Patterns of helminth and protozoan parasite infections in bighorn sheep, Ovis canadensis: sex, season and activity

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Patterns of helminth and protozoan parasite infections in bighorn sheep, *Ovis canadensis*: sex, season and activity

by

Samridhi Rijal

A THESIS

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Abstract

Males and females in sexually dimorphic species show differences in their physiology and behavior. Each sex may invest energy in mating and reproduction, rather than immune function at different times of the year since they have different priorities, i.e., securing mating access vs increasing reproductive events. This means that the two sexes may be more susceptible/less tolerant to parasites at different times of the year. Since this is an observational study, I will be using fecal egg counts (FEC) in terms of eggs per 4 grams (EP4G) as a proxy for susceptibility/tolerance. Furthermore, when individuals have higher parasite FEC, they may need to spend more time grazing and ruminating to ensure enough energy to be able to invest in immune function. That is, higher FEC may lead to individuals spending more time grazing and ruminating as parasites might be siphoning energy and nutrients away from the hosts.

I used bighorn sheep, Ovis canadensis, and five of their natural parasites, strongyle, Nematodirus spp., Marshallagia spp., lungworm and Eimeria spp., to investigate seasonality of parasites and host-sex bias and whether higher parasite FEC leads to individuals spending more time grazing.

Generalised linear mixed models suggest that parasite FECs are different for male and female bighorn sheep between winter and non-winter seasons. However, the pattern of FECs between the sexes differ based on the parasite group. Strongyle FEC was significantly higher for both sexes during non-winter with male counts being higher than female counts in both seasons. Eimeria spp. count was higher in females compared to males compared in both seasons, but non-winter counts were higher than winter counts. In contrast, FECs for Marshallagia spp., and Nematodirus spp. was significantly higher in females in non-winter, but significantly higher for males in winter.
months. Following a similar pattern, lungworm larva counts were higher for males during winter, while female counts were lower in winter.

Additionally, linear mixed models suggest that bighorn sheep individuals with higher strongyle FEC tended to increase the percent of time that individuals spent grazing, while higher *Marshallagia* spp. FEC showed a trend in decreasing the percent of time that individuals spent grazing. However, FECs did not affect the time individuals spent ruminating or laying.
Preface

This thesis is an original, unpublished independent work by the author, Samridhi Rijal. Permission for this research was granted by Alberta Tourism, Parks and Recreation, Parks Division [Permit No.: 16-044 in 2016; 17-091 in 2017 and; 18-639 in 2018] and Alberta Environment and Parks, Policy and Planning Division, Fish and Wildlife Policy Branch [Research Permit #18-639].
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On a more serious note, I would also like to thank my committee members, Dr. Mary Reid and Dr. Susan Kutz, who have asked difficult questions and helped me develop a more scientific way of thinking and; my supervisor, Professor Kathreen Ruckstuhl, who has provided support and guidance throughout the entire process. I remember hot summer and freezing winter days sitting and observing sheep with Kathreen as she shared bits of her knowledge about bighorn sheep in general, and this population, in particular. I would also like to thank Dr. Peter Neuhaus for all the times that he, willingly or unwillingly, drove us out there and even helped me collect fecal samples when I was missing an assistant!

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<tr>
<td>Abomasum</td>
<td>The fourth division of a ruminants’ stomach where digestion takes place</td>
</tr>
<tr>
<td>Analytical sensitivity</td>
<td>The ability of a test to detect parasitic elements. It is usually expressed as the minimum detectable concentration of the solution.</td>
</tr>
<tr>
<td>Caecum</td>
<td>At the junction of the large and small intestine</td>
</tr>
<tr>
<td>Ectoparasite</td>
<td>Parasite that lives on the surface of the host</td>
</tr>
<tr>
<td>Endoparasite</td>
<td>Parasite that lives within the host</td>
</tr>
<tr>
<td>EP4G</td>
<td>Eggs per 4 grams</td>
</tr>
<tr>
<td>Epi-parasite</td>
<td>Parasite that feeds on another parasite</td>
</tr>
<tr>
<td>Facultative parasite</td>
<td>Organism that could be parasitic but is not dependent on a host to complete its life cycle.</td>
</tr>
<tr>
<td>FEC</td>
<td>Fecal egg count</td>
</tr>
<tr>
<td>Infection intensity</td>
<td>The number of parasitic elements (eggs, larva or oocyst) shed in the feces of the host</td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td>A complex molecule that consists of lipids and polysaccharides</td>
</tr>
<tr>
<td>Mitogen</td>
<td>Chemical substance that stimulates cell division</td>
</tr>
<tr>
<td>Obligate parasite</td>
<td>Parasite that cannot complete its lifecycle without exploiting its host</td>
</tr>
<tr>
<td>Parasite burden</td>
<td>The number of adult parasites found within the host.</td>
</tr>
<tr>
<td>Parasite abundance</td>
<td>FEC of a parasite, including individuals with zero counts. Mean parasite abundance (EP4G) refers to the mean number of eggs/larva/oocyst in the population sample, including zero counts.</td>
</tr>
<tr>
<td>Spp.</td>
<td>Species</td>
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<tr>
<td>Virulence</td>
<td>Severity of a disease, i.e the degree of damage that a pathogen can inflict on its host in terms of reduction in host fitness</td>
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Chapter 1. General introduction
Living organisms face numerous life history trade-offs, such as: 1) growth versus reproduction, 2) reproduction versus survival, 3) quality versus quantity of offspring, among others (as reviewed in Stearns 1989; Zera and Harshman 2001; Santos and Nakagawa 2012; Wells 2018). These trade-offs have one currency in common - energy. Energy affects the survival and Darwinian fitness of an organism.

1.1. Immune reaction
One such trade-off requiring energy is maintenance of body condition (health) versus reproduction (Norris and Evans 2000). For example, immune function of female burying beetles, *Nicrophorus vespilloides*, was challenged by wounding individuals with a sterile needle before and during breeding. If the wounding happened before breeding, there was no difference in the number of larvae produced between wounded and control females. However, when immune function was challenged during breeding, the number of larvae produced decreased (Reavey et al. 2014). Similarly, blood serum extracted from pregnant ewes (*Ovis dalli dalli*), showed a trend of decreased ability in killing bacteria (*Esherichia coli*) during gestation, compared to yeld ewes (Downs et al. 2018). While these studies seem to imply that immune function and reproduction are in competition over host resources, investing in immune function does not automatically lead to reduced reproductive capacity (Tieleman 2018). In this thesis, I first explore the potential association between a Rocky Mountain bighorn sheep’s (*Ovis canadensis*) sex and sampling seasons - winter and non-winter - and fecal egg/larval/oocyst counts (FEC) based on eggs per 4 grams (EP4G) of five different gastrointestinal parasite groups (Chapter 2). I, then, investigate the potential association between infection intensities and time invested in different activities (i.e., activity budgets as a proxy for energy gain and expenditure) (Chapter 3).
In principle, trade-offs between time budgets and FEC can be affected by other internal and external factors, such as an individual’s reproductive status, stress level, energy reserves, and season. In the following paragraphs, I will explore each by providing a few examples from primary literature.

As a baseline, future food availability and current energy reserves are important factors that determine an individual’s investment into immune defence and the time that an individual spends grazing (Houston et al. 2007). That is, when food is abundant, individuals can afford to invest energy into immune function regardless of their energy reserves, but when food is scarce and the individual does not have much energy reserves, then investment in immune function may be compromised. For example, a study investigated activity levels and the total amount of time that male song sparrow’s (*Melospiza melodia morphna*) invested in food consumption when injected with a bacterial lipopolysaccharide. In winter, body mass and territorial displays of injected males decreased within 24 hours of infection. However, during the breeding season, in spring, there was no change in their aggressive territorial behavior and body mass compared to the control groups (Owen-Ashley and Wingfield 2006). Stress can also compromise immune function. In Soay sheep (*Ovis aries*), females that experienced more environmental stress, such as a shortage of food, had a higher parasite infection intensity, while in males, environmental stress was not associated with parasite infection intensity (Hayward et al. 2009). These examples imply that food availability can change an individual’s investment into immune function depending on their mating and reproduction costs and priorities.
Individuals at different stages of their life history can also allocate resources differentially. When adult and juvenile male rodents, *Meriones crassus*, were infected with a natural ectoparasite, *Xenopsylla conformis*, juveniles grew slower than controls, but there was no difference in body mass between infected and control adults (Garrido et al. 2016). In the study, flea-infected juveniles spent twice as long feeding compared to uninfected juveniles; meanwhile, infected adults spent more than twice the time grooming and less time feeding, compared to uninfected adults. Additionally, similarly aged bighorn sheep ewes (*Ovis canadensis*) have also shown a difference in their FEC based on reproductive status. For example, females that successfully weaned offspring shed more lungworm larvae than females that either did not gestate, or whose lambs died prior to weaning (Festa-Bianchet 1989). Clearly, individuals at different ages and reproductive status have different demands in terms of resource allocation, with juveniles and non-pregnant individuals likely diverting some of the energy towards immune function, and reproductively active individuals investing energy into reproduction.

Another factor that can affect immunity is prior exposure to pathogens or antigens. Prior exposure and infection by a helminth can lead to immunity against subsequent infection attempts. This can be either due to the expression of certain interleukins that lead to strong immunoglobulin responses, which are able to kill the larval stages of the parasite, or because the primary infection changes the anatomy/physiology of the host such that it is more difficult for any subsequent larvae to establish itself within the host (MacDonald et al. 2002; Sorobetea et al. 2018). The latter is thought to be the reason for *Schistosoma mansoni* eggs being found dead in the lungs after they failed to establish in the liver (Wilson et al. 1983).
Finally, it is also important to keep in mind that there are a multitude of viruses, bacteria, fungi and other parasites that can impact individual hosts. While all these are important, my thesis will focus on internal parasites that affect bighorn sheep (*Ovis canadensis*) in my study population. For more details on the effects of these specific parasites, see subsection: 1.4. Effects of parasites on bighorn sheep.

1.2. What are parasites?
Parasites can be broadly categorised into two major groups – microparasites and macroparasites. Microparasites tend to multiply within the host, have short generation time and do not have a specific infective stage; while macroparasites tend to reproduce outside the definitive host and are transmitted when the parasites reach their infective stage (Hudson et al. 2002). As such, parasites may range from being invisible to the naked eye to more than 20 meters in length; e.g. beef tapeworm (*Taenia saginata*) (Schmid-Hempel 2010)! Although able to live outside the host for a period of their life cycle, parasites are defined by the fact that they live on or within the host, and depend on host resources for some part of their survival and/or reproduction, i.e., parasites are typically metabolically dependent on their host (Goater et al. 2014), with some exceptions in ectoparasites and some brood parasites (as reviewed in Payne 1977) (facultative parasites). As such, parasites can affect their hosts in a variety of ways, which can lead to the host showing signs of illness.

Parasites fall under three main categories:

1. Protozoa
2. Arthropods
3. Helminths
However, there are other categories of parasites as well, for example, a) Microsporida are parasitic fungi that produce spores, b) myxozoa are spore-forming parasites that are metazoan, c) Nematomorphs are obligate parasites while juveniles, but the adults are free-living (Goater et al. 2011).

The type of parasites can further be divided into three different lifestyles; endo-, ecto-, and epi-parasites, based on how they access resources, i.e., from inside the host, from being on the host or by feeding on another parasite, respectively.

1. Protozoan parasites are single-celled organisms that typically multiply within a host. Parasitic protozoa can cause serious infections in their host, including diarrhea (Kotloff et al. 2013). In some cases, only individuals with high infection intensity show signs, as is the case with Monarch butterfly (*Danaus plexippus*) larva infected with the protozoan parasite (*Ophryocystis elektroscirrh*): larvae with the highest infection intensity had smaller wingspans and were less active compared to larva with lower infection intensity (Altizer and Oberhauser 1999).

2. Arthropods that are parasitic tend to be ectoparasites. For example, fleas (*Ctenicephalides felis, Ctenicephalides canis, Pulex irritans, Echnidnophaga gallinacea*) that live on dogs (*Canis lupus*) (Gracia et al. 2008); sucking lice, which belong to the Order Anoplura and tend to be host specific and; ticks, which belong to the Order Ixodida and can feed on different host species (Hopla et al. 1994).

3. Helminths are parasitic worms and can be further categorized into three groups: trematodes (flukes), cestodes (tapeworms) and nematodes (roundworms). Helminths produce eggs within their host and excrete them with host feces (Castro
1996). The eggs pass through the larval stages, at least one of which is infective. If the host consumes the infective stage larvae, the parasite can establish itself within the host and develop into reproductive adults. However, if ingested prior to the infective stage, that larva cannot establish itself in the host.

Helminths can include gastrointestinal (GI) parasites that establish themselves along the digestive tract of the host (locations within the digestive tract are parasite species-specific) (Goater et al. 2014). Some species have specifically designed structures that allows the parasite to ‘hook’ themselves along the intestine of the host so that they can extract resources from the host by pumping food into their intestine (Goater et al. 2014), while others lack an alimentary tract, so digested food enters the worm via microvilli that resemble the lumen lining of mammal intestines (Castro 1996; Goater et al. 2014). Nematodes have a muscular esophagus that helps suck food into their intestine (Goater et al. 2014). Thus, GI helminth have especially adapted to exact resources from within the digestive tract of their hosts.

For the purposes of this thesis, I will be focusing on helminths, although, there will be some mention of a protozoan parasites as well. However, when I refer to ‘parasites’, I am referring to helminth parasites, unless otherwise specified.

1.3. What effects do parasites have on their hosts?
In this section, I will discuss some of the ways in which parasites can affect their hosts. Parasites divert resources from their hosts and, thus, are expected to have a negative impact on their hosts, though these impacts may not always be obvious (Henigst et al 1961; Goater et al. 2014). Sometimes the effects of parasites are easily noticeable, i.e., they can make their hosts sick by causing a parasitic infection (Goater et al. 2014).
However, it is in the parasite’s best interests not to immediately kill their host, since that would lead to its own demise. This can be complicated if the intermediate host of the parasite is trophically linked to the definitive host, but the parasite still needs to reach the infective stage within the intermediate host before being consumed (Lu et al., 2018). In that case, it is to the parasite’s benefit that the intermediate host is weakened or alters its behavior so that it can be consumed by the definitive host. In this case, the parasite can change the morphology and behavior of the intermediate host such that the infected intermediate host has a greater chance of being consumed by its definitive host (Lewis et al 2012). This is the case with the thorny-headed helminth, *Pomphorhynchus laevis*, that parasitizes and changes the drifting behavior of its intermediate host, an amphipod (*Gammarus pulex*), so that it is more likely to be ingested by its final host, the European bullhead fish (*Cottus gobio*) (Lagruè et al 2007). For a review on potential ways to counteract parasite manipulation of intermediate hosts, see Cezilly et al. (2010).

Parasites can also manipulate their hosts when the juveniles are parasitic, while the adults are free-living and not dependent on hosts. Crickets (*Nemobius sylvestris*) infected with the nematomorph parasite, *Paragordius tricuspisidatus*, behave erratically. This can take them close to a waterbody, which the host enters, therefore allowing the aquatic worm to emerge from within the host (Thomas et al. 2002). In most cases, the host cricket drowns before or after worm emergence.

Parasitic infections can cause a range of symptoms, depending on the type of parasite (Dantzer 2001). These range from flu-like symptoms that can last for more than a month, to diarrhea, vomiting and weight loss (Schmid-Hempel 2011). Even when
considering the same host-parasite system, infection symptoms can range from mild to severe. This difference in infection severity can be attributed to variance in parasite virulence, variance in host susceptibility and immune response within the population (Ebert et al. 2000; Carius et al. 2001), and the infection intensity which can change the cumulative parasite virulence (Altizer and Oberhauser 1999; Aleuy et al. 2019).

1.3.1. Factors that affect parasite infections in hosts
A multitude of factors can influence host exposure and susceptibility to parasites. Host susceptibility can be positively (because of previous exposure and acquired immunity) or negatively (due to the declining efficiency of the immune system and changes in the structures and functions of aging organs) influenced by age (i.e., Gardner 1980). Older *Daphnia magna*, for example, are less likely to become infected with the bacterial parasite, *Pasteuria ramose*, compared to younger individuals (Izhar and Ben-Ami 2015). This could be attributed to acquired immunity, where older individuals who have, previously, been exposed to the parasite can defend themselves against it (Goater et al. 2014). However, as an individual gets older, the efficiency of the innate immune system such as macrophage production and activity declines due to intracellular changes in signalling (Gardner 1980; Albright and Albright 1998; Plackett et al. 2004). Thus, there could be an intricate interplay between innate and acquired immunity in shaping an individuals’ immune function.

Season can also influence host and parasite behavior. Changes in weather patterns such as temperature and precipitation can lead to the appearance and disappearance of infective parasitic elements and also influence host behavior since certain seasons can cause crowding around resource rich areas and increase investment in energy-heavy behaviors such as pregnancy (Dowell 2001; reviewed by Barger 1993 and; Altizer et al.
2006). Along the same vein, either one of the sexes could be more susceptible to parasitic infections (Barger 1993; Poulin 1996a). Additionally, the body size of an individual can also have implications on susceptibility and tolerance. For example, same aged snails (*Biomphalaria glabrata*) that were larger had lower infection intensity of the trematode, *Schistosoma mansoni* (Niemann and Lewis 1990).

Diet can also influence host susceptibility. When Egyptian cotton leafworm, *Spodoptera littoralis*, larvae were immuno-challenged by introducing a needle dipped in lyophilised *Micrococcus lysodeikticus* bacteria, larvae were more likely to consume protein-rich food (Cotter et al. 2011). Previous parasite exposure, as mentioned earlier, can also influence host susceptibility. For example, rainbow trout, *Oncorhynchus mykiss*, developed resistance to the trematode, *Diplostomum spathaceum* over time, with naïve hosts showing higher infection intensity compared to hosts that had been continuously exposed to the parasite through the course of the experiment (Karvonen et al 2004).

However, the underlying cause of host susceptibility is the trade-off associated with different energy demands and the result of parasite-host-co-evolutionary arms race. There is a trade-off between immune function and other life history traits, such that when the host faces an additional cost, there is an increase in infection intensity (for example, food shortages and temperature variations over the annual cycle – Lloyd 1995; Hillgarth and Wingfield 1997 and reviewed in Dowell 2001 – and mating, reproduction and ageing – Lanciani 1975; Festa-Bianchet 1989; Richner et al. 1993; Cattadori et al. 2005; Marzl et al 2005).
In this thesis, I will be focusing on three variables that could serve as proxies for energy expenditure and intake: the effects of 1) season, 2) host-sex, and 3) activity budgets. I will be using faecal egg counts of parasites as a proxy measure of infection intensity, and thus, focus on how FEC is associated with season and host-sex (chapter 2), and how different levels of FEC may correlate with time budgets of individual bighorn sheep (chapter 3).

1.3.1.1 Season
To fully understand host-parasite relationships, it is imperative to identify periods of the year when hosts are most susceptible, and factors that could be associated with increased parasite infection intensity in different individuals. There are several factors that contribute to the seasonal variation in parasite prevalence and intensity within a population. Firstly, changes in host physiology could make the host more vulnerable to parasitic infections around specific periods. In wild house finches (Carpodacus mexicanus), prevalence of the bacterium (Mycoplasma gallisepticum) consistently declined during summer and increased during fall and winter, over a period of more than 70 months (Altizer et al. 2004). It could be because winter is more energy-demanding, since additional energy is needed for thermoregulation and maintenance of the internal metabolism, while at the same time there is a shortage of available food resources. Food scarcity and lowered ambient temperatures can increase concentrations of corticosteroids in individuals and thus, increase physiological stress which can compromise the immune function if corticoid concentrations remain consistently high for long durations (as reviewed in Nelson and Demas 1996). However, Nelson and Demas (1996) also hypothesized that hosts may have developed mechanisms to counter susceptibility, for example, by increasing the size of their lymphatic organs to fend off parasites in winter. Secondly, changes in host behavior,
such as crowding around watering holes or feeding areas during winter/dry season (i.e., Dowell 2001) could result in higher exposure to parasites, and thereby infections.

Thirdly, parasites need to have reached their infective stage during this period to establish in the host (Roberts and Grenfall 1992; Lass and Ebert 2006). At specific periods of the year, weather conditions may be ideal for parasite transmission, whereas in other periods, weather conditions might not be favorable for parasite or intermediate-host development (Muir 1988). This pattern would be highly parasite-specific though. Some parasite eggs can withstand freeze-thaw cycles (for example, *Nematodirus* spp. (van Dijk and Morgan 2008)), others can withstand extreme heat, such as eggs of the fluke, *Opisthorchis viverrini*, which can withstand being heated up to 70°C for more than 10 minutes (Boueroy et al. 2019). Yet, others are unable to develop at high temperatures (for example, eggs of the roundworm (*Baylisascaris procyonis*) cannot withstand temperatures above 63 degrees Celsius (Shafir et al. 2011)). At the other extreme, when *Marshallagia marshalli* eggs were subjected to 28 months of sub-zero temperature, they were still alive and hatched after warming. In fact, even their larvae were able to withstand exposure to -30°C (Carlsson et al. 2013)!

Finally, pregnancy, birth and lactation (in mammals) are energetically demanding periods for females and can also impact parasite infection intensity, or egg shedding, which aids in the spread of parasites within the population. Fecal egg counts of nematodes in Soay sheep (*Ovis aries*), for example, were higher in females during the lambing periods only (Gulland and Fox 1992). Similarly, Soay sheep ewes that gave birth and weaned an offspring had higher fecal egg counts, compared to ewes that did
not give birth or wean an offspring (Leivesley et al 2019; also reviewed in Altizer et al. 2006).

Thus, there are numerous factors that can impact host-parasite interactions even within a season. For more detailed information about effects of seasonality, see Chapter 2.

1.3.1.2. Host sex-bias
Male hosts often have a higher parasite burden compared to females (Poulin 1996b). Two reasons were put forward for this host-sex bias: 1) physiological differences (i.e. hormones) between the sexes can increase susceptibility of one sex to parasite infections (as reviewed in Klein (2000 and 2004). Parasites can also alter the production and metabolism of sex hormones to their benefit (as reviewed in vom Steeg and Klein 2016), and 2) behavioral differences that increase exposure of one sex to parasites more than the other. This was demonstrated when male white-footed mice (Peromyscus lecopus) infected with helminth parasite, Pterygodermatites peromysci, were treated with an anthelminth and led to a decline in the parasite burden of the entire population (Luong et al. 2009). In the same experiment, when only females were treated, parasite burden of the population did not change. This could indicate that males are sources of infection and females are sinks. In contrast, treating female common voles (Microtus arvalis) against parasites, lowered parasite burden in the population, while treating males simply lowered parasite burden amongst male members (Sanchez et al. 2011). These two studies show that the link between parasites and hosts can be complicated and that some parasites may have adapted to exploiting males, while others may have adapted to exploit females.
In sexually size dimorphic species, there are more explicit physiological and behavioral differences between the sexes. Chu and Lee (2012), for example, suggest that the bias in reproductive costs between males and females leads to a ‘surplus’ of energy for males, which allows them to invest in costly secondary sexual characteristics that increase their mating success. This is because in such systems, the highest-ranking males can sire a substantial number of offspring over their lifetime (as discussed by Vanpe et al. 2008). For example, one of the highest-ranking bighorn rams sired about 26% of the total number of offspring over a period of six years (Coltman et al. 2002).

Successful lambing and weaning in bighorn ewes, on the other hand, was age-dependent; ewes aged 4-9 years were more likely to lactate, and the lamb was more likely to survive if the mother was 4+ years of age, than lambs of younger mothers (Festa-Bianchet 1988). As such, the two sexes are under different pressures at different times of the year and may invest differentially into immune function.

Of course, it could also just be the effect of body size dimorphism alone. Male bighorn sheep, for example, seem to gain mass faster than females of the same age, such that, at the end of their fourth year, rams are about 50% heavier than ewes (Festa-Bianchet et al. 1996). This could imply that parasites would prefer male hosts, because they are a bigger resource to exploit, or because males may eat more. However, an argument against a size-driven effect is that some parasites tend to exploit female resources: fleas, *N. fodiens* and *S. araneus*, more commonly infect the smaller females hosts, in 10 different rodent species (Morand et al. 2004).

Furthermore, some parasites affect females and manipulate them to have a higher proportion of female offspring. For example, female amphipods (*Gammarus duebeni*),
infected with microsporidian parasites, *Microsporidium* sp. A, and *Nosema granulosis* (Ironside et al. 2003), produced broods with only 10% males, while uninfected females produced broods with 60% male offspring. Ironside and Alexander (2015), too, showed that *Gammarus duebeni* infected by another microsporidium (*Dictyocoela duebenum*) produced similarly female-biased broods.

These examples show that multiple factors are at play, which all affect parasite host-sex biases. For more details and the interplay between host-sex and season, please refer to Chapter 2.

### 1.3.1.3. Time Budget
As mentioned in the sections above, differential energy allocation by the host may affect immune function. As Chu and Lee (2012) suggested, males and females may have different priorities and allocate resources to mating or reproduction. However, resource allocation is an important factor of immune function. This is because as an individual is developing, there are several trade-offs that affect energy balances: 1) investment into growth versus immune function (poultry selected for growth had compromised immune function, but poultry selected for immune function did not always have slower growth (van der Most et al. 2011)); 2) body maintenance versus immune function, and; 3) reproduction versus immune function. Tree swallows, *Tachycineta bicolor*, in Alaska have a lower survival rate, compared to those in Tennessee. When brood size of the birds was experimentally increased, females in Alaska increased their feeding visits to ensure maintenance of offspring quality even at the cost of their own immune function, while females in Tennessee maintained their own immune function at the cost of offspring quality (Ardia 2005).
Thus, individuals seem to balance their investment into immune function and reproduction. Since energy intake is partially within the host’s control, the host could increase the time spent grazing to increase energy intake and reallocation (Coop and Holmes 1996). Alternatively, they could also decrease their energy expenditure by reducing time spent engaged in energy-demanding activities.

These examples illustrate that there is a range of effects that energy intake and expenditure can have on mating and reproductive success, and that the host response is not always predictable. For more details on time budget and parasite infection intensity, please refer to Chapter 3.

Overall, the research described in my thesis is observational and non-invasive. This was possible because the bighorns in Sheep River Provincial Park in Alberta are used to human observers. This population has been studied for over three decades. Thus, it was assumed that our presence would have little to no effect on the behavior or ecology of this species. In fact, over the course of the research data collection period, individuals got used to our presence and frequently came as close as 5-10 meters from us while grazing. Because of the non-invasiveness of the study, I am using several variables and proxies to investigate what factors could affect an individual’s time budget, and thus, energy intake and expenditure.

In the next section, I will describe the specific parasites found in bighorn sheep and their effects on their hosts.
1.4. Which parasites are found in bighorn sheep and what effects do they have on their host?

Parasites in bighorn sheep have been categorised according to their egg morphology in this thesis, such that there are seven common parasite groups in bighorn sheep populations in Alberta. Broadly, these are strongyle (which may include multiple Ostertagia spp. and Teladorsagia spp.), Trichuris trichuris, Nematodirus spp., Marshallgia marshalli, Eimeria spp., Moniezia spp., and Protostrongyles spp. (lungworm larvae). Of the seven, Nematodirus spp., Trichuris trichuris, Marshallgia marshalli, strongyle and Eimeria spp. have a direct life cycle, whereas Moniezia spp. and lungworm larvae require an intermediate host, in the form of various species of mites (Goater et al. 2014) and gastropods (Robb and Samuel 1990), respectively. Strongyle, Trichuris trichuris, Nematodirus spp., Marshallgia marshalli and lungworm larvae are nematodes, Moniezia spp. is a cestode, and Eimeria spp. is a protozoan parasite (Kutz et al. 2012).

The full effects of these diverse parasites on bighorn sheep are still not fully understood. When specific research on bighorn sheep is not available, I will use research conducted on other ungulates, assuming similar impacts on bighorn sheep. Helminth parasites can have a range of effects on their hosts. These effects range from lowering the rate of pregnancy (Kutz et al., 2012; Aleuy et al. 2019), increasing mortality (Kutz et al. 2012) and changing the body condition of their hosts (Aleuy et al. 2019).

1.4.1. Marshallgia spp.

Marshallgia spp. belong to the subfamily Ostertagiinae and are an abomasal parasite. They are found in ungulates across the Holarctic region. As mentioned earlier, Marshallgia marshalli eggs are resistant to extreme cold weather conditions, i.e., even
Because it is more tolerant to cold conditions, *M. marshalli* egg production is higher during the colder, winter months (Irvine et al. 2000) compared to egg production of other parasites that are not cold tolerant (*Trichostrongylus colubriformis* - Wharton et al. 1984). Furthermore, the infective stage larva is most abundant during summer (Table 1) and so, *M. marshalli* is transmitted during summer.

In Dall’s sheep, there was a negative correlation between the infection intensity of *Marshallagia marshalli* and adult ewe body condition and pregnancy status (Aleuy et al. 2019). This suggests that ewes in better condition can better resist infection, or cope with it. However, in wild Svalbard reindeer (*Rangifer tarandus platyrhynchus*), treatment against *M. marshalli* did not have a significant effect on overwinter differences in body mass, which suggests that *M. marshalli* did not affect body condition of the host (Carlsson et al. 2018). Research on female reindeer from west Greenland also found that there was no association between *M. marshalli* infection intensity and pregnancy, although there was a negative correlation between intensity and indicators of body condition (Steele 2013). In the case of bighorn sheep, it is possible that since the host species is more closely related to Dall’s sheep, we would see a similar pattern of a negative association between body condition and pregnancy status, and infection intensity.

1.4.2. *Nematodirus* spp.

*Nematodirus* spp. belongs to the Subfamily Nematodirinae. *Nematodirus* spp. are found in the small intestine. *Nematodirus* spp. eggs are also resistant to extreme cold temperatures, and freeze-thaw cycles (van Dijk and Morgan 2008) (Table 1). The parasite develops into its infective stage larvae within the egg membrane (Kutz et al.
Since the eggs are resistant to extreme cold temperatures, they could produce eggs throughout winter so larval development can take place in spring and summer when the ideal environmental conditions are met. Different parasite species have their own range of temperatures in which they hatch, for example, *N. battus* requires temperatures ranging from 11 to 17°C, while *N. filicollis* has a slightly larger range of 6 to 20°C (van Dijk and Morgan 2009; Table 1). It is hypothesized that these parasite eggs require a freeze-thaw cycle (i.e. winter) to enable hatching since the majority of the infective stage larvae are found in spring, rather than during summer or fall (van Dijk and Morgan 2009).

In Norway, poor body condition of captive muskoxen was hypothesized to be due to the high intensities of *Nematodirus* spp. along with other parasites (Alendal and Helle 1983 as cited by Kutz et al. 2012). There was also a suggestion that Dall’s sheep ewes who were in better condition and who were pregnant shed fewer *Nematodirus* spp. eggs (Simmons et al. 2001 as cited by Kutz et al. 2012). *Nematodirus* species are expected to have a similar effect on bighorn sheep. However, Aleuy et al. (2018) did not find an association between *Nematodirus* spp. infection intensity and body condition or pregnancy status.

1.4.3. *Trichuris* spp.
*Trichuris* spp. belong to the subfamily Trichurinae. They tend to occupy the large intestine and caecum of their ruminant hosts. The first larval stage is the infective stage and remains in the egg until ingested by the host. The egg is resistant to desiccation (Kutz et al. 2012). Individuals infected with *Trichuris trichurus* are younger animals (Aleuy et al. 2018) and tend to experience diarrhea (Seidel and Rowell 1996), whereas older animals that have had the parasite in the past, may have acquired immunity
against it (Kutz et al. 2012). Additionally, there was a negative correlation between *Trichuris* spp. infection intensity and host body condition in terms of subcutaneous fat reserves (Aleuy et al. 2018)

1.4.4. *Protostrongylus* spp.
Lungworms, i.e., *Protostrongylus* spp., are a common parasite of bighorn sheep (Forrester and Senger 1964), and their larvae can be transmitted fecal-oral, although they do require an intermediate host in order to do so, or transplacentally (Hibler et al. 1972). They are also the most widely studied bighorn sheep parasites. Bighorn sheep with higher lungworm larva infection intensity were, previously, thought to be more susceptible to pneumonia, but this effect may be indirect (Festa-Bianchet 1991). Lungworm larvae can cause lesions in the lungs (Forrester and Senger 1964) of infected individuals. Similar lesions in the lungs of Dall’s sheep have also been attributed to *P. stilesi* (Kutz et al., 2012). Pregnant and lactating ewes have also been observed to have a higher fecal output of lungworm larvae, compared to yeld ewes (Pelletier et al. 2005). Additionally, rams that spent more time searching for estrous females during the rut also shed more lungworm larvae in their feces (Pelletier et al. 2005).

1.4.5. Strongyle
There are a few different genera of parasites that get grouped under ‘strongyle’. The most common genera found in bighorn sheep are *Ostertagia* spp. and *Teladorsagia* spp.. This group is based on the egg morphology. They belong to the subfamily Trichostrongylinae and are found in the small intestine of their hosts (Table 1). These parasites can also decrease host body condition (Kutz et al. 2012).
The only cestode of the group, *Moniezia* spp. has an indirect life cycle. It belongs to the subfamily Anoplocephalinae and occupies the small intestine of its ruminant host (Goater et al. 2014). It relies on its intermediate host, oribatid mites, for its development and transmission to its final host (Daubney 1932; Denegri et al. 1998), one of which is the bighorn sheep. One of the interesting facts about *Moniezia* species is that it requires relatively warm temperatures of 18-20°C to be sustained for 28 to 97 days to develop into cysticercoids within their intermediate host (Narsapur and Prokopiec 1979). This also implies that transmission to bighorn sheep will take place in autumn, when higher temperatures have been maintained over summer, allowing the larva to develop. Sheep and other ruminants acquire the parasite when they ingest infected mites while grazing during autumn.

*Moniezia* species have been associated with emaciation among reindeer calves in Russia (Jokelainen et al. 2019). Similarly, heavy infections of *Moniezia* spp. caused the death of translocated muskoxen calves from Canada to Iceland (Samuel and Gray, 1974). Given the impact on other hosts (Samuel and Gray 1974; Jokelainen et al. 2019), in bighorn sheep, heavy infections might lead to declining body condition of individuals. To my knowledge, no bighorn sheep death has been directly linked to *Moniezia* spp. infection.

1.4.7. *Eimeria* spp.
The only protozoan parasite found in bighorn sheep is the *Eimeria* spp. *Eimeria* spp. is an intercellular parasite and has a direct life cycle (Kutz et al. 2012). Three species have been identified in bighorn sheep and these are: *Eimeria granulosa*, *Eimeria ovina* and *Eimeria faurei* (Duszynski et al. 1977). At least a few species of *Eimeria* have
shown to be tolerant to -20°C for a few months, even though, in general, most species are unable to sporulate after being stored at -7°C for several months (Rind and Brohi, 2001) (Table 1). In reindeer, *Eimeria* spp., are present in their hosts throughout the year, but only extremely high oocyst counts (up to 800,000 EPG!) lead to clinical disease symptoms (Oksanen et al. 1990), which might be related to severe diarrhoea. This suggests that *Eimeria* spp. could lead to diarrhoea in bighorn sheep, if oocyst count is extremely high, i.e., >800,000 EP4G. However, research is limited, and results indicate that different hosts have different patterns of parasite abundances and infections, so we have to take this into consideration when making predictions.

While I had some samples with *Trichuris* spp. and *Moniezia* spp., counts were too low, and hugely zero-inflated, which meant that the distribution and assumptions for diverse statistical tests were not met. *Moniezia* spp. have a segmented body that is made up of reproductive tracts, i.e., proglottids. Each mature proglottid is full of eggs and tends to be passed out in host feces (Taylor et al. 2007). During this process, the mature proglottid could break up and release the eggs or could be contained within a single pellet, thus leading to high variance in egg counts. *Moniezia* spp. eggs were mostly found in female feces; however, the lack of sufficient data warranted removing *Moniezia* and *Trichuris* from analyses from Chapters 2 and 3.

In the next section, I will describe mating and reproductive costs in bighorn sheep as well as the hypothesis and predictions I postulate in this thesis.
Table 1. Environmental requirements, type of life cycle and the pre-patency period of the five parasites that this thesis will investigate, the seasonality, and host-sex bias. Infection intensity (FEC) and adult parasite are linked in that the studies showed that the infection intensity was correlated to the number of adult parasites listed below.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>FEC</th>
<th>Parasite burden</th>
<th>Environmental requirements</th>
<th>Direct vs indirect</th>
<th>Pre-patency period</th>
<th>Lifespan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongyle</td>
<td>NA</td>
<td>NA</td>
<td>Hatching occurs in summer (~18 - 25°C); reach infective stage within 2 weeks; can enter arrested development stage; cannot survive in extreme cold temperatures (below, i.e., -30°C)⁴,⁸</td>
<td>Direct</td>
<td>28 days⁴</td>
<td>~ 2 months⁶</td>
</tr>
<tr>
<td><em>Nematodirus</em> spp.</td>
<td>Up to 200 EPG²</td>
<td>3,500 – 6,300²</td>
<td>Maximum hatching occurred only after chilling to 4°C; Developed into infective L3 when temperatures ranged from 6-25°C; Hatching occurred at temperatures 11-13°C¹</td>
<td>Direct</td>
<td>28 days²</td>
<td>4 to 5 months²</td>
</tr>
<tr>
<td><em>Marshallagia</em> spp.</td>
<td>28 – 36 EPG³</td>
<td>1,600-2,000³</td>
<td>Eggs are resistant to extremely cold temperatures of ~20°C; Develop into</td>
<td>Direct</td>
<td>28 days⁴</td>
<td>?</td>
</tr>
<tr>
<td><strong>Protostrongylus spp.</strong></td>
<td><strong>Eimeria spp.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>-------------------------</td>
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<td></td>
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</tr>
<tr>
<td><strong>Lungworm</strong></td>
<td><strong>Indirect</strong></td>
<td>~100 days$^5$</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Protostrongylus spp.)</td>
<td>Eggs are shed in lungs, develop into L1, coughed up, swallowed and shed in feces; gastropods are intermediate hosts; develops into infective L3 which is ingested by definitive host$^5$; transplacental transmission does occur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Eimeria spp.</strong></td>
<td><strong>Direct</strong></td>
<td>~ 2 week$^7$</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Different species resistant to different range in temperatures between -30°C to +60°C$^7$; penetrate small intestine cells for two asexual multiplication cycles$^7$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$ van Dijk and Morgan 2009; $^2$ Kauzal 1937; $^3$ Morgan et al. 2005; $^4$ Hoar 2012; $^5$ Monson and Post 1972; $^6$ Armour, Jarret and Jennings 1966; $^7$ Foreyt 1990 as cited by Chartier and Paraud 2012; $^8$ Rossanigo and Gruner 1995; NA = Not available; ? = unable to find articles investigating or stating the life span of an individual worm.
1.5. Bighorn sheep (*Ovis canadensis*)
1.5.1. General Introduction
Bighorn sheep are ruminants. They are named for the large, curled horns that the males (rams) have. These horns are used to determine the social hierarchy of the males within the herd. Rocky mountain bighorn sheep are found in the mountains of Western Canada and the United States. They, generally, have a winter and summer range, with the winter range being at a lower elevation (Geist 1971). However, in this study population, females migrate between the summer and winter ranges, but the males are more sedentary and have the same summer and winter range (Ruckstuhl 1998). Some males may migrate during rut to find estrous females outside of the study site.

Before the start of the mating season, or ‘rut’, males will establish their dominance hierarchy by engaging in activities such as horn clashes. Larger, more dominant males are higher on the dominance hierarchy, while younger rams are less dominant and lower on the hierarchy. When a female is in estrus, the dominant ram will employ the ‘tending’ mating strategy, whereby he will defend the estrus female so that he may be the only one to mate with her (Hogg 1984). Less dominant males may challenge the tending ram by approaching the tended ewe, i.e., coursing (Hogg 1984). In general, females prefer to mate with a tending ram, rather than a coursing ram (Hogg 1984). For this population, the ‘rut’ takes place for approximately a month starting mid to late-November. Consequently, birthing happens from late May to mid-June. In the next section, I will discuss the mating and reproductive costs in rams and ewes, respectively.
1.5.2. Mating and reproductive costs in bighorns

Male and female bighorn sheep have different life-history trajectories because they are a sexually dimorphic species. Males invest in faster growth and larger bodies, compared to females such that the male rate of mass gain was 10% and then 16.7% higher than the female rate of mass gain at lamb and yearling age, respectively (Festa-Bianchet et al. 1996). This is probably because larger, more dominant rams monopolize mating by using the ‘tending’ strategy and thus, guarding the females against mating with other males (Hogg 1984; Pelletier and Festa-Bianchet 2006). Dominance is decided in fights and seems to depend on body size and secondary sexually selected traits, such as a large antlers or horns in ruminants (Clutton-Brock et al. 1980). These traits also seem to be selected for by females (assuming they are honest signals of male quality), and thus are under intra- and intersexual selection. During pre-rut, bighorn rams fight and establish their social hierarchy, which is largely based on their body and horn size, such that larger males with larger horns are more dominant, and able to access mating with estrous females (Geist 1971; Hogg 1987).

Consequently, body- and horn-growth in their early years (lamb to four years of age), are hypothesized to be important determinants of their fitness (two to six years of age) in male bighorn sheep. However, a shortage of available resources during ontogeny, showed that when challenged, two- to four-year-old rams invested in body maintenance, rather than in horn growth (Festa-Bianchet et al. 2004). On the other hand, the same study also showed that older rams (those five to nine years of age, and thus close to or full- grown adults) continued to invest resources into horn growth. The authors attributed this difference between the two age-classes to younger rams investing in survival, rather than potential future mating success. In late November to late December, during rut, heavier males can afford to spend more time searching for
and trying to secure mating with females since the heavier males have more fat stores than younger, lighter rams (Pelletier et al. 2009). As a result, males that invest in increasing their fat reserves before rut could benefit from being able to spend more time engaged in mating-related activities, rather than foraging (Pelletier et al. 2005). In addition, the rut is also a risky time for males in terms of parasite infection intensities. For example, fecal output of lungworm larvae (*Protostrongylus* spp.) in rams increased with the amount of time that they spent searching for estrous females, but there was no association between infection intensities and the mating tactic they used (Pelletier et al. 2005).

At the same time, in iteroparous species like bighorn sheep, females are expected to develop traits that increase lifetime reproductive success, rather than just a single-year mating success. Additionally, Festa-Bianchet et al. (1988) suggest that bighorn ewes need to have a minimum fat reserves before estrus, even though they continue to take in resources during gestation. This could be because winter conditions can be unpredictable and higher quality food sources are not available, so individuals try to gain mass during summer to ensure successful gestation through pregnancy (Martin and Festa-Bianchet 2010). Females also prioritize their own survival and future reproductive success, over the offspring’s survival and may limit milk production or access, when running out of fat reserves (Martin and Festa-Bianchet 2010).

1.5.3. Thesis Objectives
As mentioned above, understanding different components that influence host-parasite relationships are vital for our understanding of the ecological role of parasites and hosts. As such, the two main objectives of this thesis are to: 1) determine if there is a seasonal host-sex bias in parasite FEC in bighorn sheep (Chapter 2) and 2) determine if
there is an association between fecal egg counts of five parasites found in bighorn sheep and the amount of time that the infected individuals engage in grazing, laying, and ruminating activities (Chapter 3).

Studies have shown that females with offspring have a higher lungworm larva output, compared to females who did not gestate, or whose offspring died shortly after birth (Festa-Bianchet 1989). Males during the mating season also tend to have higher counts of lungworm larvae in their feces (Pelletier et al. 2005). Additionally, parasite-host-sex-biases have been documented for other species, with higher infection intensity in males or females, depending on the parasite and host species under question. The parasite FECs of this bighorn sheep population were recorded over multiple years (May 2016 – November 2018). This dataset, thus, allows me to more fully investigate the effects of host-sex bias and seasonality.

In this thesis, I used generalized linear mixed models to determine the association between host sex, season and FECs, while using linear mixed models to determine if the percent of time spent engaged in the three main activities (grazing, laying or ruminating) was associated with FECs.

Given the above review of the literature, for Chapter 2, I predicted that: a) male FEC for *Nematodirus* spp., *Marshallagia* spp. and lungworm larva will be higher in winter due to host biology (costs associated with pre-rut and rut); b) female FEC for *Nematodirus* spp., *Marshallagia* spp., lungworm larva, strongyles and *Eimeria* spp. will be higher in non-winter due to heavy energy expenditure of the host during late gestation, birthing and lactation and finally; c) strongyle and *Eimeria* spp. FEC will be higher for males in
non-winter compared to males in winter due to parasite biology (i.e., strongyle eggs and *Eimeria* oocysts cannot survive extreme winter temperatures (Table 1)).

In Chapter 3, I predicted that: a) hosts with higher FEC will spend more time grazing or ruminating, since parasites may be siphoning nutrients and energy from the host for their own reproduction. Hosts, thus, may need to increase their own energy intake by increasing foraging and ruminating and; b) hosts with higher FEC may spend more time laying to minimize energy expenditure.
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Wilson R A, Coulson P S, McHugh S M. 1983. A significant part of the ‘concomitant immunity’ of mice to Schistosoma mansoni is the consequence of a leaky hepatic portal system, not immune killing. Parasite Immunol. 5: 595-601
Chapter 2. Patterns of gastrointestinal parasite infections in bighorn sheep, *Ovis canadensis*, with respect to host sex bias and seasonality

**Introduction**

Traditional disease transmission models assume that contacts between conspecific members are random. However, parasite intensity follows the 80/20 rule, where about 20% of the population is responsible for 80% of the infection intensity (Woolhouse et al. 1996; Poulin 1996a). A meta-analysis also revealed a male bias in the transmission of parasites within populations (Poulin 1996b). These papers show that disease transmission may follow a pattern, rather than being random.

Individual hosts differ in the number of eggs /larvae /oocysts shed in the feces, i.e., the fecal egg count (FEC). Moving forward, I will refer to FEC as parasite infection abundance. For the purpose of this chapter, it is assumed that infection abundance correlates with parasite burden, as true intensity can otherwise only be measured by cutting open the affected areas of the body. This assumption is based on numerous studies that have found a strong link between infection intensities and parasite burden (strongyle, *Haemonchus contortus* and *Nematodirus* spp. in sheep – Kingsbury 1965; strongyle in domestic sheep in New Zealand – McKenna 1981; *Trichostrongylus tenuis* (strongyle) in red grouse, *Lagopus lagopus scoticus* – Seivwright et al. 2004; strongyle in African buffalo, *Syncerus caffer* – Budischak et al. 2015; however, studies have also found poor or no link between FEC and parasite burden, for example, in coyotes infected with nematodes, *Toxascaris leonina*, *Ucinaria stenocephala* and *Ancylostoma caninum*, and cestodes, *Taenia* spp. - Liccioli et al. 2012). Some individuals within the same population may shed few parasite eggs per gram (EPG) and are said to have a light infection intensity, while some individuals shed 1000s to 10,000s EPG, and are
said to have a heavy infection intensity (Salih et al. 1979; McKenna 1981; Altizer and Oberhauser 1999). Even within the same individual, there are differences in infection intensity over a one-year period. For example, parasite infection intensity of three taxa of gastrointestinal helminths, *Fasciola hepatica*, *Elaphostrongylus cervi* and strongyles, were investigated in individually marked wild red deer, *Cervus elaphus*, over 12 months (Albery et al. 2018). Infection intensity of all three helminths were highest in spring and summer, and lowest during winter. Similarly, infection intensity of *Eimeria* spp. and helminth strongyles and *Strongyloides* in springbok (*Antidorcas marsupialis*) in Namibia were highest in wetter seasons compared to drier seasons (Cizauskas et al. 2015). When four common fish in Northern Wadden Sea were investigated for parasites, the authors found 16 different macroparasites, including several species of trematodes and nematodes. Trematode intensity in these fish were highest in spring and summer, and lowest in autumn and winter; in contrast, nematode intensity was highest in winter and lowest in autumn (Schade et al. 2016).

Such variation in parasite infection intensity is common and has been attributed to: i) changing climactic conditions (as reviewed in Nelson and Demas 1996; Barber et al. 2016), ii) biology of the parasite (i.e., different adaptations of blood parasites leads to their thriving in different areas – Merila et al. 1995) and/or iii) biology of the host (Trichostrongyloidea prevalence and infection intensity increased in adult female springbok, *Antidorcas marsupialis*, only after birthing and during lactation – Turner et al. 2012). This cyclic prevalence of parasites in host populations is common (Altizer et al. 2006; Martinez-Bekker and Helm 2015) and can be attributed to external environmental factors, such as temperature and precipitation (i.e.; investigation of hunter-killed bighorn rams (*Ovis canadensis*) in the fall, suggested a positive
correlation between the amount of rainfall and the intensity of infection with lungworm larvae, *Protostrongylus stilesi* and *P. rushi* – Forrester and Littell 1976), or intrinsic factors, such as host immunity (as reviewed in: Nelson 2004), and sex (Poulin 1996b).

Parasite intensity and abundance can be influenced by host behavior and physiology which can vary over the seasons. Parasite intensity and abundance could be higher in winter (or the dry/wet season), because winter is more energy-demanding, and animals typically allocate more resources into thermoregulation, at a time when food availability or quality decreases (review by Nelson and Demas 1996). Since energy demands are high, individuals may reallocate resources from immune function to other functions, which could increase host susceptibility/response to parasitic infections. Additionally, Moyers et al. (2018) demonstrated that transmission of the bacteria, *Mycoplasma gallisepticum*, between house finches, *Harmorhous mexicanus*, at feeders was correlated with the density of visiting birds. This is likely because as the density of host species increases, encounters between individuals increase, as does the probability of infected individuals encountering naïve hosts. Since crowding around food resources are more likely to take place during winter, increased infection abundance during winter may be due to increased exposure to infected hosts and their parasites (i.e. via fecal contamination).

While naivety of hosts, age or grouping have an impact on the overall seasonality of parasite infection intensities, the sex of the individual can also be an important variable. Meta-analysis shows that male hosts generally have a higher parasite prevalence compared to female of the same species (Poulin 1996b). This has been attributed to either behavioral or physiological differences. For example, female arctic fox (*Vulpes
lagopus) prefer to eat birds, while males eat mostly rodents. Males also have a higher number of cestodes, Taenia spp. and Echinococcus multilocularis, that use rodents as an intermediate host; there was no sex-based difference in the number of directly transmitted nematodes, Ascarids, and Spirocerca lupi (Friesen et al. 2015). Bachelor Grant’s gazelle (Nanger granti) had lower helminth parasite infection intensity and were less territorial than reproductively active males (Ezenwa and Snider 2016). Furthermore, three rodent species (Apodemus agrarius, Apodemus flavicollis and Myodes glareolus) displayed a distinct male-bias in the spread of 11 species of fleas in Poland (Kowalski et al. 2015). The authors hypothesized that this male-bias in flea infestation could be attributed to the larger territory of males, which increased the likelihood of males encountering parasites. Similarly, female-biased mite-parasitism (Spinturnix spp.) in five European species of bats has been attributed to crowding in maternity roosts, compared to the solitary roosts of non-breeding female and male bats (Christe et al. 2007). These examples illustrate how difference in behavior can change exposure, which could lead to increased infection in the population.

On the other hand, physiological differences between the sexes, especially in sexually dimorphic species could also play a major role in parasite infection (Klein 2004; Zuk and McKean 1996). It has, for example, been proposed that sex hormones, such as testosterone can have immunosuppressive effects: male barn swallows (Hirundo rustica) injected with testosterone had a higher abundance of their natural ectoparasites, compared to control males (Saino et al. 1995). Similarly, testosterone-treated male red grouse (Lagopus lagopus scotica) had a higher infection intensity of the nematode, Trichostrongylus tenuis, compared to control males (Mougeot et al. 2006). Along the same vein, females and castrated male lab mice (C57BL/10, BIO.A,
BIO.A(3R), BIO.A(4R) and B10.D2 strains) were more resistant to *Plasmodium chabaudi* malaria infection, while control males, testosterone-treated females and testosterone-treated castrated males had a higher infection intensity (Wunderlich et al. 1991). This could be due to the immunosuppressive effects of the male hormone, testosterone, or immunoenhancing effects of the female hormones, discussed below.

Oestrogen and progesterone can have immunoenhancing effects. When individual mice were treated with progesterone, they were able to better resist the establishment and growth of *Taenia crassiceps* parasite larva compared to controls (Vargas-Villavicencio et al. 2006). It is thought that the process of converting progesterone to estradiol plays a central role in preventing the establishment of the *T. crassiceps* larva in the host (Vargas-Villavicencio et al. 2005). On the other hand, some parasites have specifically adapted to exploiting females. For example, microsporidian parasites, *Microsporidium* spp. A, *Nosema granulosis*, and *Dictyocoela duebenum*, primarily infect females and lead to the infected female amphipod, *Gammarus duebeni*, producing a predominantly female brood (Ironside et al., 2003; Ironside and Alexander 2015).

As discussed so far, season and host-sex related behavior and physiology influence parasite-host relationships. As a result, the interplay between behavior, sex-hormones and season could lead to a difference in host exposure and susceptibility to parasites with males suffering an overall higher infection intensity. However, although it has been accepted that host-sex does play a role in parasite prevalence and transmission (males are responsible for parasite transmission in white-footed mice – Luong et al. 2009; females are responsible for parasite transmission in common voles – Sanchez et al. 2011), few studies have investigated the relationship between host-sex and seasonal
variation in parasite infection intensity in marked individuals over a longer period of time.

Seasonality in reproduction is thought to be one of the factors that contribute to the seasonality in parasite infection intensity (Altizer et al. 2006). For example, males invest in short-term mating success by diverting energy resources from immune function to develop secondary sexual traits before mating (bighorn sheep horn development – Geist 1966; Clutton-Brock et al. 1980). Iteroparous females investing in reproduction would, on the other hand, predominantly divert resources from immune function into reproduction during gestation, birthing and lactation, not throughout the entire year (Trichostrongylus retortaeformis in rabbits - Cattadori et al. 2005).

Most studies of parasites in large mammals have been limited to studying a specific period of life history and the associated level of parasite infection intensity of one parasite taxa. This is because host-specific factors linked to life history trade-offs can dictate seasonality of parasite loads or shedding in a population (Stearns 1992). It is generally agreed that there is a trade-off between reproductive effort and immune function. During gestation and lactation, females shed more parasite eggs, compared to males (as discussed in: Nelson and Demas 1996 and for example, bighorn sheep – Festa-Bianchet 1989 and Pelletier et al. 2005; springbok (Antidorcas marsupialis) – Turner et al., 2012; spotted hyena (Crocuta crocuta) - East et al., 2015). Similarly, in males, there is trade-off between immune function and investment into mating success in the form of sexually selected traits or territory. Festa-Bianchet et al. (2004), for example, discussed the possibility that, in times of food shortage, older rams might continue investing into horn growth (which translates into mating success), while
younger rams might invest in body maintenance, at the cost of current mating success. Additionally, in sexually dimorphic species in which estrous female mate with the largest male, the male generally guards these females. Guarding is an energy-expensive activity, especially since food consumption during guarding is compromised (isopod crustacean, Lirceus fontinalis – Sparkes et al. 1996; long-tailed macaques, Macaca fascicularis – Girard-Buttoz et al. 2014; baboons, Papio cynocephalus – Alberts, Altmann and Wilson 1996; whiptail lizard, Aspodoscelis costata – Ancona et al. 2010). This indicates that investment into immunity can increase or be impaired at different times of the year for males and females of the same species.

Here, I investigate the pattern of parasite abundance of five parasites in male and female bighorn sheep. I compare the parasite abundance of bighorn sheep throughout the year (except December, due to rut) and over multiple years (a total of 29 months). I hypothesize that the parasite abundance differs between seasons, due to differences in parasite and host biology with non-winter counts being significantly higher for strongyles and Eimeria spp. (Figure 1:A) in both sexes. I also hypothesize that the pattern of parasite abundance is different between males and females depending on the energy demands that the hosts face. In winter, I predict that the parasite abundance for lungworm larva, Nematodirus spp. and Marshallagia spp. is higher for males, compared to females, due to costs related to pre-rut and rut; while in non-winter, I predict that infection intensity is higher for females, due to costs associated with birthing and lactation (Figure 1:B).
Figure 1. Prediction for strongyle and Eimeria spp. infection intensity (A) and lungworm larva, Nematodirus spp., and Marshallagia spp. (B) infection intensity during non-winter and winter seasons.

Methodology

Study area
The study was conducted in Sheep River Provincial Park, in southwestern Alberta, Canada (50.63° N, 114.38° W). Annual temperature in the park ranges from −8.4°C in winter to 15°C in summer (Sheep River and Bluerock management plan 2008). However, the weather can range from extreme cold (below -30°C), to extreme hot (up to 37°C), as was the range during the fecal sample collection period of this study.

The study population at the time of the study was approximately 35 resident rams (>2 years of age) and more than 80 ewes. Lambs are caught a few months after birth (September to November) and are individually marked with colored and numbered ear tags (Festa-Bianchet 1988a). Thus, more than 90% of the population have been individually marked, which allows us to identify them over their lifespan. I collected fecal samples from identified male and female bighorn sheep within the park from May 2016 to November 2018 for a total of 29 months. This is because for a total of six
months (August 2016, August and September 2017 and February, March and April 2018), I was either unable to find sheep within the park or unable to collect samples due to high concentrations of smoke in the study area. I followed individual male and female bighorn sheep within the park and collected fecal samples at least twice a month. I tried to collect samples from the same individuals every two weeks, so that I could follow seasonal changes in parasite infection intensity within an individual. However, the individuals in the park are not GPS-tagged, and therefore, I could not always collect feces from all of them.

When I came across sheep, I remained about 50m away from them to minimize disturbance. I only moved from the observation spot, once the sheep had moved away and were out of sight. The total number of individuals sampled was 107 (M = 40; F = 67), with most individuals having been sampled at least twice during this study, resulting in a total of 623 fecal samples.

**Fecal sample collection**
The most common way to collect fecal samples was to wait for all the sheep to bed down. Once they had settled down, I drew a laying association map, in which I noted down key landmarks to help identify the location of each individual sheep and the distance between landmarks and each sheep. If the herd moved, I left an easily identifiable mark at the original observation spot, to collect the samples at the end of my observation day. Then, I continued to follow the herd. The second most common fecal sample collection method was observing sheep defecate while they were standing or moving. If they did defecate, I noted down the location and the sheep identification. As for the laying association, I collected samples only after the herd had moved away. In a few instances, I had to go collect samples before the herd moved, since it was
getting dark. In these instances, I slowly walked towards the sheep, so as not to startle them. I was able to get up to within 5m of the sheep before they slowly got up and moved a few meters away from the location and watched me. Once all samples were collected, and I had moved back more than 5m, the sheep either went back to their original bedding spot or started grazing.

Each fecal sample was collected in a separate, individually tagged zip-lock bag. Once samples were in the bag, air was pushed out to ensure that the eggs did not continue to develop, since hatching, development or death of parasite eggs can negatively affect fecal analysis (Nielsen et al. 2010). Fecal samples were stored in a cooler box with an icepack to ensure that the temperature remained relatively cool and consistent. The fecal samples were removed from the cooler as soon as possible (15 mins to 2 hours) and stored in a refrigerator at 4°C – the ideal temperature that stops the further development of eggs (Nielsen et al. 2010; Sengupta et al. 2016).

**Fecal sample processing and fecal egg counts**

I used the Modified Wisconsin Double Centrifugation method to separate fecal parasites from digested plant parts, soil and gut cells. We followed the Standard Operating Procedure #3 from the Alberta CWHC Parasitology Lab, with slight modifications. The modifications and justification for using this specific procedure is in Appendix A. After processing the samples, the egg counts were conducted using a microscope in the lab, or the field station. All samples were processed within 5 days of collection, well within the 8 days recommended (Crawley et al. 2016).
Statistical Analyses

Moniezia spp. and Trichuris spp. had an excess count of zeroes, i.e., more than 50% of the samples had a zero count (Table 2), but, when present, could occur in the thousands (maximum FEC for Moniezia spp. was 2,221 eggs per four grams of feces (EP4G), and for Trichuris spp. it was 1,983 EP4G). Because of the skew and large variance, parasite counts for these two species were removed from any analyses.

All statistical models were run in the programming language, R, (R Core, 2019), using the statistical packages, ‘glmmTMB’ (Brooks et al. 2017) and ‘DHARMa’ (Hartig 2019); and ‘ggplot2’ (Wickham 2016).

The count data followed a negative binomial distribution, were heavily overdispersed, and zero-inflated (Denwood 2008; Chipeta 2014). Furthermore, the variance was not equal to the mean for any of the six parasites, and I thus used the negative binomial distribution family for all further analyses.

Infection intensity (FEC with eggs per 4 grams) of each parasite was analysed separately, with bighorn sheep ID as a random effect (to control for pseudo-replication). Fixed effects were sex and season. I was unable to resample individuals every two weeks, so, I pooled the data into two seasons, winter and non-winter, with data from October to March as data for the ‘winter’ season and data from April to September as data for the ‘non-winter’ season. Because winter is the coldest period of the year and includes the pre-rut, rut and post rut, I expect to see higher levels of fecal egg output in males, compared to non-winter fecal egg output due to energetic demands of mating and hypophagia (Pelletier and Festa-Bianchet 2006. Non-winter, on the other hand, also includes energetically demanding late gestation and early lactation.
I thus expect fecal egg outputs to be higher for females, compared to males in the non-winter period. Similarly, I expect that non-winter female count will be higher than winter female count.

For each parasite, I fit three models, in which the conditional model remained consistent, while the specification of the zero-inflation parameter changed. In the first model, the non-zero-inflation parameter was specified as $zi \sim 0$; in the second, the zero-inflation parameter was applied across all observations ($zi \sim 1$) and finally; in the third, the zero-inflated model included sex, season, and their interaction as fixed factors ($zi \sim 1 + sex + season + sex:season$). The latter allows parasite intensity to vary according to the season and the sex of the individual. In addition, the fixed effects were age and $age^2$ (z-score transformed to standardise the variable so that the mean is 0 and there is a parabolic increase with age), a two-level factor for sex (male and female) and a two-level factor for season (winter and non-winter).

The three models were compared using AIC values. The simplest, most parsimonious model had to be at least two AIC units smaller than any other model (Burnham and Anderson 2002; however, Richards (2008) argue that any model with $\Delta$AIC of 6 or less may be acceptable). If not, all models within a $\Delta$AIC of two were included as equal candidate models (Table 3).

**Correlation between parasite counts**

A Spearman’s rank order correlation matrix was produced to test for co-linearity between pairs of parasite species. Due to repeated sampling of same individuals over the three years, Spearman’s rank correlation was re-estimated 1000 times such that each individual only appeared once in the resulting dataset.
Table 2. Parasite data counts with the number of zeros, non-zeros, and the proportion of zeros in each parasite group. Note that the percentage of fecal samples that had zero counts for *Moniezia* spp. * or *Trichuris* spp. * is extremely high, with only 14% non-zero counts for *Moniezia* spp., and 8.7% for *Trichuris* spp.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Number of Zeros</th>
<th>Number of non-zeros</th>
<th>Proportion of Zeros</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongyles</td>
<td>146</td>
<td>477</td>
<td>0.234</td>
</tr>
<tr>
<td>Nematodirus spp.</td>
<td>158</td>
<td>465</td>
<td>0.254</td>
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<tr>
<td>Moniezia spp. *</td>
<td>536</td>
<td>87</td>
<td>0.860</td>
</tr>
<tr>
<td>Marshallagia spp.</td>
<td>112</td>
<td>511</td>
<td>0.180</td>
</tr>
<tr>
<td>Lungworm Larvae</td>
<td>209</td>
<td>414</td>
<td>0.335</td>
</tr>
<tr>
<td>Eimeria spp.</td>
<td>124</td>
<td>499</td>
<td>0.199</td>
</tr>
<tr>
<td>Trichuris spp. *</td>
<td>571</td>
<td>52</td>
<td>0.917</td>
</tr>
</tbody>
</table>

Results
Parasite infection intensity and season
The top model for strongyles, *Nematodirus* spp., and *Marshallagia* spp., all included sex, season, and their interaction as significant predictors of infection intensity. For lungworm larvae, the top model included sex and the interaction between sex and season, while the top model for *Eimeria* spp. only included sex and season as predictor variables (Table 4). As predicted, female parasite infection intensity is significantly higher during non-winter in *Nematodirus* spp., *Marshallagia* spp., lungworm larva and *Eimeria* spp. compared to female count in winter (Table 4; Figure 2). Similarly, as predicted, male parasite infection intensity is significantly higher during winter in lungworm larva, *Nematodirus* spp. and *Marshallagia* spp. compared to non-winter; however, strongyle infection intensity in males is significantly higher in non-winter, compared to winter (Figure 2).
Table 3. Parasite data distribution and model comparisons. All parasites are modeled with the same fixed effects structure in the conditional model (parasite count \(\sim\) age + age\(^2\) + season + season:sex), but vary in the fixed effects structure of the zero inflation model. Models were compared by AIC, with the most parsimonious model in each case highlighted in bold.

<table>
<thead>
<tr>
<th>Parasite group</th>
<th>% Zeros</th>
<th>Maximum count</th>
<th>Model</th>
<th>Parameters</th>
<th>AIC</th>
<th>∆ AIC</th>
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<td>Strongyles</td>
<td>23.4</td>
<td>399</td>
<td>NegBinomial</td>
<td>8</td>
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<td>28.71</td>
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<td></td>
<td></td>
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<td>4625.86</td>
<td>30.71</td>
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<td></td>
<td></td>
<td></td>
<td>NegBinomial, zi (\sim) 1 + season*sex</td>
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<td>4595.15</td>
<td>0.00</td>
</tr>
<tr>
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<td>25.4</td>
<td>88</td>
<td>NegBinomial</td>
<td>8</td>
<td>3509.80</td>
<td>0.00</td>
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<td>9</td>
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<td>1.54</td>
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<td>69</td>
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<td>3490.68</td>
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<td>12</td>
<td>3480.74</td>
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<td>Lungworm larvae</td>
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<td>8</td>
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<tr>
<td>Eimeria spp.</td>
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<td>8</td>
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<td>12</td>
<td>7483.93</td>
<td>0.00</td>
</tr>
</tbody>
</table>
Parasite infection intensity and host-sex
As predicted, parasite infection intensity in females was significantly higher than males in non-winter for lungworm larva, *Nematodirus* spp., *Marshallagia* spp. and *Eimeria* spp. (Table 4; Figure 2). In contrast, infection intensity for strongyles was higher for males in non-winter compared to females in non-winter (Figure 2). Similarly, infection intensity was significantly higher for males in winter, compared to females in winter for strongyles, *Nematodirus* spp., and *Marshallagia* spp. (Table 4; Figure 2).

Surprisingly, host age was not a significant factor for any of the five parasites.
Table 4. Parameter estimates for the most parsimonious generalized linear mixed model fitted to each parasite group. “Intensity” refers to the infection intensity (FEC) in the conditional (negative binomial), and “ZI-intensity” refers to the infection intensity (FEC) in the zero-inflated model (logit). Parameters estimates for the zero-inflation terms have been multiplied by -1 so that they are of the same sign as the conditional model. Significant p-values are bolded.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Model</th>
<th>Fixed Effect</th>
<th>Estimate</th>
<th>SE</th>
<th>z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strongyle</strong></td>
<td>Intensity</td>
<td>Intercept</td>
<td>3.01</td>
<td>0.23</td>
<td>12.86</td>
<td><strong>0.012</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sex (M)</td>
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<td>0.24</td>
<td>2.51</td>
<td><strong>0.012</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Season (Winter)</td>
<td>-2.35</td>
<td>0.33</td>
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<tr>
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Figure 2. Mean parasite FEC for each parasite where ‘F non-winter’ is female non-winter count, ‘F winter’ is female winter count, ‘M non-winter’ is male non-winter count, and ‘M winter’ is male winter count for A) strongyles, B) lungworm larva, C) Nematodirus spp., D) Marshallagia spp., and E) Eimeria spp.. Please note that the scales are different for each parasite. Bars represent ± standard error.
Correlation between parasite group FECs
There was a weak to strong positive correlation between most parasite FECs (Table 5). However, there was a negative correlation between strongyle FEC and lungworm larva count.

These numbers show that individuals with high FECs for one parasite group are more likely to also have high FECs of one or more other parasite group. This relationship is quite strong in the case of Nematodirus spp. and Marshallagia spp., i.e., as FECs increase for one, there is a marked increase in the other. In contrast, individuals with high strongyle FECs have significantly lower lungworm larvae FEC levels.

The strength of correlation, and their significance was largely unaffected by resampling of the dataset (Figure 4), confirming a correlation between parasite group FECs.
Table 5. Correlation matrix showing the correlation coefficients and p-values for all pair-wise comparisons of parasite group FECs. Correlations represent Spearman’s rank correlations on the full ‘population-level’ dataset, n = 623 samples across 107 individuals. Rho is represented as r (correlation coefficient) and non-significant p-values are bolded.

<table>
<thead>
<tr>
<th></th>
<th>Nematodirus spp</th>
<th>Marshallagia spp</th>
<th>Lungworm larva</th>
<th>Eimeria spp</th>
<th>Moniezia spp</th>
<th>Trichurus spp</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strongyle</strong></td>
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<td>r = 0.23 p &lt; 0.001</td>
<td>r = -0.14 p &lt; 0.001</td>
<td>r = 0.10 p = 0.011</td>
<td>r = -0.16 p &lt; 0.001</td>
<td>r = -0.093 p = 0.0459</td>
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<td>-</td>
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<td>r = 0.31 p &lt; 0.001</td>
<td>r = 0.30 p &lt; 0.001</td>
<td>r = 0.15 p &lt; 0.001</td>
<td>r = 0.11 p = 0.024</td>
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<tr>
<td><strong>Marshallagia spp.</strong></td>
<td>-</td>
<td>-</td>
<td>r = 0.28 p &lt; 0.001</td>
<td>r = 0.28 p &lt; 0.001</td>
<td>r = 0.19 p &lt; 0.001</td>
<td>r = <strong>0.01</strong> p = <strong>0.81</strong></td>
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<tr>
<td><strong>Lungworm larva</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>r = 0.27 p &lt; 0.001</td>
<td>r = 0.38 p &lt; 0.001</td>
<td>r = 0.11 p = 0.018</td>
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<tr>
<td><strong>Eimeria spp.</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>r = 0.28 p &lt; 0.001</td>
<td>r = <strong>-0.03</strong> p = <strong>0.482</strong></td>
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<td><strong>Moniezia spp.</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>r = 0.19 p &lt; 0.001</td>
</tr>
</tbody>
</table>


Figure 3. A sample of re-estimation of Spearman's rank correlations after randomly resampling each individual so that it only appeared in the dataset once. The resampling approach was repeated 1000 times. The distribution of the resulting correlation coefficients is presented in histograms, with the 'population-level' correlation estimated from the complete dataset (noted by the black vertical line). The relative position of the population-level coefficient in almost all cases suggests that the pseudoreplication played little to no role in conflating correlation estimates. Similarly, significance of non-significance of correlations was largely retained, as indicated by the text, which quantifies the proportion of the 1000 iterations where the p-value was < 0.05. The x-axis refers to rho, while y-axis represents the frequency.
Discussion
This study showed that host sex and season explained differences in parasite infection intensity in all five parasite groups. Seasonal trends varied between the sexes and between winter and non-winter. Strongyle infection intensity was higher for both sexes in non-winter, but in both seasons, male infection intensity was higher than female infection intensity. In contrast, *Eimeria* spp. intensity was higher for females in both periods, but non-winter counts were significantly higher than winter counts. Interestingly, *Marshallagia* spp. and *Nematodirus* spp., had a different trend – the non-winter count was higher for females, while the winter count was higher for males. Finally, lungworm larvae count in males were lower in the non-winter period and higher in the winter, while female winter counts were lower than non-winter counts (Figure 2).

The differences in infection intensity of parasites between the sexes and over season could be explained by the differences in host biology and parasite abundance (Sargison 2013). As mentioned above, *Marshallagia* spp. and *Nematodirus* spp. infection intensity was significantly higher in non-winter for females (Figure 2; Table 4) compared to winter counts for females and non-winter counts for males. This may be because birthing and lactation are energy-expensive activities. For example, energy consumption patterns in pregnant female rodents increased by 25% and at least 60% during late gestation and lactation, respectively (bank voles, *Clethrionomys glareolus* – Kaczmarski 1966; common vole, *Microtus arvalis* – Pawel 1969; cotton rats, *Sigmodon hispidus* – Randolph et al. 1977).

In bighorn sheep, birthing and lactation takes place during the non-winter months, with birthing taking place in late May to early June and lambs being weaned off of milk.
around September-November. Festa-Binachet (1998a) showed that lactating ewes shed more lungworm larva in their feces when compared to yeld ewes. It is possible that ewes reallocate resources from immune function to meet the costs related to reproduction during non-winter and as a result, have higher parasite infection intensities. This is an especially compelling argument since female non-winter counts are higher than female winter counts for all parasite groups (although, the average lungworm larvae count is relatively similar between the two seasons).

Peri-parturient rise has been reported in domestic sheep (West African Dwarf sheep – Agyei et al. 1991; Bulkhi sheep – Chaudhry et al. 2009) where infection intensity in females increases during the first few weeks before and after birthing. In this study, we did not sample females during that time. However, given the patterns present in other ungulates, it is possible that the infection intensity in females during non-winter has been under-estimated in this study. It could be a parasite’s adaptive strategy to shed more eggs in females during the non-winter months. During the first couple of years of their lives, all lambs and yearlings stay in nursery groups with their mothers, rather than males joining bachelor groups (Ruckstuhl 1998). During this time, the lambs are naïve potential hosts for parasites. Thus, Armour (1980) posited that infection intensity during non-winter months in females could increase as some of these eggs and larvae could establish within the newly born lambs and yearlings. However, in this study, age was not a significant factor explaining variation in parasite infection intensity. This could only be the case if the lambs had already acquired parasites that developed into sexually mature adults and started shedding eggs. It would be interesting to see a follow-up study that identifies the parasite species and potentially, also the genetic similarities between the eggs shed by females and their lambs.
Additionally, winter could be more costly for the males since the months categorized as winter also include the pre-rut, rut, and post-rut. The pre-rut can be costly for males as they engage in social interactions to establish their dominance hierarchy (Geist 1971; Pelletier and Festa-Bianchet 2006), while the rut is costly since the males are either engaged in mate guarding or trying to secure mating opportunities with a guarded estrous female (Hogg 1984). Studies have shown that courting and mate guarding are energy expensive activities. For example, males in multiple species have shown to forego food consumption to maximize mating success by searching for estrus females or guarding females (bighorn sheep, *Ovis canadensis* – Pelletier 2005; long-tailed macaques, *Macaca fascicularis* – Girard-Buttoz et al. 2014a; 2014b; isopod – *Lirceus fontinalis* – Sparkes et al 1996). To meet the demands of mating costs, males may reallocate resources from immune function to mating in winter and thus, have higher infection intensities in winter, compared to non-winter. Furthermore, during the same time, males also tend not to eat (Pelletier and Festa-Bianchet 2004; Pelletier et al. 2009) and therefore, decrease their energy intake while increasing energy expenditure. This could indicate that energy is reallocated from other activities, such as immune function, or body fat to mating-related activities.

Finally, seasonality can play a crucial role as well. For example, strongyle eggs are relatively easily damaged by freezing events (Foreyt 1986; Wharton and Allan 1989). In a study on nematode FECs in red deer, *Cervus elaphus*, Albery et al. (2018) posit that nematodes may decrease egg production during winter, due to their low over-winter survival, rather than being indicative of reduced parasite burden in the hosts. This
seems consistent with the results found in my study, since the females tend to migrate to the mountains to give birth, while the males of the cohort remain in Sheep River Provincial Park towards the end of May. At this time, the temperatures are warming in the Park, which may allow for strongyle egg development, but the females are still in colder areas that are not yet conducive to strongyle egg development. That is, ideal temperature for egg development in eight nematode species ranged from 18 to 28°C (Rossanigo and Gruner 1995). Two of the possibilities for strongyles are Ostertagia spp. and Teladorsagia spp. and the temperature range for the development of these parasite genera range from 5 to 25°C (Hoar 2012) and 5 to 30°C (Rossanigo and Gruner 1995), respectively.

In contrast Nematodirus spp. and Marshallagia spp. produce eggs with a thick outer shell that makes the eggs resistant to freezing (van Dijk and Morgan 2008). In fact, egg production tends to increase during winter and spring for Marshallagia marshalli (Irvine et al. 2000) and during fall for Nematodirus spp. (Fruetel 1987). This could explain the higher FECs found in males during the winter season in this study.

Some species of Eimeria can survive -20°C for a few months (Rind and Brohi 2011). However, the ideal temperature ranges from 24 to 32 degrees Celsius. At these temperatures, most species sporulate within 5 days (Engidaw et al. 2015). Given the large range of temperature tolerance of oocysts, there may be differences in oocyst production based on the species. For example, species resistant to extreme cold may continue producing oocysts, while other species reduce egg production during winter, as discussed earlier. This could explain the dip in Eimeria spp. egg counts in winter in both sexes. Thus, higher FECs in females during non-winter could be due to an interplay
between parasite and host biology, while the lower male infection intensity during non-winter may be due to parasite biology, rather than host biology.

Finally, while other parasites have a direct lifecycle, lungworm larvae have an intermediate host – snails. There are two ways of acquiring lungworms, by ingesting infected snails (Samson et al. 1987) or from their mother via transplacental transmission (Gates and Samuel 1977). Lungworms tend to develop into their infective stage during summer (Samson et al. 1987), even though the parasite is resistant to extreme cold (Uhazy et al. 1973) due to intermediate host restrictions. That is, gastropods, the intermediate hosts of lungworm larva, are only found in the field site during spring and fall (Boag and Wishart 1982). This may restrict the transmission and development of the infective larva into an adult until late autumn to early winter. This could help explain the variation between winter and non-winter infection intensities between the sexes. Lungworm larvae can reach the infective stage in the and infect the definitive hosts in non-winter months, but shed larva during the winter months. Festa-Bianchet (1988) found that lungworm larva intensity was higher in lactating ewes compared to yeld ewes; however, the study only considered lungworm intensities during March and April, so it is unclear whether lungworm larva intensity was different at other times of the year.

Another consideration that could be contributing to increased parasite infection intensity could be the quality of foraging material. Diets that have higher concentrations of protein enhance immune function (i.e. Faria et al. 2013). Plant material is the main source of protein and energy for all herbivores. However, plant nitrogen concentrations in fecal samples showed that the quality of the plant material available during winter is
lower than that available during summer (Irwin et al. 1993). Since protein is an essential ingredient for mounting an immune response (Li et al. 2007), it is possible that the decreased forage quality contributed to increased FEC in rams. Similarly, ewes migrate to the mountains for the first few months during and after lambing (late May). Crude protein content is highest in Sheep River Provincial Park in May and June (~13%) and then decreases to reach around 7% by the end of September (Ruckstuhl et al. 2000). However, fecal crude protein content was variable over the years from May to July in the female birthing range with some years having higher protein content and some years having lower protein content, compared to the wintering range (Festa-Bianchet 1988b). In general, females have access to high quality plant material only in early May before they migrate to the mountains and when they come back to the park (September), whereas, males have continuous access to high quality plant material throughout until September (delayed plant phenology). This delay in access to high quality forage could decrease female immune response such that they show a higher infection intensity, compared to the males.

Finally, my study showed several significant correlations between the FECs of different parasite groups. That is to say, individuals that show an infection intensity in one parasite are also likely to show an infection intensity in another parasite. However, it is unclear whether this correlation is due to an actual relationship between the parasites, or because compromised individuals are easier for other parasites to establish in. The first implies a strong relationship between the parasites influencing the host in a way that increases host susceptibility; the second implies that host susceptibility is constant, and it is the overall more susceptible hosts that are likely to show higher parasite infection intensities. Interestingly, there is a negative correlation between strongyles
and lungworm larvae, which could potentially indicate parasite competition. More research is needed to look at these interactions of different parasite groups within a single host.
Literature Cited


Sheep River and Blueroak management plan. 2008.
URL: https://open.alberta.ca/dataset/c3a03e84-c64c-47c3-92ae-f294a0f6d78d/resource/65287078-cad6-4fcf-96a4-b3be6844fa29/download/2008-sheep-blueroak-plan-web.pdf
Chapter 3. Association between parasite infection and time budget

Introduction
When a parasite establishes itself within a host, it diverts host resources/energy into its own growth and reproduction (Schmid-Hempel 2011; Goater et al. 2011) and is therefore dependent on the host (Combes 2001). This also means that parasites add to the energy expenditure of the host. If parasite presence activates host immune response, it can further add to the hosts’ energy expenditure (Goater et al. 2011; Graham et al. 2011). As such, parasites can, directly or indirectly, affect host morphology and physiology, including changing host size, body condition (Graham et al. 2011; as reviewed in Sanchez et al. 2018), and behavior (Poulin 1994).

To further our ecological understanding of host-parasite interactions and evolutionary history, we need to consider impacts that parasites have on host behaviors, interactions between hosts, and Darwinian fitness. Since parasites depend on hosts for their own fitness, some parasites have evolved a strategy to optimize resource extraction from the host to a degree that has little to no impact on host survival, while conferring the maximum fitness benefit to the parasite (Schmid-Hempel 2011). In fact, when a novel disease agent (virus, parasite, bacteria or fungus) is introduced to a naïve host population, parasite virulence can decrease, while simultaneously host tolerance can increase over generations. When myxoma virus was first introduced in Australia as a method for controlling rabbit (Oryctolagus cuniculus) populations, rabbit mortality was 99%; over the next seven years, however, subsequently mortality decreased to 50% and then, 30% (Kerr and Best 1998). This change in mortality was attributed to increased host tolerance, and, simultaneously, decreased virus virulence (as reviewed in Kerr 2012).
Despite this, parasites with low and non-lethal virulence can still have negative impacts on their hosts. Parasites can, for example, considerably affect the amount of energy a host invests into their immune response (McElroy and de Buron 2014). A mitogen protein, phytohaemagglutinin, was injected into house sparrows (*Passer domesticus*) to challenge their immune system, which resulted in increasing the resting metabolic rate of the ‘infected’ individuals by 29%, compared to the resting metabolic rate of the control group (Martin et al. 2002). Similarly, feral rock doves (*Columba livia*) with a high load of ecto-parasites, (Phthiraptera: Ischnocera) a feather-feeding louse, increased their metabolic rate by 8.5%, compared to individuals with a low parasite load (Booth et al. 1993). The energy invested into an immune response is thus costly, as it is taking away host resources that could otherwise be used for body growth, health, or reproduction.

However, to minimize the costs of parasitism, hosts can also alter their own behavior. There are multiple ways of achieving this, for example, infected hosts can 1) decrease additional exposure to parasites by foraging in parasite-free areas (Cooper et al. 2000; Hutchings et al. 2001), 2) increase consumption of food with greater protein content to help increase parasite resistance (Coop and Holmes 1996; Lee et al. 2005), 3) self-medicate by consuming food with anti-parasitic properties (Singer et al. 2009; and as reviewed by Hutchings et al. 2003), 4) increase overall energy intake (Coop and Holmes 1996; Kyriazakis et al. 1998), or, 5) decrease overall energy expenditure (Agnew et al. 2000; Schultz et al. 2006). The former three behaviors can benefit host fitness and thus affect genotypes; however, it is unclear whether these behaviors are related to social learning, or innate. For example, herbivores avoid grazing in areas with
more feces. The authors hypothesize that these herbivores may be relying on cues, such as presence of feces, as an indicator for the presence of infective stage helminth parasite larvae, and so, could become a parasite avoidance technique (Cooper et al. 2000). However, the mechanism behind this behavior is relatively unknown. Thus, I limit the scope of this paper to investigating the latter two methods of minimizing cost of parasitism – increasing the percent of time spent grazing (energy intake time) and/or increasing the percent of time spent laying (lowering energy expenditure).

The overall energy consumption of an individual depends on the quality and quantity of food consumed (Rolls 1995). An individual allocates the available energy from forage into different bodily functions. These functions range from body maintenance (Spotila and Standora 1985), growth (Taborsky and Grantner 1998; Chow 1987), activity levels (Hicks et al. 2018) to thermoregulation (Zhao et al. 2010), immune function (Viblanc et al. 2011) and reproduction (Kozlowski 1992; Engen and Saether 1994). When multiple demands are placed on an individual, energy can be simultaneously allocated to satisfy relevant energy demands (Perrin and Sibly 1993). However, this means that energy allocated to any one function will be lower than if that was the only function requiring energy. In Northeast Arctic cod (Gadus morhua), larger individuals start breeding at an earlier age; but, once they start reproducing, they grow at a slower rate compared to individuals that didn’t breed (Folkvord et al. 2014). This example demonstrates that there are trade-offs in energy allocation towards growth and reproduction.

Three-spined stickleback (Gasterosteus aculeatus L.) infected with a tapeworm (Schistocephalus solidus), spent significantly more time foraging compared to uninfected individuals even after 12 to 72 hours of starvation when there was a
predator threat before the feeding trial started (Giles 1987). Infected three-spined stickleback also took longer to reach satiation, compared to uninfected individuals (i.e., 300 seconds for infected fish, and just more than 100 seconds for uninfected individuals) (Cunningham et al. 1994). Interestingly, when infected with an ectoparasite (*Xenopsylla conformis*), juvenile mice (*Meriones crassus*) increased the amount of time they spent feeding (i.e., increased their energy intake) but decreased the amount of time spent grooming, and so allocated less resources into immune function (Garrido et al. 2016). Adults in the same study, on the other hand, increased the time spent grooming, but decreased the time they spent eating. The difference in the amount of time juveniles and adults spent in grooming and feeding activities, may be related to potential mating opportunities. That is, if females prefer to mate with unparasitized males, adults may spend more time grooming to ensure a lower parasite load; but since juveniles aren’t sexually mature yet, they may prefer to invest in growth, rather than immune function.

Along the same lines, infected hosts may decrease energy expenditure rather than increasing energy intake. Solitary French grunt fish, *Haemulon flavolineatum*, infected with an isopod ectoparasite, *Anilocra haemuli*, travelled only about half the distance travelled by uninfected individuals, had smaller territories, and engaged in less nocturnal foraging migration than uninfected individuals (Welicky and Sikkel 2015). Similarly, 16 days after red grouse (*Lagopus lagopus scoticus*) were infected with the nematode, *Trichostrongylus tenuis*, control groups expended 83% more energy compared to their infected conspecifics (Delahay et al. 1995). These examples show that the host can change consumption patterns or allocation of energy to minimize negative impacts that a parasite can have on them.
Mating and reproduction can further affect the intake or allocation of energy at different times of the year, even in the absence of parasites. Animals engaging in mating and reproduction related behaviors, such as mate location, competition, mate-guarding, gestation, lactation and parental care have higher energy demands than normal body maintenance (Trivers 1972). For example, while trying to secure mating success, male bighorn sheep (*Ovis canadensis*) spent substantial amounts of time and energy to locate estrus females, and often forego food consumption entirely (hypophagia) (Pelletier et al. 2009). Similarly, male isopod crustaceans, *Lirceus fontinalis*, that engaged in mate guarding had significantly lower glycogen reserves, compared to males that did not engage in mate-guarding behavior, which the authors attributed to lowered food consumption during mate guarding (Sparkes et al. 1996). Mate guarding is also associated with decreased food consumption (Girard-Buttoz et al. 2014a) and increased stress hormones, glucocorticoids (Girard-Buttoz et al. 2014b) in long-tailed macaques (*Macaca fascicularis*).

Similarly, reproduction is costly for females. The cost of reproduction hypothesis states that energy investment into current reproductive episode decreases investment into self-maintenance and therefore, future reproductive capacity (Williams 1966). Pregnant females increase their energy consumption during late gestation and lactation by about 25% and more than 60% respectively (common vole, *Micortus arvalis* – Migula 1969; bank voles, *Clethrionomys glareolus* – Kaczmarski 1966; cotton rats, *Sigmodon hispudus* – Randolph et al. 1977). In dwarf sea horses, *Hippocampus zosterae*, pregnant males increased their metabolic rate by 10 to 50% (Masonjones 2001).
Clearly, gestation is an energy-heavy activity and could lead to increased susceptibility of individuals to parasites or disease (as discussed in Zuk 1990).

Lactating bighorn ewes (*Ovis canadensis*) have a higher bite rate, compared to yeld ewes or rams, which could be attributed to the fact that lactation is an energy heavy activity (Ruckstuhl et al. 2003). Lactating ewes also have higher lungworm larva counts, compared to non-lactating ewes (Pelletier et al. 2005). Taking all of this into account, it is plausible that females and males reallocate resources from immune function to reproductive and mating success, respectively, which impact their immune function and make them more susceptible to infections.

Here, I investigate whether there is any association between the time spent engaging in various activities (time budget as a proxy for energy intake and expenditure) and helminth and protozoan fecal egg counts (FEC) in bighorn sheep. FECs of parasites were used as a proxy for parasite burden, since studies on other ungulates have shown that fecal egg counts are a good indicator of nematode parasite burden (*white-tailed deer* (*Odocoileus virginianus*) - Schultz et al., 1993; *Marshallagia* spp. in Svalbard reindeer (*Rangifer tarandus platyrhynchus*) - Irvine et al., 2001; African buffalo (*Syncerus caffer*) - Budischak et al. 2015). Taking into account the information on host-sex bias covered in chapter 2, I predict that individuals (of both sexes) with a higher FEC will spend more time engaged in grazing and/or ruminating to increase energy intake, since rumination is related to nutrient extraction, rather than any other activity. I also predict that individuals with higher FEC will spend more time laying, i.e., resting without ruminating, compared to individuals with lower FEC. In this chapter, I compare the spring, summer and autumn (May – November) parasite FECs of five parasites.
(strongyle, *Nematodirus* spp., *Marshallagia* spp., *Eimeria* spp. and lungworm), with the percent of time individuals spent engaged in the three activities – grazing, ruminating and laying.

**Methodology**

**Study area**

This study was conducted in 2018 in Sheep River Provincial Park (50.63° N, 114.38° W), in southwestern Alberta, Canada. I collected data from May to November 2018. The climate in Sheep River Provincial Park ranges from extreme cold (~30°C) to extreme hot (37°C); however, the annual average temperature in the park ranges from about –8°C in winter to 15°C in summer (Sheep River and Bluerock management plan 2008).

More than 90% of the study population have been individually tagged with colored and numbered ear tags. Lambs are caught a few months and up to a year after lambing (September to November) and individuals are marked with colored and numbered ear tags (Pelletier et al. 2005). Male-only groups of sheep are referred to as ‘bachelor’ groups, while groups that contain females, lambs and subadults are referred to as ‘nursery’ groups. During the study period, I followed bachelor or nursery groups opportunistically for 5-9 hours per day for behavioral observations and then collected fecal samples of the observed individuals. I tried to collect data from the same individuals every two weeks, to measure individual changes in time budgets and parasite FEC. However, since individuals were not GPS-tagged, I was only able to consistently measure some individuals, mainly those in bachelor groups. Since I have been observing this population since May 2016, they were used to my presence and did not react when I came up to them and stayed more than 40 meters away.
When I came across tagged individuals, I stayed away at about 50m to minimize disturbance. I moved from the original observation spot only if I lost sight of the sheep. In this case, I marked the original spot so that I could come back to get fecal samples from the places that the sheep visited. I observed sheep with binoculars (40x10) and a Swarovski spotting scope (20–40x magnification).

**Behavioral time budget observations**
The mean age of the observed individuals was five and ranged from lambs (0) to 16-year-old females. I only observed behaviors of individually tagged sheep. Since I started observations when I came across a group of sheep, start times varied from 8am to 1pm (MST). Once all animals in the group were identified, I randomly chose (6–22) focal sheep and conducted focal animal observations on them for 5–9 hours each day, recording their activities (see below for types of behavior and definitions). Over the seven months, 75 individuals were sampled a total of 236 times (males = 166, females = 70). On average, an individual was sampled 3 times/year (mean = 3.15), with some individuals sampled multiple times (>7, n = 15) and few individuals (n = 4) sampled only once. Observations lasted, on average, 6 hours and 53 mins for each focal sheep.

The beginning and end of behavioral activities was recorded to the nearest minute. If any behavior occurred for less than a minute, that activity was not included in the daily budgets, i.e., moving or nursing for less than 60 seconds (for more details on the method see Ruckstuhl 1998). Observations took place during daytime only (between 8am to 8pm). The same individual was observed only once every two weeks. Only one group was followed each day since observations and fecal collection, cumulatively, took more than 7.5 hours/day.
The activities for the time budget are in bold, followed by a description of their definition:

1. **Grazing**: individual is taking fresh forage material from plants. This is clearly visible and includes gathering of vegetation, followed by a jerk of the head (upwards), when plant parts are ripped off – they may be moving while foraging, or they may be bedded and reaching out to close-by forage, by extending their neck. This latter behavior is rare but, can on occasion last more than a minute. As with all other behaviors, if grazing stopped for more than a minute, I wrote down the time the individual switched to a new behavioral category.

2. **Resting/Laying**: individual is bedded down and not ruminating (i.e., chewing cud) or engaged in any other specific behavior, individual may have their head on the ground and may or may not have closed their eyes.

3. **Ruminating**: individual is standing or laying down and is chewing cud. This process is very visible and includes the repeated regurgitation of forage (a bolus of cud), followed by mastication, and swallowing.

4. **Moving**: individual is engaged in activity that displaces them from their initial start point/location by least 1 meter and takes place over a period of longer than 59 seconds. The individual may be walking or running.

5. **Social interaction**: activity in which two or more individuals interact with each other by coming closer than a meter’s distance of each other.

6. **Nursing**: the lamb is actively suckling on the ewes’ teats. The bout starts when the lamb is initiating a suckling bout by pushing its snout against the teats.

7. **Other**: individual is engaged in any behavior other than those described above, such as scratching their body against a tree, standing vigilant and so on.
Although I categorize these seven behavioral categories, I will only be focusing on the main activities of interest in the analyses of the effect of parasites on time budgets; grazing, laying and ruminating.

**Fecal sample collection**
As I could identify each individual, I made sure to only collect fecal samples that I was 100% sure to track back to the identified individuals. The most common way that I collected samples was by making a laying-association map while the sheep were bedded down. I noted down key landmarks to help identify the location of each individual sheep and the distance between landmarks and each sheep. Sheep often defecate while bedded or after getting up and before moving off. If the herd moved, I left an easily identifiable mark at the original observation spot, so that I could go back to it and collect the samples. After marking the spot, I followed the herd. The second fecal sample collection method was observing whether the sheep defecated while they were standing or moving. If they defecated, I noted down the location and the sheep identification. I collected samples only after the herd had moved away.

In a few instances, I had to collect samples before the herd moved, since it was getting dark and the sheep had been observed for at least 5 hours. In these instances, I walked towards the sheep slowly, so as not to startle them. I was able to get up to within 5m of the sheep before they got up, stood and moved away from the location. While the samples were being collected, individuals stood vigilant or started to graze nearby. Once all samples were collected, and I moved away, the sheep often went back to bedding or continued grazing. This showed that my presence had minimal effect on their behavior.
Each fecal sample was collected in a separate, individually tagged zip-lock bag. Once samples were in the bag, air was pushed out to ensure that the eggs did not continue to develop, since hatching, development or death of parasite eggs can affect faecal egg analysis (Nielsen et al. 2010). Fecal samples were stored in a cooler box with an icepack to ensure that the temperature remained consistent. The fecal samples were removed from the icepack as soon as possible (15 mins to 2 hours) and stored in a refrigerator at 4°C – the ideal temperature that stops further development of eggs (Nielsen et al. 2010; Sengupta et al. 2016).

**Fecal egg counts**
I conducted the Modified Wisconsin double centrifugation to get the fecal egg counts on the sample. I followed procedure based on the Standard Operating Procedure 3 from the Alberta CWHC Parasitology Lab, with some slight modifications. The procedure is described in more detail in the Appendix A. After processing the samples, the egg counts were conducted using microscopes in the lab, or at the field station. All samples were processed within 5 days of collection, within the 8 days recommendation (Crawley et al. 2016).

**Statistical Analysis**
All statistical models were run in the programming language, R, (R Core, 2019). The activity budget data was distributed normally within 95% confidence interval (Figure 4). I used the statistical package, ‘lme4’ (Bates et al. 2015) and ‘lmerTest’ (Kuznetsova et al. 2017) to build the models and ‘ggplot2’ (Wickham 2016) and JMP for data visualization. The three activities, ‘grazing’, ‘laying’ and ‘ruminating’ were the dependent variables and therefore, were analysed separately with bighorn sheep ID as random effects (to control for within individual replications through the months).
As all behavior was observed for a time ranging from 5 to 9 hours, each activity period was converted to a percentage of the total time observed. After that, I checked whether sex, month and age of individuals were confounding factors that explained variability in the length of each of the activities. I used lmer to test for the effects of age, month and sex on each activity. I used mixed effects model since same individuals were sampled multiple times during the 7 months. In this round of data exploration, activity was the dependent variable and age, sex and month were fixed effects with sheep id as random effects.

The results showed that sex and month were significant factors, while age showed a trend in ‘ruminating’ activity (Table 6; Figure 6). For the next set of statistical analysis, I added month as random effects to control for confounding effects on the linear regression for activities. However, since sex has only two categories, I added it as a fixed effect in the final model being aware of the previous steps. In each of the following three models, I kept all five parasite FECs as explanatory variables.
Spearman’s rank correlation was conducted between each activity and FECs of the five parasites in R.
Table 6. Regression coefficients of activities (grazing, laying and ruminating) with sex, age, and months (May to November) as explanatory variables. Sample size for the months are as follows: May (n = 21), June (n = 67), July (n = 27), August (n = 30), September (n = 26), October (n = 19) and November (n = 46). Significant effects are bolded.

<table>
<thead>
<tr>
<th>Explanatory variables</th>
<th>Grazing</th>
<th>Laying</th>
<th>Ruminating</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (M)</strong></td>
<td>-10.78</td>
<td>7.81</td>
<td>4.83</td>
</tr>
<tr>
<td>p-value &lt;0.001</td>
<td>p-value &lt;0.05</td>
<td>p-value &lt;0.05</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>-0.54</td>
<td>0.10</td>
<td>0.50</td>
</tr>
<tr>
<td>p-value = 0.11</td>
<td>p-value = 0.779</td>
<td>p-value = 0.071</td>
<td></td>
</tr>
<tr>
<td><strong>June</strong></td>
<td>-10.24</td>
<td>12.03</td>
<td>-3.14</td>
</tr>
<tr>
<td>p-value &lt;0.01</td>
<td>p-value &lt;0.01</td>
<td>p-value = 0.336</td>
<td></td>
</tr>
<tr>
<td><strong>July</strong></td>
<td>-8.81</td>
<td>11.19</td>
<td>-2.58</td>
</tr>
<tr>
<td>p-value &lt;0.05</td>
<td>p-value &lt; 0.05</td>
<td>p-value = 0.486</td>
<td></td>
</tr>
<tr>
<td><strong>August</strong></td>
<td>-15.36</td>
<td>23.33</td>
<td>-15.38</td>
</tr>
<tr>
<td>p-value &lt;0.001</td>
<td>p-value &lt;0.001</td>
<td>p-value &lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>September</strong></td>
<td>-1.78</td>
<td>5.29</td>
<td>-4.70</td>
</tr>
<tr>
<td>p-value = 0.669</td>
<td>p-value = 0.246</td>
<td>p-value = 0.183</td>
<td></td>
</tr>
<tr>
<td><strong>October</strong></td>
<td>15.60</td>
<td>-5.07</td>
<td>-7.31</td>
</tr>
<tr>
<td>p-value &lt;0.001</td>
<td>p-value = 0.300</td>
<td>p-value = 0.054</td>
<td></td>
</tr>
<tr>
<td><strong>November</strong></td>
<td>9.45</td>
<td>-5.07</td>
<td>-4.90</td>
</tr>
<tr>
<td>p-value &lt; 0.05</td>
<td>p-value = 0.218</td>
<td>p-value = 0.124</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5. Boxplot of the percent of time individuals spent grazing in both sexes (males (M) and females (F)) over seven months (5 = May, 6 = June, 7 = July, 8 = August, 9 = September, 10 = October, 11 = November).
Results
On average, sheep spent the majority of their daytime grazing, followed by ruminating and laying; however, males spent more time laying than ruminating, while females spent less time laying than ruminating (Table 7).
Table 7. Range of measures for the Sheep River provincial park bighorn sheep population (n = 236; individuals = 75) on their activities (in percent) and fecal egg counts (EP4G). The male and female combined values of the measures are bolded.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>St. Dev</th>
<th>Min</th>
<th>25th Percentile</th>
<th>75th Percentile</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>5.03</td>
<td>2.94</td>
<td>0</td>
<td>3</td>
<td>6.2</td>
<td>16</td>
</tr>
<tr>
<td>Male</td>
<td>5.28</td>
<td>2.39</td>
<td>0</td>
<td>4</td>
<td>6.75</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>4.46</td>
<td>3.91</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>16</td>
</tr>
</tbody>
</table>

### Activities

<table>
<thead>
<tr>
<th>Activities</th>
<th>Mean</th>
<th>St. Dev</th>
<th>Min</th>
<th>25th Percentile</th>
<th>75th Percentile</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grazing</td>
<td>42.71</td>
<td>19.38</td>
<td>0</td>
<td>30.58</td>
<td>51.18</td>
<td>95.64</td>
</tr>
<tr>
<td>Male</td>
<td>35.74</td>
<td>15.98</td>
<td>0</td>
<td>27.13</td>
<td>42.12</td>
<td>90.51</td>
</tr>
<tr>
<td>Female</td>
<td>59.24</td>
<td>16.56</td>
<td>26.32</td>
<td>47.58</td>
<td>70.70</td>
<td>95.64</td>
</tr>
<tr>
<td>Laying</td>
<td>28.66</td>
<td>19.19</td>
<td>0</td>
<td>14.74</td>
<td>40.54</td>
<td>94.69</td>
</tr>
<tr>
<td>Male</td>
<td>34.54</td>
<td>18.60</td>
<td>0</td>
<td>19.75</td>
<td>47.33</td>
<td>94.69</td>
</tr>
<tr>
<td>Female</td>
<td>14.72</td>
<td>12.09</td>
<td>0</td>
<td>4.72</td>
<td>23.00</td>
<td>45.17</td>
</tr>
<tr>
<td>Ruminating</td>
<td>20.38</td>
<td>12.60</td>
<td>0</td>
<td>10.99</td>
<td>29.50</td>
<td>54.11</td>
</tr>
<tr>
<td>Male</td>
<td>21.54</td>
<td>12.74</td>
<td>0</td>
<td>11.67</td>
<td>30.29</td>
<td>54.11</td>
</tr>
<tr>
<td>Female</td>
<td>17.61</td>
<td>11.89</td>
<td>0</td>
<td>9.11</td>
<td>24.79</td>
<td>48.25</td>
</tr>
</tbody>
</table>

### Fecal egg counts

<table>
<thead>
<tr>
<th>Strongyle</th>
<th>Mean</th>
<th>St. Dev</th>
<th>Min</th>
<th>25th Percentile</th>
<th>75th Percentile</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nematodirus</td>
<td>3.97</td>
<td>4.79</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>32</td>
</tr>
<tr>
<td>spp.</td>
<td>3.62</td>
<td>7.54</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>79</td>
</tr>
<tr>
<td>Marshallagia</td>
<td>3.48</td>
<td>6.28</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>69</td>
</tr>
<tr>
<td>spp.</td>
<td>15.80</td>
<td>55.09</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>487</td>
</tr>
<tr>
<td>Lungworm</td>
<td>169.52</td>
<td>353.54</td>
<td>0</td>
<td>11</td>
<td>136.2</td>
<td>2367</td>
</tr>
<tr>
<td>Larva</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eimeria spp.</td>
<td>169.52</td>
<td>353.54</td>
<td>0</td>
<td>11</td>
<td>136.2</td>
<td>2367</td>
</tr>
</tbody>
</table>

Correlations between activity variables

Negative correlations between time budgets were expected, since as an animal increases the time spent in one, it affects the time left for other activities. Surprisingly, there is only a weak negative correlation between the percent animals spent grazing
Correlation between activities and FEC

There was a negative correlation between time spent grazing and FEC of each parasite except lungworm (Table 8). In contrast, there was a positive correlation between ruminating and strongyle, *Nematodirus* spp., *Marshallagia* spp., and *Eimeria* spp. FEC (Table 8). There was no correlation between time spent ruminating and lungworm larvae counts. Finally, there was a positive correlation between laying and strongyles, *Nematodirus* spp., and *Eimeria* spp., and a negative correlation with lungworm larvae.
FEC (Table 8). However, the relationship is very weak (Table 8) and appears near random for both sexes (Figure 8).

Table 8. Correlations between parasite FECs and the percent time spent in different activities as calculated by using Spearman’s rank correlation. Significant relationships have bolded Spearman $\rho$- and $p$-values.

<table>
<thead>
<tr>
<th>Activity / Parasite</th>
<th>Grazing</th>
<th>Laying</th>
<th>Ruminating</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongyle</td>
<td>$\rho = -0.34$</td>
<td>$\rho = 0.29$</td>
<td>$\rho = 0.17$</td>
<td>$\rho = -0.09$</td>
</tr>
<tr>
<td></td>
<td>$p &lt; 0.001$</td>
<td>$p &lt; 0.001$</td>
<td>$p &lt; 0.01$</td>
<td>$p = 0.18$</td>
</tr>
<tr>
<td>Nematodirus spp.</td>
<td>$\rho = -0.17$</td>
<td>$\rho = 0.13$</td>
<td>$\rho = 0.18$</td>
<td>$\rho = -0.03$</td>
</tr>
<tr>
<td></td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.05$</td>
<td>$p = 0.63$</td>
</tr>
<tr>
<td>Marshallagia spp.</td>
<td>$\rho = -0.14$</td>
<td>$\rho = 0.09$</td>
<td>$\rho = 0.17$</td>
<td>$\rho = -0.07$</td>
</tr>
<tr>
<td></td>
<td>$p &lt; 0.05$</td>
<td>$p = 0.17$</td>
<td>$p &lt; 0.01$</td>
<td>$p = 0.25$</td>
</tr>
<tr>
<td>Lungworm</td>
<td>$\rho = 0.16$</td>
<td>$\rho = -0.14$</td>
<td>$\rho = 0.02$</td>
<td>$\rho = 0.06$</td>
</tr>
<tr>
<td></td>
<td>$p &lt; 0.05$</td>
<td>$p &lt; 0.05$</td>
<td>$p = 0.71$</td>
<td>$p = 0.35$</td>
</tr>
<tr>
<td>Eimeria spp.</td>
<td>$\rho = -0.24$</td>
<td>$\rho = 0.19$</td>
<td>$\rho = 0.18$</td>
<td>$\rho = -0.05$</td>
</tr>
<tr>
<td></td>
<td>$p &lt; 0.001$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
<td>$p = 0.48$</td>
</tr>
</tbody>
</table>
**Figure 7.** Scatter-graph with strongyle FEC and percent of time individuals spent grazing among female (F) and male (M) bighorn sheep.

**Model-derived estimates of FEC and activity**
Linear mixed model results indicate that, after controlling for sex and month, the FEC of none of the five parasites observed were significant predictors for the percent of time individuals spent engaged in any of the three activities – grazing, laying or ruminating (Table 9). However, there was a trend in strongyle and *Marshallagia* spp. FEC affecting the grazing time. Individuals with higher strongyle FEC spent more time grazing, while individuals with higher *Marshallagia* FEC spent less time grazing (Table 9).
Table 9. Regression results for grazing, laying and ruminating activities. Due to repeated measures, the random effects of the models were sheep ID and month. The activities, ‘grazing’, ‘laying’ and ‘ruminating’ were the dependent variables of each model, while the fixed effects were the FEC of the five parasites and sex. None of the parasite fecal egg counts were significant factors explaining percent of time individuals spent in these activities, but those that show a trend are bolded.

<table>
<thead>
<tr>
<th>Parasite /Activity</th>
<th>Grazing</th>
<th>Laying</th>
<th>Ruminating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongyle</td>
<td>0.455</td>
<td>-0.416</td>
<td>0.138</td>
</tr>
<tr>
<td>p-value = 0.051</td>
<td></td>
<td>p-value = 0.1</td>
<td>p-value = 0.48</td>
</tr>
<tr>
<td>Nematodirus spp.</td>
<td>0.243</td>
<td>-0.071</td>
<td>-0.139</td>
</tr>
<tr>
<td>p-value = 0.25</td>
<td></td>
<td>p-value = 0.75</td>
<td>p-value = 0.43</td>
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<tr>
<td>Marshallagia spp.</td>
<td>-0.476</td>
<td>0.171</td>
<td>0.157</td>
</tr>
<tr>
<td>p-value = 0.06</td>
<td></td>
<td>p-value = 0.54</td>
<td>p-value = 0.47</td>
</tr>
<tr>
<td>Lungworm Larva</td>
<td>-0.016</td>
<td>0.01</td>
<td>0.006</td>
</tr>
<tr>
<td>p-value = 0.53</td>
<td></td>
<td>p-value = 0.73</td>
<td>p-value = 0.79</td>
</tr>
<tr>
<td>Eimeria spp.</td>
<td>-0.004</td>
<td>0.005</td>
<td>-0.003</td>
</tr>
<tr>
<td>p-value = 0.21</td>
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<td>p-value = 0.25</td>
</tr>
<tr>
<td>Sex</td>
<td>-14.17</td>
<td>10.1</td>
<td>5.11</td>
</tr>
<tr>
<td>p-value &lt; 0.001</td>
<td></td>
<td>p-value = 0.002</td>
<td>p-value = 0.03</td>
</tr>
<tr>
<td>Constant</td>
<td>54.072</td>
<td>21.004</td>
<td>16.186</td>
</tr>
<tr>
<td>p-value &lt; 0.001</td>
<td></td>
<td>p-value = 0.001</td>
<td>p-value &lt; 0.001</td>
</tr>
</tbody>
</table>

**Discussion**

On average, individuals spent about 90% of their time engaged in the three main activities with the remaining 10% of the time engaged in other activities, such as vigilance, scratching, moving, and social interaction.

I discovered that there were significant but weak correlations between each activity and parasite FECs. However, when the data was input into a linear mixed model after controlling for ID and month, none of the FECs were significant factors that explained...
variation in the percent of time individuals spent grazing, ruminating and laying. There is a trend of strongyle and *Marshallagia* spp. FEC affecting the time an individual spent grazing, with individuals with higher a strongyle fecal egg count spending more time grazing, while individuals with a higher *Marshallagia* spp. count spending less time grazing. However, sex and month significantly affected the amount of time individuals spent grazing and thus, had to be controlled for in the final models. Similarly, sex and season were significant factors that affected FECs (Chapter 2). Taking both into consideration, it is possible that sex and season/month affect fecal egg outputs and the percent of time spent in certain activities, rather than a strong and direct link between FECs and specific activities.

In a previous study on bighorn sheep, increasing counts of lungworm larva led to a decrease in time spent grazing (Pelletier and Festa-Bianchet 2004). In my study, time spent grazing was negatively correlated with FEC of all parasites. Studies on other ruminants also reported decreased energy intake when individuals had a higher parasite intensity (Fox 1997; as reviewed in Gunn and Irvine 2003; Worsley-Tonks and Ezenwa 2015). Similarly, in this study, there was a weak trend of hosts with higher *Eimeria* spp. count spending more time laying compared to individuals with a lower infection intensity (Table 9). This could be to reduce the energy expenditure of the host organism, since higher infection intensities are associated with fitness costs (Aleuy et al. 2018).

Organisms may increase energy intake (*Meriones crassus*, mice - Garrido et al. 2016), reduce energy expenditure (as modelled by Houston et al. 2007) or reallocate available resources into immune function (Tree swallows, *Tachycineta bicolor*, in Tennessee –
Ardia 2005) depending on their current and future reproductive status. It thus seems likely that bighorn sheep may fall within the latter two groups and tend to reduce energy expenditure or reallocate resources into immune function, rather than increasing energy intake due to increasing FEC.

It is also possible, as Lozano (1991) proposed, that being infected with parasites encourage a host to change their food consumption patterns, rather than change their food consumption period. For example, numerous species (bumble bees (*Bombus impatiens*) – Richardson et al. 2015; dik-dik (*Madoqua kirkii*) – Ezenwa 2004; domestic sheep – Lisonbee et al. 2009; Costes-Thire et al. 2019) ingest plants with tannins that helps fight off parasites. Infected individuals are also more likely to choose foraging material that has higher protein content (Coop and Holmes 1996; Lee et al. 2005) to offset the costs of an immune response. Bighorn sheep could utilize a similar tactic, so while there is no difference in the time that they spent grazing among individuals with light and heavy infection intensities, there could be a difference in the plants that they consume, such that individuals consuming plants with higher concentrations of tannins end up with lower infection intensities.

Alternatively, bighorn sheep could engage in behavior that ensure limited exposure to parasites. Pacific chorus frogs (*Pseudacris regilla*), for example, actively moved away from locations with their trematode parasites (*Riberiroia* spp. and *Echinostoma* spp.) (Daly and Johnson 2010). Similarly, experiments show that some herbivores use fecal pellet presence as a cue for the presence of parasites and tend to prefer feeding in areas with low or no fecal pellets (sheep – Cooper et al. 2000; dik-dik (*Madoqua kirkii*) - Ezenwa 2004). Thus, it is possible that rather than changing the amount of time spent
in any activity, individuals change the area that they graze in to reduce further exposure.

Furthermore, it is also possible that the worm burden in this population is low enough that it does not have an impact on their behavior. Domestic sheep strongyle egg counts that fell within the range of 600 to 2000 FECs per gram had moderate levels of parasite burdens (4000 – 10,000), while individuals with more than 2000 FECs per gram had high levels of parasite burdens (McKenna 1987). Our bighorn sheep study population has relatively low strongyle egg counts with the highest egg counts still well below 400. As such, it is possible that the population has a low worm burden, with little to no impact on their behavior. Thus, if the FECs were higher, then we may see a change in their activity patterns or food choice.

To conclude, there are known negative consequences related to bighorn sheep FECs, body condition, activity patterns and reproductive success. In this study, I showed that FECs of some parasites (namely, Marshallagia spp., = and strongyle) showed a trend in affecting the percentage of time individuals spent grazing, but other parasites did not show any pattern detectable at these FEC levels.
Literature Cited


Sheep River and Bluerock management plan. 2008. URL: https://open.alberta.ca/dataset/c3a03e84-c64c-47c3-92ae-


Chapter 4. In conclusion...
Both the host’s and the parasite’s life history can lead to different infection intensities at
different times of the year. As I discussed in chapter 2, the pattern of parasite FEC is
complicated. Out of the five parasites investigated, males had a significantly higher FEC
in non-winter compared to females, but only in strongyles. Females, on the other hand
had a significantly higher FEC in all other parasites, *Nematodirus* spp., *Marshallagia*
spp., lungworm, and *Eimeria* spp., during non-winter. However, the winter patterns are
more complex, with females having a lower FEC than males in strongyles, *Marshallagia*
spp and *Nematodirus* spp., but a higher *Eimeria* spp., and lungworm FEC. In the latter
two cases, the overlap between male and female FECs are fairly high.

As I discussed in this thesis, these patterns in FEC could arise as a mixture of host
resource demands and parasite biology. For the hosts, non-winter is energetically
expensive for females, while winter is energetically expensive for males, due to
investment into reproduction and mating opportunities, respectively. This could mean
that individuals are allocating limited resources to reproduction and mating, rather than
to immune function, during those periods. The reallocation of these resources for
reproduction could cause an increase in infection intensity. Furthermore, specific
parasite species have co-evolved with the host and show reproductive patterns that
often show adaptations to develop in adverse conditions. *Nematodirus* spp. and
*Marshallagia* spp. eggs and lungworm larvae, for example, are resistant to freezing and
indeed, some species may require a freeze-thaw cycle to reach their infective stage.
Strongyle and *Eimeria* spp., on the other hand, are not as resistant to extreme cold
temperatures (-8 to -30°C). This could be the reason for a corresponding decline in FEC
in both host sexes during the winter season.
Since mating, reproduction and immune function are essential for a host and require energy, I had originally predicted that individuals with higher FEC may try to increase their total energy intake or decrease their energy expenditure, to deal with the extra costs incurred due to the parasites. In chapter 3, I showed that sex and month were significant predictors of the percent time that individuals spent engaged in the three main activities that would be related to energy gain or conservation of energy: grazing, ruminating and laying. However, parasite FECs were not significant predictors for these activities, although, there was a trend for some parasite species to affect activity: individuals with higher strongyles FEC tended to spend more time grazing, while individuals with higher *Marshallagia* spp. FEC tended to spend less time grazing.

As such, it seems that fecal egg counts of the five parasites are for the most part not significant factors in predicting the proportion of time an individual spent engaged in the three activities. On the other hand, these activities are significantly associated with an individual’s age, sex and month of observation, with females spending more time grazing in most months compared to males, and younger individuals spending more time grazing than adults.

4.1. Future studies
My study showed that there are differences in FECs of all parasites in the study between seasons (winter and non-winter) and that these counts differ between the two sexes. It also shows that time budgets are affected by sex and season, rather than
FECs. These two findings are raising a few more questions, that I think would be worthwhile to follow up with future research, as suggested below:

1. Studies have demonstrated that fecal egg counts is correlated to total adult parasites (Kingbury 1965; McKenna 1981; Budischak et al. 2015); however, other studies have shown weak or no correlation between the two (Liccioli et al. 2012). It may be beneficial to investigate parasite infection intensity and gastrointestinal parasite burden in bighorn sheep to determine whether there is a correlation between the two, as was done by Aleuy et al. (2018) for Dall sheep, *Ovis dalli dalli*. This could be done by partnering with hunters and investigating the parasite burden and FEC in fresh hunter kills.

2. Host-sex bias could be related to sex-hormones, so that there is a difference in terms of egg shedding, or in total adult worm burden. Some studies have suggested that high testosterone (sage grouse, *Centrocercus urophasianus* – Mousseau et al. 2006; common wall lizard, *Podarcis muralis* - Oppliger et al., 2004; freshwater snake, *Natrix piscator* - Tripathi and Singh 2014) or progesterone (feral horses, *Equus ferus* - Debeffe et al. 2016) levels negatively impact immune response; however, we know relatively little about the correlation between hormone levels and FECs. Feces are an ideal non-invasive source of both FECs and sex hormones. Such investigations could be particularly elucidating by comparing naturally occurring fluctuations in hormone levels within a sex, or even between animals of the same age class. A more invasive option would be to treat animals with sex hormones and test how these affect FECs for the various parasites.
3. Fecal and parasite build up in the study site could lead to differences in exposure between males and females and at different times of the year. In the three years that I was collecting data, the bachelor groups seemed to prefer specific areas each year (I.e., in 2016, males frequented Sandy McNabb or Windy point East. In 2017, they were mainly at Dot mountain far west. In 2018, males frequented the field below the Bighorn Lookout). It is unclear why the males seemed to switch focal areas between these years. Since some of the parasite eggs (Nematodirus spp. and Marshallagia spp.) and larva (lungworm) can survive for a few years in the external environment, it is plausible that they may be trying to avoid patches with fecal build up. Future research could try to quantify parasite densities in current versus the previous year to confirm whether parasite density is lower in that area, compared to the area that they frequented the previous year. Another way to get at the problem indirectly is to experimentally increase or decrease local fecal densities in specific patches to see whether there is active avoidance of heavily contaminated vegetation.

4. I was unable to collect fecal samples from females during early and mid-lactation. Analyses of female FEC when they are in the mountain for birthing, and through the summer would help fill the gap, to enable having equal sample distribution of male and female FEC throughout the year. This might elucidate a further pattern of host sex bias and allow us to determine the ‘costs’ of energy investment in mating/reproduction and correlated FEC values.

5. There are strong positive correlations between the FECs of a few parasites, such as between Nematodirus spp. and Marshallagia spp., and a negative correlation
between strongyles and lungworm larvae. It is unclear whether these associations are coincidental, since strongyle eggs cannot survive winter, but lungworm larva can, or whether there are negative or positive relationships between the parasite abundances within the host. That is, if *Nematodirus* spp is established within a host, is it easier for *Marshallagia* spp. to establish within the same host, or vice versa? Thus, investigating whether these parasites have an additive, or non-additive effect would help tease apart the mechanisms leading to these possible patterns of co-existence or competition we see in FECs. Short of experimentally manipulating the numbers of each parasite group within captive bighorn sheep, in very controlled conditions, I do not currently see an obvious way to control all independent variables and interactions in the wild.

6. One of the findings of the study was that the percent of time individuals spent grazing was not greatly altered based on parasite FECs. Some ungulates are known to ingest plants with anti-parasitic properties (dik-dik, *Madoqua kirkii* – Ezwnwa 2004; domestic sheep – Lisonbee et al. 2009; sheep – Costes-Thire et al. 2019). As such, it would be interesting to investigate whether bighorn sheep change their diets towards plants with high levels of secondary compounds, i.e., by ingesting more tannin-rich plants at high infection intensities, rather than spending more time feeding.

7. Parasite communities within a host can be complex and the interactions between species are not well understood. Understanding the composition of parasite communities, i.e., nemabiome, and its consequences on the host could help gain
a better understanding of the interactions between parasite species within an individual host or within a specific population.
Literature Cited
Appendix A.
Comparing faecal parasite egg counts of bighorn sheep, Ovis canadensis, using three different methods: McMaster, Wisconsin and FLOTAC

Introduction
Understanding host-parasite relationships depends on reliable fecal egg counts (FEC). FEC is often used as a non-invasive method to detect gastrointestinal parasite eggs, larvae, oocysts and cysts, that are shed in host feces (Castro 1996). FEC is also referred to as a measure of infection intensity. Infection intensity can determine the cumulative virulence of a parasite, for example, in Monarch butterflies (Danaus plexippus), only individuals with the highest infection intensity of a protozoan parasite, Ophryocystis elektroscirrho, were less active and had smaller wingspans, while when infection intensity was lower (<1000 oocyst per gram), there was no detectable difference in individual behavior or wingspan size (Altizer and Oberhauser 1999). The infection intensity can also affect host fitness by changing reproductive patterns and outcomes (Aleuy et al. 2018). Thus, keeping track of changes in infection intensity of individuals in a population can help monitor individual host and population health (Cringoli et al. 2010). To do this effectively, it is imperative that the tests are sensitive to the specific elements that the parasites shed, as well as being reproducible (i.e., able to reproduce the same results using a subset of the same sample pool), and feasible.

In the past, diagnostic tests have relied on the presence or absence of parasitic elements. However, as previously mentioned, infection intensity can influence the degree to which the host is impacted by a parasite. While ‘presence and absence’ may still be enough to test for parasites in pets and treat individuals, it is not enough if we want to understand parasite-host relationships and monitor population health (Cringoli et al., 2010). To fully understand how multiple parasites and cumulative virulence can affect the host, it is crucial that we can accurately quantify the infection intensity of
each parasite. While there are exceptions, studies support the idea that infection intensity reflects an individual’s parasite burden (domestic sheep – Kingsbury 1965; domestic sheep – McKenna 1981; red grouse, *Lagopus lagopus scoticus* – Seivwright et al. 2004; African buffalo, *Syncerus caffer* – Budischak et al. 2015; white-tailed deer, *Odocoileus virginianus* - Schultz et al. 1993; Svalbard reindeer, *Rangifer tarandus platyrhynchus* - Irvine et al. 2001)

Reliability of parasite counts and thus diagnosis depends on the precision, sensitivity and reproducibility of the tests (Banoo et al. 2006). Reproducibility of diagnostic tests refers to high consistency of test results (repeatability), when sampling from the same sample pool. For example, consistency of results from the same individual removes uncertainty and means that the diagnostic test is reproducible, and a reflection of parasite load (Levecke et al. 2012; Nikolay et al. 2014).

Wildlife populations are frequently infected with multiple parasites at once (MacIntosh et al. 2012). It is crucial, then, that the diagnostic test detects parasitic elements of most, if not all, parasites within the host. Failing to do so would mean that we get a skewed image of parasite loads and their natural host-parasite relationships. However, some diagnostic tests are better suited to detect some parasitic elements but might be less precise with others. For example, when comparing sensitivity of tests on soil-transmitted helminths, Glinz et al. (2010) found that the test was more sensitive to parasite egg detection, when samples had been preserved in sodium acetate-acetic acid-formalin or in an ether-concentration, compared to eggs from fresh fecal samples. Similarly, when conducting FEC on horses (*Equus caballus*) and Llamas (*Llama glama*) on egg-spiked samples, the Wisconsin technique was the most precise methodology for
horse FEC, McMaster was more precise for Llamas FEC, but mini-FLOTAC was more precise for both host species (Paras et al. 2018). As such, there is a clear need to compare the effectiveness of the diagnostic tests for each host species to determine which method is the most sensitive, reliable and cost-effective, when interested in the prevalence and FEC for the parasite cocktail in an individual or host population.

Diagnostic tests such as McMaster and Wisconsin are well-established methods to extract parasite parts, eggs and larvae from fecal matter. The McMaster diagnostic test has an analytical sensitivity range of 10 to 50 EPG, depending on the modification used (Vadlejch et al. 2011). Analytical sensitivity is based on the dilution of the feces and the amount of solution used in the reading chambers to detect FEC such that the researcher must multiple the FEC with the analytical sensitivity to get the eggs per gram of feces (EPG). This makes it a good test when egg counts are high, i.e., when FECs are in 1000s or 10,000s. However, when the egg counts are low (less than 10 EPG), it could fail to detect parasitic elements. The Wisconsin double centrifugation technique, on the other hand, can extract and detect parasite eggs/oocysts at lower densities, and thus is more sensitive (Egwang and Slocombe 1982). The Wisconsin method would be very good for the detection for a thorough investigation of parasite intensities, when FECs are low (Cringoli et al. 2010), but overwhelming and time-consuming, when infection intensity is high.

Weighing the processing of fecal samples to extract parasite diversity and prevalence, the clear winner of the two methods in term of time and consumables is the McMaster technique, which only requires a Sheather’s solution, counting chambers, solution mixing bottle, and a syringe. The Wisconsin test requires a centrifuge, a test tube for
each sample, a Sheather’s solution, microscope slides and coverslips. This means that between the two, McMaster may be the better option when infection intensity is high and you’re working in the field, but Wisconsin could be the better option if the infection intensity is low.

The relatively new diagnostic test, FLOTAC combines McMaster chamber use with the Wisconsin flotation method (Cringoli et al. 2010). FLOTAC also requires a mixing bottle and the reading chambers. However, it does not require a centrifuge, could be easier to set up than McMaster and more cost-effective than Wisconsin to use in the field. Since McMaster and Wisconsin are techniques that have been tested in many studies, both are recommended by the World Association for the Advancement of Veterinary Parasitology ([WAAVP] 1992). FLOTAC has yet to be accepted as a set standard.

Validation studies, similar to this one, have been used to conduct FEC of parasitic elements in different herbivores (i.e., domestic sheep (Ovis aries) infected with Moniezia expansa, Dicrocoelium dendriticum and gastrointestinal (GI) strongyles - Rinaldi et al. 2011; domestic calves (Bos taurus) infected with nematodes (groups not specified) - Levecke et al. 2011; cattle infected with GI nematodes (groups not specified) - Levecke et al. 2012; humans infected with Entamoeba coli, Schistosoma mansoni, Entamoeba histolytica/dispar and Giardia intestinalis - Barda et al. 2014; domestic cattle infected with strongyles - Bosco et al. 2014) with varying results, but with mini-FLOTAC emerging as a clear frontrunner in detecting parasitic elements. However, parasite cocktails are different between ungulate species. Mini-FLOTAC seems to be gaining popularity as an accurate, precise and feasible diagnostic test in domestic
ungulates, but we do not know how accurate it would be in detecting bighorn sheep-specific parasitic elements.

The objective of this paper was to compare bighorn sheep parasite FEC comparing the three methodologies, mini-FLOTAC (FLOTAC), modified McMaster (McMaster) and modified Wisconsin double centrifugation (Wisconsin). My aim was to determine the sensitivity, reproducibility and feasibility of each test for bighorn FECs. Once done, it would help me to select the method that is most reliable for my research (used in chapter 2 and 3). It is expected that there will be differences in the FEC between the tests, due to variations in sensitivity and repeatability of the three tests. I would predict that Wisconsin is most sensitive to parasitic elements, with FLOTAC, and McMaster showing similar levels of sensitivity. All three tests are expected to show similar levels of repeatability, that is, the correlation between replicates from the same test will be high. Finally, FLOTAC will be the most feasible option and the Wisconsin technique will be the most time-consuming method, and less than ideal in field settings.

**Methodology:**

**Study site**

This study was conducted between June to October 2016 with fecal samples collected from individually tagged wild male bighorn sheep (*Ovis canadensis*) within Sheep River Provincial Park, Alberta, Canada (50.63° N, 114.38° W). The climate in the park ranges from below -30°C to above 30°C, with averages of 15°C +/- 5°C degrees during the study period. More than 90% of the population is individually marked with a unique combination of colored and numbered ear tags.
**Fecal sample collection**

In total, I collected 58 samples over 5 months and analysed a sample twice for each of the three techniques \((58 \times 2) \times 3 = 348\) separate FECs, in total. I collected samples from tagged individuals. If individuals in the group were laying down, I drew a laying-association map, placing the individuals within the landscape (also see Chapters 2 and 3 for more details). I, then, waited until the individuals were at least 40 meters away from where they defecated, before collecting fecal samples from known bedding sites. If there was any doubt as to the origin of fecal pellets, i.e., when multiple samples were scattered close together, I did not collect any.

Alternatively, when I saw tagged individuals defecating while foraging or moving, I collected samples within 15 mins of their passing by. All samples were collected within four hours after defecation and stored in a cooler with an ice pack for less than three hours. The samples were then stored in the refrigerator at 4°C until they were processed (within two days of collection).

**Parasitological tests**

Floatation-based diagnostic tests depend on the specific gravity of the solution to detect parasitic elements. Higher specific gravity of the solution allows more different parasitic elements to float. However, higher specific gravities can also distort the appearance and shape of parasitic elements, which can make it difficult to detect (Ballweber et al. 2014). Most parasite species have eggs with specific gravities that range from 1.05 to 1.23 (David and Lindquist 1982). Thus, when the specific gravity ranged from 1.26 and 1.35, FECs of parasites were comparable (O’Grady and Slocombe 1980). As a result, all diagnostic tests in this study used Sheather’s sugar solution with a specific gravity of 1.26.
For this validation research, I am comparing three methods: McMaster, FLOTAC and Wisconsin. Each replicate of McMaster and FLOTAC methods require two grams of feces each, while Wisconsin requires four grams of feces. Since there are two replicates of each method, I measured and separated out 16 grams of feces for each individual. This was manually crushed using a wooden spatula, to mix and homogenize the distribution of parasite eggs in that sample. Once the pellets were crushed, appropriate amounts (2 grams for McMaster, 2 grams for FLOTAC and 4 grams for Wisconsin for each replicate) were measured out and separated for each test and replicate.

*Modified McMaster*

The McMaster technique is modified from Foreyt (2001). Two grams of feces were mixed with 15-ml tap water and poured through two layers of cheesecloth. The strained material was centrifuged for 5 minutes at 1500 rpm and the sediment mixed with 30-ml of Sheather’s solution (Blagburn and Butler 2006). One ml of the solution was transferred to the counting chambers. Since only one ml of the solution was used, the raw counts were multiplied by 30 and divided by the amount of feces (grams); used to get the EPG.

*Modified Wisconsin double centrifugation*

The methods I used followed the Standard Operating Procedure – 3, from the Alberta CWHC Parasitology Lab (WCVM Parasitology diagnostic techniques handbook, 2002). However, we used 4 grams of fecal sample and two-layers of cheesecloth to strain the fecal solution. The raw data were divided by the weight of the fecal sample in grams, to calculate the number of eggs per gram of feces (EPG).
**FLOTAC**

Approximately, 2 grams of feces was taken and thoroughly mixed with 15-ml of Sheather’s solution. Additional Sheather’s solution was added to a total volume of 40-ml. After mixing the additional Sheather’s solution, I poured it into both chambers of the FLOTAC using pipette tips (1 ml each). I waited 15 minutes for the parasitic elements to float to the top of the chamber as the specific gravity of the elements are lower than that of the fluid and were separated from the rest of the chamber and ready to be counted. Since only 2 ml of the solution were used, the raw counts were multiplied by 20, and divided by the amount of feces (2 grams), to arrive at the EPG value.

**Statistical analysis**

I used R (R Core Team, 2019) and the packages ‘ggpubr’ (Kassambara, 2019) and ‘ggplot2’ (Wickham, 2016) to plot graphs. To test the sensitivity of the three methods, a value of 0 was given if no eggs were detected in the sample, and 1, if at least 1 egg was counted. Then, the total number of samples that tested positive (n) was added and divided by the total number of samples (n=58) to get a sample prevalence (percentage). The sample prevalence was used as an indicator of test sensitivity in lieu of actual sensitivity because we did not perform necropsies on the sheep to check if sheep had the worm or not. Additionally, a 100% parasite prevalence was assumed when testing the sensitivity of the techniques. This was to make sure that the baseline was the same across all techniques, I.e., under this assumption, if all three tests are highly sensitive, then all tests should show 100% sensitivity of detecting the prevalence for all parasites. However, there were differences between the prevalence detected in different techniques, and Kruskall-Wallis tests were conducted on the parasite
prevalence of the three diagnostic techniques. Tests are compared to the same baseline.

I conducted a Spearman’s correlation coefficient test on the parasite infection intensity for an association between the two replicates of each parasite group, comparing the three method. Furthermore, an ANOVA was conducted at the Alpha= 5% significance threshold, for each parasite type within each method, to verify the reproducibility of each test over the two replicates

Finally, for feasibility, I discuss the amount of time it took to prepare and read each slide, and the equipment required for each method.

Results
Sensitivity
Of the 58 samples analysed, assuming a 100% prevalence, and using the sample prevalence as proxy for sensitivity, FLOTAC and Wisconsin tests showed the highest level of sensitivity, while McMaster sensitivity range was about half of the other tests for detecting strongyle eggs (Table A10). Sample prevalence of Nematodirus spp. eggs was lowest in McMaster, and highest in the Wisconsin technique (Table A10).

Kruskal-Wallis tests on the data presented in Table A10 showed a significant difference in Marshallagia spp. FEC between the three methods (Kruskal-Wallis chi-squared = 38.476, df = 2, p-value < 0.001). McMaster shows the least sensitivity with the lowest counts in each parasite group, while the Wisconsin method showed the highest sensitivity to parasitic elements. Subsequently, a Kruskal-Wallis test was performed, comparing the results of the FLOTAC and Wisconsin methods. There was a significant
difference between the two methods, FLOTAC and Wisconsin (Kruskal-Wallis chi-squared = 4.4384, df = 1, p = 0.03514), with the Wisconsin technique being more sensitive and able to detect parasitic elements, compared to either McMaster or FLOTAC techniques.

Similarly, there was a significant difference between the three methods in: lungworm larvae counts (Kruskal-Wallis chi-squared = 22.025, df = 2, p-value < 0.001); Nematodirus spp. (Kruskal-Wallis chi-squared = 33.179, df = 2, p-value <0.001) and strongyle (Kruskal-Wallis chi-squared = 72.634, df = 2, p-value < 0.001) egg counts.

Table A10. Sample prevalence in percent, calculated as the total of all positive tests for each methodology, divided by the number of replicates. The replicates came from the same sample pool of feces.

<table>
<thead>
<tr>
<th>Number of animals examined (n) = 58</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Parasite type</th>
<th>FLOTAC (%)</th>
<th>McMaster (%)</th>
<th>Wisconsin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stronglyes</td>
<td>89.70</td>
<td>36.20</td>
<td>91.40</td>
</tr>
<tr>
<td>Nematodirus spp.</td>
<td>36.20</td>
<td>5.20</td>
<td>69.00</td>
</tr>
<tr>
<td>Marshallagia spp.</td>
<td>36.20</td>
<td>6.90</td>
<td>82.80</td>
</tr>
<tr>
<td>Lungworm larvae</td>
<td>43.10</td>
<td>8.60</td>
<td>55.20</td>
</tr>
</tbody>
</table>

**Reproducibility**
Spearman correlation coefficient rank tests showed that there was significant association between replicates 1 and 2 in strongyle egg counts using the FLOTAC and Wisconsin method, but not McMaster (Figure A1). Similarly, association between replicates for Nematodirus spp. And Marshallagia spp. were highest in the Wisconsin tests, followed by FLOTAC and the least in McMaster (Figure A1). However, rather
surprisingly, the association between replicates in lungworm larvae counts was highest when using the McMaster, or Wisconsin method, while FLOTAC showed lower but significant replicability of lungworm larvae counts (Figure A2).
Figure A 1. Strength of association between the two replicates in counting strongyle (top row), Nematodirus spp. (middle) and Marshallagia spp. (bottom) eggs in the three techniques, FLOTAC (A), McMaster (B) and Wisconsin (C). Each axis presents the egg or larval count per gram of fecal samples (EPG). Please note that the scales are different for each test.
Figure A.2. Strength of association between the two replicates in Lungworm Larva counts using the three techniques, FLOTAC (A), McMaster (B) and Wisconsin (B). Based on rho (R), McMaster has the strongest correlation between replicates 1 and 2, while Wisconsin is second and FLOTAC is last. Please note that the scales are different for each test.

Feasibility
McMaster was the fastest technique in preparing an individual slide (about 15 minutes per slide). FLOTAC took about 25 minutes to prepare per slide, including 15 minutes waiting period. Preparation of slides for both techniques were simple and could easily be done in the field. However, having more than 12 slides would add to the preparation
time of both techniques. While in Wisconsin, the greater the number of samples, the less time it took per slide to prepare. This is because in Wisconsin, the greatest amount of time is spent waiting for two rounds of centrifugation (25-30 mins in total). This means that while it took 40 mins to prepare 2 slides (thus, 20 mins per slide), it took only 90 to prepare 12 slides (thus, 7.5 mins per slide).

McMaster and FLOTAC techniques have thicker flotation counting chambers, compared to Wisconsin. This added to the slide reading time as each screen had to be focused at different levels of depth to allow a thorough parasite count. While there were some adjustments required for Wisconsin slides, such adjustments were minor and did not apply to each screen view. Thus, reading was faster in Wisconsin, compare to the other two methods. However, since the Wisconsin slides contain most or all parasitic elements in the feces, the reading time for the overall slide was comparable to the other two techniques.

Finally, McMaster and Wisconsin slides could be viewed using any microscope, however, FLOTAC chambers could only be viewed under a specific microscope, due to the size and shape of the reading chambers. This severely limited ease of access (since other people were using the microscope for other projects) of this technique, as the microscope is not available at the field site and very costly to buy.

Discussion
Testing sensitivity (using sample prevalence as proxy), reproducibility and feasibility of the three techniques, McMaster, Wisconsin, and FLOTAC, allowed me to examine and evaluate each test and determine which diagnostic test would be best suited for further field research.
Based on sample prevalence, Wisconsin is the most sensitive test in detecting parasitic elements of the parasites (strongyle, lungworm, *Nematodirus* spp., and *Marshallaia* spp.). The Wisconsin technique could be picking up false positives, however, the other two methods are more likely to showing false negatives, since a) there is a stronger association between the replicates when using Wisconsin and b) Wisconsin method is a reliable diagnostic test that captured more than 60% of strongyle parasitic elements in an egg spiking experiment (Egwang and Solcombe 1982). Currently, multiple versions of the McMaster technique are used in veterinary parasitology to evaluate success of anthelminthic drugs (Coles et al. 2006). However, while the McMaster method is easy to use in the field, sensitivity of the test to parasitic elements in bighorn sheep was low compared to the FLOTAC and Wisconsin techniques. This low sensitivity could be due to the lower total egg counts in the feces, since McMaster is used when parasite egg densities are high (in the thousands or tens of thousands), while the FEC in bighorn sheep was rarely above 100 in my samples.

FLOTAC has a similar analytical sensitivity of 20 EPG. Compared to McMaster and Wisconsin, FLOTAC has medium sensitivity to parasitic elements. Other studies have shown that FLOTAC is sensitive to soil-transmitted helminth parasites (Nikolay et al. 2014) and is comparable to McMaster (Bauer et al. 2010; Rinaldi et al. 2010 and Levecke et al. 2012). In those studies, FLOTAC was measured against McMaster and Kato Katz (another field technique that requires specific equipment. It requires glycerine-malachite-green solution as well as hydrophyllic cellophane instead of Sheather’s solution) (Katz et al. 1972), but not Wisconsin. However, in this study Wisconsin showed the greatest sensitivity in detecting parasitic elements. Wisconsin is
reliable to use at lower parasite densities, but when the count is high, it lacks precision (Egwang and Slocombe 1982). In fact, reliability of McMaster and FLOTAC increased with egg count, while Wisconsin reliability decreased with increasing egg count (Rinaldi et al. 2010).

Additionally, a technique used to detect parasitic elements must be reproducible, so host health can be monitored over time. This study had two replicates from the same sample in each of the three tests and there was a strong association between replicate 1 and 2 for all egg counts using the Wisconsin and a strong association for FLOTAC for most egg counts, while the association was weaker in McMaster. This implies that if a treatment was applied to an individual, the resulting egg count from Wisconsin and FLOTAC would be reliable but results from McMaster could underestimate remnant parasites that were not killed by the treatment.

The equipment required for Wisconsin can be a bit more difficult to get access to when working in the field, however, depending on the number of samples, it can also be the most time-efficient technique. The centrifuge stocking capacity is the main limiting factor, when it comes to efficiency of Wisconsin. In this study, the centrifuge only had 12 test-tubes. When there were at least 12 samples, it took 7.5 minutes to prepare each slide. Compared to this, preparation of each sample took 15 minutes for McMaster and 25 minutes for FLOTAC. Thus, in terms of preparation time, Wisconsin was the fastest technique and FLOTAC was the slowest.

Overall, Wisconsin and FLOTAC were highly sensitive to the parasitic elements in bighorn sheep. Wisconsin and McMaster had more reproducible results. Although, it
should be noted that the reason for a high degree of reproducibility for McMaster was because of its low sensitivity. That is, while FLOTAC was not reliable in terms of being reproducible, McMaster just did not pick up the parasitic elements that the other two did, even if only in one replicate. Finally, Wisconsin seems to be the most time efficient, even though it requires higher costs for the initial equipment to be acquired. Furthermore, the more samples there are, the less time Wisconsin seems to take, while the reverse is true for McMaster and FLOTAC. As such, in the case of the four parasites, strongyle, Nematodirus spp., Marshallagia spp., and lungworms, examined in this study, Wisconsin is the one that sticks out as the most sensitive, reproducible and time efficient method.
Literature Cited


Kassambara A. 2019. ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.2.2. https://CRAN.R-project.org/package=ggpubr


Appendix B
Distribution of data collected for chapter 2 and chapter 3.

Table B 1. Data distribution for chapter 2: mean fecal egg count for males and females for each month, including sample size per month and combined sample size for each season (winter – October to March; non-winter – April to September). Please note that '-' signifies lack of data for those months, since the sample size was 0.

<table>
<thead>
<tr>
<th>Sex</th>
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<th>May</th>
<th>June</th>
<th>July</th>
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<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
<th>January</th>
<th>February</th>
<th>March</th>
<th>Sample size (n)</th>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>-</td>
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<td>-</td>
<td>2.64</td>
<td>2.50</td>
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<td>7.20</td>
<td>8.00</td>
<td>5.36</td>
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<td>4.67</td>
<td>8.00</td>
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Table B 2. Data distribution table for chapter 3: sample size, mean fecal egg count and mean percent of time spent in activity for each sex (M = male and F = female) and month from May to November 2019. Please note that '-' signifies that data is not available for that month, since sample size was 0.

<table>
<thead>
<tr>
<th>Sample size (n)</th>
<th>Sex</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
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<tr>
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<td>67</td>
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Mean fecal egg count

<table>
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<th>Sex</th>
<th>May</th>
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<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
</tr>
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<tbody>
<tr>
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<td>-</td>
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<td>0.38</td>
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Mean percent of time spent in activity (%)

<table>
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<th>October</th>
<th>November</th>
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