ANNUAL REPORT

Klinse-Za/ScottE Caribou Maternal Pen Health Evaluation

- UPDATED -

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EXECUTIVE SUMMARY

Mountain caribou across British Columbia are declining, with many populations classified as threatened or endangered. While predation is an important factor in the observed declines, little to no consideration has been given to individual or population-level health, until very recently. In this study, we launched an investigation of health in the Central Mountain Klinse-Za caribou herd, whose precipitous decline down to 16 individuals launched a suite of emergency recovery measures in 2014, including a protective maternity pen project. Our primary objectives for this study were to: 1) evaluate the baseline health parameters of the Klinse-Za/ScottE caribou herd, and 2) test for possible effects of penning on caribou health, using physiological stress, pathogen prevalence, serum biochemistry, and trace nutrients as our indices. We analyzed 488 fecal samples collected during the 2017 and 2018 calving season, and 56 blood serum samples collected during March capture in 2014 – 2018. We found that fecal cortisol levels in penned and free-ranging cows are fairly similar pre-calving, but increase significantly after calving; cortisol levels in free-ranging animals (median 136 ng/g, range 18.7 – 1273.3 ng/g) was significantly higher than in penned animals (median 90 ng/g, range 17.3 – 687.7 ng/g). We did not find a relationship between the number of times a cow has been penned and her average pre-calving or post-calving fecal cortisol level. Hair cortisol levels in the Klinse-Za were higher than in Omineca or boreal subpopulations, but also did not show any effects of increased stress with repeated penning. Hair cortisol was slightly higher among cows that had an abortion or a stillbirth compared to cows that delivered a live calf. Seroprevalence of antibodies to the herpes virus 1 in penned cows was 22.2%, *Neospora caninum* was 8%, and *Erysipelothrix rhusiopathiae* was either 13.4% or 26.7% in 2018, depending on which of two recommended thresholds was considered. Individually the pathogens did not appear strongly correlated with reproductive success. Haptoglobin levels across the 53 cow-years had a mean of 0.28 g/L and ranged from 0.15 to 1.36 g/L. We found that both the seroprevalence of pathogens and the level of serum haptoglobin was, on average, lower in the Klinse-Za herd then in boreal caribou herds in northeastern BC. Based on reference values from captive *Rangifer* and free-ranging boreal caribou, cows in the Klinse-Za herd appear to be potentially deficient in 5 out of the 7 tested trace nutrients. For two of those, zinc and iron, we found significant and marginally significant evidence for a positive correlation with reproductive success. Levels of trace nutrients do not appear to increase or decrease systematically as a function of repeated penning. In this study, we provide some data to suggest that penning is not having an imminently negative effect on the health of cows. The data we collected also contributes to building a better baseline understanding of caribou health trends in BC, and identifies priority directions and tests for future investigations.

The Klinse-Za health evaluation aligns with the Fish and Wildlife Compensation Program's Species of Interest Action Plan. The project does so by directly addressing the Species-based Action 1b-3 'Implement projects identified through approved recovery strategies, action plans, and management plans developed for woodland caribou'. The goal of this project is to reduce the current knowledge gap around caribou health and its effect on survival, a need identified both by the Environment Canada "Recovery strategy for the Woodland Caribou" (2014) in their recommendation to "monitor southern mountain caribou health and condition" and BC's "Implementation Plan for the Ongoing Management of South Peace Northern Caribou" which recommends "improving knowledge of South Peace Northern Caribou …adult and calf survival". This project also

addresses species-based Action 1b-4 to ‘Implement actions recommended by past projects related to woodland caribou’ by following up on the recommendation McNay et al (2017) to “Consider initiating other health studies (e.g., fecal pellet collections, antler-drop timing)”.

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INTRODUCTION

Background on Klinse-Za herd decline and maternal penning

The Klinse-Za caribou herd in north-central British Columbia is part of the endangered Southern Mountain (central group) population of caribou. Its range is bordered by the Parsnip reach and the Peace Arm of Williston Reservoir to the west and north, and Highway 97 to the south. The herd declined in size from 198 animals in 1995 to a low of 16 animals in 2013 (Seip and Jones 2015). At the time, the herd suffered from unsustainable levels of predation (mostly by wolves) and was predicted to have become extirpated in the near future. Maternity penning was implemented in 2014 as an emergency measure to avert extirpation of the herd. In combination with wolf removals conducted by First Nations and the BC Government, the pen project has helped to arrest the steep decline in the Klinse-Za population and the combined Klinse-Za/Scott East caribou herd size has increased at an average annual rate of ~13% from 36 individuals in 2013 to ~80 in 2019 (McNay et al. 2019).

The Klinze-Za maternal pen has now been implemented for five consecutive years. The pen operation begins in March, when 10-20 adult cows are captured, just prior to their third trimester of pregnancy. The cows are transported to a specially constructed pen, located in an alpine meadow within natural, historical calving range, where they are marked, collared, and evaluated by a vet. The pen for the first four years of the project was 7 ha in size and located on Mt. Bickford, and the current pen is 14 ha in size and located on Mt. Rochford (see Figure 1). Cows are fed, monitored, and protected while in the pen. Calves are born between early May and early-June, and are also monitored while in the pen. All cows and calves are simultaneously released when the youngest calf is at least 6 weeks old i.e., past the highest-risk period for neonatal predation.

Part of the annual monitoring associated with this project are three population surveys, carried out via radio-telemetry, which collect demographic data on population size and age-sex structure, as well as adult and calf survival and mortality.

While the objective of halting and reversing population decline within the herd has been achieved, the population is still not large enough to be self-sustaining and so the maternal pen project is likely to continue until such a time as it is deemed ineffective. Comprehensively evaluating the pros and cons of this resource-intensive and intrusive management strategy is an important step towards making truly well-informed decisions about how best to continue operating the maternal pen now that extirpation has been averted.

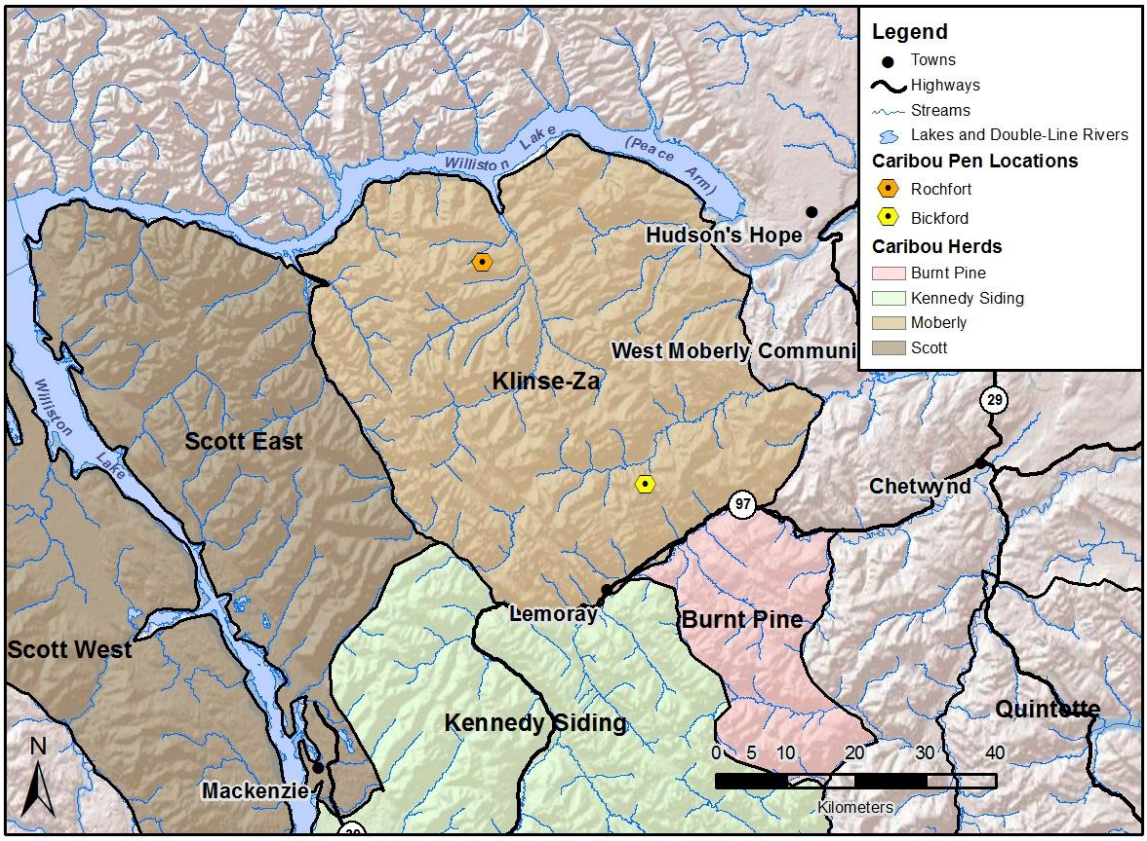

Figure 1. Location of the Klinse-Za/ScottE caribou herd range and two maternity pens in northern British Columbia.

Caribou health

Wildlife health is a growing research field and a fairly new topic for wildlife managers and practitioners. While traditionally, wildlife health work focused on detecting, preventing, or mitigating diseases and toxins, current approaches are increasingly treating health as an integrative metric that represents an animal's interaction with its environment and resilience to change (Stephen 2014, Macbeth and Kutz 2018).

To date, most studies of *Rangifer* health in Canada and Europe have been on pathogens and parasites (Curry 2009, Ducrocq 2011). However, in recent years the focus has broadened to include metrics like body condition (Brook et al. 2011) and serum biochemistry (Johnson et al. 2010), mainly in barren-ground caribou. However, McLellan et al. (2012) did investigate bone marrow content in the context of nutrition and limiting factors in mortality samples from across the range of mountain caribou in BC. Macbeth (2013) identified opportunities and challenges in using hair cortisol concentrations as an integrative measure of long-term physiological stress in caribou. A recent article documents a sudden disease outbreak in semi-domesticated reindeer in Sweden (Tryland et al. 2019), which the authors attributed to the increased stress, animal-to-animal contact, and compromised hygiene associated with corralling and feeding. While the Klinse-Za maternity pen has a much lower animal density, and the

pathogens in questions have not been detected in the area, this study nonetheless highlights some relevant risks for the penning project.

The BC Boreal Caribou Health Research Program (hereon 'BCHRP') provided the first broad investigation of caribou health in British Columbia (MacBeth et al. 2014). Over the course of 4 years (2012 – 2016), this collaborative project developed a herd health assessment model and applied it to biological samples collected from live and dead animals across 7 boreal caribou herds: the Calendar, Chinchaga, Maxhamish, Parker, Prophet, Fort Nelson, and Snake-Sahtaneh herds. Over the course of the project, they identified emerging viral, bacterial, and parasitic diseases with implications for survival and reproduction of caribou, as well as other health indices related to physiological stress, serum biochemistry and trace minerals (Bondo 2018).

Goals and objectives

In the 2018-19 year, we initiated Year 1 of the Klinse-Za/Scott East (hereon referred to as simply 'Klinse-Za') maternal pen caribou health evaluation. Despite a precipitous population decline, and an invasive and expensive herd recovery plan, an evaluation of basic herd health parameters has been lacking to date.

Investigating the health of caribou in the Klinse-Za herd is important because the penning project provides us with a unique opportunity to annually live sample, often the same animal repeatedly, a relatively large proportion of a mountain caribou herd and add to an emerging body of knowledge. Also, given the highly involved nature of maternity penning, trying to identify any effects that our management is having on animal health (either positive or negative) can help weigh the 'pros and cons' of the project more objectively. Finally, the combination of maternity penning and regular monitoring provides us with a unique dataset that can allow for deeper insights into connections between health and herd demographics.

This study has two wildlife health objectives:

1. Characterizing baseline health parameters within the Klinse-Za/ScottE herd
2. Evaluating potential effects of penning on caribou health

In Year 1 of our study of caribou health in the Klinse-Za herd, we used the herd health assessment model developed by the BCHRP as a guide for selecting priority metrics and indices.

The Klinse-Za health evaluation aligns with the Fish and Wildlife Compensation Program's Species of Interest Action Plan. The project does so by directly addressing the Species-based Action 1b-3 'Implement projects identified through approved recovery strategies, action plans, and management plans developed for woodland caribou'. The goal of this project is to reduce the current knowledge gap around caribou health and its effect on survival, a need identified both by the Environment Canada "Recovery strategy for the Woodland Caribou" (2014) in their recommendation to "monitor southern mountain caribou health and condition" and BC's "Implementation Plan for the Ongoing Management of South Peace Northern Caribou" which recommends "improving knowledge of South Peace Northern Caribou …adult and calf survival". This project also addresses species-based Action 1b-4 to 'Implement actions recommended by past projects related to woodland caribou' by following up on the recommendation McNay et

al (2017) to “Consider initiating other health studies (e.g., fecal pellet collections, antler-drop timing)”.

STUDY AREA

The study area for this project is defined by the range of the Klinse-Za herd, in north-eastern British Columbia (Figure 1). As noted above, the range is bounded by the Parsnip reach and the Peace Arm of Williston reservoir to the west and north, and Highway 97 to the south. The eastern herd boundary is somewhat gradual, but falls somewhere west of Highway 29, running from Chetwynd to Hudson’s Hope. All samples were collected within this area. Samples were stored in Mackenzie and Nanaimo, and analyzed in laboratories within and outside the province.

METHODS

Methods of sample collection

This project is operationally linked to the Klinse-Za maternity penning project and as such, sample collection is tied to the larger project operations. The various sample types used in this study have been collected under several different sampling circumstances, described below.

At capture

When pregnant cows are captured for penning, they undergo sedation for the duration of transport and handling. During that time period, the veterinarians on the capture team collect: 1) fecal pellets (typically by hand from the rectum, but sometimes from the ground), 2) several tubes of blood, and 3) a clump of hair (from rump or shoulder). If the female is being captured and handled for the first time, an ear biopsy is also taken in the course of ear-tagging. The blood collected at capture is centrifuged within a day or two of capture. If the cow is showing any signs of abnormal health, other samples may be collected (e.g., vaginal or nasal swabs in case of discharge). In situations where the animal is overheated or showing other signs of distress, sample collection may be reduced or skipped entirely in order to reverse sedation as quickly as possible.

During the penning season

Since 2017, our team has been collecting fecal pellets from both penned and free-ranging caribou three times throughout the penning season: once in April or May (pre-calving) and twice post-calving (in June and July). Pen and free-ranging sampling sessions are 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. In the pen, when possible, pellets are taken from known individuals, observed by crew members who then collect freshly dropped samples on foot. Ideally, at least one pellet sample is obtained from every cow every month. In 2017, on Mt. Bickford, this approach was feasible because the smaller size and simpler habitat configuration enabled visual observation. However,

in 2018 when the maternal pen was relocated to the larger Mt. Rochford site, visually observing a cow defecating while identifying her became very difficult. Instead, we collected a larger number of very fresh (i.e., same day) pellets during pen sampling sessions, and planned to use genetic ID methods to attach the fecal sample to the individual animal.

To collect samples from the free-ranging (i.e., not penned) cows, we use GPS data from collared individuals (usually around 10) to identify locations where caribou have recently (i.e. within last 3 days) been. We aim to collect samples from every collared female's location; depending on the sampling session, the number of locations and accessibility varies. In April/May, the cows tend to be more clustered into groups, and are predominantly in alpine habitats that are easy to access via helicopter and hiking. In June, the immediately post-calving, the cows tend to be very dispersed, and are often in forested habitat that is difficult to access, and are also at their most sensitive, so we avoid any locations where we would need to fly over repeatedly in order to locate a good landing site. In July, some cows converge into groups again and move into the alpine, while other remain in more timbered habitat. So far, we have collected at 4 to 7 sites each session. Crews access all locations by helicopter, then hike in and collect the pellets that appear freshest by hand. Fecal pellets from young of year calves are noticeably smaller than adult pellets and were not collected. We bring an ice-filled cooler on board to maintain samples as cold as possible until they can be frozen that evening. Identifying fecal samples to individual or even sex was not possible without further genetic analyses. Therefore, where collared cows are traveling in mixed groups, we might also collect samples from males and/or calves (though we mark very small pellets as 'likely calf'). For questions where we would want to relate the results of fecal pellet analyses to individual animals, we would need to use genetic ID testing.

Hair and ear biopsy samples from calves are collected when they are captured for collaring and ear-tagging in the pen; however, these samples were not used in this study since our focus was on health of adult animals.

Mortality investigations

If any collared cows die over the course of the year, a crew is dispatched to investigate the cause of mortality and to collect samples within 48 hours. Also, cows that die in the pen (n = 2 to date) are promptly collected and taken to a government or veterinary facility for a full necropsy. Several sample types, including bone marrow for fat analysis, as well as teeth for aging, and fecal pellets and hair when available, are of interest for health analysis. At this time, we were not able to analyze any of the mortality samples and have archived them for Year 2 analysis.

Sample storage, processing, and transportation

All samples collected at capture were transported back to government facilities by the provincial wildlife veterinarian on the crew. The samples that were collected during the penning season and during mortality investigations were always brought back to the WII office in Mackenzie on the day of collection and stored in -20 freezers temporarily. Eventually all samples were transported to the central provincial wildlife health facility in Nanaimo, BC, where samples were archived and stored at the appropriate

temperatures. Hair was initially stored in the freezer but upon recommendation of a veterinarian, was taken out, dried, and thereon stored in paper envelopes.

Since almost any sample type (fecal pellets, blood serum and clots, hair) can be used for a variety of tests, sub-sampling of the samples was required to send to different labs and also to keep some for long-term storage. A professional biologist and lab technician carried out the sub-sampling, taking care to minimize freeze-thaw where it can degrade sample quality.

Lab methods

The labs and analytical methods that we used for this study closely followed the protocol established for the BC Boreal Caribou Health Research Program[1] (MacBeth et al. 2014, Bondo et al. 2019). The assays that we ran were targeted at addressing three priority metrics of caribou health (Table 1): physiological stress, body condition, and pathogen exposure. For a more detailed background on individual assays, see BCHRP Year 1 report (MacBeth et al. 2014). While there are numerous assays that could be run under these umbrella metrics, 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 also chose tests that would enable comparison with the results available from the BCHRP and the building of provincial caribou health baseline knowledge. Several laboratories across Canada were used to run the different assays (see Appendix A).

A challenge that we encountered was delays in the lab analysis process. The preparation of samples took longer than expected due to a much larger number of caribou being sampled across the province, thus placing unforeseen demands on Wildlife Health staff to prepare, and then sort, capture kits. In addition, analytical labs experienced high volumes of requests, thus further delaying results.

As per the amendment that was submitted and approved, we removed the collection and analysis of weigh scale data. The primary reason was a lack of context for this time-consuming analysis. Due to the sheer number of photos that are collected by the motion-activated cameras by the feeder in the pen, extracting weight data from thousands of photos would take even more days than anticipated. At the same time, we have very few data points, either from the Klinse-Za or published numbers from any other mountain caribou herd, on the normal weight trajectory of free-ranging caribou cows throughout the calving season – which changes significantly through the third trimester, parturition, and lactation. Therefore, understanding whether the body condition of penned cows (as measured by weight) is better, worse, or comparable to free-ranging animals, is not currently possible.

[1] https://www2.gov.bc.ca/gov/content/environment/plants-animals-ecosystems/free-ranginglife/free-ranginglife-health/free-ranginglife-health-matters/caribou-health

Analytical methods

Database organization

In order to manage the large amounts of data associated with this project, we have built a database based on WII's existing wildlife information management system. In order to keep metadata linked to lab assay results, we have assigned a unique sample ID to each individual sample, which 'follows' it to any labs that it gets sent to. The metadata that can be looked up using sample ID includes: sample type, animal ID, collection date, collection circumstances (capture or mortality or pellet collection), geographic location, and other details. Once lab assay results are received, they are appended to the overall database, enabling analysis of relationships and patterns (see APPENDIX B for sample data).

Analysis and interpretation

As per Objective #1, we started our analysis by extracting basic statistics for all assay results so as to characterize baseline health parameters for the Klinse-Za herd. Specifically, we calculated the seroprevalence of the three pathogens, and the summary metrics (averages and ranges) for levels of fecal cortisol, hair cortisol, trace nutrients in the blood serum, and levels of haptoglobin in the serum. Where possible, we compared these metrics to the results for boreal caribou in northeastern BC, as reported by the Boreal Caribou Health Research Program (BCHRP; MacBeth et al. 2014, Bondo et al. 2019). We used a confusion matrix[2] to test the accuracy of a disease status, in a given year, in predicting a cow's reproductive outcome that year as a simple way of testing the strength of the relationship between seropositive status and reproductive failure. We also tested the relationship between hair cortisol measurements and three metrics of body condition at capture: weight in kgs, boy fat percentage based on ultrasound, and a qualitative assessment of body condition based on palpation. The first two variables are continuous and the latter is categorical, with animals being classified as either 'poor', 'fair', or 'good'.

Since we have knowledge of the reproductive outcomes of penned cows, we tested relationships between health results and cows' individual reproductive history, as well as any other case histories or anomalies.

As per objective # 2, we explored possible effects of penning on caribou health by: 1) comparing cortisol levels between fecal pellets collected in the free-ranging and those collected from penned cows, and 2) correlating results of health assays with penning histories of individual animals. Since there is considerable uncertainty about the effects of penning on animal health, we addressed both possible predictions, namely that penning caribou might lead to worse outcomes (e.g., more stress or higher rates of disease transmission) or better ones (e.g., improved trace nutrient levels).

[2] https://classeval.wordpress.com/introduction/basic-evaluation-measures/

RESULTS AND OUTCOMES

Background

Over the course of the penning project from 2014 - 2018, 26 individual adult female caribou have spent the calving season in the pen, with number of pen stays ranging from one to five times per animal, for a total of 56 female-years. Most years we also captured cows for re-collaring but did not take them into the pen, and in some cases health samples were also taken from these animals (n = 3). In both 2017 and 2018, we conducted three fecal pellet sampling sessions throughout the calving/penning season in both the pen and free-ranging study areas (in April, June, and July), trying to collect samples from the pen and the free-ranging as close in time as possible. The samples and lab results that were used in this study are summarized in Table 1. Some results for some years are missing, mostly due to the quantity of blood sample that was available. Besides the first section below, on fecal pellets collected throughout the penning season, the rest of the results represent findings from samples taken at capture, in March. Therefore, it's important to note that while the samples are taken from 'penned cows', they do not necessarily reflect the effects of that year's pen, since the samples are collected before the cows start living in the pen.

Table 1. A summary of all samples that were used in this study. Besides the fecal pellets, all results are based on blood samples collected at time of March capture, both for animals who were brought into the pen, and those who were re-collared in the free-ranging. Fecal pellets are mainly collected in the post-capture period, both in the wild and in the pen – the only exception are 10 fecal pellet samples collected in 2018 at time of capture for penned cows.

Metrics	Sample	Assay/test	Samples from penned animals	Samples from free-ranging animals
Stress	Fecal pellets	Cortisol metabolites	189 (2016-2018)	299 (2017-2018)
Stress	Hair	Hair cortisol concentration	93 (2014-2021)	15 (2016, 2018-2021)
Pathogens/disease	Blood serum	Neospora	53 (2014-2018)	3 (2016-2018)
Pathogens/disease	Blood serum	Erysipelothrix	54 (2014-2018)	3 (2016-2018)
Pathogens/disease	Blood serum	Herpes (IBR)	53 (2014-2018)	2 (2016-2018)
Body condition	Blood serum/plasma	Trace nutrients	53 (2014-2018)	3 (2016-2018)
Body condition	Blood serum/plasma	Haptoglobin	52 (2014-2018)	2 (2016-2018)

Fecal cortisol

Penned and free-ranging caribou samples, collected April to July

Across the fecal samples (free-ranging n = 299 and penned n = 189) we collected, cortisol levels ranged from 17.3 ng/g of dried feces to 1273.3 ng/g. Since the 2016 samples represent just a snapshot in time from penned animals, we do not include it in any further analysis besides summary statistics presented in Table 2. The 2017-2018

mean was 228.6 ng/g with a standard deviation of 235.2 ng/g, reflecting the wide spread, as well as the strong positive skew, in the data (Figure 2). The median across the 2017-18 samples was 117.6 ng/g. The mean cortisol levels across all samples were similar in 2017 and 2018, but the median was higher in 2018. It must be noted that these results do not reflect the initial stress animals may experience as a result of the capture, since we do not start pellet collection until several weeks post-capture.

Table 2. Summary of fecal cortisol data across sampling classes in the Klinse-Za herd. Note that pen samples are only from adult females, while free-ranging samples are a mix of male and female samples. All values are in ng of cortisol per g of dried feces.

Subsample	N	Min	Max	Mean (SD)	Median
All 2017 and 2018 samples	410	17.3	1273.3	228.6 (235.2)	117.6
2016 samples (June, pen only)	78	17.6	969.2	159.7 (153.9)	119.5
Free-ranging samples (2017-2018)	299	18.7	1273.3	259.2 (255.2)	136.0
Free-ranging 2017	137	23.2	1273.3	260.0 (251.7)	131.5
Free-ranging 2018	162	18.7	1016.2	257.3 (258.6)	140.9
Pen samples (2017-2018)	111	17.3	687.6	147.8 (143.4)	90.1
Pen 2017	42	18.9	418.8	125.0 (114.5)	76.0
Pen 2018	69	17.3	687.6	161.8 (157.6)	92.6
2017 (pen + free-ranging)	179	18.9	1273.3	228.3 (233.9)	112.0
2018 (pen + free-ranging)	231	17.3	1016.2	228.8 (236.8)	121.8

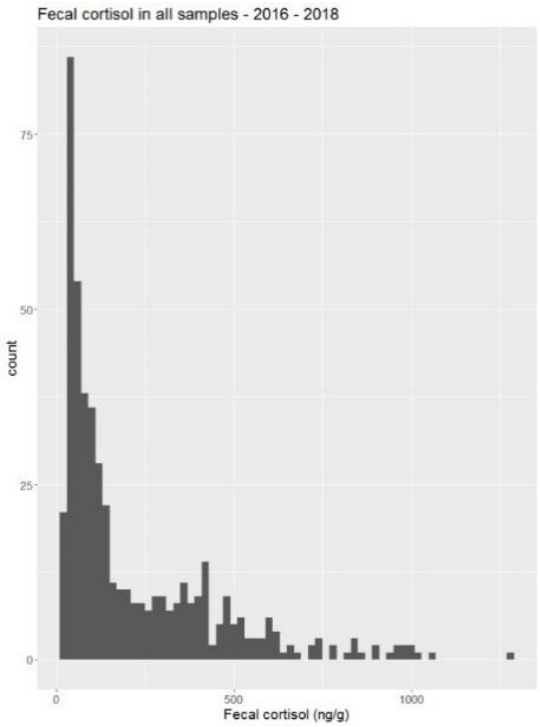

Figure 2. Histogram showing distribution of fecal cortisol values across all 488 samples.

Visually inspecting the data shows an average increase in fecal cortisol levels between the spring (April) and the summer (June and July) sampling sessions (Figure 3). Of the 40+ calves born in the Klinse-Za maternal pen, only one live calf was born in June; the rest were born in May. It is therefore likely that the observed increase in cows' fecal cortisol levels is in some way linked to the transition from pregnancy to lactation and other maternal behaviors.

Average fecal cortisol levels were higher in free-ranging caribou than in cows from the pen; median stress values across both years were 136 vs 90 ng/g, respectively (Table 2). Also, if we select only the 'very high' cortisol readings i.e., ≥ 500 ng/g, the majority of them come from samples collected in the wild (47/55 = 85%). While free-ranging samples presumably represent a mixture of female and male samples, it is unlikely to be the only factor driving the differences we observe – see discussion below for more.

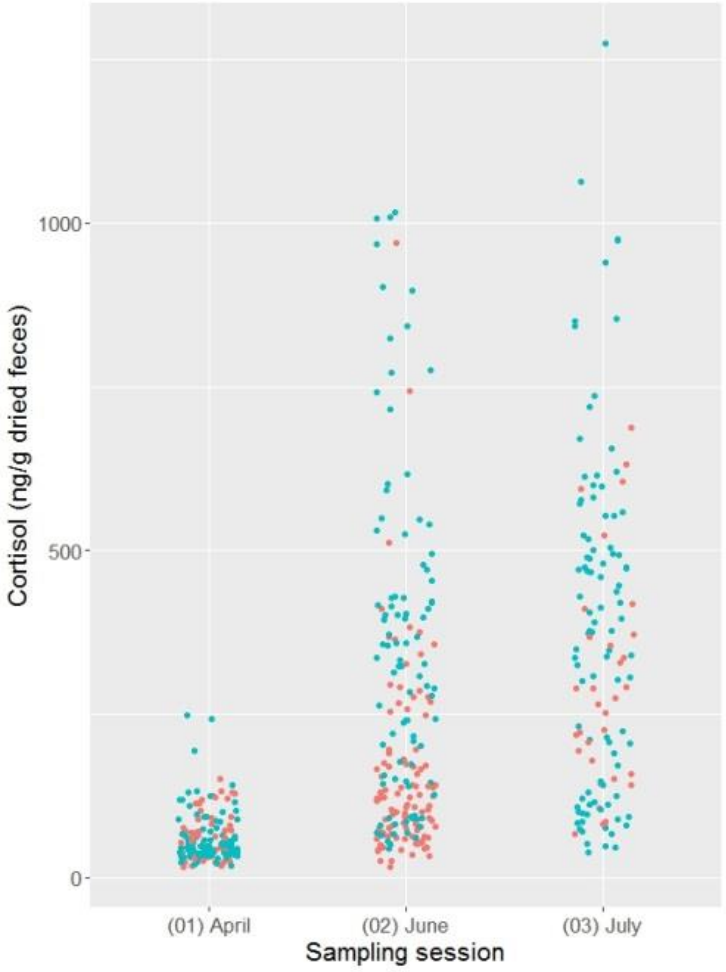

Figure 3. Raw data showing changes in cortisol levels in fecal samples between sampling sessions. Pink points represent samples collected in the pen and blue points are from samples collected in the free-ranging. Combined data from 2017 and 2018.

When we partition the fecal cortisol data by year and session (Figure 4 and Figure 5), we see that the difference between penned and free-ranging samples becomes more pronounced after calving, with some differences between years. In 2017, the average cortisol level in free-ranging samples was higher, but not significantly different in June (Figure 4), but significantly different in July. In 2018, it was the opposite: a significantly lower average cortisol level in penned cows in June, and an insignificant difference in July. At this point, with only two years of data, we cannot determine if the reversal between June and July reflects a biological pattern or simply stochastic variation. Also, while still relatively low, the average cortisol in penned cows in April 2018 was slightly higher than in free-ranging caribou; this reversal from April 2017 might reflect the change in pen location that was implemented that year, since the April sampling session took place just a month after cows were captured and transported to the unfamiliar pen at Mt. Rochford.

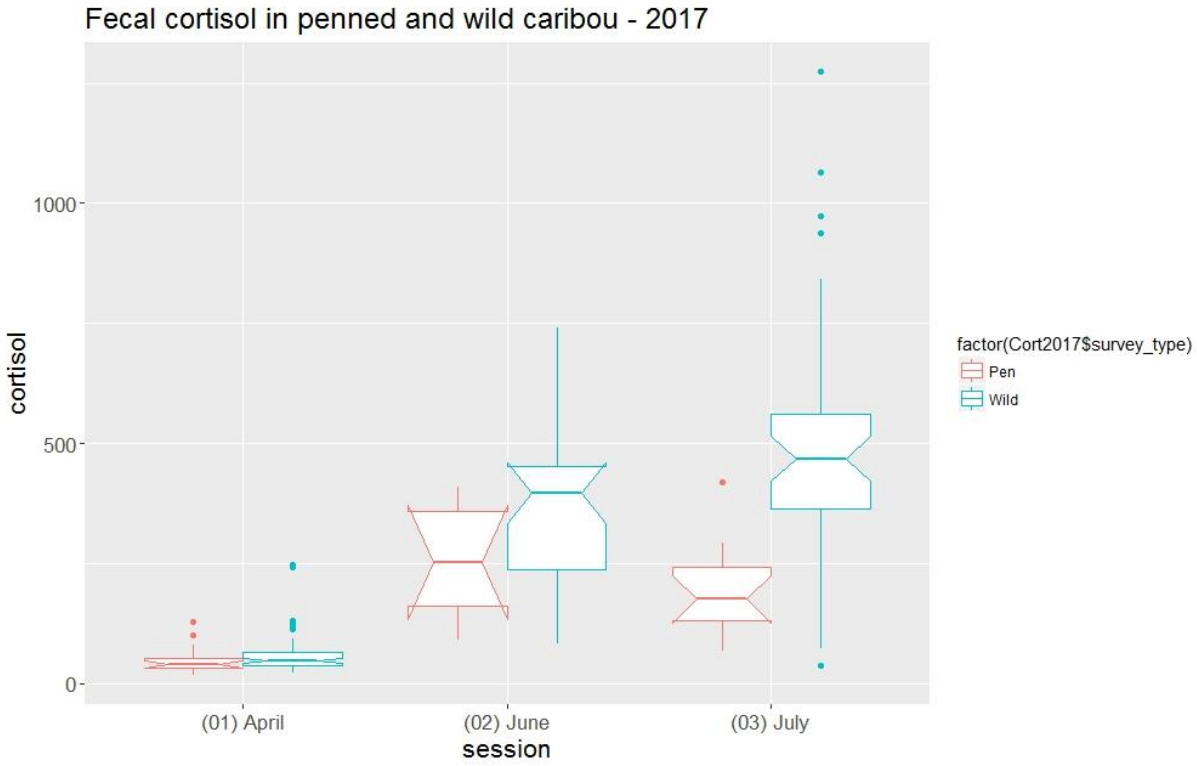

Figure 4. Differences in fecal cortisol between pellets of penned cows (pink) and free-ranging caribou (blue) – 2017.

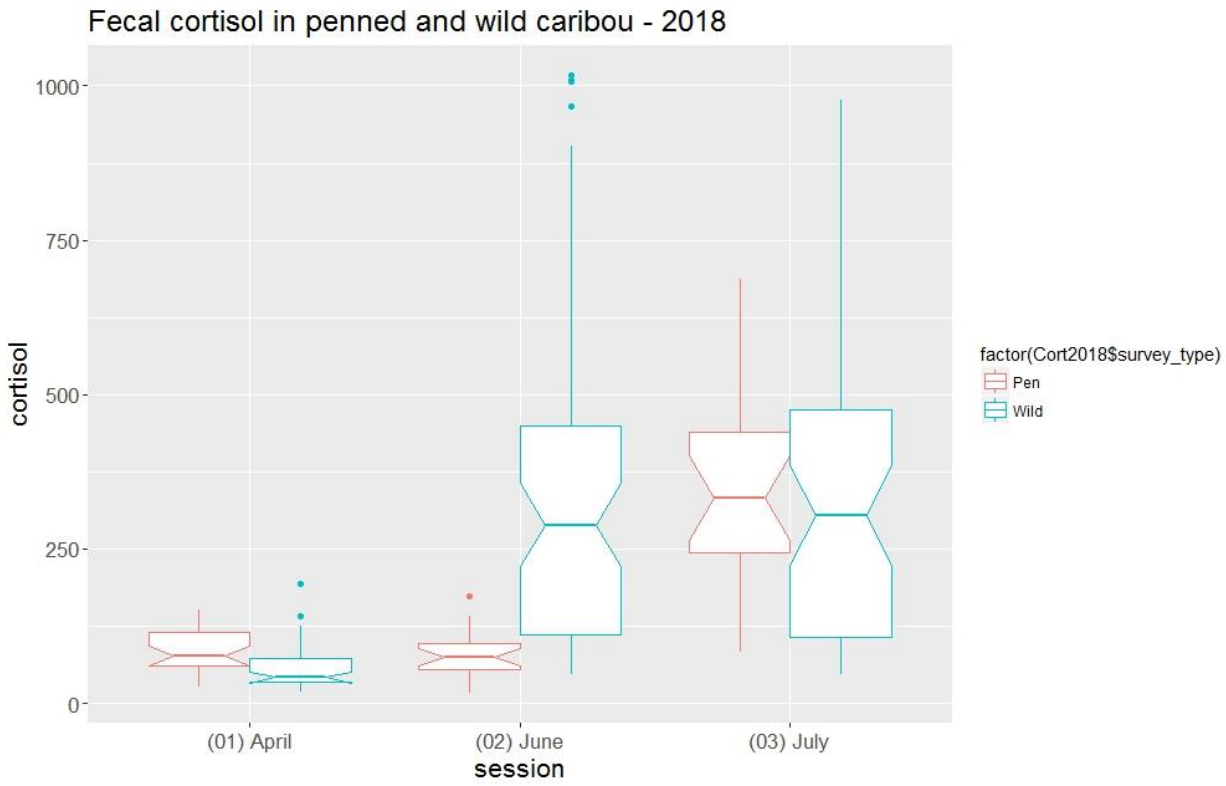

Figure 5. Differences in fecal cortisol between pellets of penned cows (pink) and free-ranging caribou (blue) – 2018.

Penned animals at capture

Since we were able to link, through field observation, the 2017 pen samples with the cows which produced them, we could track the trajectory of fecal cortisol (in snapshots) over the course of the season (see Figure 6). Since this is a small dataset, we cannot test for relationships between individual cortisol level and other metrics, but this data does suggest that the pattern of increasing fecal cortisol level throughout the calving season is commonplace. Interestingly, the only cow that was not pregnant in this dataset (C364K) still followed the general trend.

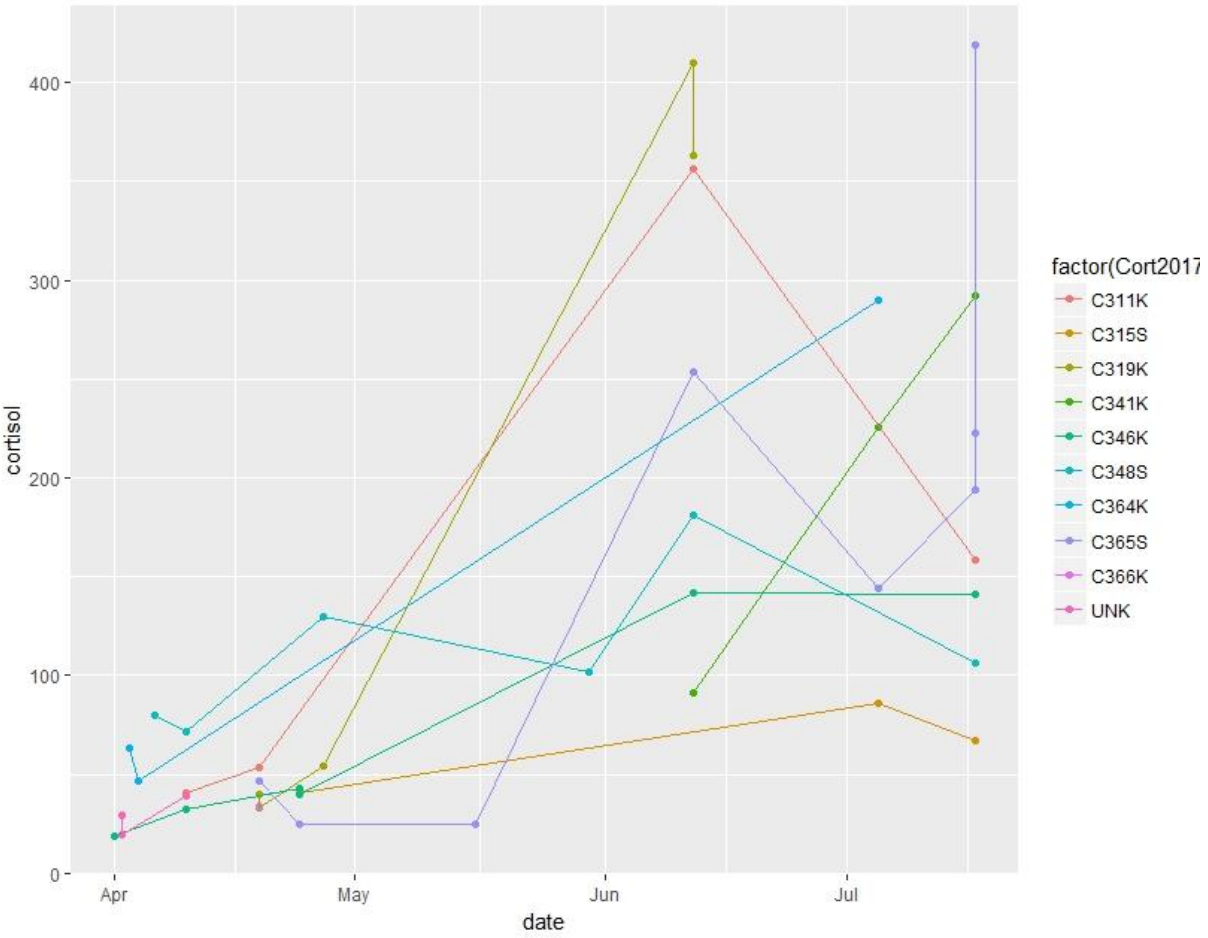

Figure 6. Fecal cortisol levels in individual penned cows over the 2017 calving season.

Using the same data, we asked if repeated penning may have influenced the physiological stress of the cows. Based on the 2017 data, fecal cortisol in penned cows does not seem to have a relationship to number of times a cow has been penned, either before (Figure 7) or after calving (Figure 8).

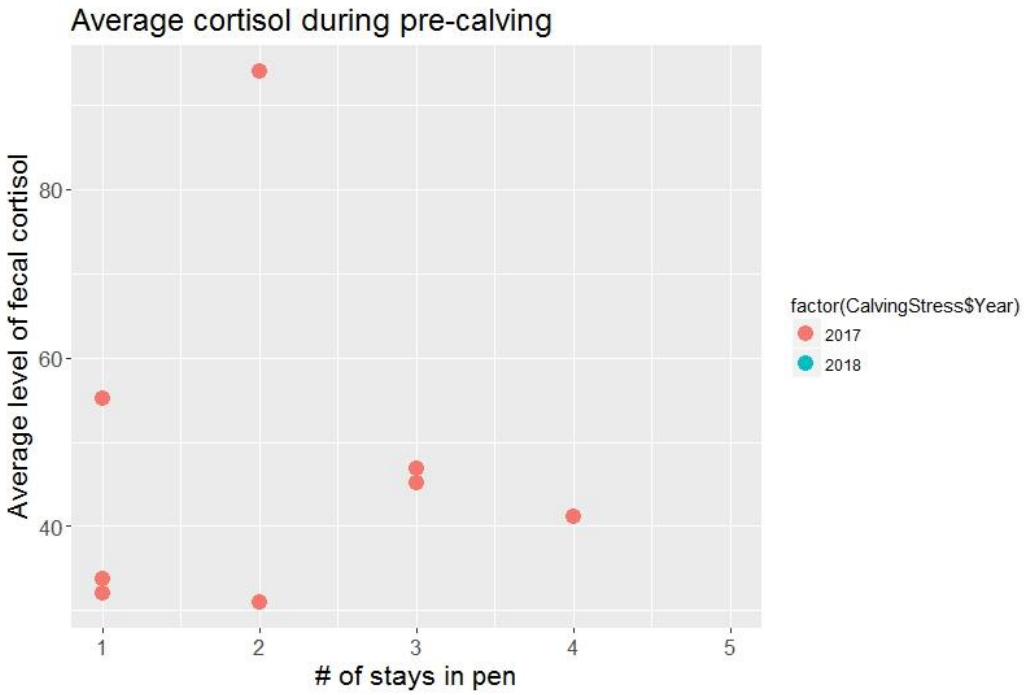

Figure 7. Average level of fecal cortisol for the pre-calving period for individual cows as a function of the number of times they have been penned.

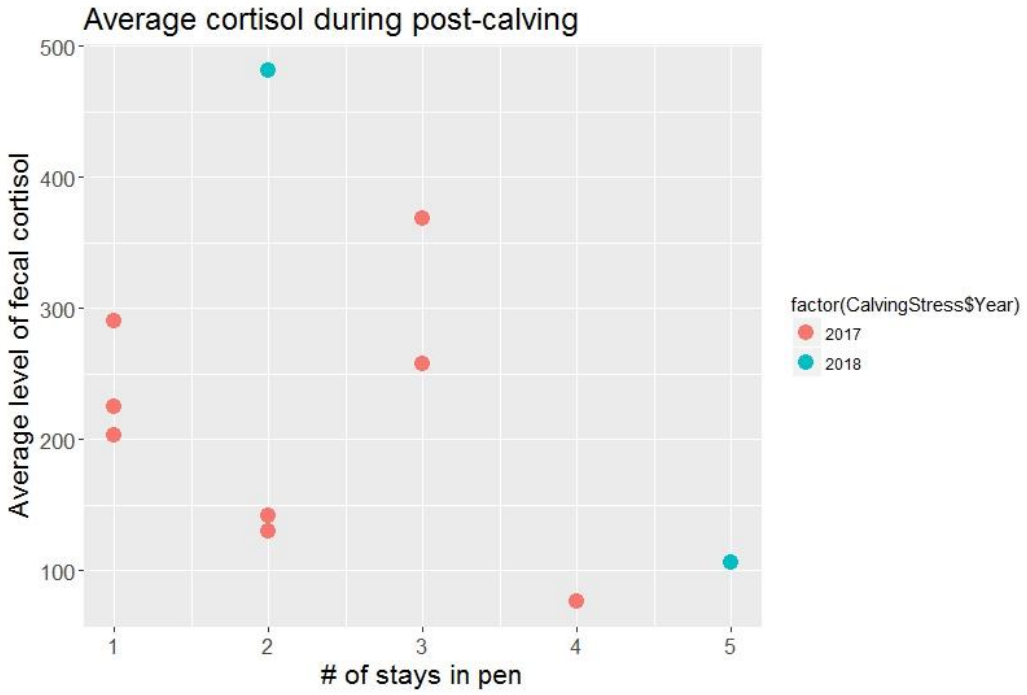

Figure 8. Average level of fecal cortisol for the post-calving period for individual cows as a function of the number of times they have been penned.

Hair cortisol

The hair cortisol concentration in our Klinse-Za samples consisted mostly of observations from penned females (n =93, mean=7.21 pg/mg) but also included fifteen observations from free-ranging animals (mean=8.0 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 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 9). We did not find evidence for cortisol relationships with caribou body mass ($\beta = -0.05$, $p=0.23$), body condition ($\beta = -1.4$, $p=0.42$), or body fat ($\beta = -0.44$, $p=0.51$).

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 9). 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 9). There was no evidence that this related to the number of times animals were captured ($\beta captures = 0.03$, $p=0.94$, Figure 9.

Pathogens

In recent studies of caribou health in BC (BCHRP Year 1 and 2), several pathogens have emerged as potential concerns. Using data from five years of Klinse-Za cow capture, we focused on three priority pathogens: protozoan *Neospora caninum*, bacteria *Erysipelothrix rhusiopathiae*, and an alphaherpes virus (as yet unidentified in *Rangifer* but most likely Cervid Herpes virus 2, CvHV-2 – the test that is being used is for Bovine Herpes virus 1, which causes Infectious Bovine Rhinotracheitis in cattle). All of these pathogens, under certain circumstances, have been associated with reduced reproductive success (reduced fertility and abortion) and in some cases, mortality (Bondo et al. 2018).

Generally, the seroprevalence of these pathogens in the Klinse-Za herd over the last 5 years appears to be relatively low (see Table 3). 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 disease outbreak or detrimental symptoms.

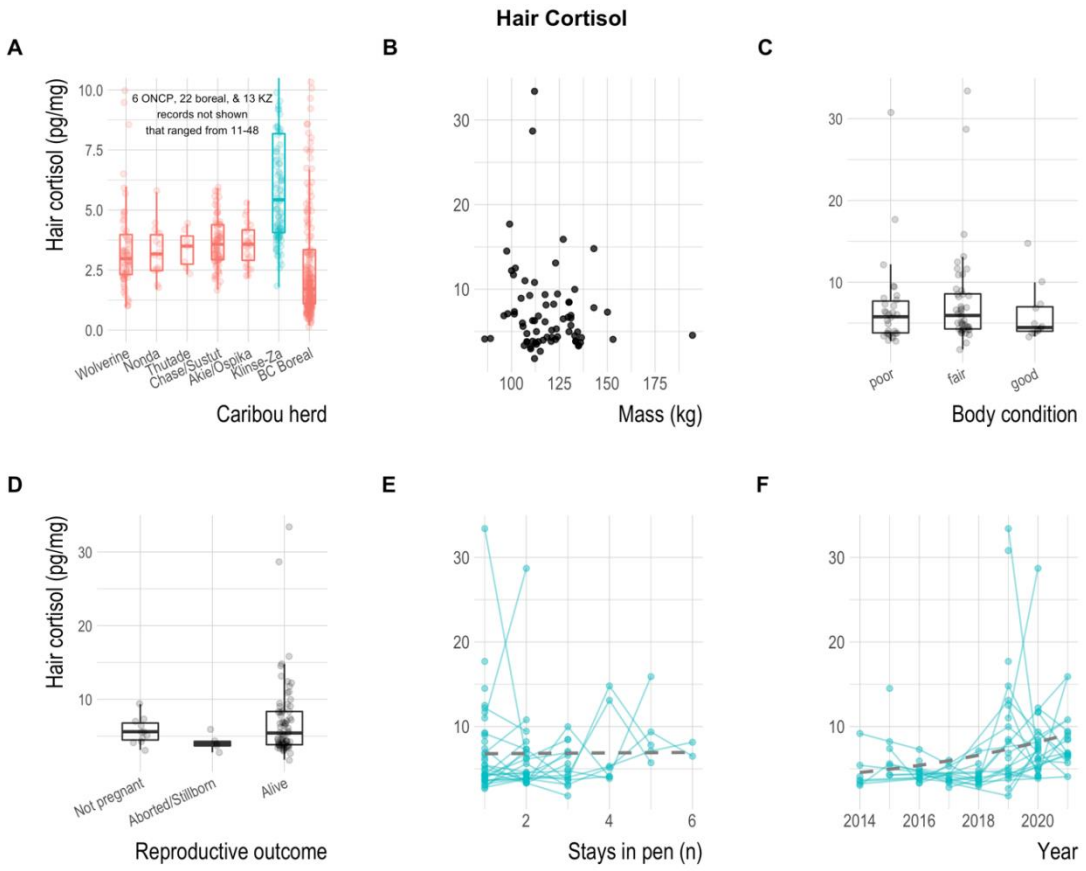

Figure 9. Hair cortisol relationships for Klinse-Za female caribou. Hair was sampled in March and generally reflects an averaged cortisol level from the entire period during hair growth (spring-fall). A) Hair cortisol levels for Klinse-Za female caribou compared to nearby mountain caribou subpopulations 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.

Nonetheless, among the cows that had compromised (< 100%) reproductive success in the pen (n=11 out of 26), six were seropositive for at least 1 pathogen, and two had co-occurring seropositive results (C332K = IBR and Erysip, C338K = IBR and Neospora). Among the cows with 100% reproductive success, 4 tested positive for IBR, none have tested positive for *Neospora,* and none were positive for *Erysipelothrix* in 2018 (see Table 4).

Herpes virus appears to have been present in the population for as long as the penning project has been ongoing, since there are some seropositive animals in every year. *Neospora*, on the other hand, does not show up in our results until 2016. Also, none of the cows that had 100% reproductive success in the pen (n=15 out of 26) ever had a seropositive result for *Neospora*.

Table 3. Seroprevalence of pathogens in Klinse-Za caribou penned cows.

	Number of animals testing positive (percentage)	Number of cow/years testing positive (percentage)
Neospora	2/25 (8%)	3/42 (7.1%)
Erysipelothrix	4/15 (26.7%) or 2/15 (13.4%)*	NA (only 1 year of data)
Herpes (IBR)	6/27 (22.2%)	13/55 (23.6%)

* Two of the animals were considered 'positive', while two were relatively close to the cut-off and considered 'borderline positive'.

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The two animals that tested definitely positive for *Erysipelothrix* in 2018 were captured in the free-ranging for re-collaring and not brought into the pen. One of them, C333K, is in the pen this year (2019), so will be observed closely for signs of abnormal health.

For the pathogens that we tested, seroprevalence in the Klinse-Za herd appears to have a slightly different profile to boreal caribou in northeastern BC (Bondo et al. 2018). The overall proportion of animals seropositive for Herpes virus in the BCHRP study, over the course of 4 years, was ~62%, though this varied by herd from 13 – 100%. In Klinse-Za seroprevalence was 22%. *Neospora* prevalence, while low in both, was relatively higher in Klinse-Za (at 8%) than the average BCHRP prevalence (2%)

With *Erysipelothrix*, seroprevalence in the BCHRP across 13 years ranged from 35 – 58% (besides one year of 14%), while for Klinse-Za in 2018 it was between 13 and 17% (depending on how to classify the 'borderline positive' cases).

We found the accuracy of the confusion matrices to be moderately high (0.65 to 0.75) (Table 5, Table 6, Table 7). However, these values seem to be largely driven by the 'true negative' cases where a seronegative cow has a live calf, rather than true positives' where a seropositive cow has a failure. In the case of the pathogens for which we had a multi-year dataset (herpes and *Neospora*), we see that seropositive cows are still more likely to produce a live calf than not.

Haptoglobin

Haptoglobin is a protein believed to play a role in fighting inflammations and infectious diseases. As such, serum haptoglobin levels can serve as an index of immune response in ruminants (Bondo 2019). Haptoglobin levels across the 53 cow-years had a mean of 0.28 g/L and ranged from 0.15 to 1.36 g/L. These values are slightly higher than those reported for boreal caribou in the BCHRP Year 1 but lower than years 2 and 3 (Table 8). In fact, the maximum value in our data (1.36 g/L) is an outlier, since the next lowest value is 0.48. 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.

Table 4. Penned cows reproductive success in relation to pathogen testing history. Reproductive success means the cow successfully got pregnant and delivered a live healthy calf.

WII_ID	Times in pen	Reproductive success	IBR positive (ever)	Neos positive (ever)	Erisyp positive (2018)
C366K	1	0%			
C332K	3	33%	yes		yes
C333K	2	50%			yes
C338K	2	50%		yes	yes
C346K	2	50%			
C364K	2	50%			
C315S	5	60%		yes	
C312K	3	67%			
C313K	3	67%			yes
C316S	3	67%	yes		
C317S	3	67%			
C311K	3	100%	yes		
C314K	2	100%	yes		
C318S	1	100%			
C319K	4	100%			
C320S	1	100%			
C331K	1	100%			
C334K	1	100%	yes		
C335K	2	100%			
C336K	2	100%	yes		
C337S	2	100%			
C341K	1	100%			
C347K	2	100%			
C348S	2	100%			
C349S	1	100%			
C365S	1	100%			

Table 5. Confusion matrix for IBR disease status and reproductive success for 52 cow-years in the pen. Spanning 2014-2018. Accuracy = 34/52 = 0.65.

AHC_IBR	Negative	Positive	Total
Success	30	9	39
Fail	9	4	13
Total	39	13	52

Table 6. Confusion matrix for *Neospora* disease status and reproductive success for 52 cow-years in the pen. Spanning 2014-2018. Accuracy – 39/52 = 0.75.

Neospora	Negative	Positive	Total
Success	38	2	40
Fail	11	1	12
Total	49	3	52

Table 7. Confusion matrix for *Erysipelothrix* disease status and reproductive success for 12 cow-years in the pen. Data only for 2018. Accuracy = 9/12 = 0.75.

Erysipelothrix	Negative	Borderline Positive	Total
Success	8	1	9
Fail	2	1	3
Total	10	2	12

Table 8. Haptoglobin serum levels measured in captured Klinse-Za cows between 2014-2018, compared to values from boreal caribou in the BCHRP.

Haptoglobin g/L	**N**	**Min**	**Max**	**Mean (SD)**	**Median**
Klinse-Za(2014-2018)	53	0.15	1.36	0.28 (0.17)	0.24
BCHRP Year 1 (2012/13)	152	0.11	0.39	0.25	-
BCHRP Year 2 (2013/14)	40	0.27	0.9	0.4	-
BCHRP Year 3 (2014/15)	32	0.37	1.15	0.57	-

Trace nutrients

Trace nutrients are vitamins, minerals and other microelements, 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 certain trace nutrients have been associated with decreased health and reproductive success (Bondo 2019). The BC Boreal Caribou Health Program identified a suite of trace nutrients that can be tested for using blood serum, and we replicated those tests with blood samples from 5 years of Klinse-Za cow captures to establish baseline levels and identify potential deficiencies.

Overall, we found that average levels of trace nutrients in the Klinse-Za herd are lower or comparable to boreal caribou that have been tested through the BCHRP, and also relative to a reference range from captive, and presumably well-fed, animals (Table 9).

Table 9. Average and range of trace nutrient levels in Klinse-Za cows, 2014-2018. Based on blood serum collected at capture for (n = 53) penned and (n = 3) free-ranging cows.

Trace Nutrient	Min	Max	Mean (SD)	Median	Mean and Range of Values in Free-ranging Boreal Caribou in NE BC	Laboratory Reference Range α
Mn (ppm)	0.0017	0.0075	0.00311 (0.001)	0.0028	0.027 (0.001-4.80)	NE
Fe (ppm)	1.6	12	3.64 (2.10)	3.05	5.61 (1.70-140.00)	NE
Co (ppb)	0.32	2.6	1.05 (0.76)	0.76	0.65 (0.27-1.70)	NE
Cu (ppm)	0.34	1	0.47 (0.10)	0.46	0.43 (0.11-0.74)	0.70-1.80
Zn (ppm)	0.17	1.4	0.74 (0.23)	0.66	1.04 (0.59-3.00)	1.10-2.50
Se (ppm)	0.045	0.34	0.072 (0.05)	0.061	0.054 (0.030-0.51)	0.050-0.140
Mo (ppm)	0.0011	0.051	0.0081 (0.0011)	0.0038	0.034 (0.0009-1.00)	NE

* Reference range obtained from captive animals, and referenced in MacBeth et al. 2016

Exploring the relationship between the levels of individual trace nutrients and reproductive success suggested that there might be a positive correlation between a cow's successfully producing a live calf on a given year, and her serum levels of zinc and iron in March (capture time) of that year. A one-way ANOVA test for both nutrients produced a significant F-value of 9 ($p = 0.004$) for zinc (Figure 10), and an insignificant F-value of 3.2 ($p = 0.08$) for iron (Figure 11).

Exploratory graphics suggested that the number of times a cow has been penned do not strongly predict levels of trace nutrients. While the fluctuations in nutrient levels vary by individual and across years, they seem to, on average, slightly decline over successive pen stays for Zn, Fe, Cu, and Se, and slightly increase on average for Co and Mn (see Appendix E for figures).

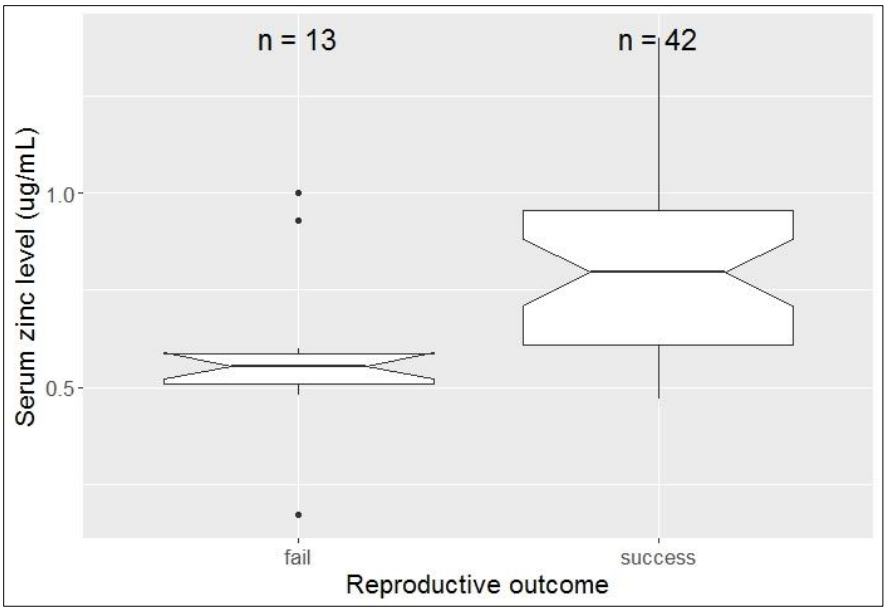

Figure 10. Boxplot depicting differences in Zinc levels between cows achieved reproductive success on a given years vs those who did not. Non-overlapping notches indicate significant difference. One-way ANOVA test confirmed significant relationship ($p = 0.004$).

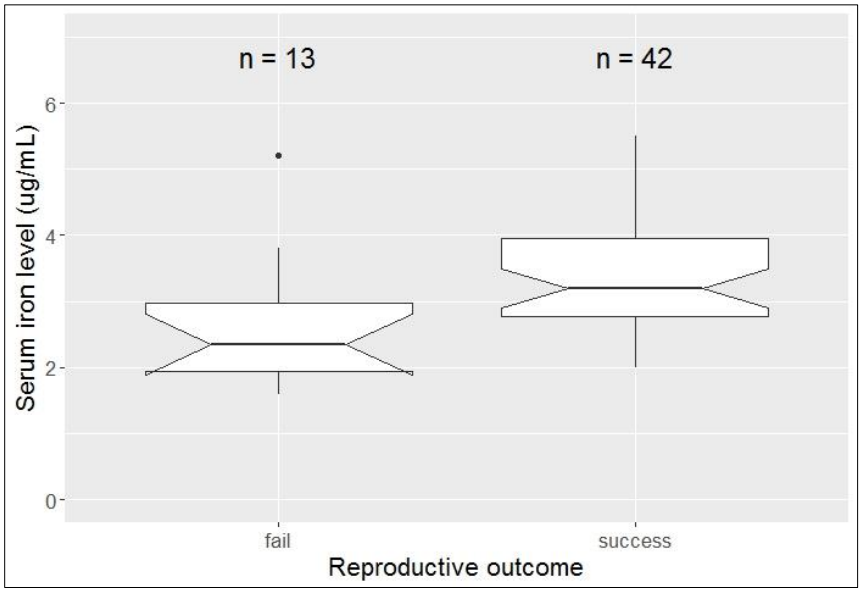

Figure 11. Boxplot depicting differences in iron levels between cows achieved reproductive success on a given years vs those who did not. Non-overlapping notches indicate significant difference. One-way ANOVA test did not show a significant relationship ($p = 0.08$). Three samples are cropped from view in the 'success' class for ease of viewing and due to being outliers (values = 11, 11 and 12 ug/mL).

DISCUSSION

In year 1 of the Klinse-Za health evaluation, we established a baseline for several health indices that will allow for monitoring the trajectory of herd health going forward. As well, the collected samples enable comparisons with other herds, using past and current samples. We have also begun to explore the potential effects of penning on the health of individuals and the herd as a whole. This year we inventoried and transported all samples to an appropriate long-term storage facility, which allows for systematic analysis and protects them from degradation. Finally, we developed a database system which allows us to link the results of lab assays to the rich metadata on the animals from whom we have collected the samples; since most animals were being monitored for population and demographic studies, we often have data such as pregnancy rates, cow and calf survival, and other information which we can explore through the health lens.

Due to the highly invasive and disruptive nature of the maternal pen operation, a key question raised since the beginning of the project has been around the level of stress that capture and penning might be having on the cows. While fecal cortisol is an index of physiological stress and might not truly reflect all dimensions of animal well-being, our preliminary fecal cortisol results this year suggest that penning does not cause significantly more physiological stress than would be naturally experienced by caribou in the free-ranging. It is possible that the free-ranging caribou experience significantly higher fecal cortisol levels because they are facing the compounded stressors of predator vigilance and avoidance, sometimes singly rather than in a herd, avoiding anthropogenic and natural disturbance, and searching for food. Some of these factors are mitigated for penned cows, who are fed, protected and always in close proximity to each other – this might be some of the reason for their lower levels of fecal cortisol, especially after calving.

The levels of fecal cortisol measured in samples collected from penned cows and free-ranging Klinse-Za caribou in April are consistent with other late winter fecal cortisol measurements in caribou; Joly et al (2015) report mean glucocorticoid levels of 118.5 and 112.1 ng/g in pregnant and non-pregnant cows in Alaska, respectively. While the utility and limitations of using fecal cortisol as an indicator of physiological stress has been explored both in the husbandry and the wildlife literature for *Rangifer* (Rehbeinder and Hau 2006, Ashley et al. 2011, 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.

While the free-ranging fecal samples almost certainly contain some pellets from males who were traveling together with the collared females that we were targeting, we do not expect this factor alone to account for the much higher levels of fecal cortisol observed in free-ranging over penned samples. Joly et al (2015) report fecal cortisol levels from free-ranging males in Alaska, during March/April, with a mean (SE) of 109.1 (2.3) ng/g – a value which is slightly lower than the median value measured in penned cows. In addition, telemetry survey data shows that normally, only one or two (usually juvenile) males are traveling with groups of females, and so presumably their fecal samples also comprise the minority of the data. Samples from yearlings (male and female) who are traveling with the cows are also presumably mixed into the results from the free-ranging

fecal pellet collections. There is no published data to suggest whether we would expect their overall level of cortisol to be lower, similar or higher than adult cows. However, since these animals are not yet reproducing, we can assume that their samples are not driving the difference observed in post-calving cortisol between samples from the pen and the free-ranging.

The other difference in sampling between free-ranging and penned animals is the time elapsed between defecation and sample collection; therefore, free-ranging pellets are often more desiccated than those collected from penned animals. However, since all samples undergo oven drying prior to analysis, this factor is also unlikely to affect the final result.

A link between hair cortisol concentration and body condition indicators emerged from our early exploration of this integrative measure of health. Despite working with a modest dataset, we identified a significant negative relationship between body weight in March and hair cortisol concentration. Hair cortisol, by its nature, represents an indicator of long-term stress, given that it incorporates circulating hormones over the course of its growth cycle (MacBeth 2013) – in our case, the growth cycle from shedding the previous May to the day of capture in March. Body condition in March also reflects the status of caribou cows during the most nutritionally challenging part of the year (Parker et al. 2009). It thus makes sense for animals with higher body weights, and presumably better condition, to have experienced less stress over the preceding year. Interestingly, the actual body condition metric, a qualitative assessment collected by experienced handlers during capture using palpation, which also had more data points, did not yield a significant relationship. Perhaps the very general body condition categories (‘poor’, ‘fair’ and ‘good’) did not provide sufficient variation to detect a relationship. The lack of correlation between fecal cortisol and hair cortisol from samples taken at capture highlights the different time horizons that these two metrics represent. When compared with boreal and mountain caribou hair cortisol concentrations, the Klinse-Za samples had a slightly lower mean and a narrower range, which might either represent lower overall levels of stress, or simply an artefact of a smaller sample size. In any case, the hair cortisol data suggests that caribou in the Klinse-Za are not experiencing higher levels of physiological stress than other caribou herds in BC.

At this time, seropositive status for Herpes virus, Neospora and Erysipelothrix does not seem to, independently, explain the reproductive failures that we have observed in penned Klinse-Za cows. However, there may be covariates, such as age, trace nutrient status, comorbidity, habitat disturbance, or serum biochemistry (Bondo et al. 2018) which might increase the explanatory power of infectious diseases for reproductive loss. We have undertaken only a preliminary investigation at this point and we feel it would be premature to make the sweeping generalization that caribou health, in general, has no effect on reproductive success. The nutrition hypothesis involves a prediction that caribou are unlikely to raise calves to juvenile status in successive years and there is some evidence of that in the Klinse-za population. This could explain why calf production in the free-ranging population (which is biased towards cows having previous year successes from the mat pen). If the prediction has any support, then we have to ask the question why and it could be a cumulative effect from nutritional status and/or a number of health-related factors including stress, pathogens, trace nutrients, and others – as we have pointed out. We think we need to finish our lab assays and carry out comprehensive modelling before we can say that there are no linkages between health and population dynamics.

The fact that serum haptoglobin levels were, on average, lower in Klinse-Za than in the BCHRP seems generally congruent with the lower seroprevalence of pathogens observed in the Klinse-Za herd. Also, the haptoglobin level in caribou who were known, from post-mortem examinations, to have carried moderate - high levels of parasitic and infectious disease (n = 5) ranged from 0.49 - 5.6 g/L (Bondo et al. 2018) and was completely non-overlapping with the levels measured in the Klinse-Za herd, except for one measurement of 1.36 g/L in 2018. However, this test 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.

Given the importance of nutrients to a range of physiological functions related to survival and reproduction (Hidiroglou 1979), understanding their relationships with fitness in caribou could help explain patterns of individual fitness, as well as inform management protocol. Cows in the Klinse-Za herd had levels of serum trace minerals that were either comparable or lower than those of BCHRP caribou samples or laboratory reference values, with some possible links to reproductive failure. Since penned cows are fed supplementary pelleted feed throughout the calving season, fortifying the diet with certain lacking minerals might be considered (White 1993, Spears 2003).

RECOMMENDATIONS AND FUTURE WORK

In this study, we have helped answer some basic questions about health in the Klinse-Za herd, and have added important data points for developing baselines for woodland caribou health. At the same time, we have laid the groundwork for continuing and deepening the investigation.

Fecal cortisol reflects a relatively short-term physiological stress response (between 1 and 2 days, Ashley et al. 2011), and as such our data represents snapshots in time. In order to better evaluate potential long-term effects of penning (single or repeated), or other disturbances in the cows' environment, we can analyze the level of cortisol in caribou hair as a more integrative measure, which reflects blood cortisol level on an annual time-frame (MacBeth 2013).

Another year, at least, of fecal cortisol data collection would provide a 3-year dataset that would allow for better statistical analysis. Additionally, genetically identifying the pellets collected in the free-ranging would allow for the results to be linked to individual animals, and analyze their fecal cortisol levels with their penning and reproductive history, which might be especially interesting for cows who were sampled both in and out of the pen (on different years).

Because penned caribou are experiencing a heavily altered feeding regime during calving and lactation, and since preliminary analysis suggests that penning may have a 'concentrating' effect on the ranging behavior of Klinse-Za caribou (unpubl. data), characterizing the diet of Klinse-Za caribou should be a component of future health research. We recommend an analysis of fecal crude protein content as a widely used index of diet quality in ruminants (Wofford et al 10984, Festa-Bianchet 1988).

Simultaneously sampling the forage availability across the herd's range would provide important data for understanding diet quality.

Since the Klinse-Za/Scott East herd size consisted of just 36 individuals prior to the commencement of emergency recovery actions, it fell below the 50 individuals believed necessary to avoid inbreeding depression in mammalian populations (Frankham et al. 2014). As such, the possibility of inbreeding (Ralls et al. 1988, lacy 1997) is very real in this herd and merits consideration. Genomic techniques using blood clots (Kanai et al. 1994) allow for the investigation of genetic relatedness, which could be used to evaluate the level of inbreeding in the herd. This information could be relevant to understanding the reproductive failures (i.e., failed/aborted pregnancies and still births) observed in the herd[3], and/or could be used to guide management action such as translocations of breeding individuals.

Developing a thorough understanding of woodland caribou health is going to require ongoing sample collection, research and collaboration. The Klinse-Za herd is just one of the many threatened caribou populations in British Columbia. Over the past several decades of caribou studies across the province, samples have been collected and some have been preserved in archival storage. Comparisons with other herds from other regions or ecotypes, which have faced different types or levels of anthropogenic or natural disturbance, and were part of different predator-prey systems, will provide invaluable context for the results of the Klinse-Za study.

[3] Generally, a decrease from 90% pregnancy to 75% successful parturition (n = 56) (Unpubl. Data, Free-ranginglife Infometrics Inc.; Mackenzie, BC)

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APPENDIX A. ANALYTICAL LABORATORIES USED FOR ANALYSIS OF HEALTH SAMPLES

Laboratory/facility	Contact	Test
Toronto Zoo, ON	Gabriela Mastromonaco, PhD	Fecal cortisol
Prairie Diagnostic Services Inc., SK	Dale Godson, DVM, PhD	Neospora
University of Calgary, AB	Angie Schneider	Erysipelothrix
Animal Health Centre, BC Ministry of Agriculture	Tomy Joseph, DVM, M.Sc., Ph.D	AHC_IBR
University of Saskatchewan, SK	Dr. David Janz	Hair cortisol
University of Guelph, ON	Helen Kocmarek	Haptoglobin
University of Guelph, ON	Nick Schrier	Trace nutrients

APPENDIX B. SAMPLE DATABASE EXAMPLE

WLH ID	WII Animal ID	Date	Year	Neospora	AHC_IBR	ERYSIP	Mn (ng/mL)	Fe (ug/mL)	Co (ng/mL)	Cu (ug/mL)	Zn (ug/mL)	Se (ug/mL)	Mo (ng/mL)	Haptoglobin g/L	stay_in_pen	precalf_ avg_cort	postcalf_a vg_cort	Calf_result
14-4954	C311K	2014-03-27	2014	Negative	Positive	positive	3.6	12	1.1	0.43	1.4	0.066	2	0.43	1			Alive
14-4955	C312K	2014-03-27	2014	Negative	Negative	positive	2.6	3.8	0.53	0.44	1.1	0.06	1.9	NRH	1	850.67		Alive
14-4956	C313K	2015-03-18	2015	Negative	Negative	negative	2.3	3.5	0.46	0.4	0.58	0.18	<MDL	0.28	2			Not pregnant
14-4958	C315S	2015-03-18	2015	Negative	Negative	positive	2.3	1.6	0.66	0.48	0.6	0.077	2.6	0.21	2			Aborted/ Stillborn
14-4954	C311K	2016-03-17	2016	Negative	Positive	positive	2.2	2.9	2.3	0.35	0.78	0.074	<MDL	0.2	2			Alive
14-4955	C312K	2016-03-16	2016	Negative	Negative	positive	3.1	2.6	2.2	0.49	1	0.073	7.8	0.18	2			Alive
16-8272	C347K	2016-03-16	2016	Negative	Negative	negative	3.2	5.5	2	0.47	0.87	0.062	14	0.34	1			Alive
16-8274	C348S	2016-03-16	2016	Positive	Negative	negative	3.9	11	2.6	0.55	0.84	0.055	4.5	0.37	1			Alive
16-8278	C351K	2016-03-17	2016	Negative	Negative	positive	2.4	2.8	2.2	0.45	0.9	0.045	<MDL	0.21	1			Free-ranging
14-4956	C313K	2017-03-23	2017	Negative	Negative	positive	4.3	3.2	0.45	0.46	0.74	0.055	1.1	0.21	free-ranging			Free-ranging
17-9709	C315S	2017-03-23	2017	Positive	Negative	positive	2.8	3.9	0.38	0.53	0.61	0.061	1.5	0.26	4	41.1	76.7	Alive
17-10493R	C312K	2018-03-14	2018	Negative	Negative	Negative	4.2	1.8	0.52	0.4	0.17	0.062	1.6	0.38	3			Not Pregnant
17-10496R	C313K	2018-03-14	2018	Negative	Negative	Borderline Positive	4.1	3.9	0.66	0.36	0.61	0.053	<MDL	0.21	3			Alive

APPENDIX C. DATA ON REPODUCTIVE SUCCESS AND DISEASE BY YEAR

AHC_IBR (herpes)												
	Negative					Negative Total	Positive				Positive Total	Grand Total
Row Labels	2014	2015	2016	2017	2018		2014	2015	2016	2018		
Aborted/Stillborn		3	2	1	1	7		1			1	8
Alive	7	3	7	5	8	30	3	2	3	1	9	39
np		1			1	2		1	1	1	3	5
Free-ranging			1	1	1	3						3
Grand Total	7	7	10	7	11	42	3	4	4	2	13	55

Neospora										
	Negative					Negative Total	Positive		Positive Total	Grand Total
Row Labels	2014	2015	2016	2017	2018		2016	2017		
Aborted/Stillborn		4	1	1	1	7	1		1	8
Alive	10	5	10	4	9	38	1	1	2	40
np		2			2	4			0	4
Free-ranging			1	1	2	4			0	4
Grand Total	10	11	12	6	14	53	2	1	3	56

Erysipelothrix				
Row Labels	Borderline Positive	Negative	Positive	Grand Total
Aborted/Stillborn		1		1
C317S		1		1
Alive	1	8		9
C313K	1			1
C315S		1		1
C319K		1		1
C335K		1		1
C336K		1		1
C337S		1		1
C347K		1		1
C349S		1		1
C364K		1		1
np	1	1		2
C312K		1		1
C332K	1			1
Free-ranging			2	2
C333K			1	1
C338K			1	1
Grand Total	2	10	2	14

APPENDIX D. PHOTO OF COW WITH HIGH HAPTOGLOBIN READING



APPENDIX E. TRACE NUTRIENTS OVER SUCCESSIVE PEN STAYS

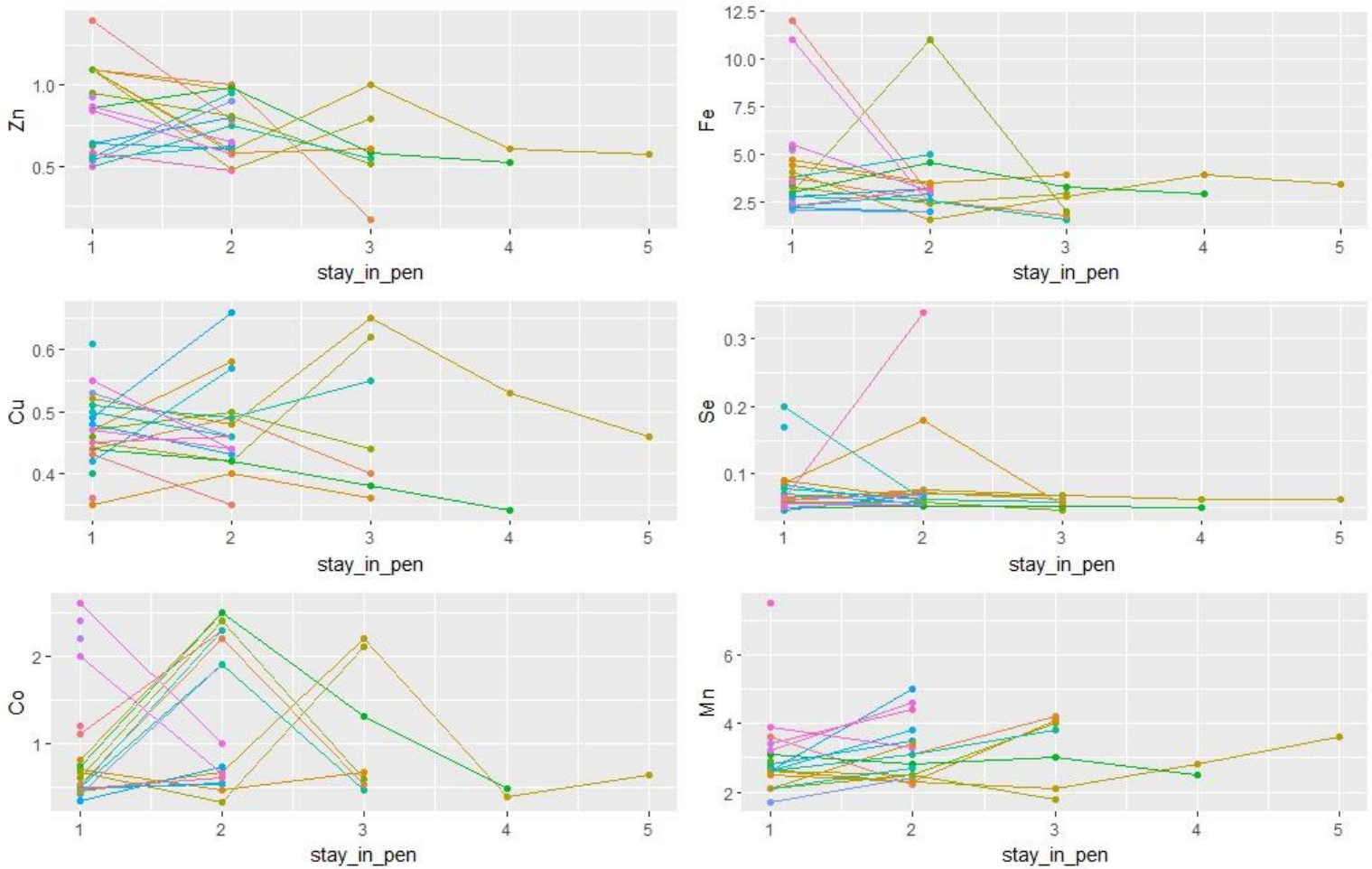

Figure 12. Serum trace nutrients level over the course of successive pen stays. Colors represent individual cows. X axis denotes which penning stay this is for a given cow (i.e. it does not represent year of penning program).