

Original Article

Macroparasitism influences reproductive success in red squirrels (*Tamiasciurus hudsonicus*)

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Life-history theory predicts that all organisms have finite energy reserves. In order to optimize fitness, individuals must make trade-offs in allocating energy among survival, growth, and reproduction. Parasites have the ability to negatively impact host fitness and shift the balance of energy trade-offs. The aim of our study was to determine the relationships among parasite load and reproductive success in a free-living population of North American red squirrels (*Tamiasciurus hudsonicus*) in Algonquin Park, Ontario. We hypothesized that heavily parasitized individuals must allocate more energy toward immune function. As energy storage is finite, this will inevitably reduce the resources available for reproduction. Here, we show that parasite richness can compromise fitness through decreasing reproductive success. However, ectoparasite intensity increased with increasing reproductive success in males but not females, suggesting a possible trade-off between secondary sexual characteristics in males and immunosuppressive qualities of testosterone. Our study provides unique evidence of the costs parasites exact on their hosts. It is among the relatively few studies conducted within an ecological context incorporating host fitness in relation to multiple parasite infections. *Key words*: fitness, life-history traits, mammals, parasite richness. [*Behav Ecol* 22:1195–1200 (2011)]

INTRODUCTION

Life-history theory predicts that organisms have finite energy reserves, and thus, to optimize fitness, individuals must make trade-offs in allocating energy among survival, growth, and reproduction (Stearns 1976; Roff 2002; Morand et al. 2006; Schwanz 2008). Shifts in the balance of these trade-offs and negative impacts on fitness can be caused by ubiquitous parasitic infections (reviewed in Morand et al. 2006; Thomas et al. 2009). By extracting energy and nutrients from hosts (Khokhlova et al. 2002), and indirectly by increasing immune activity (Wedekind 1992; Lochmiller and Deerenberg 2000), parasites can directly influence the life-history dynamics of their hosts. Mortality, restricted growth, reduced reproductive function, and poor offspring quality are among the many costs of parasitism (e.g., Lochmiller and Deerenberg 2000; Zuk and Stoehr 2002; Michalakis 2009). These costs have been observed across species (Fox and Hudson 2001; Hurd 2001; Neuhaus 2003; Schwanz 2008).

Secondary impacts of parasite infection can impair growth and reproductive functions either by direct damage or a decrease in available nutrients (Sheldon and Verhulst 1996; Norris and Evans 2000; Zuk and Stoehr 2002; Schwanz 2008). Ectoparasites can damage the skin and inject salivary toxins into feeding wounds as well as become vectors of microparasite infections (reviewed in Degen 2006). In particular, mites cause irritation to the skin, fur, and feathers, whereas fleas and ticks feed off of host blood and tissue (reviewed in Degen 2006). Endoparasites can compete with the host for nutrients and, in the case of intestinal helminth worms, can damage the gastrointestinal tract as well as instigating an immune reaction (reviewed in Degen 2006). As a result, the amount of energy

available to the host is reduced (Munger and Karasov 1989; Kristan and Hammond 2004), the host loses body mass and experiences reduced body condition (Khokhlova et al. 2002; Meagher and Dudek 2002). In order to compensate, hosts may divert any remaining energy into maintenance, therefore limiting reproductive activities (reviewed in Agnew et al. 2000; Lochmiller and Deerenberg 2000).

Although the negative impacts of parasites are well recognized, the response to infection may differ between the sexes (e.g., Moore and Wilson 2002; Ferrari et al. 2004; Scantlebury et al. 2010). Physiological differences in hormones and immunity as well as reproductive strategies between females and males may lead to sex-biased parasitism (Moore and Wilson 2002). Typically, males inhabit larger territories, which inevitably can lead to increased susceptibility to infection (e.g., Brown et al. 1994; Ferrari et al. 2004).

However, there are also many examples, where no costs of parasite infection were observed on host fitness traits (Pacejka et al. 1998; Bouslama et al. 2001; Dowling et al. 2001; Schmid-Hempel 2003; Voordouw et al. 2008). The recent increase of field-based investigations of the cost of multiple parasite infection on host energy trade-offs illustrates the need to understand these dynamics within an ecological context (Neuhaus 2003; Yacob et al. 2006; Scantlebury et al. 2007; Heylen et al. 2009).

The North American red squirrel (*Tamiasciurus hudsonicus*) is a small, polygynandrous semiarboreal rodent inhabiting coniferous dominant forests of North America. Previous studies have identified red squirrels to be the host of both ecto- and endo-macroparasites (fleas, *Orchopeas cadens* and *Opisodasys pseudoarctomys*; helminth worms, *Strongyloides* sp.; Gorrell and Schulte-Hostedde 2008). Here, we determine the relationships among parasite load (internal and external parasites) and reproductive success in a free-living population of red squirrels. We hypothesized that in order to compensate for the costs of infection, energy should be diverted from reproductive function. Hence, we predicted that high levels of parasite infestation would lead to reduced reproductive success.

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MATERIALS AND METHODS

All methods and procedures were conducted according to an animal use protocol approved by the Laurentian University Animal Care Committee (2009-03-01).

Field methods

A marked population of red squirrels was sampled within a 23-ha grid of mixed deciduous forest at the Wildlife Research Station in Algonquin Provincial Park, Ontario (lat 45°30'N, long 78°40'W) between late April to late August, 2009. Tomahawk live traps (Tomahawk Live Trap Co., Tomahawk, WI) were set 40 m apart and approximately 1.5–2 m from the ground on a wooden platform (60 × 15 × 1.5 cm). Each trap was equipped with a sheet of aluminum covering the top and sides and polyester bedding inside to discourage predators and to reduce exposure of trapped animals to inclement weather (Gorrell and Schulte-Hostedde 2008). Traps were set at dawn 5–6 days each week, baited (10 g mixture of peanut butter and oats with a thin slice of apple) and checked twice daily (early and late morning).

On capture, each red squirrel was transferred into a handling bag where it was sexed, aged, weighed (Pesola scale; mass ± 0.1 g), and marked with a numbered ear tag (model 1005-1; National Band and Tag, Newport, KY) for future identification and long-term tracking. All tools and tissue were disinfected prior to tagging. Age category was determined based on mass (gram) (adults > ~150 g), and sex was verified by the presence/absence of testes as well as by the distance between genitals and anus when males were not scrotal.

DNA collection and analysis

Parentage analysis

Reproductive success was determined by parentage assignment using molecular techniques to identify number of offspring per adult. Tissue samples for genetic analyses were taken from 74 North American red squirrels (adult females, $N = 23$ and adult males, $N = 21$; offspring, $N = 30$) using sterilized dissecting scissors from the distal tip of the ear (~1 mm), placed in an Eppendorf tube, and preserved on ice immediately after collection. Ear tissue was transferred to a -20 °C freezer until DNA extraction could be performed.

DNA was extracted from ear tissue using a QIAGEN DNeasy Tissue Kit (QIAGEN Inc., Mississauga, Ontario, Canada). Eight microsatellite loci (Thu00, Thu02, Thu09, Thu 10, Thu21, Thu23, Thu36, and Thu70) were used to determine maternity and paternity from tissue samples using primers developed for red squirrels (Gunn et al. 2005; Bonanno and Schulte-Hostedde 2009). The polymerase chain reaction (PCR) protocol, including cocktail, PCR cycling reactions, and annealing temperatures for microsatellites was followed as noted Gunn et al. (2005) and Bonanno and Schulte-Hostedde (2009). PCR products were sent to MOBIX, the McMaster Institute for Molecular Biology and Biotechnology, at McMaster University for genotyping. Parentage was assigned using the likelihood-based approach and simulation procedures of CERVUS 3.0 (Kalinowski et al. 2007; Bonanno and Schulte-Hostedde 2009). Simulations were run for 10 000 cycles with the following parameters: number of candidate parents ($N = 44$), the proportion of candidate parents sampled for mothers (1) and for fathers (0.90), the proportion of loci typed (0.99), and the rate of typing error (0.01). All assignments were based on 80% (relaxed) and 95% (strict) confidence criteria (Kalinowski et al. 2007; Bonanno and Schulte-Hostedde 2009).

Parasite collection and analysis

Ectoparasite identification

Ectoparasites were collected with a metal flea comb (teeth spacing < 300 µm—1/10th the size of the smallest fleas; Burgham Ltd., Toronto, Ontario, Canada) by combing 5 times down the mid back to the base of the tail, 5 times along each hind leg, and 5 times down the tail from base to end. Ectoparasites collected were preserved in 70% ethanol. Sample collections were limited to once every 4 weeks to minimize the manipulative effects of parasite removal on body condition and survival (Gorrell and Schulte-Hostedde 2008). Sampling methods were consistent for all individuals.

Each individual parasite was weighed (± 0.000001 g, Sartorius MC21S). All internal soft tissue was chemically cleared (Hoyer's solution; Poorbaugh and Gier 1961; Santos-Flores and Dodson 2003) from specimens that were then mounted between a microscope slide and cover slip for identification (Holland 1949). Parasites were identified using a Siphonaptera key based on geographic range, host species, and anatomy developed from Gorrell and Schulte-Hostedde (2008). The number of fleas and mites was used to determine a relative external infection load per individual (Ectoparasite intensity).

Endoparasite burden: fecal egg counts

Helminth worm eggs from fecal samples were collected either from the animal directly while the animal was in the hand, after capture or from the trap platform. The platform was cleaned after each capture to ensure that each fecal sample was from the most recently captured red squirrel. Each fecal sample was stored in 70% ethanol and then frozen for later quantification of intestinal helminth egg load (Pauli et al. 2004; Gorrell and Schulte-Hostedde 2008; Hillegass et al. 2008).

An index of endoparasite load was determined by counting helminth eggs per gram (EPG) of feces using a modified centrifuge McMaster technique (Rossanigo and Gruner 1991; Pauli et al. 2004; Hillegass et al. 2008). Feces were blended into Fecasol (liquid zinc sulphate [1:10 dilution] floatation solution). The mixture was strained through a sieve to separate matter, poured into a 1.5 ml Eppendorf tube, and centrifuged at 3000 revolutions per minute for 5 min. Each tube was left for 10 min to allow for eggs to float to the top layer of tube, after which 1 ml of solution was extracted from the tube and pipetted into McMaster slide chambers. The slide was then examined under a microscope ($\times 10$ magnification; Olympus BX41) where the eggs were counted in two 1 cm grids. The EPG was calculated by multiplying the sum of the number of eggs observed within each chamber by 100 (Pauli et al. 2004; Gorrell and Schulte-Hostedde 2008; Hillegass et al. 2008). Endoparasite eggs were identified to genera using 2 online archives and identification guides; Diagnosing Medical Parasites: A Public Health Officers Guide to Assisting Laboratory and Medical Officers (http://www.afpmb.org/bulletin/vol29/Diagnosing_Medical_Parasites.pdf) and RVC/FAO Guide to Veterinary Diagnostic Parasitology (Gibbons et al. 2009; <http://www.rvc.ac.uk/review/Parasitology/Index/Index.htm>).

Data handling and statistical analyses

All statistics were conducted using Statistica 7 (StatSoft, Tulsa, OK). Only adults were considered in analyses. Variables were $\log(x + 1)$ or $\log_{10}(x + 1)$ transformed where necessary to improve normality. Nonparametric statistics were used for variables that did not transform to a normal distribution. Factorial Analyses of variance (ANOVAs) were used to examine differences between the sexes and seasonal fluctuations. Analyses of covariance (ANCOVAs), with sex as a categorical

factor, were used to test the relationships among reproductive success and parasite load for each individual.

Because males could have mated with unsampled females located outside our trapping perimeter, our measure of male reproductive success could have been biased. In order to assess the presence of “edge effects,” we ran 2 analyses of trapping frequency and reproductive success to assess the possibility of bias in trapping location. Trappability was used as an index for number of times caught and calculated from the residuals of the regression between number of captures and number of trapping opportunities available to each individual squirrel (date of last capture—first capture + 1 = number of days a squirrel was active in trapping area; Patterson and Schulte-Hostedde, 2011).

Males were assigned to either “interior” or “peripheral” trapping location, which was used as an independent variable in the 2 tests. An ANOVA was used to assess if males trapped on the edge of our trapping perimeter were caught less than those individuals caught within the interior. Trappability was used as a dependent variable. Secondly, in order to determine whether our measures of reproductive success were biased, we used a General linear model (GLM) with the number of offspring as a dependent variable and the trappability for both interior and peripheral males as an independent variable.

Measures of parasite load

Five measures of parasite load were calculated for each individual. Ectoparasite load was calculated by intensity (total mites and fleas), richness (presence and/or absence of both mites and fleas), as well as biomass (total mass of all mites and fleas per sample). Similarly endoparasite load included intensity (total number of intestinal eggs found per fecal sample) and richness (total number of different types found per sample). To address the issue of missing data and to prevent pseudoreplication, seasonal averages (April–August) of measures of parasite load were calculated for each individual.

RESULTS

Reproductive success: parentage assignment

Reproductive success was measured by the number of offspring from each individual assigned through parentage analyses. Seventy-four North American red squirrels (adult females, $N = 23$ and adult males, $N = 21$; offspring, $N = 30$) were genotyped. Three loci were not in Hardy–Weinberg (HW) equilibrium due to high occurrence of homozygotes. However, these loci were retained in the analysis as Cervus is robust when HW disequilibrium is present and because loci were scored reliably. All 30 offspring were assigned to a mother with 95% confidence level. Known mothers were then used to increase the confidence in paternity assignment. Twenty-two of the 30 offspring were assigned to a father at a 95% confidence level, and all 30 were assigned at an 80% confidence level.

Neither test of edge effects were significant, indicating that males trapped on the periphery did not have lower trappability scores than interior males (ANOVA; $F_{1,11} = 3.326$, $P = 0.10$). Similarly, reproductive success was not dependent on trappability (GLM; $F_{1,11} = 1.643$, $P = 0.23$).

Parasite load

Mites and 2 species of fleas (*Or. cadens* and *Op. pseudarctomys*) were found on squirrels. Little is known about the specific life histories of the fleas found in our study. Both *Op. pseudarctomys* and *Or. cadens* have been found on other rodents (Northern flying squirrels, *Glaucomys sabrinus*; Perez-Orella and Schulte-Hostedde 2005).

Mites were not identified to species. Many species of mites are not parasitic. However, mites that are parasitic are known to extract a cost from the host (Gangloff et al. 2008). Mites collected from Northern flying squirrels (*G. sabrinus*) in the same trapping area were found to be parasitic (Perez-Orella and Schulte-Hostedde 2005).

Ectoparasite intensity did not change over the trapping season ($\chi^2 = 7.505$, degrees of freedom = 4, $P = 0.11$) and did not differ between the sexes ($Z = -1.415$, $P = 0.16$). This variable did not respond to data transformation, thus a non-parametric Kruskal–Wallis ANOVA was applied to investigate changes over time and a Mann–Whitney U test was used to identify any sex differences. Spearman correlations revealed that ectoparasite intensity was correlated with both ectoparasite mass ($r_s = 0.92$, $P < 0.001$) and ectoparasite richness ($r_s = 0.98$, $P < 0.001$), thus to reduce the number of tests, only ectoparasite intensity was used in ANCOVAs.

Helminth eggs were identified to genus (*Strongyloides* sp., *Tricuris* sp., *Toxacara* sp., and *Ascaris* sp.). Seven unknown genera were also found in fecal samples. Endoparasite intensity and richness were not correlated to any ectoparasite measure (Endoparasite intensity: Ectoparasite intensity, $N = 22$, $r_s = 0.36$, $P = 0.10$; richness, $N = 22$, $r_s = 0.38$, $P = 0.08$; mass, $N = 22$, $r_s = 0.41$, $P = 0.06$; Endoparasite richness: Ectoparasite intensity, $N = 28$, $r_s = 0.03$, $P = 0.88$; richness, $N = 28$, $r_s = -0.01$, $P = 0.96$; mass, $N = 28$, $r_s = 0.09$, $P = 0.64$) or each other ($N = 21$, $r^2 = 0.09$, $P = 0.18$). Similar results were observed for log endoparasite load in that no sex differences were found in intensity or species richness (intensity, $F_{1,59} = 0.073$, $P = 0.79$; richness, $F_{1,59} = 0.274$, $P = 0.60$). Endoparasite intensity increased from May to August ($F_{4,37} = 5.602$, $P = 0.001$). Endoparasite richness did not differ among months ($F_{4,56} = 1.767$, $P = 0.15$).

Parasite load and reproductive success

There was a trend for greater reproductive output as ectoparasite intensity increased ($F_{1,35} = 3.541$, $P = 0.07$). Although no sex interaction was found ($F_{1,17} = 0.092$, $P = 0.77$), the slopes appeared different between females and males. Further investigation through separate simple regressions of male and female reproductive success–ectoparasite intensity relationships determined that males, and not females, had significant result (Males, $F_{1,12} = 8.485$, $P = 0.01$, Figure 1a; Females, $F_{1,16} = 0.571$, $P = 0.46$; Figure 1b). Endoparasite richness was negatively related to reproductive success in both males and females ($F_{1,23} = 5.315$, $P = 0.03$, Figure 2). No interactions of sex were observed ($F_{1,23} < 0.000$, $P = 0.99$). No relationship between reproductive success and endoparasite intensity was observed ($F_{1,23} = 0.137$, $P = 0.71$; sex interaction, $F_{1,23} = 0.042$, $P = 0.84$).

DISCUSSION

Our study shows evidence that parasite infection negatively impacts reproductive success and thus fitness in both males and females. Interestingly, our results indicate increasing male reproductive success with increasing ectoparasite intensity but a decrease in both male and female reproductive success with increasing endoparasite richness. No relationship was found between female reproductive success and ectoparasite intensity. It seems that even within macroparasites, different costs and/or benefits to the host may be caused by different types of parasite (reviewed in Morand et al. 2006; Hillegeass et al. 2008). Ectoparasites and endoparasites rely on different host resources that may result in different costs to the host. For example, all fleas are obligatory hematophagous parasites (Medvedev and Krasnov 2006), whereas endoparasitic worms rely on intestinal fluids and other nutrients within

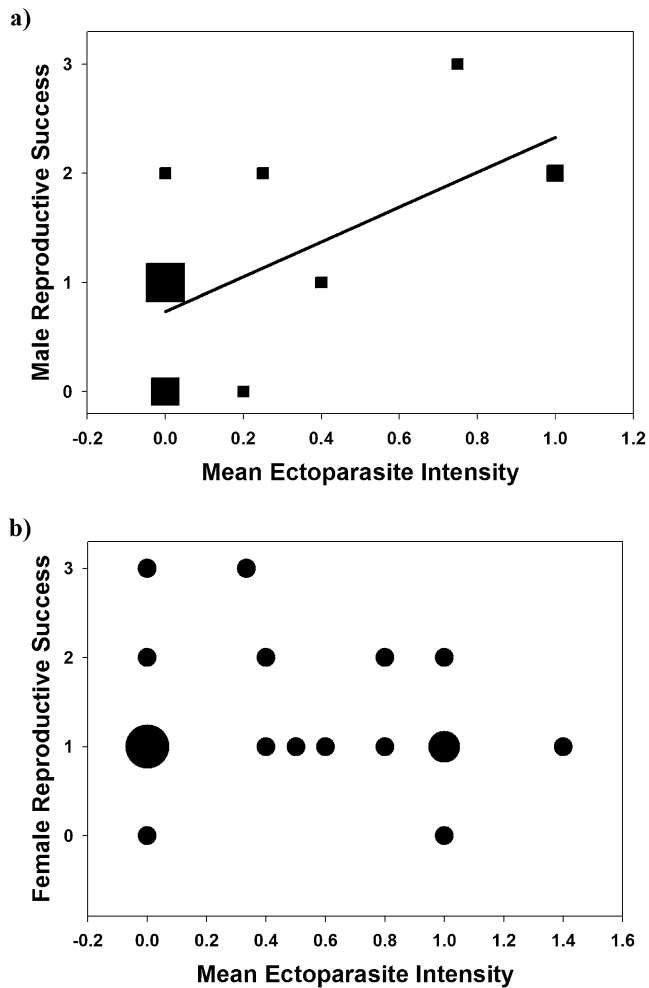


Figure 1
Scatterplots of reproductive success and mean ectoparasite intensity for (a) male and (b) female adult red squirrels collected in Algonquin Park in 2009. Male mean ectoparasite intensity increased with greater number of offspring ($F_{1,12} = 8.485$, $P = 0.01$). No relationship was observed between female mean ectoparasite intensity and number of offspring ($F_{1,16} = 0.571$, $P = 0.46$). Data points are proportionally representative. Larger data points reflect more individuals, and the smallest data points represent only one individual.

the host's organs that may extract a greater cost on host health (reviewed in Degen 2006).

However, it is unlikely that ectoparasites are improving host reproductive success directly. There are few examples where reproduction is seen to increase with the onset of infection (reviewed in Schwanz 2008). These cases involved microparasite infection that may be associated with higher virulence and more severe and immediate impacts on host survival (Minchella and LoVerde 1981; Sorci and Clobert 1996; Polak and Starmer 1998). Rather, the positive relationship between ectoparasite load and male reproductive success could be a consequence of a trade-off between activity and exposure to parasites (Poulin 1996; Krasnov et al. 2005). Increased conspecific contact was observed in male wild mice (*Peromyscus leucopus*) with elevated testosterone levels (Gear et al. 2009). More dominant, reproductively active males may have an increased risk of exposure to infected individuals (Gear et al. 2009). Male reproductive success is limited by the number of mates acquired (Bateman 1948) and so males may spend more time

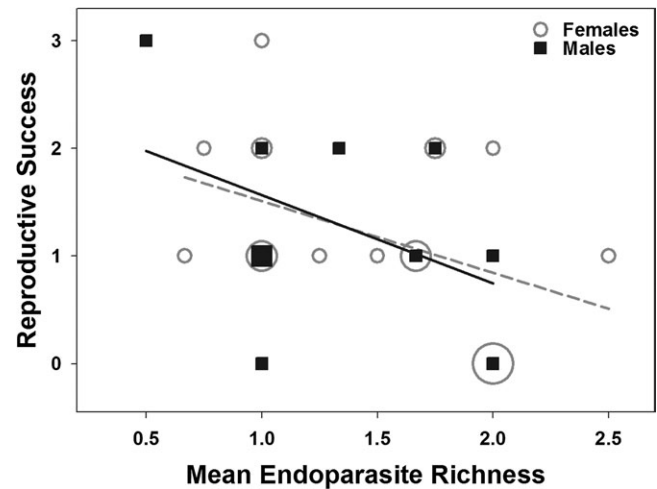


Figure 2
Scatterplot of reproductive success (i.e., number of offspring) and endoparasite richness for male (closed square) and female (open circle) adult red squirrels collected in Algonquin Park in 2009. Displayed is the relationship between endoparasites richness and number of offspring. Endoparasite richness negatively predicted number of offspring ($F_{1,23} = 5.315$, $P = 0.03$). No sex interaction was observed ($F_{1,23} < 0.000$, $P = 0.99$). Data points are proportionally representative with larger data points reflecting more individuals.

searching for mates than would females (Radespiel 2000). However, mate searching not only occurs at the expense of other life history traits (i.e., immunity) but also increases the exposure and risk of infection (Poulin 1996; Krasnov et al. 2005). Nonetheless, increased contact also increases the chances of successfully finding a mate or defending territory.

An alternative explanation for the impact of ectoparasites on male reproductive success could be the immunocompetence hypothesis (Hamilton and Zuk 1982; Folstad and Karter 1992). Testosterone is the primary androgen in males and is necessary in the development of secondary sexual traits and facilitation of aggressive interactions (reviewed in Folstad and Karter 1992). As a result, males with high levels of testosterone may be more attractive to females and better able to defend territories (e.g., Kruczek 1997). Yet, testosterone suppresses the immune system, which inevitably leads to increased vulnerability to disease and parasite infection (Folstad and Karter 1992; MacIntosh et al. 2010). The impacts of testosterone have more often been investigated within males. Male red grouse (*Lagopus lagopus scoticus*) injected with testosterone implants had greater nematode intensities than did controls (Seiwright et al. 2005). Similarly, male voles (*Clethrionomys glareolus*) and wood mice (*Apodemus sylvaticus*) with high testosterone levels had higher tick (*Ixodes ricinus*) attachment rates than control individuals (Hughes and Randolph 2001).

Contrary to previous findings, female reproductive success was not influenced by ectoparasites (see Neuhaus 2003; Hillegass et al. 2010). By contrast, both females and males with greater endoparasite richness had fewer offspring. Previous investigations have revealed that females can harbor more endoparasites, but males may harbor more ectoparasites which could explain why one type of parasite impacted reproductive success and not another (Hillegass et al. 2008). Females typically have smaller home ranges than males (Ims 1987), which could mitigate exposure to ectoparasites but increase the risk of endoparasites that are more easily transferred through contaminated food (Ferrari et al. 2004; reviewed in Hillegass et al. 2008). However, we found no sex differences in either type of parasite in our study. Although

sex-biased parasitism has been well investigated (e.g., Krasnov et al. 2005; Perez-Orella and Schulte-Hostedde 2005; Hillegass et al. 2008), that female life-history traits are impacted by one type of parasite and not another is a gap in the literature and requires further investigation.

No relationship was observed between endoparasite intensity and reproductive success. Previous studies have suggested that endoparasite richness may be a more reliable indicator of internal parasite burden than endoparasite intensity (Schulte-Hostedde and Elsasser 2011; Morand and Poulin 2000). A common index for endoparasite intensity is determined by the number of helminth eggs present in a known volume of fecal matter (eggs per gram of feces, EPG; Morand and Poulin 2000; Gorrell and Schulte-Hostedde 2008). EPG is assumed to represent an estimate of the number of adult worms present in the gastrointestinal tract. However, it is unknown how many eggs each worm releases. Consequently, the use of helminth eggs may be inaccurate as an index of parasite intensity (Morand and Poulin 2000). Species richness, on the other hand, is a count of the number of different species present (or, in our case, different types of parasites). Unlike parasite intensity, parasite richness may more accurately indicate the parasite burden on the host as it does not vary across time or between populations as much as parasite intensity (reviewed in Morand and Poulin 2000). In American mink (*Neovision vison*), individuals with greater endoparasites richness but not intensity had larger spleen mass (Schulte-Hostedde and Elsasser forthcoming). The spleen has been identified as a critical organ in immunity; thus, an increase in spleen mass may indicate increased immune response as well as greater energy allocation to the immune system (e.g., Corbin et al. 2008).

Based on our study, future research should examine the effects of confounding environmental factors such as variation in food abundance or predator prevalence on individual body condition (Thomas et al. 2007), immune status, and parasite abundance. Additionally, little is known about hormonal impacts on individual condition or how hormones interact with parasite infection in wild populations (Romero 2002; Mougeot et al. 2006). Investigations assessing levels of stress, and other immunocompromising hormones (i.e., testosterone) may reveal a greater understanding of infection susceptibility. Ultimately, an experimental approach in which body condition and immune status are manipulated will be most fruitful in determining the causality behind the patterns we have uncovered.

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