Limiting factors on a small herd of Rocky Mountain bighorn sheep residing in the Kicking Horse Canyon, near Golden, British Columbia

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ABSTRACT: This case study identified limiting factors on a herd of about 15 Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) residing in the Kicking Horse Canyon, near Golden, British Columbia, using noninvasive techniques. These included observations on recruitment and highway mortalities plus fecal analysis to test inbreeding, parasite loads, and cortisol levels. Herd health results showed heterozygosity at over 65% of the loci tested. Dorsal spine larvae and a range of gastro-intestinal parasites were present, and baseline cortisol levels were higher than those documented in other bighorn sheep studies. The widespread presence of a dorsal spine larvae, possibly *Muellerius sp*, could be of concern as stress potentially increases in this herd because of additional anthropogenic influences associated with upcoming highway widening. Recruitment varied between 0.17 and 0.33 juveniles per ewe between 2015 and 2020. The Trans-Canada Highway (HWY 1) occupies almost 20 % of the study area, and BC Ministry of Transportation and Infrastructure data indicates that highway mortality is not uncommon. The data from this case study suggests that low recruitment and highway-related mortality are the principal factors limiting this herd of bighorn sheep and that fencing placement and breaching of one-way "escape" gates and jump-outs may contribute to highway usage. Several conclusions are presented which could result in improved outcomes for this herd.

Biennial Symposium of Northern Wild Sheep and Goat Council 22:106-112; 2020

KEY WORDS: British Columbia, cortisol, fecal analysis, highway mortality, inbreeding, limiting factors, noninvasive techniques, parasites, recruitment, fencing

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INTRODUCTION

Identification of limiting factors can inform management decisions which may alleviate threats faced by free-ranging wildlife. Using non-invasive methods, this case study illuminates our understanding of the threats faced by a small group of sheep residing in a restricted canyon in southeastern British Columbia (BC), which is bisected by the Trans-Canada Highway (HWY 1) and a heavily-used Canadian Pacific Railway train line. Inbreeding (Coltman *et al.* 1999; Luikart *et al.* 2008), parasite loads (Flanagan 2009), stress hormones (Coburn *et al.* 2010; Miller *et al.* 1991; Millspaugh and Washburn 2004), recruitment (Enk *et al.* 2001), and highway mortality (Neumann *et al.* 2012) are all evaluated in this case study of the Golden Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*) herd.

Inbreeding, defined by a reduction of alleles and an increase in homozygous loci, can be associated with inbreeding depression (Rioux-Paquette *et al.* 2011) and may significantly affect birth weight, survival, reproductive success, resistance to disease, predation, and environmental stress (Fitzsimmons *et al.*1995; Keller and Waller 2002; Luikart *et al.* 2008; Luikart and Allendorf 1996). Due to the physical isolation of the Golden sheep herd from other bighorn sheep herds, lower than average genetic diversity would be expected.

Parasite loads fluctuate seasonally (France 2015) and some level of intestinal parasites is not uncommon; bighorn sheep are known to remain in good health despite the presence of various parasites although the presence of *Mycoplasma ovipneumoniae* (M. ovi) has been linked to pneumonia outbreaks and related die-offs (Besser *et* *al.* 2013; Cassirer *et al.* 2018; Festa-Bianchet 1989).

High stress levels can also negatively impact bighorn sheep, leading to lowered resistance to disease. Long-term or chronic exposure to stressful events is thought to cause an increase in cortisol and a lowering of immune functions (Miller *et al.* 1991) with reduced reproductive success (Coburn *et al.* 2010). Given the high level of anthropogenic influence and the frequency of interactions between bighorn sheep and vehicles, a high stress level would be predicted for the Golden herd.

The rate of recruitment required to sustain a population varies considerably. Relatively high survival rates have been documented in bighorn sheep adults (Overstreet *et al.* 2014), such that even minimal recruitment could increase herd size; estimates of 0.30 juveniles per adult ewe have been made for bighorn sheep (Buechner 1960; Jorgenson 1992). Based on reports and observations of the Golden herd, recruitment is likely to be low.

Finally, highway-mortality has the potential to significantly impact animal numbers and herd viability. Bighorn sheep near Radium, BC preferred winter ranges close to human habitation over steeper habitat, leading to a variety of management concerns (Dibb 2010). Demarchi (2004) noted that, "roads and railways occupy habitat, dissect migration routes, and result in direct mortality. Furthermore, salt used for road maintenance can attract and hold sheep in highway corridors. In some cases, significant numbers of adults have been lost in single seasons" to vehicle traffic. Various other studies (Huwer 2015; Keller and Bender 2007) have documented the impacts of highways on wildlife, which is expected to be high in this study. Urbanization and human developments impact habitat selection and have the potential to attract wildlife with high quality forage, water, and possible protection from predation, while also exposing them to disease transmission, stressful interactions, and highway mortality (France 2015; Rubin *et al.* 2002). Fencing with escape gates and jump-outs has been the principal method employed to keep sheep and other wildlife off of HWY 1 in this area.

METHODS

Rocky Mountain bighorn sheep are a blue-listed (vulnerable) species in BC whose numbers have been in steady decline. Several small populations exist in eastern British Columbia (Poole 2019; Teske 2015), including the Golden herd, numbering approximately 14 individuals and residing in the Kicking Horse Canyon. The 620-hectare study area encompasses the annual range of the nursery group and is located on the western extreme of the Rocky Mountains in southeastern BC (51°N, 117°W; 800 - 1300 m elevation). It extends east from the town of Golden (population 4,000) along HWY 1 to the Yoho Bridge (7 kms), south of the highway to the Kicking Horse River and north of the highway for approximately 300m (Figure 1).

Sixty freshly excreted bighorn sheep fecal samples were photographed, mapped and collected into plastic bags which were labelled and stored in a cooler in the field. Thirty samples were collected between February 24 and July 19, 2019; an additional thirty samples were collected in 2020, between February 7 and April 9. Samples were refrigerated or frozen depending on the testing to be done.

Extracted DNA from fecal samples was typed at 13 (U. of Alberta) to 28 (Wildlife Genetics International) microsatellite loci following procedures described in Deakin *et al.* (2020), Paetkau (2003) and Woods *et al.* (1999). All loci selected were believed to be neutral except for MMP9 (Luikart *et al.* 2008). Since marker loci used varied, a measure of individual heterozygosity was calculated (H_I = number of heterozygous loci/total number of loci typed for each individual animal). Observed heterozygosity (H_O) was calculated for each of the loci tested by dividing the number of heterozygous individuals by the total number of individuals sampled. Parasite loads were analyzed using the Baermann test and the fecal flotation test (Foreyt 2001) by the Washington Animal Disease Diagnostic Lab (WADDL) at Washington State University. Fecal cortisol was measured at the Toronto Zoo following the procedure described in

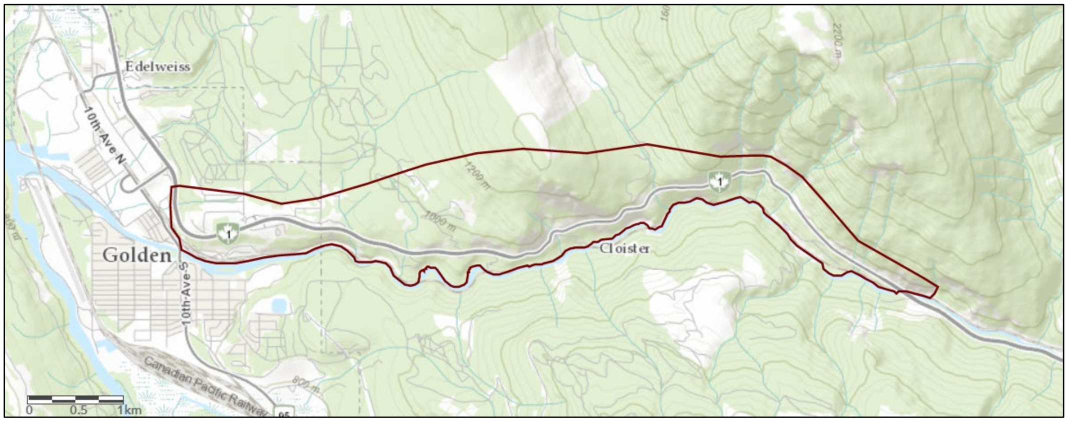

Figure 1. Study area (620 ha), located in and to the east of Golden, BC, Canada. The maple leaf with the number one represents the Trans-Canada highway (HWY 1). The red line is the border of the study area.

Miller *et al.* (1991) and Dulude-de Broin *et al.* (2019).

Reproductive success was documented through observation from a vehicle. Between 2016 and 2018, the number of ewes and lambs was documented on 53 trips through the study area, between 14:00 and 17:00 between May and August each year. If animals were sighted, they were filmed if possible. In 2019 and 2020, bighorn sheep were observed and filmed daily during lambing season and throughout the year (79 trips in 2019 and 198 trips in 2020) to determine lambing areas, lambing dates, reproductive success, fall recruitment and to observe use of the highway corridor and escape structures.

Highway mortality was assessed based on known (observed or reported) losses and road-kill data collected by the Ministry of Transportation and Infrastructure (MOTI; WARS 2020).

RESULTS

Genetic analysis of the fecal samples identified nine individual members of the Golden bighorn sheep herd. The average proportion of the loci that were heterozygous (H_I) was 0.641 ± 0.102 and the average number of alleles per locus was 2.88 ± 1.05. The average observed heterozygosity at each locus (H_O) was 0.66 ± 0.25. Baermann tests found dorsal spine larvae (DSL) in 88.24% of 17 samples with a mean value for larvae per gram of 14.99 ± 25.90 and a range = 1-81. Two samples believed to be from lambs contained no parasites. Although the DSL were not able to be identified to species, the likelihood is high that they are *Muelleris capilaris* (Laura A. Williams, personal communication, June 4, 2020). Fecal flotation tests found gastrointestinal parasites of the genera *Strongyles, Eimeria, Nematodirus, Capillaria, Wyominia, Moniezia* and *Trichuris ovis,* in 65% of 17 samples, with a prevalence ranging from 5% - 35% depending on the genus. Although one individual had three different isolates, all other samples contained 2 or fewer. Stress hormone results showed a range of values from 15.23 ng/g to 245.79 ng/g with standard deviation of 42.99 ng/g (n = 34). Between 2015 and 2020, recruitment ranged from 0.17 to 0.30 juveniles/adult female, based on 6 ewes able to give birth each year. Several adult females died on the highway which kept the adult ewe number constant during this period. Highway mortality data from MOTI (WARS, 2020) and citizen reporting (Mike Nickle, personal communication, June 15, 2019) documented 10 deaths between 2000 and 2020. Between 2015 and 2020, highway mortality was the only known cause of mortality for this herd; three lambs died between June and September, 2020 and at least 2 of these deaths were highway-caused (Helen Schwantje, personal communication, July 22, 2020). Bighorn sheep were observed accessing the Trans-Canada highway corridor by breaching one-way gates and jump-outs and going around fence end points to access winter and spring ranges (www.wildsight.ca/goldensheep).

DISCUSSION

The various non-invasive techniques employed in this case study addressed several of the potential limiting factors faced by the Golden herd. The individual heterozygosity (H_I) for the 9 unique individuals for which analysis was completed was similar to that documented in other studies (Hedrick and Wehausen 2014; Hogg *et al.* 2006; Wehausen and Ramey 2004), and the observed heterozygosity (H_O) for this herd is higher than expected and higher than that found in Alberta by Deakin *et al.* (2020) for the same loci. The analysis at the MMP9 locus showed especially high H_O which may indicate less susceptibility to lung infection than in animals with low heterozygosity at this locus (Luikart *et al.* 2008). In contrast, the fixed allele (all samples homozygous) at the MAF36 locus may warrant further study.

The presence of dorsal spine larvae in 88% of the fecal samples is consistent with other studies and is not a cause for concern, unless M. ovi is also detected along with environmental stressors (Butler et al. 2018).

Seven or more types of gastrointestinal parasites infect the Golden bighorn sheep herd and may degrade body condition, change behavior, and lower immune response (Foreyt 2001; Miller *et al.* 2012), although levels of concern have not been established (Hoar *et al.* 1996; Jenkins and Schwantje 2004) and no individuals harbored more than 3 unique species of gastrointestinal parasites.

Stress, as indicated by cortisol level, may be relatively high (up to 250 ng/g) in this herd. Results from other studies show levels between 20-50 ng/g (France 2015; Goldstein *et al.* 2005) although different methods of extraction can affect these values and limit the value of comparison between studies. These animals may be under stress due to their interactions with HWY 1. Variability in hormone levels between studies makes this data primarily useful as a baseline for future comparison.

Low recruitment is of considerable concern for this herd. Ewes give birth and lambs survive for the initial months but are threatened once they leave the lambing area and enter the highway corridor. The proximity of the historic lambing area to HWY 1 makes highway mortality almost inevitable, unless changes are made to force sheep to go under the nearby Yoho bridge versus over the roadway.

Highway accidents appear to be a significant source of mortality for both the Golden herd and the Radium herd (Dibb 2010), located 100 kms south of Golden. While human activity may lead to reduced habitat quality and/or quantity, it may also cause sheep to abandon usage areas (Bunch *et al.* 1999; DeForge 1972; DeForge 1981; Hamilton *et al.* 1982). That has not yet been the case with the Golden herd, members of which spend a lot of their time near, or on, the highway likely seeking food, water, minerals, and easy passage. Grain spills from transport trucks seeking to reduce their weight occur 3-5 times per summer in the canyon and attract sheep which feed on these protein-rich grains. Planting of preferred plants like alfalfa, *Medicago sativa*, and wheatgrass, *Agropyron* spp., make roadside areas even more desirable than they already are due to their SW facing aspects. Patches of preferred shrubs like scrub birch, *Betula nana*, chokecherry, *Prunus virginiana*, and prickly rose, *Rosa acicularis*, close to the highway also attract sheep as do “road salts”. Using HWY 1 to access different parts of their home range likely saves energy, despite the traffic, and fencing placement forces sheep to use the highway to reach desirable areas. These incentives make the highway corridor appealing. Bighorn sheep are currently able to gain access to fenced portions of HWY 1 by jumping up at jump-out structures and opening one-way gates from the back making highway mortality a serious concern.

Impacts of highway mortality are well-documented and have been found to be speed-related (Hardy *et al.* 2006; Neumann *et al.* 2012). Lower speeds have been shown to reduce highway mortality (Bond and Jones 2013) and various forms of signage, including signs associated with remote cameras have been effective in reducing speeds (Hardy *et al.* 2006). Fencing has effectively kept animals off of roadways (Huijser *et al.* 2008); Members of the Golden sheep herd have learned how to gain access to fenced portions of HWY 1 by breaching escape structures. These jump-outs and one-way gates should be modified to reduce the chance of breaching or removed from fenced sections of highway. Modifications could include creating a depression at the base of the jump-out, installing a bar at the top (Siemers *et al.* 2013) or fabricating gates using inflexible materials for the gate tines. Effective strategies to reduce collision rates on highways exist (Huijser *et al.* 2008), but they cost money and require planning and cooperation.

ACKNOWLEDGEMENTS

This project was made possible by financial support from the CSRD-Area A and the Public Conservation Assistance Fund plus encouragement from the Town of Golden, Tourism Golden, Parks Canada (Dr. Seth Cherry and Emma Carroll), Wildsight Golden, and BC Ministry of Forests, Lands, Natural Resource Operations, and Rural Development (Ariana McKay and Irene Teske).

The following labs completed fecal analysis and offered much appreciated advice: Dr. Coltman's lab at the University of Alberta (Dr. David Coltman and Dr. Samuel Deakin), Wildlife Genetics International (Dr. David Paetkau), Toronto Zoo (Dr. Gabriela Mastromonaco and Paula Mackie) and WADDL at the Washington State University (Trevor Alexander and Dr. Laura Williams).

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