

UNIVERSITY OF CALGARY

Hair Biomarkers to Support Barren-ground Caribou Health Monitoring and Management

by

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A THESIS

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Abstract

Barren-ground caribou (*Rangifer tarandus groenlandicus*) are a keystone species of Canada, whose population health is a current and future management priority. Many of these historically numerous populations, including the Bluenose-East (BNE) and Dolphin and Union (DU) herds, have severely declined in the last two decades, thus there is an impetus to understand the health status of these populations. Considering the challenges associated with monitoring Arctic wildlife, hair is a practically advantageous sample type that is currently opportunistically collected. I evaluated two biomarkers derived from caribou hair (trace element and cortisol concentrations) in the context of opportunistic monitoring and review the literature to understand how to best orient *Rangifer* health research into management and conservation. First, I reviewed the most abundant health literature on caribou, the *Rangifer* infectious disease literature, and documented numerous barriers to health information dissemination and implementation. I then outlined practical solutions to facilitate solutions-oriented *Rangifer* health research. Second, I examined two biomarkers pertinent to caribou health, hair trace element and hair cortisol concentrations that provide seasonal measures of nutrition and contribute to allostatic load, respectively. I demonstrated that these biomarkers vary between anatomic sampling locations and provided recommendations for future hair collection protocols. Furthermore, I uncovered associations of these biomarkers with sex, season, year, and sampling source that have implications for future monitoring and biomarker interpretation. This work has advanced our understanding of two biomarkers derived from caribou hair, outlined future research avenues to improve the robustness of these monitoring tools, and demonstrated broadly how to better translate caribou health research into management and conservation frameworks.

Preface

This thesis consists of manuscripts that have been either published in a peer-reviewed journal or are in preparation for submission to a journal. Filip Rakic conceptualized and designed the studies, collected the data, analyzed the data, and wrote the manuscripts with guidance from his immediate academic supervisor, thesis committee, and co-authors. Written permission has been obtained from publishers and all co-authors for the reproduction of manuscripts in this thesis.

Published manuscripts

Chapter 2 – Rakic F, Pruvot M, Whiteside DP, and Kutz J. 2022. A Scoping Review of the Rangifer Infectious Disease Literature: Gap Between Information and Application. *Journal of Wildlife Diseases*. In press. DOI: 10.7589/JWD-D-21-00165.

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Dedication

*To Violeta and Zdravko Rakić,
without all the sacrifices you've made,
this would have never been possible.*

-Love Pačić

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List of Abbreviations and Nomenclature

| Abbreviation | Definition |
|---------------------|--|
| ACTH | Adrenocorticotrophic hormone |
| AICc | Akaike's Information Criterion for small sample sizes |
| AMAP | Arctic Monitoring and Assessment Program |
| BNE | Bluenose-East caribou herd |
| Capt. | Captured animal |
| CARMA | CircumArctic <i>Rangifer</i> Monitoring and Assessment |
| Cd | Cadmium |
| CI | Confidence Interval |
| CoCoPop | Condition, Content, and Population search strategy |
| COSEWIC | Committee on the Status of Endangered Wildlife in Canada |
| Cu | Copper |
| DF | Degrees of Freedom |
| ECC | Environment and Climate Change Canada |
| EIA | Enzyme immunoassay |
| ELISA | Enzyme-linked immunoassay |
| F-Test | Hypothesis testing using the F distribution |
| GC | Glucocorticoid |
| GLS | Generalized least squares regression |
| GNWT | Government of the Northwest Territories |
| HCC | Hair cortisol concentrations |
| HEC | Hair element concentrations |
| HNO ₃ | Nitric acid |
| HPA | Hypothalamic-pituitary-adrenal |
| Hunt. | Hunted animal |
| kg | Kilogram |
| ICP-MS | Inductively coupled plasma mass spectrometry |
| L | Litre |
| lme | Linear mixed effect model |
| Log | Logarithmic transformation |
| LOQ | Limit of quantification |
| Max | Maximum value |
| Med | Median value |
| Mg | Mercury |
| mg | Milligram |
| min | Minimum value |
| MI | Mosquito index |
| Mo | Molybdenum |
| n | Sample size |
| NRC | National Research Council of Canada |
| NU | Nunavut |
| OA | Open Access |
| OI | Oestrid index |
| p-value | Null hypothesis significance testing (alpha =0.05) |
| Pb | Lead |

| | |
|--------------------|--|
| pg | picogram |
| PRISMA | Preferred Reporting Items for Systematic Reviews and Meta Analyses |
| r | Pearson's correlation coefficient |
| r_s | Spearman's rank correlation coefficient |
| R^2 | Coefficient of determination |
| R^2_c | Conditional coefficient of determination |
| ROC | Receiver operating characteristic |
| Se | Selenium |
| TK | Traditional knowledge |
| Zn | Zinc |
| 10X | 10 times |
| $^{\circ}\text{C}$ | Degrees Celsius |
| μl | Microliter |
| % | Percent |
| < | Less than |
| > | Greater than |
| = | Equal to |

CHAPTER 1: INTRODUCTION

1.1. BACKGROUND

1.1.1. Wildlife disease

The promotion and maintenance of healthy wildlife populations is recognized as a collective societal goal in Canada (Hanisch et al., 2012; Stephen 2019). Healthy wildlife populations directly relate to topics of societal concern such as the conservation of biodiversity, maintenance and safety of food production systems, and protection of public health (Daszak et al., 2000; Deem et al., 2001; OIE, 2010). Wildlife management considers the manipulation of three dimensions: wildlife populations, their habitats, and interactions with people, to achieve stakeholder values (Decker et al., 2012). For management, wildlife health has been primarily considered in the context of disease-causing agents (Hanisch et al., 2012) or contaminants (Stephen, 2014), since wildlife health was conceptualized in relation to species of economic importance and/or human food safety (Deem et al., 2001). Although these viewpoints are a restricted definition of health, most of the wildlife health literature speaks to the control of infectious diseases (Stephen et al., 2018). This applies to *Rangifer* health research, in which the bulk of health-related information pertains to disease or disease-causing agents (Wittrock et al., 2019).

Caribou (*Rangifer tarandus*) are considered a keystone species in the Arctic, that under an ecosystem services framework, provide numerous ecological, economic, cultural, and spiritual benefits to peoples of the North (Lyver, 2005; McGregor et al., 2010; Festa-Bianchet et al., 2011; Côté et al., 2012). The wealth of information on *Rangifer* infectious diseases has yet to be condensed or summarized in a formal review, which is a potential barrier to future effective *Rangifer* research, management, and conservation. Further, the completion of a formal review is a pragmatic first step in the initiation of a graduate research project (Pickering & Bryce, 2013). Among the modes of knowledge synthesis available, scoping reviews are the most appropriate in bodies of research in which past synthesis work is lacking (Young et al., 2014; Munn et al., 2018), such as the field of *Rangifer* infectious disease. Lastly, understanding how to integrate disease information into management through a review is also pertinent to other health indicators of interest, such as nutrition and stress.

1.1.2. Wildlife health monitoring

Contemporary wildlife management is centered on adaptive management strategies, that utilize interdisciplinary and local inputs alongside biological science expertise (Riley, 2002; 2003). Although wildlife disease is an important aspect of conservation, a disease-centric approach is limited in its use and applicability for decision-making (Hanisch et al., 2012). Alternatively, we may define wildlife health as multiple determinants of health that acknowledge the role of the biotic, abiotic, and social environments, and moves beyond the sole historical goal of disease prevention (Wittrock et al., 2019). Indicators are identifiable factors that relate to these broad determinants of health, such as allostatic load, that may be used to inform upon management or conservation of a species (Peacock et al., 2020). Allostatic load may be defined as the cumulative physiological compensation of an organism because of a disturbance. A metric of health would be a measure of these indicators: in the case of allostatic load, we may apply cortisol concentration as a metric or biomarker that contributes to overall allostatic load. These biomarkers of health are commonly quantified via the continued collection of epidemiological data from wildlife populations and a large source of wildlife epidemiological data is derived from monitoring programs (Mörner et al., 2002).

Consistent monitoring and collection of epidemiological data from wildlife populations is a first step in understanding current and future population health status (Mörner et al., 2002). Furthermore, the first pillar identified in the “Pan-Canadian Approach to Wildlife Health” is consistent health monitoring of wildlife (Stephen, 2019). Conceptually, health monitoring may be understood as the continuous collection of epidemiological data to understand temporal trends (Artois et al., 2009), which can be used to inform management and conservation. Wildlife health sampling for monitoring, however, is logistically and technically challenging, especially for wildlife populations that inhabit remote environments, are of conservation concern, or are cryptic (Ryser-Degiorgis, 2013). To overcome these barriers, partnerships between local communities, government officials, and researchers have developed participatory epidemiological programs that improve the consistency and breadth of wildlife health monitoring (Sirpa et al., 2017). In Arctic Canada, these programs often center on partnerships with local indigenous hunters who regularly participate in subsistence harvesting of wildlife (Tomaselli et al., 2019). These

partnerships provide a dual benefit, providing consistent biological samples to monitoring programs while simultaneously supporting Indigenous ways of life.

1.1.3. Caribou health monitoring

A holistic *Rangifer* health framework has been previously outlined by Macbeth & Kutz (2018), that describes a mix of intrinsic (genetics, behaviour, life stage) and extrinsic (climate, disease, nutrition, habitat etc.) health indicators that contribute to individual and population health, an adapted version of this framework is illustrated in Figure.1.1.

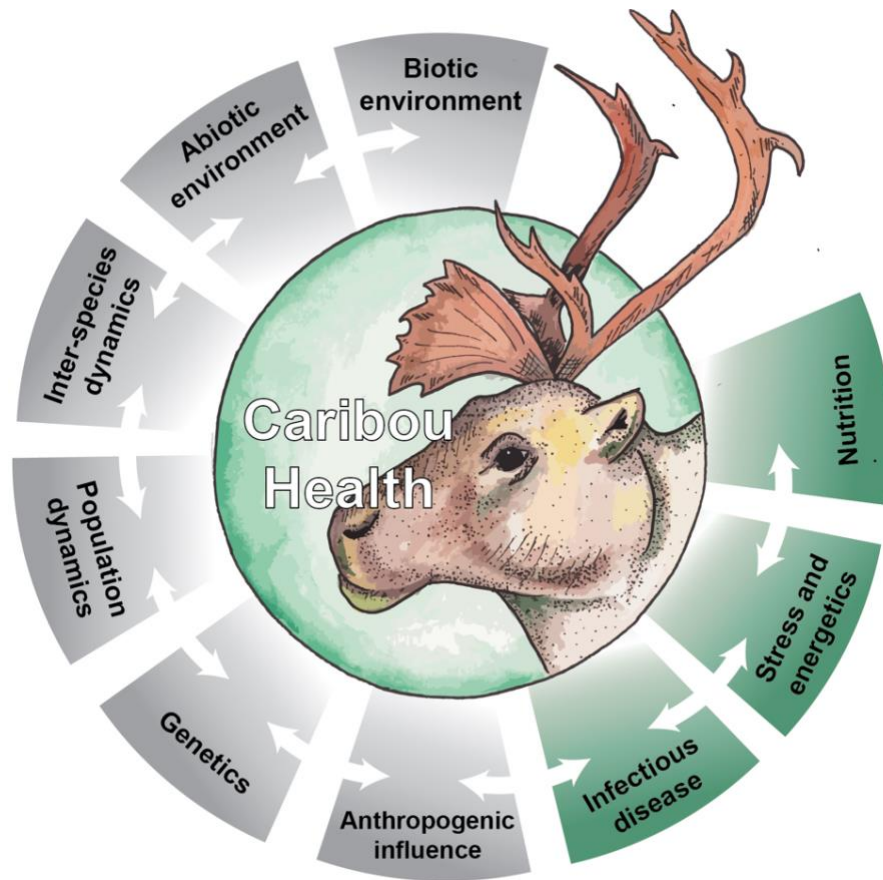


Fig.1.1. Illustration of the conceptual model of key health indicators of caribou. Green indicators are investigated in Chapters 2, 3, and 4. Grey indicators are important indicators of health not covered in this thesis. Conceptual diagram adapted from Wittrock et al. (2019), and Tryland and Kutz (2018).

Biological samples play a large role in understanding a variety of these health indicators, and the Circumarctic Rangifer Monitoring & Assessment Network (CARMA, 2008), has developed protocols for the sampling of caribou (Brook et al., 2011). Caribou biological samples are derived from two primary sources that utilize opportunistic sampling. The first, is samples

derived from partnerships with local communities who regularly harvest caribou, hunters complete a standardized set of samples in a 'hunter kit' that is then submitted for reimbursement (Tomaselli et al., 2019). The second, is samples taken by government biologists during capture or collaring activities, whereby additional samples are taken from the animal opportunistically during handling.

Among the array of biological samples collected through these sampling sources, hair samples present a plethora of advantages. Hair is an extremely stable medium compared to other biological samples such as blood, feces, soft tissues, or urine, that can be easily transported and stored at room temperature (Felicetti et al., 2003; Jaspers et al., 2010). Hair also may be collected passively and non-invasively using hair snares, which is especially useful in sampling elusive or cryptic mammals (Beir et al., 2005; Henry et al., 2011; Ellis et al., 2012). Hair is already commonly collected as a part of ongoing wildlife monitoring programs in Canada (CARMA 2008; Macbeth et al., 2010). Furthermore, archived hair samples that are years to decades to centuries-old, may still be used to successfully quantify certain biomarkers, such as contaminants and select steroid hormone concentrations (Kintz, 2004; Webb et al., 2010). Due to these advantages and uses, hair is a strong candidate for wildlife health monitoring programs, especially those involving local community partnerships for sample collection.

1.1.4. Overview of hair biology

Hair may be defined as a keratinized fibril, that is produced by follicles embedded just below the epidermis, while the hair shaft lies above the skin, illustrated in Figure.1.2. A single hair is associated with a pili erector muscle, sebaceous glands, as well as sweat glands (Pragst & Balikova, 2006). The hair shaft is predominantly composed of proteins (65-91 %) and contains trace amounts of elements and hormones (0.25%-0.91%) (Harkey, 1993), that may be used to measure health biomarkers pertinent to wildlife.

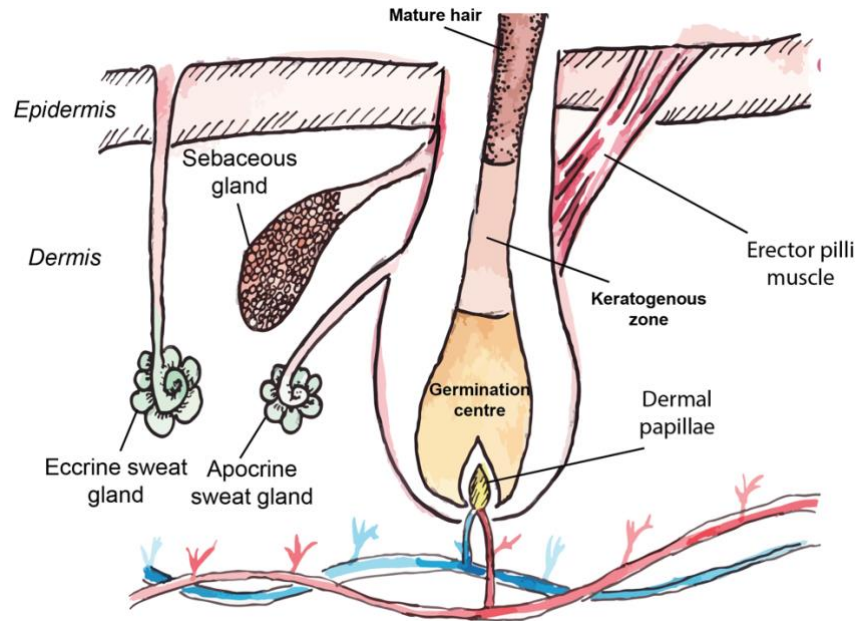


Figure 1.2. Illustration outlining anagen phase hair anatomy. Structures included are dermal layers (epidermis, dermis), associated glands (sebaceous, eccrine sweat, apocrine sweat), associated muscles (erector pilli), and hair sections (germination section, keratogenous zone, and the mature follicle).

The hair shaft/fibre originates from a hair follicle that is connected to a capillary system and is metabolically active during hair growth (Pragst & Balikova, 2006). Hair grows in a three-stage cycle, an active growth anagen phase, a transitional catagen phase, and a resting telogen phase (Harkey, 1993), illustrated in Figure.1.3. During growth (anagen phase), the hair follicle is surrounded by matrix cells that form a germination centre, this is the site of rapid mitosis and new hair synthesis (Stenn & Paus, 2001). The germination centre is composed of matrix cells (keratinocytes and melanocytes) that subsequently differentiate into the three layers of the hair shaft (Pragst & Balikova 2006). During maturation, cells die and form protein fibrils, and once they mature, the hair shaft is a metabolically inactive tissue and disassociated from the dermal papilla (telogen phase). Lastly, during shedding or molt, hair enters a final exogen phase, in which the hair fibre dissociates from dermal tissue (Higgins et al., 2002). Hair follows a cyclical pattern of active growth and senescence that is species-specific.

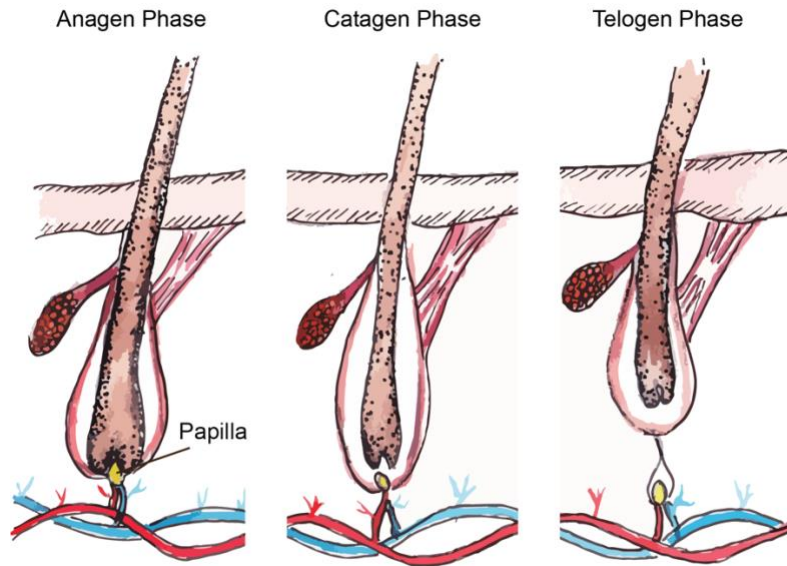


Figure 1.3. Illustration outlining hair growth cycle. Three main phases are outlined, 1) anagen phase (active growth phase), 2) catagen phase (intermediate phase), and 3) telogen phase (shedding phase).

The precise molecular mechanism in which health biomarkers of interest, such as hormones, drugs, contaminants, and trace minerals are incorporated into the hair shaft remains unclear (Blank & Kidwell 1995; Pragst & Balikova 2006; Kidwell & Smith 2007). Substances are believed to diffuse into the hair shaft passively from the blood supply during active growth, however, deposits from associated glandular systems also contribute to hair shaft composition (Combs, 1972; Henderson, 1993; Di Francesco et al., 2020). Hair may be used to quantify numerous health metrics of interest such as contaminant exposure, trace element concentration, isotope diet analysis, genetic individual identification, cryptic species detection, capture-mark-recapture estimates, and cortisol concentration, all relevant to wildlife health monitoring.

1.1.5. Overview of hair biomarkers

Hair as a biomonitoring tool in wildlife has been used to monitor contaminants, trace element status, diet via isotopes, cortisol as one measure of allostatic load, and lastly determination of genetic makeup. These various applications are summarized in Table 1.1.

Table 1.1. Overview of hair biomarkers used in wildlife monitoring.

| Biomarkers | Overview |
|----------------------------|---|
| <i>Hair contaminants</i> | Toxic contaminant exposure and bioaccumulation may alter the reproductive success, growth, and lifespan of wildlife (Brunström et al., 1991; Leonard et al., 1995; Wolfe et al., 1998; Letcher et al., 2010; Vermeulen et al., 2015). Heavy metal contaminants, such as lead (Pb), cadmium (Cd), and mercury (Hg), have been successfully quantified in hair of free ranging wildlife and demonstrate promising associations with organ standards (O’Hara et al., 2001; Roug et al., 2015; Dainowski et al., 2015). Among domestic animal systems, hair has been used as a successful marker of dietary intake (Gorbani et al., 2015) and environmental exposure to heavy metals (Patra et al., 2006; McKinney et al., 2017). Organochlorine persistent pollutants, such as Dichlorodiphenyltrichloroethane, polychlorinated biphenyls and chlordanes have been investigated in wildlife hair (Kim et al., 1996), and hair analyses have been similarly positively associated with tissue concentrations in small mammals (D’Havé et al., 2006). Many of these contaminants have been successfully measured in wildlife or domestic animal hair, however, reference ranges of hair for toxic exposure for heavy metals and POPs have yet to be developed for many species, including <i>Rangifer</i> . |
| <i>Hair Trace Elements</i> | Trace minerals are elements that are required in extremely low concentrations to maintain bodily homeostasis and physiological function (Kincaid 2000; Flueck et al., 2012; Weise and Carver, 2017). In livestock systems, experimental dietary trials demonstrated that hair concentrations of select trace minerals were positively associated dietary supplementation (Anke 1966; Cunningham et al., 1989; Gorabni et al., 2015). In wild ungulates, hair demonstrates good correlations with organ standards to monitor Se (selenium) and Mo (Molybdenum), making a hair a promising tool for wildlife monitoring of those elements (O’Hara, 2001; Roug et al., 2015; Jutha et al., In Press). Similarly, to contaminants, reference ranges for trace minerals in <i>Rangifer</i> hair have yet to be developed. |
| <i>Hair Isotopes</i> | Foraging preferences and diet analysis may be undertaken through stable isotope measurements in wild animal tissues, including hair (Kelly, 2000). Plants exhibit distinct carbon and nitrogen stable isotope ratios, creating unique signatures that may be identified when incorporated into tissues (Dawson et al., 2002). Experimentally, hair isotope analysis has been validated in domestic animals that were fed an isotopically varied diet (Ayliffe et al., 2004; Zazzo et al., 2007). In wildlife, hair isotopes are predominantly used to investigate the diet ecology of herbivores, with a special interest in dietary niche breadth among sympatric species (Stewart et al., 2003; Drucker et al., 2010). Hair isotope studies commonly employ low impact sampling, such as hair snares/traps to infer diet in difficult to access species (Jones et al., 2006; Hopkins et al., 2012). Lastly, hair isotope analysis can also be done segmentally and sequentially, to inform upon changes in diet during the hair growth period at relatively high temporal resolution (Mosbacher et al., 2016; Burnik Šturm, 2017; Rogers et al., 2020). Therefore, hair for the purpose of isotope analysis may be collected passively and opportunistically and is readily available to inform upon diet of and between species. |
| <i>Hair Cortisol</i> | Changes in cumulative stressors and resulting allostatic load, are associated with numerous individual and population level impacts in free-ranging wildlife (Bonier et al., 2009; Busch and Hayward 2009). Allostatic load may be monitored through biomarkers of HPA axis activity (Baker et al., 2013; Ewacha et al., 2017). Hair cortisol concentration (HCC) is a cumulative indicator of HPA axis activity, that reflects circulating glucocorticoids from weeks to months, depending on the length of the hair growth period (Gormally & Romero, 2020). Experimentally, HPA axis stimulation through repeated ACTH injection was reflected in heightened quantified HCC in muskoxen (<i>Ovibos moschatus</i>) compared to controls (Di Francesco et al., 2021). Limitations of experimental |

Genetic sampling

methodology used (Ashley et al., 2011) has resulted in no robust validation of HCC in *Rangifer*.

Hair and feces are both commonly used for non-invasive genetic sampling of wildlife (Waits et al., 2005). Monitoring abundance of carnivores or other difficult to detect species can be challenging, and many methods, such as track counting, camera traps etc, do not allow for consistent identification of individuals, making genetic tagging valuable (Mowat et al., 2002; Waits et al., 2005). Moreover, among extremely elusive carnivores, such as ocelots, (*Leopardis paradalis*), hair genetic tagging has been used to monitor at the individual level (Weaver et al., 2005). Beyond individual identification and detection, hair genetic extraction may be further applied to mark-capture-recapture studies for population size estimation, as well as range distributions (Mowat et al., 2000; Garth et al., 2002). Ultimately, non-invasive hair sampling is a valuable tool in individual identification, population size estimation, and range distribution analysis of wild mammals, namely carnivores.

1.1.6. Caribou hair

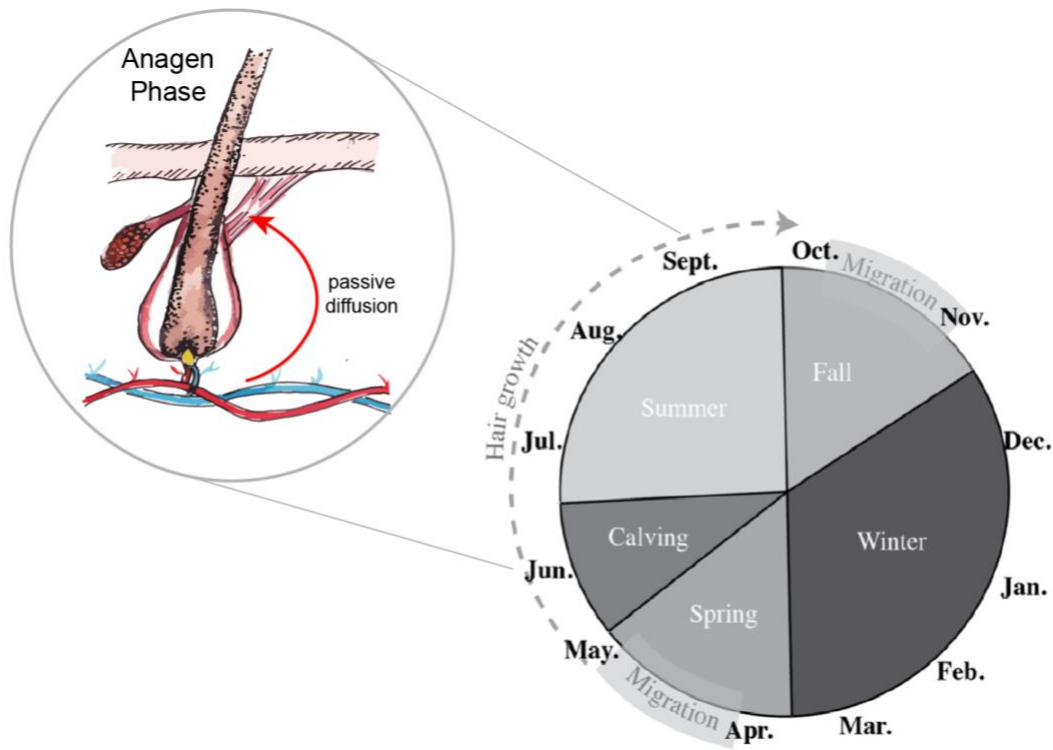


Figure 1.4. The annual cycle of caribou hair growth and graphical representation of trace elements and cortisol incorporation into the hair shaft. Definitions of seasons, as well as periods of migration, are denoted. The hair growth period is believed to begin in May and ends in late September/early October. Months are represented by 3 letter abbreviations.

Rangifer pelage consists of an outer layer of thick and hollow guard hairs, as well as a sparser wool undercoat (Timijarvi et al., 1984). Adult animals will generally have a dark brown

body, and a lighter ventral side (Cuyler & Øritsland, 2002), however, coat colour is variable between *Rangifer* ecotypes and sub-populations (Miller 2003). The coat grows in a seasonal cycle that includes a single annual molt and results in two distinct pelages: a short summer coat, and a long winter coat. Although not well described in the literature, caribou molting begins in May, hair growth occurs in June-August, and the new coat is established by October (Timisjarvi 1984; Cuyler & Øritsland, 2002). Guard hair growth timing can be individually variable, an example being that mature bulls will generally display a mature coat first before the onset of the rut (Drucker et al., 2010). Lastly, as the annual coat ages, it tends to become bleached and damaged and change to a stark white colour by the end of the winter (Macbeth, 2013). Hair collected and analyzed by the end of growth is believed to represent an integrated measure of health metrics (cortisol, trace elements, contaminants) that were incorporated into the hair shaft from June-October (Combs 1987; Russell et al., 2015)

1.2. THESIS OVERVIEW

1.2.1 Study objectives

Hair trace element concentrations (HECs), as well as hair cortisol concentrations (HCCs), are two valuable biomarkers of barren-ground caribou health. Both biomarkers have been validated to an extent for use in ungulates, however, their applications in the field have yet to be explored, and sources of variation associated with opportunistically sampling are relatively unknown. The overarching goal of this MSc research was to explore two biomarkers derived from hair and pertinent to individual and population health of caribou and situate findings in relation to the reality of opportunistic biomonitoring of this species. The objectives of this research were to:

1. Broadly review and characterize the most abundant and available health literature on *Rangifer*, infectious diseases, and explore how this information relates to management and conservation.
2. Establish baseline data for HECs in the Bluenose-east herd of barren-ground caribou and explore intrinsic and extrinsic sources of variation associated with opportunistic sampling.

3. Establish baseline data for HCCs of two herds of barren-ground caribou, and relate findings to a seasonal stressor, biting insect harassment.

1.2.2 Chapter outline

Chapter 2 presents the results from a scoping review of the infectious disease literature of *Rangifer* and relates findings to caribou management. This work served to compile the infectious disease literature for *Rangifer* and begin to understand how we may facilitate and increase the consideration of health in management. These findings are pertinent to the use of other metrics of health in management frameworks as well.

Chapter 3 explores the intrinsic and extrinsic sources of variation associated with the opportunistic sampling of caribou hair and HEC analyses of the BNE caribou herd. This work also established baseline values for the BNE herd and continues to develop HECs as a health monitoring tool in wildlife.

Chapter 4, HCC of two barren-ground caribou populations (BNE and DU) are quantified and related to the environmental stressor of changing biting insect activity. Additionally, potential variations associated with neck and rump body region sampling were investigated, as well as differences among herds, seasons, and sample sources, that have implications for the use and interpretation of this biomarker in future monitoring.

Chapter 5. discusses the challenges, limitations and future research opportunities associated with this thesis.

Appendix D compares hair cortisol values from two partner laboratories in a blinded study and

Appendix E includes additional serological results from the BNE herd for 6 pathogens of interest

1.2. 3 Chapter contributions

Chapter 2 Filip Rakic (FR) and Susan Kutz (SK) conceptualized the study. FR conducted searches and SK acted as the second reviewer for screening and data extraction. Mathieu Pruvot (MP) and Douglas Whiteside (DW) provided methodological considerations and reviewed the article. FR completed the analysis and generated the coding framework. FR wrote the manuscript which was edited by all co-authors.

Chapter 3 FR conceptualized and initiated the study with guidance from SK and MP. FR completed laboratory analysis and was aided by Regina Krohn (RK). Samples were analyzed in partnership with the Alberta Centre for Toxicology. FR completed statistical analysis and coding with guidance from MP. FR wrote the manuscript with guidance from SK that was edited by all co-authors.

Chapter 4 FR conceptualized the study and initiated collaboration with the CARMA network and was aided by SK, Javier Fernandez-Aguilar (XF), and MP. FR coordinated sample shipment from Kugluktuk and Yellowknife with aid from SK. Samples were analyzed in partnership with the Toronto Zoo Reproductive Physiology Laboratory. FR completed statistical analysis and generated the coding framework which was reviewed by MP and XF. FR wrote the manuscript which was edited by SK.

CHAPTER 2: A SCOPING REVIEW OF THE *RANGIFER* INFECTIOUS DISEASE LITERATURE: GAP BETWEEN INFORMATION AND APPLICATION

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2.1. ABSTRACT

The role and impact of infectious diseases in wildlife population dynamics are increasingly recognized, yet disease information is variably incorporated into wildlife management frameworks. This is particularly relevant for *Rangifer tarandus* spp. (caribou or reindeer), a keystone circumarctic species experiencing widespread population declines. The primary objective of this review was to characterize the available peer-reviewed literature on infectious diseases of *Rangifer* using a scoping review methodology. Three databases of peer-reviewed literature (Web of Science, BIOSIS previews, and Scopus) were searched and 695 articles met the criteria for initial review. After screening for relevance and language, 349 articles, published between 1967 and 2020, remained. Over half of the excluded articles (181/346, 52%) were excluded because they were not published in English; the majority of these (120) were in Russian. From the 349 included articles, 137 (39%) pertained to wild *Rangifer* populations (as opposed to semi-domesticated or captive). Articles on infectious disease in wild *Rangifer* were published in 40 different journals across various disciplines; the most common journals were disease and parasitology-oriented, accounting for 55% of included articles. Most studies were descriptive (87%), followed by experimental (9%). Of pathogen taxa investigated, helminths were most common, comprising 35% of articles. *Rangifer* sub-species were not equally represented in the literature, with barren-ground caribou (*R. t. groenlandicus*) $n=40$, and woodland caribou (*R. t. caribou*) $n=39$, having the greatest abundance and diversity of infectious disease information available. Few studies explicitly examined individual or population-level impacts of disease, or related disease to vital population rates, and only 27 articles explicitly related results to management or conservation. Findings from this review highlight an unbalanced distribution of studies across *Rangifer* ecotypes, a preference for dissemination in disease-specialized publication venues, and an opportunity for investigating population-level impacts that may be more readily integrated into caribou conservation frameworks.

Keywords:

Infectious disease, *Rangifer*, Scoping Review, Wildlife Health, Wildlife Management

2.2. INTRODUCTION

Governments, natural resource managers, and Indigenous rightsholders are invested in issues of wildlife health as they directly relate to population sustainability, public health, and economic growth (Lanfranchi et al., 2003; Stephen, 2014). Wildlife population health is an integral but seldom explicitly stated factor in wildlife management and conservation strategies, most notably for species of conservation concern (Deem et al., 2001; Ryser-Degiorgis et al., 2015; Stephen, 2017). Current concerns regarding health and disease in wildlife populations may be further amplified by anthropogenic climate change, causing novel and unforeseen interactions in ecological systems and possible increased negative health outcomes (Kutz et al., 2005; Price et al., 2019). The integration of health monitoring into wildlife management and conservation is, therefore, a fundamental aspect of modern natural resource management policy and practice (Hanisch et al., 2012; Decker et al., 2016).

Rangifer tarandus (reindeer or caribou) is a keystone species (Klein et al., 1999; Bernes et al., 2015; Røed et al., 2019). Despite substantial research and conservation efforts, multiple free-ranging wild *Rangifer* populations have declined precipitously across the circumpolar Arctic (Gunn et al., 2010; Government of the Northwest Territories (GNWT), 2015; Committee on the Status of Endangered Wildlife in Canada (COSEWIC), 2015; 2016). Health and disease are among the multiple stressors identified as potential drivers of *Rangifer* declines (Carlsson et al., 2018; Macbeth & Kutz 2018); changes in infectious disease dynamics are capable of large-scale effects on populations (Tompkins et al., 2011; Canessa et al., 2019). Negative impacts of infectious disease are potentially amplified by climate change, precipitating complex and unpredictable shifts in host-pathogen interactions and subsequent impacts (Kutz et al., 2004; Lafferty, 2009; Gallana et al., 2013; Dobson et al., 2015). Infectious disease and health information is increasingly mentioned in conservation planning documents for *Rangifer* (Environment and Climate Change Canada (ECCC), 2012; 2017); however, infectious disease information has yet to be fully mobilized or integrated into cumulative effects models (Adamczewski et al., 2009; Boulanger et al., 2011; Russell et al., 2021), or other frameworks used for wildlife management decision making (COSEWIC, 2016; GNWT, 2019).

To explore and understand the barriers associated with fully utilizing *Rangifer* infectious disease knowledge in conservation and management plans and/or actions, a scoping literature review was undertaken. We aimed to understand why a seemingly abundant and readily accessible peer-reviewed literature on *Rangifer* infectious diseases is generally not fully considered in *Rangifer* management and conservation. Our objective was to characterize and summarize the *Rangifer* infectious disease literature to understand the types of information available (which pathogens, sub-species, and study types), the pathogen characteristics studied (impacts, pathogen life history, pathology, diagnostics, etc.), the publication venues, potential barriers to access, and ultimately understand how this information may relate to future conservation and decision making.

2.3. MATERIALS AND METHODS

We used the scoping review methodological framework outlined by Arksey & O'Malley (2005), coupled with the PRISMA-Scr (PRISMA extension for Scoping reviews) protocol by Tricco et al., (2018) to explore the peer-reviewed literature. A list of definitions, search queries, and search protocols are available in Appendix A.

2.3.1. Developing and defining the research question

Rangifer was defined as all recognized reindeer and caribou sub-species that are free-ranging and not domesticated. Infectious agents were defined as any organism that lives in or on another living organism (host) and derives its nourishment therefrom (Porta, 2014). These infectious agents were split into two broad classes of micro- (viruses, bacteria, fungi, protozoans, prions) and macro-(arthropods and helminths) parasites (Carlsson et al., 2018).

2.3.2. Literature search strategy

The review question was structured using the Condition, Context, Population (CoCoPop) search strategy common in epidemiological reviews (Munn et al., 2018b). Three search strings pertaining to the population [*Rangifer/caribou/reindeer*], conditions [*Diseas*/Path*/Parasit*/Infect*/Health**], [*Virus*/Bacter*/Protozoa*/Prion**], [*Helminth*/Nematod*/Trematod*/Cestod*/Arthropod**], and a free-ranging context (Appendix A), were used to query three multidisciplinary databases: BIOSIS Previews, Scopus, and Web of

Science. All databases were searched in English across titles, abstracts, and keywords. Solely primary peer-reviewed literature was included, and final searches were completed on 8 May 2020.

2.3.3. Screening and selecting articles

Following final searches, records were exported, compiled, and imported into Covidence Systematic Review Software (Covidence, 2020) for screening. To be included in the full review, articles had to satisfy a two-step screening process: an initial title and abstract screening, followed by full record screening (Arksey & O'Malley, 2005). Relevant articles included any English primary peer-reviewed research article directly about *Rangifer* infectious disease. Articles had to: (i) explicitly mention *Rangifer*/reindeer/caribou within the title or abstract, (ii) pertain to a macroparasite, microparasite, or syndrome of *Rangifer*, (iii) be written in or translated to English, (iv) be available through the University of Calgary library, library holdings and/or interlibrary loan service. Primary research was defined as original research in which authors either generated their own data or interpreted or reanalyzed existing data sets. Following initial inclusion, articles not about free-ranging wild *Rangifer* populations were excluded from data extraction. The screening protocol was validated by three reviewers, each completing a 50-article subset of articles eligible for review. Following validation, a single reviewer completed the remaining screening.

2.3.4. Data extraction

To be consistent in data extraction and charting, two reviewers pre-tested an extraction template using a 30-article subset. Information collected included: author(s), publication date, journal, study location, pathogen group (arthropod, helminth, protozoan, bacteria, virus, prion, multi-pathogen), *Rangifer* sub-species (*R. t. caribou*, *groenlandicus*, *granti*, *pearyi*, *tarandus*, *platyrhynchus*, *fennicus*), level of domestication (free-ranging, semi-domesticated, domestic), study type (experimental, descriptive, traditional knowledge, modelling, synthesis), and health information generated (pathogen prevalence, pathogen intensity, pathogen taxonomy, pathogen life history, intrinsic risk factors, extrinsic risk factors, animal behaviour, individual impacts, populations impacts, treatment, pathology, mortality investigation). Extracted data were inputted into Microsoft Excel.

2.3.5 Data analysis and characterization

Extracted data were formatted, analyzed, and summarized in Microsoft Excel and in R statistical software using the tidyverse packages (Wickham et al., 2019). Descriptive analysis consisted of an analysis of frequencies and distributions of publishing journals, methodologies employed, parasite taxa studied, and health information generated across time, sub-species, and infectious agents. Health information was further categorized into broader health categories: 1) Pathogen Occurrence (pathogen occurrence, prevalence, intensity); 2) Pathogen Life History (pathogen life history, taxonomy, and experimental infection); 3) Risk factors (intrinsic and extrinsic risk factors); 4) Pathogen Impacts (individual, population level, and *Rangifer* behaviour impacts); 5) Diagnostics (diagnostic and treatment information); and 6) Pathology (pathology and mortality investigations). Definitions of categories are available in Appendix A. Some articles contributed to multiple health information categories; therefore, total health information may exceed the total number of articles in the review. Further, statistical significance of any hypothesis testing in the reviewed documents was not a factor in their final inclusion into the relevant health category.

Lastly, articles were assessed for an explicit mention of conservation or management applications of research findings in the abstract, objectives, or discussion of the article. Wildlife management was defined as the “implementation of practices to purposefully influence interactions among and between peoples, wildlife, and habitats to achieve impacts valued by stakeholders” (Riley et al., 2002). Articles were considered to relate to management or conservation if they explicitly stated and related findings to conservation, management, policy, and/or harvest and related synonyms (Appendix A). To maintain clear and objective criteria in data extraction, research with implicit, not directly stated management or conservation applications, was evaluated as not fitting the inclusion criteria for management or conservation applications.

2.4. RESULTS

Of 3,116 articles screened for relevance, 695 were included in the full-text review, of which 349 met our inclusion criteria (Figure.2.1). Language inaccessibility represented the largest reason for article exclusion: 181 non-English, non-translated articles were excluded, the majority of which were in Russian and had no publicly retrievable full article translation (120 articles, 66.3%). Of the 349 included articles, 137 (39.2%) pertained to wild *Rangifer* populations and these were analyzed further; the remaining 212 articles pertained to semi-domesticated or captive populations. Publications included in this final data extraction were dated from 1967 to 8 May 2020. The number of publications slowly increased overtime at a rate of five additional publications per year per decade.

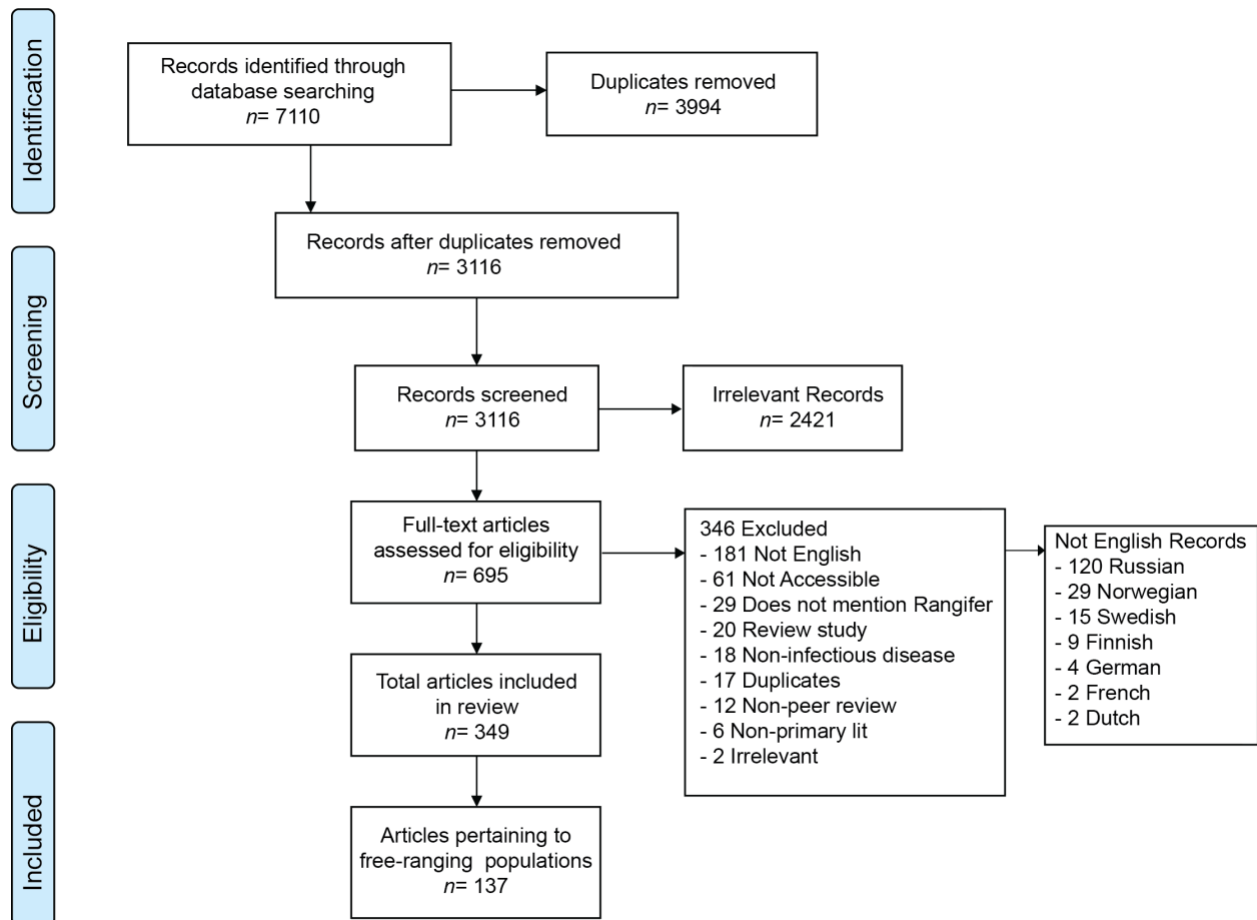


Figure.2.1. Search flow diagram (modified from the PRISMA flow diagram (Page et al. 2021)) depicting a scoping review literature search of *Rangifer tarandus* (caribou or reindeer) infectious diseases. A total of 3116 papers were considered for initial inclusion, which were combined results from Scopus, BIOSIS Previews and CAB Abstracts (searches occurred on 8 May 2020). First and second screening resulted in

349 papers being considered for data extraction, 137 of which pertained to wild populations of caribou or reindeer (*Rangifer tarandus* ssp.).

2.4.1. Publication venue

The *Rangifer* infectious disease literature is published in diverse journals. The 137 research articles related to wild *Rangifer* populations were published in 40 distinct journals. Approximately half of the articles were published in six journals: The Journal of Wildlife Diseases (24.8%), Canadian Journal of Zoology (10.2%), *Rangifer* (8.0%), Journal of Parasitology (5.8%), and The International Journal for Parasitology: Parasites and Wildlife (5.1%). The 40 journals were assigned into eight categories based on journal scope: Disease and Epidemiology, Parasites and Parasitology; Ecology and Zoology; Veterinary Medicine; Management/Conservation; Arctic Ecosystems; Pathogen Specific; and Multidisciplinary. The bulk of journals publishing wild *Rangifer* health information were disease and epidemiology or parasites and parasitology journals, accounting for 59.1% of the articles. Of the 40 journals, two were paid-for access (5.0 %), 27 hybrid access (67.5%), and 11 open access (27.5%).

2.4.2. Geographic locations and study type

Most of the articles were focused on wild *Rangifer* populations in Canada, Norway, and Alaska. Descriptive studies were the most common across all study locations, regardless of time or pathogen group being investigated. Temporal trends in study types suggested that a greater diversity of study types has emerged in recent decades (Figure.2.2). Descriptive research was the most common ($\geq 60\%$) study type in all time periods from 1969 to 2020. Experimental research occurred from the 1960s to the 2020s, representing 10-25% of the studies, whereas experimental and predictive modelling began to take place in the late 1990s and has represented $\leq 5\%$ of the research since. Traditional knowledge (TK) and synthesis research in *Rangifer* infectious disease were published only in the last decade, accounting for a relatively small proportion ($\leq 5\%$) of the articles included. The majority of TK research about infectious diseases of *Rangifer* had occurred in the Canadian Arctic.

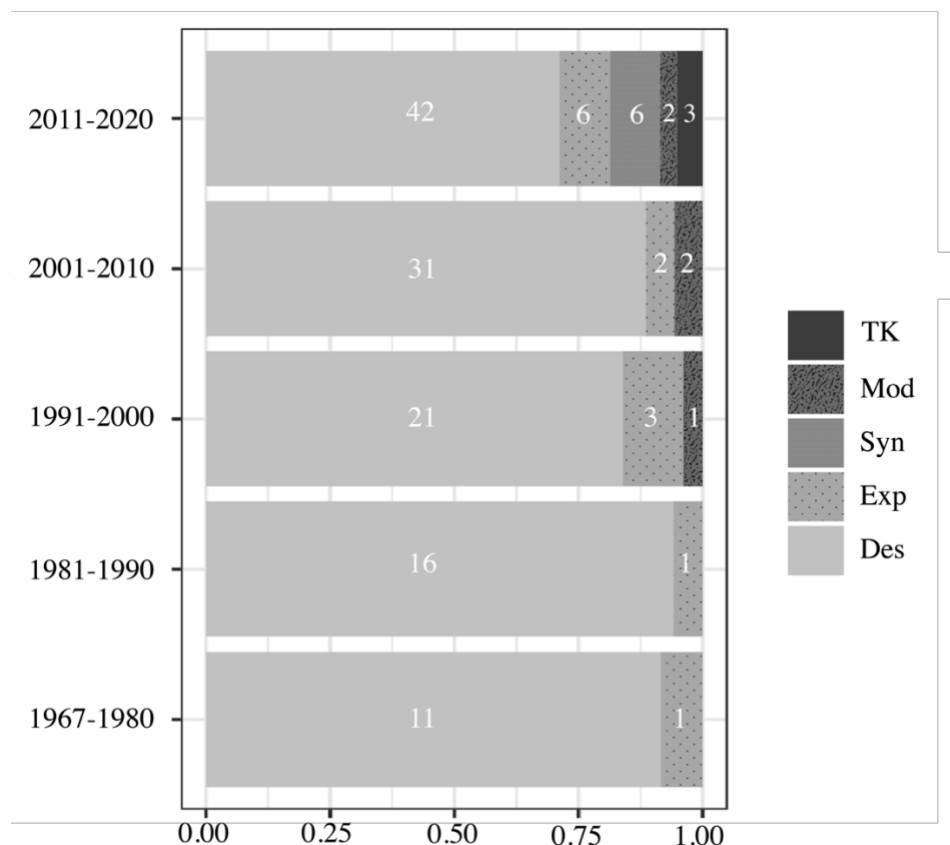


Figure.2.2. Summary proportion bar graph of scoping review results depicting 137 English language *Rangifer tarandus* disease publications pertaining to wild populations published between 1967 and 2020. Included articles are summarized based on primary methodology used. The x-axis denotes the proportion of a methodology used in relation to the total number of articles published in that decade. The y-axis denotes five groupings based on decade. Study types include Experimental (Exp), Descriptive (Desc), Traditional Knowledge (TK), Ecological Modelling (Mod), and Synthesis studies (Syn). White numbers denote the number of articles within each category.

2.4.3. Infectious disease information

The types of pathogens studied varied among the six *Rangifer* sub-species and five-time periods (Figure.2.3A). In general, helminth taxa were the most studied, and research output pertaining to helminths increased over time (Figure.2.3B). Protozoans and bacteria were studied consistently, and prions were the least studied taxa, group. Research investigating multiple pathogen groups simultaneously, multi-pathogen order research, increased over time. The greatest abundance and diversity of infectious disease information available pertained to woodland caribou and barren-ground caribou, with scant literature on infectious disease for Peary caribou and forest reindeer.

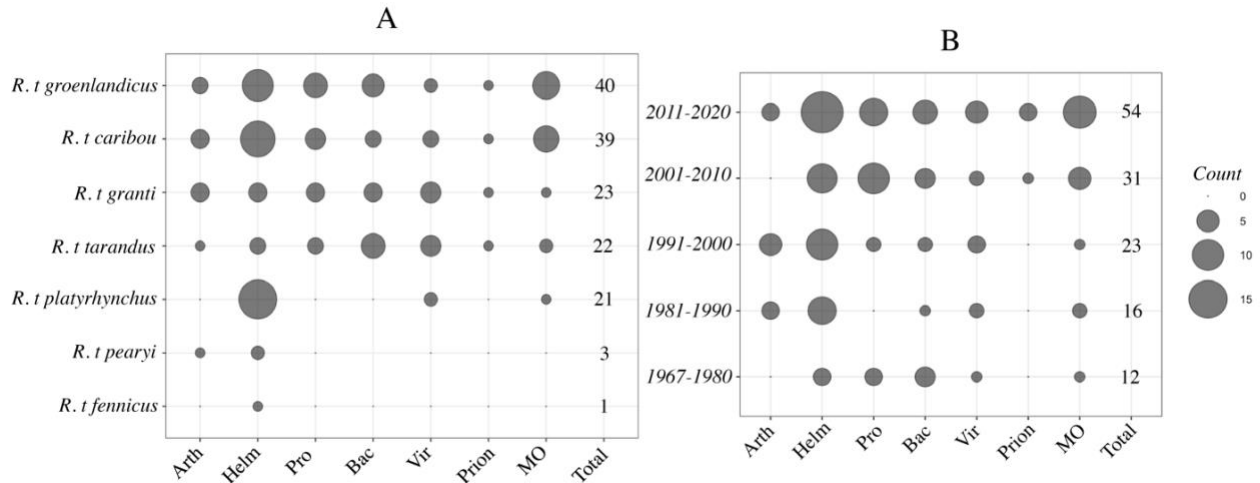


Figure.2.3. Bubble chart summary of scoping review results depicting 137 English language research articles of disease in free-ranging *Rangifer tarandus* in relation to pathogen order investigated (x-axis). Pathogen classifications are Arthropods (Arth), Helminths (Helm), Protozoa (Pro), Bacteria (Bac), Viruses (Vir), Prions and Multi-Pathogen order (MO) studies. A) pathogen category in relation to *Rangifer tarandus* (*R.t.*) sub-species, y-axis, seven categories; B) pathogen orders investigated in relation to publication year (five categories by decade). The size of the points denotes the number of research articles, the ‘Total’ column is the sum of research articles per sub-species and date category.

The type of infectious disease information generated by studies was dominated primarily by descriptive studies on ‘pathogen occurrence’ then ‘pathogen life history’ and ‘pathogen risk factors’ for all the subspecies. Population level impacts accounted for 5% of available health articles among all sub-species and pathogen types. Among those information categories, helminths then multi-pathogen studies were the most common pathogen taxa studied (Figure.2.4. A and B). Arthropod research was most often focused on occurrence, with no research on diagnostics, treatments, or gross pathology. In general, viral, and bacterial studies were less common, especially with respect to population level impacts of these pathogens.

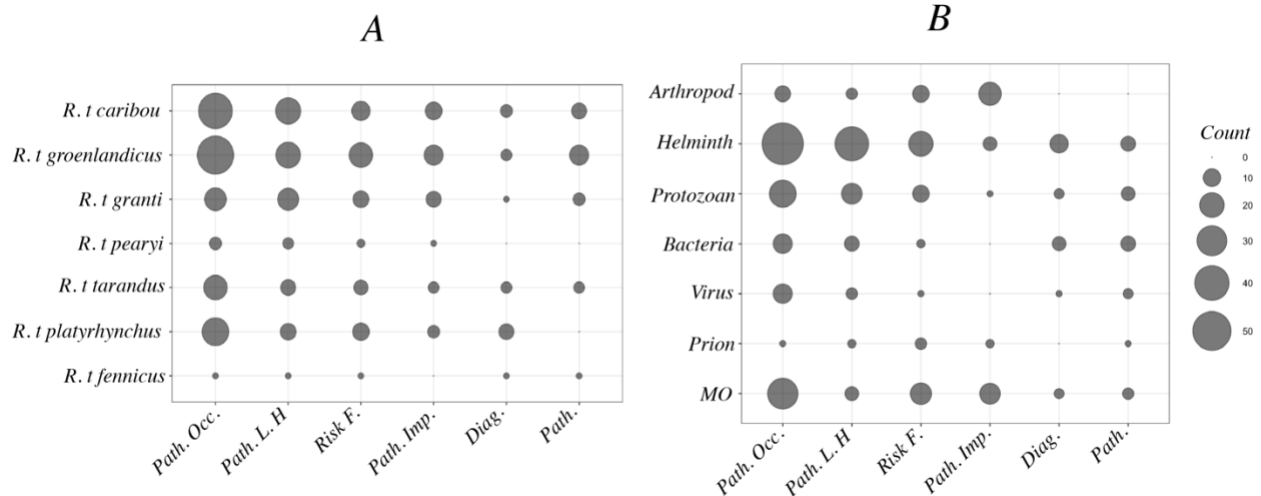


Figure.2.4. Bubble chart summary of scoping review results depicting 137 research articles on *Rangifer tarandus* (caribou or reindeer) disease, and the type of infectious disease information produced (x-axis). Health information categories are: (i) Pathogen occurrence information (Path. Occ); (ii) Pathogen life history information (Path. L.H.); (iii) Risk factors information (Risk. F); (iv) Rangifer impact information (Path. imp) (v) Diagnostic and treatment information (Diag.); and (vi) Pathology information (Path.). A single article may be eligible, and included, in multiple health categories. A) information in relation to *Rangifer tarandus* (*R.t.*) sub-species (y-axis), seven categories; B) infectious disease information in relation to pathogen classifications (Y-axis). MO corresponds to multi-pathogen order studies. The size of the points denotes the number of research articles.

2.4.4. Statement on conservation and management applications

A total of 27 articles (19.7%) explicitly mentioned and related research findings to management, conservation, decision making or policy implications in wild caribou/reindeer. Management or conservation applications were predominantly introduced in the discussion (24 articles), followed by the abstract (seven articles), and lastly in the objectives (four articles); five articles mentioned applications within numerous sections of the paper. One-third of articles (18/54) published between 2011 to 2020 mentioned a management or conservation application of research findings.

2.5. DISCUSSION

Our results demonstrate that there is a growing body of literature on infectious disease in wild *Rangifer*; however, this literature is not evenly distributed across *Rangifer* subspecies or pathogen taxa. In addition, the type of information generated as well as the publication venues may limit its value and accessibility for management or conservation benefits. Considering the global enigmatic declines of *Rangifer* across its circumpolar range (Vors & Boyce, 2009; Festa-

Bianchet et al., 2011; COSEWIC, 2016) and the impacts of infectious disease on wild populations (Deem et al., 2001; Ryser-Degiorgis et al., 2015; Stephen, 2017), these findings are directly relevant to future successful *Rangifer* management actions that consider and mobilize all available knowledge pertinent to population recovery.

2.5.1. Communication

Results from the full-text review process highlighted language accessibility, as well as journal access level, as potential barriers to *Rangifer* health communication. A large proportion of *Rangifer*'s range occurs in Russia, including a sub-species of conservation concern, the Eurasian Forest reindeer (*R. t. fennicus*), whose range extends between Finland and Russia (Kojola et al., 2009). Medvedev, (1995) was the sole identified English language research article on the Russian population of *R. t. fennicus* and pertained to health broadly rather than disease. Although English is currently generally considered to be the 'lingua franca' of international scholarly communication (Liu & Li, 2018), non-English articles still make up a significant portion of the natural sciences and veterinary medicine literature (Liu, 2017), as mirrored by our findings. The use of translation services or technologies was beyond the scope of this review.

The exclusion of 120 Russian language articles limits our conclusions around *R. t. fennicus*. Approximately 1.3 million free-ranging reindeer inhabit Russia, including the Taimyr population, one of the largest wild reindeer populations globally (Syroechkovski, 1999). Thus, free-ranging reindeer in Russia represents a significant portion of *Rangifer* populations globally that are not captured within this review. The sole consideration of English language articles within reviews can pose a significant source of bias (Grégoire et al., 1995; Egger et al., 1997). Language restriction during screening rather than searching can increase the transparency of results (Pieper & Puljak, 2021), yet this does not account for non-English articles not indexed in English. To overcome these barriers to free information exchange internationally, increased collaboration with Russian *Rangifer* research colleagues is encouraged.

The intended journal audience, as well as the lack of Open Access (OA) adoption, may represent other barriers to communicating research on *Rangifer* health. Research was published predominantly in pathology and parasitology focused journals catering to veterinary medicine,

wildlife disease, or parasitologist audiences. Journals intended for managers and conservationists made up a very small portion of research articles. Manlove et al., (2016) found that significant discipline segregation between veterinary medicine and ecology still exists. This review suggests that *Rangifer* research may be similarly siloed, despite ongoing calls for interdisciplinary research in the field (Joseph et al., 2013; Allen-Scott et al., 2015). Access to infectious disease information pertinent to wildlife managers may be further complicated by the publishing model, as the ability to freely view literature has been identified as a significant barrier to access for natural resource managers (Pullin et al., 2004; Cvitanovic et al., 2015; Canessa et al., 2019). Only 27.5 % of papers included in our review were published in exclusively OA journals, which may represent a potential barrier for stakeholders seeking *Rangifer* health and disease information. Access and exposure to *Rangifer* disease information, therefore, may be limited due to the journal type and their access level.

2.5.2. Pathogens of interest

Helminths were the most studied pathogens in *Rangifer*, accounting for 36% of research articles. The number of helminth research papers increased over time, and this was the sole pathogen group with published research in all *Rangifer* sub-species. Strongylate nematodes were the most studied helminth type, with a few studies investigating potential individual and population level impacts on *Rangifer* (Albon et al., 2002; Hughes et al., 2008; Irvine et al., 2019). Most helminth articles, however, were focused on describing occurrence, distributions, and life history traits. In the few articles that did assess helminth impacts, most assessed individual, not population, impacts (Figure.2.4). Albon et al., (2002) researching Svalbard reindeer is one of the few studies demonstrating experimental and observational evidence of population level impacts of a pathogen in *Rangifer*.

Researchers exploring the effects of co-infections of multiple pathogens, as well as macroparasites, were previously identified as priorities in northern wildlife health (Hoberg et al., 2008). The number of multi-pathogen and arthropod research papers increased over time but focused primarily on life-history traits, rather than investigating impacts. Prions were the most data-deficient pathogen type. The increased number of prion related publications in the most

recent date category (Figure.2.3) can be attributed to the potential emergence and threat of chronic wasting disease in *Rangifer* (Mitchell et al., 2012; Benestad et al., 2016).

2.5.3. Conservation and management

While descriptive studies of specific pathogens are fundamental to our understanding of ecology of disease in wildlife populations, they often do not fit into wildlife management frameworks or priorities, and are, therefore, not easily mobilized into decision making (Lafferty & Gerber, 2002; Braunisch et al., 2012). Wildlife adaptive management frameworks value diverse and heterogenous sources of information and evidence (Deem et al., 2001; Joseph et al., 2013; Dressel et al., 2018), yet wildlife health research output has been categorized as predominantly non-translational, meaning it is not intended for the end-users of the information (Peters et al., 2019). Our review suggests that the *Rangifer* infectious disease literature is dominated by investigations pertaining to pathogen occurrence, life history, and epidemiology. Yet, information on demographic metrics of populations and population level impacts of disturbances are the highest ranked among conservationists as information that is directly relevant to managers or end-users (Braunisch et al., 2012). Investigations that focus on individual mortality or morbidity may not fulfill conservation biology needs (Lafferty & Gerber, 2002) if the linkages between individual and population level impacts are not understood or articulated. We recognize that research on pathogen life-history is important in understanding the effect of environmental changes on disease (Kutz et al., 2005; Kutz et al., 2013; Forde et al., 2016); however, these investigations may not be as readily integrated into decision making.

A research-to-practice gap is increasingly recognized in natural resource management and conservation (Laurance et al., 2012; Cvitanovic et al., 2015). Factors contributing to this gap include barriers to literature access (Pullin et al., 2004; Barrett & Rodriguez, 2021); a lack of directly transferable information (Canessa et al., 2019); and a lack of collaboration and involvement of practitioners in research (Laurance et al., 2012). Further, a recent review of natural resource managers (Barrett & Rodriguez, 2021) demonstrated that although managers view peer-reviewed literature as contributing to better management, this information is not presented in a readily accessible or usable form. Our review identified similar potential research-to-practice barriers for *Rangifer* infectious disease research, but these barriers may be decreasing.

For example, a third of articles on *Rangifer* infectious disease published in the last decade have explicitly related results to management or conservation, demonstrating an intention to inform policy and practice. A first step to remedy a potential research implementation gap is the inclusion and consultation of practitioners in the research (Knight et al., 2008; Braunisch et al., 2012; Barrett & Rodriguez, 2021). Increased collaboration between wildlife managers and Indigenous rights holders and wildlife health experts is facilitating the identification of the *Rangifer* health research gaps that need to be addressed to support management (Tomaselli et al., 2018; Peacock et al., 2020); these growing collaborations around this intersectional topic may remedy knowledge transfer difficulties.

2.5.4. Traditional knowledge approaches

Modern approaches to wildlife management and conservation are pivoting to encompass the integration of knowledge sources across disciplines, including local and Traditional knowledge (TK), to inform decisions and policy (Riley et al., 2002, 2003; Kutz & Tomaselli, 2019). Despite a substantial body of peer-reviewed literature on TK about caribou (Lyver & Łutsël K'É Dene, 2005; Parlee et al., 2005; Kendrick & Manseau, 2008), TK approaches represented a very small proportion of research articles included in the review. These results suggest that either TK studies containing disease information for *Rangifer* are not easily queried and/or are not catalogued as infectious disease related, or that few TK studies explicitly investigating disease in *Rangifer* have been undertaken or published. These findings do not extend to the TK research body of the semi-domesticated reindeer held by the Sami people of Fennoscandia, as this review focused on free-ranging *Rangifer* populations. Skewed differences in the abundance of health information between sub-species may be attributed to differences in conservation status and associated access, which may be remedied through the integration of TK into research methods. For example, the deficits in infectious disease literature for Peary caribou and forest reindeer may be attributed to restricted access due to conservation status, as harvest sampling is one of the main methods for sampling *Rangifer* for health surveillance (Brook et al., 2009; Tomaselli et al., 2018). Documenting and applying TK is an effective strategy for confronting areas of knowledge deficit for rare and endangered species and in remote areas (Brook & McLachlan, 2008; Biró et al., 2014; Kutz & Tomaselli, 2019). Such an approach may be readily applicable to improving knowledge of infectious diseases.

2.5.5. Limitations and future directions

Our results are the product of a scoping review methodology which characterized and summarized the varied body of research around *Rangifer* infectious disease. This type of review differs from more commonly employed systematic reviews (Levac et al., 2010; Chang, 2018) where statistical tests and meta-analyses are more common (Pham et al., 2014). North American *Rangifer* populations are well represented by this review; however, a large body of Eurasian literature was not assessed due to the inclusion (peer-reviewed, primary, English language literature on wild *Rangifer*) and exclusion (language, grey, and semi-domesticated reindeer literature) criteria, perhaps reducing the generalizability of conclusions. Nevertheless, the identification of extensive Russian ($n= 120$), and semi-domesticated reindeer ($n=212$) literature illustrates additional valuable sources of information on *Rangifer* health.

Although a large body of literature on the most common Eurasian subspecies, the semi-domestic reindeer (*R.t. tarandus*), was eliminated in our review, that literature is well-summarized elsewhere (Laaksonen & Paulsen, 2015; Riseth et al., 2020) and is potentially relevant to their free-ranging counterparts. Particularly, the effects of warbles, gastrointestinal nematodes, brain worm (*Elaphostrongylus rangiferi*) and cervid herpesvirus 2 are well described in semi-domestic reindeer (Das Neves et al., 2010; Ballesteros et al., 2012; Davidson et al., 2020); this literature can be drawn upon to understand the ecology and effects in wild *Rangifer* populations. For instance, cervid herpesvirus 2 serological monitoring tools, developed and validated using Norwegian semi-domesticated reindeer populations (Das Neves et al., 2009), are now used to monitor alphaherpesvirus exposure in free-ranging *Rangifer* populations (Evans et al., 2012; Bondo et al., 2019; Carlsson et al., 2019).

Our initial objective was to synthesize the peer-reviewed literature; thus we did not consider grey literature in this review, and many provincial, federal, and non-government organization documents were not considered. There are known benefits and detriments to including grey literature in systematic reviews. The inclusion of grey literature may broaden the scope of a review and more truly represent all the available evidence for a topic (Hopewell et al., 2005; Benzie et al., 2006). Further, grey literature management plans are the most employed

source of information used by natural resource managers (Barrett & Rodriguez, 2021). Conversely, accessing grey literature may pose a bias in itself, because of a lack of consistent indexing, categorization, and reporting standards between grey literature sources (Godin et al., 2015). Moreover, studies that have included grey literature in reviews report a significant increase in the required time and resource allocation (Benzies et al., 2006; Mahood et al., 2014). The inclusion of grey literature might have altered our results. Government reports and management plans for *Rangifer* sub-species of conservation concern may have contained information that is not represented by primary literature. An alternative to the barriers of consistently accessing the grey literature may be expert consultation (Benzies et al., 2006; Mahood et al., 2014) where ‘key documents’ related to the search criteria are included based on input from *Rangifer* managers. Some of the more recent grey literature on *Rangifer* directly considers infectious diseases as key considerations for management (COSEWIC, 2017; Lenart, 2021), further supporting the importance of engaging wildlife managers in *Rangifer* infectious disease research.

Our review findings may guide future synthesis studies of the *Rangifer* disease literature. For example, our finding that the largest and most diverse body of research on *Rangifer* disease were studies on helminth parasites, highlights this group of parasites as a good topic for a systematic review. Additionally, relatively well-researched pathogens of *Rangifer* such as strongylid nematodes, *Elaphostrongylus rangiferi*, *Besnoitia tarandii*, *Brucella suis* biovar 4, and keratoconjunctivitis syndrome, are good candidates for targeted reviews that directly address the potential impacts of these infectious agents on *Rangifer* populations. Future reviews of specific well-studied *Rangifer* pathogens should endeavour to investigate the quality of information and incorporate meta-analyses to investigate impacts, as exemplified in Koltz et al. (in press). Lastly, including managers in the research process as well as tailoring information for the application (i.e summarizing impacts, and forecasted change) would ensure the translatability of future synthesized information (Barrett & Rodriguez, 2021).

2.6. CONCLUSION

Pertinent stakeholders, rightsholders, and users of *Rangifer* are invested in positive health outcomes for this species in the face of global uncertainty and population declines. We identified a potential gap between information generated by *Rangifer* infectious disease researchers and ecological applications valued by managers and conservationists. Traditional knowledge documentation, participatory epidemiology, and modelling of host-pathogen dynamics and population level outcomes are underrepresented yet are important methodologies that could address this gap. Results from this review also highlight the need for increased practitioner involvement in research that will contribute to the effective management, conservation, and stewardship of this keystone species.

2.7. ACKNOWLEDGEMENTS

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**CHAPTER 3. INVESTIGATING TRACE ELEMENT
CONCENTRATIONS IN HAIR OF BARREN-GROUND
CARIBOU (*RANGIFER TARANDUS GROENLANDICUS*):
OPPORTUNITIES AND CHALLENGES**

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Chapter intended to be submitted to the journal *Science of the Total Environment* or *Biological Trace Element Research*

3.1. ABSTRACT

Trace elements can significantly influence the individual and population health of wildlife. Trace element imbalances, such as mineral deficiency, or heavy metal toxicity, may directly alter the reproductive success, growth, and lifespan of ungulates. Hair has been used to monitor trace element concentrations in free-ranging ungulate populations, including caribou (*Rangifer tarandus*) however, the impact of key covariates (sex, sample source, year) is largely unknown. We studied two herds of caribou, the Dolphin and Union herd (DU) (*R. t. groenlandicus x pearyi*), and the Bluenose-East herd (BNE) (*Rangifer tarandus groenlandicus*), to evaluate sources of hair element variation that may arise from the common opportunistic sampling approaches used to monitor these populations. To determine if trace elements concentrations varied in hair from different anatomical sites, we compared paired hair samples (n=18 pairs) of neck and rump hair collected from harvested DU caribou in 2019. We then drew on a larger sample size of hair samples (n=137) from the BNE herd to test the effects of sex, year of collection, age, sampling source, and anatomic sampling site. Eighty-three hair samples from hunted BNE caribou and 54 hair samples from live-captured BNE caribou were collected between 2012-2019. All hair samples were analyzed using inductively coupled plasma mass spectrometry. We found that concentrations of Zn, Se and Cu and Mo in hair for the same individual were significantly different between neck and rump sampling sites ($p < 0.05$). No differences in hair element concentrations were detected between sexes and age classes. Zn ($p < 0.01$), Pb ($p < 0.05$), Mo ($p < 0.05$), and Cu ($p < 0.05$) concentrations varied significantly between years; Zn and Cu increased over time and were best modeled as linear and 2nd degree polynomial functions of year, respectively. Pb and Mo decreased over time and were best modeled as linear and 3rd degree polynomial functions of year, respectively. Samples from hunted and captured caribou significantly differed in Se ($p < 0.01$) and Mo ($p < 0.05$) concentrations. Hair is a promising and practical tool for caribou health monitoring and is readily applicable to other ungulate species.

Keywords: Trace elements, Minerals, Heavy metals, Caribou, Hair, Wildlife Monitoring

3.2. INTRODUCTION

Barren-ground caribou (*Rangifer tarandus groenlandicus*) play important an important role in Northern ecosystems (Bernes et al., 2015) and communities (Tomaselli et al., 2018). Many of these historically numerous populations, including the Bluenose-East herd (BNE) and Dolphin and Union (*R. t. tarandus x pearyi*) herds have severely declined in the last two decades. Specifically, the BNE herd has declined by 84% from 2010 to 2018 (Adamczewski et al., 2017; Boulanger et al., 2019), and DU population has declined by 90% from 1997 to 2018 (COSEWIC 2017; Leclerc and Boulanger 2020), and both populations are of conservation concern in Canada. Caribou health may be understood as multi-factorial (Macbeth & Kutz, 2018), and nutrition is included as a key indicator of population health status. Trace elements, including essential minerals and toxic heavy metals, are elements that at deficient or toxic concentrations may negatively impact the individual and population health of wildlife (Ekin et al., 2004; Frank, 2004). In free-ranging ungulates, imbalances of these elements are associated with poor reproduction and population decline (Flueck, 1994; O'Hara et al., 2001; Frank 2004), thus understanding trace element concentrations of caribou is an important component in understanding overall population health status.

Concentrations of elements in the hair shaft are believed to be reflective of the cumulative circulating element concentrations that diffuse into the hair shaft during the hair growth period (Combs, 1987). Guard hair growth in *Rangifer*, although not precisely understood or documented in each ecotype, generally occurs in the summer/early fall (June-October) (Cuyler & Øritsland, 2002; Macbeth et al., 2013). This period in which trace elements are incorporated into the hair shaft coincides with a critical period of the barren-ground caribou annual cycle, as maximizing nutritional gain and body condition during the Arctic growing season is linked to successful pregnancy (Cameron et al., 1993; Cameron and Ver Hoef, 1994) and calf survival (Couturier et al., 2009). Caribou are considered 'capital breeders' that rely on stored resources acquired during the growing season to support fetal development and lactation (Taillon et al., 2012; 2013), and HECs may serve as a seasonal biomarker of this period.

To interpret hair element concentrations, we must relate concentrations in hair to known physiological standards (liver, kidney), sources of natural variability (sex, age), and any potential source of bias, such as those associated with sampling approach (captured, hunted). Certain

elements have demonstrated a significant response to dietary manipulation in livestock, such as Se (Combs, 1982), Zn (Beeson et al. 1977; Deeming & Weber 1977; Ward & Savage 1994) and Cu (Combs 1982; Noël et al., 2015) and have good evidence for biological relevance of hair concentrations. Other elements have demonstrated promising associations between hair concentrations and those of livers or kidneys. Examples include Se in mule deer (*Odocoileus hemionus*) (Roug et al., 2015), and mountain caribou (*R. t. caribou*) (Jutha et al., in press), Mo in mountain caribou (Jutha et al., in press), and Pb, as well as Cd in cattle (*Bos taurus*) (Patra et al., 2007). Consequently, we focused on three trace minerals (Se, Mo, Cu) and three metals (Zn, Pb and Cd) in this study with evidence of response to dietary manipulation or promising associations with organ standards in other species.

Intrinsic factors, such as sex, age, and reproductive status are associated with variations in renal and hepatic trace element concentrations in ungulates (Hermoso de Mendoza García et al, 2011; Gamberg et al., 2016; Gamberg et al., 2020), and may similarly impact hair concentrations. Females will more rapidly bioaccumulate heavy metals due to smaller body mass coupled with elevated metabolic requirements during late-stage pregnancy and lactation (Danielsson and Frank, 2009; Hermoso de Mendoza García et al., 2011). Conversely, females may have reduced hepatic Cu during late-stage pregnancy (Rombach et al., 2003; Gamberg et al., 2016) or decreased protein and nitrogen during lactation (Barboza & Parker, 2008). Pb and Cd tend to accumulate in tissues with age (Burger et al., 2007; Hermoso de Mendoza García et al., 2011), and these patterns have been previously reported in caribou (Gamberg et al., 2020). Therefore, age and sex have the potential to be significant covariates for hair element concentrations of caribou.

Extrinsic factors, such as forage availability and climate are associated with annual variation in renal element concentrations (Danielsson and Frank, 2009; Gamberg et al., 2020). Trace element content of plant tissues are species and life stage specific (Staal and Sæbø, 1983; Shaw and Reynolds, 1985), and Arctic warming has been associated with changes in plant community composition (Myers-Smith, 2011; Zamin et al., 2017) and plant phenology (Walker et al., 2006; Zamin et al., 2017). Simulations of directional warming in the Arctic forecasts the expansion of *Betula spp.* (Euskirchen et al., 2009), shrubs with markedly different trace element concentrations compared to the other dominant caribou summer forage, lichens (Oster et al.,

2018). Changes in forage intake through time should be reflected in hair element concentrations. However, caribou may be able to compensate for these incremental changes in plant community composition through behavioural plasticity and forage selection (McGreer et al., 2015), resulting in similar hair element profiles through time.

Lastly, wildlife health monitoring can be technically and logistically challenging (Mörner et al., 2001; Stephen et al., 2019), and opportunistic and/or convenience sampling approaches are frequently used (Duncan et al., 2008; Vaz et al., 2020; Fox et al., 2021). These approaches may introduce a selection bias (Nusser et al., 2008), however opportunistic sampling of wildlife is still encouraged to support ongoing monitoring of populations (Jessup, 2003; Ryser-Degiorgis et al., 2013). In this study, we used two sources of opportunistic sampling of caribou: samples collected during live captures for radio-collaring, as well as samples collected by local hunters during subsistence hunts. Hair collected during captures and mortality investigations is done predominantly for the purpose of genotyping (CARMA, 2008) that do not specify an anatomic sampling location. Select trace elements in hair, such as Se, differ between anatomic sampling sites in mountain caribou (Jutha et al., in press), but not in cattle (Szigeti et al., 2015). Potential differences in hair trace element concentrations between body regions must be better understood if we are to co-opt opportunistically collected hair for trace element analyses.

The overarching goal of this research was to report upon hair trace element concentrations in barren ground caribou and investigate key covariates associated with opportunistic health sampling of these populations. We used samples taken from the Dolphin and Union (DU) and the Bluenose-east (BNE) herds of barren-ground caribou to investigate and understand possible extrinsic and intrinsic sources of variation in hair trace elements. First, we examined paired neck and rump hair samples from the DU herd collected in 2019 to investigate possible differences between anatomic sampling sites. Second, we used archived hair samples collected by hunters and by government biologists to describe the distribution of hair trace element concentrations in the BNE herd from 2012-2020. We expected that: (1) hair element concentrations would differ between anatomic sampling sites of hair collection, (2) hair element concentrations would differ between sexes, and heavy metal concentrations would be elevated in females, (3) hair mineral concentrations would vary inter-annually and demonstrate an increase

of element concentrations associated with shrub forage (Zn), and (4) trace elements would differ between captured and harvested animals.

3.3. MATERIALS AND METHODS

3.3.1. Study area and sample collection

Barren-ground caribou occupy large ranges of several hundred thousand kilometres and migrate seasonally. Generally, these populations over-winter in the treeline and then migrate to open tundra for gregarious calving in the spring (Bergerud et al., 2008). The BNE herd ranges from east of Great Bear Lake, Northwest Territories (NWT), to west of Kugluktuk, Nunavut (NU), Canada, while the DU herd ranges on Victoria Island and nearby mainland NU and NWT (COSEWIC, 2016). These herds of barren-ground caribou are important to and harvested by the communities of the Sahtù and Tlichò (Tłı̨chò) settlement areas, and Ulukhaktok (NWT), as well as the communities of Kugluktuk and Iqaluktuutiaq (NU). Animals from the BNE herd were sampled across their annual distribution from both sources (Figure.3.1).

Eighteen paired hair samples (lateral neck and dorsal rump) from caribou of the DU herd were collected by a veterinarian accompanying Inuit harvesters on a community hunt from Kugluktuk, Nunavut in 2019. Eighty-three hair samples with a sufficient mass of hair for analysis were collected from the neck of BNE caribou and submitted in sampling kits by hunters in 2017-2019; these caribou were collected year-round. Samples requested and submitted varied among years and followed a modified CircumArctic *Rangifer* Monitoring and Assessment (CARMA) protocol (CARMA, 2008). Fifty-four hair samples were taken during live animal capture by biologists of the Government of the Northwest Territories (NWT) in March between 2012-2020. All hunter samples were collected from the lateral neck, while capture samples were collected from between the shoulder blades (back), lateral neck, and dorsal rump depending on the sampling year.

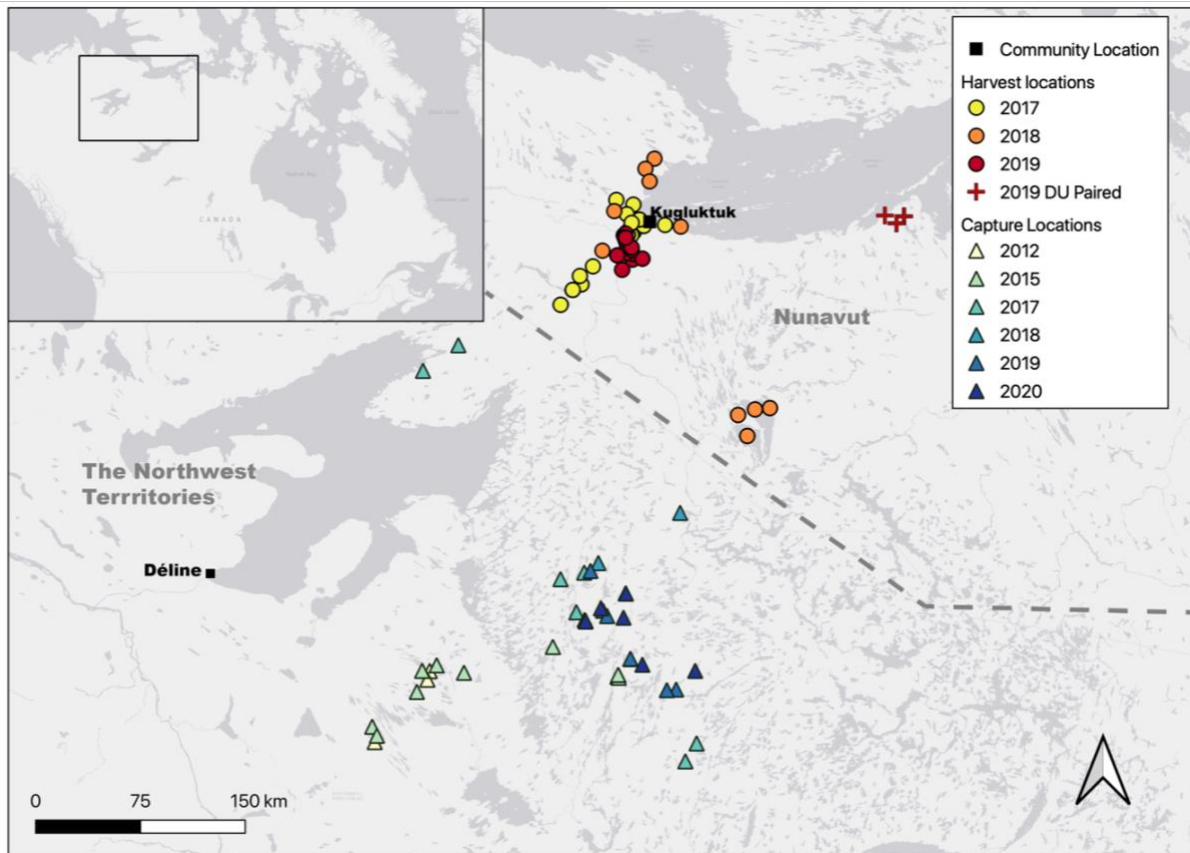


Figure.3.1. Map of sample collection sites for Bluenose-East (BNE) caribou from submitted hunter sampling kits (circles), and capture/collaring (triangles) activities by the Government of the Northwest Territories from 2012-2020. Dolphin and Union (DU) caribou paired hair sampling locations from 2019 hunts indicated by red crosses.

3.3.2. Sample processing

Hair samples were either shed from the skin (captures) or a piece of skin with hair attached was submitted frozen. For hide samples, 150-200mg of hair was shaved 2-3mm from the skin surface using a stainless-steel razor. Hair was washed in a 500mL glass beaker with 200-300 mL of 99% lab grade acetone (Acetone (Certified ACS), Fisher Chemical™), followed by three washes in 200-300 mL of Type 1 ultrapure water, and a final acetone wash, with each wash lasting 2-3 minutes in duration and consisting of swirling with plastic tweezers (modified from International Atomic Energy Agency, 1976). In the presence of noticeable surface contamination (blood, feces), samples underwent the washing protocol a second time.

Washed hair was air-dried prior to placement into paper envelopes, and then oven dried at 50°C for 24 hours. If present, visible hair follicles were removed from washed and dried

samples using stainless steel Metzenbaum scissors. 80 ± 10 mg of washed and dried hair was weighed and placed in a digester vessel (TMF vessel, 100mL; Milestone™) and 2mL of 70% HNO₃ (Nitric Acid (TraceMetal™ Grade), Fisher Chemical™) was added. Hair was digested within vessels using a high-pressure microwave digester (ETHOS EZ Microwave Digestion System, Milestone, Sorisole, Italy). The digester temperature gradually increased to a peak of 220°C over an hour, and the maximum temperature was held for 10 minutes. Following digestion, materials were left to cool to room temperature for 60 minutes.

Digested samples were decanted into 15mL Falcon™ round bottom polypropylene test tubes, the digester vessels were then rinsed twice with 1mL of Type 1 water and decanted into the polypropylene test tubes, which were then stored at 5°C. Within each digestion cycle, a 30-50mg of DORM-3 certified reference material (NRC, 2007) and an acid blank control (2 mL HNO₃ and 2 mL of type 1 water) were included and were treated the same as hair samples. Hair trace element concentrations were determined at the Alberta Center for Toxicology, University of Calgary. There, digested samples were further diluted 10X using Type 1 water and a 15-element panel analysis was carried out using inductively coupled plasma mass spectrometry (ICP-MS, 8800 Triple Quadrupole ICP-MS, Agilent). Two controls (NIST SRM 1640a and Multi-element Standard (SCP Science)) were tested prior to sample injection and at the end of the run. The acceptable criteria for controls were $\pm 20\%$ of the certified values for all elements. A duplicate ICP-MS read of one digested hair sample was included within every digestion cycle.

3.3.3. Determination of hair growth year

Samples included both non-growing (collected between October-June) and potentially growing (collected between July-October) caribou guard hair. Depending on when the hair was collected it may be representative of current or previous year hair growth. For non-growing hair, samples collected in October-December were current year hair growth, and samples collected in January-June were categorized as previous year hair growth (i.e., a 2018 January hair sample is representative of 2017 growth). Samples collected in July-September were categorized as current year growth or previous year growth based on length. The length of all hair samples collected between July-September were measured from above the skin surface to the hair tip three times, and the averaged length was used. An optimal cut-off between the length of non-growing winter

and growing summer hair was calculated using receiver operating characteristic (ROC) analysis (Brown and Davis, 2006), using the “pROC” R package (Robin et al., 2021). Said cut-off was calculated using the length of known non-growing winter hair (n=32) collected in January-February, and the length of the growing summer hair (n=16) collected in July-September.

3.3.4. Statistical analysis

If most samples for an element ($\geq 80\%$) were above LOQ, all sample results for that element were included at the reported quantification including the few samples that were below LOQ, as was the case for Mo and Pb. Values that fell below the limit of detection of ICP-MS were treated as half of the LOQ value (Vikøren et al., 2005). Elements in which most samples ($\geq 80\%$) fell below the LOQ were excluded from statistical analyses (Appendix B). Lastly, duplicate values within each ICP-MS digestion cycle were averaged for the purpose of analysis.

3.3.4.1. Body region differences (DU dataset)

To assess differences between anatomic sampling sites (neck and rump hair), we used linear mixed effect models using the lme4 (Bates et al., 2015) package in R version 3.6.0 (R Core Team, 2020). To account for repeated sampling of the same individual, we used animal ID as a random effect in all models. Correlations between element concentrations in neck and rump hair were measured using a Pearson’s rank correlation coefficient (r) in the absence of outliers, and Spearman’s rank correlation (r_s) if outliers were detected (McCrum-Gardner, 2007). A Rosner’s test from the EnvStats package (Rosner, 1975; Millard, 2013) was used on HECs to detect outlier values. The normality of the distribution of values was assessed using a Shapiro-Wilk test.

3.3.4.2. Effects of Age (BNE dataset)

The effects of age were determined solely using hunted samples, as captured animals were all adults. Age of hunted animals was determined from tooth eruption patterns of hunter submitted jaws, and animals were classified as sub-adults (< 3 years) or adults (> 3 years) at the time of harvest (Miller, 1972; CARMA). Age information was missing for 37 hunted animals, because of this reduced sample size the effects of age were modelled separately. Linear models

that fit year (2016-2019), sex (male/female), and age (sub-adult or adult) were fit, and details of models can be found in Appendix B.

3.3.4.3. Effects of Key Covariates (BNE dataset)

We fit several multi-variable regression models to assess the effects of sex (male or female), sampling source (hunted or captured), year (2012-2019), and anatomic sampling site (neck, rump, back). Samples from 11 hunted animals and 8 captures either had missing sex (n=11) or anatomic sampling site information (n=8), and were removed from all further analyses, resulting in a total sample size of 118 animals. The covariates of sampling source and anatomic sampling site are confounded because each sampling source only included a subset of possible anatomic hair sampling sites. Therefore, these covariates were examined separately in models. A box-cox transformation (Sakia, 1992) was applied to all trace element concentrations to determine optimal data transformation and satisfy linear regression assumptions. Models were compared using Akaike's Information Criterion corrected for small sample size (AICc; Burnham & Anderson, 2004), and the best fit model was selected based on the lowest AICc value. The sampling year was either modelled as categorical, linear, or polynomial (second and third-order) for each element. Details for all models for each element are presented in Appendix B. Shapiro-Wilk and Breusch-Pagan tests were used to assess normality and homoscedasticity of best fit model residuals, respectively. All statistics were completed using R (v3.6.0) statistical software (R Core Team, 2020).

3.4. RESULTS

A sufficient mass of hair was available for analysis from 83 hunted (2017-2019) and 54 live-captured BNE caribou (2012-2020), as well as 18 paired (neck and rump) samples from hunted DU caribou (2019). Elements of interest, Zn, Se, Cu, Mo and Pb all had >80% of values above the limit of quantification (LOQ) of ICP-MS and are presented in Table 3.1.

Concentrations of all elements included in the panel (15 total) are available in Appendix B.

Table.3.1. Trace element concentrations (mg/kg) in hair of Bluenose-East caribou from hunted (Hunt) 2016-2019, and captured (Capt) 2012-2019, animal sampling sources determined by ICP-MS. n denotes sample size, med denotes median, min is the minimum, max is the maximum, and <LOQ is the number of samples below the limit of quantification, n=137.

| | | 2012 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | | | | |
|-----------|------|-------|-------|-------|-------|--------|--------|--------|-------|--------|-------|--------|
| | | Capt | Capt | Capt | Capt | Hunt | Capt | Hunt | Capt | Hunt | | |
| Zn | n | 3 | 17 | 3 | 9 | 40 | 2 | 16 | 11 | 11 | 9 | 16 |
| | med | 56.02 | 69.77 | 78.53 | 76.82 | 74.97 | 100.71 | 90.31 | 72.01 | 89.56 | 75.76 | 91.379 |
| | min | 44.06 | 44.20 | 59.29 | 40.94 | 44.80 | 94.08 | 74.92 | 54.30 | 67.32 | 60.79 | 73.04 |
| | max | 80.59 | 91.47 | 79.92 | 95.49 | 146.82 | 107.35 | 117.99 | 89.66 | 133.71 | 89.72 | 143.23 |
| | <LOQ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Se | med | 0.28 | 0.31 | 0.36 | 0.28 | 0.39 | 0.39 | 0.40 | 0.31 | 0.37 | 0.30 | 0.36 |
| | min | 0.24 | 0.24 | 0.26 | 0.23 | 0.27 | 0.37 | 0.20 | 0.23 | 0.26 | 0.26 | 0.29 |
| | max | 0.37 | 0.47 | 0.40 | 0.39 | 0.61 | 0.40 | 0.64 | 0.41 | 0.50 | 0.34 | 0.45 |
| | <LOQ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mo | med | 0.06 | 0.04 | 0.03 | 0.04 | 0.03 | 0.04 | 0.04 | 0.04 | 0.04 | 0.03 | 0.02 |
| | min | 0.04 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.01 | 0.01 | 0.005 |
| | max | 0.06 | 0.08 | 0.05 | 0.06 | 0.13 | 0.05 | 0.14 | 0.09 | 0.06 | 0.05 | 0.05 |
| | <LOQ | 0 | 0 | 0 | 0 | 12 | 0 | 1 | 0 | 5 | 0 | 10 |
| Cu | med | 5.25 | 6.18 | 6.42 | 6.23 | 6.48 | 6.02 | 7.14 | 5.89 | 6.64 | 5.69 | 6.16 |
| | min | 3.88 | 3.95 | 4.47 | 4.68 | 4.28 | 5.76 | 5.47 | 4.23 | 4.44 | 5.16 | 4.97 |
| | max | 6.79 | 6.96 | 6.72 | 7.51 | 8.81 | 6.29 | 8.74 | 6.82 | 9.43 | 6.92 | 9.13 |
| | <LOQ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pb | med | 0.16 | 0.20 | 0.16 | 0.08 | 0.10 | 0.19 | 0.07 | 0.12 | 0.03 | 0.07 | 0.05 |
| | min | 0.10 | 0.03 | 0.14 | 0.04 | 0.02 | 0.12 | 0.03 | 0.04 | 0.01 | 0.03 | 0.01 |
| | max | 0.29 | 0.97 | 0.36 | 0.13 | 0.39 | 0.26 | 2.14 | 1.40 | 1.90 | 0.25 | 9.68 |
| | <LOQ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 1 | 2 |

3.4.1. Hair year classification

The optimal cut-off between winter and summer hair lengths was determined to be 3.4 cm. Of the 83 submitted hair samples from hunter kits, 59 samples collected between January to early August, were assigned as being the previous year's growth. Specifically, 41 hair samples collected in June/May 2017 were assigned as 2016 growth, 15 samples collected in January/February 2018 were assigned as 2017 growth, and three samples collected in early August 2019 were assigned as 2018 growth. The remaining 24 samples, collected from late August to October, were assigned as being the current year's hair growth.

3.4.2. Body location differences (DU dataset)

The majority (>80%) of paired DU samples of Pb hair concentrations were below LOQ, and therefore, body location differences for this element could not be assessed. As determined by

a Shapiro-Wilk test, Zn ($p=0.68$), Se ($p=0.49$), Cu ($p=0.47$) hair concentrations were normally distributed and not transformed, Mo ($p<0.001$) neck hair concentrations were not normal and were subsequently square root transformed for analysis.

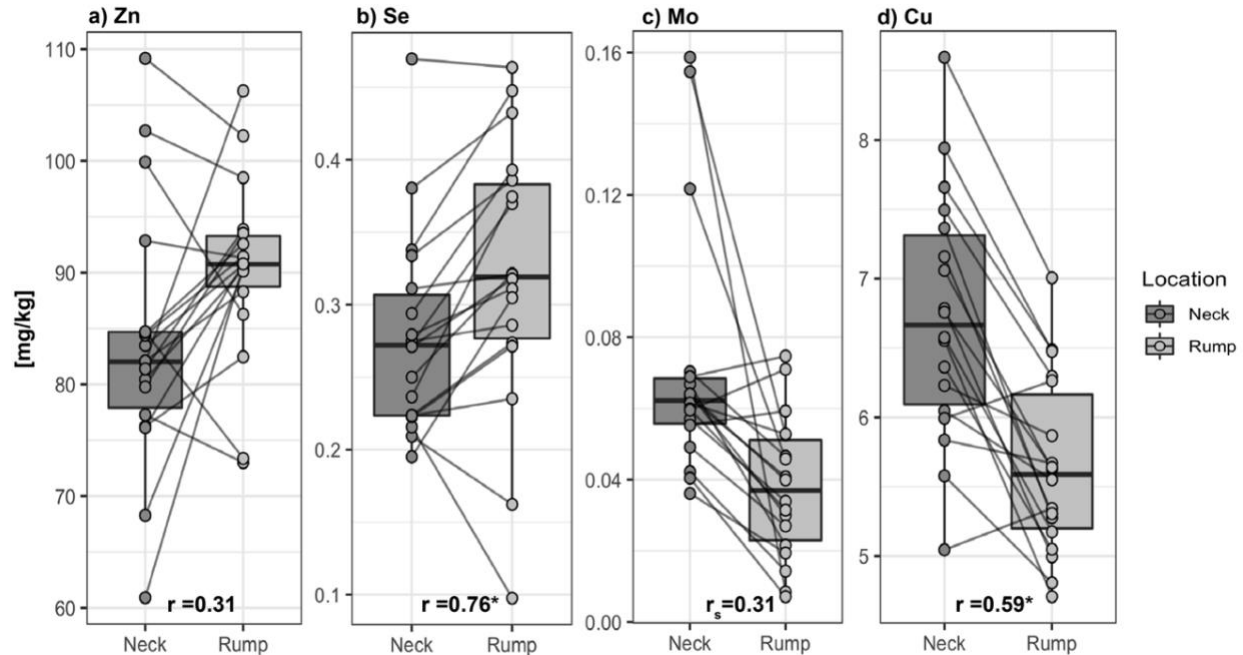


Figure.3.2. Trace element concentrations (mg/kg) in paired Dolphin and Union caribou neck and rump hair collected in 2019. r indicates Pearson’s correlation coefficient, r_s Spearman’s correlation coefficient, and * denotes a p -value <0.05 . Lines connect data points from the same individual.

To detect differences between body locations. Paired hair samples significantly differed between neck and rump concentrations for Zn, Se, Mo, and Cu ($p<0.05$), $n=18$, Figure.3.2. Nested ANOVAs were used to test the inclusion of sex; solely the best fit Zn model included sex as a covariate (Appendix B). Best fit models adhered to the assumptions of normality and constant variance of residuals. Paired sample element concentrations were significantly correlated for Se ($p<0.001$, $r=0.76$), and Cu ($p<0.001$, $r=0.59$), but not Zn ($p=0.21$, $r=0.31$) nor Mo ($p=0.07$, $r_s=0.31$). The best fit body region models had varying conditional R^2 values for Zn ($R^2_c=0.38$), Se ($R^2_c=0.72$), Cu ($R^2_c=0.56$), and Mo ($R^2_c=0.11$). Three outliers were detected within Mo hair concentrations using a Rosner’s test, and a Spearman correlation was used. In a sensitivity analysis, when Mo outliers were removed, neck and rump values were significantly correlated ($p=0.02$, $r=0.61$). Molybdenum concentrations were significantly more variable ($p=0.02$) within neck values compared to rump as determined by an F-test. Variances of other elements (Zn, Se, Cu) were not significantly different between body locations ($p>0.05$).

3.4.3 Age analysis (BNE dataset)

Thirty-five hunted animals from the BNE herd with known ages (10 sub-adults, 25 adults) were included for analysis. Results from a Mann-Whitney-Wilcoxon test showed no significant difference between median adult and sub-adult hair concentrations of Zn ($p=0.397$), Se ($p=0.653$), and Cu ($p=0.957$), Mo (0.815), and Pb ($p=0.397$). Within linear models (summarized in Appendix B), age and year were included as covariates in the best fit Zn, Se, Cu and Mo models. Age estimates included zero within 95% confidence intervals for every element and were thus not significant in any case.

3.4.4. Effects of Covariates (BNE dataset)

The best fit models for Zn and Se included year as a continuous variable with a linear relationship to hair concentration and included body sampling location (for Zn) or sampling source (for Se) (Table.3.2). The best fit models for Cu and Mo concentrations included a polynomial function of year (second and third order, respectively), and sampling source as explanatory variables. Lastly, the best fit model for Pb included the variables of sex, source, and year (linear continuous). The best fit models displayed normally distributed and homoscedastic residuals, except for the residuals of the best fit Pb model which was heteroscedastic.

Table.3.2. Summary of best fit model covariates for Zn, Se, Cu, Mo, and Pb, with representative best fit model (model), R^2 , covariates in best fit model, slope estimate (if applicable), 95% Confidence Interval, and associated p-value.

| element | model | R^2 | covariates | estimate | 95% CI | p-value |
|-----------|-------|-------|---------------------------|----------|---------------|---------|
| Zn | Zn4 | 0.25 | Year | 0.014 | 0.008: 0.02 | <0.001 |
| | | | Location(neck) | 0.007 | -0.026: 0.04 | 0.69 |
| | | | Location(rump) | -0.041 | -0.08: -0.006 | 0.02 |
| Se | Se7 | 0.23 | Year | -0.007 | -0.029: 0.01 | 0.52 |
| | | | Source(hunter) | 0.234 | 0.156 :0.31 | <0.001 |
| Cu | Cu8 | 0.13 | Year (poly ²) | NA | NA | <0.05 |
| | | | Source (hunter) | 0.30 | 0.013: 0.59 | 0.041 |
| Mo | Mo10 | 0.21 | Year (Poly ³) | NA | NA | <0.05 |
| | | | Source (hunter) | -0.13 | -0.25: -0.020 | 0.02 |
| Pb | Pb1 | 0.11 | Year | -0.20 | -0.38: -0.016 | 0.03 |
| | | | Sex (male) | -0.47 | -1.11: 0.16 | 0.14 |
| | | | Source (hunter) | -0.61 | -1.29: 0.07 | 0.08 |

Based on best fit models, HECs were not significantly different between sexes. The best fit model for Pb concentration included sex, however, the effect was not statistically significant ($p=0.076$). In a sensitivity analysis, the single Pb outlier (9.68 mg/kg) in a 2019 hunted female animal was removed, and neither model performance nor output was markedly altered.

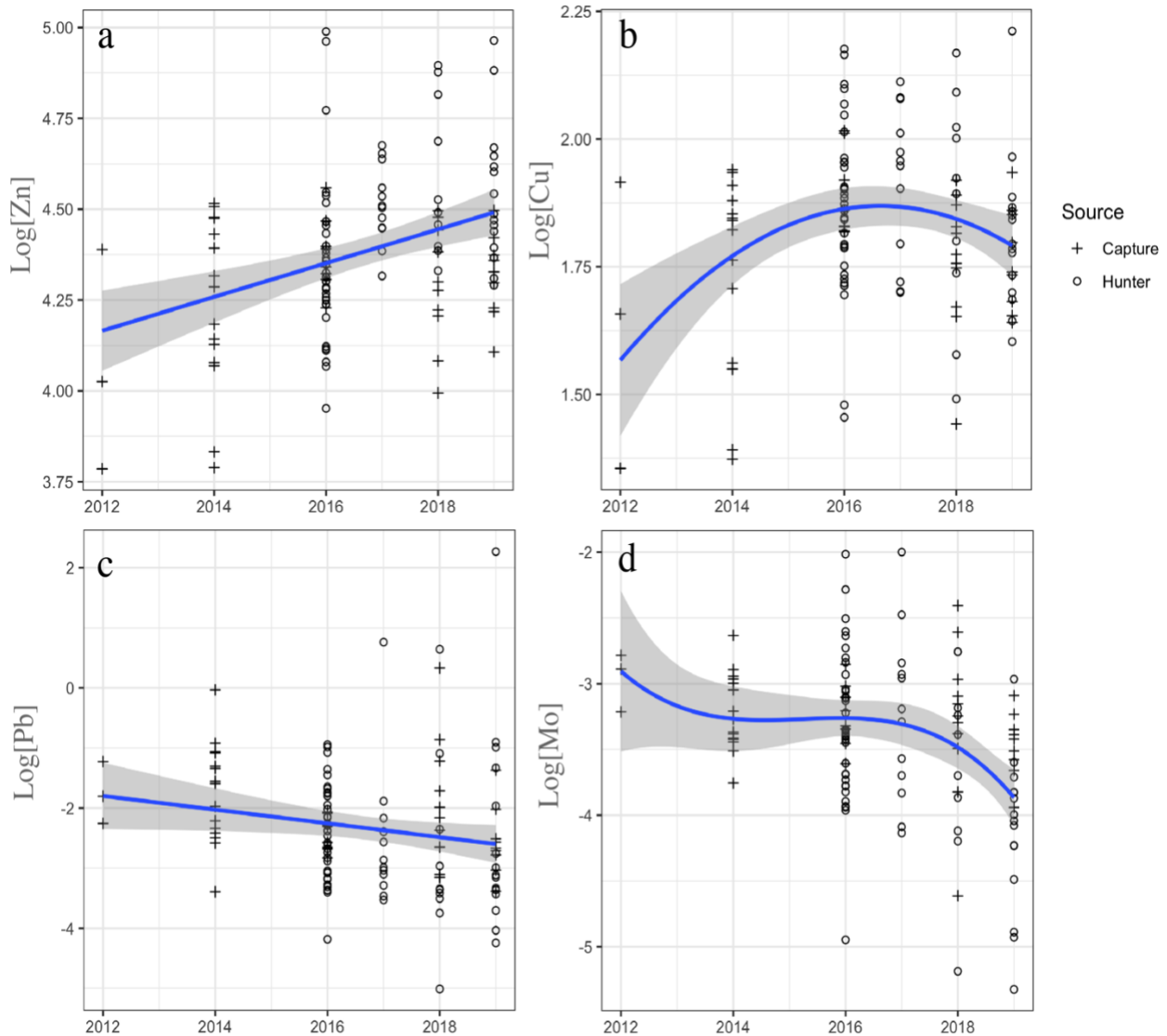


Figure.3.3. Log concentration of trace elements in BNE caribou hair between 2012 and 2019. Log zinc (Zn) (a), log copper (Cu) (b), log lead (Pb) (c), and log molybdenum (Mo) (d) concentrations (mg/kg) with corresponding linear model trend (blue line) and 95% confidence intervals (grey shading), $n=118$. Sampling sources are denoted by circles (hunter collected), and crosses (capture collected).

Best fit models for Zn, Cu, Pb and Mo indicated that HECs varied significantly from the 2012 to 2019 growth years (Table 3.2, Figure.3.3). Zn concentrations (Figure.3.3-a), increased

linearly from 2012 onward. Cu concentrations were best fitted with a second-order polynomial function (Figure.3.3-b), increasing over time, and appearing to peak in 2016/2017. Pb concentrations decreased linearly from 2012 to 2019 (Figur.3.3-c) and were the lowest in 2019. Lastly, Mo concentrations decreased following a third-order polynomial function of time (Figure.3.3-d).

The best fit models for Zn concentration included body location and hair collected from the rump was significantly lower ($p=0.03$) in concentrations compared to other body locations (neck, back). No other elements included body location in best fit models, but the 2nd best fit models for Cu and Mo included body location, replacing source as a covariate, but body location was not significant in its effect ($p>0.05$).

Se, Cu, Mo and Pb all included sampling source (hunted or captured) as a covariate within best fit models. Sampling source had a statistically significant effect within Mo, Se, and Cu models; hunted samples had higher Se and Cu concentrations, while capture samples had higher hair Mo concentrations. Although included as a covariate in the best fit model, the effect of sampling source was not significant for Pb models.

3.5. DISCUSSION

3.5.1. Hair Trace Element Concentrations

In this study we report hair trace element values of barren-ground caribou for the first time, and investigate key covariates associated with opportunistic health sampling of these populations. Concentrations for Zn, Se, Cu and Mo of BNE caribou hair fell within ranges previously reported in mountain caribou (*R.t caribou*) (Jutha et al., in press), moose (*Alces alces*) (O'Hara et al., 2001), and mule deer (*Odocoileus hemionus*) (Roug et al., 2015). A persistent outlier value of 9.68mg/kg of Pb in hair was detected in a hunted female. These values have been reported in the hair of cattle grazing within heavily polluted environments (Patra et al., 2007) or in top predators (Dietz et al., 2006), but not in free-ranging ungulates. Animals hunted with lead tipped ammunition exhibit elevated Pb concentrations in tissues (Johansen et al, 2004; Tsuji et al., 2009), and the effects are more pronounced near the wound channel (Gerofke et al., 2008), however no wound channel was present in any submitted samples. Alternatively, blood, feces,

and urine contamination are known to impact hair cortisol concentrations (Macbeth 2013) and were present on the Pb outlier sample, however, impacts on HECs are unknown. Within future hair collection protocols of *Rangifer*, emphasis should be placed on limiting surface contamination coupled with dry storage, and outlier values should be re-considered in the presence of visible surface contaminants.

Specified reference ranges of hair element concentrations are lacking for most wild ungulate species, including *Rangifer* (Poppenga et al., 2012). Concentrations of elements within blood, organ, and hair values differ significantly in the time scale they represent and their subsequent biological relevance. Organ values are representative of a mix between lifetime uptake, storage, as well as current metabolic status (O'Hara et al., 2001; Gamberg et al., 2020). Blood values are representative of immediate circulating concentrations that are strongly associated with current diet (Herdt and Hoff, 2011; Poppenga et al., 2012). Hair values on the other hand, are a cumulative indicator of circulating blood concentrations during the hair growth period and are representative of seasonal diet intake and metabolic use (O'Hara et al., 2001). Summer nutrition is a key factor governing the health of arctic ungulates (Couturier et al., 2009; Shively et al., 2019); hair, developing during this critical period, provides a unique annual marker of summer nutrition, which may reflect environmental availability of key trace elements relevant to caribou health.

3.5.2. Annual Trends in Hair Element Concentrations

We observed annual differences in concentrations of Pb, Zn, Cu, and Mo in BNE hair that may be attributed to differences in environmental availability of elements, changes in forage type, or alteration of resource selection by caribou. Directional and rapid climate change in the Arctic is associated with non-uniform shifts in plant community composition and encroaching shrubification across the caribou range (Myers-Smith et al., 2001; Tape et al., 2012; Reichle et al., 2018). A common shrub, dwarf birch (*Betula glandulosa*), growth and cover has increased by 25% since 2006 on parts of the barren-ground caribou range in the NWT (Andruko et al., 2020). Major taxonomic forage groups (graminoids, shrubs, lichens) significantly differ in trace element content of tissues (Oster et al., 2018). Toxic heavy metals (Pb, Cd) are generally elevated in lichen forage because of slow growth and high surface area (Crête et al., 1992; Nash and Gries,

1995; Robillard et al., 2002). Certain metals, such as Pb, are elevated in graminoids compared to shrubs while other metals, such as Zn, are elevated in shrubs (*Salix spp.*, *Betula spp.*) compared to graminoids (Ohlson and Staaland, 2001). During the summer and fall, the barren-ground caribou diet is most diverse and includes a mix of all taxonomic forage groups (Webber et al., 2022).

Two heavy metals were detected to vary linearly, Pb decreased linearly, and Zn increased linearly in BNE hair from 2012-2019. These trends may be reflective of an increased proportion of shrubs in the diet, or alternatively, may reflect trends in Arctic atmospheric deposition of heavy metals. Atmospheric Pb is decreasing ubiquitously in Arctic environments (McConnell and Edwards, 2008; AMAP 2015), while environmental Zn concentrations are more variable (AMAP 2002; Li and Cornett 2011; CCME 2018). Point source contamination, such as dust from diamond mining operations, elevate Pb and Zn levels of immediately surrounding forage (Watkinson et al., 2021), however caribou strongly select against these areas (Boulanger et al., 2012; Tłjcho, 2013; Plante et al., 2018). Lastly, Zn is under homeostatic control and physiological regulation of concentrations may complicate interpretation of hair values (Koh and Judson, 1986; Alonso et al., 2002). These findings are consistent with previous studies demonstrating a high variability of hair Zn values in ruminants (Miller, 1970).

Cu and Mo varied significantly interannually and inversely, but not following a linear trend. Of interest is a non-linear increase of Cu concentration in BNE hair. This is consistent with reported increasing renal Cu in the BNE caribou herd from 2005-2016, despite other barren-ground caribou populations demonstrating evidence of declines in renal Cu concentrations during the same timeframe (Gamberg et al., 2020). Cu deficiency is associated with reduced immune function in cattle (Scaletti et al., 2003; Scaletti and Harmon, 2012), as well as overall population decline in free ranging ungulates (Flynn et al., 1977; Barboza and Reynolds, 2004). In caribou, hepatic Cu is essential for nourishing the developing fetus (Gamberg et al., 2020) and is, therefore, a key element to monitor for health. However, hair Cu concentrations may only accurately represent Cu concentrations when an animal is deficient (Kellaway et al., 1978; Krohn and Kutz, unpublished data) and it is difficult to interpret hair Cu values of animals that are within normal ranges. Further, hair growth corresponds to a summer diet of Cu-rich grasses and

sedges that may result in stable circulating concentrations. Lastly, Cu and Mo exhibit an antagonistic relationship (O'Hara et al., 2001; Alonso et al., 2002). Both elements varied non-linearly from 2012-2019 but in opposite directions: Cu increased overtime and Mo decreased over time. Experimentally, Mo-rich fodder in cattle is associated with decreased Cu blood concentrations (Cunningham and Hogan, 1958), and molybdenosis may further exacerbate a sub-clinical Cu deficiency in wild ungulates (Flynn et al., 1977; O'Hara et al., 2001): this relationship may be partially contributing to the observed element trends from 2012-2019.

3.5.3. Body location differences

For the most part, hair collection in caribou has been undertaken for the purpose of genotyping, and a consistent anatomic sampling site was not specified (CARMA, 2008). Our results demonstrate that trace element concentrations significantly differed between anatomic sampling sites and these differences were not consistent between elements (Figure.3.2.). These results are supported by studies in mountain caribou (Jutha et al., in press), that demonstrated different correlations with kidneys or livers depending on anatomic sampling location (rump or shoulder). Conversely, differing body locations of cattle did not demonstrate a significant difference in hair element concentrations (Szigeti et al., 2015). Detected differences between anatomic sampling locations of caribou may be attributed to differences in hair growth rate, hair type, and hair colour. *Rangifer* hair grows non-uniformly (Cuyler and Øritsland, 2002), potentially resulting in trace elements being incorporated into the hair shaft differently during the growing season depending on body location. Furthermore, caribou pelage consists of guard hairs and a wool-like undercoat (Macbeth, 2013). This undercoat is very fine and adherent, and the full removal via hand sorting is unlikely for trace element analysis (Jutha et al., in press) nor hair cortisol analyses (Ashley et al., 2011; Macbeth, 2013; Carlsson et al., 2016). However, variability in the undercoat may explain differences in element concentrations between anatomic sampling regions in caribou that are not detected in cattle. Lastly, the colour of caribou pelage differs markedly between body regions and between ecotypes; coat colour is known to significantly alter Se concentrations in the hair of cattle (Christodouloupoulos et al., 2003), as well as pigs (*Sus scrofa domesticus*) (Kim and Mahan, 2001), and may be a significant source of variation in caribou hair trace element analysis that is not accounted for.

3.5.4. Sampling source covariate

In our study, as with many other wildlife investigations, we relied on different opportunistic sampling approaches to obtain a maximum sample size. Our results demonstrate that samples from hunted and captured caribou significantly differed in element concentrations, but these differences were element dependent. Captured animal hair was collected from numerous sampling locations and hunted animals from multiple age classes, thus age and sampling site are confounded within the sampling source variable. Although an analysis of the effects of age demonstrated no effect (Appendix B), non-adult animals were most often hunted and differences in age may be partially contributing to differences between sampling sources. Se, and Cu concentrations are well correlated between the neck and rump and follow opposite trends, Se is elevated in the rump while Cu is diminished. Yet, hunted samples were significantly elevated in both Cu and Se, thus differences in sampling sources are unlikely solely explained by anatomic site. These sampling sources inherently differ in purpose, e.g., hunted food versus capture for population studies, utilizing different methods (Dumond, 2007; Cattet 2011; GNWT, 2018), and sampling from vastly differing geographic areas (Figure.3.1.). Therefore, in the context of caribou hair collection, sampling sources most likely differ in ways beyond the inconsistent sampling of body regions and ages, and these sources (hunts or captures) are potentially selecting from differing subgroups of the herd. These differences must be recognized and accounted for when analyzing samples derived from different sampling sources and drawing conclusions therefrom.

3.5.5. Sex differences

Hair trace element of BNE caribou did not differ between sexes, yet renal mineral concentrations, most notably Cu, vary significantly between sexes in caribou (Gamberg et al, 2020). Specifically, female renal Cu concentrations are diminished during late gestation, with Cu being sequestered to the fetus to support fetal development. Furthermore, caribou exhibit sex differences in habitat selection with unique forage characteristics (Jakimchuk et al., 1987; Barboza et al. 2018). Similarly, concentrations of heavy metals of livers or kidneys in female wild ungulates tend to be elevated in females compared to males (Hermoso de Mendoza García et al., 2011; Danielsson and Frank, 2009), and these sexual differences may also be influenced by season (Crête et al., 1989). *Rangifer* bulls usually molt and develop new coats earlier (Cuyler

and Øritsland, 2002), which may translate to differing hair element concentrations due to differing hair growth rates. Contrary to these expectations, we did not detect sex differences in metal (Pb, Zn) nor mineral (Cu, Se, Mo) concentrations in BNE hair. The prolonged growing season of hair may not be sensitive to sex-specific physiological changes in caribou as seasonally circulating Cu levels may be more stable compared to Cu concentrations in the liver that are more dynamic and variable.

3.6. CONSIDERATIONS AND CONCLUSIONS

Hair sampling for the purpose of trace element analysis of caribou provides unique benefits that have the potential to complement traditional monitoring approaches. Nevertheless, we have identified several important considerations for hair collection protocols in caribou if these samples are to be used for trace element investigations, as well as key covariates to consider in data analyses. We highlight the need for a standardized hair collection protocol in caribou sampling that minimizes the confounding effect of varying anatomic sampling sites. Hide sampling should be further considered in the context of hunter-based sample collection that ensures the maintenance of hide integrity, economic value, and cultural use (Di Francesco et al., 2021), as well as minimizes any exogenous contamination with blood, feces, or urine. In our paired hair dataset, outlier values were only detected in neck samples, and rump samples have a more consistent variance for future sampling. Lastly, although passive hair collection (e.g., snags), has been used as a non-invasive sampling approach for other hair health indicators (Terwissen et al., 2013), results from our research imply that hair trace element analysis may not be suitable for passive hair collection where anatomic sampling site is unknown.

A unique quality of hair concentrations is that they can provide a marker of nutrition or heavy metal exposure that is cumulatively reflective of the hair growing season. This has the potential to complement traditional organ or environmental sampling approaches. Hair may be particularly beneficial for measuring longer-term trends in trace element concentrations without being confounded by seasonal dynamics. Hair from the BNE caribou did not reveal the fine-scale changes in nutrition that are typically captured in organ samples, such as sex-specific seasonal variation of renal Cu (Gamberg et al., 2020), possibly due to the timing of hair growth and the stability of circulating concentrations during the hair growth period. Thus, hair and serum/organ

samples remain complementary as they provide information from different temporal resolutions. A lack of hair concentration reference ranges for *Rangifer* still presents a major barrier to health indicator interpretation and knowledge use, which extends to other wild ungulate species monitoring. When considering hair's practical advantages, increased adoption of hair element analysis in wild ungulate management is a promising tool for consistent health monitoring of summer nutrition and heavy metal exposure.

3.7. ACKNOWLEDGEMENTS

We would first like to thank the hunters from the community of Kugluktuk, Nunavut and the Kugluktuk Angoniatit Association for completing and submitting the samples necessary for this research to happen. We would also like to thank Judy Williams, Kirstyn Falck, and Brett Elkin of the Government of the Northwest Territories for providing the captured hair samples and offering capture insights. Our thanks are also extended to Angie Schneider and James Wang for their support in the laboratory. This study was supported financial by the W. Garfield Weston Scholarship Foundation/Association of Canadian Universities for Northern Studies, the Northern Scientific Training Program, Alberta Graduate Excellence Scholarship and University of Calgary Graduate Scholarship. Further, we would like to thank our research group funding agencies, Environment and Climate Change Canada, Polar Knowledge Canada, Irving Maritime Ship Building, the Government of Nunavut, and the Government of the Northwest Territories, and the Northern Contaminants Program.

**CHAPTER 4: ANNUAL, SEASONAL, AND INTER-HERD
VARIATION OF HAIR CORTISOL IN BARREN GROUND
CARIBOU (*RANGIFER TARANDUS GROENLANDICUS*) FROM
2012-2020**

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4.1. ABSTRACT

The measure of glucocorticoids in keratinized tissues such as hair, provides a seasonal measure of hypothalamic-pituitary-adrenal (HPA) axis activity that is being used to monitor wildlife populations. The barren-ground caribou (*Rangifer tarandus groenlandicus*) is a species of conservation concern in Canada that is experiencing increased cumulative stressors associated with warming in the Arctic. Caribou guard hairs predominantly grow from June-October, and hair cortisol concentration (HCC) provides one measure of allostatic load during that period. The objectives of this research were threefold: first, we assessed the impact of body region (neck, rump) sampling sites on HCC of caribou; second, we sought to assess key covariates possibly impacting HCCs of caribou; and third, was to examine the association between HCCs and indices of biting insect activity on the summer range (oestrid Index, mosquito Index). Linear mixed-effect models were used to assess the effect of body region, while generalized least squares regression (GLS) models were used to examine the impacts of key covariates and indices of biting insect harassment. Fixed effects included in the models were: herd (Dolphin and Union, Bluenose-East), sex (male/female), month category (1-6), sample source (hunted/captured) and indices of biting insect activity (oestrid index, mosquito index). The top 4 models that fit within 2 AICc of one another were averaged for final estimates. Body region was not a significant predictor of HCC ($p=0.384$). Year was a significant predictor of HCC ($p=0.026$) and decreased linearly for both herds. HCC was seasonally variable ($p<0.001$) and peaked in spring (May/June). DU caribou had significantly higher cortisol concentrations compared to the BNE ($p<0.001$). Indices of biting insect harassment were variably incorporated into top models and were not significantly associated with HCC. This study identified essential covariates impacting the HCC of caribou that must be accounted for in future monitoring, sampling, and statistical analyses.

Keywords: Hair Cortisol, Biting Insect Harassment, Caribou, Wildlife Health, Wildlife Monitoring

4.2. INTRODUCTION

The barren-ground caribou (*Rangifer tarandus groenlandicus*) is a keystone species in Canada, however, multiple populations have experienced enigmatic declines in the last decade (Vors & Boyce 2009; COSEWIC 2016). These declines are believed to be associated with an increase in cumulative stressors, such as climate change and increased anthropogenic development in the Arctic environment (Festa-Bianchet et al., 2011; Fauchald et al., 2017; Parlee et al., 2018). Challenges to physiologic homeostasis and resulting allostatic load are capable of being monitored by biomarkers of HPA axis activity, such as glucocorticoids (GC) (Baker et al., 2013). Among wildlife, prolonged and chronic elevation of GC concentrations have been documented to result in deleterious impacts on the individual health such as reduced immunocompetence and decreased reproduction that may have population impacts (Bonier et al., 2009; Busch & Hayward 2009). The dominant GC circulating in *Rangifer* is cortisol (Koren et al., 2012), and historically cortisol concentrations have been used to investigate stress in *Rangifer spp.* (Macbeth, 2013; Ashley et al. 2011, Carlsson et al., 2016; Ewacha et al., 2017).

Hair cortisol concentrations (HCC) have been categorized as a long-term health indicator that is representative of chronic or long-lasting stress experienced over weeks to months (Gormally et al., 2020). Cortisol is incorporated into the hair shaft passively from the bloodstream during the anagen (active) hair growth phase, reflecting the HPA axis activity at that time (Russell et al., 2012). The HPA axis activity encompasses both circulating free cortisol as well as locally synthesized cortisol being incorporated into the hair fibre (Russell et al., 2012). Experimentally, a single ACTH injection in *Rangifer* did not result in a detectable difference in HCC (Ashley et al., 2011). However, studies in other species where multiple ACTH injections were administered over a prolonged period to better simulate long-term stressors, resulted in significantly higher cortisol in hair (Dulude-de Broin et al., 2019; Di Francesco et al., 2021). In the field, HCCs have been applied to monitor the stress response of wildlife to environmental disturbance (Bryan et al., 2013), anthropogenic disturbances (Ewacha et al., 2017), and parasitism (Madslie et al., 2020), among other stressors.

Changes in the Arctic environment such as warmer temperatures and increasing precipitation are forecasted to favour increased biting insect abundance and subsequent biting insect harassment on the tundra (Witter et al., 2012). The impacts of biting insects such as black

flies, warbles, bots, and mosquitos have been documented in caribou, primarily concerning modifying behavioural activity budgets, i.e, increased time spent running or exhibiting avoidance behaviours and reduced time foraging (Colman et al., 2003). Altered activity budgets can be physiologically taxing, and prolonged harassment may lead to reduced body condition (Weladji et al., 2003) and decreased odds of a successful pregnancy (Cuyler et al., 2012). Biting insect activity indices derived from meteorological data are available for multiple barren-ground caribou ranges and provide a ratio of hours of high insect harassment to total hours from June 15th-September 1st (Witter et al., 2014).

Rangifer pelage consists of guard hairs and a woollen undercoat. Guard hair growth may be understood as two cycles. The first is active guard hair growth, which although not specifically documented, is generally understood to occur between June-October (Cuyler & Øritsland, 2002; Macbeth, 2013). The second, is a non-growing quiescent phase, that occurs between November to May. *Rangifer* pelage also has an annually persistent undercoat, whose period of growth is not documented, and is partially removed for HCC analysis (Ewacha et al., 2017). Lastly, *Rangifer* exhibit a single annual molt in May/June (Macbeth 2013) and hair collected at this time is generally shed (Rakic personal obs). Shed hair is dissociated from the follicle, but still contains a hair bulb (Higgins et al., 2009).

In this study, we analyzed hair cortisol in samples from both hunter-based and government capture/collaring sampling operations from two herds of barren-ground caribou, the Bluenose-East (BNE) and Dolphin Union (DU) populations. The primary objective of this research was to first, assess the use of hair cortisol as a biomonitoring tool by examining key covariates possibly impacting the HCC of caribou, and second, examine the association between hair cortisol concentrations and indices of biting insect activity on the summer range (Oestrid Index, Mosquito Index). There were three primary research questions associated with this study:

(1) Is there a significant difference between HCCs of neck and rump hair sampled from the same individual in caribou?

(2) What factors associated with the opportunistic sampling of caribou, (sex, season, herd, and sample source) are significant in their impact on hair cortisol variation in barren-ground caribou?

(3) Is hair cortisol associated with a source of chronic environmental stress during the hair growth period, specifically, is HCC positively associated intensity of biting insect harassment?

4.3. METHODS

4.3.1. Study area and sample collection

Caribou hair samples from the Bluenose-East (BNE) and Dolphin and Union (DU) herds were collected and archived from two opportunistic health sampling sources. First, samples were collected by subsistence hunters from 2012 to 2021 from the communities of Déline, Norman Wells, and Ulukhaktok of the Northwest Territories (NWT), as well as the communities of Kugluktuk and Iqaluktuutiaq, Nunavut (NU). Second, convenience samples that were collected by the Governments of the Northwest Territories (GNWT) and Nunavut (GN). Paired neck and rump samples (n=34) from the same individual adult female animals were also collected during 2021 DU caribou capture/collaring to investigate the impacts of body region on HCC.

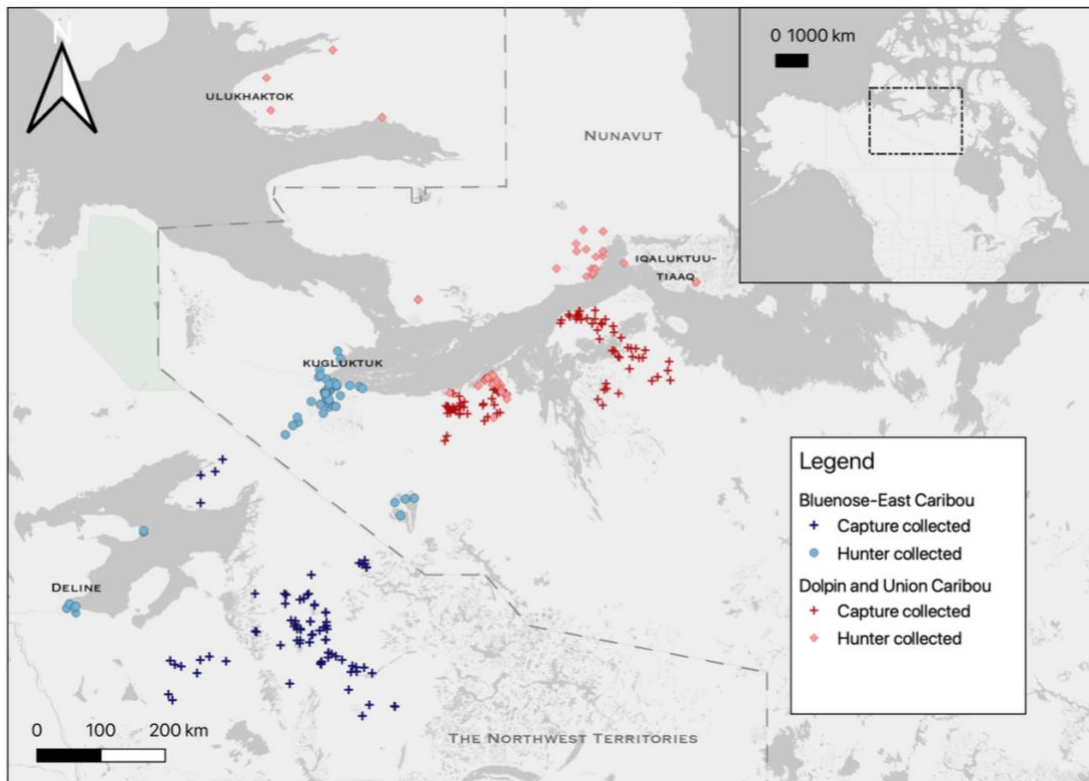


Figure.4.1. Map of Bluenose-East (blue) and Dolphin and Union (red) sampling locations, as collected by hunters (circles) and captures (crosses) between 2012-2020.

Hunted samples were collected year-round, but predominantly during common harvesting periods, the summer season for the BNE, and Fall/Spring for the DU. Hair samples from hunted animals were collected almost exclusively from the neck body location. Captured samples were taken during the month of March for the BNE and the month of April for the DU herd. Captured hair samples from the BNE were taken from various anatomical locations, such as the neck, rump, and shoulder, while DU captures were taken consistently from the neck or rump. Captured samples were generally of shed hair, that contained a hair bulb. Summaries of total samples are presented in Table.4.1.

4.3.2. Sample Processing and laboratory analysis

All hair cortisol quantification was performed by the Endocrinology Laboratory of the Toronto Zoo. Hair decontamination, preparation and steroid extraction were completed as described by Mastro Monaco et al., (2014) and used in Di Francesco et al., (2021). Briefly, hair follicles and undercoat if present, were removed, such that solely the guard hair remained. Samples were washed by immersing them in 669ml plastic containers filled with distilled water, samples were rubbed by hand for 2 minutes and then dried in a paper towel. Hand washed hair was then placed in 20ml glass vials, vortexed with 15ml of distilled water for 10s, then soaked for 5m. The liquid was removed, and a second 15 ml of distilled water was added, vortexed for 10s, and then immediately removed. Finally, 15ml of 100% methanol was added, vortexed for 10s and removed immediately. Washed hair was dried in a paper towel and then stored in paper envelopes at room temperature.

Washed and dried hair was cut into 5mm pieces and weighed into 7ml scintillation vials. 50mg of hair were extracted with 100% methanol, for a ratio of 0.01g of hair/ml of methanol, on a rotator plate (MBI Lab Equipment orbital shaker, 100rpm) at room temperature for 24 hours. Samples were centrifuged for 5 min (at 2400 x g), and the supernatant was pipetted into new glass 7ml vials. Supernatants (hair extracts) were then stored at -20C. Samples were brought to room temperature and 1500 µl of hair extract were evaporated in a fume hood and the dried extract was reconstituted using 150 µl of EIA buffer, forming a 10X concentration. Cortisol was quantified using EIAs previously described by Majchrzak et al., (2015) and Kummrow et al.,

(2011) and used in Di Francesco et al., (2021), and all samples and standards were run in duplicate.

4.3.3. Data processing

Date of sample collection, location, and sex of animal were obtained from submitted hunter forms and government databases. Based on harvest/capture date, ordered month categories (1=January/February, 2=March/April, 3=May/June, 4=July/August, 5=September/October, 6=November/December) were created. Year was classified based on hair growth year, not the year of sample collection or analysis. Depending on the date of hair collection, hair was either representative of current year growth or previous year growth, requiring hair to be individually classified. Hair collected between January-June was classified as previous year hair growth and samples collected between October-December as current year growth. Hair collected between July-September was classified based on a cut-off length determined by Rakic et al., (Chapter 3). Samples below the limit of hair cortisol quantification, reported as <1.00 were treated as low values and assigned 0.5 pg/mg. Only adults and sub-adults were included in statistical analyses with 8 calves removed from analyses. Animals of unknown sex were excluded (n=10). Resulting in a sample size of 407 animals for analysis. Lastly, hair cortisol concentrations were log-transformed to satisfy assumptions of linear regression.

4.3.4. Biting Insect Harassment Indices

Both an Oestrid activity index (OI) and mosquito activity index (MI) developed by Witter et al. (2012) were used in this study, according to a user guide developed by Witter, (2014). Daily OI and MI index estimates were obtained from the CircumArctic Rangifer Monitoring and Assessment Network (CARMA), from the BNE and DU summer ranges. Indices were restricted to the period of biting insect activity from June 15th – to September 1st (Witter 2014), and the average activity for that period was calculated. If a caribou was harvested between June-September, then the mean insect harassment index was calculated up to the date of harvest for that individual animal (i.e., an animal harvested on August 18th, would have an average harassment from June 15th-August 18th).

4.3.5. Body location differences

We compared hair cortisol concentrations in hair from the rump and neck by first comparing median values using a Wilcoxon signed-rank test. Second, an F-test was used to compare possible differences in inter and intra-body location variation. Third, correlations between neck and rump hair concentrations were measured using Pearson's and Spearman's correlations. In a sensitivity analysis, these correlations were repeated upon removal of outlier values (n=2). Last, the effects of body region (rump or neck) were assessed using a linear mixed-effects model (lme4 R package (Bates et al., 2015)). Animal ID was used as a random effect to account for repeated sampling of the same individual (Paterson & Lello, 2003) and body region (neck/rump) was a fixed effect explaining the log hair cortisol concentration of 34 animals. All animals were female adults that were sampled using the same method in 2021.

4.3.6. Covariates predicting HCC

Using the full dataset of all DU and BNE hair (n=407), we assessed the association of multiple covariates on hair cortisol concentration. Models were fit with six fixed effects: sex (male/female), herd (BNE/DU), month category (1-6), Oestrid index (OI), mosquito index (MI), and sample source (hunted/captured). Month categories were tested as categorical or linear continuous variables. Year as a categorical factor (2012-2020) was a random effect within all models. Mixed effect linear regression was fit using the lme() function. However, following log-transformation, box-cox transformation, and weighted regression, persistent heteroskedasticity of best fit models was detected. To overcome these barriers to analysis, generalized least squares regression (GLS), which accommodates violations of standard regression assumptions (Beale, et al. 2010), was done using the gls() function, from "nlme" package. Based on multiple top models including different covariates and being within 2 AIC_c of one another, model averaging was used to generate covariate estimates, confidence intervals, and statistical significance. The global model was fit using the dredge() function from 'MuMIN' (Bartoń, 2020) such that all possible combinations of fixed effects and interactions were fit. The set of top models was averaged using the model.avg() function and averaged effect sizes of included fixed effects were extracted. We considered variables to be informative if their confidence intervals did not include zero.

4.4. RESULTS

A sufficient mass of hair for analysis was collected from 201 BNE caribou (84 hunted, 117 captured) and 206 DU caribou (116 hunted and 90 captured). Samples from the BNE herd were approximately equal between sexes (F=106, M=97), however, for the DU herd, predominantly female caribou were sampled (F=171, M=35). Hair cortisol ranged from <1.00 pg/mg to 51.89 pg/mg (Table 1). Numerous hair samples (n= 19) had low cortisol levels below LOQ (<1.00 pg/mg) and were assigned a value of 0.50 pg/mg, one-half of the LOQ, for analysis. Hair samples below LOQ were predominantly from the BNE herd.

Table.4.1. Sample size (n), median, and range (min, max) of guard hair cortisol concentration (pg/mg) from captured (Capt) and hunted (Hunt) barren-ground caribou from the Bluenose-East (BNE) and Dolphin and Union (DU) herds.

| | | | 2012 | 2014 | 2015 | 2016 | 2017 | 2018 | 2019 | 2020 |
|------------|------|----------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| <i>BNE</i> | Capt | n | 3 | 21 | 17 | 17 | 15 | 15 | 29 | - |
| | | med | 2.53 | 2.21 | 3.15 | 1.55 | 2.08 | 2.48 | 2.25 | - |
| | | min | 1.52 | 1.06 | 1.17 | 0.50 | 0.50 | 1.15 | 0.50 | - |
| | | max | 3.28 | 6.36 | 8.91 | 6.34 | 4.79 | 6.11 | 4.54 | - |
| | Hunt | n | - | - | - | 48 | 12 | 8 | 16 | - |
| | | med | - | - | - | 4.29 | 2.61 | 1.56 | 1.83 | - |
| | | min | - | - | - | 1.66 | 0.50 | 0.50 | 0.50 | - |
| | | max | - | - | - | 51.89 | 15.21 | 3.03 | 8.06 | - |
| <i>DU</i> | Capt | n | - | - | - | - | 50 | - | - | 40 |
| | | med | - | - | - | - | 8.96 | - | - | 5.21 |
| | | Min | - | - | - | - | 2.91 | - | - | 2.83 |
| | | max | - | - | - | - | 15.88 | - | - | 13.29 |
| | Hunt | n | - | - | - | - | 40 | 40 | 22 | 14 |
| | | med | - | - | - | - | 8.69 | 5.63 | 2.96 | 2.48 |
| | | Min | - | - | - | - | 4.73 | 1.54 | 0.50 | 1.41 |
| | | max | - | - | - | - | 24.76 | 12.01 | 6.81 | 3.94 |

4.4.1. Body location HCC

Thirty-four paired hair samples from the neck and rump of captured DU caribou in 2021 were analyzed for hair cortisol concentration. Cortisol concentrations were not correlated between body regions using a Pearson's ($r=0.17$, $p=0.35$) and Spearman's ($r_s=0.27$, $p=0.12$) correlations. However, in a sensitivity analysis, two outlier values were removed, resulting in

cortisol concentrations between body regions significantly correlating with one another ($r=0.44$, $p=0.01$; $r_s=0.38$, $p=0.03$). Neck hair variation (5.89 pg/mg) of cortisol concentration tended to be greater than that of the rump (2.71 pg/mg), but these variances were not statistically different ($F=1.45$, $p=0.29$). Median values did not significantly differ between neck and rump (Wilcoxon $p=0.23$) and results from linear modelling (Appendix C) demonstrate that body region was not a significant covariate predicting hair cortisol concentration ($p=0.38$). Linear models adhered to the assumptions of linear regression analysis.

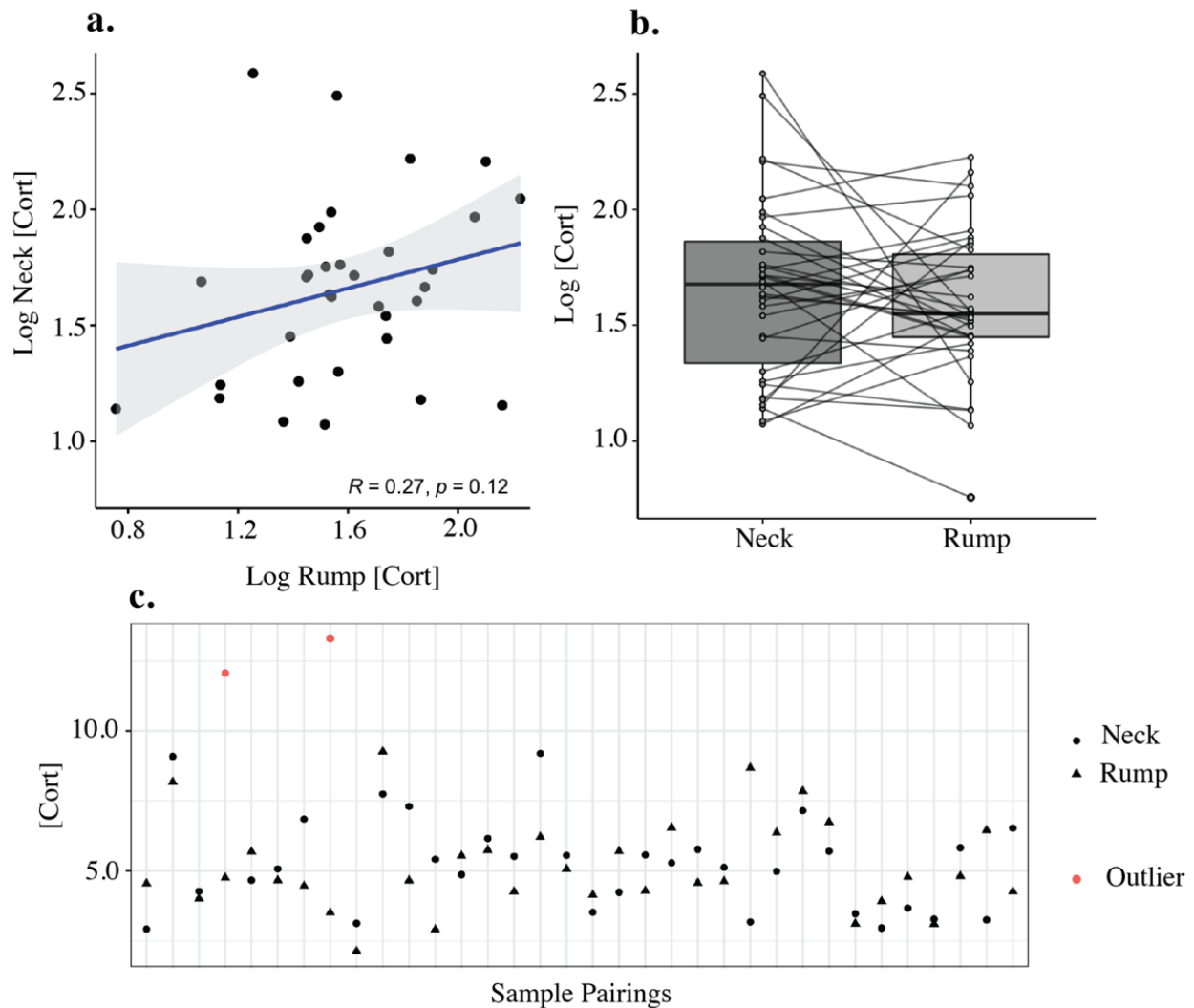


Figure.4.2. Comparison of log cortisol concentration (pg/mg) of caribou hair between paired neck and rump samples of 34 unique DU caribou from 2021. (a.) scatter plot comparing neck and rump concentrations, linear regression line (blue), associated 95% confidence interval (light grey), Pearson correlation (R), and associated p -value. (b.) boxplot comparing median neck and rump concentrations, lines connect datapoints from the same individual. (c.) scatter plot showing hair cortisol (pg/mg) between neck (circle) and rump (triangle) pairings, and outliers (red).

4.4.2. Predicting HCC in Barren-ground Caribou

Of 128 total possible combinations of models to predict caribou hair cortisol concentration, the 4 top models were within 2 AIC_c of the top model (Appendix C). Model averaging identified sex, herd, month categories, year and source as being significant predictors of HCC. Indices of oestrid and mosquito activity were not significant predictors of HCC. Interaction terms, although included in dredged models, were not present within top fit models in which averages were derived. Pseudo R² values of top models were equal to 0.29.

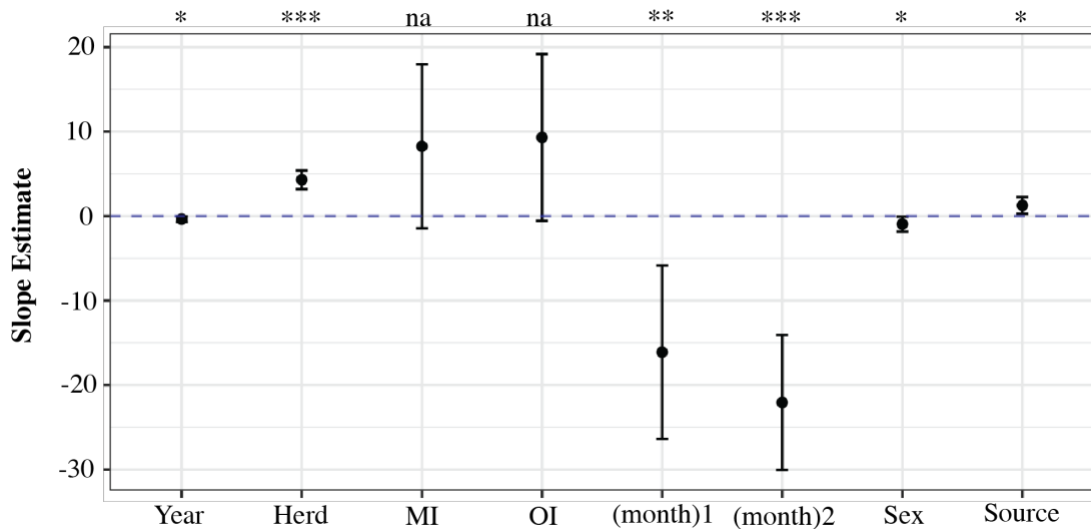


Figure.4.3. Forest plot of covariates (x-axis) predicting hair cortisol concentration in caribou with the associated generalized least squares regression slope estimate (y-axis). Confidence intervals, and statistical significance (na= $p > 0.05$, * = $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$) are denoted. Estimates were averaged from four top models ($\Delta AIC_c < 2$). Covariates included are Year (2012-2020), Herd (Du reference), MI (mosquito index), OI (oestrid index), month category (1-6), Sex (male reference) and sample source (Hunter reference). (Month) 1 and (month) 2 describe estimates from a 2nd order polynomial function.

4.4.3. Covariate Effects

Five covariates whose 95% confidence intervals did not include zero were informative; year (2012-2020), herd (DU/BNE), sex (male/female), month category (1-6), and source (hunted/captured), Figure.4.3. All these covariates were included in all the top 4 averaged models. Herd was a significant covariate in the 4 averaged models ($p < 0.001$), and the DU herd had consistently higher HCC values compared to the BNE. Month category followed a 2nd degree polynomial trend, Figure.4.4, with HCC concentrations increasing from January/February, peaking in May/June, and subsequently decreasing to November/December.

Sex was a significant predictor of HCC, female animals had significantly higher HCC values compared to males. However, in a sensitivity analysis outlier value ($n=3$, two female, one male, $HCC > 20$ pg/mg) were removed and the effects of sex were no longer statistically significant. Further, upon removal of outliers, the effects of sample source became borderline significant ($p=0.051$), while other covariate estimates remained similar. Year was significant predictor of HCC and decreased linearly for both herds, 2012-2019 for the BNE, and 2017-2020 for the DU, and was included in 3 of the top 4 models. Lastly, the mosquito index (MI) was included in one, and the oestrid index (OI) was included in two of the top 4 models but were not significant predictors of HCC values.

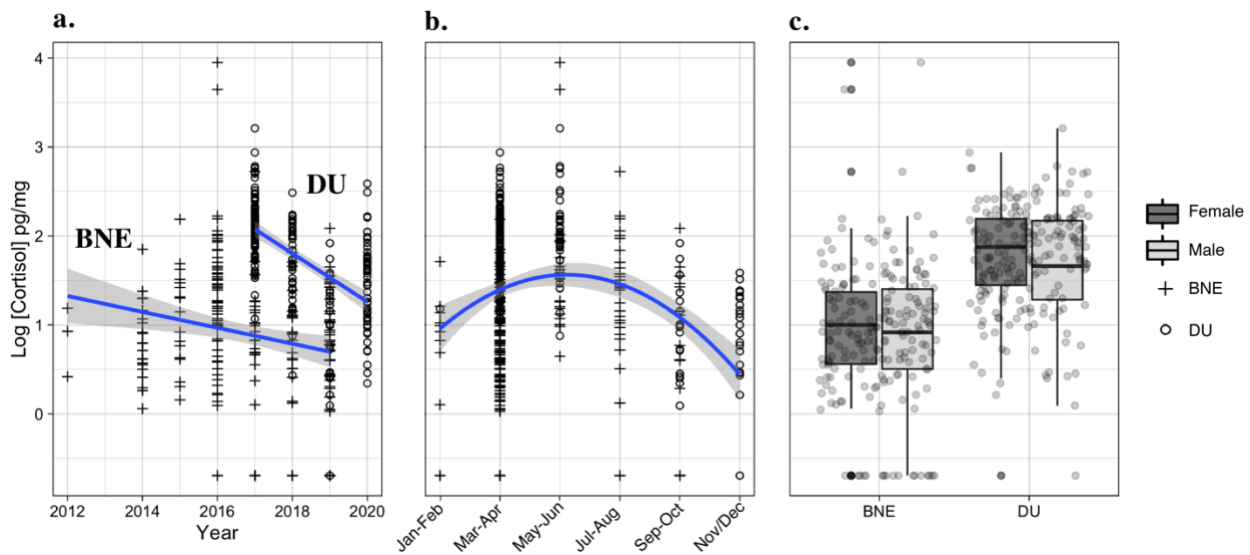


Figure 4.4. Combined log hair cortisol values (pg/mg) for all animals included in statistical modelling by (a.) Year (circles = DU herd, crosses = BNE herd), (b.) season, and (c.) herd. Thick horizontal lines of boxplots correspond to medians (dark grey = female, light grey = male). Blue lines correspond to regression lines and grey shading 95% confidence intervals of model fit.

4.5. DISCUSSION

Hair cortisol concentration is a promising integrated measure of seasonal HPA-axis activity that may be applied to monitor barren-ground caribou and other free-ranging ungulates. We report a large range of possible values for barren-ground caribou, ranging from <1.00 to 51.80 pg/mg; similar ranges of hair cortisol values have been reported in another arctic ungulate, muskoxen (*Ovibos moschatus*) (Di Francesco et al., 2017). These results demonstrate that inter-individual variability of HCC in caribou may be high and elevated values may be representative of an individual experiencing a chronically elevated allostatic load (Macbeth, 2013; Malcolm et

al., 2013). High values may also be associated with exogenous surface contamination, specifically, blood or fecal contamination that is not fully removed by the washing procedure or perhaps, penetrating the hair shaft hair when left in an aqueous environment (Eser et al., 1997; Macbeth, 2013). However, high outlier values, with concentrations ranging from 18 to 51.80 pg/mg reported in this study, were derived from clean samples with no apparent surface contamination or hair follicles present.

4.5.1. Annual Trends and Biting Insect Harassment

We did not detect a significant association between indices of biting harassment and HCC of barren-ground caribou. This result was unsuspected given the known behavioural impacts of biting insect harassment on these populations (Toupin et al., 1996; Weladji et al., 2003), and the capacity of HCC to reflect impacts of disturbances in caribou (Ewacha et al., 2017). We were limited by solely having access to and considering mosquito or oestrid presence, when black flies (*Simuliidae spp.*) and horse flies (*Tabanidae spp.*) may also be significant stressors (Toupin et al., 1996). Statistically, these indices are proxies derived from weather data that only document the total hours of moderate-high activity (Witter et al., 2012) and as a result, we have a limited range of values in which to derive a quantitative association. Biologically, biting insect harassment as a stressor is endemic to these populations and barren-ground caribou may have evolved a tolerance strategy such that the HPA-axis response is limited (Cizauskas et al., 2015). The tolerance strategy hypothesis is well documented in explaining the lack of association between HCC and parasite infection (Carlsson et al., 2016; Trevisan et al., 2017; Di Francesco et al., 2022). Although not statistically significant, there was a trend towards a positive association between HCC and biting insect activity, further study using derived biting insect abundance data rather than indices is warranted.

We detected significant annual trends in HCC of barren-ground caribou, HCC significantly and linearly declined in both herds during the sampling period (Figure.4.4.). Annual changes in HCC may be reflective of changes in population-level stressors experienced by barren-ground caribou. During this same time frame, both populations declined in size; the BNE herd declined by 84% from 2012 to 2019 (Adamczewski et al., 2017; Boulanger et al., 2019), and the DU herd declined by 90% from 1997 to 2018 (COSEWIC, 2017; Campell et al., 2021)

and have now plateaued. Although it is believed that an increase in cumulative stressors may be playing a role in caribou decline (Vors & Boyce, 2009; Festa-Bianchet et al., 2011; Gunn et al., 2014), these changes are not reflected by markers of HPA-axis activity for 2012-2020. Conversely, there may be a lag between biomarkers and population dynamics. Density-dependent effects have been documented in *Rangifer* populations (Solberg et al., 2001), and density dependence alongside environmental factors significantly impacts population dynamics of caribou (Tews et al., 2007). Thus, it may be that caribou population decline is followed by a relaxation of density-dependent constraints which are reflected in markers of HPA-axis activity. This hypothesis is somewhat supported in woodland caribou (*R.t. caribou*) which exhibit reduced HCC in response to increasing home range size (Ewacha et al., 2017), these findings are also corroborated by those in red deer (*Cervus elaphus*) (Casilini et al., 2016). Further, pregnancy rates are negatively associated with herd size in this ecotype (Courturier et al., 2008). Lastly, these populations are believed to naturally cycle (Gunn 2003; Hanke et al., 2021), and our findings support a decrease in HPA axis activity during periods of population decline.

4.5.2. Herd differences

The DU herd had significantly higher HCCs compared to the BNE herd. The DU herd is a distinct designatable unit of caribou, as defined by morphological, behavioural, and genetic characteristics (COSEWIC, 2011). Ecologically, DU caribou range on Victoria Island and seasonally migrate to the mainland across sea-ice (GNWT, 2018b). On the other hand, the BNE herd ranges on the mainland and seasonally migrate between the treeline and tundra (COSEWIC, 2016). Therefore, differences in baseline HCC may be a reflection of the prominent ecological and behavioural differences between these herds HCC. Moreover, ungulate populations inhabiting differing local environments have been documented to exhibit differing population HCC (Casilini et al., 2016). Morphologically, DU caribou are considerably smaller in size and have a lighter white/grey pelage compared to the BNE. Numerous studies have reported hair colour as a significant predictor of HCC (Burnett et al., 2014; Heimbürge et al., 2019), and in some cases, elevated values noted in lighter coloured hair (Bennett & Hayssen, 2010; Bowland et al., 2020). Other studies have reported elevated cortisol in darker pelage (Macbeth et al., 2010). However, it is unclear how melanin content would mechanistically influence HCC, as glucocorticoids have limited capacity to molecularly interact with melanin (Raul et al., 2004).

Nevertheless, coat colour differences between caribou ecotypes may present a confounding factor in comparing hair cortisol concentrations between populations and may be contributing to the differences reported by this study.

4.5.3. Sex differences

Varied results have been reported concerning sex-differences of HCCs in ungulates, as sex-unique physiology or behaviours during active hair growth may be species-specific. Studies have reported no difference in HCC between sexes in red deer (*Cervus elaphus*) (Caslini et al., 2016) and white-tailed deer (*Odocoileus virginianus*) (Potratz et al., 2019), elevated HCCs in male moose (*Alces alces*) (Madslie et al., 2020) and muskoxen (*O. moschatus*) (Di Francesco et al., 2017), and higher HCCs in female rocky mountain goats (*Oreamnos americanus*) (Dulude-de-Broin et al., 2019) and alpine ibex (*Capra ibex*) (Prandi et al., 2018). In the present study, female barren-ground caribou had higher HCC compared to males, however, these findings do not hold when outlier values (n=3) are removed from the dataset. No difference between sexes was observed for HCC in *Rangifer* by Ashley et al., (2011) and Macbeth (2013). Therefore, it is more likely that barren-ground caribou do not exhibit a biologically relevant difference in HCC between sexes as this association is not statistically rigorous in the absence of outliers.

4.5.4. Differences Between Body Regions

Previous research examining differences in hair cortisol concentration between body regions in *Rangifer* has reported conflicting results (Ashley et al., 2011; Carlsson et al., 2016). Similarly, the broad literature on body region differences in hair cortisol demonstrates both significant (Macbeth et al., 2010; Carlitz et al., 2015; Acker et al., 2018), and non-significant (Macbeth et al., 2012; Schell et al., 2017) effects of body region on hair cortisol concentration. Results from this research demonstrate that hair cortisol concentrations between neck and rump regions may not be significantly different from one another. Further, between body region variance of concentration was negligible when considering in-group body region variance, like findings for qiviut cortisol differences among body regions in muskoxen (Di Francesco et al., 2021). Therefore, our results further support conclusions by Carlsson et al. (2016) demonstrating no significant difference in hair cortisol concentration between body regions for *Rangifer*. Yet, for future investigations, rump hair did demonstrate a lower variance in values compared to neck

hair, which may suggest that the rump is the most appropriate sampling location for future consistent sampling.

The lack of correlation of hair cortisol values between body regions of the same animal, warrants further consideration. Differences in hair cortisol concentration between body regions have been previously attributed to various factors, such as hair colour (Ashley et al., 2011; Acker et al., 2018), grooming behaviour (Acker et al., 2018), local cortisol production (Macbeth et al., 2010) and heterogenous molting (Macbeth et al., 2010; Heimbürge et al., 2020). Differences in molting and hair length are the most likely cause for the lack of correlation between body regions detected in this study. Heterogenous molting and hair growth is commonly hypothesized explanation for differences in HCC among body regions (Macbeth et al., 2010; Heimbürge et al., 2019). However, the precise phenology of shedding is rarely well described for many wild ungulate species (Nowak et al., 2020), including *Rangifer* (Ashley et al., 2011). Using moose shedding patterns as a proxy, hair shedding begins in the ventral and cranial areas, and proceeds to the dorsal and caudal, and the margins of the rump are generally the last area to be shed (Samuel et al., 1986), resulting in neck hair being reflective of earlier in the growing season. Solely neck hair presented outlier values, and upon removal of these pairings, neck and rump hair were significantly correlated with one another.

4.5.5. Seasonal variation

The month of hair collection was a significant predictor of HCC in caribou, and these values tended to increase from January/February and peak in May/June (Figure.4.4.). Quantified HCC values are believed to be representative of internal cortisol of the hair shaft following a wash procedure. Internal cortisol would be reflective of circulating free cortisol as well as local cortisol synthesis taking place during the hair growth period that is incorporated passively into the hair shaft (Russell et al., 2012; Ito et al., 2005; Keckeis et al., 2012), which is June-October for caribou (Cuyler & Øritsland, 2002). The proportional contribution of circulating or locally produce cortisol incorporated into the hair shaft is not known (Keckeis et al, 2012; Sharpley et al., 2012). We found that HCC increased within the hair shaft during the non-growth quiescent phase, when guard hairs are disconnected from the blood supply. This signifies that although assumed that cortisol is solely incorporated into the hair shaft during active growth (Gormally et

al., 2020), caribou guard hair cortisol is variably deposited beyond the period of active growth. These findings are supported by those in grizzly bears (*Ursus arctos*), that also reported a change in HCC outside of the hair growth period (Cattet et al., 2014).

Caribou guard hair grows in a distinct season and the density of pelage is stable year-round (Cuyler & Øritsland, 2002). Although guard hairs seem to be stable in density, there is a possibility that a select percentage of hair is shed and replaced continuously. In muskoxen, 20% of guard hairs are continuously replaced on the rump year-round (Flood, 1989). This hypothesis is supported by our results being synchronous with seasonal specific stressors in caribou, a peak of HCC in May/June corresponds to spring migration to calving grounds following a strenuous winter (Taillon et al., 2012). Seasonal spring peaks in cortisol have been documented in another migratory capital breeder, humpback whales (*Megaptera novaengliae*) (Mingramm et al., 2020), as well as boreal snowshoe hares (*Lepus americanus*) who exhibit markedly higher HCC in summer coats compared to winter (Lavergne et al., 2020). Although a proportion of guard hairs may be continuously shed and replaced outside of molt, and this may explain seasonal variation in HCC, these patterns have yet to be documented in the *Rangifer* pelage. Lastly, although there is a wool undercoat present year-round in *Rangifer* pelage (Cuyler & Øritsland, 2002), most of this undercoat was removed before analysis and is unlikely driving the detected seasonal pattern.

Excreted cortisol may be a potential explanation for this detected monthly variation of hair cortisol beyond the hair growth period. Sweat from apocrine glands contains quantifiable concentrations of cortisol (Russell et al., 2014), and any cortisol-containing fluid can diffuse into the hair shaft (Otten et al., 2020). The capacity of external substances to diffuse into the hair shaft may also be related to hair integrity. Indeed, damaged, or distal hair may be more susceptible to external cortisol penetration from sweat or sebum (Heimbürge et al., 2020). Conversely, it has also been hypothesized that hair cortisol may be capable of washout in response to grooming or environmental exposure (Acker et al., 2018). Our results support the former hypothesis, such that damaged hair is more susceptible to external cortisol penetration, resulting in an increase in guard hair cortisol concentration in the spring during strenuous migration. Further, caribou pelage undergoes a marked change during the winter from grey/brown in the fall to white in winter, alluding to a change in composition that has yet to be quantified. Significant annual variation of HCC beyond the hair growth period demonstrates that

season is a key covariate to consider when analyzing the HCC of caribou and perhaps other ungulates.

4.5.6. Limitations and future directions

This study has highlighted numerous limitations and challenges associated with hair collection in caribou for hair cortisol analysis. Most pressing is the need for precise documentation of *Rangifer* molt and hair growth. This need is confirmed by our detection of HCC variation beyond the hair growth season. Future investigations should endeavour to document the precise timing of molt and heterogeneity of molt patterns between body regions. Moreover, annual variation in the undercoat also requires investigation. *Rangifer* pelage consists of guard hairs and a wool-like undercoat, and this undercoat is not completely removed for hair cortisol analysis (Macbeth, 2013, Ashley et al., 2011; Carlsson et al., 2016). Guard hair and undercoat HCC of other species are weakly correlated (Macbeth et al., 2010; Dulude-de-Broin et al., 2019), and the season of guard hair and undercoat growth may not be perfectly synchronous in ungulates (Nixon et al., 1991). Therefore, it remains unclear to what extent the undercoat contributes to quantified HCC and if undercoat growth is seasonal or continuous.

Studies utilizing hair as a health indicator should endeavour to consistently collect from a standard sample location (potentially the rump), and to separate guard hairs from the undercoat before laboratory analysis. Further, sample storage and uncontrolled exogenous contamination may have possible biased results presented in this study. Due to the nature of hunter-based sampling in the field, numerous samples were submitted frozen with significant blood/feces/urine present on the hair surface. The presence of aqueous solutions can alter the permeability of the hair shaft (Eser et al., 1997). Consequently, during sample thawing, if exogenous contamination is present, these external contaminants can leech into the hair shaft (Macbeth et al., 2012; Cattet et al., 2014), and once incorporated into the shaft, will not be removed by surface washing (Kidwell & Blank, 1996). Further, repeated freeze-thawing could provide multiple opportunities for a leeching event to occur in the presence of liquid. Conversely, outlier values of HCC reported in this study were derived from clean hair. The capacity to which surface contamination confounded our results in this study is unknown,

however, care was taken to solely thaw samples for the period required to shave hair, and to select uncontaminated hair from a sample.

4.6. CONCLUSION

Barren-ground caribou are a keystone species in Canada that support ecosystem function (Festa-Bianchet et al., 2011) and Indigenous ways of life (Polfus et al., 2016). Many populations of barren-ground caribou have drastically declined in the past decade (COSEWIC 2016; COSEWIC 2017), with few herds showing signs of recovery. Monitoring the health of free-ranging wildlife population is challenging, and sample acquisition presents a large barrier to consistent monitoring (Ryser-Degiorgis, 2013). Hair is a practical sample type due to its ease of collection and relative stability. Hair provides a unique measure of long-term HPA-axis activity that is temporally matched with a sensitive period in the caribou life cycle and is thus a promising conservation tool for monitoring seasonal allostatic load. In this study, we investigate and identify numerous key factors that may significantly influence HCC in caribou. We report hair cortisol values for two herds of barren-ground caribou from 2012 to 2020 using opportunistically derived hair samples. We found that: body regions do not significantly differ in HCC, HCC in barren-ground caribou has linearly decreased from 2012 to 2020, DU animals have higher HCC compared to BNE, and HCC may be seasonally variable outside of the hair growth season. These results point to the potential for HCC to be used as a monitoring tool for barren-ground caribou and outline key factors to consider in monitoring implementation and interpretation.

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CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

5.1. APPLICATIONS TO WILDLIFE MONITORING

Wildlife health is a key aspect of successful wildlife management and conservation (Deem et al., 2001; Stephen, 2014; Decker et al., 2016) and consistent monitoring of wildlife populations via the continuous collection of epidemiological data is key to understanding the “health status” of a population (Mörner et al., 2002). This thesis provides evidence for the utility and practical use of two biomarkers of barren-ground caribou health derived from hair. The first is hair trace element concentrations (HECs), which may serve as proxies for nutritional status and heavy metal exposure (Combs, 1972; Jutha et al., in press). The second is hair cortisol concentrations (HCCs), which provide a seasonal measure of allostatic load (Dantzer et al., 2014). By furthering our understanding of these biomarkers concerning opportunistic sampling, we have progressed their utility as metrics used to inform upon management and conservation of caribou. While many studies have begun to analyze these biomarkers, few studies have oriented research questions to the realities of opportunistic sampling of these populations, this thesis aimed to fill this gap in our understanding.

5.2. SUMMARY OF THESIS FINDINGS

Through this research, I have summarized the *Rangifer* infectious disease literature and assessed hair trace element and hair cortisol concentrations of barren-ground caribou. This work significantly contributes to our understanding of how to orient health research to be incorporated into decision making, and simultaneously assessed two biomarkers of barren-ground caribou health. This thesis generated three primary research chapters.

In Chapter 2, we summarized the *Rangifer* infectious disease literature using a scoping review methodology to understand why health information was not fully incorporated into management and conservation frameworks. We summarized the most abundant health literature of *Rangifer*, the infectious disease literature, and found that information may not be readily usable by practitioners. Specifically, this literature primarily consisted of descriptive studies on gastrointestinal parasites that focused on North American populations. Although these studies are valuable to the clinical and ecological understanding of pathogens, this information is not readily

integrated into management frameworks (Lafferty and Gerber, 2002; Laurance et al., 2012). We highlight key measures, such as the inclusion of managers into the research process (Barret and Rodriguez 2021), that may facilitate solutions-oriented *Rangifer* health research (Peters et al., 2019).

In Chapter 3, we established the first values of hair trace element concentrations in Bluenose-East caribou and explored significant sources of variation associated with opportunistic health sampling of this population. We found that anatomic body regions significantly differed in hair trace element concentrations, and these findings are supported by those in mountain caribou (*R.t. caribou*) by Jutha et al., (2022, in press). These differences have direct implications for future hair sampling protocols of *Rangifer* and the ability to institute passive sampling procedures (e.g. hair snags). Further, contrary to our hypothesis and previous work in renal concentrations (Gamberg et al., 2020), we did not detect a difference in HECs between the sexes. However, we were able to detect significant annual changes in trace elements of concentrations in hair from 2012 to 2019. These results point to hair trace element analyses as a promising biomarker of seasonal element uptake, that offers a different temporal resolution of element concentrations compared to acute measures (blood, organs).

In Chapter 4, we examined hair cortisol concentrations of the Bluenose-East and Dolphin and Union herds of barren-ground caribou. Although a species-specific validation of HCC in caribou is lacking (Ashley et al., 2011), these analyses are pertinent to our understanding of changing caribou allostatic load through time (Macbeth and Kutz 2019). Dissimilar to trace elements, we found that rump and neck sampling sites did not significantly differ in HCC concentrations, these findings are supported by investigations conducted by Carlsson et al. (2016). We found that herd, season, year, and sex were significant predictors of HCC in barren-ground caribou, but not indices of biting insect harassment with current sample size. These results opposed our initial hypothesis, given the known behavioural and energetic impacts of biting insect harassment (Toupin et al., 1996; Weladji et al., 2003). Of note, is a linear downward trend of HCC in barren-ground caribou from 2012 to 2020, suggesting a reduction of HPA-axis activity despite a continued population decline during that period (COSEWIC 2016; COSEWIC 2017). Lastly, we detected a variation in HCC outside of the hair growth period, an effect

previously documented by Cattet et al. (2014) in grizzly bears (*Ursus arctos*) that has large ramifications for the use and interpretation of this health biomarker.

Ultimately this thesis significantly contributed to our understanding of caribou health research by investigating two biomarkers of caribou health to be used in future monitoring and by furthering our understanding of how to best orient aspects of *Rangifer* health research into management and conservation.

5.3. STUDY LIMITATIONS

As in any research, this thesis had multiple significant study limitations. These limitations were primarily related to study design, sampling protocol, and understanding of caribou hair growth. To begin, our studies relied on an opportunistic sampling approach, which is commonplace in wildlife health research (Duncan et al., 2008). However, these opportunistic sampling schemes impose multiple limitations on the rigour of conclusions (Ryser-Degiorgis et al., 2013). The two sampling sources used to monitor health in caribou, hunted animals for subsistence and captures for demographic investigations, inherently differ in purpose, procedure, and scope. However, to gain an adequate sample size for current and future caribou health investigations, both sample sources are and will continue to be commonly relied upon (Jessup, 2003). Results from this thesis confirmed that sample sources do differ significantly in two hair biomarker outcomes, HECs and HCCs, without a clear mechanistic explanation as to why. In this light, there may be a difference between animals that are sampled for different purposes, and sample source must be accounted for in analysis and interpretations combining sources to achieve maximal sample size.

Current caribou hair sampling protocols impose numerous biases and limitations on research. First, is the inconsistent sampling between anatomic body locations. Hunters generally sampled consistently from the neck, while captured animals were sampled for numerous body locations during the study period, such as the neck, rump, back, hip and shoulder. Further, although labelled consistently as neck hair, hunted samples are collected from variable neck locations. Caribou pelage becomes significantly longer below the head, forming a “beard” (Laaksonen and Paulsen 2015) and when labelled as a neck sample, hunters either collected long beard hair or shorter neck hair further down the body, potentially confounding results, and

interpretations. This research suggests that rump hair offers a range of values of both biomarkers with lower variance than neck hair, potentially due to uniform length. Therefore, as suggested for future muskox hair sampling (Di Francesco et al., 2021), rump hair may be the preferred sampling location in barren-ground caribou for biomarker analyses and future consistent monitoring protocols.

Hair samples were either collected on the hide, shaved, or were pulled when shed. Shed hair still presents a prominent telogen phase hair bulb (Macbeth 2013), which must be individually removed for analysis. This process is meticulous and taxing and may result in a significant portion of follicles remaining despite best efforts (Cattet et al., 2017; Sergiel et al., 2020). Remaining hair follicles can increase quantified hair cortisol levels (Sergiel et al., 2020), and the impacts of follicles on hair trace element analyses are unknown. However, the impact of hair follicles although statistically significant, has a relatively low magnitude (0.3 pg/mg), when considering the range of possible values (1-44 pg/mg) of HCC (Sergiel et al., 2020). Furthermore, external contamination, such as blood/feces/urine was present in many submitted hunter samples and these external contaminants can alter quantified hair biomarkers (Macbeth 2013; Otten et al., 2020). When submitted frozen, care should be taken to immediately remove any liquid present on the hair samples, shave the hair, and store the hair dry. Storing samples on the hide frozen with contaminants present, and repeated freezing and thawing allows for possible permeability of the hair shaft while in the presence of a liquid (Kidwell and Smith, 2007). Ideally, samples should be submitted free of external contaminants, and either frozen on the hide or shaved and dry in a paper envelope.

Lastly, the lack of precise documentation and understanding of the caribou growth cycle severely limited this study. This information deficiency of hair growth is common in ungulate species whose coat is not part of the commercial textile industry (Nowak et al., 2020). In my thesis this knowledge gap of caribou hair growth imposes three problems. The first, is in our understanding of the differences between anatomic sampling sites that may be due to heterogenous molting. The second, is in the determination of hair growth year for samples collected between July-September; hair that is shed, shaved, or plucked and submitted may pose difficulties in ascertaining the appropriate growth year solely on hair length alone. Lastly, our understanding of the wool undercoat is unclear whether the wool undercoat is stable or

continuously growing in *Rangifer* pelage (Cuyer & Øritsland 2002; Laaksonen & Paulsen, 2015). This latter point is of concern since the wool undercoat in *Rangifer* is not fully separated from guard hairs for cortisol or trace element analyses, and impacts from the undercoat on these quantified biomarkers are unknown.

5.4. FUTURE DIRECTIONS

Results from this thesis highlight numerous future research avenues and study opportunities. The scoping review demonstrates the need for future research to access and synthesize the remaining significant portion of the *Rangifer* disease literature (non-English and semi-domesticated) and we suggest an attempt to collaborate with Russian colleagues to increase our understanding of *Rangifer* disease across the complete circumpolar distribution. Furthermore, results from our scoping review have implications for solutions-focused wildlife health frameworks, adapted from human medicine and outlined by Peters et al., (2019). Results from our review show that the *Rangifer* infectious disease literature is predominantly Phase 0 or Phase 1, basic discovery, and description of pathogens, with a need to move to Phase 2, the understanding of impacts of disease and creation of candidate solutions to be incorporated into wildlife management and conservation. The first step in increasing translatability would be the inclusion of managers in wildlife health research investigations (Laurence et al., 2012), and increased understanding of pathogen impacts (Braunisch et al., 2012).

An in-depth study examining caribou molt pattern, guard hair growth, and wool hair growth is a key future research priority if the hair is to be used for the health monitoring of caribou. In moose, (*Alces alces*) the precise molt pattern and phenology have been previously documented using standardized photography in experimental animals (Samuel et al., 1986) and similar work is possible in *Rangifer*. Furthermore, an examination of hair follicle density as well as follicle growth stage would provide clear evidence for continuous or discrete hair growth in *Rangifer*, as has been completed in muskoxen (Flood, 1989). Lastly, consultation with caribou hair experts, such as traditional knowledge holders, and individuals working with caribou pelts frequently, may corroborate experimentally generated evidence of caribou hair growth (Stern and Humphries, 2022).

Last, is the need for physiological validation of both HEC and HCC concentrations in *Rangifer*, to confirm the biological relevance of these biomarkers (Koren et al., 2019). Mechanisms for which trace elements and GCs enter the hair shaft are poorly understood and/or are species-specific, physiological validation is the process in which we determine the biological relevance of concentrations and the temporal context they represent (Di Francesco et al., 2021). HEC as a biomarker in wildlife has been validated to an extent by correlating hair concentrations with serum, kidney, or liver concentrations of select elements (Roug et al., 2015; Jutha et al., in press). However, given that serum values represent an acute measure, and liver/kidney are storage organs, correlations may be overly spurious rather than reflecting a true robust biological relationship. HEC values presumably represent both dietary intake and metabolic expenditure during the period of hair growth. Previous validations of elements of interest, such as Se have been accomplished through sequential Se dietary supplementation during hair growth (Combs, 1972). We could then take standardized hair and blood samples during dietary supplementation to understand the relationship between circulating element concentrations and hair uptake. Lastly, the use of radiolabeled elements, such as ^{65}Zn (Strain et al., 1971) may further elucidate species-specific mechanisms of element uptake and retention in the hair shaft. Next, is the requirement of an HCC physiological validation that can be accomplished through repeated ACTH injection during the *Rangifer* hair growth period, as was conducted in muskoxen (Di Francesco et al., 2021), rather than a single injection that had previously been conducted in *Rangifer* (Ashley et al., 2011). Stimulation of the HPA-axis through injection of ACTH is the current “gold standard” for HCC validation (Koren et al., 2019). Experimental injection of radiolabeled cortisol has also been sought out (Keckeis et al., 2012), to further understand the proportion to which circulating, and locally produced cortisol make up quantified HCC. By conducting experimental validations of hair biomarkers, we may better support quantified values as being truly biological meaningfully representations of HPA axis activity or circulating element concentrations during the hair growth period.

5.5. CONCLUDING STATEMENT

In this thesis, I have summarized a significant portion of the caribou health literature and used hair as a sample type to: (1) examine trace element concentrations, (2) examine cortisol concentrations, and (3) confront the sources of variation that arise from opportunistic health

sampling of caribou. By establishing baseline data, and then examining key sources of variation governing these values, this work has provided key steps in future integrated caribou health monitoring. Although much research is still required, this work has broadened our understanding of the limitations and challenges associated with using these health biomarkers to monitor barren-ground caribou. Further, we have identified proactive steps to integrating health information into conservation and management frameworks for this keystone species of conservation concern.

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APPENDIX A

Chapter 2 (Scoping Review) Supplementary Material

Title and Abstract Screening Protocol

- a. Download title and abstract RIS files and import them into Covidence Software
- b. First Screening Inclusion Criteria:
 - i. Written in English
 - ii. Peer reviewed primary published literature
 - iii. *Rangifer*/Caribou/Reindeer must be explicitly mentioned in the title or abstract
 - iv. Study must be related to
 1. Free-roaming, semi-domesticated and domesticated populations
 2. Pathogens that impact *Rangifer*
 - a. Micro-parasites: bacteria, protozoans, viruses, and prions
 - b. Macro-parasites: arthropods, nematodes, cestodes, and trematodes
 - v. Related (directly or potentially) to *Rangifer* health in terms of infectious disease
 - vi. Morphological, descriptive, ecological, and evolutionary accounts of infectious agents may be included if they are present in *Rangifer* hosts
 - vii. Diagnostics studies of infectious agents may be included if they pertain to *Rangifer* hosts in some capacity
 - viii. If unclear, studies will be retained as a maybe and move unto full text screening
 - ix. Available from the University of Calgary library or interlibrary loan service
- c. Exclusion Criteria
 - i. Non peer reviewed literature
 - ii. Non primary literature
 1. Conference proceedings
 2. Grey literature
 3. Review papers
 - a. Review papers defined as pure summaries of previous work
 - iii. Caribou/*Rangifer*/Reindeer was the name of a place, geological feature, plant species (etc.)
 - iv. Studies pertaining to *Rangifer* clinical conditions that are not associated with an infectious agent
 - v. Non peer reviewed literature

Full Text Screening Protocol

- d. Full text importation into Covidence software of remaining screened titles
- e. Exclusion criteria
 - i. Full text not available through University of Calgary library or interlibrary loan services
 - ii. Full text not written in English

- iii. Full text study population is fully domestic, with research findings having no application towards free-roaming counterparts
- iv. Full text study does not pertain to *Rangifer* in some capacity (explicitly stated impact)
 - 1. Infectious agents are not related to *Rangifer* within the full text
- v. Full text study is a duplicate
- vi. Full text is a review study (Summarizes work, with a lack of novel contribution)
- vii. Full text is a human clinical case, with no explicit statements of impacts on *Rangifer*

Inclusion and Exclusion criteria for the evaluation of management/conservation applications within infectious disease articles of free-ranging *Rangifer* populations Protocol

To be considered a paper that applies results towards management/conservation of *Rangifer*, the article must explicitly state the following terms: Management/managers, conservation/conservationists, co-management, decision makers, policy/practitioners, stakeholders/rights holders, harvest/TAC/etc, within the:

- a. Abstract, in the context of relating results from the study to management/conservation, and not solely as background information
 - Ex. “We believe these results are relevant to wildlife managers....”
- b. Objectives/Aims/Goals/Research questions/Purpose, generally found in the last paragraph of the introduction
- c. Discussion/Conclusion, in the context of relating results from the study to management/conservation, and not solely as background information
 - Ex. “These results have important implications for wildlife conservation”

Table.A.1. Journal categories, individual journal names, and accompanying summary statistics for the 137 wildlife research articles. ‘Journals’ denotes the number of journals within each category, ‘Count’

denotes the number of articles and ‘%’ is the proportion of articles from the total. ‘Open’ access level was categorized as completely open access (OA), hybrid open access (H) and paid for access (PA).

| Journal Category | Journals | Count | % | Specific Journals | Count | % | Open |
|--|----------|-------|------|--|-------|------|------|
| Disease and Epidemiology | 5 | 38 | 27.7 | Journal of Wildlife Diseases | 34 | 24.8 | H |
| | | | | Transboundary and Emerging Diseases | 1 | 0.7 | H |
| | | | | Infection Ecology & Epidemiology | 1 | 0.7 | OA |
| | | | | Eurosurveillance | 1 | 0.7 | OA |
| | | | | Journal of Comparative Pathology | 1 | 0.7 | H |
| Parasites and Parasitology | 9 | 43 | 31.4 | Journal of Parasitology | 8 | 5.8 | H |
| | | | | International journal for Parasitology: Parasites and Wildlife | 7 | 5.1 | OA |
| | | | | International journal for Parasitology | 6 | 4.4 | H |
| | | | | Parasitology | 6 | 4.4 | H |
| | | | | Parasites and Vectors | 5 | 3.7 | OA |
| | | | | Veterinary Parasitology | 5 | 3.7 | H |
| | | | | Parasitology International | 2 | 1.5 | H |
| | | | | Parasitology Research | 2 | 1.5 | H |
| | | | | Systematic Parasitology | 2 | 1.5 | H |
| | | | | Ecology and Zoology | 11 | 37 | 27.0 |
| Rangifer | 11 | 8.0 | OA | | | | |
| Canadian Field Naturalist | 2 | 1.5 | H | | | | |
| Global Change Biology | 2 | 1.5 | H | | | | |
| Journal of Animal Ecology | 2 | 1.5 | H | | | | |
| Functional Ecology | 1 | 0.7 | H | | | | |
| Molecular Ecology | 1 | 0.7 | H | | | | |
| Acta Zoologica Fennica | 1 | 0.7 | PA | | | | |
| Journal of Biogeography | 1 | 0.7 | H | | | | |
| Oecologia | 1 | 0.7 | H | | | | |
| Proceedings of the Royal Society of Biological Sciences Series B | 1 | 0.7 | H | | | | |
| Veterinary Medicine | 6 | 8 | 5.8 | BMC Veterinary Research | 2 | 1.5 | OA |
| | | | | Canadian Veterinary Journal | 2 | 1.5 | PA |
| | | | | Acta Veterinaria Scandinavica | 1 | 0.7 | OA |
| | | | | Veterinary Record | 1 | 0.7 | H |
| | | | | Veterinary Research | 1 | 0.7 | OA |
| | | | | Veterinary Research Communications | 1 | 0.7 | H |
| Management/Conservation | 3 | 4 | 2.9 | Journal of Wildlife Management | 2 | 1.5 | H |
| | | | | Human dimensions and Wildlife | 1 | 0.7 | H |
| | | | | Biological Conservation | 1 | 0.7 | H |
| Arctic Ecosystems | 2 | 3 | 2.2 | Arctic | 2 | 1.5 | H |
| | | | | Polar Biology | 1 | 0.7 | H |
| Pathogen specific | 3 | 3 | 2.2 | Canadian Journal of Microbiology | 1 | 0.7 | H |
| | | | | Frontiers in Microbiology | 1 | 0.7 | OA |
| | | | | Prion | 1 | 0.7 | OA |
| Multidisciplinary | 1 | 1 | 0.7 | PLoS One | 1 | 0.7 | OA |

Table.A.2. Definitions and metadata used to populate database during data extractions of articles

| Code | Sub-Code | Description | Definition |
|------|----------|---------------------------|---|
| Loc. | | Country location of study | Country location as related to where the <i>Rangifer</i> population occurs, tissues derived from, or health information most immediately applicable, i.e. not necessarily the location of the host institution. |

| Code | Sub-Code | Description | Definition |
|--------|----------|-----------------|--|
| Access | | | |
| | Y | Open Access | Completely open access publishing model, articles may be accessed by anyone at any time. |
| | N | Paid for Access | Traditional publishing model, in which articles may only be accessed through institutional credentials or paid for access. |
| | H | Hybrid | Hybrid publishing model, which mixes traditional paid for publishing with open access options. |

| Code | Sub-Code | Description | Definition |
|----------|----------|-----------------------|--|
| Stud.typ | | Study Type | Way in which health information was generated through a specific scientific methodology. |
| | Des. | Descriptive | Study utilizes quantitative descriptive methods, produces descriptive data. |
| | Exp. | Experimental | Study utilizes quantitative experimental methods, produces experimental data. |
| | TK. | Traditional Knowledge | Study utilizes qualitative methods, produces narrative data. |
| | Mod. | Modelling | Study utilizes mathematical modelling methods, produces predictive quantitative data or exploratory data beyond statistical inference. |
| | Syn. | Synthesis | Study synthesizes past work and incorporates it into novel data analyses and/or interpretations. |

| Code | Sub-Code | Description | Definition |
|-------------|----------|----------------------------|--|
| Journal.Cat | | | |
| | Dis. | Disease and Epidemiology. | Journal scope states and identifies infectious disease and epidemiology in animals as their primary publishing focus. |
| | Paras. | Parasites and parasitology | Journal scope identifies fundamental and medical parasitology, parasite epidemiology, as their primary publishing focus. |
| | Eco. | Ecology and Zoology | Journal scope states and identifies zoology and animal ecology as their primary research focus, not in relation to wildlife management and conservation. |
| | Vet. | Veterinary medicine | Journal scope states or identifies veterinary science and medicine as their primary publishing focus. |

| | | | |
|--|--------|-----------------------------|--|
| | Multi. | Multidisciplinary | Journal scope states interdisciplinary and multidisciplinary research as their publishing focus. |
| | Manag. | Management and Conservation | Journal scope state and identify wildlife management and conservation as their primary publishing focus, most likely in relation to fundamental ecological research of wildlife. |
| | Arct. | Arctic Ecosystems | Journals scope identify their publishing focus as relating to the polar and subpolar localities. |
| | Path. | Pathogen Specific | Journal scope is related to a single pathogen type, Ex. Prion. |

| Code | Sub-Code | Description | Definition |
|----------|----------|------------------|---|
| Path.ord | | Pathogen species | Order of pathogen being assessed in the study, categories derived from (Carlsson et al, 2019). |
| | MO | Mixed order | Multiple species and or orders of pathogens were assessed within the study (Multispecies within the same order excluded). |
| | Syn | Syndrome | Clinical syndrome involving multiple pathogen species were assessed within the study, etiology unclear of disease |
| | Bac | Bacteria | Solely bacteria pathogen species were assessed within the study, multiple bacteria species may be considered. |
| | Pro | Protozoa | Solely protozoa pathogen species were assessed within the study, multiple protozoan species may be considered. |
| | Helm | Helminth | Solely helminth pathogen species were assessed within the study, multiple helminth species may be considered. |
| | Arth | Arthropod | Solely arthropod pathogen species were assessed within the study, multiple arthropod species may be considered. |
| | Vir | Virus | Solely viral pathogen species were assessed within the study, multiple viral species may be considered. |
| | Pri | Prion | Solely prion pathogen species were assessed within the study, multiple prion species may be considered. |
| | Fung | Fungus | Solely fungus pathogen species were assessed within the study, multiple fungus species may be considered. |

| Code | Sub-code | Description | Definition |
|---------|----------|------------------------|---|
| Lev.dom | | Level of domestication | Level of domestication of host <i>Rangifer</i> population or individual within the study. |
| | Wild | Free-ranging wildlife | Host study population/individual are/is free ranging wildlife. |
| | Semi | Semi-domesticated | Host study population/individual are semi-domesticated (mostly applicable to reindeer herding). |
| | Dom | Domesticated | Host study population are completely domesticated within an agricultural, zoo or research facility context. |

| Code | Sub-code | Description | Definition |
|------|----------|-------------|------------|
|------|----------|-------------|------------|

| | | | |
|---------|-------|---|--|
| Sub.spp | | <i>Rangifer</i> sub species designation | The sub- population of <i>Rangifer</i> assessed in the study, denotes the unique and scientifically proven diversity among <i>Rangifer</i> . |
| | Mix | Reindeer and Caribou | Mix of reindeer and caribou assessed within study, subspecies unclear, and or mix of sub-species. |
| | R-TT | Eurasian Tundra reindeer | <i>Rangifer tarandus tarandus</i> |
| | R-TF | Eurasian wild boreal forest reindeer | <i>Rangifer tarandus fennicus</i> |
| | R-TP | Svalbard reindeer | <i>Rangifer tarandus platyrhynchus</i> |
| | R-UN | Reindeer, unknown | <i>Rangifer tarandus</i> , specific sub-species unknown or unclear for the study |
| | C-TGG | Grant's Caribou | <i>Rangifer tarandus granti</i> |
| | C-TG | Barren-ground caribou | <i>Rangifer tarandus groenlandicus</i> |
| | C-TC | Woodland caribou | <i>Rangifer tarandus caribou</i> |
| | C-TP | Peary Caribou | <i>Rangifer tarandus pearyi</i> |
| | C-UN | Caribou, unknown | <i>Rangifer tarandus (caribou)</i> , specific sub species unknown |

| Code | Sub-Code | Description | Definition |
|-------------|----------|---------------------------|---|
| Health.info | | | |
| | P.occ | Pathogen occurrence | The distribution and occurrence of pathogens as related by time, place and hosts. |
| | P.pre | Pathogen prevalence | Proportion of a population that is infected or exposed by a pathogen, gives an estimate of how widespread infection is. |
| | P.int | Pathogen intensity | A quantitative measure of the pathogen intensity or abundance is given. |
| | P.tax | Pathogen taxonomy | Phylogenetic and taxonomy information is provided, these include morphological and molecular descriptions. |
| | P.eco | Pathogen ecology | Fundamental life history traits of the pathogen are provided, such as development, behaviour, life cycle, transmission etc. |
| | In.risk | Intrinsic risk factors | Intrinsic risk factors associated with disease, infection, or exposure (sex, weight, genetics) . |
| | Ex.risk | Extrinsic risk factors | Extrinsic risk factors associated with disease, infections, or exposure (climate, season, habitat, contaminants) that interact with infectious disease. |
| | R.behav | <i>Rangifer</i> behaviour | Behavioral factors associated with infection, exposure, or disease (selection, habitat choice etc). |
| | In.imp | Individual level impacts | Individual level impacts of infectious agents on the host (Body condition, reproduction). |
| | Pop.imp | Population level impacts | Population level impacts of infectious agents on the host population (demographics, decline). |

| | | | |
|--|--------|-------------------------|---|
| | Diag. | Diagnostic | Diagnostic methodology paper, provides information on pathogen exposure/presence/effects. |
| | Treat. | Treatment | Clinical treatment or management treatment related to individual or population impacts of pathogens. |
| | Path. | Pathology | Provides information on the physical manifestations of infection – gross or cellular anatomical lesions as well as cellular/physiological processes leading to lesions. |
| | Mort.i | Mortality investigation | Description of infectious disease outbreak or mortality event, descriptive account . |

| Code | Sub-code | Description | Definition |
|------------|-----------|---------------------------|--|
| Health.cat | | | |
| | Path. Occ | Pathogen Occurrence | Umbrella health category that includes studies of pathogen occurrence, pathogen prevalence and pathogen intensity health information categories. |
| | Path.l | Pathogen Life History | Umbrella health information category that is an amalgamation of the pathogen taxonomy, pathogen ecology, and experimental infection health information categories. |
| | Risk. F | Risk Factors | Combination of the intrinsic risk factors, extrinsic risk factors health information categories. |
| | Rang.B | <i>Rangifer</i> Behaviour | <i>Rangifer</i> behaviour health information category, unchanged. |
| | Path.Imp | Pathogen Impacts | Combination of the individual impacts and population impacts health information categories. |
| | Path. | Pathology | Combination of the pathology and mortality investigation health information categories |
| | Treat. | Treatment | Treatment health information category, unchanged. |

Table.A.3. Search string entered into databases to search for peer-reviewed literature relevant to *Rangifer* infectious disease.

| Group | Search strings |
|----------------|---|
| Rangifer | Caribou OR Rangifer OR Reindeer |
| Disease | Diseas* OR Path* OR Parasit* OR Infect* OR Health |
| Microparasites | Virus* OR Bacter* OR Protozoa* OR Prion* |
| Macroparasites | Helminth* OR Nematod* OR Trematod* OR Cestod* OR Arthropod* |

Table.A.4. *Rangifer* infectious disease study types by location for wild populations. Total denotes the total articles, Proportion denotes the proportion compared to all articles included, and

study type (Experimental, Descriptive, Traditional Knowledge, Modelling and Synthesis) percentages are compared to the total for that country.

| | Total | Proportion (%) | Experimental (%) | Descriptive (%) | Traditional Knowledge (%) | Modelling (%) | Synthesis (%) |
|---------------|-------|----------------|------------------|-----------------|---------------------------|---------------|---------------|
| Canada | 72 | 50.3 | 6.9 | 86 | 4.2 | 4.2 | 6.9 |
| Norway | 29 | 20.2 | 31 | 79 | 0 | 6.9 | 0 |
| Alaska | 25 | 17.5 | 0 | 100 | 0 | 4 | 0 |
| Greenland | 7 | 5 | 0 | 100 | 0 | 0 | 0 |
| Finland | 4 | 3 | 0 | 100 | 0 | 0 | 0 |
| Iceland | 3 | 2 | 0 | 100 | 0 | 0 | 0 |
| Belgium | 1 | 0.7 | 0 | 100 | 0 | 0 | 0 |
| Sweden | 1 | 0.7 | 0 | 100 | 0 | 0 | 0 |
| South Georgia | 1 | 0.7 | 0 | 100 | 0 | 0 | 0 |

APPENDIX B

Chapter 3 Supplementary Material

Table.B.1. Trace element concentrations in hair (mg/kg, dry weight) of Bluenose-East caribou determined by ICP-MS. Sample size (n), related to number of samples above the limit of quantification (LOQ). Samples below the limit of detection were treated as true zeros.

| Element | n | > LOQ | Median | Min | Max |
|-----------------|-----|-------|--------------|---------|----------|
| Arsenic (As) | 0 | 0% | Not detected | 0 | 0 |
| Calcium (Ca) | 137 | 100% | 379.138 | 185.898 | 1278.99 |
| Cadmium (Cd) | 0 | 0% | Not detected | 0 | 0 |
| Cobalt (Co) | 41 | 29% | 0.039 | 0.010 | 0.155 |
| Chromium (Cr) | 21 | 15% | 0.309 | 0.0499 | 1.024 |
| Copper (Cu) | 137 | 100% | 6.310 | 3.807 | 9.425 |
| Iron (Fe) | 137 | 100% | 20.949 | 7.247 | 400.259 |
| Potassium (K) | 68 | 49% | 150.829 | 20.941 | 1519.387 |
| Magnesium (Mg) | 87 | 63% | 74.527 | 34.182 | 670.849 |
| Molybdenum (Mo) | 109 | 80% | 0.035 | 0.005 | 0.135 |
| Manganese (Mn) | 137 | 100% | 0.681 | 0.153 | 11.318 |
| Sodium (Na) | 39 | 28% | 384.845 | 8.980 | 1436.451 |
| Selenium (Se) | 137 | 100% | 0.351 | 0.196 | 0.636 |
| Lead (Pb) | 131 | 96% | 0.093 | 0.015 | 9.680 |
| Zinc (Zn) | 137 | 100% | 79.921 | 40.944 | 146.821 |

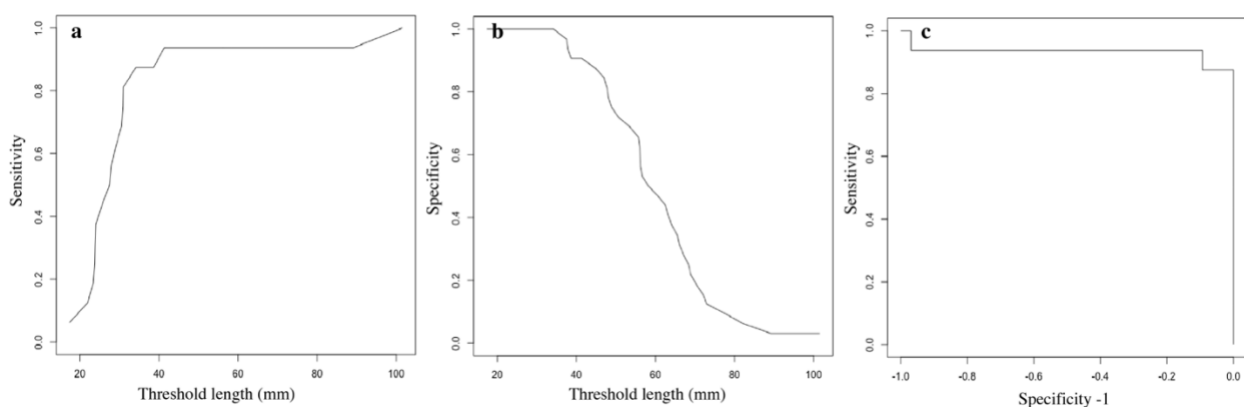


Figure.B.1. Determination of hair growth year cut-off. Panel “a” sensitivity (y-axis), at differing hair length thresholds in mm (x-axis), Panel “b” specificity (y-axis), and Panel “c” ROC curve of sensitivity in relation to specificity-1.

Details of linear mixed model with log element concentration as the dependent variable and coefficient estimates

Model Estimates (reference category for location is neck)

Table.B.2. Summary of best fit model covariates for models comparing paired neck and rump concentrations of Zn, Se, Cu, Mo, and Pb, with representative best fit model, slope estimate, standard error (SE), degrees of freedom (DF), t-value, and p-value.

| Model | Covariates | estimate | SE | DF | t-value | p-value |
|-------|-----------------|----------|-------|----|---------|---------|
| Zn1 | Intercept | 82.88 | 2.778 | 17 | 29.82 | <0.001 |
| | Location (rump) | 6.63 | 2.83 | 17 | 2.33 | 0.03 |
| | Sex | 2.41 | 4.14 | 16 | 0.58 | -0.57 |
| Se | Intercept | 0.277 | 0.02 | 17 | 14.19 | <0.001 |
| | Location(rump) | 0.04 | 0.014 | 17 | 2.93 | 0.0094 |
| Cu | Intercept | 6.72 | 0.183 | 17 | 36.70 | <0.001 |
| | Location (rump) | -1.083 | 0.17 | 17 | -6.34 | <0.001 |
| Mo | Intercept | 0.262 | 0.013 | 17 | 19.46 | <0.001 |
| | Location (rump) | -0.075 | 0.018 | 17 | -4.202 | <0.001 |

Table.B.3. Details of best fit models for paired neck and rump samples include number of observations, number of groups, within-groups variance, between-group variance, marginal R², conditional R², and Intraclass Correlation (ICC).

| | Zn | Se | Cu | Mo |
|--|-------|-------|------|-------|
| Number of observations | 36 | 36 | 36 | 36 |
| Number of groups | 18 | 18 | 18 | 18 |
| Intercept variance (within) | 32.45 | 0.005 | 0.34 | 0.004 |
| Error variance (between) | 72.29 | 0.002 | 0.26 | 0.003 |
| Marginal R² (fixed only) | 0.11 | 0.06 | 0.33 | 0.31 |
| Conditional R² (whole model) | 0.38 | 0.74 | 0.71 | 0.39 |
| ICC | 0.31 | 0.72 | 0.56 | 0.11 |

Details of linear models with log element concentration as the dependent variable and coefficient estimates (excluding age)

Table.B.4. Comparison of multiple linear models, for Zn, Se, Cu, Mo, and Pb and the corresponding AIC, Δ AIC, and degrees of freedom (DF), and best fit model (highlighted). Year of sampling collection (Year), Sex (male or female), and sampling source (hunted or captured) were included as potential parameters.

| Element | $\hat{\beta}$ | # | n | Model | AICc | Δ AICc | DF |
|-----------|---------------|------|-----|-----------------------|-----------|---------------|-----|
| Zn | -0.3 | Zn4 | 118 | Year+Hair loc | -354.7463 | 0 | 109 |
| | -0.3 | Zn9 | 118 | poly(Year,2)+Hair loc | -352.8238 | 1.9225 | 108 |
| | -0.3 | Zn2 | 118 | Sex+Year+Hair loc | -352.6653 | 2.081 | 108 |
| | -0.3 | Zn7 | 118 | Year+Source | -351.6627 | 3.0836 | 111 |
| | -0.3 | Zn11 | 118 | poly(Year,3)+Hair loc | -350.7364 | 4.0099 | 107 |
| | -0.3 | Zn1 | 118 | Sex+Year+Source | -349.6586 | 5.0877 | 110 |
| | -0.3 | Zn8 | 118 | poly(Year,2)+Source | -349.49 | 5.2563 | 110 |
| | -0.3 | Zn10 | 118 | poly(Year,3)+Source | -347.4345 | 7.3118 | 109 |
| | -0.3 | Zn5 | 118 | Sex+Year | -340.4524 | 14.2939 | 110 |
| | -0.3 | Zn6 | 118 | Sex+Source | -338.7127 | 16.0336 | 111 |
| | -0.3 | Zn3 | 118 | Sex+Hair loc | -336.5348 | 18.2115 | 109 |
| Se | -0.4 | Se7 | 118 | Year+Source | -37.67332 | 0 | 111 |
| | -0.4 | Se8 | 118 | poly(Year,2)+Source | -37.27283 | 0.40049 | 110 |
| | -0.4 | Se6 | 118 | Sex+Source | -37.25897 | 0.41435 | 111 |
| | -0.4 | Se1 | 118 | Sex+Year+Source | -35.49966 | 2.17366 | 110 |
| | -0.4 | Se4 | 118 | Year+Hair loc | -35.4946 | 2.17872 | 109 |
| | -0.4 | Se3 | 118 | Sex+Hair loc | -35.21997 | 2.45335 | 109 |
| | -0.4 | Se9 | 118 | poly(Year,2)+Hair loc | -35.15059 | 2.52273 | 108 |
| | -0.4 | Se10 | 118 | poly(Year,3)+Source | -35.08469 | 2.58863 | 109 |
| | -0.4 | Se2 | 118 | Sex+Year+Hair loc | -33.28188 | 4.39144 | 108 |
| | -0.4 | Se11 | 118 | poly(Year,3)+Hair loc | -32.92211 | 4.75121 | 107 |
| | -0.4 | Se5 | 118 | Sex+Year | -6.598769 | 31.0746 | 111 |
| Cu | 0.8 | Cu8 | 118 | poly(Year,2)+Source | 253.8017 | 0 | 110 |
| | 0.8 | Cu9 | 118 | poly(Year,2)+Hair loc | 254.7959 | 0.9942 | 108 |
| | 0.8 | Cu10 | 118 | poly(Year,3)+Source | 255.7331 | 1.9314 | 109 |
| | 0.8 | Cu11 | 118 | poly(Year,3)+Hair loc | 256.7649 | 2.9632 | 107 |
| | 0.8 | Cu6 | 118 | Sex+Source | 257.3066 | 3.5049 | 111 |
| | 0.8 | Cu7 | 118 | Year+Source | 257.3683 | 3.5666 | 111 |
| | 0.8 | Cu4 | 118 | Year+Hair loc | 259.1763 | 5.3746 | 109 |
| | 0.8 | Cu3 | 118 | Sex+Hair loc | 259.2595 | 5.4578 | 109 |
| | 0.8 | Cu1 | 118 | Sex+Year+Source | 259.4809 | 5.6792 | 110 |
| | 0.8 | Cu2 | 118 | Sex+Year+Hair loc | 261.3356 | 7.5339 | 108 |
| | 0.8 | Cu5 | 118 | Sex+Year | 267.0924 | 13.2907 | 111 |
| Mo | 0.2 | Mo10 | 118 | poly(Year,3)+Source | 27.89761 | 0 | 109 |
| | 0.2 | Mo11 | 118 | poly(Year,3)+Hair loc | 29.9854 | 2.08779 | 107 |
| | 0.2 | Mo8 | 118 | poly(Year,2)+Source | 31.11603 | 3.21842 | 110 |
| | 0.2 | Mo9 | 118 | poly(Year,2)+Hair loc | 33.17169 | 5.27408 | 108 |
| | 0.2 | Mo7 | 118 | Year+Source | 35.99226 | 8.09465 | 111 |
| | 0.2 | Mo5 | 118 | Sex+Year | 36.92236 | 9.02475 | 111 |
| | 0.2 | Mo4 | 118 | Year+Hair loc | 37.39291 | 9.4953 | 109 |
| | 0.2 | Mo1 | 118 | Sex+Year+Source | 38.16783 | 10.27022 | 110 |
| | 0.2 | Mo2 | 118 | Sex+Year+Hair loc | 39.60387 | 11.70626 | 108 |

| | | | | | | | |
|-----------|-------|------|-----|-----------------------|----------|----------|-----|
| | 0.2 | Mo6 | 118 | Sex+Source | 49.68538 | 21.78772 | 111 |
| | 0.2 | Mo3 | 118 | Sex+Hair loc | 50.65191 | 22.753 | 109 |
| Pb | -0.25 | Pb1 | 118 | Sex+Year+Source | 470.3989 | 0 | 110 |
| | -0.25 | Pb7 | 118 | Year+Source | 470.4251 | 0.026 | 111 |
| | -0.25 | Pb5 | 118 | Sex+Year | 471.4669 | 1.068 | 111 |
| | -0.25 | Pb4 | 118 | Year+Hair loc | 472.1008 | 1.7019 | 109 |
| | -0.25 | Pb2 | 118 | Sex+Year+Hair loc | 472.1554 | 1.7565 | 108 |
| | -0.25 | Pb8 | 118 | poly(Year,2)+Source | 472.5357 | 2.1368 | 110 |
| | -0.25 | Pb6 | 118 | Sex+Source | 472.8979 | 2.499 | 111 |
| | -0.25 | Pb9 | 118 | poly(Year,2)+Hair loc | 474.1543 | 3.7554 | 108 |
| | -0.25 | Pb10 | 118 | poly(Year,3)+Source | 474.2189 | 3.82 | 109 |
| | -0.25 | Pb3 | 118 | Sex+Hair loc | 474.8213 | 4.4224 | 109 |
| | -0.25 | Pb11 | 118 | poly(Year,3)+Hair loc | 475.8754 | 5.4765 | 107 |

Table.B.5. Summary of Mann-Whitney- Wilcoxon tests between adult (n=25) and sub-adult (n=10) Zn, Se, Cu, Mo, and Pb hair concentrations.

| Element | N | W | p-value |
|---------|----|-----|---------|
| Zn | 35 | 101 | 0.397 |
| Se | 35 | 138 | 0.653 |
| Cu | 35 | 123 | 0.957 |
| Mo | 35 | 118 | 0.815 |
| Pb | 35 | 149 | 0.397 |

Table.B.6. Multiple linear regression models of Zn, Mo, Pb, Se, and Cu, with corresponding AIC and Δ AIC. Age of animals (adult or non-adult), year of sampling collection (Year), Sex (male or female), were included as potential parameters. Associated Shapiro test of residuals and Breusch pagan test p-values are also included.

| | $\hat{\beta}$ | # | n | Model | AICc | Δ AICc | Shapiro | Bptest |
|-----------|---------------|------|----|------------------|-----------|---------------|---------|--------|
| Zn | -0.5 | Zn13 | 35 | Year + Age | -577.2048 | | 0.322 | 0.49 |
| | | Zn14 | 35 | Sex + Age | -576.0176 | | | |
| | | Zn15 | 35 | Sex + Year | -575.7441 | | | |
| | | Zn12 | 35 | Sex + Year + Age | -574.4938 | | | |
| Se | 1.25 | Se13 | 35 | Year + Age | -99.2781 | | 0.956 | 0.21 |
| | | Se15 | 35 | Sex + Year | -99.12183 | | | |
| | | Se14 | 35 | Sex + Age | -98.63146 | | | |
| | | Se12 | 35 | Sex + Year + Age | -96.64858 | | | |
| Cu | -0.75 | Cu13 | 35 | Year + Age | -119.3341 | 0 | 0.817 | 0.137 |
| | | Cu15 | 35 | Sex + Age | -118.8993 | | | |
| | | Cu12 | 35 | Sex + Year + Age | -117.4187 | | | |
| | | Cu14 | 35 | Year + Sex | -114.5016 | | | |
| Mo | log | Mo13 | 35 | Year +age | 72.61234 | 0 | 0.46 | 0.702 |
| | | Mo15 | 35 | Sex + Year | 72.94725 | | | |
| | | Mo12 | 35 | Sex + Yar + Age | 75.34558 | | | |
| | | Mo14 | 35 | Sex + Age | 88.44762 | | | |
| Pb | log | Pb15 | 35 | Sex + Year | 121.9339 | 0 | 0.001 | 0.11 |

| | | | | | | | |
|--|------|----|-----------------|----------|--|--|--|
| | Pb13 | 35 | Year + Age | 122.7286 | | | |
| | Pb14 | 35 | Sex + Age | 123.2978 | | | |
| | Pb12 | 35 | Sex + Yar + Age | 124.6298 | | | |

Table.B.7. Summary of best fit age model parameters for Zn, Se, Cu, Mo, and Pb, with representative model # (Model), R2, Parameters, Estimate, CI and p-value.

| Element | Model | Parameter | Estimate | 95% CI | p-value |
|---------|-------|-------------|---------------------|--|---------|
| Zn | Zn13 | Year | -5.69 ⁻⁵ | -1.91 ⁻⁵ : 3.05 ⁻⁵ | 0.644 |
| | | Age (adult) | -2.45 ⁻⁵ | -2.24 ⁻⁵ : 7.13 ⁻⁵ | 0.295 |
| Se | Se13 | Sex (Male) | -0.01 | -0.03: 0.012 | 0.339 |
| | | Year | -0.01 | -0.05: 0.03 | 0.555 |
| Cu | Cu13 | Year | -0.02 | -0.04 : -0.002 | 0.03 |
| | | Age (adult) | -0.01 | -0.05: 0.02 | 0.378 |
| Mo | Mo13 | Year | -0.53 | -0.79 : -0.25 | <0.001 |
| | | Age (adult) | -0.21 | -0.72 : 0.30 | 0.41 |
| Pb | Pb15 | Year | 0.22 | -0.32 : 0.76 | 0.41 |
| | | Sex (male) | 0.09 | -0.93 : 1.11 | 0.86 |

APPENDIX C

Chapter 4 Supplementary Material

Details of linear mixed model with log-hair cortisol concentration as the dependent variable and coefficient estimates

Table.C.1. Model estimates (reference category for body location is neck) for mixed effect models of body location effects.

| | Coef | SE | DF | t-value | p-value |
|-----------|--------|--------|----|---------|---------|
| Intercept | 1.66 | 0.061 | 33 | 27.24 | <0.001 |
| Rump | -0.066 | 0.0744 | 33 | -0.88 | 0.384 |

Table.C.2. Model details for mixed effect linear model of body location effects.

| | |
|----------------------------------|-------|
| Number of observations | 68 |
| Number of groups | 34 |
| Intercept variance (between) | 0.032 |
| Error/Residual variance (within) | 0.094 |
| Marginal R ² | 0.09 |
| Conditional R ² | 0.26 |
| ICC | 0.253 |

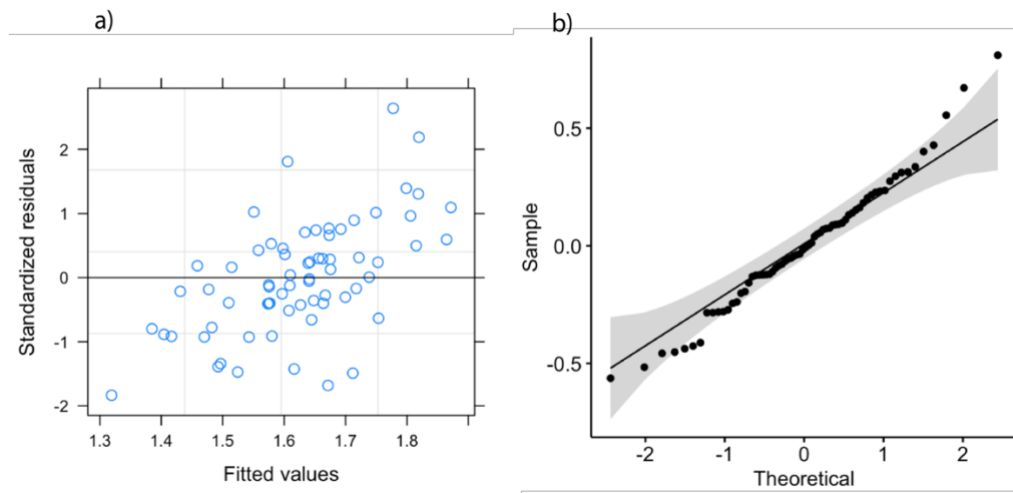


Figure.C.1. Summary of model fit for body location. A) corresponds to the residual's vs fitted values. While B) corresponds to a qqplot of the residuals.

Details of linear mixed effect model predicting hair cortisol concentration in caribou

Best fit Model: `lme(log_cort ~ Sex+ Herd + MI + OI + poly(month_cat, 2), random~1|Year)`

Random effects: year of hair growth

Fixed effects: Sex (Male/Female)+ herd (DU/BNE) + MI (continuous) + OI (continuous) + month(continuous)

Table.C.3. Model estimates for covariates impact caribou HCC using mixed effect linear regression.

| | Coef | SE | DF | t-value | p-value |
|---------------------|-------|-------|-----|---------|---------|
| Intercept | 0.81 | 0.663 | 393 | 1.21 | 0.22 |
| Sex (Male) | -0.15 | 0.07 | 393 | -2.21 | 0.03 |
| Herd (DU reference) | 0.91 | 0.27 | 393 | 3.37 | <0.001 |
| MI | 3.33 | 5.43 | 393 | 0.61 | 0.54 |
| OI | -4.62 | 3.82 | 393 | -1.21 | 0.23 |
| Poly (month, 1) | -2.19 | 0.71 | 393 | -3.08 | 0.002 |
| Poly (month,2) | -3.94 | 0.68 | 393 | -5.80 | <0.001 |

| | |
|----------------------------|------|
| Number of observations | 407 |
| Marginal R ² | 0.41 |
| Conditional R ² | 0.56 |

Summary of model fit

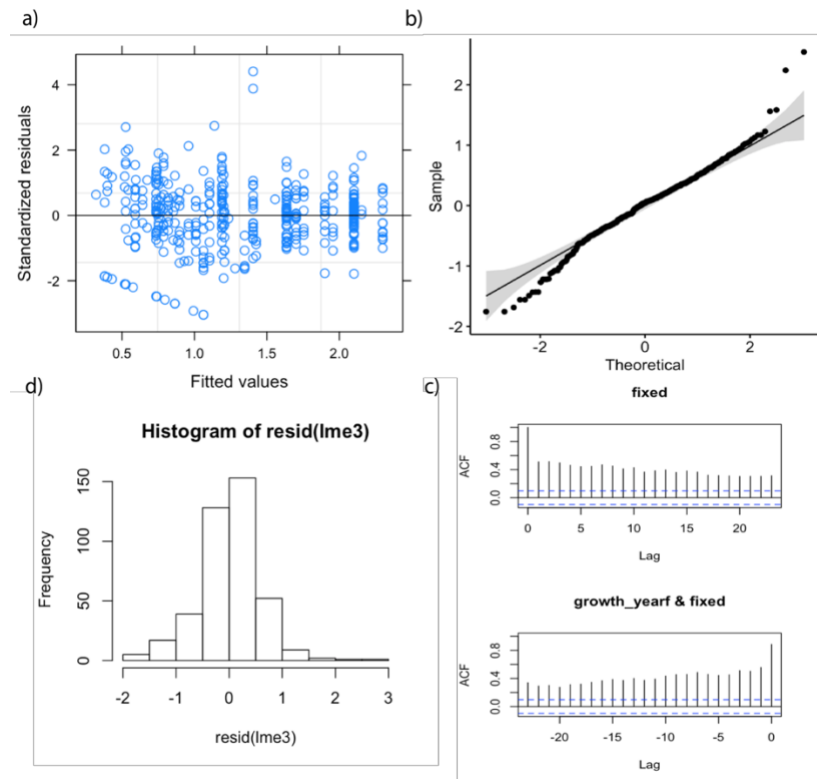


Figure.C.2. Summary of model fit for body location. A) corresponds to the residuals vs fitted values. While B) corresponds to a qqplot of the residuals. C) plots of autocorrelation and D) histogram of residuals.

Details of Averaged models predicting hair cortisol concentration in caribou

Global Model = log_HCC ~ Sex + Herd + poly(Month,2) + MI + OI + Year

Table.C.4. Summary of top dredged models out of 64 total possible combinations. No top models included an interaction between parameters. Model number (#), included parameters (+ = included, - = excluded), df, AIC and change in AIC (delta)

| # | Year | Herd | MI | OI | Month | Source | Sex | df | AIC | delta |
|---|------|------|----|----|-------|--------|-----|----|----------|-------|
| 1 | + | + | + | - | + | + | + | 9 | 2260.953 | 0.00 |
| 2 | + | + | - | + | + | + | + | 9 | 2260.982 | 0.03 |
| 3 | + | + | - | - | + | + | + | 8 | 2261.709 | 0.76 |
| 4 | + | + | - | + | + | + | + | 8 | 2262.650 | 1.69 |

Table.C.5. Summary of averaged model parameters obtained from dredging global model predicting log caribou hair cortisol concentration, n=407.

| | Estimate (95%) | Std. Error | Z -value | p-value |
|----------------|---------------------------|------------|----------|---------|
| Year | -0.382(-0.69 : -0.04) | 0.177 | 2.157 | 0.03 |
| Herd (DU) | 4.583(3.19: 5.40) | 0.568 | 8.041 | <0.001 |
| OI | 15.221(-0.56 :19.17) | 11.815 | 1.286 | 0.198 |
| Poly(month,1) | -16.1 (-26.4 : -5.83) | 5.22 | 3.073 | 0.002 |
| Poly(month, 2) | -23.321(-30.05 : -14.08) | 4.069 | 5.715 | <0.001 |
| Sex (Male) | -0.930 (-1.83 : -0.07) | 0.454 | 20.47 | 0.041 |
| MI | -6.431(-1.44 : 17.95) | 15.845 | 0.406 | 0.685 |

APPENDIX D

Preliminary Findings from an Interlaboratory Comparison of Caribou Hair Cortisol Analysis

D.1. Background

Hair cortisol concentration (HCC) has become an increasingly utilized tool to monitor trends in long-term HPA-axis activity of wildlife (Macbeth 2013, Dantzer et al., 2014). There are numerous advantages to the use of hair as a sample type to monitor chronic stress in wildlife, especially for difficult to access species inhabiting remote areas (Di Francesco et al., 2017). Within barren-ground caribou (*Rangifer tarandus groenlandicus*), hair cortisol concentration has been measured primarily by two laboratories, Laboratory A and Laboratory B. These two laboratories quantify cortisol using differing EIAs, as well as differing hormone extraction protocols.

Both laboratories have been used in the past to quantify hair cortisol for wild ungulate health research (Ashley et al., 2011; Carlsson et al., 2016; Dulude-de Broin et al., 2019). Laboratory A follows a protocol developed by C. Munro, and is described by Kummrow et al., 2011. Laboratory B uses an altered commercially available ELISA kit (Oxford EA-65 Cortisol EIA Kit), as described by Macbeth et al., 2010, summarized in Table.D.1. As a result, compiled HCC datasets of barren-ground caribou include values from laboratories utilizing differing Enzyme Immuno Assays (EIAs). In the context of long-term health monitoring of caribou, assay consistency is key to understanding temporal trends. The objective of this study is to examine interlaboratory consistency in quantifying caribou hair cortisol concentrations in the hopes of establishing a conversion factor.

D.2. Materials and Methods

Interlaboratory differences in hair cortisol quantification have been previously investigated in human medicine (Russell et al., 2015), and this study will utilize a similar methodological approach. The differences in EIA procedures are summarized in Table.D.1.

Table.D.1. Summary of differences in hair cortisol quantification procedures between Laboratory A and Laboratory B.

| Procedure | Laboratory A | Laboratory B |
|------------------------------|--|---|
| Hair mass | 0.05g | 0.05g |
| Hair follicle removal | Yes | Yes |
| Washing Method | Distilled water hand wash (2 mins), distill water soak (5 mins), vortex distilled water (10 sec), vortex methanol (10 sec) | 5 X methanol wash for 3 minutes in slow rotator |
| Pulverization | Cut into 5mm pieces | Ground into fine powder using a ball mill |
| Extraction solvent | 100% methanol | 100% methanol |
| Extraction duration and temp | 24 hours, 20-25 °C | 24 hours, 20-25 °C |
| Evaporation | 1500 ul evaporated 25°C | Dried at 38°C |
| Reconstitution solvent | Phosphate buffer | Phosphate buffer |
| EIA used | C. Munro | Oxford EA-65 Cortisol Kit |

Hair samples were derived from archived hair from two herds of barren-ground caribou, the Bluenose-East (BNE) and Dolphin and Union (DU) populations. Samples from 20 unique animals representing the range of possible values of caribou hair cortisol were selected based on quantile ranges. Five samples with a sufficient mass of hair remaining (>60mg) were taken from the 75-100%, 75-50%, 50-25%, and 0-25% quantiles.

If on the hide, 60-80mg of hair were shaved 2-3mm from the skin surface using a stainless-steel razor. If hair was archived off the hide, hair was checked for the presence of follicles, and follicles were removed by cutting them off with stainless steel metzenbaum scissors. If present, the wool undercoat was hand sorted out of the sample to the best of our ability. Hair length (short (<3cm), average (3-5cm) and long (>5cm), colour (brown, white, grey), and contamination level (clean, category 1-3) were denoted. Contamination level of blood/feces/urine were determined based on categories established by Macbeth (2013), categories are: 0%, (clean), 0-25% (category 1), 25-75% (category2), and 75-100% (category 3) covered in biologicals.

Hair samples were placed in paper envelopes and left to dry overnight (12 hours) at room temperature. Dried hair samples were placed in a 500mL beaker and homogenized.

Homogenization consisted of swirling the hair using plastic tweezers for 1-2 minutes, until an even distribution of hair was achieved. Hair was subsampled from the homogenized beaker, 100mg of homogenized hair was placed and weighed in paper envelopes suitable for storage. Four distinct sub-samples (100mg) were collected. Samples were shipped out to partner research laboratories on the same date, and hair was analyzed by partner research laboratories within two months of one another. Laboratory A checked hair and removed follicles, while Laboratory B returned the shipment for follicle removal.

To statistical comparison, duplicate values were averaged for comparisons between laboratories. Analyses were carried out using R statistic software version 3.6.0 (R. Core Team, 2020). A Wilcoxon signed rank test was used to test for difference in central tendency. Next, an F-test was used to compare differences in variation of values. Correlations between laboratories were examined using a Pearson’s correlation (r). Lastly, the linear relationship between laboratory results were examined using a linear regression, via the `lm()` function in R using log-transformed HCC values. Average values were log-transformed to adhere to the assumptions of linear regression.

D.3. Results and Discussion

Laboratory A reported HCC ranges of 2.02 to 19.59 pg/mg, and magnitude of difference between blinded duplicated values were 0.1-3.1 pg/mg (Table.D.2.). Laboratory B reported HCC ranges of 5.00 to 43.3 pg/mg, and the magnitude of difference between blinded duplicates were 0.69 to 25.37 pg/mg.

Table D.2. Results from interlaboratory comparison of barren-ground hair cortisol concentration. Animal ID, approximate undercoat presence, contamination category is denoted. Participating laboratories and blinded duplicate (A and B), and difference between duplicates (diff) hair concentrations in pg/mg are reported.

| # | Animal ID | Undercoat | Contam | Laboratory A | | | Laboratory B | | |
|---|-----------|-----------|--------|--------------|-------|------|--------------|-------|------|
| | | | | [A] | [B] | diff | [A] | [B] | diff |
| 1 | DU105 | <5% | 1 | 10.31 | 9.84 | 0.47 | 9.16 | 15.00 | 5.84 |
| 2 | BNE-28-18 | NA | 3 | 19.59 | 17.48 | 2.11 | 31.4 | 43.3 | 11.9 |
| 3 | BNE-10-17 | <5% | 2 | 6.44 | 5.94 | 0.5 | 10.0 | 7.92 | 2.08 |
| 4 | BNE-92-17 | 20-25% | 1 | 10.22 | 7.12 | 3.1 | 11.3 | 21.8 | 10.5 |
| 5 | BNE-10-19 | 20-30% | 3 | 6.00 | 5.20 | 0.8 | 16.8 | 15.6 | 1.2 |
| 6 | BNE-14-19 | NA | 1 | 4.47 | 3.81 | 0.66 | 8.43 | 7.64 | 0.79 |

| | | | | | | | | | |
|----|------------|--------|---|------|------|------|------|-------|-------|
| 7 | DU-129 | <5% | 1 | 5.88 | 7.19 | 1.31 | 36 | 12.5 | 23.5 |
| 8 | BNE-86-17 | <5% | 1 | 7.14 | 7.24 | 0.1 | 11.5 | 26.00 | 14.5 |
| 9 | BNE-23-18 | 15-20% | 2 | 5.38 | 6.44 | 1.06 | 8.01 | 8.63 | 0.62 |
| 10 | BNE-78-17 | NA | 1 | 6.42 | 6.6 | 0.18 | 35.2 | 9.83 | 25.37 |
| 11 | BNE-51-17 | NA | 1 | 8.01 | 7.36 | 0.65 | 13.7 | 18.03 | 4.33 |
| 12 | DU-257 | <20% | 1 | 5.53 | 5.42 | 0.11 | 8.53 | 7.84 | 0.69 |
| 13 | BNE-26-18 | <5% | 1 | 4.17 | 3.96 | 0.21 | 10.5 | 14.3 | 3.8 |
| 14 | DU-317 | <5% | 1 | 4.49 | 4.61 | 0.12 | 13.3 | 10 | 3.3 |
| 15 | BNE-12-19 | 5-10% | 1 | 2.99 | 3.97 | 0.98 | 15.1 | 8.09 | 7.01 |
| 16 | BNE-46-18 | 5-10% | 3 | 3.15 | 3.63 | 0.48 | 12.4 | 8.51 | 3.89 |
| 17 | BNE-34-18 | <10% | 1 | 5.66 | 4.45 | 1.21 | 6.15 | 7.11 | 0.96 |
| 18 | BNE-043-19 | 10-20% | 1 | 2.02 | 2.75 | 0.73 | 5.42 | 8.4 | 2.98 |
| 19 | BNE-25-18 | 5-10% | 1 | 4.24 | 3.18 | 1.06 | 5.02 | 9.03 | 4.01 |
| 20 | BNE-003-19 | <5% | 1 | 3.87 | 3.48 | 0.39 | 8.75 | 5.00 | 3.75 |

Duplicate samples sent to Laboratory A were well correlated ($r=0.97$ (0.91-0.99), $p<0.001$), Figure.D.1, and most duplicate samples were very similarly quantified by the Laboratory A. 90% ($n=18$) of samples had a difference that was less than or close to the Limit of Quantification of the assay (1.00 pg/mg), and 10% of samples had a difference that was greater than the LOQ ($n=2$); both samples were clean without surface contamination, and follicles were removed.

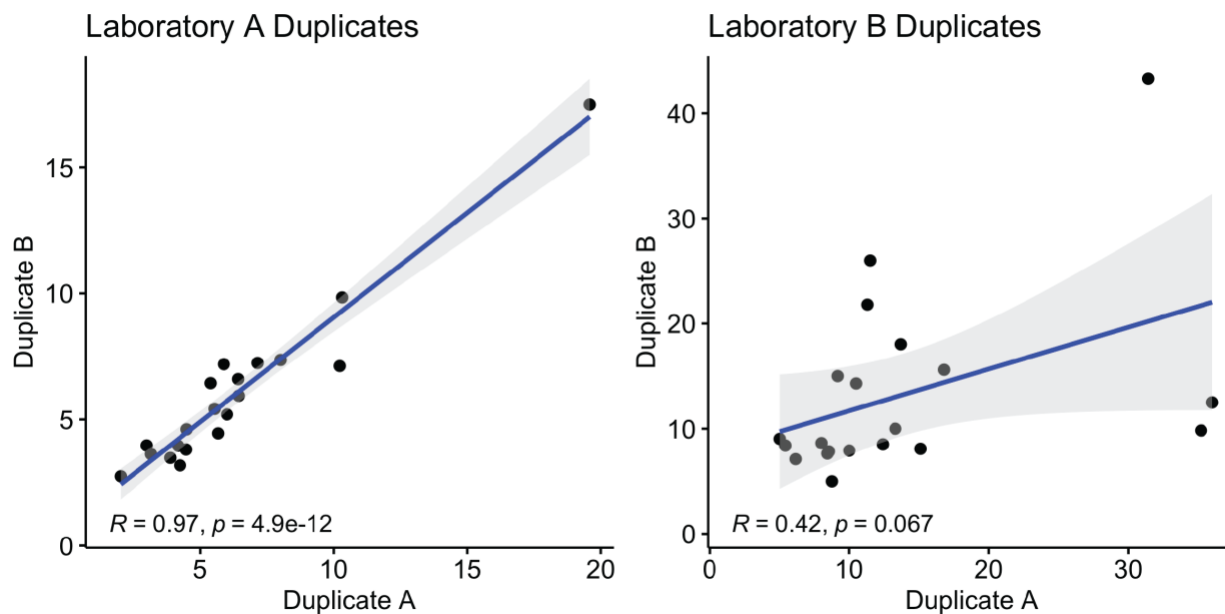


Figure.D.1. Correlations between blinded duplicate samples (n=20 pairs), concentration of duplicate A (x-axis), concentrations of duplicate B (y-axis), for the participating laboratories quantifying caribou hair cortisol concentration.

Duplicate samples sent to Laboratory B were not significantly correlated with one another ($r=0.43$ (-0.03 – 0.73), $0=0.07$). Most duplicate samples had a greater difference than 1.00 pg/mg (80%, $n=16$) and 20% ($n=4$) of samples had a difference less than 1.00 pg/mg. Further, $n=2$ samples had a difference between duplicates greater than 20 pg/mg. Duplicates with the greatest difference occurred in samples with low contamination (category 1), and no to little undercoat present (<5%).

Difference between duplicates may be due to variable presence of follicles, surface contamination and undercoat. The variable presence of follicles may have the capacity to alter quantified HCC (Sergiel et al., 2020), and although they were removed by hand, there is a possibility that some persisted variably between duplicates. However, although statistically significant, the magnitude of difference caused by follicles has been reported as 0.21 pg/mg, which is not able to explain the magnitude of difference between duplicates reported in this study. Similarly, variable surface contamination can alter derived HCC (Macbeth, 2013), however, the maximum magnitude of this effect when exposed to cortisol spiked slurry of 1200 ng was 6pg/mg. Furthermore, the greatest difference between blinded duplicate occurred in clean samples, with little to no undercoat present. Lastly, the undercoat is known to have markedly lower cortisol concentrations compared to guard hairs, but these hair types are significantly correlated with one another (Dulude-de-Broin et al., 2019), and duplicate samples were independently categorized as having the same level of undercoat abundance by partner laboratories.

Median values were significantly different between laboratories, and Laboratory B had reported significantly higher values on average (Wilcoxon $p<0.001$). Laboratories also had a had significantly different variance in reported values ($F=4.97$, $p=0.001$). When using average values between duplicates, laboratories were significantly correlated with one another ($r=0.80$, $p<0.001$), Figure D.2.

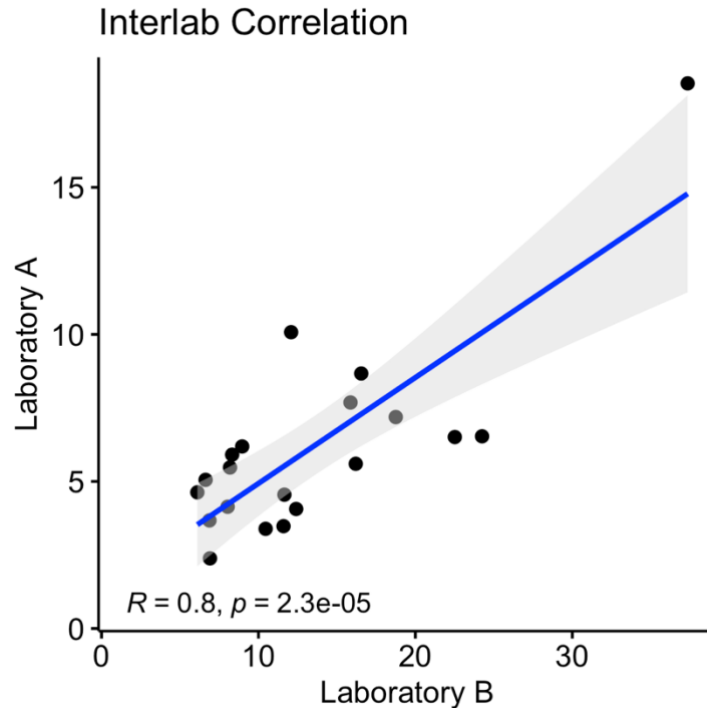


Figure.D.2. Correlations between averaged (n=20) samples sent to the Laboratory B (x-axis) and Laboratory (y-axis) A for caribou hair cortisol concentrations.

In a linear model, laboratories were a significant predictor of reported cortisol concentration ($p < 0.001$), and models adhered to the assumptions of linear regression (normal and equally distributed residuals). Using Laboratory A concentrations as the independent variable, the equation of the log transformed regression is $[\text{Lab A}] = 0.13 + 0.64[\text{Lab B}]$. As both the dependent and independent variables were log transformed, we may interpret that for every 1% increase in HCC derived by Lab B equates to a 0.64% increase in HCC derived by Lab A.

Therefore, when using averaged samples, it is possible to derive a conversion factor that will allow for the use of all available HCC data of barren-ground caribou.

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APPENDIX E

Preliminary Results of Serological Investigation of BNE Caribou

E.1. Introduction

Multiple studies have evaluated the seroprevalence of numerous pathogens of concern that impact barren-ground caribou, including the Bluenose-East (BNE) herd (Kutz et al., 2001; Stieve et al., 2010; Bondo et al., 2019; Carlsson et al., 2019). The last serosurvey to occur for the BNE population was between 2007-and 2009 during the international polar year (Carlsson et al. 2019), and a contemporary serosurvey for this population is warranted at this time. Therefore, the first objective of this study was to complete provide seroprevalence data for 6 key pathogens within the BNE from 2012 to 2020.

Second, is that certain pathogens, such as herpesvirus, are capable of latent infection or flares up, associated with elevated stressors (Das Neves et al., 2009). Pathogens that are transmitted through direct contact or aerosols, such as herpesvirus, may exhibit sex differences in *Rangifer*. A higher incidence of exposure in males is attributed to unique male behaviours resulting in increased direct contact with other individuals (Lillehaug et al., 2003), such as direct sexual competition and promiscuity. Sexual differences in exposure have yet to be documented in barren-ground caribou, despite being tested for in the past (Carlsson et al., 2019).

Further, patterns of elevated risk of co-exposure to herpesvirus and pestivirus have been previously reported in barren-ground caribou herds in the past, including the BNE. Adult animals are 2.6 times more likely to be seropositive for pestivirus, if they are already seropositive for herpesvirus or vice versa (Carlsson et al., 2019). These patterns of co-infection for pesti/herpes have also been previously reported in reindeer in Norway (Kautto et al., 2012), and Sweden (Tryland et al., 2012). The mechanistic explanation for these co-infections is that both viruses are associated with immunosuppression and are therefore associated with an increased risk for a secondary infection (Biswas et al., 2013).

This study employed both filter paper (FP) from hunters as well as serum collected from captures. BNE sera were tested for seroprevalence of 6 key pathogens of caribou using serological diagnostic tools (Table 1.). Following a descriptive summary of seroprevalence, we then sought to explore possible differences in seroprevalence between sexes, sample sources, year, and co-exposure.

E.2. Material and methods

FP and serum samples were run analyzed using a mix of commercially available and ELISAs that have been validated for FP in *Rangifer* (Curry et al., 2011; 2014), and are presented in Table.E.1.

Table.E.1. Diagnostic tests employed for bacteria, viruses, and protozoans from filter paper (FP) and serum samples of BNE caribou from 2012-2020.

| <i>Pathogen</i> | <i>Sample type</i> | <i>Method</i> | <i>Laboratory</i> |
|---|--------------------|---|--|
| <i>Pestivirus (Bovine Diarrhea virus (BVDV))</i> | FP/ serum | SERELISA BVD p80 Ab Antibody Test Kit; IDEXX | Kutz Research Group, University of Calgary |
| <i>Alphaherpesvirus (Bovine herpesvirus-1, BHV-1)</i> | FP/Serum | SERELISA BHV-1 gB Ab Mono Blocking; Synbiotics | Kutz Research Group, University of Calgary |
| <i>Brucella suis biovar 4</i> | FP/Serum | C-ELISA | CFIA-Ontario Health Laboratory (Fallowfield), Ottawa, ON |
| <i>Erysipelothrix rhusipathiae</i> | FP/Serum | Sandwich ELISA | Kutz Research Group, University of Calgary |
| <i>Neospora caninum</i> | FP/Serum | <i>Neospora caninum</i> Antibody Test Kit, cELISA; VM DR Inc., Pullman, WA, USA | Kutz Research Group, University of Calgary |
| <i>Toxoplasma gondii</i> | FP/Serum | ID Screen Toxoplasmosis Indirect Multispecies ELISA Kit | Kutz Research Group, University of Calgary |

Animals that were labeled as calves or had missing sex information were removed from the total dataset of 263 (22 animals were removed), resulting in a dataset of n=241 for analysis. Sample seroprevalence confidence intervals were calculated using a proportion test (“prop.test”). We utilized a logistic regression using the glm () function from R, fit with a “logit” link to analyze seroprevalence data. Solely alphaherpesvirus (Herpes) and Pestivirus (Pesti) had enough positive samples to undergo statistical modelling. The general approach was to first fit a global model with all explanatory variables of interest. Then, a stepwise likely-hood ratio (LRT) test was performed to identify parameters of significance. This approach is common in GLM models with binomial data (Cordeiro, 1983). Significant parameters identified by LRT were then added in different combinations and best fit models were compared using AICc. Model fit was tested using a Hoslem Test from the “ResourceSelection” package.

Table.E.2. GLM model summaries for alphaherpesvirus (Herpes) and Pestivirus (Pesti) seroprevalence. Global models are indicted in model #, sample size (n), Model combinations and associated AICc values. Best fit models, as determined by AICc are highlighted in green.

| Path | Model # | n | Model | AICc |
|--------|-------------|-----|--------------------------------------|----------|
| Herpes | H2 | 241 | Herpes ~ Sex + Source | 266.7661 |
| | H1 (global) | 241 | Herpes ~ Sex + Source + Year + Pesti | 268.4360 |
| | H3 | 241 | Herpes ~ Sex + Pesti | 272.5337 |
| | H4 | 241 | Herpes ~ Sex + Source | 273.7936 |
| | H6 | 241 | Herpes ~ Source + Year | 273.9052 |
| | H5 | 241 | Herpes ~ Source + Pesti | 274.5925 |
| Pesti | P2 | 241 | Pesti ~ Herpes + Year | 302.1561 |
| | P4 | 241 | Pesti ~ Year + Sex | 302.7628 |
| | P6 | 241 | Pesti ~ Source + Year | 303.031 |

| | | | | |
|--|-------------|-----|--------------------------------------|----------|
| | P5 | 241 | Pesti ~ Source + Herpes | 303.041 |
| | P3 | 241 | Pesti ~ Herpes + Sex | 303.4205 |
| | P1 (global) | 241 | Pesti ~ Year + Sex + Source + Herpes | 305.1112 |

E.3. Results and Discussion

Seroprevalences (% positive/total sample) for all investigated pathogens are like those previously reported in barren-ground caribou (Kutz et al., 2001; Bondo et al., 2019; Carlsson et al., 2019). Of interest is that only certain pathogens were detected within certain sample sources. Only captures were positive for *Brucella suis* biovar 4, and solely hunted animals were positive for *Toxoplasma gondii* exposure. The differences in seropositivity between sample sources are difficult to mechanistically explain but they may indicate that these sources differ in the sub-population being selected. Spatial factors were not included in this analysis and these sources sample from vastly different geographic areas.

Table.E.3. Bluenose-East (BNE) caribou seroprevalence. Observed seroprevalence of caribou screened for: Alphaherpesvirus (HERP), Pestivirus (PESTI), *Neospora caninum* (NEO), *Toxoplasma gondii* (TOXO), *Eysipelothrix rhusiopathiae* (ERYSIP), and *Brucella suis* biovar 4 (BRUC). Sample size (n), sample seroprevalence (%), 95% confidence interval (CI), and number of positive samples (p).

| | | 2013 | 2013 | 2014 | 2015 | 2016 | 2017 | | 2018 | | 2019 | | 2020 |
|---------------|----|-----------|----------|----------|-----------|-----------|-----------|-----------|----------|-----------|-----------|-----------|-----------|
| | | Capt | Capt | Capt | Capt | Capt | Capt | Hunt | Capt | Hunt | Capt | Hunt | Capt |
| HERP | n | 31 | 3 | 7 | 26 | 15 | 17 | 59 | 8 | 25 | 15 | 24 | 33 |
| | % | 77.5 | 33.3 | 100 | 76.9 | 66.7 | 76.5 | 79.7 | 75 | 36 | 80 | 54.2 | 97.0 |
| | CI | 58-90 | 18-87 | 56-100 | 56-90 | 39-87 | 50-92 | 67-89 | 35-96 | 19-57 | 51-95 | 33-74 | 82-99 |
| | p | 24 | 1 | 7 | 20 | 10 | 13 | 47 | 6 | 9 | 12 | 13 | 32 |
| PESTI | % | 29.0 | 0 | 71.4 | 26.9 | 33.3 | 11.8 | 28.8 | 37.5 | 36 | 26.7 | 33.3 | 42.4 |
| | CI | 15-48 | 0-69 | 30-95 | 12-48 | 13-61 | 2-38 | 10-74 | 9-55 | 26-61 | 18-42 | 19-57 | 16-55 |
| | p | 9 | 0 | 5 | 7 | 5 | 2 | 17 | 3 | 9 | 4 | 8 | 14 |
| NEO | % | 0 | 0 | 0 | 0 | 0 | 0 | 3.4 | 0 | 8.0 | 0 | 0 | 0 |
| | CI | 0-14 | 0-69 | 0-44 | 0-16 | 0-25 | 0-23 | 0-13 | 0-40 | 1-28 | 0-25 | 0-17 | 0-13 |
| | p | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 0 |
| TOXO | % | 0 | 0 | 0 | 0 | 0 | 0 | 13.6 | 0 | 20.0 | 0 | 33.3 | 0 |
| | CI | 0-14 | 0-69 | 0-44 | 0-16 | 0-25 | 0-23 | 6-25 | 0-40 | 8-41 | 0-25 | 16-55 | 0-13 |
| | p | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 5 | 0 | 8 | 0 |
| ERYSIP | % | 9.7 | 33.3 | 0 | 19.2 | 6.7 | 5.9 | 5.1 | 12.5 | 4.0 | 6.7 | 8.3 | 9.1 |
| | CI | 3-27 | 2-87 | 0-44 | 7-40 | 0-34 | 0-31 | 0-53 | 0-34 | 2-25 | 1-15 | 0-22 | 1-28 |
| | p | 3 | 1 | 0 | 5 | 1 | 1 | 3 | 1 | 1 | 1 | 2 | 3 |
| BRUC | % | 0 | 0 | 28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| | CI | 0-14 | 0-69 | 5-70 | 0-16 | 0-25 | 0-23 | 0-76 | 0-40 | 0-17 | 0-25 | 0-17 | 0-17 |
| | p | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

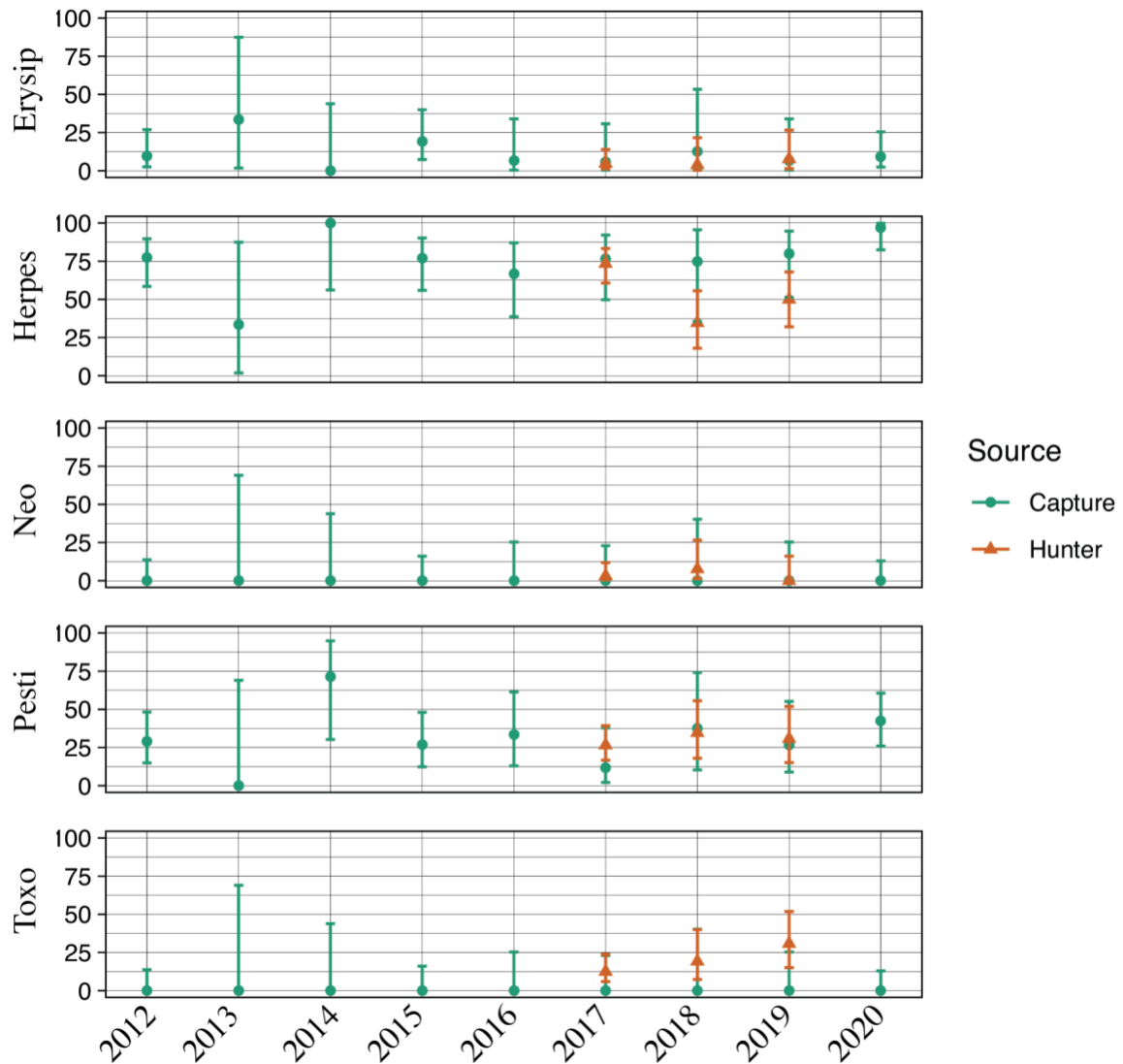


Figure.E.1. Sample source differences for seroprevalence of *Erysipelothrix rhusiopathiae* (Erysip), alphaherpes virus (Herpes), *Neospora caninum* (Neo), Pestivirus (Pesti), and *Toxoplasma gondii* (Toxo) from 2012-2020. Seroprevalence values calculated combined from all animals, sexes, and seasons. Confidence intervals the product of a proportion test. Of the included pathogens solely Alphaherpesvirus and pestivirus had a sufficiently high seroprevalence to model associations in sex, season, year and co-exposure, and solely herpesvirus had statistically significant findings.

Table.E.4. Table summarizing model output for some alphaherpesvirus and pestivirus models within the BNE herd from 2012-2020. Sample size (n), best fit model (Model), covariates, slope estimate, and 9%

CI determine by a proportion test (Estimate-CI), p-value, and checking of model fit using Hoslem Test are shown.

| <i>Pathogen</i> | <i>n</i> | <i>Model</i> | <i>Covariates</i> | <i>Estimate (CI)</i> | <i>p-value</i> | <i>Hoslem-test</i> |
|-----------------|----------|--------------|-------------------|-----------------------|----------------|--------------------|
| <i>Herpes</i> | 241 | H2 | Sex (male) | 0.96 (0.33 : 1.62) | 0.003 | P= 0.98 |
| | | | Source (Hunter) | -0.82 (-1.44 : -0.21) | 0.008 | |
| <i>Pesti</i> | 241 | P2 | Herpes | 0.33 (-0.31 : 1.00) | 0.323 | P=0.80 |
| | | | Year | 0.07 (-0.04 : 0.19) | 0.214 | |

Year and season were not significant parameters in any best fit model. Sex was a significant parameter, such that males were twice as likely to be seropositive for herpesvirus (OR=2.6, 1.9-5.1). These results differ from those previously reported in the BNE by Carlsson in 2007-2009 (Carlsson et al., 2019). However, sexual differences in seroprevalence for herpesvirus have been reported in reindeer populations in the past (Das Neves et al., 2010).

Sample source was also a significant parameter, such that hunted animals were less likely to be seropositive for herpesvirus (OR=0.4, 0.2-0.7). This difference between sample sources may be the product of two separate confounding factors that could not be accounted for. The first is season, as the stressors experienced by barren-ground caribou vary seasonally, such as insect harassment and predation (Toupin 1996, Witter et al., 2012). Viruses capable of latent infection, such as herpesvirus, may experience flare ups in response to stressors, and an increase in subsequent antibody production (Das Neves et al., 2009), therefore, seasonality may present a significant source of variation into analyses that should be accounted for. The second is animal age, there is very limited information for age of hunted animals (demographic information missing), and therefore age could not be included as a parameter in statistical analysis. Captured samples were known to be all adults, while hunted animals could have been a range of age classes. It is known that herpesvirus exposure and chance of positive seroprevalence increases with age (Das Neves et al., 2010). Therefore, diminished seroprevalence in hunted animals may be attributed to the fact that this sample set had a greater proportion of juvenile animals.

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APPENDIX F

BAERMANN FECAL SEDIMENTATION

Table.F.1. Summary of Protostrongylid nematode larvae in BNE caribou feces (5g) using a modified Baermann fecal sedimentation technique. Table includes year of collection, source (hunter collected or government capture), sample size (n), mean count of PA (*Parelaphostrongylus andersoni*) larvae, mean prevalence of PA larvae, Mean count of VE (*Varestrongylus eleguniensis*) larvae, and mean prevalence of VE larvae.

| Year | Source | n | Mean Count PA | Mean Prev. PA (%) | Mean Count VE | Mean Prev. VE (%) |
|------|---------|----|---------------|-------------------|---------------|-------------------|
| 2016 | Hunter | 0 | | | | |
| | Capture | 12 | 0.1 | 10 | 2.6 | 25 |
| 2017 | Hunter | 44 | 0.8 | 14 | 3.05 | 36 |
| | Capture | 0 | | | | |
| 2018 | Hunter | 21 | 0.43 | 28 | 1.05 | 24 |
| | Capture | 14 | 0.64 | 7 | 8.4 | 14 |
| 2019 | Hunter | 23 | 0 | 0 | 0.56 | 22 |
| | Capture | 4 | 1.25 | 25 | 28 | 75 |
| 2020 | Hunter | 0 | | | | |
| | Capture | 27 | 2.04 | 11 | 19.70 | 52 |

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APPENDIX G

JAW EXAMINATION

Table.G.1. Summary of jaw examination from hunted Bluenose-east caribou collected in 2018-2019. Sample ID, Age (from tooth eruption matter), incisor width (Inc.width), Diastema length (Dias.), Anterior jaw length (Ant.j), Posterior jaw length (Post.j), Total jaw length (Tot.j) and the second molar height (M2).

| # | Sample ID | Age | Inc.width (cm) | Dias. (cm) | Ant.j (cm) | Post.j (cm) | Tot.j (cm) | M2 (cm) |
|----|------------|-----------|----------------|------------|------------|-------------|------------|---------|
| 1 | BNE-03-18 | Adult | 1.9 | 11.7 | 16.5 | 13.1 | 29.6 | 1.3 |
| 2 | BNE-04-18 | Adult | 2.1 | 11.7 | 17 | 14 | 31 | 1 |
| 3 | BNE-07-18 | Adult | NA | 11.5 | 15.7 | 13 | 28.7 | 1.1 |
| 4 | BNE-15-18 | Adult | 1.9 | 9.7 | 14.7 | 11 | 25.7 | 1.1 |
| 5 | BNE-23-18 | Adult | 1.8 | 9.2 | 14 | 11.7 | 25.7 | 1.1 |
| 6 | BNE-25-18 | Adult | NA | 7.7 | 12.1 | 11.1 | 23.2 | NA |
| 7 | BNE-26-18 | Sub-Adult | 1.2 | 7.2 | 12.7 | 8.5 | 21.2 | 0.7 |
| 8 | BNE-29-18 | Adult | 1.7 | 9 | 14.4 | 11.7 | 26 | 1.4 |
| 9 | BNE-30-18 | Adult | 2.3 | 12 | 17.2 | 13.4 | 30.6 | 1 |
| 10 | BNE-31-18 | Adult | NA | 10.1 | 15.5 | 12.4 | 27.9 | NA |
| 11 | BNE-33-18 | Adult | 1.3 | 9.1 | 14 | 11.6 | 25.6 | 1.3 |
| 12 | BNE-34-18 | Adult | 2 | 9.7 | 14.7 | 12.1 | 26.7 | 1.2 |
| 13 | BNE-35-18 | Adult | 2.3 | 10 | 14.5 | 12.4 | 28 | 1.3 |
| 14 | BNE-36-18 | Adult | 2 | 9.8 | 14.5 | 11.6 | 26.1 | 1 |
| 15 | BNE-38-18 | Adult | 2.2 | 9.8 | 14.6 | 12.1 | 26.7 | 1 |
| 16 | BNE-40-18 | Adult | 2.2 | 10.7 | 15.8 | 12.6 | 28.9 | 0.9 |
| 17 | BNE-41-18 | Adult | 2 | 11.5 | 16.3 | 12.7 | 29.0 | 1 |
| 18 | BNE-42-18 | Sub-adult | 1.1 | 8.2 | 12.4 | 8.6 | 21 | 0.7 |
| 19 | BNE-43-18 | Adult | 2 | 9 | 14.4 | 11 | 25.4 | 1.2 |
| 20 | BNE-48-18 | Sub-adult | 1.8 | 8.2 | 13.3 | 9.6 | 22.9 | NA |
| 21 | BNE-49-18 | Adult | 2.1 | 10 | 14.6 | 11.9 | 26.5 | 1.1 |
| 22 | BNE-50-18 | Sub-Adult | 2.1 | 9.2 | 16 | 9.7 | 25.7 | 1.1 |
| 23 | BNE-003-19 | Adult | 2.4 | 10.2 | 15 | 12.6 | 27.4 | 0.9 |
| 24 | BNE-008-19 | Sub-adult | 1.1 | 8.3 | 11.9 | 8.2 | 21.2 | NA |
| 25 | BNE-012-19 | Adult | 2.2 | NA | 16.9 | 13.5 | 30.3 | 0.8 |
| 26 | BNE-013-19 | Adult | 2.3 | 11.5 | 15.5 | 13.9 | 29.9 | 0.98 |
| 27 | BNE-014-19 | Adult | 2.5 | 13.3 | 17.3 | 14.3 | 32.2 | 0.6 |
| 28 | BNE-016-19 | Sub-adult | 2 | 8.3 | 13.5 | 10.9 | 24.6 | 1.4 |
| 29 | BNE-024-19 | Sub-adult | 1.9 | 8.9 | 14.1 | 10.2 | 24.5 | 0.8 |
| 30 | BNE-027-19 | Adult | 2 | 11.9 | NA | NA | 28.2 | 0.5 |
| 31 | BNE-033-19 | Sub-adult | 1.9 | 9.4 | 14.1 | 11.7 | 25.8 | 0.8 |
| 32 | BNE-040-19 | Sub-adult | 1.9 | 8.4 | 13.5 | 10.3 | 23.7 | 0.9 |

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APPENDIX H

METATARSUS EXAMINATION

Table.H.1. Summary of Metatarsus examination of BNE caribou harvested between 2018-2019. Information includes Sample ID, Year of Collection, General leg observation, Metatarsus length in cm, circumference of midpoint in cm and % fat percentage of dried marrow.

| # | Sample ID | Year | Observation | Length (cm) | Circumf (cm) | Fat % |
|----|------------|------|-------------|-------------|--------------|-------|
| 1 | BNE-005-19 | 2019 | Normal | 26.8 | 10.1 | 86.8 |
| 2 | BNE-006-19 | 2019 | Normal | 27.2 | 9.2 | 94.0 |
| 3 | BNE-008-19 | 2019 | Normal | 23 | 7.3 | 90.2 |
| 4 | BNE-009-19 | 2019 | Normal | 23.5 | 7.9 | 89.1 |
| 5 | BNE-010-19 | 2019 | Normal | 26.6 | 9 | 89.3 |
| 6 | BNE-011-19 | 2019 | Normal | 27.9 | 9.5 | 90.2 |
| 7 | BNE-012-19 | 2019 | Normal | 29.2 | 11.4 | 91.7 |
| 8 | BNE-014-19 | 2019 | Normal | 30.6 | 12.1 | 93.6 |
| 9 | BNE-016-19 | 2019 | Normal | 26.4 | 8.4 | 90.9 |
| 10 | BNE-019-19 | 2019 | Normal | 27.2 | 9.9 | 93.5 |
| 11 | BNE-025-19 | 2019 | Normal | 27.9 | 10.1 | 95.2 |
| 12 | BNE-026-19 | 2019 | Normal | 27.3 | 9.6 | 95.1 |
| 13 | BNE-027-19 | 2019 | Normal | 26 | 9 | 88.9 |
| 14 | BNE-028-19 | 2019 | Normal | 28.8 | 11.1 | 94.9 |
| 15 | BNE-031-19 | 2019 | Normal | 24.1 | 7.8 | 92.0 |
| 16 | BNE-033-19 | 2019 | Normal | 26 | 8.6 | 94.3 |
| 17 | BNE-034-19 | 2019 | Normal | 25.8 | 8.6 | 88.2 |
| 18 | BNE-040-19 | 2019 | Normal | 25.6 | 6.8 | 61.9 |
| 19 | BNE-041-19 | 2019 | Normal | 26.4 | 9.8 | 94.3 |
| 20 | BNE-042-19 | 2019 | Normal | 26 | 9.2 | 94.2 |

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APPENDIX I

KIDNEY EXAMINATION

Table.I.1. Summary of Kidney examination of BNE caribou harvested predominantly in 2019. Information includes Sample ID, Year of collection, kidney + fat mass, Kidney mass alone, Fat mass alone and % Fat.

| # | Sample ID | Year | Kidney + Fat (g) | Kidney (g) | Fat (g) | Fat % |
|----|------------|------|------------------|------------|---------|-------|
| 1 | BNE-37-18 | 2018 | 232.22 | 145.58 | 86.64 | 59.5 |
| 2 | BNE-41-18 | 2018 | 250.15 | 201.43 | 48.72 | 24.2 |
| 3 | BNE-003-19 | 2019 | 101.51 | 95.76 | 5.76 | 6.0 |
| 4 | BNE-005-19 | 2019 | 153.72 | 124.16 | 29.56 | 23.8 |
| 5 | BNE-006-19 | 2019 | 198.03 | 138.03 | 60 | 43.5 |
| 6 | BNE-008-19 | 2019 | 75.67 | 56.03 | 19.64 | 35.05 |
| 7 | BNE-009-19 | 2019 | 80.92 | 60.91 | 20.01 | 32.85 |
| 8 | BNE-011-19 | 2019 | 190.89 | 153.57 | 37.32 | 24.3 |
| 9 | BNE-012-19 | 2019 | 260.04 | 202.24 | 57.8 | 28.6 |
| 10 | BNE-013-19 | 2019 | 240.74 | 172.07 | 68.67 | 39.9 |
| 11 | BNE-014-19 | 2019 | 393.58 | 256.42 | 137.16 | 53.5 |
| 12 | BNE-016-19 | 2019 | 117.99 | 91.49 | 26.5 | 28.9 |
| 13 | BNE-017-19 | 2019 | 271.21 | 155.55 | 115.66 | 74.3 |
| 14 | BNE-018-19 | 2019 | 183.75 | 130.65 | 53.12 | 40.66 |
| 15 | BNE-024-19 | 2019 | 145.21 | 142.87 | 2.34 | 1.6 |
| 16 | BNE-025-19 | 2019 | 184.12 | 145.21 | 38.91 | 26.8 |
| 17 | BNE-026-19 | 2019 | 260.24 | 157.47 | 102.77 | 65.3 |
| 18 | BNE-027-19 | 2019 | 118.7 | 98.36 | 20.34 | 20.7 |
| 19 | BNE-028-19 | 2019 | 319.055 | 252.03 | 67.03 | 26.6 |
| 20 | BNE-031-19 | 2019 | 150.42 | 79.83 | 70.59 | 88.4 |
| 21 | BNE-033-19 | 2019 | 130.66 | 88.45 | 42.21 | 47.7 |
| 22 | BNE-40-19 | 2019 | 91.36 | 70.92 | 20.44 | 28.8 |
| 23 | BNE-041-19 | 2019 | 179.79 | 123.59 | 56.2 | 45.5 |
| 24 | BNE-042-19 | 2019 | 131.52 | 111.44 | 20.08 | 18.02 |
| 25 | BNE-043-19 | 2019 | 178.54 | 127.22 | 51.32 | 40.3 |

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APPENDIX J

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