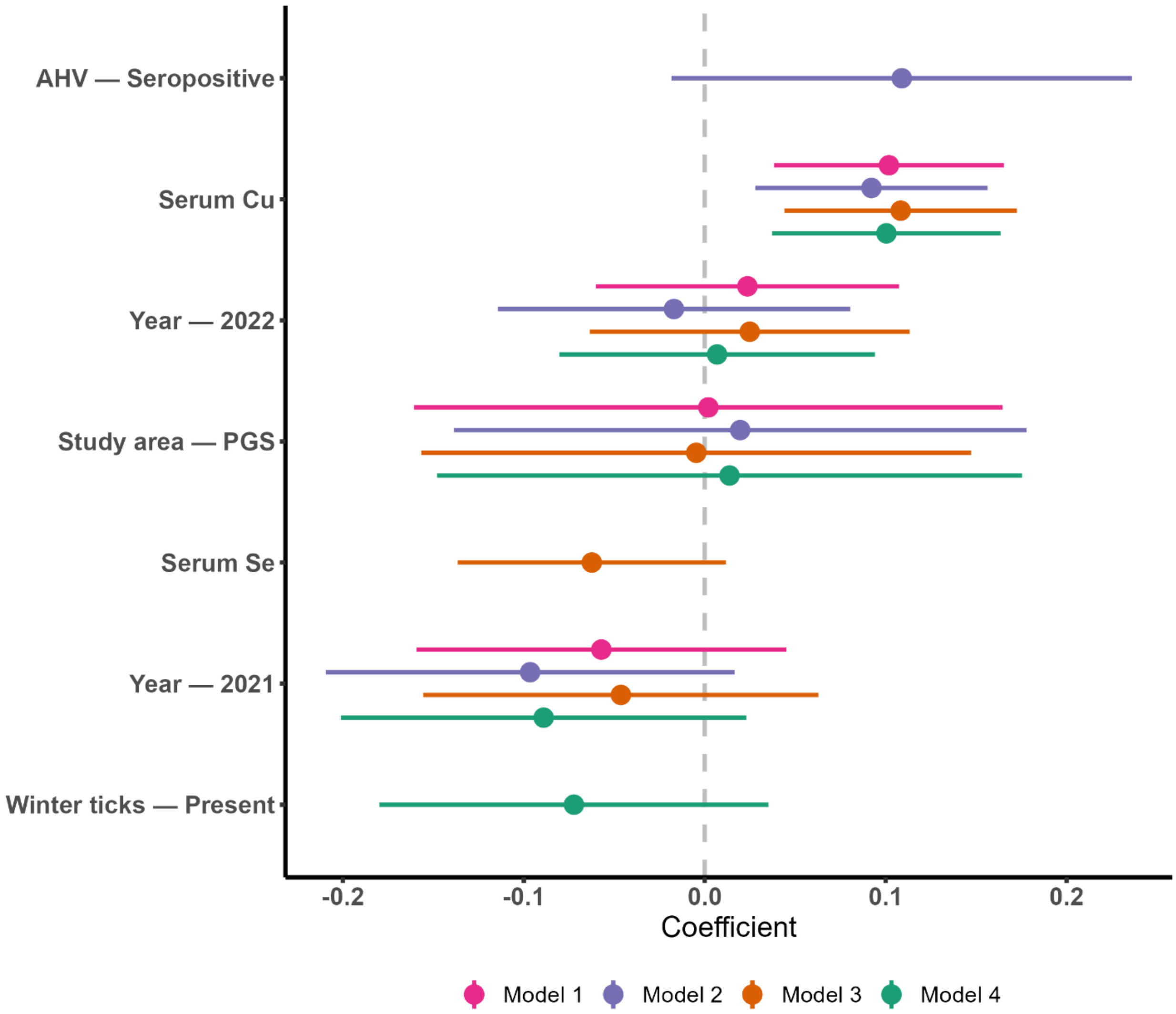

Figure 3.3. Coefficient estimates and 85% confidence intervals (CI) for independent variables in the top-ranked generalized linear mixed effects models (< 2 ΔAICc) explaining haptoglobin concentrations in female moose (*Alces alces*) serum. Serum samples (*n* = 58; two outliers removed) were collected during capture events in the winters of 2020–2022 from two study areas (Prince George South and the Bonaparte Plateau) in central British Columbia, Canada. An independent variable has an influential relationship with the mineral if the CI does not overlap 0. Continuous predictors serum Cu and serum Se were standardized to a mean of zero and a standard deviation of one prior to analysis. Coefficients were ordered from most positive to most negative based on standardized estimates. AHV = Alphaherpesvirus.

For the remaining immune biomarkers (*i.e.*, IL-1β, IL-10, and IL-4), our predictor variables did not explain more variation in the data than the null model, which included study area and year. For IL-4, the first ranked model included serum Zn as a predictor, which was positively associated with IL-4 concentrations (β = 0.45, SE = 0.28, 85% CI = [0.06, 0.85]), and the confidence interval did not encompass zero. The null model, however, ranked second and was within two AICc units, suggesting the addition of Zn did not meaningfully improve the model. For cytokines IL-1β and IL-10, the null model was the first ranked model. Female moose in the PGS study area had higher concentrations of IL-4 (β = 1.15, SE = 0.75, 85% CI = [0.07, 2.23]) and IL-10 (β = 1.21, SE = 0.82, 85% CI = [0.04, 2.39]) compared to females in the BP study area. However, IL-1β concentrations were similar between study areas (β = 0.68, SE = 0.69, 85% CI = [−0.32, 1.67]). No year-to-year variation was detected for IL-1β (2021: β = −0.46, SE = 0.41, 85% CI = [−1.05, 0.13]; 2022: β = −0.16, SE = 0.38, 85% CI = [−0.71, 0.40]), IL-10 (2021: β = −0.34, SE = 0.49, 85% CI = [−1.05, 0.36]; 2022: β = 0.23, SE = 0.47, 85% CI = [−0.45, 0.90]), or IL-4 (2021: β = −0.51, SE = 0.44, 85% CI = [−1.13, 0.12]; 2022: β = −0.03, SE = 0.41, 85% CI = [−0.62, 0.57]).

Discussion

Immunity plays a central role in helping animals cope with physiological and parasite-related challenges. Measures of immune function can therefore offer valuable insights into individual health and population resilience (Ohmer *et al.*, 2021). In this study, we demonstrated that serum concentrations of immune biomarkers in adult female moose reflected variation in both physiological condition and parasite exposure. We found that moose with greater fat reserves had greater concentrations of IL-12, suggesting that sufficient energetic reserves could enable greater investment in immune function. In addition, we found that total globulin levels were elevated in individuals exposed to micro- and macro-parasites, consistent with immune activation. We also found that Zn concentration was associated with both IL-12 and total globulin levels, and that Cu was associated with haptoglobin, underscoring the potential role of trace minerals in modulating immune responses. Importantly, we believe this study represents the first to characterize cytokine expression in moose, establishing baseline values for a suite of immune biomarkers within this demographic—including cytokines, acute phase proteins, and total globulins—and revealing variation across years, populations, and immune biomarkers. Collectively, these findings underscore the complex and context-dependent interactions among nutrition, parasitism, and immune function in wild ungulates, and provide a foundation for future efforts to monitor moose health in the face of environmental change.

Our findings suggest that energetic reserves could relate to immune investment in wild adult female moose, consistent with theoretical expectations that individuals in better nutritional condition can afford the costs of mounting stronger immune responses (Downs and Stewart, 2014; Ohmer *et al.*, 2021). Specifically, we found that female moose with more body fat had greater concentrations of interleukin-12 (IL-12), a pro-inflammatory cytokine central to cell-mediated immunity. Interleukin-12 promotes Th1 differentiation and enhances interferon-gamma (IFN-γ) production, both of which are critical for controlling intracellular pathogens (Graham *et al.*, 2007; Zimmerman *et al.*, 2014). The production of IL-12 is metabolically costly, as it contributes to inflammation, which can incur physiological damage if not tightly regulated. Thus, our results support the idea that individuals with greater energy stores may be better able to invest in demanding immune functions. Similar patterns have been reported in other mammals, such as northern elephant seals (*Mirounga angustirostris*), where body reserves positively influenced levels of pro-inflammatory cytokines across life history stages (Peck *et al.*, 2016). This relationship could also potentially relate to reproductive history, as another study conducted on this same moose population found that females in better condition were less likely to have raised a calf in the previous year (Jefferies, 2024). This may suggest that females who avoided the energetic costs of lactation may have had additional energy available to invest in their immune system. Another alternative explanation is that elevated IL-12 could partly reflect increased adipose tissue, as fat cells are known to secrete cytokines including IL-12 (Clemente-Suárez *et al.*, 2023). Overall, this finding provides an important initial insight into the potential ways by which energy balance may shape immune function in wild moose, highlighting the need for further research to better understand the mechanism underlying this relationship in natural populations.

We found that total globulin concentrations were elevated in moose with winter tick infestations and in individuals seropositive for an alphaherpesvirus, consistent with immune activation following pathogen infection or exposure. Globulins represent a broad class of serum proteins involved in immune defense—such as antibodies and complement proteins—and often increase during prolonged or repeated immune challenges as the body responds to foreign antigens (Alberghina *et al.*, 2011; Couch *et al.*, 2017). Macro-parasitic infections, including winter tick infestations, induce type 2 immune responses that promote antibody production (Jolles *et al.*, 2015), suggesting that infested adult female moose could have exhibited an adaptive immune reaction reflected in elevated globulin levels. This finding aligns with previous work on moose calves, where gamma-globulin concentrations increased shortly after the onset of winter tick engorgement (Glines and Samuel, 1989), highlighting the immune challenge posed by tick infestation. However, it remains unclear whether this immune response can effectively reduce or clear tick infestations, or whether it primarily helps moose cope with the physiological impacts of parasitism. Similarly, alphaherpesvirus seropositivity indicates prior viral exposure and the long-term persistence of specific antibodies (das Neves *et al.*, 2010), which would also be reflected in total globulin levels. This relationship may also help to explain the temporal variation in total globulins observed across years, as alphaherpesvirus seroprevalence increased from 52% in 2020 to 83% in 2022, paralleling increases in globulin concentrations. These findings suggest that total globulin concentrations could serve as reliable biomarkers of general immune activation and parasite exposure in moose, a consideration that becomes increasingly important as the prevalence of parasitic infections rises under changing environmental conditions.

We found associations between trace minerals and several immune biomarkers, which may support the known roles of trace mineral status in immune regulation. Trace minerals are critical cofactors for numerous enzymes that support immune function, including antioxidant defense, cell proliferation, and the maintenance of physical barriers, including skin and mucous membranes (Kincaid, 2000; Underwood, 2012; Paul and Dey, 2015). Both Cu and Zn play pivotal roles in regulating innate and adaptive immunity by modulating cytokine production, lymphocyte activity, and controlling inflammation. In line with these functions, we found a strong positive association between serum Zn and the cytokine IL-12, and weaker evidence for a positive relationship between Zn and IL-4, although the latter model did not clearly outperform the null model. We also found a positive association between Cu and haptoglobin. Conversely, the negative association between Zn and globulin concentrations could potentially indicate that individuals with lower Zn levels are more vulnerable to chronic or persistent infections, as elevated globulins reflect sustained immune activation. Zinc deficiency could impair the ability to control infections effectively (Scott and Koski, 2000), leading to prolonged immune responses and increased globulin production. Because serum minerals were measured at a single time point, it is possible that animals experiencing immune challenges may have depleted mineral levels as a consequence of increased immune activity, rather than mineral status directly influencing immune responses. Nonetheless, our results highlight possible links between trace mineral status and immune function in moose, underscoring the need for further investigation into the interactions among mineral deficiencies and immune challenges in wild populations.

Given the energetic demands of reproduction, we expected pregnant females to show different immune responses due to trade-offs around energy availability (Stearns, 1989; Sheldon and Verhulst, 1996); however, we found that immune biomarker levels were similar between pregnant and non-pregnant individuals. This may, in part, reflect the timing of sample collection, as females were in early pregnancy and may not yet have incurred substantial energetic costs associated with pregnancy. Late gestation and active lactation are more energetically demanding phases when trade-offs with costly functions might be more pronounced (Clutton-Brock *et al.*, 1989; Christe *et al.*, 2000; Beasley *et al.*, 2010). In addition, some moose testing positive for pregnancy may not have carried a fetus to term or produced a viable calf, limiting the reliability of a single serum test as an indicator of reproductive investment. Furthermore, the immune biomarkers we measured may not have captured the specific facets of immune function modulated during pregnancy. For example, a study on Dall sheep (*Ovis dalli dalli*) found that pregnant individuals had reduced bacterial killing ability, a functional measure of innate immunity, but showed no relationship between pregnancy and haptoglobin (Downs *et al.*, 2018), highlighting that different immune measures vary in sensitivity to reproductive status.

Future research should include a broader range of immune biomarkers and target sampling during critical reproductive stages, such as late gestation and post-parturition, to better capture shifts in immune investment across the reproductive timeline. Given the constraints of wildlife research, including limited access to individuals, logistical challenges of long-term monitoring, and the stress associated with capture, sampling moose during the spring and summer to better capture reproductive stages was not feasible for this project. However, non-invasive approaches may help address these gaps. For example, Albery *et al.* (2019) used fecal IgA concentrations to assess immune function across seasons and reproductive states in wild red deer (*Cervus elaphus*). Similar methods could be applied to moose to better understand how immune investment shifts across reproductive stages and energetic demands throughout the year.

Although the cytokines measured in this study have distinct roles in immune regulation in model species, we found that all were strongly positively correlated. This pattern suggests that multiple cytokine pathways may be activated simultaneously, reflecting a broad, coordinated immune response rather than discrete or polarized signaling (Graham *et al.*, 2007; Zimmerman *et al.*, 2014). Such upregulation could result from environmental or physiological stressors such as infection, injury, or chronic inflammation that activate several immune pathways concurrently. Despite strong correlations among cytokines, we found no associations between any cytokine and other immune biomarkers such as haptoglobin or globulins, nor were haptoglobin and globulins correlated with each other. This lack of association likely reflects fundamental differences in the timing and function of these immune responses. Cytokines are typically produced in rapid, short-lived bursts at the site of immune activation and can quickly return to baseline levels (Graham *et al.*, 2007; Zimmerman *et al.*, 2014). In contrast, haptoglobin is synthesized in the liver in response to cytokine signaling (Gruyse *et al.*, 2005; Peck *et al.*, 2016; Libera *et al.*, 2022), introducing a delay in its expression that depends on the timing and intensity of upstream signals. Globulins, which include circulating antibodies and other serum proteins, reflect longer-term or repeated immune stimulation and tend to change more gradually over time (Alberghina *et al.*, 2011; Couch *et al.*, 2017). Given that our measurements were taken from single-point serum samples, we may have missed temporal fluctuations and dynamic changes in these biomarkers, underscoring the importance of repeated longitudinal sampling to capture the complexity of immune responses fully. The absence of co-variation among immune biomarkers may also reflect the nature of immune challenges currently experienced by this population, which may not elicit strong or temporally aligned responses across immune axes. These findings highlight the complexity and individuality of immune responses in free-ranging moose and emphasize the importance of assessing multiple immune components to characterize systemic immune activity in ecological studies.

To our best knowledge, this study provides the first record of serum cytokines measured in free-ranging moose. Incorporating cytokines alongside traditional immune biomarkers expands the range of immunological responses that can be measured in wild animals and enhances our ability to assess individual immune status. We found that a commercial porcine multiplex assay exhibited strong cross-reactivity with moose serum, with nine of thirteen cytokines detectable in at least 80% of samples. The assay yielded consistent, replicable signals across individuals, and even those cytokines with lower detection frequencies displayed clear variation in fluorescence intensity—suggesting biologically meaningful differences. Individual cytokine concentrations varied with physiological metrics and across study areas, further supporting the sensitivity of these biomarkers to ecological and individual factors. However, this assay has not yet been formally validated for moose using functional tests such as immune stimulation tests. Future research should prioritize validation efforts to confirm assay specificity and sensitivity in this species, which will be essential for advancing cytokine-based immune monitoring in wildlife (Levin *et al.*, 2014; Borque *et al.*, 2020).

In summary, our study revealed potential relationships between immunity, parasite exposure, and host physiology in free-ranging moose. The immune system plays a critical role in protecting animals from infection, maintaining physiological homeostasis, and promoting population resilience (Schmid-Hempel, 2021). Therefore, understanding factors linked with immunity is essential for predicting infection outcomes and overall health of wild populations, which can inform targeted monitoring and management strategies (Ohmer *et al.*, 2021). Although our analysis was exploratory in nature, we identified preliminary relationships with immune biomarkers that warrant further investigation; for example, cytokines may be particularly responsive to nutritional stressors in female moose, while globulins may better reflect chronic immune stimulation, such as persistent parasite exposure, suggesting these biomarkers could serve as useful indicators in ongoing health assessments. Our findings also provide valuable baseline data for immune biomarkers in adult female moose and underscore the importance of continued monitoring to evaluate how environmental change may affect wildlife health. Future research employing longitudinal sampling across multiple seasons and infection stages would be valuable to better capture the dynamic relationships between immune profiles, infection progression, parasite clearance, and fitness outcomes. Such approaches would provide critical insights into the functional role of immunity in mediating host-pathogen interactions under natural environmental conditions and support adaptive management efforts. Ultimately, this study provides a foundation for future efforts to understand the causes and consequences of immune variation in moose and other large herbivores inhabiting rapidly changing landscapes.

CHAPTER 4: Conclusions

Research summary and implications

The rapid pace of environmental change is profoundly altering the landscapes and ecological conditions on which wildlife depend, with important consequences for their nutrition, health, and population dynamics (Acevedo-Whitehouse and Duffus, 2009). Changes in climate and habitat quality, in particular, can affect the availability and nutritional value of forage, thereby influencing the physiological condition and fitness of large herbivores (Parker *et al.*, 2009; Stephenson *et al.*, 2020). Monitoring health biomarkers (*i.e.*, measurable indicators that provide insight into wildlife health) offers a valuable approach to understanding how large herbivores physiologically respond to environmental stressors (Stephen, 2014; Thacker *et al.*, 2019; Wittrock *et al.*, 2019; Aleuy *et al.*, 2023). For example, measuring essential mineral levels and immune biomarkers can reveal early signs of nutritional deficiencies or subclinical disease, thereby guiding adaptive conservation strategies that promote population resilience.

Health biomarkers may also help elucidate the mechanisms behind recent population changes observed in some wildlife species. For example, in the early 2000s, a widespread mountain pine beetle (MPB) outbreak caused significant pine tree mortality across much of British Columbia, leading to intensified salvage logging that dramatically transformed the landscape. Concurrently, some moose populations experienced steep declines of up to 70% (Kuzyk *et al.*, 2018), hypothesized to be caused by rapid landscape changes. Initial findings from long-term research and monitoring suggest that bottom-up factors (*i.e.*, food quality and/or availability) may have contributed to these declines based on evidence of starvation and health-related mortalities combined with suboptimal pregnancy rates (Thacker *et al.*, 2019). The mechanisms linking environmental variation, including food quality and quantity, with moose health and population declines, however, are poorly understood. Considering the complex interplay of natural disturbances, human activities, and climate effects shaping moose habitat and population trends, a more thorough understanding of the factors that are associated moose health, and ultimately population numbers, is critical.

Accordingly, my thesis aimed to identify the environmental and physiological conditions associated with essential mineral concentrations and immune responses in adult female moose from two study areas affected by landscape disturbances and shifts in climatic regimes. In Chapter 2, I examined associations between hair mineral concentrations and environmental variables using GPS collar data, spatial datasets, and hair samples collected during winter captures as part of the BC Provincial Moose Research Project. I found that essential mineral concentrations in moose hair varied in relation to climatic conditions, habitat composition, and landscape disturbances. Specifically, female moose with greater concentrations of Se and Zn experienced increased precipitation, suggesting a potential link between climate-driven vegetation changes and mineral uptake. Mineral concentrations of K and Mg were greater in female moose spending more time in deciduous forests, consistent with the nutritional characteristics of these habitats. In addition, moose with access to recently burned areas had greater Zn concentrations in their hair, indicating a potential connection between post-fire environments and forage quality and availability. I also documented the concentrations of a suite of macro minerals, trace minerals, and heavy metals in the hair of female moose from central British Columbia, revealing notable variation between study populations and across years. This chapter demonstrates that hair mineral analysis can serve as a potential indicator of environmental variation and provides baseline data important for ongoing health monitoring in moose.

In Chapter 3, I measured concentrations of several immune biomarkers in the serum of female moose and explored how these markers related to physiological condition and parasite exposure. Moose with more fat reserves had greater concentrations of IL-12, suggesting that individuals in better body condition may be able to allocate more resources toward immune function. Total globulin levels were elevated in moose exposed to both micro- and macro-parasites, indicating immune activation in response to parasitic challenges. Additionally, I observed associations between zinc concentrations and both IL-12 and total globulin levels, whereas copper levels were linked to haptoglobin, highlighting the possible role of trace minerals in modulating immune responses. Altogether, these results underscore the intricate links between nutrition, parasitism, and immune function in wild ungulates, laying crucial groundwork for future monitoring of moose health under changing environmental conditions.

Collectively, my findings offer important insights into patterns of moose responses to environmental change. Mineral status and immune biomarkers were dynamic and reflected variation in environmental and physiological conditions, underscoring their value for monitoring wildlife health. This work provides a foundation for future research aimed at refining how health biomarkers can be integrated into long-term monitoring frameworks, particularly in systems experiencing rapid landscape change. For example, patterns linking mineral concentrations to habitat type, disturbance history, and climatic conditions highlight opportunities to investigate how habitat composition, such as the extent of deciduous stands or post-fire regeneration, affects forage quality and nutritional status. Similarly, the observed associations between trace minerals, body condition, and immune biomarkers point to promising avenues for exploring the nutritional underpinnings of disease susceptibility and resilience in wild ungulates. Building on these preliminary relationships, future studies could clarify the pathways connecting environmental pressures, nutrition, and immunity, and ultimately inform adaptive management and conservation strategies that support resilient moose populations.

Limitations and future directions

One limitation of my research is that I examined broad-scale relationships between habitat composition and trace mineral concentrations in moose hair using the proportion of GPS collar points within different habitat types. This approach provides valuable insights into habitat use patterns but does not directly reflect the specific plant species consumed by moose within those habitats. In addition, diet is not the only factor affecting mineral status; other environmental sources such as water intake and the use of natural mineral licks may also contribute to variation in mineral levels (Spears, 1994; Ayotte *et al.*, 2006). Consequently, my findings are limited to generalizations about habitat-level influences on mineral exposure rather than precise connections that could link diet composition and other environmental inputs with essential mineral uptake. Future research that integrates habitat use data with dietary analyses, mineral profiling of forage, and assessments of alternative mineral sources would help clarify the mechanisms driving the spatial patterns observed in this study and enhance our understanding of how fine-scale foraging choices and environmental factors shape mineral status in moose populations.

Further, my analysis is limited by a lack of understanding of the degree to which trace minerals in hair reflect levels found in the whole body. Although hair sampling is increasingly used in wildlife research due to its minimally invasive nature, the relationship between mineral concentrations in hair and those in storage organs remains poorly understood, and reference intervals have not been established, leading to ambiguity in how this metric should be interpreted. Studies validating hair mineral concentrations have yielded conflicting evidence regarding their correspondence with organ levels; for instance, Jutha *et al.* (2022) found that hair concentrations reflected Co, Mo, and Se in liver and/or kidney, but not Cu, Fe, Mn, or Zn. To date, no studies have assessed the correlation between hair and organ mineral levels specifically in moose. In this study, paired samples were not available because only minimally invasive, live-animal sampling was conducted during captures. However, this represents a critical knowledge gap and a valuable area for future research, as establishing these relationships in moose would improve the interpretation and application of hair mineral analysis for wildlife health monitoring. Such validations could be accomplished through targeted harvest-based sampling and coordinated surveillance efforts (Jutha *et al.*, 2022).

An additional limitation of my research lies in the interpretation that higher mineral concentrations in hair may be assumed to reflect better health or improved nutritional status in moose. However, elevated mineral levels do not necessarily indicate optimal physiological functioning, as both deficiencies and excesses of certain minerals can have adverse physiological consequences. Although many minerals are essential for key biological functions, excessive accumulation can lead to toxicity, metabolic disturbances, or impaired organ function. For example, while trace minerals such as Se are essential for immune function and antioxidant defense, chronically elevated levels can result in adverse effects including hair loss, hoof deformities, and reproductive impairments (Puls, 1988; Flueck *et al.*, 2012; Raisbeck, 2020). Moose, like other wildlife, must maintain mineral intake within a narrow optimal range, and the balance among different minerals can further complicate how they are absorbed and utilized in the body (Underwood, 2012). In this study, I was not able to determine whether the observed hair mineral concentrations fell within normal, deficient, or potentially harmful thresholds, underscoring the need for future research to establish reference ranges and to investigate how mineral levels relate to health indicators, fitness outcomes, and survival in moose populations.

Similarly, interpreting immune biomarkers also presents challenges because higher levels of immune activity do not necessarily indicate better health or effective parasite resistance. Immune responses are energetically costly and can reflect ongoing infection, inflammation, or physiological stress rather than successful parasite clearance or recovery. Elevated immune biomarkers may signal trade-offs where animals allocate resources toward immune function at the expense of other vital processes such as growth or reproduction (Stearns, 1989; Sheldon and Verhulst, 1996). Although some immune biomarkers in this study were associated with parasite exposure, whether these responses correspond to effective parasite management or simply immune activation remains unclear. Furthermore, my sample included only moose with repeated measures across multiple years, which limited my ability to directly link immune variation to survival outcomes. Without longitudinal data tracking immune biomarkers alongside fitness measures such as survival or reproductive success, I was unable to assess whether elevated immunity reflects beneficial or detrimental health states. This limitation highlights the need for future research that integrates immune profiling with long-term monitoring across multiple seasons and parasite infection stages to better understand the effects of immune function and parasite exposure on wild moose populations.

Finally, a major limitation of my research, and of the broader provincial moose research project, is that only adult female moose were captured and collared for long-term monitoring. As a result, males and calves were not represented in the data. Male moose and calves may exhibit different behaviours, such as variations in diet, habitat use, and movement patterns (Bowyer, 2004; Oehlers *et al.*, 2011), which could influence their exposure to environmental conditions and lead to distinct mineral or immune profiles that were not captured by this study. Additionally, within the group of adult females, detailed age classifications were not available, which limited the ability to examine age-related variation in mineral status and immune profiles (Nussey *et al.*, 2012; Draghi *et al.*, 2023). Expanding future research to include males, calves, and more precise age distinctions among females would provide a more comprehensive understanding of how different individuals within the moose population interact with their environment and how these differences may influence health and fitness outcomes, ultimately affecting overall moose population performance.

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APPENDIX A: Supplemental Information for Chapter 2

Table A.1. Full candidate models and model selection statistics used to explain potassium (K) concentrations in the hair (n = 60) of female moose (*Alces alces*) in two study areas in central British Columbia, Canada. All models included a random intercept for Individual ID to account for repeated measures. Study area and year were included as fixed effects in every model.

Model	**df**	**logLik**	**AIC$_c$**	**ΔAIC**	**AIC$_c$ w_i**
Study area + Year + Deciduous forest + Mixed forest	7	-368.80	756.42	0.00	0.59
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian	9	-367.76	760.01	3.59	0.10
Study area + Year + Temperature + Precipitation	7	-370.97	760.76	4.34	0.07
Study area + Year + Deciduous forest + Mixed forest + Temperature + Precipitation	9	-368.23	760.95	4.53	0.06
Study area + Year + Wetland + Riparian + Temperature + Precipitation	9	-368.30	761.09	4.67	0.06
Study area + Year + Forest stand age + Temperature + Precipitation	8	-370.29	762.18	5.76	0.03
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Forest stand age	10	-367.63	762.76	6.34	0.02
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire	10	-367.76	763.02	6.60	0.02
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Temperature + Precipitation	11	-366.24	763.13	6.71	0.02
Study area + Year + Wildfire + Temperature + Precipitation	8	-370.95	763.50	7.08	0.02
Study area + Year + Wildfire + Forest stand age + Temperature + Precipitation	9	-370.28	765.06	8.64	0.01
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age	11	-367.62	765.89	9.47	0.01
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age + Temperature + Precipitation	13	-365.64	768.61	12.19	0.00
Study area + Year (null)	5	-379.97	773.51	17.09	0.00
Study area + Year + Forest stand age	6	-379.89	775.94	19.52	0.00
Study area + Year + Wildfire	6	-379.94	776.03	19.61	0.00
Study area + Year + Wetland + Riparian	7	-379.61	778.05	21.63	0.00
Study area + Year + Wildfire + Forest stand age	7	-379.87	778.56	22.14	0.00

Table A.2. Full candidate models and model selection statistics used to explain magnesium (Mg) concentrations in the hair (n = 60) of female moose (*Alces alces*) in two study areas in central British Columbia, Canada. All models included a random intercept for Individual ID to account for repeated measures. Study area and year were included as fixed effects in every model.

Model	**df**	**logLik**	**AIC$_c$**	**ΔAIC**	**AIC$_c$ w_i**
Study area + Year + Deciduous forest + Mixed forest	8	-284.27	587.36	0.00	0.44
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian	10	-282.52	589.53	2.17	0.15
Study area + Year + Temperature + Precipitation	8	-285.99	590.81	3.46	0.08
Study area + Year + Deciduous forest + Mixed forest + Temperature + Precipitation	10	-283.30	591.08	3.73	0.07
Study area + Year (null)	6	-289.26	592.11	4.76	0.04
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire	11	-282.44	592.37	5.02	0.04
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Forest stand age	11	-282.51	592.52	5.17	0.03
Study area + Year + Wetland + Riparian	8	-286.86	592.54	5.19	0.03
Study area + Year + Forest stand age + Temperature + Precipitation	9	-285.82	593.24	5.89	0.02
Study area + Year + Wildfire + Temperature + Precipitation	9	-285.99	593.59	6.23	0.02
Study area + Year + Wetland + Riparian + Temperature + Precipitation	10	-284.66	593.81	6.45	0.02
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Temperature + Precipitation	12	-281.63	593.91	6.55	0.02
Study area + Year + Wildfire	7	-289.24	594.63	7.27	0.01
Study area + Year + Forest stand age	7	-289.25	594.66	7.31	0.01
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age	12	-282.43	595.50	8.15	0.01
Study area + Year + Wildfire + Forest stand age + Temperature + Precipitation	10	-285.82	596.13	8.77	0.01
Study area + Year + Wildfire + Forest stand age	8	-289.22	597.27	9.92	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age + Temperature + Precipitation	14	-281.63	600.59	13.24	0.00

Table A.3. Full candidate models and model selection statistics used to explain copper (Cu) concentrations in the hair (n = 60) of female moose (*Alces alces*) in two study areas in central British Columbia, Canada. All models included a random intercept for Individual ID to account for repeated measures. Study area and year were included as fixed effects in every model.

Model	**df**	**logLik**	**AIC$_c$**	**ΔAIC**	**AIC$_c$ w_i**
Study area + Year (null)	6	-75.44	164.46	0.00	0.29
Study area + Year + Temperature + Precipitation	8	-73.39	165.60	1.14	0.16
Study area + Year + Wildfire	7	-74.94	166.03	1.58	0.13
Study area + Year + Deciduous forest + Mixed forest	8	-73.86	166.55	2.09	0.10
Study area + Year + Forest stand age	7	-75.41	166.98	2.52	0.08
Study area + Year + Wetland + Riparian	8	-74.73	168.28	3.82	0.04
Study area + Year + Forest stand age + Temperature + Precipitation	9	-73.38	168.36	3.90	0.04
Study area + Year + Wildfire + Temperature + Precipitation	9	-73.39	168.37	3.91	0.04
Study area + Year + Deciduous forest + Mixed forest + Temperature + Precipitation	10	-72.04	168.57	4.11	0.04
Study area + Year + Wildfire + Forest stand age	8	-74.93	168.69	4.23	0.03
Study area + Year + Wetland + Riparian + Temperature + Precipitation	10	-72.92	170.32	5.87	0.02
Study area + Year + Wildfire + Forest stand age + Temperature + Precipitation	10	-73.38	171.25	6.79	0.01
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian	10	-73.61	171.71	7.26	0.01
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire	11	-73.25	174.00	9.54	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Forest stand age	11	-73.26	174.02	9.56	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Temperature + Precipitation	12	-71.77	174.19	9.73	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age	12	-72.98	176.59	12.14	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age + Temperature + Precipitation	14	-71.66	180.66	16.20	0.00

Table A.4. Full candidate models and model selection statistics used to explain iron (Fe) concentrations in the hair (n = 59) of female moose (*Alces alces*) in two study areas in central British Columbia, Canada. All models included a random intercept for Individual ID to account for repeated measures. Study area and year were included as fixed effects in every model.

Model	**df**	**logLik**	**AIC$_c$**	**ΔAIC**	**AIC$_c$ w_i**
Study area + Year (null)	6	-230.49	474.60	0.00	0.39
Study area + Year + Wildfire	7	-230.23	476.65	2.04	0.14
Study area + Year + Forest stand age	7	-230.31	476.82	2.21	0.13
Study area + Year + Deciduous forest + Mixed forest	8	-229.32	477.53	2.93	0.09
Study area + Year + Temperature + Precipitation	8	-229.73	478.34	3.74	0.06
Study area + Year + Wildfire + Forest stand age	8	-230.03	478.94	4.34	0.04
Study area + Year + Wetland + Riparian	8	-230.11	479.09	4.49	0.04
Study area + Year + Wildfire + Temperature + Precipitation	9	-228.98	479.64	5.04	0.03
Study area + Year + Deciduous forest + Mixed forest + Temperature + Precipitation	10	-228.22	481.02	6.42	0.02
Study area + Year + Forest stand age + Temperature + Precipitation	9	-229.68	481.04	6.44	0.02
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian	10	-228.58	481.74	7.14	0.01
Study area + Year + Wildfire + Forest stand age + Temperature + Precipitation	10	-228.95	482.48	7.87	0.01
Study area + Year + Wetland + Riparian + Temperature + Precipitation	10	-229.01	482.61	8.01	0.01
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire	11	-228.30	484.22	9.61	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Forest stand age	11	-228.51	484.63	10.03	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Temperature + Precipitation	12	-227.65	486.08	11.47	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand	12	-228.22	487.23	12.62	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age + Temperature + Precipitation	14	-226.86	491.27	16.67	0.00

Table A.5. Full candidate models and model selection statistics used to explain manganese (Mn) concentrations in the hair ($n = 60$) of female moose (*Alces alces*) in two study areas in central British Columbia, Canada. All models included a random intercept for Individual ID to account for repeated measures. Study area and year were included as fixed effects in every model.

Model	**df**	**logLik**	**AIC$_c$**	**ΔAIC**	**AIC$_c$ w_i**
Study area + Year (null)	6	-150.82	315.23	0.00	0.30
Study area + Year + Temperature + Precipitation	8	-148.95	316.72	1.49	0.14
Study area + Year + Wetland + Riparian	8	-149.10	317.03	1.80	0.12
Study area + Year + Wildfire	7	-150.72	317.60	2.37	0.09
Study area + Year + Forest stand age	7	-150.82	317.78	2.56	0.08
Study area + Year + Deciduous forest + Mixed forest	8	-149.67	318.16	2.93	0.07
Study area + Year + Wildfire + Temperature + Precipitation	9	-148.80	319.20	3.97	0.04
Study area + Year + Forest stand age + Temperature + Precipitation	9	-148.95	319.49	4.26	0.04
Study area + Year + Wetland + Riparian + Temperature + Precipitation	10	-147.61	319.71	4.48	0.03
Study area + Year + Wildfire + Forest stand age	8	-150.72	320.26	5.03	0.02
Study area + Year + Deciduous forest + Mixed forest + Temperature + Precipitation	10	-148.41	321.31	6.08	0.01
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian	10	-148.62	321.73	6.50	0.01
Study area + Year + Wildfire + Forest stand age + Temperature + Precipitation	10	-148.80	322.08	6.85	0.01
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire	11	-148.46	324.43	9.20	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Forest stand age	11	-148.59	324.69	9.46	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Temperature + Precipitation	12	-147.55	325.74	10.51	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age	12	-148.45	327.53	12.30	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age + Temperature + Precipitation	14	-147.50	332.34	17.11	0.00

Table A.6. Full candidate models and model selection statistics used to explain molybdenum (Mo) concentrations in the hair ($n = 60$) of female moose (*Alces alces*) in two study areas in central British Columbia, Canada. All models included a random intercept for Individual ID to account for repeated measures. Study area and year were included as fixed effects in every model.

Model	**df**	**logLik**	**AIC$_c$**	**ΔAIC**	**AIC$_c$ w_i**
Study area + Year (null)	6	122.60	-231.61	0.00	0.32
Study area + Year + Forest stand age	7	123.49	-230.83	0.78	0.22
Study area + Year + Wildfire	7	122.62	-229.08	2.53	0.09
Study area + Year + Temperature + Precipitation	8	123.80	-228.78	2.82	0.08
Study area + Year + Wetland + Riparian	8	123.58	-228.34	3.27	0.06
Study area + Year + Deciduous forest + Mixed forest	8	123.55	-228.28	3.33	0.06
Study area + Year + Wildfire + Forest stand age	8	123.54	-228.25	3.36	0.06
Study area + Year + Forest stand age + Temperature + Precipitation	9	124.22	-226.83	4.77	0.03
Study area + Year + Wildfire + Temperature + Precipitation	9	123.98	-226.36	5.24	0.02
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian	10	124.77	-225.05	6.56	0.01
Study area + Year + Wetland + Riparian + Temperature + Precipitation	10	124.51	-224.52	7.08	0.01
Study area + Year + Wildfire + Forest stand age + Temperature + Precipitation	10	124.42	-224.36	7.25	0.01
Study area + Year + Deciduous forest + Mixed forest + Temperature + Precipitation	10	124.08	-223.67	7.94	0.01
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Forest stand age	11	125.37	-223.25	8.36	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire	11	124.82	-222.14	9.47	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age	12	125.46	-220.28	11.32	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Temperature + Precipitation	12	125.16	-219.68	11.92	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age + Temperature + Precipitation	14	126.01	-214.68	16.93	0.00

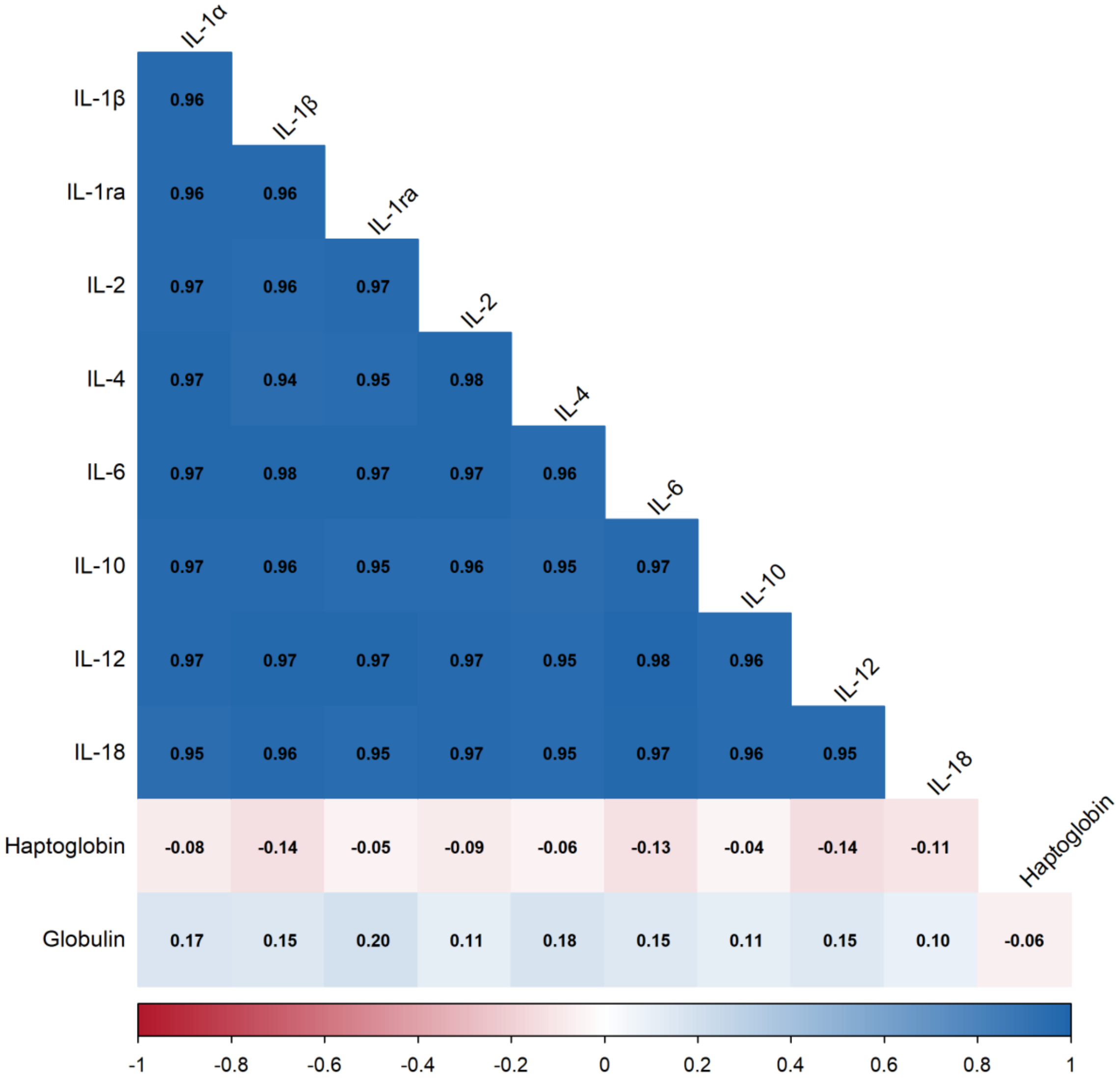
Table A.7. Full candidate models and model selection statistics used to explain selenium (Se) concentrations in the hair (n = 60) of female moose (*Alces alces*) in two study areas in central British Columbia, Canada. All models included a random intercept for Individual ID to account for repeated measures. Study area and year were included as fixed effects in every model.

Model	**df**	**logLik**	**AIC$_c$**	**ΔAIC**	**AIC$_c$ w_i**
Study area + Year + Temperature + Precipitation	8	78.44	-138.05	0.00	0.51
Study area + Year + Wildfire + Temperature + Precipitation	9	78.47	-135.34	2.71	0.13
Study area + Year + Forest stand age + Temperature + Precipitation	9	78.46	-135.31	2.74	0.13
Study area + Year + Deciduous forest + Mixed forest + Temperature + Precipitation	10	79.65	-134.81	3.24	0.10
Study area + Year + Wetland + Riparian + Temperature + Precipitation	10	79.45	-134.41	3.64	0.08
Study area + Year + Wildfire + Forest stand age + Temperature + Precipitation	10	78.48	-132.48	5.57	0.03
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Temperature + Precipitation	12	80.86	-131.09	6.96	0.02
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age + Temperature + Precipitation	14	80.91	-124.48	13.57	0.00
Study area + Year + Deciduous forest + Mixed forest	8	71.47	-124.11	13.94	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian	10	73.34	-122.20	15.85	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire	11	74.05	-120.61	17.45	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Forest stand age	11	73.40	-119.30	18.75	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age	12	74.06	-117.49	20.56	0.00
Study area + Year (null)	6	61.88	-110.18	27.87	0.00
Study area + Year + Forest stand age	7	62.64	-109.12	28.93	0.00
Study area + Year + Wetland + Riparian	8	63.61	-108.39	29.66	0.00
Study area + Year + Wildfire	7	61.96	-107.77	30.28	0.00
Study area + Year + Wildfire + Forest stand age	8	62.74	-106.66	31.39	0.00

Table A.8. Full candidate models and model selection statistics used to explain zinc (Zn) concentrations in the hair (*n* = 60) of female moose (*Alces alces*) in two study areas in central British Columbia, Canada. All models included a random intercept for Individual ID to account for repeated measures. Study area and year were included as fixed effects in every model.

Model	**df**	**logLik**	**AIC$_c$**	**ΔAIC**	**AIC$_c$ w_i**
Study area + Year + Wildfire + Temperature + Precipitation	9	-162.98	347.56	0.00	0.37
Study area + Year + Temperature + Precipitation	8	-164.53	347.89	0.33	0.31
Study area + Year + Wildfire + Forest stand age + Temperature + Precipitation	10	-162.98	350.44	2.89	0.09
Study area + Year + Forest stand age + Temperature + Precipitation	9	-164.53	350.66	3.10	0.08
Study area + Year + Deciduous forest + Mixed forest + Temperature + Precipitation	10	-163.72	351.92	4.37	0.04
Study area + Year (null)	6	-169.23	352.04	4.48	0.04
Study area + Year + Wetland + Riparian + Temperature + Precipitation	10	-164.19	352.87	5.31	0.03
Study area + Year + Wildfire	7	-169.09	354.34	6.78	0.01
Study area + Year + Forest stand age	7	-169.21	354.57	7.02	0.01
Study area + Year + Wetland + Riparian	8	-168.36	355.55	7.99	0.01
Study area + Year + Deciduous forest + Mixed forest	8	-168.72	356.27	8.71	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Temperature + Precipitation	12	-163.14	356.91	9.35	0.00
Study area + Year + Wildfire + Forest stand age	8	-169.08	356.98	9.43	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age + Temperature + Precipitation	14	-161.56	360.45	12.89	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian	10	-168.00	360.50	12.94	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire	11	-167.86	363.23	15.67	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Forest stand age	11	-168.00	363.50	15.94	0.00
Study area + Year + Deciduous forest + Mixed forest + Wetland + Riparian + Wildfire + Forest stand age	12	-167.86	366.36	18.80	0.00

APPENDIX B: Supplemental Information for Chapter 3



	IL-1α	IL-1β	IL-1ra	IL-2	IL-4	IL-6	IL-10	IL-12	IL-18	Haptoglobin
IL-1β	0.96									
IL-1ra	0.96	0.96								
IL-2	0.97	0.96	0.97							
IL-4	0.97	0.94	0.95	0.98						
IL-6	0.97	0.98	0.97	0.97	0.96					
IL-10	0.97	0.96	0.95	0.96	0.95	0.97				
IL-12	0.97	0.97	0.97	0.97	0.95	0.98	0.96			
IL-18	0.95	0.96	0.95	0.97	0.95	0.97	0.96	0.95		
Haptoglobin	-0.08	-0.14	-0.05	-0.09	-0.06	-0.13	-0.04	-0.14	-0.11	
Globulin	0.17	0.15	0.20	0.11	0.18	0.15	0.11	0.15	0.10	-0.06

Figure B.1. Spearman correlation matrix showing paired correlation coefficients between immune biomarkers concentrations measured in serum samples (*n* = 60) from adult female moose sampled in winter (2020–2022) from two populations in central British Columbia, Canada: the Bonaparte Plateau (BP; *n* = 31) and Prince George South (PGS; *n* = 29). Each coloured box represents the strength and direction of the paired correlation, with colours ranging from red (negative correlation) to blue (positive correlation). The correlation coefficient ranges from -1 (perfect negative correlation) to 1 (perfect positive correlation), with 0 indicating no correlation. Immune biomarkers GM-CSF, IFN-γ, IL-8, and TNF-α were excluded from this analysis due to having less than 80% detectable samples.