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Assessing health and fitness correlates for endangered mountain caribou demographically supported by maternal penning

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Title: **Assessing health and fitness correlates for endangered mountain caribou demographically supported by maternal penning**

Running head: **KZ health paper #1**

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Abstract

The health of wildlife is a key factor in population demography, linking habitat and physiology. Southern mountain caribou are an endangered group that could benefit from integrated approaches to wildlife health, but little is known about their health. Here, we use an Indigenous-led conservation initiative to assess the health of southern mountain caribou temporarily protected in a maternal pen to increase population growth. We collected health metrics from 46 female caribou between 2014-2021 and compared health metrics between penned and non-penned animals, and between successful and unsuccessful reproductive attempts. Results suggest that Klinse-Za caribou were generally healthy relative to neighboring subpopulations and that repeat capture and penning did not create accumulated health issues. We provide evidence linking trace nutrients and stress to reproductive success but find no evidence for relationships between reproductive success and pathogens or inflammation. This work provides a baseline assessment of southern mountain caribou health that can inform future trends and provide guidance on maternal penning activities in support of caribou recovery.

Keywords: cortisol, disease, endangered species, fitness, one health, rangifer, stress

Introduction

Wildlife health is an expanding field of research with emerging interest from wildlife managers and practitioners. While traditionally wildlife health focused on detecting, preventing, or mitigating diseases and toxins in animal populations, current approaches are increasingly treating health as an integrative metric representing an animal's collective interactions with its environment, people, and resilience to change (Stephen 2014, Bondo et al. 2019). This integrated approach is often referred to as One Health, which acknowledges that animal, people, and environmental health are intimately connected and creating positive outcomes for one supports the other (Zinsstag et al. 2011). Although a novel term, the One Health paradigm is not new. Indigenous Peoples have long viewed the health of environments, wildlife, and people as one (Jack et al. 2020), and actively stewarded landscapes as such (Kimmerer 2015, Knight et al. 2022).

One subspecies that has suffered from a recent lack of integrated approaches to health is the woodland caribou (*Rangifer tarandus caribou*). Woodland caribou are distributed across the forested landscapes in northern North America, and have been central to many northern Indigenous Peoples' culture and food security for millennia (Sharp and Sharp 2015, Parlee and Caine 2018). Woodland caribou historically lived in areas with low primary productivity and low densities of other ungulate species, affording caribou refuge from high densities of predators such as wolves, cougars, and wolverine (Serrouya et al. 2011). However, woodland caribou populations have declined precipitously in the last century due to intense habitat modification and climate change following colonization of the continent (Serrouya et al. 2021, Nagy-Reis et al. 2021). Industrial resource extraction and climate change have altered caribou habitat making it favourable for higher densities moose, deer, and elk in caribou habitat (Serrouya et al. 2011, 2021, Dawe and Boutin 2016, Fisher et al. 2020). Predator populations also increased in response to these surging prey densities and severely altered the predator-prey dynamic that caribou evolved under (Holt 1977, Serrouya et al. 2011, Dawe and Boutin 2016, Wallingford et al. 2020). Such changes have since caused caribou population declines and extirpations due to unsustainable predation rates (Wittmer et al. 2005). While the direct causes of caribou population declines are well understood, the interactions of these changes with caribou health and how individual well-being may influence population-level demographics are unclear.

To date, most studies of caribou health in Canada and Europe have focused on pathogens and parasites (Ducrocq et al. 2008, Curry 2009). In recent years the perspective has broadened to include metrics like serum biochemistry (Johnson et al. 2010), body condition, and Traditional Ecological Knowledge (Brook et al. 2011), but this work mainly focused on barren-ground caribou. Integrated measures of health are becoming more common, as seen in northern British Columbia where Bondo et al. (2019) collected information on boreal caribou pathogens, physiological stress, serum biochemistry and trace minerals to assess overall health and emerging threats. While baseline health information about caribou is slowly accumulating, there is a paucity of data and no such studies have been conducted on mountain caribou. Health and population demography are rarely considered together, but in one example Tryland et al. (2019) linked a sudden disease outbreak in semi-domesticated reindeer in Sweden to the increased stress, animal-to-animal contact, and compromised hygiene associated with corralling and feeding. Health considerations for mountain caribou in Canada are especially important in the southern portion of their distribution where populations are considered endangered due to rapid declines (Boutin and Merrill 2016).

Southern Mountain Caribou are at the southernmost edge of the woodland caribou distribution. Unfortunately, such a distribution means that this globally unique population of caribou face extensive anthropogenic habitat disturbance, invading white-tailed deer, and greater winter variability compared to more northerly distributed caribou (Wallingford et al. 2020, Nagy-Reis et al. 2021). This endangered group of caribou is composed of 38 subpopulations, once distributed between southwestern Canada and Northern USA (Environment Canada 2014). However, many subpopulations are in steep decline and over the last two decades, twelve subpopulations have been extirpated and the species is now extinct in the contiguous USA (Environment Canada 2014, Moskowitz 2019, Lamb et al. 2022). Adding to the concerns for this group of caribou, recent evidence suggests that woodland caribou may be nutritionally stressed (Heard and Zimmerman 2021, Cook et al. 2021, Denryter et al. 2022). As a result of the decline of southern mountain caribou, Indigenous Peoples who have long lived amongst abundant populations of caribou have voluntarily curtailed or completely halted cultural practices such as caribou hunting; an infringement of the treaty rights to a subsistence livelihood promised by Canada (Lamb et al. 2022).

An ambitious Indigenous-led effort, focused on recovering a subpopulation of southern mountain caribou to an abundance that could one day support a hunt, provided a unique opportunity to assess the health of these caribou. To avert the extirpation of the once abundant Klinse-Za caribou subpopulation, West Moberly First Nations, Saulteau First Nations and partners began a recovery program that included wolf population reductions, habitat restoration, and maternal penning (Lamb et al. 2022). The goal of this program was to increase survival of adults and young, thereby allowing the subpopulation to begin recovering from a low of 38 animals in 2013 (McNay et al. 2022). The annual live capture of the Klinse-Za caribou subpopulation, and their subsequent stay in the maternal pen from March through July gave us an opportunity to monitor health in a detailed manner that is rarely possible for wild animals. By bringing adult females into the maternal pen, a ~10 ha enclosure in the wild where they can safely birth and rear their young calves, we are able to connect results of health assays with reproductive and other demographic outcomes. Given the highly involved nature of maternity penning, we sought to identify any effects this management effort might have on caribou health—either positive or negative—to guide future initiatives and asses the overall effectiveness of maternal penning as a conservation strategy.

In this study we attempt to: 1) assess potential health implications from maternal penning, including repeat captures, 2) characterize the current health of Klinse-Za caribou in relation to nearby subpopulations and published references ranges, and 3) evaluate interactions between individual health and reproduction for Klinse-Za caribou.

Methods

Study area

The study area for this project is defined by the range of the Klinse-Za caribou subpopulation, in north-eastern British Columbia, Canada (Figure 1). The Klinse-Za range is located in the western Peace region in British Columbia and characterized by both steep and rolling sections of the Rocky Mountains. Anthropogenic disturbance is concentrated towards the eastern side of the subpopulation range (though present at some level throughout), lower elevations, and includes both permanent infrastructure such as paved roads, electronic transmission lines and gas pipelines, as well as dynamic features like cutblocks, forest service roads, a large hydroelectric reservoir, snowmobile trails and more. Additional details on the biogeoclimatic conditions within the area, history, and habitat protections are detailed in Lamb et al. (2022) and McNay et al. (2022).

Maternal pen primer

The Klinze-Za maternal pen has operated annually since 2014 (McNay et al. 2022) and we consider health metrics for the 2014-2021 period. Each year, the pen operation is initiated in March by capturing 10 to 18 adult females just prior to their third trimester of pregnancy. The adult females are transported to a pen (7-14 ha), located in an alpine meadow within the caribou's historic calving range, where they are marked, collared, and evaluated by a licensed veterinarian, and health samples collected. Female caribou in the pen are fed, monitored, and protected by Indigenous Caribou Guardians. Calves are typically born between early May and mid-June and are regularly monitored while in the pen. All females and calves are simultaneously released when the youngest calf is at least 6 weeks old and is therefore past the highest-risk period for neonatal predation (Adams et al. 2019). Concurrently during the penning season, three aerial surveys of the free-ranging population are carried out via radio telemetry, collecting demographic data on population size and age-sex structure, as well as adult and calf survival and mortality.

Sample collection

At capture

We captured caribou in March of each year between 2014-2021. Capture was conducted by aerial net gunning, as described in (McNay et al. 2022). Adult female caribou were the target of most captures, but calves of both sexes were sometimes brought into the maternal pen with their mothers. We aimed to have an approximately similar number of marked adult Klinse-Za females in the pen as we left in the free-ranging population. While initially most marked animals were brought into the pen, as the population grew, the sample of free-ranging females increased as well. Captured adult females headed for the maternal pen were sedated with intranasally administered medetomidine. Once in the maternal pen, veterinarians on the capture team collected: 1) fecal pellets (typically by hand from the rectum, but sometimes from the ground), 2) 30 millilitres of blood, 3) a clump of hair from the shoulder, and 4) three metrics of body condition: weight in kgs, and a qualitative assessment of body condition based on palpation of the rump, and in some years a body fat percentage based on ultrasound. If the female was captured and handled for the first time, we also took a skin biopsy sample in the course of ear-tagging. The blood collected at capture was centrifuged within one to two days of capture to collect blood components (i.e., serum, plasma, buffy coat, and red cells).

In addition to the data from the Klinse-Za animals, we also analyzed blood and hair samples from caribou that were captured in nearby subpopulations as part of the Omineca Northern Caribou Project (ONCP) between 1998 and 2013 (Unpubl.Data, Wildlife Infometrics Inc., Mackenzie, British Columbia). Samples from six caribou subpopulations (Chase, Wolverine, Thutade, Akie/Ospika, Nonda, and Scott) were subsampled and sent for laboratory analysis in tandem with the Klinse-Za samples to provide additional comparison groups. The ranges of the ONCP subpopulations during the study years were relatively less disturbed than the Klinse-Za currently is, providing a chance to contrast these animals' health metrics with those of caribou experiencing lower levels of anthropogenic disturbance. Given that established values for optimal parameter ranges don't exist for most caribou health metrics, we compared the health metrics of penned caribou to surrounding subpopulations, including the ONCP herds as well as previously published data from boreal caribou in BC (Bondo et al. 2019).

During the penning season

Between 2017-2019, we collected fecal pellets from both penned and free-ranging caribou three times throughout the penning season: once pre-calving (in April or May) and twice post-calving (in June and in July). Pen and free-ranging sampling sessions were carried out within 10 days of each other. In 2016, we carried out a one-time, two-day effort to collect fecal pellets in the pen (June 23 and 24, 2016), but were not able to follow up with free-ranging sampling that year. Initially, we attempted to collect pellets from known females in the pen through close observation. However, due to the close proximity of the females to one another, we could not assign individual ID to pellet samples so we collected as many high quality, fresh pellets as we could during the sessions to capture samples from as many animals as possible.

To collect samples from the free-ranging (i.e., non-penned) females, we used GPS data from collared individuals (ca. n=10 annually) to identify locations where caribou had been within the previous three days. We aimed to collect samples from every collared female's location, however, depending on the sampling session, the number of locations and accessibility varied. We collected at 4 to 7 sites each session. Crews accessed all locations by helicopter and hiked in to hand-collect the pellets that appeared freshest. Fecal pellets from calves were noticeably smaller than adult pellets and were not collected. We brought an ice-filled cooler on board to keep samples as cold as possible until they could be frozen that evening. Identifying fecal samples to individual or sex was not possible in the field. Therefore, where collared females were traveling in mixed groups, we may have also collected samples from males and/or yearling calves. Upon completion of fecal sampling in 2019, we genotyped all samples to identify sex.

Please refer to permits and ethics approvals in Appendix 1 for the legal and permitting authorities used in the recovery actions by the First Nations, provincial government, contractors, and universities.

Lab methods

The lab and analytical methods that we used for this study closely followed the protocol established for the Boreal Caribou Health Research Program (BCHRP;(Schwantje et al. 2014, Bondo et al. 2019)). We chose to address four priority 'classes' of caribou health (Table 1): nutrition, inflammation, physiological stress, and pathogen exposure. For a more detailed background on individual metrics, see (Schwantje et al. 2014). While there are numerous physiological metrics that could be evaluated under these umbrella classes, we selected the tests that, based on current best knowledge, could help provide information related to animal survival and reproductive success, which are closely tied to population persistence and recovery. We collaborated with several laboratories across Canada to run the different physiological assays (see Appendix A).

Nutrition

Trace minerals are inorganic micronutrients typically obtained from the diet that are necessary for the healthy physiological functioning of animals (Hidiroglou 1979). In domestic and some free-ranging ungulates, deficiencies in trace minerals such as selenium or iron have been associated with decreased health and reduced reproductive success (Flueck 1994, Bondo et al. 2019, Newby and DeCesare 2020). The BCHRP identified a suite of trace minerals that can be evaluated using blood serum, and we replicated those tests with blood samples from seven years of Klinse-Za cow captures to establish baseline levels and identify potential deficiencies. In addition, we assessed levels of fecal nitrogen from both penned and free-ranging caribou (discussed below) as a measure of diet quality (Leslie Jr. et al. 2008).

Inflammation

Haptoglobin is an acute phase protein produced in response to inflammation and infection. As such, serum haptoglobin levels can serve as an index of immune response in ruminants (Bondo et al. 2019).

Stress

Glucocorticoids, released in response to stressors through activation of the hypothalamic-pituitary-adrenal (HPA) axis, are frequently used to quantify physiological stress in animals and can be measured through various biological media (i.e., blood, saliva, feces, urine, hair) (Sheriff et al. 2011). Measuring glucocorticoids in hair, hereafter 'hair cortisol', can provide a longer-term record (weeks to months) of cumulative physiological stress than other endocrine measures, as it's thought to be incorporated into the hair shaft during periods of hair growth (Macbeth 2013, Spong et al. 2020).

Fecal glucocorticoid metabolites can be used as an integrated measure of physiological stress over a shorter period of time, usually hours or days (Millspaugh and Washburn 2004). We used fecal pellets collected throughout the penning season to compare the short-term stress and diet quality experienced by penned versus free-ranging

Klinse-Za caribou. Specifically, we compared fecal glucocorticoid metabolites (hereafter 'FGM') as a measure of physiological stress.

For the genetic analysis of fecal pellets, we contracted the services of Wildlife Genetics International (wildlifegenetics.ca). We swabbed all spring and summer fecal pellet samples collected in the pen and from free-ranging caribou. The lab work was focused on individual identification of fecal samples (verified by skin and/or hair samples from known individuals), and sex identification.

Pathogens

In recent studies of caribou health in boreal BC subpopulations (Bondo et al. 2018), several pathogens have emerged as potential concerns. We focused on three priority pathogens: protozoan *Neospora caninum* (hereafter, 'Neospora'), bacterium *Erysipelothrix rhusiopathiae* (hereafter, 'Erysipelothrix'), and an alphaherpes virus – as yet unidentified in caribou but most likely Cervid Herpes virus 2, CvHV-2, identified using a test for Bovine Herpes virus 1, which causes Infectious Bovine Rhinotracheitis in cattle (hereafter, 'Alphaherpesvirus'). We also tested for *Toxoplasma gondii* (hereafter 'Toxoplasma'), a protozoan parasite that infects a wide range of mammalian species, including cervids (Dubey et al. 2007). All of these pathogens, under certain circumstances, have been associated with reduced reproductive success (reduced fertility and abortion) and in some cases, mortality (Dubey et al. 2007, Bondo et al. 2019). Seroprevalence signifies an exposure to the pathogen and the presence of a certain level of antibodies in the blood serum of the individual – it does not necessarily indicate active disease or pathology.

Analytical methods

The primary health metrics we consider in this analysis are trace minerals, fecal nitrogen, haptoglobin, glucocorticoid concentration (hair and feces), and pathogen exposure. We focused on comparing, where possible, health metrics between penned and free-ranging Klinse-Za animals, between Klinse-Za and nearby caribou subpopulations, within the Klinse-Za subpopulation through time, and between successful and unsuccessful reproductive attempts. We only assessed connections between health metrics and reproductive success for penned females due to 1) the paucity of free-ranging health samples and 2) uncertainty in calf outcomes for many free ranging animals, especially when calves are killed soon after birth but before our weekly flights. We conducted all analyses in program R (R Core Team 2021).

We assessed statistical significance between groups using Kruskal-Wallis (2 groups) and Dunn (>2 groups) tests. For analyses requiring accounting for multiple variables we used generalized linear mixed models (glmm) and generalized linear models (glm). We determined whether a glmm was needed by comparing, via an analysis of variance, model fits between glmm and glm. The glmm was fit with a random intercept for year, and capture location when appropriate, while the glm was fit without either random term. When evidence suggested that the glmm was warranted we used the more complex glmm formulation, otherwise we used the simpler glm.

Results and outcomes

Over the course of the penning project from 2014 - 2021, 42 individual adult female caribou spent at least one, and up to six, calving seasons in the pen, for a cumulative 102 monitoring years. Most years, we also captured and re-captured females for marking and (re)collaring without translocation into the pen, and in some cases health samples were also taken from these animals (n=18 individuals, 22 animal-years). Aside from the fecal pellets that we collected throughout the penning season, the remaining results represent findings from samples taken at capture in March. Therefore, it is important to note that while the samples are taken from 'penned females', they do not necessarily reflect the effects of captivity, since the samples are collected prior to penning and often reflect the conditions experienced by the female the minutes/hours before capture in most cases (blood), the days before capture (fecal pellets), or during the previous summer and fall (hair). For females that have been previously penned, a portion of the hair growth would have happened while in the maternal pen the previous summer.

We tested for correlations between multiple health metrics and found multiple instances where the metrics covaried, highlighting the integrated nature of health (Appendix 2). We tested for correlations between haptoglobin and trace minerals following the results of (Newby and DeCesare 2020). Haptoglobin was positively correlated with iron (r=0.63) and zinc (r=0.25), but not the other trace minerals considered. In addition, hair cortisol and haptoglobin levels were correlated (r=0.2) and higher rates of pathogens correlated with higher zinc (r=0.14—0.35) and lower hair cortisol (r=-0.13—-0.2).

Nutrition

We found that average levels of blood serum trace minerals in the Klinse-Za subpopulation were comparable to boreal caribou evaluated by Bondo et al. (2019), and also relative to a published reference range (Puls 1994)(Figure 2). Klinse-Za animals had trace minerals levels that were generally on par with those of Omineca caribou, although copper, iron, manganese, and zinc appear to be lower for Klinse-Za ($p<0.001$). Compared to the BC boreal caribou, Klinse-Za caribou had lower zinc and manganese ($p<0.001$) and higher levels of selenium and molybdenum ($p<0.001$). For the three minerals we had reference ranges for, Klinse-Za values fell at the lower end of the range for selenium and below the reference range for copper and zinc. Pregnant caribou had higher levels of zinc, iron, and cobalt in their blood serum collected in March of that year (Figure 3, zinc: $H=8.74$, $p=0.003$, iron: $H=8.25$, $p=0.004$, cobalt: $H=4.79$, $p=0.029$).

Of the caribou that were pregnant, those that produced a live calf had moderately higher levels of iron in their blood serum (Figure 3, iron: $H=2.16$, $p=0.06$).

Exploratory graphs suggested that some trace minerals may be either changing through time, through successive penning, or both (Figure 3). To discriminate between annual trends and successive penning, we fit a model using only penned females for each trace nutrient and assessed nutrient level in response to annual changes through time, pen visit number, female age class, and reproductive outcome. While successive penning and time are clearly correlated for each individual ($r=0.99$), at the population level time and penning visits were less correlated ($r=0.45$), offering an opportunity to statistically decouple the two. Of the two temporal variables, year or times penned, year was more often related to changes in nutrients through time. There was evidence of declines through time for cobalt ($\beta=-0.235$, $p=<0.001$) and copper ($\beta=-0.014$, $p=0.028$), and for increases in iron ($\beta=1.43$, $p=<0.01$). Selenium is a nutrient given to females parenterally by intramuscular injection of Dystosel (Zoetis Canada Inc., Kirkland, QC) at capture and this was the only nutrient for which we detected evidence of change through successive penning and it was increasing (β=0.0226, p=0.007). Given the evidence of year as the major driver for changes through time, especially for declining minerals, we fit a second model with all animals that included year, location (penned or not), age class, and reproductive outcome. Results from this analysis (Figure 3) suggested that cobalt and copper were significantly decreasing through time across the population ($p<0.05$) and iron was increasing ($p<0.01$), and compared to young animals, mature animals had lower cobalt (β=-0.309, $p<0.01$).

Fecal nitrogen levels across all samples varied from 1.5 to 4.7 % of fecal dry weight (Figure 7). Overall, the level of fecal nitrogen was higher for caribou pellets collected inside the pen versus outside the pen (2.78% (SE=0.04) and 2.59% (SE=0.05), respectively, H=44.2, $p<0.001$). Using a sample of genotyped samples of known sex from the free-ranging population we tested whether fecal nitrogen differed by sex, after controlling for day of year, location, their interaction, and a random intercept for year. We found little evidence that sex influenced fecal nitrogen levels (sex(male): β=0.013 (se= 0.09), z=0.14), and the estimated effect was small (0.013), thus we pooled male and female samples together in the data.

Free-ranging animals appeared to have lower levels of fecal nitrogen than penned animals in April, but by July the levels were similar between the two groups (Figure 7). A glmm with year as a random intercept suggested that fecal nitrogen was higher in the pen (β=0.70, z=6.2), increased through the year (β=0.015 (se= 0.001), z=13.3), and increased slightly faster through the year for free-ranging animals (day of year*location(pen): β=-0.006 (se= 0.002), z=-4.16). In addition, a negative trend in the random effect for year was observed, so we included year in a post-hoc glm. The glm provided similar inferences and provided weak evidence for an annual decrease in fecal nitrogen overall (year β=-0.06 (se= 0.04), p=0.12), and was most notable for penned animals where fecal nitrogen levels were higher than in free ranging samples (β=1.4 (se= 0.21), $p<0.001$) but was slowly declining through time (year*pen: β=-0.18 (se= 0.05), $p<0.001$).

Inflammation

Haptoglobin levels across 102 adult female serum samples had a mean of 0.28 g/L, and ranged from 0.15 to 1.36 g/L. These values were higher than those reported for 151 boreal caribou (0.14-0.19 g/L, Bondo et al. 2019). The maximum value in our data (1.36 g/L) is a likely an outlier since the next highest value is 0.65. This cow was re-collared in the wild in March 2018 – she had a large, hairless, very bruised, and scabbed-over wound on her back (picture in Appendix D), which might explain the high inflammation markers in her blood.

Haptoglobin levels were similar between females regardless of pregnancy status (H=1.71, p=0.18), and viability of their calves (H=1.06, p=0.29) (Figure 4). Assessing haptoglobin changes through time for penned and free-ranging animals provided weak evidence for haptoglobin decreases through time in penned animals and free ranging animals (year: β=-0.07, p=0.04, year*pen: β=0.07, p=0.04), with haptoglobin in penned animals decreasing less per year than free ranging animals. However, this result was sensitive to the removal of the one free-ranging outlier, and when removed the interaction effect flipped and suggested that free ranging haptoglobin was increasing through time while the effects in the pen remained similar to the previous model (year: β=0.04, p=0.14, year*pen: β=-0.04, p=0.15). Removing the outlier only left 11 samples for free-ranging animals, likely contributing to the statistical instability of free-ranging results (Figure 4). Assessing haptoglobin levels for only penned animals with a model that included year and stays in pen provided no evidence of decreases through time (β=-0.005, p=0.40), and some evidence for additional decreases in haptoglobin with increasing stays in pen (β=-0.016 p=0.05).

Stress

The hair cortisol concentration in our Klinse-Za samples consisted mostly of observations from penned females (n =75, mean=4.76 pg/mg) but also included twelve observations from free-ranging animals (mean=5.58 pg/mg). We excluded one potential outlier value of 213.4 pg/mg from a free-ranging female in 2019 because this value was >6 times larger than the next largest value (33.4 pg/mg) and the same individual had a hair cortisol level of 11.7 pg/mg when it was captured the following year.

Klinse-Za caribou hair cortisol concentrations were significantly higher than those measured in Omineca (Z=6.12, p<0.001) and boreal (Z=11.7, p<0.001) subpopulations (Figure 5). We did not find evidence for cortisol relationships with caribou body mass (β = -0.05, p=0.23), body condition (β = -1.4, p=0.42), or body fat (β = -0.44, p=0.51). We compared levels of hair cortisol between pregnant and non-pregnant adult females in the pen but found no effect (H=0.004, p=0.95) (Figure 5). We detected a weak effect difference in hair cortisol between adult females that produced a live calf versus those that aborted or had a stillborn calf ($Z=1.84$, $p=0.06$).

A moderate increase in average hair cortisol across all animals was detected through time ($\beta_{year} = 0.66$, $p<0.01$, Figure 5). There was no evidence that this related to the number of times animals were captured ($\beta_{captures} = 0.03$, $p=0.94$, Figure 5).

Across all the fecal samples (free-ranging n = 501 and penned n = 301) collected April to July, FGM levels ranged from 17.3 ng/g of dried feces, to 1273.3 ng/g. The mean across all samples was 183.0 ng/g. These results do not reflect the initial stress animals may have experienced as a result of capture, since we did not start pellet collection until several weeks post-capture. Using a subset of genotyped samples of known sex, we tested whether FGM levels differed by sex, after controlling for day of year, location, and their interaction. We included a random intercept for year. We did not find evidence that sex influenced FGM levels (sex(male): $\beta=9.9$ (se= 11.9), $p=0.83$), thus we retained both male and female samples in the data and analysed them together.

Visually inspecting the data showed an increase in FGM levels between the spring (April) and the summer (June and July) sampling sessions, coinciding with the calving period (Figure 7). Of the 65 calves born in the Klinse-Za maternal pen between 2014-2020 (McNay et al. 2022), five live calves were born in June, one in July, and the rest were born in May. While penned and free-ranging animals had similar FGM values prior to early May, FGMs in free-ranging animals began to increase faster than in penned animals and remained higher through the duration of sampling, which ended in late July. A glmm with a random intercept for year confirmed this trend, where a significant interaction between day of year and location suggested that FGMs in free-ranging caribou rose faster through the year compared to penned animals (day of year: β=4.6 (se= 0.2), z=19.1, day of year*penned: β=-1.8 (se= 0.4), z=-4.4).

Pathogens

Erysipelothrix seroprevalence in the Klinse-Za across animals and across years was 65% (60/93). The bacterium appears to have been well-established in the subpopulation prior to the beginning of sampling (10 of 11 females captured in 2014 were seropositive). Among boreal caribou in 2012-2014, the seroprevalence was 24%, which is lower than the Klinse-Za in the first four years of our study (Bondo et al. 2019), however seroprevalence in nearby mountain subpopulations ranged from 18-76% (Figure 8).

Alphaherpesvirus prevalence across all animals and years was 15% (14/91) and was generally lower than among boreal caribou – across different subpopulations and years, Alphaherpesvirus prevalence in boreal caribou ranged from 22 – 86% (Bondo et al. 2019). Nearby mountain subpopulations generally had higher prevalence, which ranged between 18-100% but sample sizes were small (Figure 8). Alphaherpesvirus was detected in Klinse-Za animals every year except 2019, suggesting it was present in the population prior to the initiation of the penning project. Alphaherpesvirus seroprevalence appears long-lasting; each of the six females who tested positive for this virus had a positive test result for samples taken in subsequent years, except one individual (C311K), who had a negative result in 2019 after three positive results (in 2014, 2016 and 2017), and a positive result again in 2020.

Neospora does not show up in our results until 2016, and has only been detected in two individuals, accounting for the four positive samples: once in C348S (in 2016) and three times in C315S (in 2016, 2017 and 2020). Unlike Alphaherpesvirus, both animals had negative results following the initial positive result. Interestingly, C315S was sampled in both February and March of 2020 – the February sample was positive, while in March it was negative for Neospora. The level of seroprevalence in Klinze-Za was on par with the boreal caribou, which was around 2% (Bondo et al. 2019). Three nearby mountain subpopulations had no seroprevalence, while a fourth (Nonda) had 15% (4/26) prevalence.

Tests for Toxoplasma on 100 samples from 40 females across seven years all came back negative, hence we did not further analyze these data. This is consistent with boreal caribou results, which also did not identify any evidence of exposure to Toxoplasma in the boreal caribou subpopulations (Bondo et al. 2019).

We did not detect any effects of seropositive disease on reproductive outcomes – i.e., on pregnancy status or successful delivery of a live calf. Kappa values measuring the balanced accuracy of the confusion matrices ranged from -0.01 to 0.06, confirming the lack of an effect (Figure 8, C). Neospora, which has the strongest established correlation with reproductive loss, was only associated with one adverse outcome; C315S was seropositive in 2016 and aborted her pregnancy at some point during the third trimester. Interestingly, even though she did not test positive in March of 2015, she delivered a stillborn calf at full term in May.

Discussion

Southern mountain caribou are an endangered ecotype of woodland caribou whose recovery is at the nexus of ecological, legal, and economic issues (Hebblewhite 2017, Lamb et al. 2022). Averting the loss of southern mountain caribou and providing conditions for self-sustaining populations is a legal requirement under Canada's Species at Risk Act (Government of Canada 2002) and an ecological state promised by Canada to Indigenous Peoples during the signing of many numbered treaties (Government of Canada 1899 p. 8, 2002) . While the habitat, climate, and predation-related challenges to caribou recovery are well described (Wittmer et al. 2005, Serrouya et al. 2019, Laurent et al. 2021, Nagy-Reis et al. 2021, DeMars et al. 2021), health indicators can potentially help inform the recovery of caribou if health metrics can be linked to demographic effects with population-level implications. Here we provide baseline information on southern mountain caribou health metrics and leverage a unique opportunity, provided through short term recovery actions, to assess the impacts of maternal penning and repeat captures on caribou health.

Our results suggest that Klinse-Za caribou are generally healthy compared to nearby subpopulations that are less disturbed or live in different ecosystems, and relative to available reference values (Puls 1994). We did not detect any health concerns from maternal penning or repeat captures. Data and theory on large ungulate ecology repeatedly affirm the fundamental role of nutrition in reproductive outcomes such as pregnancy, parturition, and calf survival (Cook et al. 2004, Parker et al. 2009). Here we assessed the influence of trace nutrients, haptoglobin, hair cortisol, and pathogens on reproductive outcomes for penned females. We assessed links between health metrics and reproductive outcomes which suggested increased zinc, iron, and cobalt levels were correlated with pregnancy, and provided weak evidence that calf viability (i.e., carried to term and not aborted/stillborn) increased with higher iron levels and lower hair cortisol. The iron result is consistent with a study of pregnancy in moose, where serum Fe was the strongest single predictor of pregnancy status (Newby and DeCesare 2020). Unlike Flueck (1994) who found increased reproduction following selenium supplementation, we did not find evidence that selenium was limiting Klinse-Za caribou reproduction. Disease and haptoglobin levels did not appear to influence reproductive outcomes. Finally, we showed that maternal penning and calving likely interact to influence short-term cortisol levels, whereby females in the pen had lower fecal cortisol metabolites post calving than free-ranging animals, despite having similar levels pre-calving; we posit that this is likely due to penned caribou having to be less vigilant of predators during this vulnerable period for calves (Bøving and Post 1997), but may also be related to increased food availability and quality in the pen. Collectively, we provide evidence that caribou health is an underutilized approach that can support caribou recovery through better understanding of how health influences demography at the population level.

Due to the invasive and possibly disruptive nature of the maternal pen operation, a key question raised at the beginning of the project was how the stress of capture and penning might be affecting female caribou. Recognizing that FGM values can index physiological stress but do not necessarily reflect all dimensions of animal well-being, our fecal cortisol results nonetheless suggest that penning did not cause significantly more physiological stress than would be naturally experienced by caribou in the free-ranging population. Rather, across and within years, the level of fecal cortisol metabolites in penned animals was lower than, or equal to, that observed in free-ranging individuals. An important methodological consideration is the potential for fecal sample collection method to affect the results, as the samples from penned caribou were collected within a day of defecation, whereas the free-ranging samples were collected from one to three days post-defecation. While laboratory studies of captive species (tigers (Parnell et al. 2015) and sheep (Scherpenhuizen et al. 2020)) showed a gradual decrease in FGMs with exposure to environmental conditions, a field study of in-situ FGMs in mountain-dwelling ungulates found that values declined over time and with exposure (Donini et al. 2022). As such, we take the higher FGMs observed in free-ranging Klinse-Za caribou pellets to represent a meaningful physiological difference between the two groups, rather than a sampling artefact (Parnell et al. 2015, Scherpenhuizen et al. 2020).

We also tested for the effects of repeat penning on 1) trace mineral levels that could be altered due to confinement and feeding, 2) haptoglobin, an index of ongoing immune response and inflammation, and 3) hair cortisol, a longer-term measure of stress than fecal cortisol (Ewacha et al. 2017). Repeat penning marginally increased selenium levels, a mineral supplementally given at capture, while other trace minerals were unaffected. There was weak evidence that repeated penning reduced haptoglobin levels, signalling reduced infection and inflammation, perhaps due to more balanced nutrition in the pen, less stress from predation, or consistent access to food,. Hair cortisol levels did not increase with successive pen visits, suggesting that repeated captures and stays in the pen were not chronically stressing the animals, beyond the stress experienced at capture and shortly thereafter. Maternal penning has proved to be an effective part of the Klinse-Za's comprehensive recovery program, which also includes the reduction of wolf density and habitat restoration (Lamb et al. 2022). While the pen has allowed calf survival to increase by nearly 50% and facilitated modest increases to adult female survival (McNay et al. 2022), the influence of repeat captures on caribou wellbeing has historically been of concern. Here we show that, based on the available data, females who were repeatedly captured and stayed in the maternal pen did not accrue negative health outcomes.

Klinse-Za caribou health metric results generally fell within the ranges recorded in other subpopulations and reference values, with a few exceptions. Trace minerals levels were similar among mountain caribou subpopulations, but Klinse-Za was lower in zinc, manganese, iron, and copper than most subpopulations in the Omineca mountains. We do not yet know the exact cause of this difference but note that the Nonda subpopulation also had similarly low values to Klinse-Za, suggesting Klinse-Za was not a complete exception. The mountain subpopulations generally had higher levels of selenium and molybdenum than boreal subpopulations, perhaps due to differences in the nutrition available in mountain habitats in central BC versus the boreal habitats of northeast BC (Denryter et al. 2022).

Hair cortisol, by its nature, is an indicator of long-term stress, since it incorporates circulating hormones over the course of its growth cycle (Macbeth 2013) – in our case, the growth cycle is from shedding the previous spring or early summer to the end of growth in the fall. Klinse-Za caribou had higher levels of hair cortisol compared to nearby subpopulations of mountain caribou. These subpopulations often occupied less disturbed landscapes which may explain this pattern, given that disturbance level has been linked to higher cortisol concentrations in other caribou herds (Ewacha et al. 2017). Despite the higher hair cortisol concentrations in the Klinse-Za herd, we did not find a relationship between hair cortisol concentration and body mass in March, body condition assessed during capture using palpation, or pregnancy. But we did detect a weak effect on calf viability, whereby females with lower stress levels were more likely to deliver a live calf, suggesting potential fitness links between stress and demography. Within the Klinse-Za herd, penned animals had lower hair cortisol concentrations compared to free-ranging individuals, suggesting no adverse effects of penning on their long-term stress levels.

Serum haptoglobin levels were, on average, lower in Klinse-Za caribou than in the BC boreal subpopulations, which was congruent with the lower seroprevalence of pathogens observed in the Klinse-Za subpopulation. Haptoglobin levels in BC boreal caribou ranged from 0.49 - 5.6 g/L for the five animals that were known from post-mortem examinations to have carried moderate to high levels of parasitic and infectious disease (Bondo and Schwantje 2018), a range of haptoglobin that was non-overlapping with the 98 levels measured in the Klinse-Za subpopulation, except for one measurement of 1.36 g/L in 2018. However, this assay is still not well-validated for caribou and so at this stage, establishing a baseline is the priority. Once this is developed, a quantitative, predictive model could help more precisely characterize the relationship between disease and serum haptoglobin.

Seropositive status for Erysipelothrix, Alphaherpesvirus, Neospora was low across our samples, relative to the BC boreal caribou samples. All animals were seronegative for Toxoplasma, the same as in the boreal (Bondo et al. 2019). Erysipelothrix had the highest seroprevalence in the first four years of penning, and it appears that immunity waned after an exposure at some point before 2014. From these data, exposure to pathogens in the Klinse-Za subpopulation does not seem to, independently, explain the reproductive failures (defined as not being pregnant or being pregnant but not delivering a live, healthy calf) that we have observed in penned Klinse-Za females. However, there may be covariates, such as age, trace nutrient status, comorbidity, habitat disturbance, or serum biochemistry (Bondo et al. 2018) which might collectively increase the explanatory power of infectious diseases for reproductive loss (Appendix 2).

The levels of FGMs measured in samples collected from penned females and free-ranging Klinse-Za caribou in April are consistent with late winter FGM measurements in caribou elsewhere; Joly et al. (2015) report mean FGM levels of 118.5 and 112.1 ng/g in pregnant and non-pregnant females in Alaska, respectively. While the utility and limitations of using FGMs as an indicator of physiological stress has been explored both in the husbandry and the caribou literature (Rehbinder and Hau 2005, Wasser et al. 2011), very few studies have carried out systematic field sampling over the course of the year, and so baseline values for free-ranging populations are lacking. As such, we cannot say if the high values observed during the June and July sessions, particularly for the free-ranging samples, fall within a normal range for caribou. Since we observe a marked increase in FGMs between the pre-calving sampling session (April) and post-calving (June and July), it is possible that the observed increase in the females' FGM levels is linked to the transition from pregnancy to lactation and other maternal behaviors. However, because both male and female free-ranging caribou show parallel increases in FGMs during this time, it may be the compounded stressors of predator vigilance and avoidance as well as nutritional demands in a period when spring forage can be sparse. To decouple these effects, we suggest future investigations include thyroid hormone levels—measuring T3 (triiodothyronine) via blood samples or feces. T3 is a hormone which is associated with metabolism and is a marker for nutritional stress, and has been validated in several North American cervids (Martinez and Hewitt 1999, Goheen and Jesmer 2013).

Fecal nitrogen results from our samples are generally consistent with nitrogen levels from other *Rangifer* studies. Newton et al. (2015) recorded 2.7 – 3.7% fecal nitrogen content for their study area near Hudson Bay, with samples from inland subpopulations having significantly lower values (~2.8%) than coastal samples (~3.5%). A study of diet and behaviour in Svalbard reindeer documented fecal nitrogen content ranging from 2.5 – 3.6%, with most (40/47) samples having values ≤ 3.2% (Karbø 2019). Our samples showed more variation, ranging from 1.5 – 4.1%, but the mean was very similar to the inland Hudson Bay subpopulations. Comparing fecal nitrogen between penned and free-ranging caribou, we found that the penned females had significantly higher fecal nitrogen in April, but that difference became insignificant by June or July. We interpret this to indicate that in spring, free-ranging caribou have limited access to high quality forage as spring vegetation is only emerging. The penned animals, by contrast, have daily access to pelleted feed and supplementary lichen. By June and July, however, natural forage is abundant and thus the diet quality among penned and unpenned animals, at least as indicated by protein content, is expected to be very similar. One possible implication of this temporary improvement in diet quality for penned animals is the timing with respect to pregnancy. Pregnant females are in the pen during the third trimester of their pregnancy, which incurs the greatest energetic costs during gestation (Parker et al. 2009).

Not surprising then, related studies have found higher reproductive success among penned caribou (McNay et al. 2022).

Collectively, these results provide insights into how habitats and management interventions affect individual physiology, which in turn can influence population demographics. This study provides evidence that maternal penning and repeat captures are not harming the health of caribou and provides baseline knowledge for mountain caribou health parameters. As penning-type measures continue to be considered in the suite of recovery actions for caribou (Boutin and Merrill 2016), our work highlights the importance of further investigation into the links between trace nutrients and reproductive outcomes, as well as the interplay between health metrics and their collective effects on demography. In penning situations, it might be possible to identify limiting nutrients and provide these as supplements to test if removing this limitation increases pregnancy or calf viability.

Acknowledgements

This project is closely integrated with the Klinse-Za maternity pen project and therefore owes much to the people and organizations that support this large and complex endeavor. Since many of the samples used come from the capture session, we would like to acknowledge the support provided by the ~25 individuals (helicopter pilots, veterinarians, government biologists, First Nations Lands Office staff, contract biologists) during the capture and transport of adult females that occurs each March, from 2014 to present. This “team” performs the delicate job of handling each caribou in the most professional and humane manner possible. Especially, we thank the Nîkanêse Wah tzee Stewardship Society and it's Directors for their continued, unwavering support for the recovery of caribou in the traditional territories of Treaty 8 First Nations. For help with project conception, development, and technical guidance we are deeply grateful to Drs Helen Schwantje, Bryan Macbeth, and Owen Slater. In carrying out the finicky work of organizing samples and data we would like to thank the staff members of Wildlife Infometrics Inc. and the BC Wildlife Health branch, especially Shari Willmott and Amelie Mathieu for making the long trek to get samples from Mackenzie, and to Shari and Meave for expert and patient management of databases and physical samples. We thank Kristin Bondo, Susan Kutz, and the BC Health Program for sharing the BC boreal caribou data with us. We are very grateful for the financial support of the Nîkanêse Wah tzee Stewardship Society, Habitat Conservation Trust Foundation, Nîkanêse, Canadian Mountain Network, Liber Ero Fellowship, Mitacs Canada, Fish and Wildlife Compensation Program (FWCP) (The FWCP is a partnership between the Province of B.C., Fisheries and Oceans Canada, First Nations and public stakeholders and enhance fish and wildlife in watersheds impacted by BC Hydro dams.), Yellowstone to Yukon Conservation Initiative, and Environment and Climate Change Canada. Your contributions have allowed us to finally launch something that has been years of questions and pen-side conversations in the making.

Data availability statement

All data and analyses will be made available on github or a similar repository upon acceptance

Tables

Table 1. A summary of all samples that were used in this study. The samples were collected either at time of capture, immediately prior to the commencement of penning, or during the spring/summer fecal pellet sampling program. Further information on the diagnostic tests used can be found in Appendix 1.

Health class	**Sample type**	**Health metric**	**Samples from penned animals (years)**	**Samples from free-ranging animals (years)**
Stress	Fecal pellets	Glucocorticoid metabolites	305 (2016-2019)	501 (2017-2019)
Stress	Hair	Cortisol	93 (2014-2021)	15 (2016, 2018-2021)
Pathogens	Blood serum	Neospora	100 (2014-2021)	18 (2017-2021)
Pathogens	Blood serum	Toxoplasma	100 (2014-2021)	17 (2017-2021)
Pathogens	Blood serum	Erysipelothrix	102 (2014-2021)	18 (2017-2021)
Pathogens	Blood serum	Alphaherpesvirus	101 (2014-2021)	17 (2017-2021)
Nutrition	Blood serum	Trace minerals	96 (2014-2021)	17 (2017-2021)
Inflammation	Blood serum	Haptoglobin	99 (2014-2021)	18 (2017-2021)
Nutrition	Fecal pellets	Fecal nitrogen	262 (2016-2019)	210 (2017-2019)

Table 2. Average and range of trace nutrient levels in female Klinse-Za caribou, 2014-2021. Based on blood serum collected at capture for (n = 94) penned and (n = 16) free-ranging females.

Trace nutrient	**Min**	**Max**	**Mean (SD)**	**Median**	**Mean and range of values in free-ranging boreal caribou in NE BC**	**Reference range***
Mn (ppm)	0.0013	0.0075	0.003	0.0027	0.027 (0.001-4.80)	-
Fe (ppm)	1.6	110	6.3	3.5	5.61 (1.70-140.00)	-
Co (ppb)	0.25	2.6	0.73	0.5	0.65 (0.27-1.70)	-
Cu (ppm)	0.25	1.4	0.46	0.45	0.43 (0.11-0.74)	0.70-1.80
Zn (ppm)	0.17	1.4	0.7	0.65	1.04 (0.59-3.00)	1.10-2.50
Se (ppm)	0.045	0.55	0.074	0.062	0.054 (0.030-0.51)	0.050-0.140
Mo (ppm)	0.51	51	7	2.7	0.034 (0.0009-1.00)	-

* Reference range obtained from 100 caribou and reindeer samples, referenced in (Puls 1994).

Figures

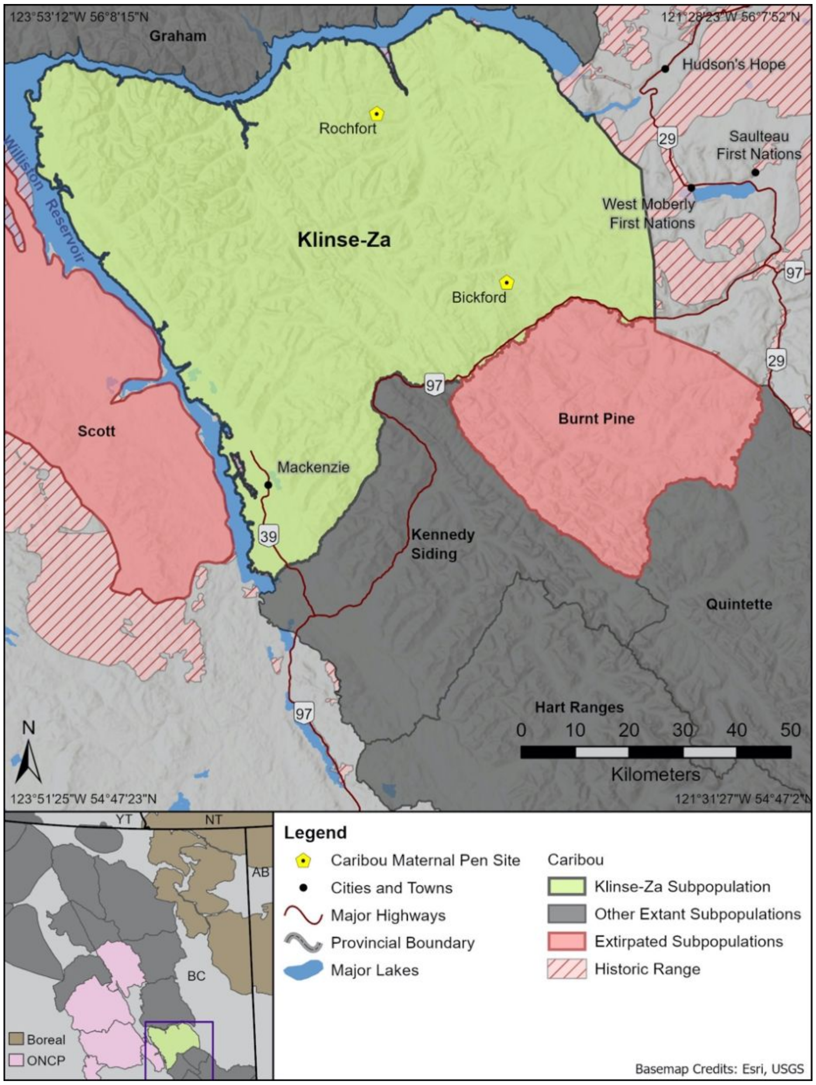

Figure 1. Location of the Klinse-Za caribou subpopulation range and two maternity pens in northern British Columbia. We used samples from caribou in the BC Boreal and Omineca Northern Caribou Project (ONCP) subpopulations as to compare our results to. The location of these subpopulations is shown in the inset.

706 Figure 2. A) Serum trace mineral levels for Klinse-Za caribou (in blue) compared to
707 nearby mountain caribou subpopulations and BC boreal caribou from (Bondo et al. 2019)
708 (in pink). Shaded regions for Cu, Se, and Zu represent the reference ranges for 100
709 caribou and reindeer reported in (Puls 1994). B) estimated coefficients and 95%
710 confidence intervals from a generalized linear model. All subpopulation values were
711 modelled relative to Klinse-Za, thus values above 0 had higher levels than Klinse-za and
712 below 0 had lower levels.

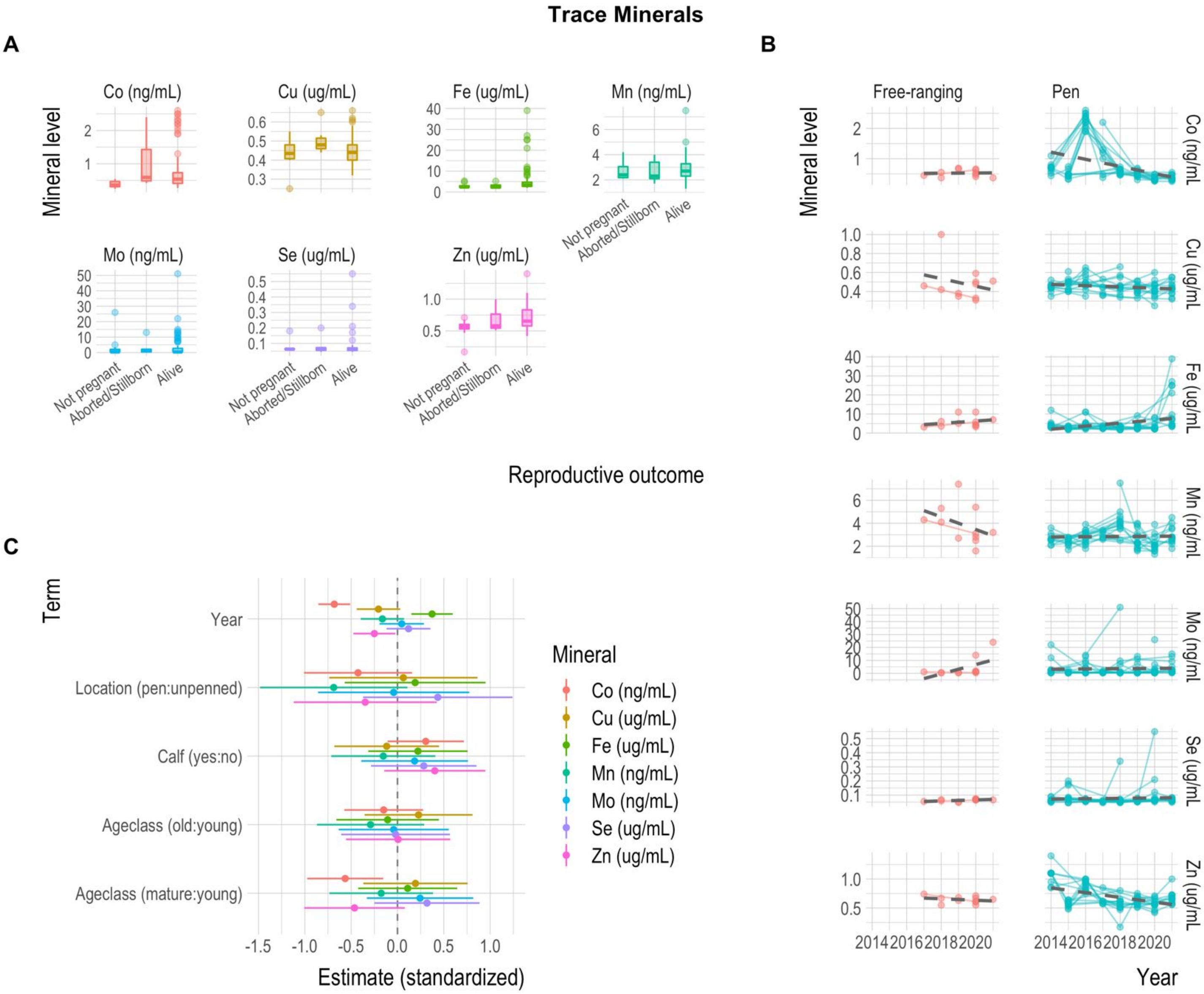

Figure 3. A) Spring reproductive outcomes and serum trace mineral levels measured in March for Klinse-Za caribou. B) Annual trends in trace minerals for penned and free-ranging Klinse-Za caribou, values for individuals sampled in multiple years are connected by a line, C) Estimates and 95% confidence intervals for covariates fit to each trace mineral in a linear model.

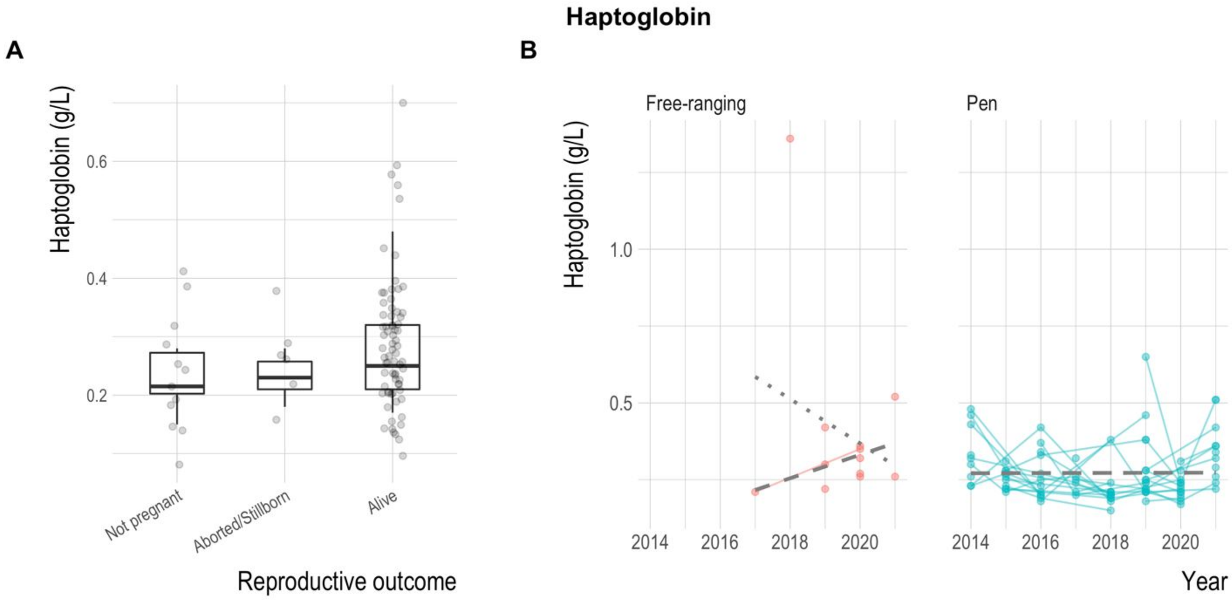

Figure 4. A) Haptoglobin levels measured in March and resulting spring reproductive outcome for Klinse-Za caribou, B) Annual trends in haptoglobin levels between penned and free-ranging animals. The trend line for free-ranging animals was sensitive to the inclusion (dotted lined) or exclusion (dashed line) of a likely outlying value at 1.36 g/L.

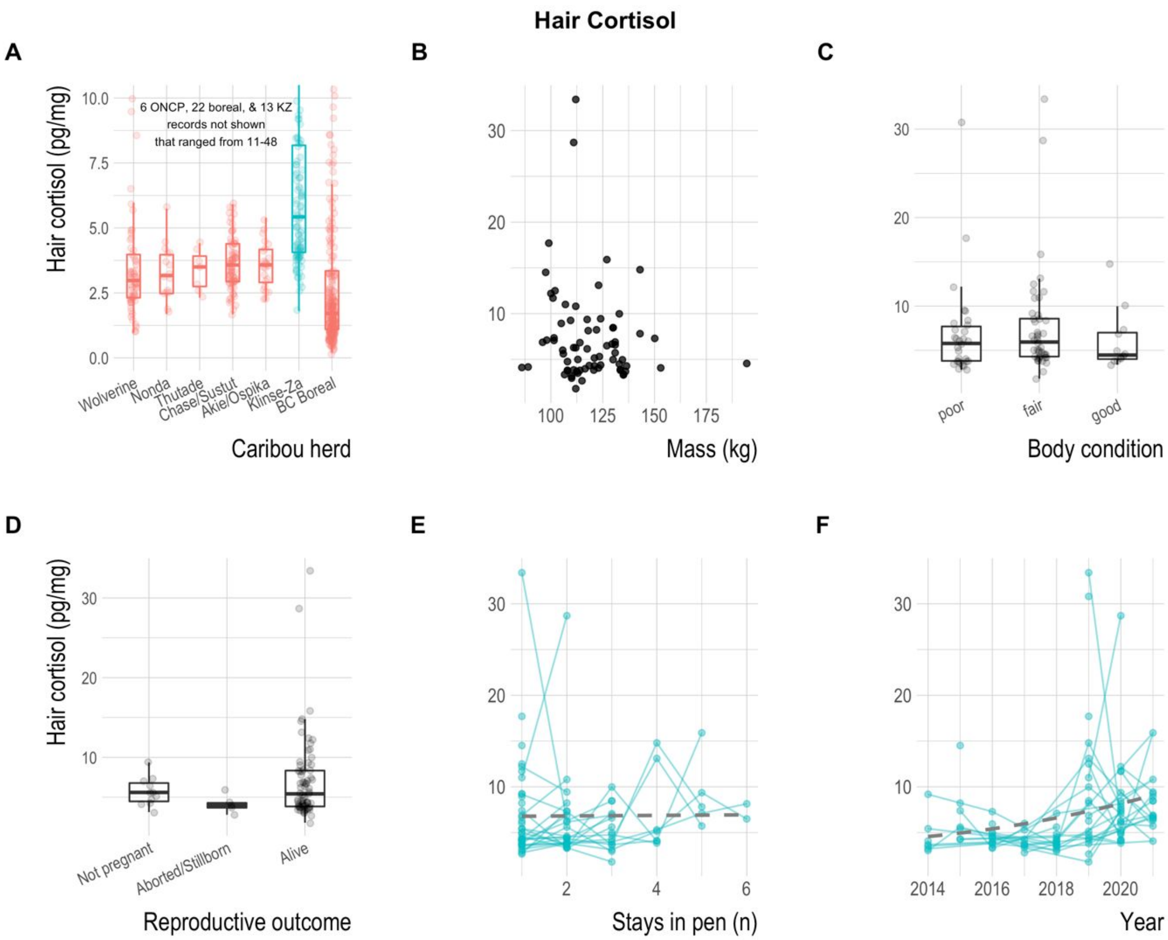

Figure 5. Hair cortisol relationships for Klinse-Za female caribou. Hair was sampled in March and generally reflects an averaged cortisol level from the entire period during hair growth (spring-fall). A) Hair cortisol levels for Klinse-Za female caribou compared to nearby mountain caribou subpopulations and BC boreal caribou from (Bondo et al. 2019). Hair cortisol was weakly related to two body condition metrics for: B) mass, and C) expert-based body condition score assessed via palpation. D) Spring reproductive outcomes and hair cortisol concentrations. E&F) hair cortisol concentration through time, either through repeated stays in the pen or by year.

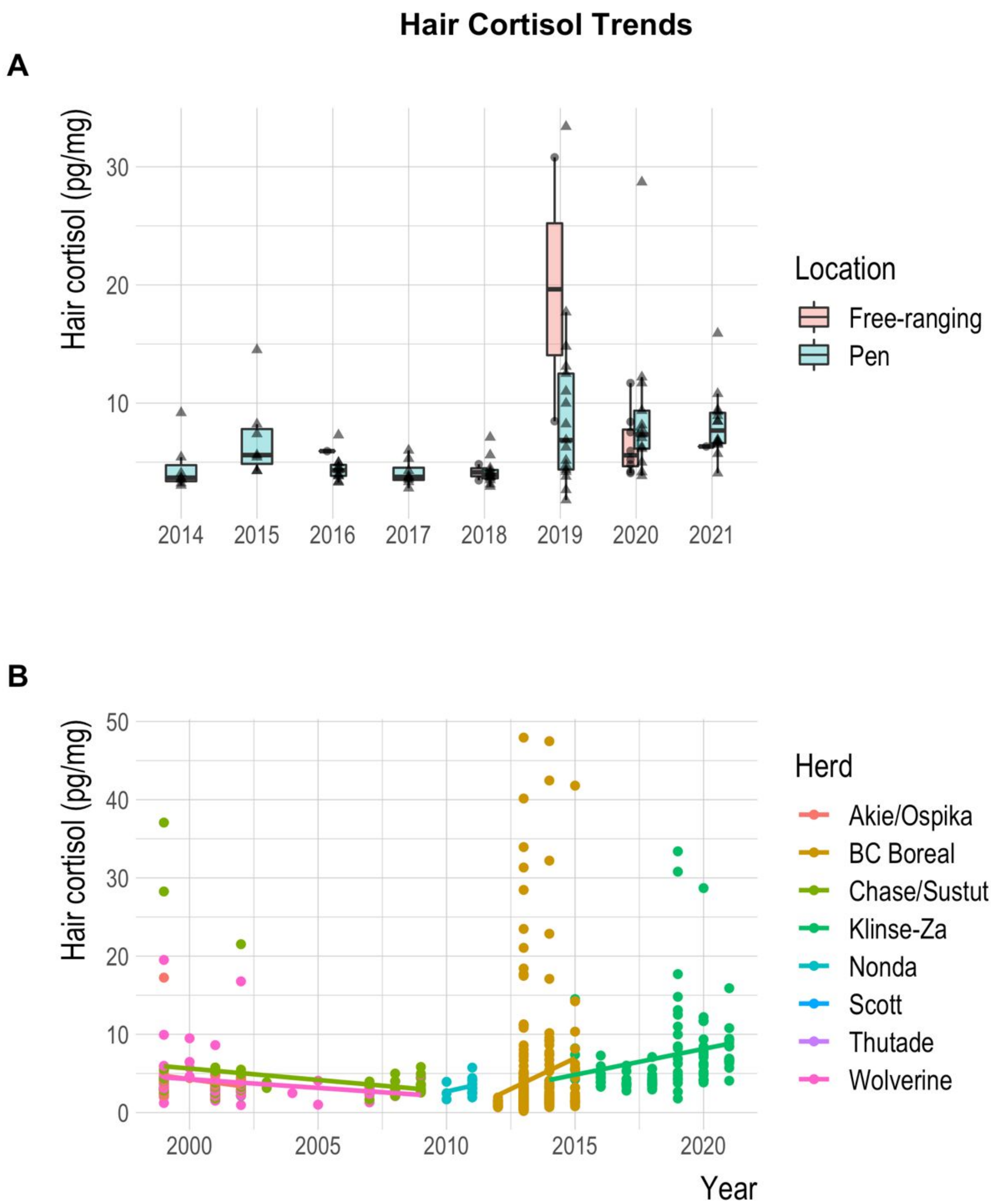

Figure 6. Hair cortisol trends through time for A) Klinse-Za female caribou inside vs outside maternal pen, and B) for Klinse-Za female caribou compared to nearby mountain caribou subpopulations and BC boreal caribou from (Bondo et al. 2019).

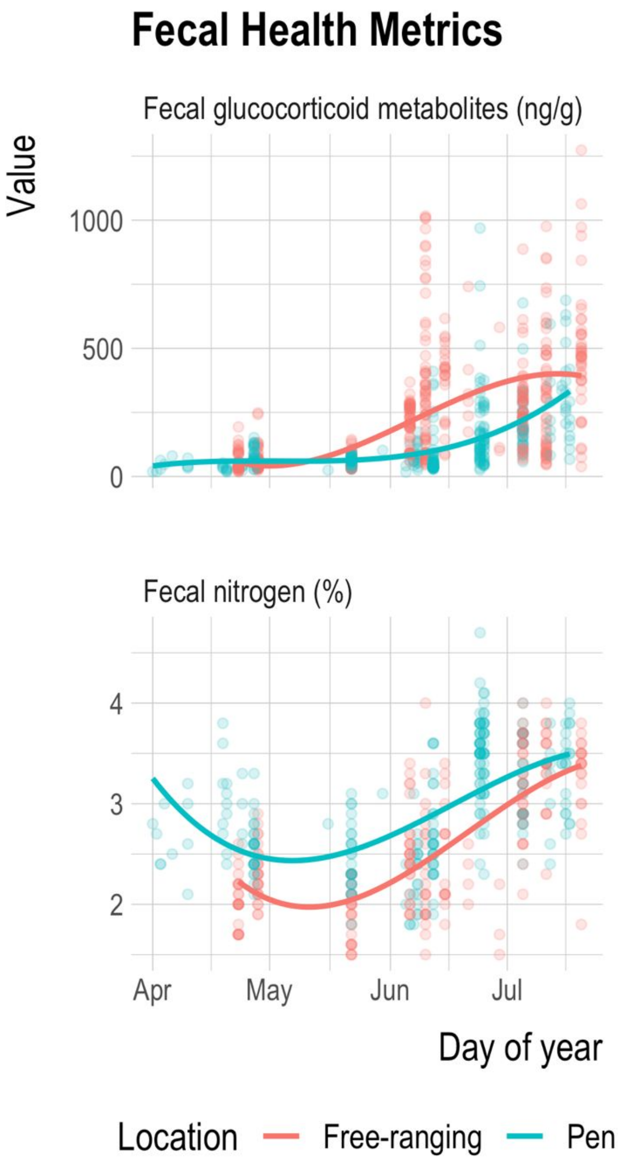

Figure 7. Fecal glucocorticoid metabolites and nitrogen levels collected between April and July for penned and free-ranging Klinse-Za caribou between 2016-2019.

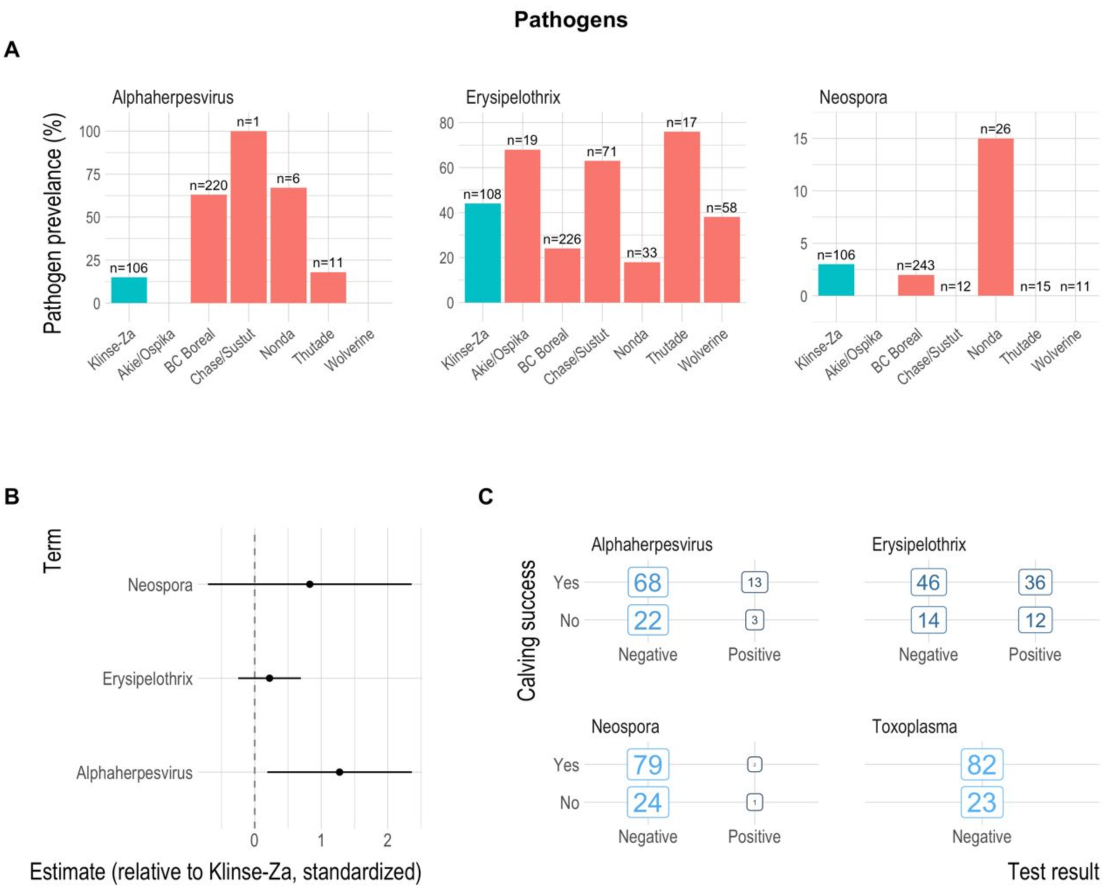

Figure 8. Pathogen seroprevalence for Klinse-Za female caribou compared to nearby mountain caribou subpopulations and BC boreal caribou from (Bondo et al. 2019) shown as A) bar chart, B) all pooled and compared to Klinse-Za seroprevalence in a linear model, 95% confidence intervals shown. C) confusion matrices for disease seroprevalence and calving success, all of which had little predictive power (kappa=-0.04 to 0.02).

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APPENDIX 1. PERMITS AND ETHICS APPROVAL

Procedures for capturing caribou, care while in captivity, and monitoring radio-collared caribou complied with guidelines established by the Canadian Council on Animal Care (2003, 2017), with standards for live animal capture and handling and monitoring established by BCMOELP (1998). All activities were approved under BC Wildlife Act Permits FJ14-93094, FJ18-421458, FJ21-623574, FJ22-682329 and FJ22-655188).

Aerial wolf reductions were carried out by contractors to the Province of BC, as well as internal government staff, under the authority of the BC Wildlife Act between 2015-2021. The aerial wolf reduction considered here was permitted and received animal care approval through the Provincial Animal Care Review process for Scientific Permits (Wildlife Act Permit #'s: FJ15-169004, FJ15-165140, FJ-169006, FJ17-264123, FJ17-253645, FJ17-253804, FJ18-286980, FJ18-416476, FJ19-597709). The BC Animal Care Committee is chaired by the Provincial Wildlife Veterinarian and follows published animal care guidelines (CCAC 2003, 2017, BCMOELP 1998, Underwood and Anthony 2013). The aerial wolf reduction was exempt from the prohibitions in s.27 of the BC Wildlife Act against herding and hunting wildlife from an aircraft (exempt under s.3(1)(c)(ii) and 3(1)(c)(iii) of the Permit Regulation, B.C. Reg. 253/2000 from the prohibitions in s.27). Indigenous trapping and harvesting of predators, including wolves, was carried out between 2013-2021 under the authority enshrined in treaty rights on traditional territory. Maternity penning was permitted and underwent Provincial Animal Care Review (Wildlife Act Permit #'s: FJ14-93094, FJ18-421458, and FJ22-682329; and Special Use Permit #'s: S25789, S26697, S26316 and Free Use Permit #: 20767). Registered trapping by BC trappers was conducted under the authority of the Wildlife Act. No university personnel were involved in planning or conducting wolf reduction, operating maternity pens, or capturing caribou, thus obviating the need for university animal care review or approvals.

APPENDIX 2. ANALYTICAL LABORATORIES AND METHODS USED FOR ANALYSIS OF HEALTH SAMPLES

Health Class	Health Metric	Sample Type	Method	Laboratory
Pathogen	Alphaherpesvirus [Bovine herpesvirus-1, Infectious Bovine Rhinotracheitis (IBR)]	serum	LSIVet Bovine IBR gB Blocking ELISA (Life Technologies Inc., Paris, France)	Animal Health Centre, Abbotsford, British Columbia, Canada
Pathogen	Toxoplasma gondii	serum	ID Screen Toxoplasmosis Indirect Multispecies ELISA Kit (Innovative Veterinary Diagnostics, Grabels, France)	Prairie Diagnostic Services Inc., SK
Pathogen	Neospora canidum	serum	Indirect ELISA with a posteriori western blot	Prairie Diagnostic Services Inc., SK
Pathogen	Erisypelothrix rhusiopathiae	serum	Indirect ELISA	University of Calgary, AB
Inflammation	Haptoglobin	serum	Photometric (+/- calculated) tests (Roche Diagnostics, Indianapolis, Indiana, USA) using bovine clinical diagnostic panel	University of Guelph, ON
Stress	Hair Cortisol Concentration	hair (200 guard hairs with bulbs removed)	ELISA Oxford EA-65 Cortisol Competitive EIA kit (Oxford Biomedical, Lansing, Michigan, USA)	University of Saskatchewan, Toxicology Laboratory, Saskatoon, Saskatchewan, Canada
Stress	Fecal Glucocorticoid Metabolites (FGM)	feces (3-5 pellets)	ICP-MA (Bruker 820 S; Bruker Ltd. Milton, Ontario, Canada)	Toronto Zoo, ON

Nutrition	Serum Trace Mineral Levels (Mn, Fe, Co, Cu, Zn, Se, Mo)	serum	Inductively coupled plasma mass spectrometry using Bruker 820 MS (Bruker Ltd., Milton, Ontario, Canada)	in-house at BC Wildlife Health Program Laboratory, Nanaimo, British Columbia, Canada
Nutrition	Fecal Nitrogen	feces	Elemental analyzer	Northern Analytical Laboratory Services (at UNBC)
Reproducti on	Pregnancy	serum	ELISA test measuring pregnancy-specific protein B (BioPRYN wild test, BioTracking Inc., Moscow, Idaho, USA)	
NA	Sex	fecal pellet 'swab' (epitheli al cells)	Microsatellite analysis with a ZFX/ZFY sex marker, using QIAGEN DNeasy Blood and Tissue kits.	Wildlife Genetics International. Nelson, BC, Canada.

APPENDIX 3. CORRELATIONS BETWEEN HEALTH METRICS

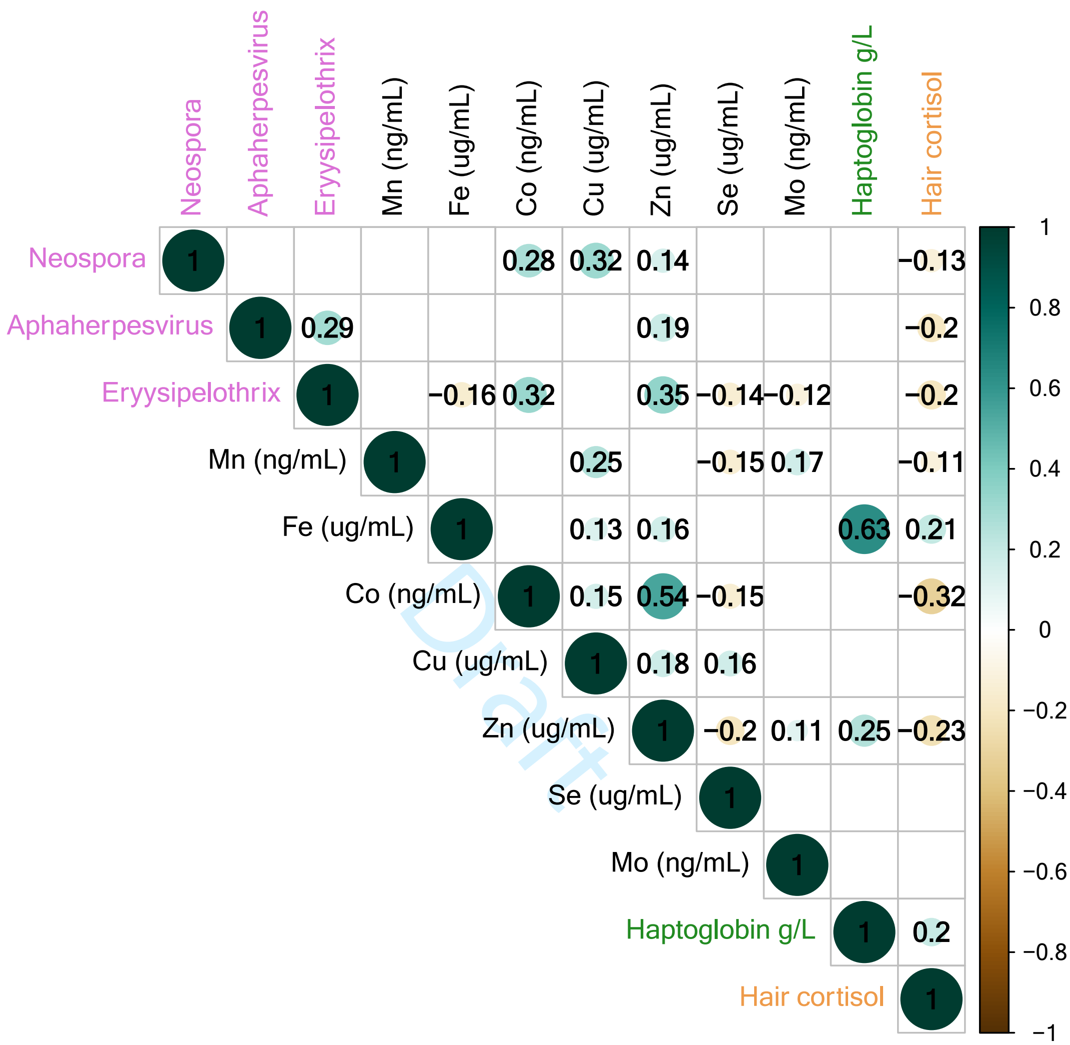

Figure S1. Correlation between health metrics from Klinse-Za caribou 2016-2021. Pairs with absolute correlation coefficients values >0.1 are shown.

APPENDIX 4. PHOTO OF COW WITH HIGH HAPTOGLOBIN READING

Figure 1. A photo of caribou cow C338K during capture in April 2018.