Fecal counts of lungworm larvae and reproductive effort in bighorn sheep, *Ovis canadensis*

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Because parasite resistance and reproduction require metabolic resources, life-history models predict a tradeoff between current reproduction and parasite load. These tradeoffs have been widely studied in birds, but few studies have been conducted on mammals. We monitored lungworm (*Protostrongylus* spp.) larvae counts in bighorn sheep (*Ovis canadensis*) over four years to examine how individual differences in fecal output of lungworm larvae (LPG) by yearlings and adults were affected by season, sex, body mass, age and reproductive effort. We also compared lamb mass at six months and LPG. Overall, we found that LPG varies seasonally, peaking in females prior to lambing and in males during the rut. Age had no effect on LPG for either sex. During autumn, we found no effect of age or mass on LPG for sheep one year and older. Lamb body size or sex did not affect LPG. Females that weaned a lamb had higher counts than females that did not produce a lamb or females whose lamb died during summer. For rams, social rank and testosterone levels were not related to LPG but LPG increased with time spent searching for estrous ewes during the rut. Our results suggest a tradeoff between parasite resistance and reproductive effort in bighorn sheep of both sexes.

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A basic assumption of life-history theory is that because resources are limited individuals face tradeoffs in energy allocation to reproduction, maintenance and other processes, including parasite resistance (Roff 1992, Stearns 1992). Recent research suggests that parasite resistance is metabolically costly (Lochmiller and Deerenberg 2000, Bonneaud et al. 2003, Hanssen et al. 2004) and may involve a tradeoff with other life-history traits (Stearns 1992). Parasites can affect their host's life-history by increasing the costs of reproduction or by decreasing reproductive potential. Alternatively, if the host's ability to resist infection is weakened by reproductive effort, increased parasite infection or greater parasite reproductive output could be manifestations of reproductive costs. Studies comparing parasite infection with individual reproductive effort would be

particularly useful to divide causes and consequences of different levels of parasite infection. Reproductive effort has been associated with increased parasitism in fish (Skarstein et al. 2001), lizards (Sorci et al. 1996) and in several species of birds (Gustafsson et al. 1994, Norris et al. 1994, Oppliger et al. 1996, Merila and Andersson 1999) but very few studies have investigated the relationship between individual reproductive effort and parasites in ungulates (Festa-Bianchet 1989, Mulvey et al. 1994, Wilson et al. 2004), and no study has included data on males.

Experimental studies on birds have reinforced the idea that high reproductive effort may lead to an increase in the intensity of parasitism (Gustafsson et al. 1994, Oppliger et al. 1996, Saino et al. 2002) and therefore parasites could be an additional reproductive cost. On the

other hand, studies of birds suggested that high ectoparasite burdens can decrease an individual's ability to reproduce (Oppliger et al. 1994). In mammals, a study on bighorn sheep ewes (*Ovis canadensis*), revealed a positive association between parasites and host reproduction. The fecal output of lungworm (*Protonstrongylus* spp.) larvae was higher in spring for pregnant ewes and for females that had weaned a male lamb (sons are costlier to produce in this species; Hogg et al. 1992, Bérubé et al. 1996) during the previous year, compared to ewes that were not pregnant or that had weaned a daughter (Festa-Bianchet 1989). Therefore, because lactation is costly for female mammals (Robbins 1993), we predicted that in autumn, lactating ewes should shed higher number of lungworm larvae than non-lactating ones.

If there is a tradeoff between parasite resistance and reproductive effort, male reproductive effort should be related to parasite output during the rut. Measuring male reproductive costs, however, is difficult. There is no single physiological measure of reproductive effort that takes into account all factors likely to affect reproductive cost (Stearns 1992), but evidence of tradeoffs between parasite output and reproductive behaviour of marked individuals should provide insights on reproductive costs. Bighorn rams in our study used two mating tactics. 'Tending' is a form of mate guarding where one male defends one estrous female against other rams for 12 to 36 hours (Hogg 1984, 1987, Pelletier 2005). Only the most dominant ram among those courting a ewe can tend her. If a more dominant ram approaches, it will take over the tending role. 'Coursing' is a mating tactic used by subordinates, where one or several males attempt to physically displace the dominant ram and initiate a rapid chase of the female, attempting to force copulations before the tending ram regains control (Hogg 1984, 1987, Pelletier 2005). During the rut, rams can also spend much time searching for estrous females. Alone or in groups of two or three, searching males spend most of their time walking (Pelletier 2005). We predicted that each male's involvement in coursing, searching or tending should affect its lungworm output during the rut. Specifically, we expected that searching would be costlier than coursing or tending because searching rams incur higher foraging constraints (Pelletier 2005).

Several others factors can lead to heterogeneities in worm burden, including host sex, body condition, age, behaviour, genetics and season (reviewed by Clayton and Moore 1997, Wilson et al. 2001). Males usually exhibit a higher susceptibility to parasites and diseases than females, possibly because of sex differences in immunity (Cohn 1979, Grossman 1985, Zuk 1996). Mature individuals tend to have lower parasite counts than juveniles, while individuals in poor condition often show an increase in parasites (Wilson et al. 2001). Because few studies have monitored lifetime changes in parasite levels in free-ranging marked individuals, it is not clear if lower parasite infections for older individuals are true age-related changes or result from greater survival of individuals with lower infections. For Soay sheep (*Ovis aries*), fecal egg counts of stongyles increase shortly after exposure (at six months of age) then decline as sheep age due to immune responses (Wilson et al. 2004).

Bighorn sheep (*Ovis canadensis*) are infected by two species of nematode lungworms, *Protostrongylus stilesi* in the lung parenchyma and *P. rushi* in the bronchi and bronchioles (Anderson 2000). Both species have an indirect life cycle; larvae are shed in feces and infect snail intermediate hosts (Anderson 2000). The peak of excretion of larvae by ewes and very young males is in late winter (Uhazy et al. 1973, Festa-Bianchet 1991), but little is known about seasonal differences in larval output for adult rams.

Here we examine whether individual differences in fecal output of lungworm larvae in bighorn sheep are related to reproductive effort and to individual characteristics such as body mass and age. We predicted that peak parasite output should be associated with the period of highest reproductive expenditure relative to resource availability for each sex: the rut for males and late pregnancy for females. Although the greatest expected energy cost for females occurs during lactation (Robbins 1993), bighorn ewes have access to high quality forage during lactation and gain mass (Festa-Bianchet et al. 1996), while in late gestation they feed on low-quality overwintered forage (Festa-Bianchet 1988b). Therefore, the instantaneous negative effect on parasite resistance is likely greater during late pregnancy than during lactation. We then investigate the relationship between reproductive effort and LPG at the individual level for both sexes. We compared autumn larval counts for ewes that did and did not wean a lamb, expecting that those that weaned a lamb should have higher counts. For rams, we compared the degree of participation in three mating tactics during the rut (searching, coursing and tending) with LPG, expecting higher LPG for the most active individuals. To investigate the relation between larval output and individual characteristics, we examined the effects of body mass, age and sex on LPG. We also compared body mass and LPG in lambs. Finally, we compared ram social rank and testosterone levels to LPG.

Methods

Study area and population

We studied a population of marked bighorn sheep wintering in the Sheep River Provincial Park in southern Alberta from September 2000 to December 2003. Since 1981, more than 95% of these sheep have been marked with Allflex ear tags and the population dynamics have been monitored (Festa-Bianchet 1986, Loison et al.

1999). In autumn, lambs aged 4–6 months are captured by chemical immobilization (Festa-Bianchet and Jorgenson 1985, Jorgenson et al. 1990) and therefore the exact age of each marked animal is known. Since the beginning of the study, chest girth (cm) was measured for 423 captured lambs and lambs were weighed at capture.

Reproductive effort

Lamb-ewe relationships were established by observing three or more successful suckles. We considered that a ewe had successfully weaned a lamb if the lamb survived to 1 October. Females never seen with a lamb or whose lamb died before October were considered to be non-lactating. Of 56 lambs that died during summer in 2000–2003, 54 disappeared before the end of August. Body mass of free-ranging sheep was also measured with an electronic platform scale baited with salt (Bassano et al. 2003). Adult sheep did not gain mass during October and November (Pelletier and Festa-Bianchet 2004), thus we did not adjust body mass according to date of weighing. For sheep weighed more than once, we used the average mass. During the rut, we located all males present in the park twice each day and determined if they were searching, coursing or tending by observing their behaviour and the presence or absence of females near them (see Pelletier 2005 for further details of behavioral observations). We used those twice daily observations to estimate the proportion of time a male was involved in each rutting tactic.

Parasite counts

Fecal samples were collected from known individuals between the 13th and the 17th day of each month from September 2000 to December 2003, excluding January, February and March. The number of samples collected varied monthly depending of the number of sheep present in the park. For each sample, date, time, identity, sex and age of the sheep were noted. Samples were dried and stored in paper bags. Lungworm first stage larvae per gram of dried feces (LPG) were counted using the Beaker technique (Forrester and Lankester 1997a, 1997b). Because L_1 larvae of the two species of lungworms present no morphological differences (Samuel and Gray 1982), larval counts included both species. All counts were done within six months of collection by the same observers for all the study period. The hour of collection did not affect LPG ($F_{1,1228} = 0.128$, $P = 0.721$), thus, it was excluded from further analysis.

Testosterone analysis

Fecal samples collected from rams in September to December from 2001 to 2003 were analyzed for testosterone content (nanograms per gram of feces), determined by radioimmunoassay (Pelletier et al. 2003).

Male social rank

A detailed explanation of how social rank was obtained is provided in Pelletier et al. (2003, 2004). Briefly, rank was determined using agonistic encounters during the pre-rut. Each year, a matrix was built based on all encounters, and Matman 1.0 (de Vries et al. 1993) was used to first test the significance of the linearity in the matrix and then rank individual rams.

Statistical analyses

Reproductive components and LPG
Our data included repeated counts of lungworm larvae for the same individuals within and between years. Therefore, we used linear mixed models including sheep identity as a random effect (Paterson and Lello 2003). Because year of collection may affect LPG, we included it in each model before fitting other terms. To investigate the effect of seasonality on LPG, we used lungworm counts of 2000–2003 and fitted a model including year, age, sex and season and their second order interactions as independent variables. We considered 3 periods: April and May, when larval counts peak for females (Festa-Bianchet 1991), June to October, the period with the lowest LPG, and November and December, when the rut takes place (Pelletier 2005), and males were expected to show a peak in larval output (Gaudernack et al. 1984, Halvorsen et al. 1985). To avoid the confounding effect of seasonality in larval output, all further analyses were limited to larval counts from samples collected in September and October, except for the analysis of LPG change during the rut for rams that included data from December.

To investigate how weaning a lamb affected the larval output of ewes we used fecal larval counts from September and October from 2000 to 2003. We used mixed effect models with year and reproductive status (presence or absence of lamb) as fixed factor and ewe identity as random term. We also tested for an effect of the month of collection, ewe age and the interaction between age and reproductive status. Because age did not affect LPG in any analyses, however, we excluded it from the final models.

For rams, we compared the proportion of time spent searching for estrus ewes, coursing and tending with the change in fecal larval output during the rut. Because we

were interested in how ram behavior during the rut affected larval output, we calculated for each ram the change in LPG between the 15th of November (just before the beginning of the rut) and the date when 50% of ewes had been observed in estrus. Because the times spent in each activity are not independent, we used separate mixed models to investigate the effect of each behaviour on parasite output. Year was always fitted first as a fixed factor and ram identity as a random effect. Because this analysis required multiple comparisons (3), we applied a Bonferroni correction and considered 0.016 as the critical p-value for this analysis (Sokal and Rohlf 1981). We could not simply use mating tactics as categories because one ram can use more than one tactic within the same rut.

To investigate the relation between LPG and body mass, we only examined autumn (September–October) larval counts because body mass was only measured in autumn. Because bighorn sheep show a strong seasonality in body mass (Festa-Bianchet et al. 1996), comparisons of autumn mass with spring or summer larval counts may be inappropriate. We fitted a model including year, sex, mass, age and their second order interactions as independent variables. Sheep ID was included as a random effect.

To compare lamb body mass and LPG, we used the chest girth measurement (cm) taken at capture. Chest girth was highly correlated with body mass for those lambs with both measurements available (N =69, r =0.90, p <0.001). Chest girth was available for most captures (N =423 since 1981), while mass was rarely measured. Lamb chest girth increased during autumn (females N = 222, r =0.45, b =0.100 cm day^{-1}, P < 0.001; males N =201, r =0.42, b =0.107 cm day^{-1}, P <0.001), therefore, we adjusted girth to November 20 using the slopes of sex-specific linear regressions of girth on capture date. Because data on lamb mass were only available in autumn, we restricted our analysis to autumn larval counts. We fitted a mixed model including lamb identity as random effect and year, sex, chest girth and the interaction between chest girth and sex as fixed terms.

For adult rams, the effects of social rank and testosterone level on LPG were examined using mixed effect models including identity as a random term.

Because lungworm larval counts have a skewed distribution (Uhazy et al. 1973, Festa-Bianchet 1991), they were transformed with natural logarithm (ln (LPG +1) before statistical analysis, (Sokal and Rohlf 1981). To facilitate interpretation, however, all graphs and averages refer to untransformed data. Normality and homogeneity of variance were checked for all models. All analyses used S-PLUS 6.0 for Windows (Mathsoft inc.).
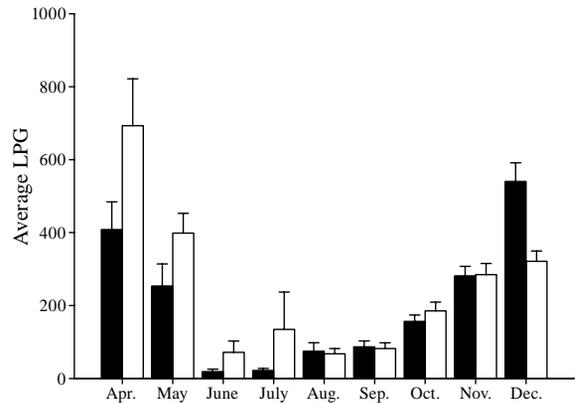

Fig. 1. Average LPG±SE for male and female bighorn sheep one year and older from April to December, 2000–2003, Sheep River Provincial Park, Alberta. Black bars refer to males and open bars refer to females.

Results

Reproductive components and LPG

Population level

Fecal output of lungworm larvae for sheep one year and older showed strong seasonality (Fig. 1). Consistent with an earlier study of this population (Festa-Bianchet 1988a), lungworm prevalence was 100% (N =104 sheep one year and older and 48 lambs). Variability in LPG from individual fecal samples was high in all seasons (range in spring =0–3372; in summer =0–1458 and in early winter =0–3169). Season had a significant effect on LPG, with a significant interaction with sex (Table 1). Females had higher counts than males in spring (mean±SE for untransformed LPG: females = 518±62.8, males =310±47.7), both sexes had low counts in summer (females =126±13.4, males =95± 8.8) and males had higher counts than females during early winter (females =302±21.0, males =385±27.3). There was no consistent effect of sex on LPG, because males and females had high counts in different seasons (Fig. 1). There were no significant effects of age or of the interaction between age and season (F$_{2,1105}$ =0.77, P =0.460). Although all subsequent analyses are re-

Table 1. Linear mixed effect model for the seasonality in fecal counts of *Protostrongylus* spp. larvae for bighorn sheep yearlings and adults at Sheep River Provincial Park, Alberta, 2000–2003. The model included 1220 samples from 104 individuals and explained 25.16% of the variance. 'Periods' were May–April, June–October and November–December.

Variables	F-value	p-value	df
Intercept	2860.78	<0.001	1, 1107
Year	2.69	0.045	3, 1107
Period	224.61	<0.001	2, 1107
Sex	0.411	0.523	1, 102
Age	0.771	0.380	1, 1107
Period × sex	14.36	<0.001	2, 1107
Age × sex	3.33	0.068	1, 1107

stricted to samples collected in September and October to avoid these complex seasonal effects, in all cases analyses using unadjusted values for samples collected from September to December gave very similar results.

Individual level

Lactating females had higher larval counts in September–October than non-lactating females ($F_{1,121} = 5.95$, $P = 0.016$, $N = 168$ samples from 43 ewes) and this difference was maintain until December (Fig. 2). Maternal LPG was not affected by the sex of the lamb weaned ($F_{1,55} = 1.11$, $P = 0.296$) or by age ($F_{1,120} = 0.35$, $P = 0.556$). Restricting the analysis to females three years and older confirmed that lactating ewes had higher LPG than not lactating ones ($F_{1,109} = 12.11$, $P < 0.001$). In males, only the time spent searching for estrus ewes affected the change in LPG during the rut ($F_{1,18} = 10.73$, $P = 0.004$, Fig. 3) and the relation remain significant after accounting for age effects (age: $F_{1,17} = 8.40$, $P = 0.010$ and searching: $F_{1,17} = 11.41$, $P = 0.003$). The time spent coursing ($F_{1,18} = 0.13$, $P = 0.72$) and tending ($F_{1,18} = 2.38$, $P = 0.14$) had no significant effects. The critical P-value for these comparisons was 0.016 after Bonferroni correction (sample size was 47 observations on 28 different rams). Year did not affect the change in LPG during the rut ($F_{3,15} = 1.30$, $P = 0.310$) and there was no interaction between searching time and age ($F_{1,16} = 0.23$, $P = 0.639$).

Individual characteristics and LPG

Body size and sex

In September and October, LPG was not affected by sex ($F_{1,58} = 1.77$, $P = 0.189$), body mass ($F_{1,123} = 0.58$, $P = 0.450$) or by the interaction between sex and mass ($F_{1,123} = 1.10$, $P = 0.295$, $N = 188$ counts for 60 individuals one year and older). Larval counts for 6 months
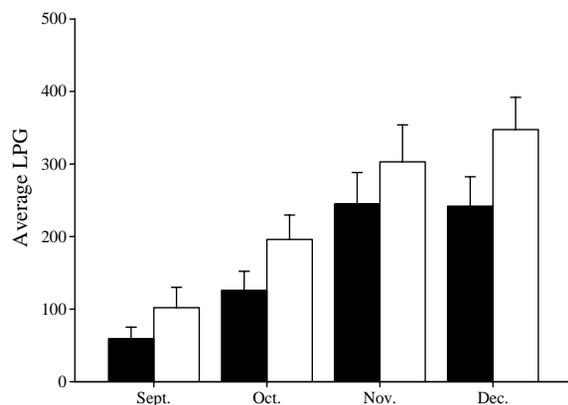

Fig. 2. Average LPG+SE for lactating (open bars) and non-lactating (black bars) bighorn ewes from September to December, Sheep River Provincial Park, Alberta, 2000–2003.
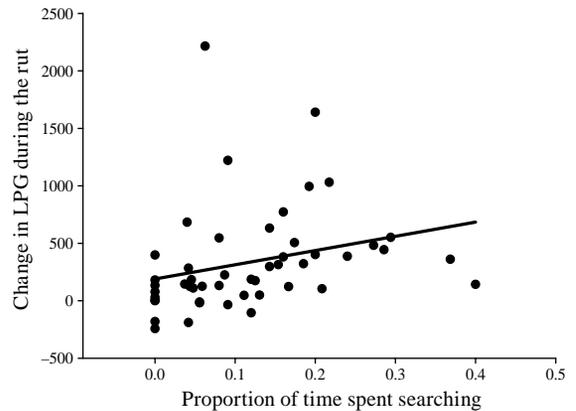

Fig. 3. Change in number of lungworm larvae during the rut compared to the proportion of time spent searching for ewes by bighorn rams one year and older at Sheep River Provincial Park, Alberta, 2000–2003. Sample size was 47 observations from 28 different individuals. Differences in searching behavior explained 15.3% of the total variance according to a linear mixed model.

old lambs ($N = 137$ samples from 46 individuals) were unrelated to sex ($F_{1,39} = 0.13$, $P = 0.720$, mean untransformed LPG$\pm$SE, females $= 259 \pm 37.1$, males $= 298 \pm 28.9$) or to chest girth ($F_{1,39} = 0.06$, $P = 0.804$) and there was no interaction between chest girth and sex ($F_{1,39} = 0.63$, $P = 0.431$).

Social rank and testosterone

For rams aged one year and older, we compared social rank and LPG during the pre-rut (September and October). Because age and social rank are highly correlated (Pelletier et al. 2003), we used the residuals of the regression between age and social rank. We found no effect of age ($F_{1,140} = 0.31$, $P = 0.579$), residual of social rank ($F_{1,140} = 0.01$, $P = 0.947$) nor any interaction between age and residual of rank ($F_{1,140} = 0.36$, $P = 0.547$) on LPG ($N = 197$ counts for 51 rams). We also compared testosterone level and LPG during the pre-rut for males one year and older and found no effects of age ($F_{1,110} = 0.25$, $P = 0.621$), testosterone level ($F_{1,110} = 1.67$, $P = 0.199$) nor any interaction between age and testosterone level ($F_{1,110} = 3.18$, $P = 0.078$) on LPG ($N = 158$ counts for 45 rams).

Discussion

Much of the variability in lungworm larval counts can probably be attributed to stochastic factors such as changes in daily fecal production and individual variation in either the initial dose of larvae received transplacentally (Hibler et al. 1972, 1974) or in the number of infected snails ingested during summer. By monitoring the larval output of marked individuals in several fecal samples collected over different months and years, however, we can identify biologically meaningful

variables that affect larval counts (Festa-Bianchet 1991). Mixed models can reveal biological processes that underlie parasite infection, because they avoid the pseudoreplication often encountered with parasitological data and instead account for possible individual differences in susceptibility (Paterson and Lello 2003).

At the population level, we found strong sex-specific seasonal cycles in lungworm excretion. In both sexes, the lowest counts were found during summer but the peak in excretion was in late gestation for females and during the rut for males. At the individual level, we also found an effect of reproductive effort on lungworm larval output. Lactation for females and searching behavior for males led to greater LPG. Individual characteristics such as age or mass, chest girth or sex of lambs, or rank and testosterone level for rams, were not correlated with lungworm output. The lack of sexual difference in LPG among lambs is somewhat surprising, given that by the following spring male lambs have higher LPG than female lambs (Festa-Bianchet 1991), and may be due to small sample size. Alternatively, sexual differences in LPG among weaned lambs may only be evident in years of low resource abundance, or may be masked by greater maternal care for sons than for daughters (Hogg et al. 1992, Bérubé et al. 1996). Reproductive effort was the only variable associated with LPG in our study, suggesting that tradeoffs between reproductive effort and parasite resistance may be important for bighorn sheep.

The sexual difference in seasonality in parasite output was consistent with other studies on ungulates (Gaudernack et al. 1984, Halvorsen et al. 1985). Peak larval excretion for each sex coincided with the period of greatest relative reproductive effort: late gestation for ewes and rut for rams. Our results therefore suggest that bighorn sheep of both sexes may face a trade-off between immunity and reproduction (Festa-Bianchet 1989). Results at the individual level also suggest a relationship between reproductive effort and parasite excretion, because lactating ewes showed higher LPG during autumn and the increase in LPG for rams during the rut was related to individual level of activity. These results are consistent with earlier findings by Festa-Bianchet (1989) who reported that pregnant ewes had higher larval counts than not pregnant ones in late winter and provide support for the hypothesis that *Protostrongylus* larval output is driven by tradeoffs between reproductive effort and parasite resistance. Therefore, changes in larval counts are more likely to be associated with differences in reproductive output by a sheep's lungworms than with differences in the intensity of infection.

Comparisons with similar species are difficult because little is known about the relationship between reproductive effort and parasites in mammals. Our results are consistent with several studies of birds where reproductive effort of females was manipulated by enlarging brood size. Oppliger et al. (1996) showed that female great tit (*Parus major*) with larger broods suffer from higher prevalence of *Plasmodium spp.* Gustafsson et al. (1994) also found a significant interaction between experimentally increased reproductive effort and parasite infection in collared flycatchers (*Ficedula albicollis*). A positive relationship between host reproductive effort and parasite load has also been reported for barn swallow (*Hirundo rustica,* Møller 1993). In our study, bighorn rams that spent more time searching for estrus ewes during the rut experienced a greater increase in lungworm larval output during the rut. Thus, several observational and manipulative studies suggest that parasitism increases in response to host reproductive effort.

Our measure of reproductive effort for rams may not be sufficiently precise to detect differences between coursing and tending rams. Nevertheless, we suggest that changes in larval output are more likely to be associated with differences in the reproductive effort of individual rams than with differences in mating tactics. Because the numbers and age structure of both potential competitors and potential mates may change from year to year, the number of fights or coursing chases can also change drastically from one year to another. Although more studies are needed to investigate male reproductive effort and to determine how male reproductive effort is affected by changes in demography, our results suggest that differences in parasite activity are related to male reproductive effort but not necessarily to male reproductive success. Tending is by far the most successful mating tactic for bighorn rams (Hogg and Forbes 1997), but it was not associated with an increase in larval output. Alternatively, an increase in the number of lungworm larvae for searching rams could be due to higher exposure to parasites. Reinfection of bighorn sheep by *Protostrongylus* spp. occurs by ingestion of infected snails (Anderson 2000). The rut is late in November, early December. In most years, at that time the ground is covered with snow and it seems unlikely that snails would still be active aboveground. Moreover, during the rut, males decrease the time spent foraging, especially when they are searching (Pelletier 2005) Therefore, it seems unlikely that reinfection may explain the increase in larval output by rams during the rut.

Our study reveals that for bighorn sheep, reproductive effort plays an important role in *Protostrongylus* larval output for both sexes. Parasites are costly for their host because they may use resources that could be channeled into other functions. Consequently, parasites are likely to increase the cost of host reproduction, leading to tradeoffs between reproductive effort, other life-history traits and parasite resistance.

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