Ecology of parasites in northern canids: impacts of age, sex, behavior, life history, and diet

by

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Abstract

Host behavior, age, sex, diet, and condition, as well as variation in parasite specificity, drive variation in parasite infection, and ultimately determine the host parasite community. The objectives of this thesis were to 1) examine intraspecific variation in arctic fox parasites, 2) determine relationships between diet and parasites in sympatric arctic and red fox, and 3) compare wolf parasites and diet. Male arctic fox had more cestodes than females and juveniles had more nematodes than adults, likely due to diet and exposure. Red fox carried fewer parasites than arctic fox, likely due to diet, evolved resistance behaviors and higher immune investment, but diet affected cestode abundance in both species. Wolves that ate more white-tailed deer had more cestodes, suggesting increasing deer populations could enhance parasite transmission to moose. However, body condition was unaffected by parasites, suggesting northern canids may have not reached a threshold of infection.

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Table of Contents

List of Tables

List of Figures

Thesis Format

The thesis is presented in a manuscript format. Chapter 1 is the general introduction and presents the overall theme and pertinent literature. Chapters 2, 3 and 4 are written in the manuscript format with an abstract, introduction, methods, results, discussion, and literature cited. Conclusions and summary of the overall findings and implications of the research are presented in Chapter 5.

The nature of the contribution to all the papers (Chapter 2-4) by the student included collecting, analyzing, and preparing samples, analyzing the data, and writing the manuscripts.

Chapter 1: Thesis Introduction

The movement of energy within an ecosystem occurs through interactions between organisms and the environment. Parasitism is the most common consumer strategy (Lafferty et al. 2008) and has the potential to modify the ecology and evolution of all interactions (Price et al. 1986). Parasites strongly influence ecosystem dynamics by impacting energy flow, population dynamics, interspecific competition, overall biodiversity, and the structuring of many food webs (Hudson et al. 2006; Lafferty et al. 2006). Parasites obtain resources from one or more hosts, which may reduce host fitness, alter host behavior and influence the structure of the community (Price et al. 1986; Schmidt and Roberts 2009). Parasitic life cycles can be complex and include multiple hosts; host specificity and mode of transmission vary considerably (Schmidt and Roberts 2009). Transmission of parasites can be horizontal, often involving indirect contamination of a food resource, or vertical, often through the consumption of a prior host (Schmidt and Roberts 2009). Host response to parasite infection or infestation includes alterations in resource allocation, altered investment in immune function, modified reproductive effort, reduced mating success, premature aging and increased vulnerability to predators or other stressful conditions (Lafferty et al. 2006; Thomas et al. 2009). Host characteristics including social group formation, pair bonding and mate choice, and habitat selection are used to minimize parasite impact (Price et al. 1986).

Biotic and abiotic factors impact hosts and ultimately determine host exposure (Lutermann et al. 2012). The variation in parasite communities is linked to the heterogeneity of the individuals in a population, their exposure to infective stages of the

parasites, and their susceptibility upon exposure to the parasites (Shaw and Dobson 1995). Variation in behavior, the condition and genetics of a host, seasonality, sex, and age all may contribute to intraspecific variation in parasites (Wilson et al. 2002).

Sex-biased parasitism has been documented in multiple vertebrate species, particularly species that have polygamous mating systems (Poulin 1996; Hillegass et al. 2008). Sexual selection can be intense in these populations, with males and females investing differently in reproduction (Zuk and McKean 1996; Soliman and Marzouk 2001). Steroid hormones, such as testosterone, progesterone and oestrogen, have been shown to impact the immune system, and parasite development and growth (Grossman 1985; Folstad and Karter 1992; Wilson et al. 2002; Viljoen et al. 2011). Oestrogens enhance humoral immunity while suppressing cell-mediated responses (Wilson et al. 2002). Male-biased parasite loads are common, particularly in species where sexual selection is intense (Zuk and McKean 1996; Soliman and Marzouk 2001; Hillegass et al. 2008; Lutermann et al. 2012). The 'immunocompetence handicap hypothesis' (ICHH) suggests that there is a trade-off between testosterone and immune function (Folstad and Karter 1992). The ICHH postulates that testosterone suppresses the immune system; therefore, males with higher testosterone levels will be more vulnerable to parasite infection and infestation (Folstand and Karter 1992).

Age can be a major influence on the parasites of a host (Wilson et al. 2002). If a host is unable to develop any acquired immunity against the parasites, numbers of parasites tend to increase with their age due to continued exposure over time (Wilson et al. 2002). If the rate of acquiring parasites is the same as the mortality rate of parasites, parasite load will reach an asymptote at a certain age (Hudson and Dobson 1995). The

development of immunity by a host against parasites it is exposed to should decrease the establishment, growth, and survival of parasites it acquires over time (Hudson and Dobson 1995). If immunity develops, older hosts may have fewer parasites than their younger conspecifics after an initial increase (Hudson and Dobson 1995). Changes in diet, morphology, and physiology that a host experiences as it ages will also affect the host's parasites. These differences can explain some of the observed differences in parasites of conspecifics of different ages.

The relationship between host density and parasite communities can have cascading effects on biodiversity (Thomas et al. 2009). The number and species richness of parasites will increase with host density (Arneberg et al. 1998; Morand and Poulin 1998). Stien and others (2009) demonstrated a strong relationship between the abundance and distribution of intermediate hosts (sibling voles, *Microtus rossiaemeridionalis*) and the gastrointestinal parasite community of arctic foxes (*Vulpes lagopus*) on the Svalbard archipelago. In the regions where the sibling voles and arctic fox overlapped, the gastrointestinal parasite community reflected this interaction (Stien et al. 2009). Arctic foxes in this area were found with parasite species transmitted by the consumption of sibling voles (Stien et al. 2009). As the abundance of sibling voles decreased and other food sources, such as reindeer (*Rangifer tarandus platyrhynchus*), became increasingly important, the prevalence of gastrointestinal parasites that used sibling voles as intermediate hosts decreased (Stien et al. 2009).

Changes in climatic conditions have dramatic effects on the function and structure of ecosystems (Kutz et al. 2009). Climate plays an important role in determining the abundance and diversity of parasites, which subsequently affects host-parasite dynamics

(Kutz et al. 2009). The Arctic ecosystem is undergoing climate warming at an unparalleled rate (Kutz et al. 2009). The region is characterized by low biodiversity making it particularly vulnerable to range expansion (Kutz et al. 2009). Range expansion, or the introduction of novel parasites into the Arctic, may have dramatic effects on formerly unexposed hosts (Price et al. 1986). Range expansion of parasites and their hosts' increased transmission rates, and longer transmission periods associated with climate warming, impact parasite dynamics in the Arctic (Kutz et al. 2009). Parasites previously range-limited by free-living life stages and invertebrate intermediate hosts are expected to expand into more northern areas (Kutz et al. 2009). For example, the Svalbard archipelago, Norway, did not have rodents until the anthropogenic introduction of the sibling vole (Henttonen et al. 2001). With the introduction of this new rodent, previously absent parasites, such as *Echinococcus multilocularis*, were introduced to arctic fox populations (Henttonen et al. 2001). Although this parasite is commonly found in arctic foxes in other areas, the absence of a suitable intermediate host prevented the establishment of *E. multilocularis* on the archipelago (Henttonen et al. 2001). The introduction of a new intermediate host brought with it a novel parasite, which has subsequently infected arctic fox populations within the region (Henttonen et al. 2001; Stien et al. 2009). The introduction of this parasite also altered predator-prey dynamics as massive infections within the intermediate hosts interfered with movement and escape potential of the vole, and increased their susceptibility to predation by the foxes (Henttonen et al. 2001). Further range expansion of hosts may serve as a reservoir for native parasites, subsequently impacting the viability of other native host species (Kutz et al. 2009).

Interactions between species may be modified by the presence of parasites, affecting the outcome of interspecific competition (Price et al. 1986). The community of parasites within a region drives the overall biodiversity of the ecosystem (Hudson et al. 2006). Parasites can dramatically influence the structure, dynamics and function of food webs (Lafferty et al. 2008), although most previous food web studies have failed to include this important consumer strategy (Lafferty et al. 2006). For example, the introduction of the rinderpest virus caused a rapid 20% reduction in abundance and diversity of ungulates found on some African savannahs (Sinclair 1979). Carnivores starved due to the lack of prey (Sinclair 1979). Without grazing, the grass grew taller and caused an increase in the frequency of fire for the region (Sinclair 1979). It further reduced resources for tree-feeding species including giraffes (*Giraffa camelopardalis*) (Sinclair 1979). Many species of parasites are trophically transmitted through the indirect contamination of a food source, but more commonly by the consumption of the prior host (Schmidt and Roberts 2009; Stein et al. 2009). Interactions between predator and prey are a key determinant factor of transmission intensity in parasites that move between trophic levels (Raoul et al. 2010). In Scandinavia, the arctic fox population was substantially impacted by an infestation of sarcoptic mange, a disease caused by mites (*Sarcoptes scabiei*) burrowing under the skin of the host (Smith and Almberg 2007; Thomas et al. 2009). The decline in the fox population markedly impacted populations of their rodent prey (Thomas et al. 2009).

Research topics

This thesis is an investigation of the relationships between parasite communities and age, sex, behavior, life history, and diet of northern canids. I further examine the

costs of being parasitized on the host, specifically looking at the relationship between body condition and parasites.

Chapter 2 is an investigation of how age and sex may impact the parasites of the socially monogamous arctic fox. I investigate any differences in diet and life history that may explain the observed differences between sexes or age. Arctic fox are a socially monogamous species and both parents take care of their young (Macpherson 1969). No differences between males and females in morphology, home range size, or diet have been documented (Angerbjorn et al. 2004; Eide et al. 2004). Sexual selection is not high in this socially monogamous species, making this an interesting system to examine any sex-biased parasitism. Arctic fox also live on average between 2-3 years and can have large litters, of up to 25 individuals when food is abundant (Macpherson 1969; Audent et al. 2002). The short lifespan of the arctic fox and its large annual investment in reproduction also make it an interesting species to examine the effects of host age (increased exposure over time versus the investment in acquired immunity) on parasites. The objectives of this chapter were to examine if parasites varied with sex or age of the host and to examine the factors, such as diet, that correspond with these differences.

In *Chapter 3* I shall examine the differences in the parasite community of the sympatric arctic and red foxes and how differences in diet, behavior, and life history may be driving these differences. The arctic fox is an important circumpolar predator that often relies on lemmings (*Dicrostonyx* and *Lemmus* spp.) but consumes other food sources such as migratory birds, eggs, ringed seals (*Pusa hispida*), and caribou (*Rangifer tarandus caribou*) carcasses when available (Chesemore 1968; Roth 2002). Switching to alternative prey will increase exposure to a different array of parasites. Interactions

between predator and prey are a key determinant factor of transmission intensity in parasites that move between trophic levels (Raoul et al. 2010). The southern range of the arctic fox is potentially limited by the presence of red fox, a potential predator that also competes with arctic foxes for food and den space (Hersteinsson and Macdonald 1992; Post et al. 2009). Parasite transmission between species is another mechanism by which these species could interact. Red fox have a large distribution, covering a broad diversity of prey types and climatic conditions. Their large range has exposed this species to a high diversity and density of parasites over evolutionary time (Larivèire and Pasitschniak-Arts 1996). Red fox have likely evolved physiological and behavioral defenses against parasites. Interactions between these two species could increase parasite abundance and diversity in arctic foxes where sympatric and, as red fox expand northward with the warming climate, this overlap will increase. *Alaria alata*, for example, can be transmitted to the red fox through ingestion of frogs, snails, or other paratenic (of the parasite) hosts, but has not yet been found in the arctic fox (Gibson et al. 2005; Schmidt and Roberts 2009). If the overall range of foxes affects the evolution of behavioral and physiological defense against helminths, then helminth prevalence and intensity should be low in red fox because they have a large, heterogeneous southern distribution and high in arctic foxes because they have a smaller Arctic distribution in comparatively homogeneous habitats. Secondly, if the diet of the fox affects their parasite exposure, then a fox with a diet (using stable isotope ratios as a proxy for diet) that is higher in small mammals or caribou should have a higher prevalence, intensity, and diversity of helminths than observed in a fox whose diet consists of birds or seals.

In *Chapter 4* I will examine the relationship between wolves' (*Canis lupus*) diet and parasite community in an area with declining moose populations. Wolves are a top predator, hunting largely ungulate prey, such as white-tailed deer (*Odocoileus virginianus*), moose (*Alces americanus*), elk (*Cervus canadensis*), and caribou (*Rangifer tarandus caribou*). Wolves provide top-down regulation in ecosystems in which they occur (Miller et al. 2012). As an important top predator, wolves host a diverse gastrointestinal parasite community, which should vary in relationship to their diet (Craig and Craig 2005; Bryan et al. 2012). Parasites also may impact population dynamics and interactions between wolves and their prey. Heavy parasite infections in wolf prey (e.g. *Echinococcus granulosus* in moose) can affect the ability of wolves to regulate prey populations (Joly and Messier 2004). Moose that are heavily infected with *Echinococcus granulosus* are unable to sustain long periods of exertion due to the negative impact of the parasite on moose lung capacity (Joly and Messier 2004). Reduced lung capacity predisposes them to an increased predation risk by wolves (Joly and Messier 2004). As wolves serve as the definitive host for this parasite, consuming a large proportion of infected moose will then increase the prevalence of the parasite within the wolf population and could impact population dynamics within the region (Joly and Messier 2004). Wolves serve as the definitive hosts for many species of parasites and understanding that parasite community is key to determining the impacts of parasitism on the population dynamics of their prey. Wolves' diet and parasites have been investigated at a population level, but this is the first time this relationship will be investigated at an individual level, as stable isotopes allow the long term diet of a wolf to be related to its parasite community.

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Chapter 2: Host age and sex affect the helminth community of the arctic fox

Abstract

Differences in host behavior, age, sex, and condition, as well as variation in parasite specificity, drive variation in parasite infection, and ultimately determine host exposure. The circumpolar arctic fox (*Vulpes lagopus*) is a socially monogamous predator with no difference in morphology or home range size between sexes. Sexual selection is low in this species and both parents invest heavily in their young. Offspring leave their maternal den a few months after birth. Older foxes may have increased exposure to parasites over time, leading to greater numbers of parasites, unless they are able to develop immunity or have differences in diet. To compare differences in parasites due to host age, sex, or diet we identified and enumerated parasites in arctic fox carcasses collected from local trappers at Churchill, Manitoba, Canada. Tissues were sampled to measure stable isotope ratios as a proxy for diet. Males had more cestodes but numbers of nematodes did not differ between sexes. Stable isotope ratios of nitrogen were lower in males, suggesting greater reliance on small mammals, but diet did not differ based on age. Likewise, the overall number of cestodes, which are only transmitted through prey, did not differ based on age. However, the species of cestodes found in adults and juveniles differed; e.g., *Echinococcus multilocularis* was present only in juveniles. The trophically transmitted nematode, *Spirocerca lupi,* was more prevalent in adults, but overall, nematodes were more abundant in juveniles, likely because pups spend more time at dens in high densities, increasing exposure. Intraspecific differences in arctic fox parasites are best

explained by variation in diet and foraging pattern, rather than hormone-mediated reduction in immunity.

Introduction

Intraspecific variation in parasite load (intensity or abundance) can be substantial, with many individuals having few parasites compared to a small number with many parasites (Wilson et al. 2002). Differences in the behavior, condition, and genetics of hosts, as well as parasite genetics, may contribute to these observed differences (Wilson et al. 2002). The sex and age of the host may also have substantial effects on parasite diversity and abundance (Folstad and Karter 1992; Wilson et al. 2002).

Intrinsic biological differences between males and females may cause one sex to be more prone to infection by parasites (Poulin 1996). Many species have evolved sex-based differences in diet, behavior (e.g. home range size), morphology (e.g. larger body size), and physiology (e.g. hormone differences), due to differences in reproductive strategies (Folstad and Karter 1992; Wilson et al. 2002; Arneberg et al. 2008b; Hillegass et al. 2008; Hosken and House 2011). Differences in reproductive investment by males could increase their vulnerability to parasites (Hillegass et al. 2008). Male-biased parasite loads are common in vertebrates, particularly in species where sexual selection is intense (Zuk and McKean 1996; Soliman and Marzouk 2001; Hillegass et al. 2008; Lutermann et al. 2012). If males are larger, sex-biased parasite loads may be a result of these body size differences (Moore and Wilson 2002). Differences in home range size and interactions with conspecifics (such as in a polygynous mating system) could also increase male exposure to parasites compared to females (Nunn and Dokey 2006). However, sex-biased parasite loads are still observed when taking home range and body size differences into

account (Moore and Wilson 2002). The 'immunocompetence handicap hypothesis' (ICHH) suggests a trade-off between immune function and testosterone (Folstad and Karter 1992). Testosterone suppresses the immune system, making males with higher testosterone levels more vulnerable to infection by parasites (Poulin 1996).

The age of a host often influences its parasites; parasite numbers tend to increase with age, as the host may have continual exposure to parasites over time (Wilson et al. 2002). In the absence of vertical transmission of the parasite, or reproduction of the parasite within the host, the host will continue to acquire parasites from the environment, increasing parasite loads over time (Hudson and Dobson 1995). If parasite mortality and acquisition remain constant then the parasite load will only increase to an asymptote (Hudson and Dobson 1995). Older individuals may also have more parasites if survival of younger individuals with many parasites is reduced in comparison to older individuals with similar loads (Soliman and Marzouk. 2001). However, if the host acquires immunity in response to exposure to parasites, its immune system should decrease parasite establishment, maturation, reproduction, and survival and parasite loads should eventually decline after an initial increase (Hudson and Dobson 1995).

Very few studies have focused on sex-biased parasite loads in species with monogamous mating systems; most studies examine sex differences in parasites in polygamous animals where sexual selection is intense and males and females invest differently in reproduction (e.g., Porteous and Pankhurst 1998; Hillegass et al. 2008; Lutermann et al. 2012). Arctic fox (*Vulpes lagopus*) are important circumpolar predators that forage alone during most of the year (Angerbjorn et al. 2004). Unlike polygamous mating systems where sexual selection is often high and sexes invest differently in

reproduction, sexual selection is not high in this socially monogamous species. Female arctic fox breed once annually and the sex ratio is nearly even (Macpherson 1969). Male arctic fox may be slightly larger than females, but only in particular parts of their range (Angerbjorn et al. 2004) and not in northern Manitoba (see Chapter 3). Both parents take care of their young and remain territorial during the breeding period (Macpherson 1969). Although extra pair paternity does occur, it is not common, and the social father will still invest in the pups of its partner (Cameron et al. 2011). Differences in diet can impact the parasite exposure of arctic foxes. As the interactions between predator and prey are key determinants of transmission intensity of many parasites, consumption of different types of prey (e.g. birds versus mammals) will expose arctic foxes to a different array of parasites (Raoul et al. 2010). No differences between male and female arctic fox in their diet or foraging have been documented (Angerbjorn et al. 2004). Home range size has not been documented to differ between males and females (Eide et al. 2004). The lack of intense sexual selection on arctic foxes makes this species an interesting comparison to polygamous species for understanding differences in parasites between sexes.

Whereas no sex bias in parasites are predicted for arctic foxes, age differences could exist based on their life history. Arctic fox live an average of 2-3 years, reaching sexual maturity within 9-10 months, and often breed their first year (Audet et al. 2002). Arctic fox litter size varies with food availability, but litters can reach up to 25 pups (Audet et al. 2002). For the first few months after birth, the pups are confined to their natal dens, where they remain while both parents forage for them (Audent et al. 2002). The short lifespan and large annual investment in reproduction made by the arctic fox also make it an interesting species to examine for effects of age on parasites. As diet also

affects parasites, age-related differences in diet must also be considered. Adult arctic fox may cache eggs from the summer bird-breeding season for use in fall and winter (Samelius et al. 2007). As juveniles remain on the den during their first summer, they may not have access to cached eggs and may rely more heavily on lemmings and other rodents, which are important intermediate hosts for cestodes (Rausch and Fay 1988; Loos-Frank 2000).

The objectives of this study were to (i) determine if parasites vary with sex or age in arctic fox and (ii) examine if differences in diet correspond to these differences. Since male and female arctic foxes have no known differences in morphology, diet, or behavior, and are socially monogamous, males and females should have similar parasite loads. If juveniles and adults have no differences in morphology or diet and immunity does not develop over time, then adults should have more parasites due to increased exposure over time. Alternatively, if juveniles and adults have no differences in morphology or diet but they can develop immunity, then juveniles should have more parasites. Further, if there are differences in diet between juvenile and adults, with adults having more access to cached eggs, then juveniles should have more cestodes.

Methods

Study Area – Arctic fox were obtained from local trappers from Churchill, Manitoba, Canada located on the west edge of Hudson Bay (58° N, 94° W). This region is the southernmost edge of the arctic tundra, near the transitional zone between the tundra and the boreal forest. The climate of this coastal area is strongly influenced by Hudson Bay, which remains frozen for 7-8 months every year (Rouse 1991). Collared lemmings

(*Dicrostonyx richardsonii*) are an important prey source for arctic fox year-round. There are also large seasonal fluctuations in alternative prey availability. Many bird species, including Lesser Snow Goose (*Chen caerulescens caerulescens*), Canada Goose (*Branta canadensis interior*), and many different species of shorebirds (e.g., Dunlin (*Calidris alpina*), Least Sandpiper (*Calidris minutilla*), Whimbrel (*Numenius phaeopus*), Hudsonian Godwit (*Limosa haemastica*), etc.), migrate into the region to breed annually, and constitute an important food source during summer (Roth 2002). Scavenged caribou (*Rangifer tarandus*) and ringed seals (*Pusa hispida*) can also serve as important food sources (Macpherson 1969; Campbell 1994; Angerbjorn et al. 2004).

Endoparasite Sampling – We collected arctic fox carcasses from local fur trappers in two trapping seasons (December 2010- February 2011 and December 2011- February 2012). We determined the endoparasite community through standard necropsy techniques (Munson 2000). Canine teeth were x-rayed to distinguish adults from juveniles based on the size of the pulp cavity (Grue and Jensen 1976). Teeth with a small pulp cavity were sent to Matson's Laboratory (Milltown, Montana) for age analysis using counts of cementum layers (Fancy 1980). Juveniles were considered to be foxes born the previous spring (Roth 2003). We measured spine length (to 1 mm) and mass (to 0.1 kg). Lungs, liver, kidneys, spleen, stomach, and heart were collected from each individual to examine for parasites or other pathology.

Each intestine was split longitudinally and scraped to remove all parasites. The remaining tissue was examined under a dissecting microscope to ensure removal of all parasites. Sedimentation and counting was used to examine all remaining tissue (Eckert

2003). All parasites were identified to species (when possible) and weighed (dry mass, 0.1g). All parasites were preserved in 70% ethanol. Prior to fixation, the scolex of each cestode was sliced off and squashed between a cover slip and glass slide. Scolex squashes were used to identify species using a compound microscope (based upon the number, shape and size of rostellar hooks following Abuladze (1964), Rausch and Fay (1988) and Loos-Frank (2000)). Nematodes were identified following Georgi (1974) and Anderson (1992). The mass of all remaining tissue was measured and all tissue was preserved in 70% ethanol. *Echinococcus* spp. were identified by characteristic morphology (see Thompson 1995). *Echinococcus multilocularis* is a very small cestode that was abundant in infected foxes. However, specimens were in poor condition (broken into pieces), making their abundance difficult to quantify. Due to their disproportionately large intensity (some with over 10 000), this species was excluded from cestode intensity and abundance. Parasite taxa were quantified by using three measurements: prevalence (presence or absence of parasite in a fox), intensity (number of parasites per infected fox), and abundance (number of parasites per fox) (Rozsa et al. 2000).

Diet Analysis – Stable isotope ratios were used to compare the diets of different sexes and ages of arctic fox (Roth 2002). As many parasites are transmitted through the consumption of infected prey, the gastrointestinal parasite community within an arctic fox should be related to its diet (see Chapter 3). Differences in stable isotope ratios of the prey are transferred to the consumer, so the stable isotope ratios of the arctic foxes will reflect their diet (Chisholm et al. 1982). For example, an arctic fox that has consumed terrestrial food sources will have lower $^{13}C/^{12}C$ ratios compared to those that consume

purely marine sources (Chisholm et al. 1982). An intermediate signature would be produced in foxes that consumed a mixed diet.

Muscle samples were obtained for stable-isotope analysis. Muscle tissue represents the few months prior to death. Our samples were collected during December-February and so our muscle samples represent the early winter diet, as it likely will have gone through complete turnover in 1-2 months (Hobson and Clark 1992; Dalerum and Angerbjorn 2005). Muscle samples were freeze-dried and powdered (Roth 2002). Lipids were removed from the samples using a soxhlet apparatus (Roth 2002) due to lipid variation affecting measurements of $\delta^{13}C$ (Rau et al. 1992). Stable isotope ratios for all samples were measured using a continuous flow isotope ratio mass spectrometer at the University of Windsor. Stable isotope values are presented in parts per thousand (‰) relative to Pee Dee Belemnite (carbon) and atmospheric N_2 (nitrogen) standards (Roth 2003; Adams et al. 2010) as follows:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] * 10^3$$

where *X* is ^{13}C or ^{15}N and R is the ratio of the heavy isotopes to light isotopes (e.g. $^{13}C/^{12}C$ or $^{15}N/^{14}N$).

Statistical Analysis – Morphological differences (i.e., body mass and spine length) between males and females' as well as between juveniles and adults' were compared using analysis of variance (ANOVA). Values of body mass were not normally distributed so the data were log-transformed. None of the parasite data had a normal distribution so parasite communities were compared between sex and age using a non-parametric analysis. We used Wilcoxon Rank Sums to compare differences in median intensity and

abundance. We used Fisher's Exact Test to compare differences in prevalence. Stable isotope values were compared between sexes and ages to detect differences in diet using multivariate analysis (MANOVA) (Hummel and Sligo 1971). Statistical analyses were performed in JMP® 10 (SAS Institute Inc. 2012) and R statistical software (http://www.R-project.org).

Results

We necropsied three arctic foxes in 2011 and 55 foxes in 2012. Although 55 arctic foxes were collected in 2012, six were eliminated from parasite analysis because the gastrointestinal tracts were destroyed from foraging by other animals. Arctic fox males (n=31) and females (n=21) did not differ in spine length (ANOVA, $F_{1,51}$=0.94, p=0.34) or body mass (ANOVA, $F_{1,51}$=1.01, p=0.32). There were no differences between juveniles (n=32) and adults (n=20) in spine length (ANOVA, $F_{1,51}$=0.23, p=0.64) or body mass (ANOVA, $F_{1,51}$=0.042, p=0.84). All adult arctic fox in our sample were one year old.

Four species of cestodes were found in the 52 arctic fox examined. *Taenia polyacantha arctica* was the most prevalent (76.9%), with *T. crassiceps* (29%), *Echinococcus multilocularis* (19.2%), *T. multiceps* (17.3%), also present. The majority arctic fox individuals only hosted one species of cestode (53.8%), with two species in some (34.6%), and a small number having three or more cestode species (7.7%). Ascarid nematodes were present in all arctic fox (100%). *Spirocerca lupi* was found in cysts on the external wall of the stomach in 55.8% of the arctic fox. One cyst was found in the

mesentery near the stomach. No macroparasites were found in the lungs, livers, kidneys, spleens, or hearts.

Males had a higher intensity and abundance of cestodes than females (intensity: Wilcoxon Rank Sums, Z= -1.95, p=0.05; abundance: Wilcoxon Rank Sums, Z= -2.40, p=0.02; Figure 2.2). We found no difference in cestode prevalence between males and females (Fisher's Exact Test, *P*=0.16). *T. polyacantha arctica* was more prevalent in males (Fisher's Exact Test, *P*=0.008), but *T. crassiceps* tended to be more prevalent in females (Fisher's Exact Test, *P*=0.07). No difference was detected between prevalence of *T. multiceps* (Fisher's Exact Test, *P*=0.29) in males versus females (Figure 2.1). There was no difference between sexes in gastrointestinal nematode abundance (Wilcoxon Rank Sums, Z=-0.065, *P*=0.94) (Figure 2.2). There was no difference in prevalence (Fisher's Exact Test, *P*=1.0), intensity (Wilcoxon Rank Sums, Z=-0.49, *P*=0.62), or abundance (Wilcoxon Rank Sums, Z=-0.12, *P*=0.91) of *S. lupi* between males (54.8%) and females (57.1%).

Cestode prevalence (Fisher's Exact Test, *P*=1.0), intensity (Wilcoxon Rank Sums, Z=-1.10, *P*=0.27), or abundance (Wilcoxon Rank Sums, Z=-1.15, *P*=0.25) did not differ between juveniles and adults. *T. crassiceps* was found more often in adults than juveniles (Fisher's Exact Test, *P*=0.044) (Figure 2.1). *E. multilocularis* was only present in juveniles (Figure 2.1). Juveniles had more nematodes than adults (intensity and abundance: Wilcoxon Rank Sums, Z=-2.30, *P*=0.021) (Figure 2.3). *S.lupi* tended to be more prevalent in adults (Fisher Exact Test, *P*=0.088) but no differences were detected in intensity (Wilcoxon Rank Sums, Z=-1.4, *P*=0.16) or abundance (Wilcoxon Rank Sums, Z=0.81, *P*=0.42).

Interactions between sex and age on parasite prevalence and intensity were examined for both cestodes and nematodes. Cestode intensity did not differ between adults and juveniles in males (Wilcoxon Rank Sums, Z=-1.16, *P*=0.25) or females (Wilcoxon Rank Sums, Z=0.16, *P*=0.87) (Figure 2.3). There was no difference in gastrointestinal nematodes (both abundance and intensity) between males and females in either juveniles (Wilcoxon Rank Sums, Z=0.56, *P*=0.56) or adults (Wilcoxon Rank Sums, Z=0.19, *P*=0.82).

Arctic fox males and females differed in muscle stable isotope values (MANOVA, $F_{2,49}$=4.47, p=0.017). Females had higher $\delta^{13}C$ values for muscle (ANOVA, $F_{1,50}$=6.2, *P*=0.016) and also had higher mean $\delta^{15}N$ than males in muscle (ANOVA, $F_{1,50}$=7.2, *P*=0.0098) (Figure 2.4). No differences between ages were detected (MANVOA, $F_{1,50}$=0.11, *P*=0.75), but an interaction between sex and age was found with muscle stable isotope ratios (MANOVA, $F_{2,49}$=5.12, *P*= 0.0097).

Discussion

The parasites in arctic fox varied by host sex and age. Cestodes were much more abundant in males, although nematode loads did not differ between sexes. Nematodes were more abundant in juveniles but no age relationship was observed for cestodes. The inequality in parasites between females and males has been previously documented in many different species, with males commonly having more parasites, including nematodes and cestodes (Poulin 1996). As male and female arctic foxes have no differences in home range size or morphology, the difference in parasites between the two is likely due to either differences in diet or physiology, as proposed by the ICHH.

The ICHH suggests that the immune system is inhibited by testosterone, increasing the individuals' vulnerability to parasitic infection or infestation (Zuk 1990; Zuk and McKean 1996). However, in monogamous species, where males have less pressure to compete for available females, differences between male and female infection susceptibility should be less than in polygamous species. The lack of severe competition (which should be linked to testosterone differences in males) suggests such a male bias in parasite load may not occur in monogamous species (Zuk 1990). Further, the relationship between parasites and sex has also not been consistent across monogamous species. Saeed and Kapel (2006) found differences between male and female red foxes, with males having a higher prevalence of the nematode, *Toxocara canis,* although these trends were strongly influenced by seasonal and age factors. Differences in the presence of nematode parasites, such as *Toxocara canis*, between males and females were not observed in our arctic fox. Porteous and Pankhurst (1998) found a female bias in Patagonian mara (*Dolichotis patagonum*), with females carrying more nematodes. In addition, we observed no difference in gastrointestinal nematodes or *S. lupi* between male and females (Figure 2.1). If the differences in immunity were driving the male bias in vulnerability to parasite infection there should be differences in both cestodes and nematodes (Hepworth et al. 2010; Jacobs and Zuk 2012). Poulin's (1996) meta-analysis on sexual inequalities on helminth infections in various vertebrate taxa demonstrated a male bias in vulnerability to infection by nematodes. Testosterone has been shown to inhibit multiple parts of the immune response and should affect both nematodes and cestodes (Hepworth et al. 2010; Jacobs and Zuk 2012). The lack of a consistent

relationship between parasites and sex in our study strongly suggests that this parasite difference in parasite load should then be due to other factors, likely diet.

Differences in diet between male and female arctic foxes were reflected in their stable isotopes values (Figure 2.4). Females had higher mean $\delta^{15}N$ values than males. As small mammals have much lower $\delta^{15}N$ values (see Table 3.2, Chapter 3) lower $\delta^{15}N$ values in males versus females indicates that males had a diet including more small mammals. As small mammals are important intermediate hosts for *E. multilocularis*, *T. crassiceps*, *T. polyacantha arctica*, (Loos-Frank 2000) a diet with higher proportion of these food sources should expose an arctic fox to more larval stages and would explain this higher cestode intensity and abundance (Figure 2.1). Further, differences in diet should only affect cestodes but not nematodes, which is consistent with our observations.

Recruitment of new naive hosts during the breeding season may contribute to patterns of parasite communities (Lutermann et al. 2012). Consistent with this idea, juvenile arctic foxes carried more nematodes than adults (Figure 2.2). Many of these ascarid nematodes can be transferred through the indirect contamination of a food resource or an area by infected feces (Schmidt and Roberts 2009), and transmission is related to host density (Arneberg et al. 1998a). Arctic fox have large litters and pups are confined to the den for the first few months of their lives (Macpherson 1969). High densities of pups feeding at dens where feces concentrate may explain the higher nematode numbers in juveniles. As all individual foxes were infected with nematodes, prevalence did not differ between ages as previously documented (Saeed and Kapel 2006; Stien et al. 2009). As *S. lupi* is a trophically transmitted nematode, its higher prevalence in adults is likely due to continual exposure over time.

Cestode load did not differ between juveniles and adults, consistent with their similar diet. However, the cestode *E. multilocularis* was relatively prevalent in juveniles but absent in adults, contrary to previous studies that found no difference in sex or age with respect to the prevalence of *E. multilocularis* (Stien et al. 2009). As individuals do not develop immunity to *E. multilocularis* (Budke et al. 2004) and may become infected again, the absence of this parasite in adults is surprising. Furthermore, we found no differences in diet that would explain this difference (Figure 2.4).

T. polyacantha arctica was the most prevalent cestode found and was more prevalent in males (Figure 2.1), likely leading to the overall male bias in cestodes. *T. crassiceps* was more abundant in adults, contrary to previous reports of no age related differences in the prevalence of *T. crassiceps* in arctic fox in Svalbard (Stein et al. 2009). These differences likely may be due to disparities in the prevalence of *T. crassiceps* between the two regions.

Intraspecific variation in parasites in this arctic fox population can best be explained by diet and feeding behavior, which creates differential exposure between different ages and sexes. Social monogamy apparently reduced sex differences in immunity predicted by the ICHH, as there were only differences in cestodes but not nematodes between males and females. Arctic fox populations are cyclic, with periods of high and low population size (Macpherson 1969). The changes in age structure over time due to these cyclic populations likely will impact the population's parasites.

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Tables and Figures

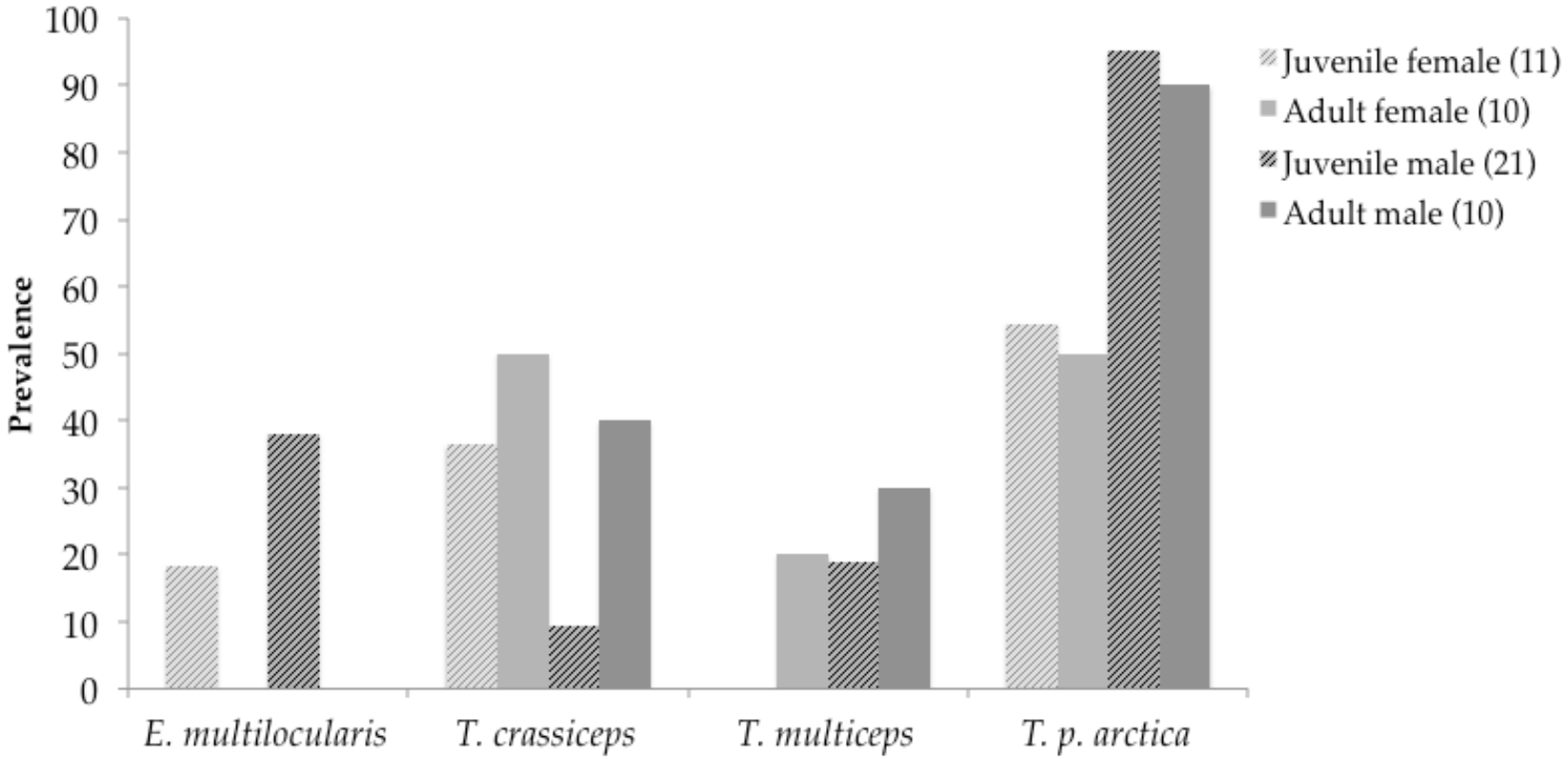

Figure 2.1. Prevalence (percentage of fox infected with cestodes) of cestode species (*Echinococcus multilocularis*, *Taenia crassiceps*, *Taenia multiceps*, and *Taenia polyacantha arctica*) in arctic fox (*Vulpes lagopus*) from Churchill, Manitoba in winter 2011 and 2012. Sample size for each group is included in the legend.

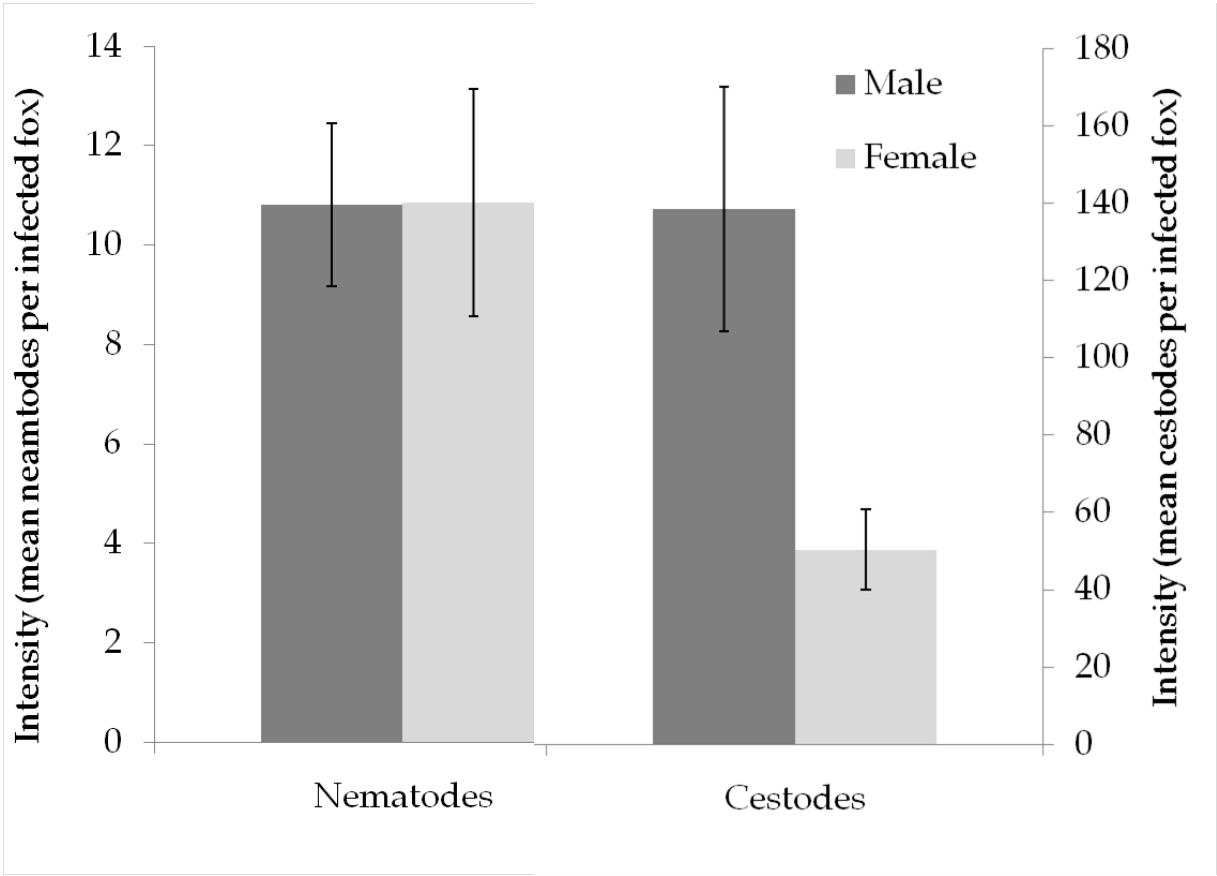

Figure 2.2. Intensity (mean ± SEM number parasites per infected individuals) of cestodes and nematodes in both female (n=21) and male (n=31) arctic fox (*Vulpes lagopus*) from Churchill, Manitoba. Fox were sampled in December 2010 - February 2011 and December 2011 - February 2012. Female and males differed in cestode intensity and abundance. Intensity and abundance of nematodes between sexes did not differ.

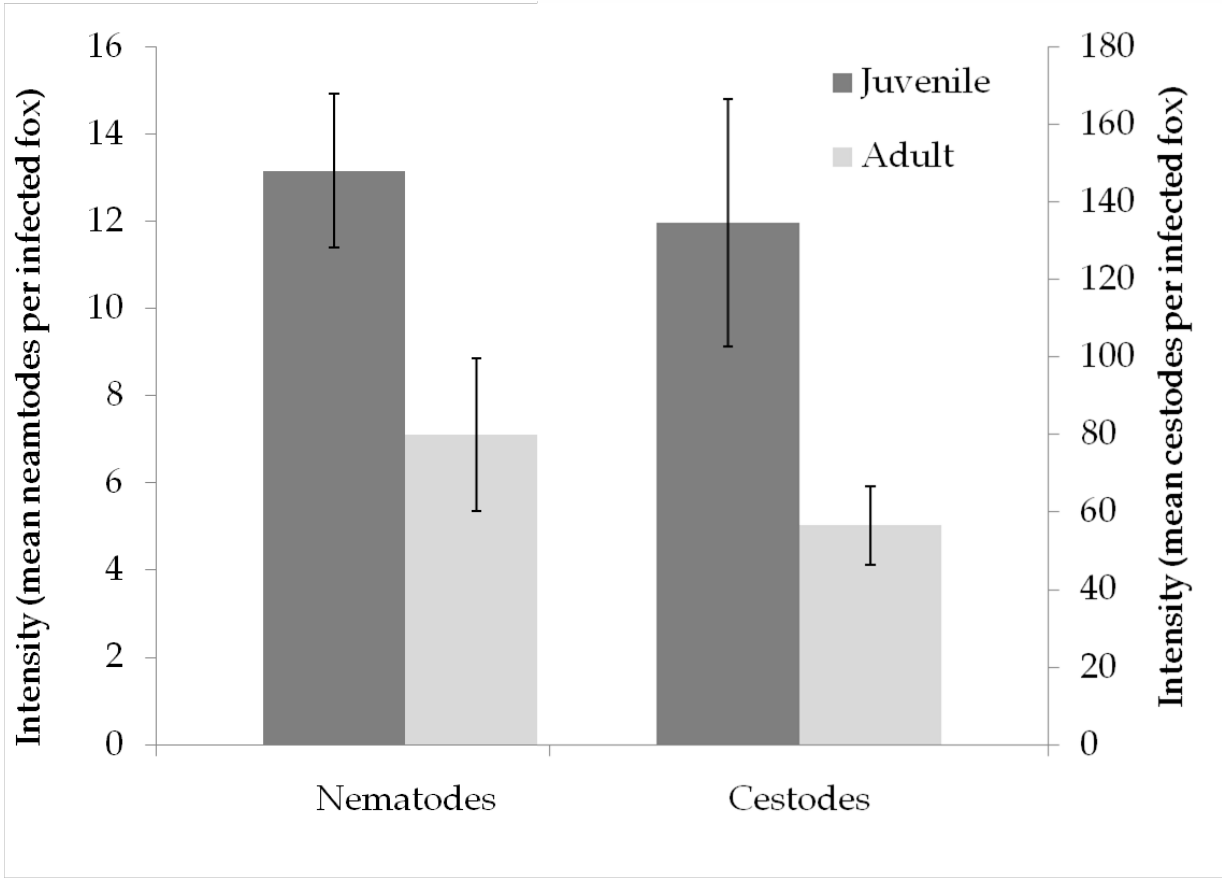

Figure 2.3. Intensity (mean ± SEM number parasites per infected individuals) of cestodes and nematodes in both juvenile (n=32) and adult (n=20) arctic fox (*Vulpes lagopus*) from Churchill, Manitoba. Fox were sampled in both December 2010 - February 2011 and December 2011 - February 2012. Juvenile and adults differed in intensity and abundance of nematodes. Cestode intensity or abundance did not differ.

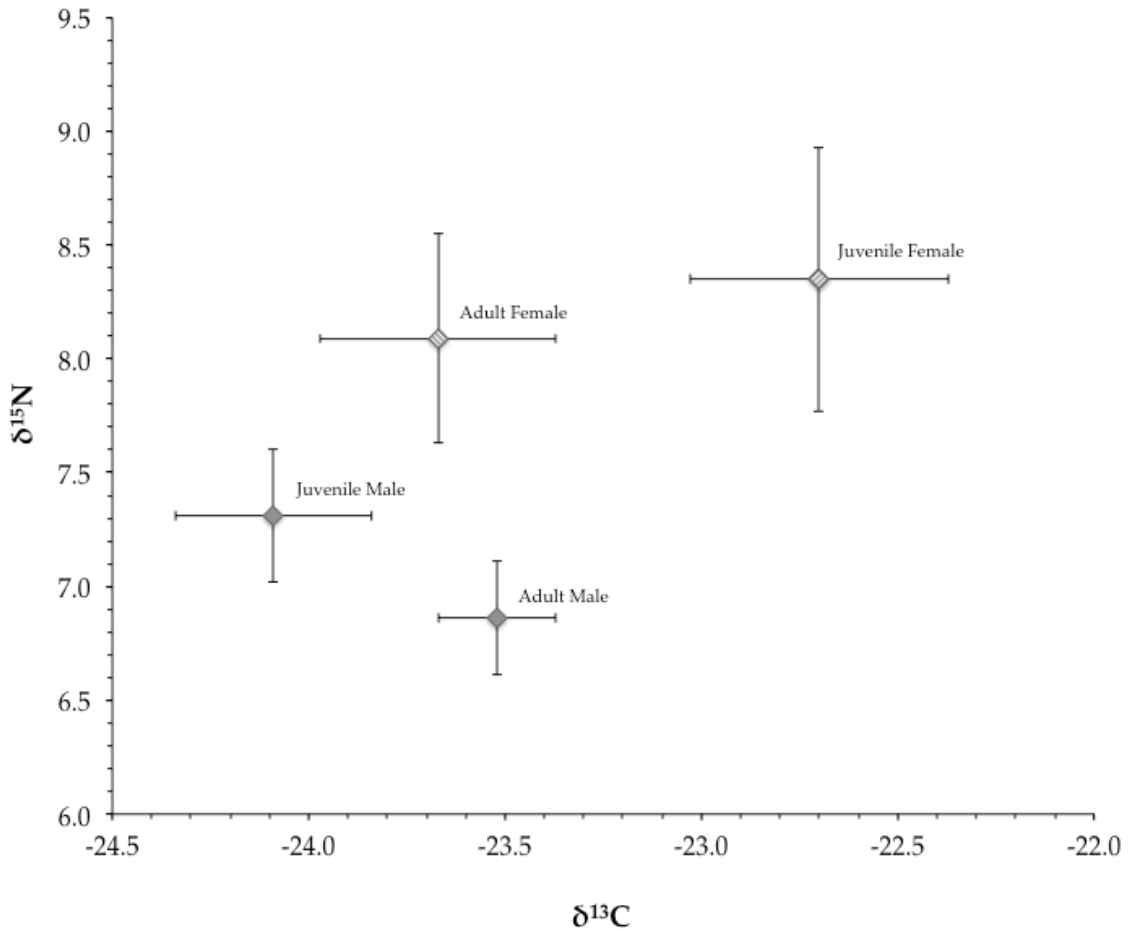

Figure 2.4. Stable isotope ratios of arctic fox (*Vulpes lagopus*, mean ± SE) muscle tissue, separated by sex and age, from Churchill, Manitoba, Canada. Arctic fox were sampled in December 2010-February 2011 and December 2011-February 2012.

Chapter 3: Parasites in sympatric arctic and red foxes related to diet, behavior, and life history

Abstract

Climate change can allow species to expand into regions where they previously were absent. Range expansion of parasites and their hosts has the potential to introduce novel parasites to the Arctic, which could affect endemic species, changing host abundance and trophic interactions, thereby impacting the entire ecosystem. The arctic fox is a circumpolar predator whose southern range overlaps with red fox, its competitor and predator, in areas where arctic tundra meets the boreal forest. Red fox has one of the largest ranges of any land animal, covering a broad diversity of prey types and climatic conditions, thus exposing this species to a high diversity of parasites. Red fox likely have evolved behavioral and physiological defenses against parasites. In contrast, biodiversity within the arctic fox range is low, reducing the diversity and density of parasites that the foxes have been exposed to during their evolutionary history. As red fox expands northward with the warming climate, interactions between these species could alter parasite abundance and diversity in arctic foxes. Differences in their life history, diet, habitat use, and behavior also could affect each fox's susceptibility to parasites. To compare parasites in sympatric arctic and red foxes we examined fox carcasses collected from local trappers in Churchill, Manitoba, Canada and compared parasites to fox diet estimates from stable isotope analysis and comparisons of defecation behavior at fox dens. Arctic fox had more parasites than sympatric red fox. This difference was reflected in diet estimates expressed as stable isotope ratios ($\delta^{13}C$) of fur and muscle. Cestode abundance was negatively related to $\delta^{13}C$ in arctic and red fox, indicating rodent

consumption increased cestode loads. Red fox defecated at dens in winter less than arctic fox, a potential adaptation for reducing exposure to nematodes, which are frequently transferred through indirect contamination. Differences in litter size between two the species may also explain higher nematodes in arctic fox, and other life-history differences may also contribute to these large differences in parasite community. The condition of arctic and red foxes was not impacted by the abundance of parasites, although further stressors in the environment may change this trend. As climate change could affect prey diversity and abundance, impacts on small mammals may further alter fox parasites and influence ecosystem dynamics in the region. Further range expansion by many invertebrate hosts and previously limited parasites that use red foxes in their southern distribution could also impact arctic foxes and have cascading effects.

Introduction

The Arctic ecosystem is undergoing climate warming at an unparalleled rate (Anisimov et al. 2001). Increasing temperatures in the Arctic are influencing the function and structure of the Arctic ecosystem (Anisimov et al. 2001; Parmesan 2006; Kutz et al. 2009). Climate plays an important role in determining the abundance and diversity of species within a region, including hosts and parasites (Parmesan 2006). The Arctic is characterized by low biodiversity making it particularly vulnerable to changes in the biotic community (Kutz et al. 2009; Hoberg et al. 2012). Increasing temperatures may bring novel species to the region and increase competition for food and space. The expansion of these species into the region may introduce new parasites and hosts (Hoberg et al. 2012) that were previously limited by the temperature limits of free-living life

stages and the range of invertebrate intermediate hosts (Brooks and Hoberg 2007; Kutz et al. 2009). These novel parasites may have dramatic effects on formerly unexposed hosts (Price et al. 1986; Parmesan 2006).

Red fox (*Vulpes vulpes*) is a generalist predator that is expanding northward with increasing temperatures (Post et al. 2009). Red fox have one of the largest ranges of any land animal in the world, throughout which they encounter broad diversity of habitats, prey types, and climatic conditions (Macdonald and Reynolds 2004). This broad habitat diversity has exposed this species to a high diversity and density of parasites (Larivèire and Pasitschniak-Arts 1996; Macdonald and Reynolds 2004) to which it has likely evolved both behavioral and physiological defenses. At the northern edge of their range, where boreal forest meets arctic tundra, red fox are competitors and predators of arctic fox (*Vulpes lagopus*), an important circumpolar predator (Hersteinsson and MacDonald 1992; Larivèire and Pasitschniak-Arts 1996; Audet et al. 2002). Arctic foxes have encountered a low diversity and density of parasites during their evolutionary history because overall biodiversity in the Arctic is low, and therefore may be susceptible to the introduction of novel parasites. Susceptibility to parasites may also depend on differences in their life history, diet, habitat use, and behavior (Schmidt and Roberts 2009; Stein et al. 2009).

Interactions between arctic and red foxes could increase parasite loads for both species in regions of overlap and, as red fox expand northward with the warming climate, parasite exposure for arctic foxes will increase (Lafferty et al. 2008; Schmidt and Roberts 2009). Red foxes serve as host to many species of parasites that are range-limited due to other factors (e.g., temperature limits on their free-living stages or lack of invertebrate

intermediate hosts) that could be novel additions to the Arctic ecosystem (Kutz et al. 2009). *Alaria alata*, for example, can be transmitted to the red fox through ingestion of frogs, or other paratenic hosts, but has not yet been found in the arctic fox (Gibson et al. 2005; Schmidt and Roberts 2009). However, congeneric parasites of *Alaria* have been found within the arctic fox and interactions between fox species could transmit *A. alata* and many other novel parasites to the arctic fox if suitable intermediate hosts exist or move into the region (Gibson et al. 2005; Schmidt and Roberts 2009).

Arctic and red foxes exhibit dietary overlap where their ranges overlap (Hersteinsson and Macdonald 1992; Barth et al. 2000) and both have been known to host various parasites that are transmitted trophically, through the ingestion of infected prey (Gibson et al. 2005; Schmidt and Roberts 2009). As the interactions between predator and prey are key determinants of transmission intensity of many parasites, the consumption of different types of prey (e.g. mammals versus birds) will expose foxes to a different array of parasites (Raoul et al. 2010). Conversely, the presence of different parasite species should reflect differences in the diet of arctic and red foxes (Rausch et al. 1983; Skirnisson et al. 2010). For example, the presence of *Taenia crassiceps*, which uses small rodents as intermediate hosts, would be indicative of small rodents in the fox's diet (Stien et al. 2009). However, the congeneric *Taenia multiceps* use ungulates, such as caribou, and lagomorphs, such as arctic and snowshoe hares, as intermediate hosts and would indicate the consumption of ungulates or lagomorphs by either fox species (Rausch et al. 1983; Gibson et al. 2005). Ringed seals (*Pusa hispida*) occasionally serve as host to *Trichinella nativa*, which is transferred between top predator hosts through the consumption of infected muscle tissue (Forbes 2000; Skirnisson et al. 2010). Therefore,

presence of *T. nativa* within a fox would indicate a diet that includes other carnivores such as ringed seals or conspecifics (Skirnisson et al. 2010). Birds, including the migratory Lesser Snow Goose (*Chen caerulescens caerulescens*) and Canada Goose (*Branta canadensis interior*), and their eggs do not serve as common intermediate hosts for parasites that infect both arctic and red foxes (Gibson et al. 2005). A handful of less common parasites can be transmitted between birds and foxes. Some insect-eating bird species can serve as intermediate hosts for *Mesocestoides* spp. (Gibson et al. 2005), although this species is uncommon in North America (Skirnisson et al. 1993). The presence of this parasite within a fox would indicate a diet including birds, as well as other amphibians and mammals. As birds, and particularly eggs, are not common intermediate hosts, a fox whose diet largely includes these food sources should have fewer parasites.

Fox dens are re-used over time (Macpherson 1969), which may assist the growth and maintenance of any parasite strongly associated with the host species (Thomas et al. 2009), particularly those that are transmitted horizontally, through the contamination of food or other resources via infected feces, such as many species of nematodes (Anderson 1992; Thomas et al. 2009). Previous studies documented the evolution of selective defecation behaviors by many species, including several species of ruminants that defecate in particular areas and feed in other areas (Ezenwa 2004). This behavior reduces their chance of infection, because some of their feces (or feces of intraspecifics) may contain the eggs of parasites (Thomas et al. 2009). Because foxes re-use den locations year after year, foxes may have evolved behaviors to avoid infection due to the ingestion of feces with infectious eggs.

Parasites are considered to be stressors on their hosts and may be detrimental to the condition of arctic and red foxes (Marcogliese and Pietrock 2011). The response of hosts to parasites can often include alterations in resource allocation, altered investment in immune function, reduced mating success, and increasing vulnerability to other stressful conditions (Lafferty et al. 2006; Thomas et al. 2009). Nevertheless, the effects of parasitism on foxes are poorly understood.

The objective of this study was to examine the relationships between diet and parasite communities between sympatric arctic and red foxes. We focused on helminthes (parasitic worms, including species of nematodes and platyhelminthes) as most transfer through trophic interactions or contamination of food resources by feces with nematode eggs. If the overall range of foxes (with differences in biodiversity and abundance of organisms) affects the evolution of behavioral and physiological defense against helminthes, then helminth prevalence and intensity should be low in red fox and high in arctic foxes. Secondly, if diet affects the fox's parasite exposure, then a fox whose diet is high in small mammals or caribou should have more parasites than a fox whose diet consists of birds or seals. Further, we examined the relationship between helminth abundance and the condition of foxes. If parasites affect the body condition of foxes, we would expect to see foxes in poorer condition with a high abundance of parasites. Understanding the connections between climate, the community and diversity of parasites, and impacts on their hosts and subsequently ecosystem dynamics is essential, particularly in light of dramatic changes in the Arctic.

Methods

Study Region - Arctic and red fox carcasses were collected near Churchill, Manitoba, on the west edge of Hudson Bay (58° N, 94° W). This region is a transitional zone between the arctic tundra and boreal forest, where the southern range of the arctic fox overlaps the northern range of the red fox. Collared lemmings (*Dicrostonyx richardsonii*) and red-backed voles (*Myodes gapperi*) represent the two primary rodent species found throughout the year on the tundra and within the boreal forest, respectively. Collared lemming populations also fluctuate annually, although the amplitude of these fluctuations may have declined in the last few decades (Scott 1993). Many alternative food sources fluctuate seasonally in this region. Lesser Snow Goose (*Chen caerulescens caerulescens*), Canada Goose (*Branta canadensis interior*), and several shorebirds (e.g., Dunlin (*Calidris alpina*), Least Sandpiper (*Calidris minutilla*), Whimbrel (*Numenius phaeopus*), Hudsonian Godwit (*Limosa haemastica*)) nest in the area during the spring and summer months (Reiter and Anderson 2008). Caribou are also seasonally found within this region but move south inland to the boreal forest during winter (Campbell 1994). Ringed seals may be available to arctic foxes during winter. Foxes have been known to scavenge on seal carcasses left by polar bears (*Ursus maritimus*) and prey on seal pups in spring (Smith 1976).

Endoparasite Sampling - We collected arctic and red fox carcasses from local fur trappers in 2011 and 2012. We determined the endoparasite community within each individual through standard necropsy techniques (Munson 2000). Canine teeth were x-rayed to distinguish adults from juveniles (individuals born the previous spring (Roth

2003)) based on the size of the pulp cavity (Grue and Jensen 1976; Fancy 1980). Large pulp cavities (width exceeding 40% of the total width of the cavity) indicate a juvenile (Grue and Jensen 1976). Canine teeth with small pulp cavities were sent to the Matson's Laboratory (Milltown, Montana) for sectioning, examining the cementum layers, and age analysis (Fancy 1980). Juveniles were considered to be foxes born the previous spring (Roth 2003). We measured body length (spine length and tail length) and mass. Lipid deposits serve as energy reserves and insulation, along with support and padding for some organs (Prestrud and Nilssen 1992). The residuals of a regression of body mass on spine length are commonly used as a measure of body condition (Schulte-Hostedde et al. 2001). Body mass residuals were calculated for each individual. Additionally, we extracted kidneys and used the kidney-fat index as a second measure of body condition (Finger et al. 1981). Hearts, lungs, and livers (including bile duct) were collected from each individual to examine for parasites or other pathology.

Each intestine was split longitudinally and scraped to remove all parasites. The remaining tissue was examined under a dissecting microscope to ensure removal of all parasites. Sedimentation and counting was used to examine all remaining tissue (Eckert 2003). All parasites were identified to species (when possible), weighed, and preserved in 70% ethanol. Prior to fixation, the scolex of all cestode species (except *Diphyllobothrium* spp., see Scholz et al. 2009) was sliced off and squashed between a cover slip and glass slide. Scolex squashes were used to identify species using a compound microscope (based upon the number, shape and size of rostellar hooks, following Abuladze (1964), Rausch and Fay (1988) and Loos-Frank (2000)). Nematodes were identified following Georgi (1974) and Anderson (1992). The mass of all remaining tissue was measured and

all tissue was preserved in 70% ethanol. *Echinococcus* spp. were identified by characteristic morphology (see Thompson 1995). *Echinococcus multilocularis* is a comparatively very small cestode that was abundant in foxes in which they were present. However, they were in poor condition (broken into pieces) within all the analyzed foxes making them very difficult to quantify. Their intensity was roughly estimated by using dilution counts (Thompson et al. 2006). The dry biomass of *E. multilocularis* was approximated using the mean mass of 20 individuals. Due to their disproportionately large intensity (some with over 10 000), this species was excluded from cestode intensity and abundance. Parasite taxa were quantified using three measurements: prevalence (presence or absence of parasite in a fox), intensity (number of parasites per infected fox), and abundance (number of parasites per fox) (Rozsa et al. 2000).

Diet Reconstruction - To compare the parasite community to the prey consumed by individual foxes we estimated the diet of the foxes using stable-isotope analysis ($\delta^{15}N$ and $\delta^{13}C$) (Chisholm et al. 1982; Roth 2002). For example, fox that have consumed terrestrial food sources will have lower $^{13}C/^{12}C$ ratios compared to those that consume purely marine sources (Chisholm et al. 1982). An intermediate signature would be produced in foxes that consume a mixed diet.

We used two different tissue types, fur and muscle, for stable isotope analysis. Fur is an inert tissue and represents the diet during its growth (Roth 2002). In arctic fox, this fur (which represents a mixture of guard hair and under fur) will have grown during the arctic fox's autumn molt and samples will represent the diet of the fox near the end of the summer period (Roth 2002). In red fox, fur and guard hair are replaced at different times

and rates during their summer molt (Maurel et al. 1986). Red fox guard hair is replaced between May-July whereas the under fur is grown between October-November (Maurel et al. 1986). To compare these growth periods, red fox under fur and guard hair were separated in the 2012 samples. We compared the stable isotopes of under fur to those of guard hair from the same individual (paired t-test). Since the under fur and guard hair did not differ isotopically ($\delta^{13}C$: t = -1.00, *df*=17, *P*=0.33; $\delta^{15}N$: t=-1.28, *df*=17, *P*=0.22), we assumed the 2011 red fox samples could be used as a representation of the fall diet. Each fur sample was washed with soap and water to remove any debris or oil, dried at 60°C, and homogenized with scissors.

The muscle tissue will have gone through complete turnover in the past few months so the tissue will represent the early winter diet (Hobson and Clark 1992; Dalerum and Angerbjorn 2005). Muscle samples were freeze-dried and powdered (Roth 2002). Lipids were removed from the samples using a soxhlet apparatus (Roth 2002) due to the strong influence variation in lipids may have on the measurements of $\delta^{13}C$ (Rau et al. 1992). The stable isotope ratios for all samples were measured using a continuous flow isotope ratio mass spectrometer at the University of Windsor and University of Manitoba. Differences in stable isotope values due to location were accounted for by rerunning nine of the same samples at both facilities. Samples run at University of Windsor had nitrogen values 0.56‰ less than University of Manitoba and so values were adjusted to account for this difference. Stable isotope values are presented in parts per thousand (‰) relative to Pee Dee Belemnite (carbon) and atmospheric N_2 (nitrogen) standards (Roth 2003; Adams et al. 2010) as follows:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] * 10^3$$

where *X* is ^{13}C or ^{15}N and R is the ratio of the heavy isotopes to light isotopes (e.g. $^{13}C/^{12}C$ or $^{15}N/^{14}N$).

Isotopic discrimination factors have been experimentally determined in feeding studies in both arctic and red foxes (Roth and Hobson 2000; Lecomte et al. 2011). Arctic and red fox have very similar isotopic discrimination factors for fur (arctic fox: $\Delta C=2.2\pm0.4$, $\Delta N=3.3\pm0.7$; red fox: ΔC 2.6±0.1, ΔN 3.4±0.1). However, arctic fox have different isotopic discrimination factors for muscle (arctic fox: ΔC 0.37±0.76, ΔN 1.79±0.41; red fox: ΔC 1.1±0.1, ΔN 3.3±0.1) (Roth and Hobson 2000; Lecomte et al. 2011). Due to these differences, 0.73 was added to all $\delta^{13}C$ values of arctic fox, and 1.51 to all $\delta^{15}N$ values of arctic before comparisons were made. Stable isotope values for ringed seals were obtained from Ramsay and Hobson (1991). Carbon stable isotope ratios for ringed seals were modified to account for temporal change (see Long et al. 2005).

Fox den monitoring - We visited all known arctic and red fox dens (107 dens in 2011 and 114 dens in 2012 in and around Wapusk National Park) via snowmobile in April. Each den was examined for the presence feces, urine, presence of a tunnel, and fur. Feces were collected when present. Dens on the tundra tend to be occupied by arctic fox although red fox have occasionally been found using them. Fur was used to identify the fox species when possible to account for this possibility. The remaining fox dens, located in forested areas, were considered to be more likely used by red fox.

Small Mammal Sampling- Small mammals serve as intermediate hosts for many species of parasites infective to canids. To understand the role diet plays in parasite transmission,

the community of larval stages of parasites within intermediate hosts needs to be evaluated. The presence or absence of larval parasites within intermediate hosts assists in making better predictions based on the presence or absence of parasites within the fox. To evaluate the parasite community within lemmings and other small mammals within the study region, small mammals were collected during summer using museum special snap-traps following protocols established by the Arctic Wildlife Observatories Linking Vulnerable EcoSystems project (ArcticWOLVES, http://www.cen.ulaval.ca/arcticwolves/) as approved (University of Manitoba's Animal Care and Use Committee (F10-016)). Three traps were set every 15 m along 300m transects, covering different habitat types. We baited each trap with peanut butter and checked once daily for a period of three days. Twenty-nine transects were set up in August 2011 and June –July 2012 in different habitats found around CNSC and Wapusk National Park, including dry hummock, beach ridge, wet sedge fen, and forested regions. Two grids (8x8 with 15 m spacing) were also set up within Wapusk National Park, using the same trapping technique with three baited snap-traps at each post. Small mammals were collected from the traps and frozen for later necropsy. Each individual was examined for the presence of different parasites. If larval cestodes were found within the carcasses, a scolex squash (as described above) was performed for the identification of the species. Muscle and fur samples were taken from each individual for stable isotope analysis, as described above. Red squirrels (*Tamiasciurus hudsonicus*) and snowshoe hare (*Lepus americanus*) were collected from local trappers and analyzed in the same manner.

Statistical Analysis - Statistical analyses were performed in JMP® 10 (SAS Institute Inc. 2012) and R statistical software (http://www.R-project.org). Biological measurements (spine length and body mass) were compared between sexes by species using ANOVA to detect differences. If females and males were different in either biological measurement, they were separated when determining the residuals of body mass on spine length. Mean stable isotope values were compared between species by year to detect differences in diet using a combination of multivariate and univariate analysis (Hummel and Sligo 1971). Mean stable isotope values of each prey species were compared using an ANOVA and Tukey-Kramer HSD post hoc test to determine if prey species differed significantly. Differences in the diversity of cestodes between arctic and red foxes were examined using the Shannon-Weaver diversity index and compared using a Student's t-test. The prevalence (proportion of foxes infected) of cestodes and nematodes was compared between species and years using logistic regression. Parasite communities are generally aggregated so log(x+1) transformations were used to normalize the data (Rozsa et al. 2000). To detect any differences in abundance (median parasites of all foxes) and intensity (median parasites of all infected foxes) between fox species or year we used Wilcoxon Rank Sums tests, as many of these data did not have a normal distribution even after transformation. The relationship between diet (stable isotope ratios) and parasite abundance was examined using linear regression. A diet that lacks suitable intermediate hosts should lack associated parasites and therefore, a relationship should appear between these foxes' parasites and their stable isotopes ratios. The regression between cestode abundance and diet ($\delta^{13}C$ and $\delta^{15}N$) for red foxes did not meet all the assumptions of the model (lack of heterogeneity of variance) and so a Poisson regression was used to

examine this relationship. Similarly, we used a Poisson regression to compare cestode biomass and diet ($\delta^{13}C$ and $\delta^{15}N$). We used logistic regression to examine differences in defecation behavior between arctic and red foxes. Only occupied dens (with tunnels through the snow) were included in the analysis. The kidney fat index and residuals from the regression of spine length against body mass were used as indices of body condition and were compared to values of the parasite community to examine the potential effects of parasites on the condition of foxes. Arctic and red foxes were examined separately to account for species differences. Correlations between body condition and the abundance of parasites (overall, cestode, and nematode abundances) were examined using Spearman's ρ.

Results

We necropsied 22 foxes in 2011 (19 red fox and 3 arctic fox) and 73 foxes in 2012 (18 red fox and 55 arctic fox) (see Table 3.1). The sex ratio was almost even, but there were slightly more male arctic foxes and slightly more female red foxes (Table 3.1). Juveniles represented a higher proportion of the population of both species (Table 3.1). All arctic fox were either juvenile or one year old. Red fox ranged in age from juvenile to nine years.

Multiple species of nematodes and platyhelminths were present in both the arctic and red fox. Taeniidae cestodes, including *Taenia crassiceps, Taenia multiceps, Taenia polyacantha arctica* and *Echinococcus multilocularis,* were present, although *E. multilocularis* was only present in arctic foxes. The cestode *Diphyllobothrium* sp. was found in one red fox. A spiurid nematode, *Spirocerca lupi,* was found encysted on the

stomach walls of arctic foxes; however, none were found in red foxes. Ascarid nematodes, *Toxascaris leonina* and *Toxocara canis*, and ancylostomatidae nematodes, were present in both arctic and red foxes. No larval parasites were found in any of the collared lemmings (n=7) and southern red-backed voles (n=6) examined. There was no difference (Student t-test, t ratio = -1.84, df=55, *P*=0.071) in parasite diversity (Shannon-Weaver diversity indices) between arctic (H=0.31) and red fox (H=0.07). No parasites were found in the hearts, lungs, and livers (including bile duct).

Arctic fox overall gastrointestinal parasites (prevalence: χ^2 =0; intensity: Wilcoxon Rank Sums, Z= 0.12, *P*=0.91; abundance: Wilcoxon Rank Sums, Z= 0.12, *P*=0.91), cestodes (prevalence: Fisher's Exact Test, *P*= 1.0; intensity: Wilcoxon Rank Sums, Z= 0.45, *P*=0.65; abundance: Wilcoxon Rank Sums, Z= 0.55, *P*=0.58), and nematodes (prevalence: χ^2 =0 ; intensity: Wilcoxon Rank Sums, Z= 0.94, *P*=0.35; abundance: Wilcoxon Rank Sums, Z= 0.94, *P*=0.35) did not differ between 2011 and 2012. Red fox also had no differences in overall gastrointestinal parasites (prevalence: Fisher's Exact Test, *P*=0.48; intensity: Wilcoxon Rank Sums, Z= -0.83, *P*=0.40; abundance: Wilcoxon Rank Sums, Z= 1.26, *P*=0.21), cestodes (prevalence: Fisher's Exact Test, *P*= 0.12; intensity: Wilcoxon Rank Sums, Z= 0.35, *P*=0.73; abundance: Wilcoxon Rank Sums, Z= 1.56, *P*=0.12), and nematodes (prevalence: Fisher's Exact Test, *P*= 0.50; intensity: Wilcoxon Rank Sums, Z= -0.27, *P*=0.79; abundance: Wilcoxon Rank Sums, Z= 0.91, *P*=0.36) between years. For this reason, data from each year were grouped for each species.

Arctic fox and red fox differed in their parasite communities. The prevalence of cestodes in arctic foxes was higher than in red fox (Logistic Regression, χ^2 = 52.91,

P<0.0001) (Table 3.2). Arctic foxes also had more individuals with nematodes (Logistic Regression, χ^2 = 21.40, *P* <0.0001) (Table 3.2). Arctic fox also had higher median intensity (Wilcoxon Rank Sums, Z= -3.69, *P* =0.0002, Figure 3.1) and median abundance (Wilcoxon Rank Sums, Z= -7.49, *P*<0.0001, Figure 3.1) of cestodes and higher median intestinal nematode abundance (Wilcoxon Rank Sums, Z= -3.91, *P*<0.0001 Figure 3.1). There was no difference in median intestinal nematode intensity (Wilcoxon Rank Sums, Z=-1.52, *P*=0.13, Figure 3.1).

Arctic fox had a higher proportion of dens with feces in comparison with red foxes (Logistic Regression, χ^2 = 18.3, *P*=0.0004), with a greater difference between species in 2011 (interaction between year and species, Logistic Regression, χ^2 = 5.97, *P*=0.015). There was no main effect of year (Logistic Regression, χ^2 = 0.18, *P*=0.67). Arctic and red fox differed in their muscle stable isotope ratios (Table 3.3, MANOVA, $F_{2,92}$=0.32, *P*<0.0001). Both δ^{13}C (ANOVA, $F_{1,93}$=19.51, *P* <0.0001) and δ^{15}N (ANOVA, $F_{1,93}$=26.61, *P* <0.0001) muscle values differed between species. Fox species also differed in fur stable isotope ratios (MANOVA, $F_{2,92}$=0.33, *P* <0.0001), particularly in δ^{15}N (ANOVA, $F_{1,93}$=17.96, *P* <0.0001; Table 3.3). However, arctic fox δ^{13}C values for fur did not differ from red fox fur values (ANOVA, $F_{1,93}$=1.91, *P* =0.17) (Table 3.3). Cestode abundance was negatively related to arctic fox fur δ^{13}C (Linear Regression, R^2=0.11, $F_{1,50}$=6.48, *P*=0.014, Figure 3.2) but unrelated to δ^{15}N of fur (Linear Regression, R^2=0.0081, $F_{1,50}$=0.41, *P*=0.53). Additionally, there was no relationship between muscle δ^{13}C values and δ^{15}N values and cestode abundance in the arctic fox (δ^{13}C: Linear Regression, R^2=0.027, $F_{1,50}$=1.40, *P*=0.24; δ^{15}N: Linear Regression, R^2=0.013, $F_{1,50}$=0.66, *P*=0.42). In red fox, cestode abundance was negatively related to muscle δ^{13}C

values (Generalized Linear Model, χ^2= 19.75, P<0.0001, n=37, Figure 3.2) but unrelated to δ^{15}N of muscle (Linear Regression, R^2=0.047, $F_{1,35}$=1.722, P=0.20) or to either the δ^{13}C or δ^{15}N values of fur (δ^{13}C: Linear Regression, R^2=0.027, $F_{1,35}$=0.99, P=0.33; δ^{15}N: Linear Regression, R^2=0.0064, $F_{1,35}$=2.40, P=0.13).

Nematode abundance was negatively related to fur δ^{15}N values in red fox (Linear Regression, R^2= 0.15, $F_{1,35}$=6.39, p=0.016, Figure 3.3), but unrelated to fur δ^{13}C values (Linear Regression, R^2=0.011, $F_{1,35}$=0.38, P=0.54), muscle δ^{13}C values (Linear Regression, R^2=0.00055, $F_{1,35}$=0.019, P=0.89), or muscle δ^{15}N values (Linear Regression, R^2=0.060, $F_{1,35}$=2.22, P=0.15). Nematode abundance in arctic fox was unrelated to muscle δ^{13}C values (Linear Regression, R^2=0.030, $F_{1,50}$=1.58, P=0.22) muscle δ^{15}N values (Linear Regression, R^2=0.0019, $F_{1,50}$=0.094, P=0.76), fur δ^{13}C values (Linear Regression, R^2=4.51x10^{-7}, $F_{1,50}$=0, P=1.0), or fur δ^{15}N values (Linear Regression, R^2=0.0052, $F_{1,50}$=0.26, P=0.61)

The biomass of cestodes was not related to muscle δ^{13}C values (Poisson Regression, χ^2 = 1.31, P=0.25) or muscle δ^{15}N values (Poisson Regression, χ^2 = 0.67, P=0.41) in the arctic fox. No relationship was found between the biomass of cestodes and fur δ^{13}C values (Poisson Regression, χ^2 = 2.33, P=0.13) or fur δ^{15}N values (Poisson Regression, χ^2 = 0.27, P=0.60) in the arctic fox. The biomass of cestodes was not related to the muscle δ^{13}C values (Poisson Regression, χ^2 = 0.0017, P=0.97) or muscle δ^{15}N values (Poisson Regression, χ^2 = 0.0018, P=0.97) in red fox. Further, no relationship was found between the biomass of cestodes and fur δ^{13}C values (Poisson Regression, χ^2 = 0.00046, P=0.98) or fur δ^{15}N values (Poisson Regression, χ^2 = 0.00073, P=0.98) in the red fox. No relationship was found between the cestode intensity and cestode biomass for

either the arctic fox (Poisson Regression, $\chi^2 = 0.50$, P=0.48) or red fox (Poisson Regression, $\chi^2 = 0.018$, P=0.89).

Arctic fox spine length and body mass did not differ between sexes (spine length: ANOVA, $F_{1,52}$=0.32, P =0.29; body mass: ANOVA, $F_{1,52}$= 0.34, P=0.28). Red fox had sex differences in in spine length, with males having longer spines (one-way ANOVA, $F_{1,36}$= 3.87, P =0.0029), and body mass (one-way ANOVA, $F_{1,36}$= 11.61, P =0.0008). Because of these differences, the residuals of body mass on spine length were calculated separately for male and female red fox. The relationship between fox body condition and parasite community was examined using two separate indices, kidney fat index (KFI) and residuals of spine length and body mass, against overall parasite, cestode, and nematode abundance. No correlations were observed between overall parasite abundance and KFI (arctic fox: Spearman's $\rho = 0.0099$, P=0.94; red fox: Spearman's $\rho = -0.14$, P=0.41) or residuals of spine length on body mass (arctic fox: Spearman's $\rho = -0.22$, P=0.12; red fox: Spearman's $\rho = 0.056$, P=0.74). Further, no correlation was observed between the abundance of cestodes and the KFI (arctic fox: Spearman's $\rho = 0.052$, P=0.72; red fox: Spearman's $\rho = -0.081$, P=0.63) or residuals of spine length on body mass (arctic fox: Spearman's $\rho = -0.18$, P=0.21; red fox: Spearman's $\rho = 0.028$, P=0.87) in arctic and red foxes. The same was true for both fox species between the abundance of nematodes and the KFI (arctic fox: Spearman's $\rho = -0.091$, P=0.52; red fox: Spearman's $\rho = -0.13$, P=0.45) or residuals of spine length on body mass (arctic fox: Spearman's $\rho = -0.12$, P=0.38; red fox: Spearman's $\rho = 0.049$, P=0.77).

Discussion

Arctic foxes had a greater prevalence and intensity of parasites than red foxes. These parasite differences are likely caused by differences in the behavior, life history, and diet of arctic and red foxes. The differences in behavior, including selective defecation, and life history are likely influencing nematode prevalence in the two species. The prevalence and abundance of nematodes were much higher in arctic foxes, although the intensity of nematodes did not differ. Very few red fox dens had feces present whereas feces were commonly encountered at arctic fox dens. By reducing the feces at their den, the fox reduces the probability of becoming infected with nematodes, especially for pups. Using a single location or a location distant from a commonly used area to defecate reduces the chance of transmission (Schmid-Hempel and Ebert 2003; Ezenwa 2004; Thomas et al. 2009). Nematodes are frequently horizontally transmitted through indirect contamination by feces containing eggs. Ascarid nematodes, such as *Toxascaris leonina* and *Toxocara canis*, both species found within these foxes, and ancylostomatid nematodes, such as *Uncinaria stenocephala*, are transmitted through the accidental consumption of infected feces (Anderson 1992). The differences in defecation behavior support our hypothesis that red fox has evolved behaviors that reduce the chance of contracting certain parasites.

Another explanation for the differences in nematode prevalence between the two species is differences in their life history. Litter size in arctic foxes (mean 6-12 pups) is larger than observed for red foxes (mean 3-6 pups) (Macpherson 1969; Larivière and Pasitschniak-Arts 1996; Audet et al. 2002). The fox pups remain at their maternal den for the first 3-4 months and defecate all over their dens (Macpherson 1969). The density of

hosts, including foxes, is strongly linked to the prevalence and load of parasites (Arneberg et al. 1998). A higher density of arctic fox pups on the den may explain the increased parasite abundance and prevalence that is observed compared to red fox, with fewer individuals using the same den site.

Arctic and red foxes have been considered to be direct competitors, consuming similar prey (Hersteinsson and Macdonald 1992; Barth et al. 2000). However, the stable isotope ratios from arctic and red foxes within this region indicate that there are differences in diet. Large differences in the parasite communities of these species suggest that these differences in diet are an important driver of differences in the parasite communities. The presence of the cestode *T. multiceps*, which was found almost exclusively in the arctic foxes, reflects caribou or hare in their diet as *T. multiceps* requires hares or ruminants as intermediate hosts (Rausch et al. 1983; Gibson et al. 2005). Similarly, the nematode *S. lupi*, found only in arctic foxes, reflects a diet of invertebrates or small mammals as *S. lupi* is transmitted through insects and small mammals. *S. lupi* has been recorded to infect red foxes (Wirsing et al. 2007; Ferrantelli et al. 2010). Therefore, its absence in red fox is likely due to dietary differences.

The diets of red and arctic foxes were linked to their gastrointestinal parasite community, with lower $\delta^{13}C$ stable isotope values related to a decreasing abundance of cestodes. Although the relationship between $\delta^{13}C$ stable isotope values and cestode intensity is only a trend in red fox, the low sample size suggests we may have just lacked sufficient statistical power to document this relationship. Further, the $\delta^{13}C$ values differ between red foxes with and without cestodes. Collared lemmings, southern red-backed voles, and snowshoe hares, all intermediate hosts for *Taenia* spp., have lower $\delta^{13}C$ values

(Rausch and Fay 1988; Loos-Frank 2000). Goose eggs (which may be cached for use in fall and winter) and ringed seals have a higher $\delta^{13}C$ values. This relationship between lower $\delta^{13}C$ values and higher cestode intensity supports our hypothesis that a diet higher in mammals, such as collared lemmings and voles, should be related to a higher cestode abundance. Conversely, a fox diet that is higher in goose eggs is related to a decreased abundance of cestodes.

The lack of relationship between $\delta^{15}N$ values and cestode abundance in both arctic and red foxes was not expected as collared lemmings and southern red-backed voles have much lower nitrogen stable isotope ratios than eggs and birds. The lack of relationship may be explained by the use of alternative prey sources, such as caribou, by the foxes. Caribou is an intermediate host for *T. multiceps* (Rausch et al. 1983; Gibson et al. 2005) and has a much higher nitrogen stable isotope ratio than collared lemmings or southern red-backed voles. A fox that consumed caribou and became infected with *T. multiceps* would have cestodes as well as higher nitrogen stable isotope ratios.

Trophic transmission can sometimes play a role in the vertical transmission of nematodes that are more commonly horizontally transferred through indirect contamination (Anderson 1992). Rodents (e.g. voles or lemmings) can serve as a paratenic hosts (i.e. an intermediate host where no development of the parasite takes place) for ascarid nematodes (Saeed and Kapel 2006; Schmidt and Roberts 2009; Stien et al. 2009). In red foxes, there was a negative relationship between $\delta^{15}N$ values and the abundance of nematodes. Small mammals (e.g. collared lemmings and southern red-backed voles) have lower $\delta^{15}N$ values; therefore, red foxes that have a diet with a higher proportion of small mammals may also have a higher nematode abundance. The

relationship between $\delta^{15}N$ values and nematodes suggests that these small mammals may be serving as paratenic hosts in this region. Stien and others (2009) suggested voles served as an important paratenic host for ascarid nematodes that were transmitted to arctic foxes in the Svalbard archipelago.

Body condition of the arctic and red foxes showed no relationship with the parasite community. The intensity of infection in the foxes may be too low for any symptoms of the infection to be present, as there may be a threshold before any symptoms appear (Schmidt and Roberts 2009). Additionally, arctic and red foxes have coevolved with many of these parasites and have been in a constant so-called mutual arms race with these parasites through evolutionary time (Van Valen 1973). Healthy populations often are able to manage their parasites (Bryan et al. 2012). Selective pressure would be high against foxes that are strongly affected by their parasites. Additional stressors on these populations, such as decreased food availability or harsh weather conditions, may change this trend as the additions of further stressors in combination with existing parasite community are more debilitating than either in isolation (Marcogliese and Pietrock 2011).

Available resources are a constant limiting factor, with organisms having to make energy trade-offs between immune investment and other activities, such as reproduction and growth (Ricklefs and Wikelski 2002; Schmid-Hempel and Ebert 2003; Johnson et al. 2012). Species that are ‘fast-lived’, characterized by rapid growth and short life spans, should invest very little in immune defense and instead invest in growth and earlier reproduction (Thomas et al. 2009; Johnson et al. 2012). ‘Slow-lived’ species, which have longer life spans and slower growth rates, invest more resources in immune responses as

their parasite exposure increases (Schmid-Hempel and Ebert 2003). The difference in investment between immunity versus reproduction and growth may be one additional explanation for the differences between the two species. Although there are differences in diet between arctic and red foxes, immunity investment may assist in explaining the stark difference in prevalence and intensity of cestodes between the species. Arctic foxes have a mean lifespan of 2-3 years and produce a high number of offspring per litter (Audet et al. 2002). Comparatively, red fox live longer, 4-6 years (Saunders 1988). Although some previous researchers have reported a higher diversity, prevalence or intensity of infection in 'slow-lived' species due to their increased exposure (Poulin and Morand 2004; Blackwell et al. 2010), others support our observation here, with 'fast-species' being more prone to infection (Johnson et al. 2012).

The effects of climate change are more pronounced in northern regions (Anisimov et al. 2001; Parmesan 2006). Ringed seals provide an important food source for arctic fox, particularly when lemmings are scarce (Roth 2003). As the climate continues to warm, the time foxes have to forage on the ice will be reduced (Anisimov et al. 2001). The reduction in access to this alternative food source may have substantial impacts on the parasite communities within the foxes. Reduction in the availability of seal carcasses likely will lead to a reduction in arctic fox populations, as seals are an important food source during the winter period (Roth 2003). The reduction in arctic fox densities may lower parasite prevalence throughout the region. Alternatively, increasing temperatures may increase the availability of seal pups due to more spring rain leading to the collapse of seal dens (Stirling and Derocher 1993). Increased availability of this alternative prey source would decrease both the prevalence and load of *Taenia* spp. in foxes that have a

high proportion of this food source in their diet. Further, collared lemming population cycles are dampening, reducing an important intermediate host for both *T. crassiceps* and *T. polyacantha arctica* (Loos-Frank 2000; Ims et al. 2008). Additionally, as the climate warms, range expansion may occur introducing both previously absent intermediate hosts (particularly invertebrate) and parasites, normally limited by their free-living life stages (Kutz et al. 2009). The presence of novel parasites within the region may have dramatic effects on the arctic foxes (Price et al. 1986). Although we did not note any differences in diversity of parasites between the two years or species, range expansion may have substantial impacts on parasite diversity and impact the viability of native species to this region, particularly affecting the arctic foxes within this region due to their limited range (Kutz et al. 2009).

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Tables and Figures

Table 3.1. Arctic and red fox (*Vulpes lagopus* and *V. vulpes*) mean biological measurements and sex ratios of carcasses sampled in 2011 and 2012 from Churchill, Manitoba. Juveniles were considered individuals born the previous spring (Roth 2003).

Species	Sample Size	Spine Length (mm±SE)	Body Mass (kg±SE)	Female: Male	Proportion of Juveniles
Arctic fox	52	408 ± 3.7	2.5 ± 0.06	21:31	0.62
Red fox	37	498 ± 6.0	3.9 ± 0.14	22:15	0.65

*Although 55 arctic foxes were collected in 2012, 6 were eliminated from parasite analysis due to the destruction of their gastrointestinal tract from foraging by other animals

Table 3.2. Prevalence of parasites in arctic and red foxes (*Vulpes lagopus* and *V. vulpes*) from Churchill, Manitoba. Foxes were sampled in December 2010 - February 2011 and December 2011 - February 2012.

Parasite Taxa	Arctic Fox	Red Fox
Cestodes	96.2%	21.6%
Echinococcus multilocularis	19.2%	0%
Intestinal Nematodes	100%	64.9%
Spirocerca lupi	55.8%	0%

Table 3.3. Stable isotope ratios (mean ± SE) of arctic and red foxes and common prey items from Churchill, Manitoba, Canada. Ringed seal values from Ramsay and Hobson (1991), all adjusted for shifts in atmospheric carbon (Long et al. 2005). Goose eggs and goslings from both Lesser Snow Goose (*Chen caerulescens caerulescens*) and Canada Goose (*Branta canadensis interior*) were grouped together due to no significant difference in stable-isotope ratios. Shorebirds stable isotope ratios were from Dunlin (*Calidris alpina*), Least Sandpiper (*Calidris minutilla*), Whimbrel (*Numenius phaeopus*), and Hudsonian Godwit (*Limosa haemastica*). Shorebirds were grouped together due to no significant difference between stable-isotope ratios. Tukey-Kramer HSD post-hoc test results are reported for all prey species. Prey values that are not connected by the same letter are significantly different.

	Tissue	n	$\delta^{13}C$		$\delta^{15}N$	
Arctic Fox						
2011	muscle	3	-25.74 ± 0.07		5.53 ± 0.06	
2012	muscle	49	-23.47 ± 0.14		7.72 ± 0.21	
2011	fur	3	-23.84 ± 0.37		7.59 ± 0.16	
2012	fur	49	-22.76 ± 0.12		7.47 ± 0.20	
Red Fox						
2011	muscle	19	-23.93 ± 0.30		7.31 ± 0.36	
2012	muscle	18	-23.84 ± 0.24		7.93 ± 0.14	
2011	fur	19	-23.01 ± 0.19		8.93 ± 0.26	
2012	guard hair	18	-23.14 ± 0.11		8.04 ± 0.16	
2012	under fur	18	-23.17 ± 0.11		8.00 ± 0.16	
Prey						
Caribou	muscle	4	-22.24 ± 0.43	BC	4.68 ± 0.29	CD
Collared lemmings	muscle	7	-25.87 ± 0.17	C	-1.67 ± 0.29	E
Goose eggs	eggs	21	-23.00 ± 0.47	B	8.64 ± 0.21	A
Goslings	muscle	8	-25.54 ± 0.09	C	6.12 ± 0.52	BC
Ringed seal	muscle	27	-19.01 ± 0.04	A	-	-
Shorebirds	muscle	14	-24.65 ± 0.76	BC	6.61 ± 0.45	AB
Snowshoe hare	muscle	1	-27.59 ± na	BC	0.45 ± na	DE
Southern red-backed voles	muscle	6	-24.44 ± 0.39	BC	2.79 ± 1.13	D

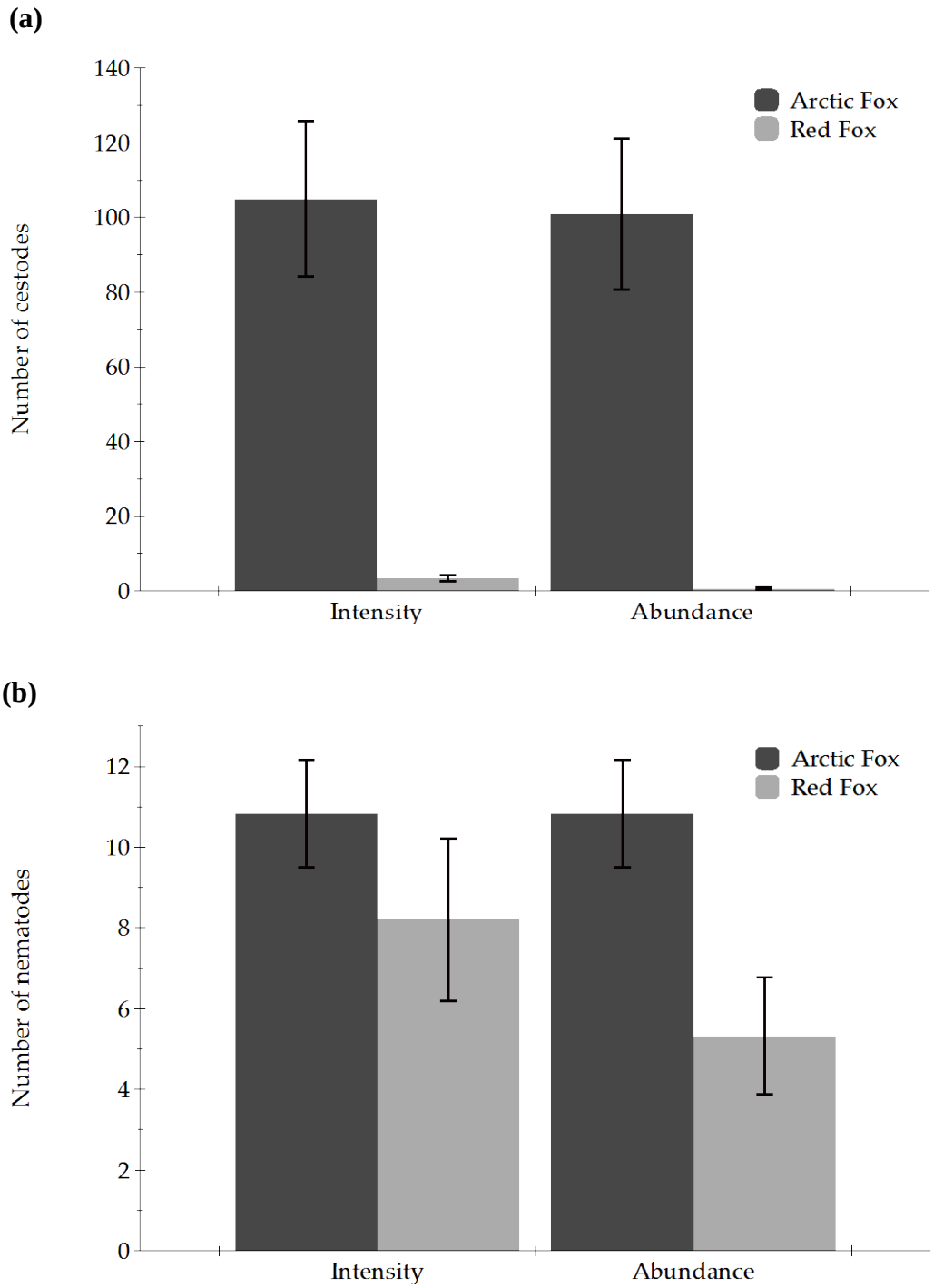
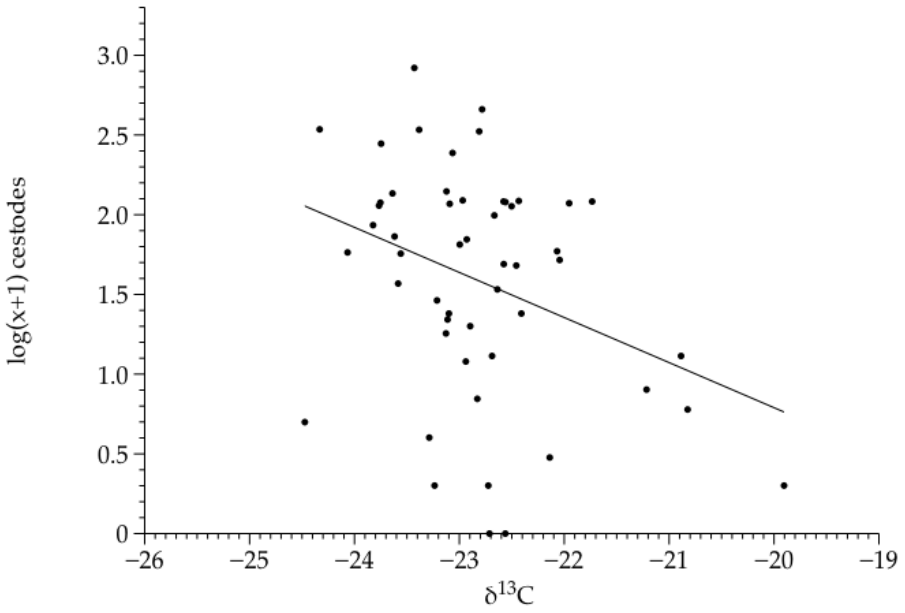


Figure 3.1. Parasite community differences in arctic (n=52) and red fox (n=37) (*Vulpes lagopus* and *V. vulpes*) from the Churchill, Manitoba, Canada. Fox (a) cestode and (b) intestinal nematode intensity (mean ± SE number of cestodes for infected individuals) and abundance (mean ± SE number cestodes for all individuals). Fox were sampled in December 2010- February 2011 and December 2011- February 2012.

(a)



(b)

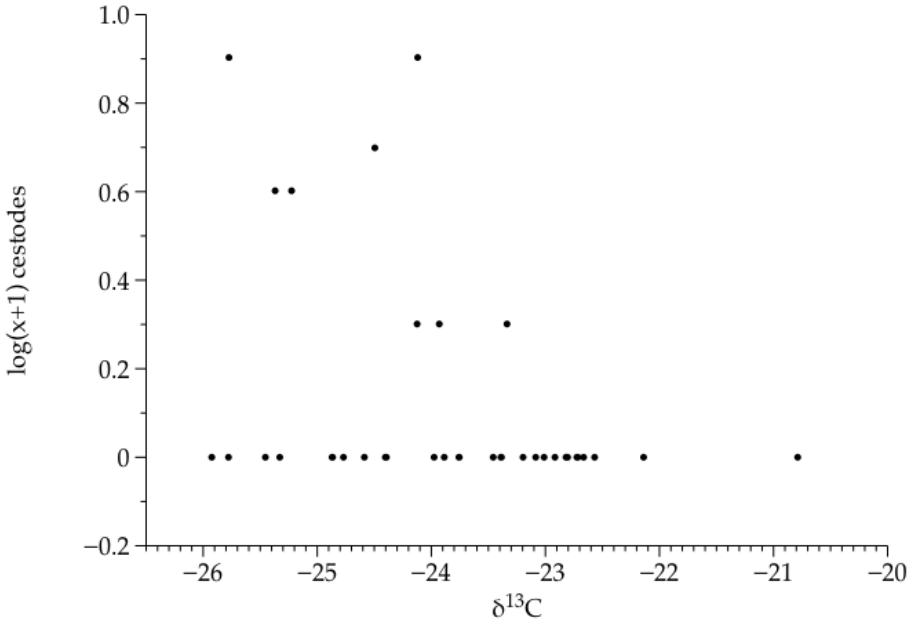


Figure 3.2. Relationship between cestode abundance (number of cestodes per fox) and (a) $\delta^{13}C$ value of fur from arctic fox (*Vulpes lagopus*) (n=52) and (b) muscle from red fox (*Vulpes vulpes*) (n=37) harvested in 2011 and 2012 in Churchill, Manitoba, Canada.

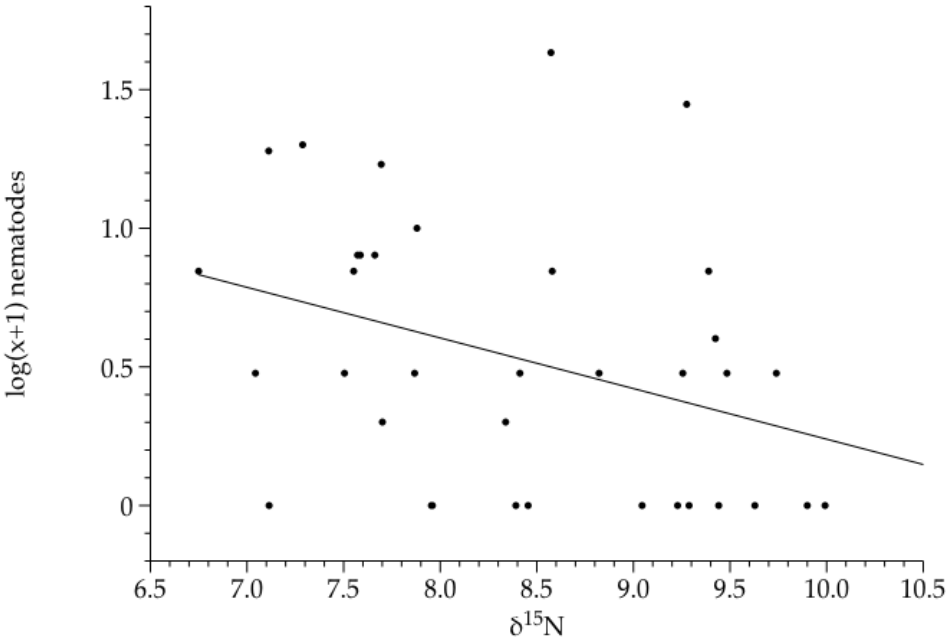


Figure 3.3. Relationship between nematode abundance (number of nematodes per fox) and $\delta^{15}N$ value of fur from red fox (*Vulpes vulpes*) (n=37) harvested in 2011 and 2012 in Churchill, Manitoba, Canada.

Chapter 4: Gray wolf diet affects helminth parasite infections in a multiple-ungulate system in eastern Manitoba

Abstract

Predators can significantly impact prey populations not only through direct predation, but also by acting as definitive hosts for their parasites and completing parasite life cycles. Understanding the impacts of parasitism on the population dynamics of ungulates requires knowing how the parasite community of their predators (e.g., wolves, *Canis lupus*) is affected by diet and prey availability. Moose (*Alces americanus*) are important prey for wolves in many regions, but in eastern Manitoba moose populations have experienced considerable declines in recent years. However, white-tailed deer (*Odocoileus virginianus*), another important prey for some wolf populations, have increased in abundance following changes in the landscape. White-tailed deer are suitable intermediate hosts for many parasites found in both moose and wolves. As host density can influence parasite transmission, increases in the density of white-tailed deer may increase the prevalence of their parasites, and through wolf movements and activities may impact sympatric moose. We examined parasites in wolf carcasses from eastern Manitoba collected from hunters, trappers, and road kill in winter 2011 and 2012. Taeniidae cestodes, including *Taenia hydatigena* and *Echinococcus granulosus,* were present in most wolves (75%), reflecting a diet primarily comprised of ungulates, but nematodes were unexpectedly rare. We reconstructed the diet of each wolf using stable isotope ratios of muscle samples from wolves and their prey in Bayesian stable isotope mixing models. White-tailed deer dominated the wolf diet but caribou and moose still

represented important prey in both years. Wolves that ate more moose tended to have fewer cestodes, while more cestodes were found in wolves with a higher percentage of deer in their diet. If white-tailed deer populations continue to increase, the prevalence and intensity of parasites in the wolf population could also continue to increase, potentially exposing moose to additional parasite risk. A rise of parasite populations in the ecosystem could affect moose by increasing their predation risk, and combined with increased predation pressure by rising wolf populations' could further reduce moose populations.

Introduction

Predators can significantly impact prey populations by means other than direct predation, such as completion of parasite life cycles, altered habitat selection, and decreased competition (Chaneton and Bonsall 2000; Joly and Messier 2004; Dussault et al. 2005). Parasites transmitted through diet can affect host population dynamics, overall biodiversity, and food web structure (Price et al. 1986; Hudson et al. 2006; Lafferty et al. 2008). As an important top predator, wolves (*Canis lupus*) host a diverse gastrointestinal parasite community, which should vary in relationship to their diet (Craig and Craig 2005; Bryan et al. 2012). As definitive hosts of many parasites, understanding wolves' parasite community is key to understanding the impacts of parasitism on the population dynamics of their prey.

Moose (*Alces americanus*) populations in eastern Manitoba have declined considerably in the past few years, but the role of wolves in this decline is unclear (Leavesley 2010). White-tailed deer (*Odocoileus virginianus*) populations in eastern

Manitoba have been increasing following changes in the landscape, including habitat fragmentation and conversion to agriculture (Ransom 1967; Miller et al. 2003). Not only do populations of white-tailed deer provide an alternative prey source for wolves, potentially altering predation pressure on moose, white-tailed deer also serve as a host to *Parelaphostrongylus tenuis*, a meningeal worm that does not cause illness in white-tailed deer but can serve as a fatal neurological disease in other ungulates, particularly moose (Miller et al. 2003; Latham et al. 2011). *Fascioloides magna*, a large liver trematode, can be carried by both ungulate species; however, the moose is an unsuitable host and *F. magna* can lead to extensive damage to a moose's liver (Lankester 1973). White-tailed deer and moose also serve as hosts to larval parasites, including *Taenia hydatigena* and *Echinococcus granulosus* that use wolves as their definitive host (Rausch 1995; Loos-Frank 2000). As white-tailed deer populations increase, parasite species found in both white-tailed deer and moose may increase in abundance due to density-dependent transmission, negatively affecting both species (Messier and Rau 1989; Morand and Poulin 1998).

Parasite infection in moose can influence the ability of wolves to regulate moose populations (Joly and Messier 2004). Heavy infection of *Echinococcus granulosus* in older moose can impact their lung capacity and ability to sustain a long period of exertion, likely predisposing them to increased predation risk by wolves (Joly and Messier 2004). As white-tailed deer are also suitable intermediate hosts for *E. granulosus*, increases in white-tailed deer populations may increase the prevalence of *E. granulosus* within the ecosystem. Wolf movements, increased density and activities may further increase transmission of many parasite species and may impact the moose

population if moose and white-tailed deer occur within the same region (Samuel et al. 1976; Messier and Rau 1989; Joly and Messier 2004).

Wolf diet has previously been reconstructed using scat or stomach analysis or kill rates, including observations and backtracking, for identifying prey items reflecting only the most recent wolf diet (Smith et al. 2004; Sand et al. 2005; Floyd et al. 1978; Latham et al. 2011).The most recent prey may not necessarily represent the intermediate host for parasites now living within the wolf, as they may have been contracted through the consumption of another food item at some point in the past. Previous studies linking diet and parasite communities have been conducted at the population level (Bryan et al. 2012), but studies comparing the diets of individuals with their parasites are lacking. Stable isotope ratios better represent the long-term contribution of multiple prey species to the wolf diet (Milkakovic and Parker 2011) and have the potential to give us better insight into the important prey items for the transmission of these trophically transmitted parasites. Using stable isotopes to examine the diet of wolves with varying parasite communities provides a novel opportunity to examine links between diet of an individual and the transmission of parasites.

The objective of this study was to compare the parasites of wolves in eastern Manitoba to the wolves' diet. Several life history traits of moose and deer may impact their degree of parasitism and the probability of infecting a predator. Moose are mostly solitary and occur in lower densities than white-tailed deer, which may form groups, particularly in winter (Dussault et al. 2005). Increased densities of possible hosts, such as white-tailed deer or moose, will increase the parasite abundance in the region (Arneberg et al. 1999). Moose may avoid areas used by wolves, whereas some white-tailed deer do

not (Nelson and Mech 2000; Dussault et al. 2005). Further, moose represent a larger biomass compared to white-tailed deer. Wolves will need to consume more individual white-tailed deer than moose to sustain themselves, increasing the chances they will consume infected prey. Thus, a wolf consuming more white-tailed deer should have more parasites.

By definition, parasites are stressors on their hosts and may be detrimental to the health and condition of both wolves and ungulates (Combes 1996; Marcogliese and Pietrock 2011). However, the impacts of parasitism on wolves are poorly understood (Paquet and Carbyn 2003). If parasites negatively impact their hosts, wolves with more parasites should be in worse condition, and increases in parasites within the ecosystem due to density-related increases of prey may further alter population dynamics.

Methods

Study Area – Wolf carcasses were collected by Manitoba Conservation from hunters, trappers, and road kill in southeastern Manitoba, within Game Hunting Area 26 (50-51°N lat, 95°W long; Manitoba Conservation). This 16 600 km^2 area lies within boreal forest in the Precambrian shield. White-tailed deer, moose, and caribou (*Rangifer tarandus*) constitute the ungulate prey available in this area, along with smaller alternative prey choices including snowshoe hare (*Lepus americanus*), beavers (*Castor canadensis),* and various birds including grouse (*Bonasa umbellus* and *Falcipennis canadensis*).

Endoparasite Sampling - All wolves were killed during the winter (November through February) as part of the legal fur trade, legal hunting, or road kill and were frozen until

analysis. Wolf carcasses were used and handled as approved by the University of Manitoba Animal Care Fort Garry Campus Protocol Management and Review Committee (Abbreviated protocol for minimal animal involvement, 2011-04-08). Wolves were sexed and body length was measured.

We determined the endoparasite community within each individual using standard necropsy techniques (Munson 2000). Lipid deposits, including fat, serve as energy reserves and insulation as well as support and padding for many organs (Prestrud and Nilssen 1992). Fat surrounding the kidneys is often used as an index to evaluate the body condition of the individual (Finger et al. 1981). We extracted the kidneys and used the kidney-fat index to estimate body condition (Finger et al. 1981; Lajeunesse and Peterson 1993). We collected lungs, liver (including the bile duct), and heart from each individual to inspect for parasites or other pathology. We removed intestines from each individual and analyzed them separately. Each intestine was split longitudinally and scraped to remove all parasites. The remaining tissue was strained and examined using the sedimentation and counting technique (Eckert 2003).

We identified all parasites to species (when possible) and weighed each dry individual. All parasites were preserved in 70% ethanol. Prior to fixation, the crown of hooks on the scolex of all cestode species was sliced off and squashed between a cover slip and glass slide. Identification of each specimen was made based on the shape, number, and size of the rostellar hooks (Loos-Frank 2000). Any *Echinococcus* spp. were identified using characteristic morphology (see Thompson 1995). Trematodes were preserved in AFA (alcohol, formalin, and acetic acid) and then transferred to 70% ethanol. We grouped parasites by different levels of taxonomic resolution, i.e. all

gastrointestinal parasites (species in Phylum Platyhelminthes and Nematoda), cestodes (Class Cestoda), trematodes (Class Trematoda), *Taenia* (species *Taenia hydatigena*) and *Echinococcus* (species *Echinococcus granulosus*).

Parasites were quantified using three descriptors: prevalence (presence or absence of parasite in a wolf), intensity (number of parasites per infected wolf), and abundance (number of parasites per wolf) (Rozsa et al. 2000). *Echinococcus granulosus* is comparatively small cestodes that was very abundant in wolves. However, their poor condition (broken into pieces) within all analyzed wolves made them very difficult to quantify. Their intensity was roughly estimated by using dilution counts (Thompson et al. 2006). The dry biomass of *E. granulosus* was approximated using the mean mass of 20 individuals. Due to their disproportionately large intensity, this species was excluded from cestode intensity and abundance. To insure this exclusion did not bias our results, we compared the biomass of all cestodes (including both *T. hydatigena* and *E. granulosus*) to our estimates of cestode abundance (excluding *E. granulosus*). Biomass and abundance were strongly related (linear Regression, $R^2 = 0.80$, $P<0.0001$), suggesting our abundance estimates without *E. granulosus* were unbiased. Nematodes (Phylum Nematoda) were excluded from this analysis because most species present in wolves are directly transferred from host to host (not trophically transmitted).

Stable Isotope Analysis - We used stable isotope analysis to determine the proportions of wolf diet acquired from different prey species (Roth 2002). Differences in stable isotope ratios of prey transfer to the consumer, allowing the predator's diet to be reconstructed (Chisholm et al. 1982). For example, wolves that have consumed a mainly ungulate diet

will have lower $^{13}C/^{12}C$ ratios compared to those that consume purely marine sources, such as salmon (Chisholm et al. 1982; Szepanski et al. 1999). An intermediate signature would be produced in wolves that consumed a mixed diet. Muscle samples obtained from wolf carcasses for analysis will have developed during the few months prior to the death of the wolf so samples represented the diet of the wolf in late autumn (Hobson and Clark 1992; Dalerum and Angerbjorn 2005). Samples were collected from potential prey items from the same region for comparison with wolf values. We sampled muscle from moose (n=12) and white-tailed deer (n=27) and hair from caribou (n=7); muscle and hair stable isotopes of ungulates do not differ significantly (Milakovic and Parker 2011). Stable isotope ratios for snowshoe hare in Manitoba were from Roth et al. (2007) (carbon isotope ratios were corrected for temporal change; see Long et al. 2005). Each muscle sample was freeze-dried and powdered with mortar and pestle (Roth 2002). Lipids have substantially different carbon isotope ratios than other compounds (DeNiro and Epstein 1978; Tieszen et al. 1983), and variations in lipid concentration can strongly influence $\delta^{13}C$ measurements (Rau et al. 1992). For these reasons, lipids were removed from muscle samples using a Soxhlet apparatus with petroleum ether as solvent for 8 hours (Roth 2002). Each sample was dried in a drying oven to remove the solvent (Roth 2002). Hair samples were washed with soap and water to remove any debris or oil, dried at 60°C, and homogenized with scissors. Stable isotope ratios were measured using a continuous flow isotope ratio mass spectrometer at University of Windsor. Stable isotope values are presented in parts per thousand (‰) relative to Pee Dee Belemnite (carbon) and atmospheric N_2 (nitrogen) standards as follows:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] * 10^3$$

where X is ^{13}C or ^{15}N and R is the ratio of the heavy to light isotope (e.g., $^{13}C/^{12}C$ or $^{15}N/^{14}N$).

To estimate the diet of wolves, we incorporated the stable isotope ratios of wolves and their prey into the Stable Isotope Analysis in R (SIAR) package (Parnell and Jackson 2011; R Core Development Team 2011) to calculate proportions of different prey sources in the diet. Although beavers (*Castor canadensis*) may be an important seasonal component of wolf diets (Floyd et al. 1978; Fuller 1989; Urton and Hobson 2005; Latham et al. 2011; Milakovic and Parker 2011), they were considered to be largely inaccessible to wolves during the winter (the period represented by wolf muscle) as they forage under the ice and reside in their lodges (Novakowski 1967; Mech 2007). To correct for trophic discrimination between diet and consumer tissues, we used discrimination values from a study on captive red foxes (*Vulpes vulpes* – Roth and Hobson 2000), which have previously been used as a close replacement in wolf models (Urton and Hobson 2005; Darimont et al. 2009; Milakovic and Parker 2011). Discrimination values added to prey values (Inger et al. 2011) were $\Delta^{13}C = 1.1‰$ and $\Delta^{15}N = 3.3‰$, with standard deviations of 0.1‰ for both values (Roth and Hobson 2000).

Statistical Analysis – Mean stable isotope values for each prey source were compared using univariate ANOVAs and Tukey's HSD post hoc test to determine if prey species differed significantly. Multivariate methods (MANOVA) were performed on wolf stable isotope ratios to detect if there was a difference between the two years or between sexes

and for any interaction effects, as the stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ are not independent of each other and should be compared together (Hummel and Sligo 1971). We normalized the parasite data using a log(x+1) transformation (Rozsa et al. 2000). To determine the relationship between diet and wolf parasites, we compared the $\delta^{13}C$ and $\delta^{15}N$ to different measurements of the parasite community using linear regression. The kidney fat index was used as an index of body condition and was compared to values of the parasite community to examine if parasites negatively impacted the condition of the wolves. All analyses were carried out using JMP® 10 (SAS Institute Inc. 2012) and R statistical software (http://www.R-project.org).

Results

We examined 32 wolves (12 from 2011 and 20 from 2012). The sex ratio was close to even, with 17 females and 15 males. Spine length ranged from 0.65-1.09m, but body mass could not be measured consistently due to variation in the state of the carcasses (i.e. presence or absence of pelt, presence or absence of head (severed at the atlas)).

Most wolves (81%) harboured gastrointestinal parasites, but parasite diversity was very low, with only four genera present (*Taenia hydatigena, Echinococcus granulosus, Alaria* sp., and *Dioctophyma renale*). Identification to species was impossible for *Alaria* sp., a trematode, given the condition of the wolf carcasses. *Taenia hydatigena* was the most prevalent parasite (72%), followed by *Echinococcus granulosus* (47%) and *Alaria* sp. (16%) (Figure 4.1). Nematodes were found only in one individual that hosted *Dioctophyma renale* in its abdomen near the right kidney.

Wolves from 2011 and 2012 did not differ in parasite community quantifiers (overall prevalence (χ^2= 0.48, *P*=0.65), intensity ($F_{1,23}$=2.75, *P* =0.11), and abundance ($F_{1,31}$=2.47, *P* =0.13); cestode prevalence (χ^2= 0.70, *P* =0.43), intensity ($F_{1,22}$=1.98, *P* =0.17), and abundance ($F_{1,29}$=2.32, *P* =0.14); trematode prevalence (χ^2= 1.01, *P* = 0.63), intensity ($F_{1,3}$=0.14, *P* =0.74), and abundance ($F_{1,30}$= 0.30, *P* =0.59); *Echinococcus*: prevalence (χ^2= 0.075, *P* = 1.0)). Likewise, stable isotope ratios of wolf muscle did not differ between years (MANOVA, Exact $F_{2,28}$= 0.48, *P*= 0.63). Therefore, years were pooled for subsequent analyses. Males and females also did not differ in parasite quantifiers (overall prevalence (χ^2= 0.83, *P* =0.65), intensity ($F_{1,23}$= 0.042, *P* = 0.84), and abundance ($F_{1,30}$=0.0001, *P* = 0.99); cestode prevalence (χ^2=0.67, *P* = 0.69), intensity ($F_{1,21}$= 0.0013, *P* = 0.97), and abundance ($F_{1,29}$= 0.26, *P* = 0.61); trematode prevalence (χ^2= 2.26, *P* = 0.33), intensity ($F_{1,3}$= 2.36, *P* = 0.22), and abundance ($F_{1,30}$= 0.42, *P* = 0.52); *Echinococcus* prevalence (χ^2= 1.14, *P* = 0.48)) or stable isotope ratios (MANOVA, Exact $F_{2,28}$= 0.62, *P*= 0.55).

All prey sources differed in either δ^{13}C and δ^{15}N or both. Most prey differed in δ^{13}C values ($F_{4,74}$=68.9, *P*<0.0001), although snowshoe hare did not differ from moose or deer (Table 1). Prey sources differed in δ^{15}N ($F_{4, 74}$=19.71, *P*<0.0001), with moose significantly depleted in ^{15}N compared to all other prey species (Table 1). All prey sources comprised similar proportions of wolf diet in both 2011 and 2012, with white-tailed deer and snowshoe hares representing slightly more of the diet (75% confidence interval: 27-51% and 9.9-48% respectively, Figure 4.2).

$\delta^{13}C$ values were more negative in wolves that had cestodes (Logistic regression, R^2=0.21, χ^2 = 7.49, *P*=0.0062; Figure 4.3), *T. hydatigena* (Logistic regression, R^2=0.13, χ^2 = 4.88, *P*=0.027), or any intestinal parasite (Logistic regression, R^2=0.27, χ^2 = 8.32, *P*=0.0039). However, $\delta^{15}N$ values did not differ between wolves which had parasites and those that did not (Logistic regression, overall prevalence: R^2=0.003, χ^2 = 0.09, *P*=0.7597; cestode prevalence: R^2=0.03, χ^2 = 0.99, *P*=0.32; *T. hydatigena*: R^2=0.05, χ^2 = 1.83, *P*=0.18). No isotopic difference was found between wolves which had *Echinococcus* and those that did not ($\delta^{13}C$: R^2= 0.02, χ^2 = 0.73, *P*= 0.39; $\delta^{15}N$: R^2=0.0001, χ^2 = 0.004, *P*=0.95). Likewise, the presence of trematodes (only *Alaria* sp.) did not affect $\delta^{13}C$ and $\delta^{15}N$ values (($\delta^{13}C$: R^2= 0.04, χ^2 = 0.91, *P*= 0.34; $\delta^{15}N$: R^2=0.07, χ^2 = 1.8, *P*=0.18).

Wolf $\delta^{13}C$ values were negatively related to cestode abundance (Linear regression, R^2=0.164, $F_{1,28}$=5.01, *P*=0.026; Figure 4.4) and overall parasite abundance (Linear regression, R^2= 0.09, $F_{1,29}$=3.01, *P*=0.09); however, $\delta^{15}N$ values did not affect overall abundance (Linear regression, R^2= 0.007, $F_{1,29}$=0.19, *P*=0.66) or cestode abundance (Linear regression, R^2=0.04, $F_{1,28}$=1.12, *P*=0.30). Trematode abundance was not related to either $\delta^{13}C$ or $\delta^{15}N$ values (Linear regression, $\delta^{13}C$: R^2=0.004, $F_{1,29}$=0.14, *P*=0.71; $\delta^{15}N$: R^2=0.04, $F_{1,29}$=1.22, *P*=0.28).

The kidney fat index was unrelated to overall parasite abundance (Spearman ρ = 0.034, *P*=0.86, Figure 4.5), or the abundance of cestodes (Spearman ρ = -0.16, *P*=0.39) or trematodes (Spearman ρ = 0.15, *P*=0.40).

Discussion

The gastrointestinal parasite community of wolves was affected by diet; wolves that had consumed a higher proportion of white-tailed deer had more cestodes (Figure 4.3, 4.4). Relationships between wolf diet and parasite composition have been found previous studies, but for the first time that we can link the diet of the individual to the presence and abundance of cestodes (Craig and Craig 2005; Bryan et al. 2012). Furthermore, it also suggests that white-tailed deer may be serving as a more important intermediate host for the transmission of Taeniidae cestode species within this region. Only $\delta^{13}C$ was correlated with the cestodes within the wolves (Figure 4.3, 4.4). No relationship was found between $\delta^{15}N$ and any parasite descriptor; however, the variation in $\delta^{15}N$ of prey was quite high, particularly in moose and white-tailed deer, explaining this lack of relationship. The $\delta^{13}C$ for each prey source was significantly different, so despite the high variation in $\delta^{15}N$ the differences in isotopic signatures were enough to differentiate between prey sources.

No relationship was observed between the stable isotopes of both $\delta^{13}C$ and $\delta^{15}N$ and the prevalence of *E. granulosus*. As a trophically transmitted parasite, the lack of relationship was slightly surprising. Previous studies have suggested that hosts, such as wolves, cannot develop immunity to *E. granulosus* (Roberts et al. 1986). However, there has been some suggestion immunity does develop in regions where the parasite is very prevalent (Lahmar et al. 2001). Further, a study by Budke and others (2004) suggested that there was likely immunity to *E. granulosus* in dogs, based on the modeling of abundance and prevalence data, contrary to infection with *E. multilocularis* which

showed no parasite induced host immunity. If the wolves had developed immunity, it may explain this disconnect between diet and *E. granulosus* prevalence.

Wolves in southeastern Manitoba relied on both white-tailed deer and moose as major ungulate prey species (Figure 4.2). Concurrently, ungulate prey species are not evenly distributed throughout this area and it is not uncommon for only one or two ungulates to inhabit some areas of this region. The study area is small enough that wolves would be able to move within these areas and have access to all ungulates. However, caribou are only found in a very small portion of this region, explaining their low proportion in the wolves' diet.

Nematodes were almost completely absent from the wolves in this region. Many species of nematodes have been previously documented in wolves, including *Toxocara canis* and *Toxascaris leonina.* Both species have been documented in populations of wolves within close proximity to those studied here (i.e. southwestern Manitoba, Canada and northeastern Minnesota, USA) (Byman et al. 1977; Samuel et al. 1978; Craig and Craig 2005). These species of nematodes are not limited latitudinally and are distributed in northern areas (Craig and Craig 2005), so their absence in this population was unexpected. Age-related immunity (acquired immunity) or good health may be factors reducing or eliminating nematodes from adult wolves (Bryan et al. 2012). If the wolves were exposed as young pups, this early exposure could impart immunity to further infection (Bryan et al. 2012). Further, the wolves in this study were seen to be in generally good health, as observed by the high amounts of subcutaneous and visceral fat deposits seen during necropsy. Their good health may explain the absence of nematodes (Bryan et al. 2012).

The condition of the wolves, represented by the kidney fat index, was not significantly related to the parasite community, indicating that parasites were not detrimental to these wolves (Figure 4.5). This result is contrary to the previous understanding of the influence of parasites on their host (Marcogliese and Pietrock 2011). Parasites, by definition, should have a negative impact on their host at some point (Combes 1996). There could be a few alternative explanations for this trend. Wolves that are strongly impacted by their parasites may be selected against. Additionally, there is a threshold of infection or infestation that needs to be reached before the parasites negatively impact their host (Schmidt and Roberts 2009). If this threshold has not been reached in this population, it may explain the lack of relationship between parasite abundance and body condition. As parasite populations are usually reflected as a negative binomial, only a few hosts should have high enough populations of parasites to be negatively impacted. Further, the wolves within this region may be in good health overall due to a high abundance of food and few other stressors (Marcogliese and Pietrock 2011). It has been suggested that healthy populations could be able to manage their parasite communities, possibly being able to rid themselves of parasites (Bryan et al. 2012). The lack of nematodes within these wolves may be a further indication of this population's ability to manage their parasite community. The addition of further stressors on the population, such as decreased food or anthropogenic development, may reverse this trend as parasites in combination with other stressors are more debilitating than either one acting alone (Marcogliese and Pietrock 2011).

Continued increases in the white-tailed deer population likely will lead to the increase of the prevalence and intensity of gastrointestinal parasites in the wolf

population. As the wolves in this region are currently not being affected by their gastrointestinal parasites, the potential impacts of this increase in parasites likely will not be detrimental for this population in the short term. There may be a critical point at which the parasite abundance in the wolves becomes high enough that it will start to harm the wolf population. Further, food stress may be created through decreasing moose populations, which in turn will reduce food availability for wolves in the region. The additional stressor may negatively impact the wolf population, affecting the population dynamics within the region. If white-tailed deer act as reservoirs of infection (Samuel et al. 1976), an increase in parasite populations in the ecosystem could also affect moose, making them more vulnerable to predation, and further reducing moose populations. At the same time, since wolves do not appear to be impacted by these additional parasites, further increases in white-tailed deer populations will supplement their population and moose will face not only increased parasites but increased predation, putting their population further at risk.

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Tables and Figures

Table 4.1. Stable isotope ratios (mean ± SE) of wolves and common prey items from southeastern Manitoba, Canada. Snowshoe hare values were from Roth et al. (2007) and adjusted for temporal shifts in atmospheric carbon (Long et al. 2005). Prey values that are not connected by the same letter are significantly different (Tukey-Kramer HSD, $p<0.05$).

	Tissue	n	$\delta^{13}C$		$\delta^{15}N$	
Wolf	muscle	32	-25.35 ± 0.13	-	6.40 ± 0.21	-
Caribou	hair	13	-23.95 ± 0.10	A	3.73 ± 0.17	AB
Moose	muscle	12	-26.10 ± 0.14	B	1.40 ± 0.27	D
White-tailed deer	muscle	23	-27.09 ± 0.16	C	4.11 ± 0.23	A
Snowshoe hare	muscle	13	-26.64 ± 0.13	BC	2.79 ± 0.33	BC

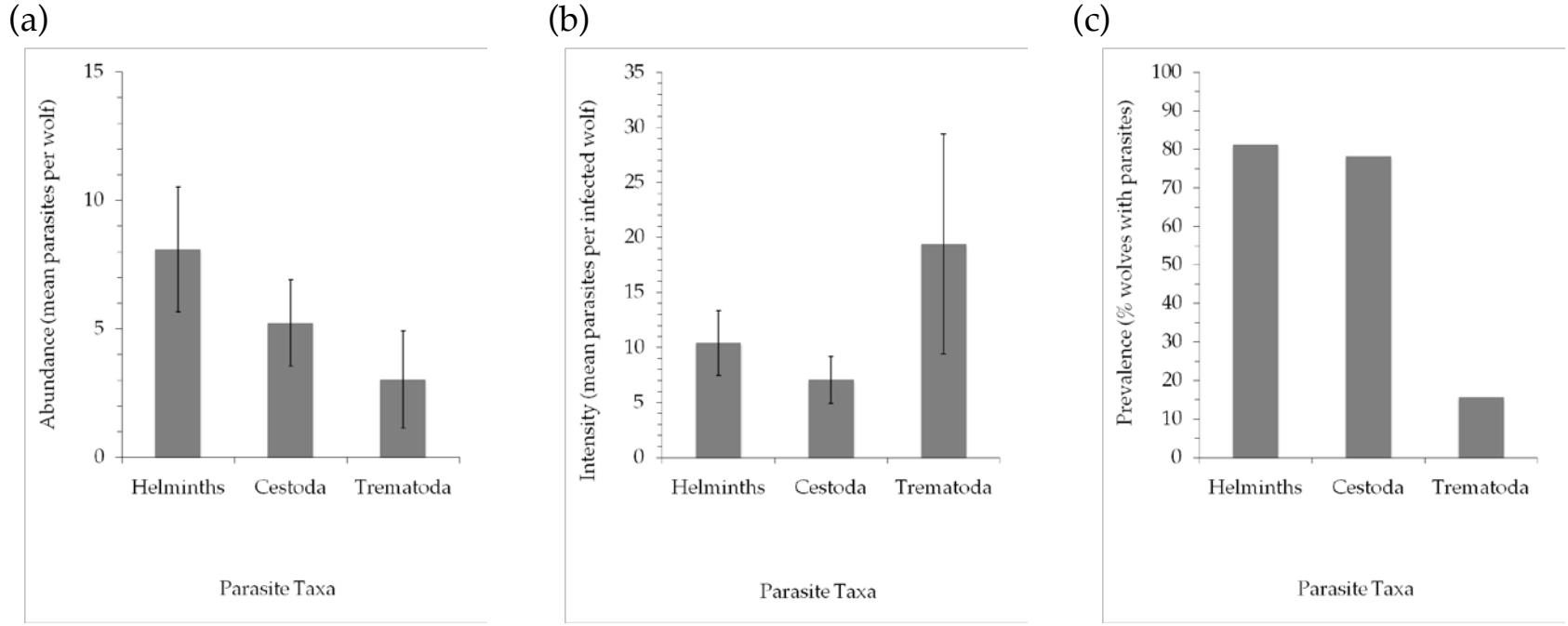

Figure 4.1. Gastrointestinal parasites in gray wolves (*Canis lupus*) from southeastern Manitoba, Canada in 2011 (n=12) and 2012 (n=20): (a) prevalence (percentage of wolves with parasites), (b) intensity (mean number of parasites per infected wolf), and (c) abundance (mean number of parasites per wolf). Error bars represent standard error.

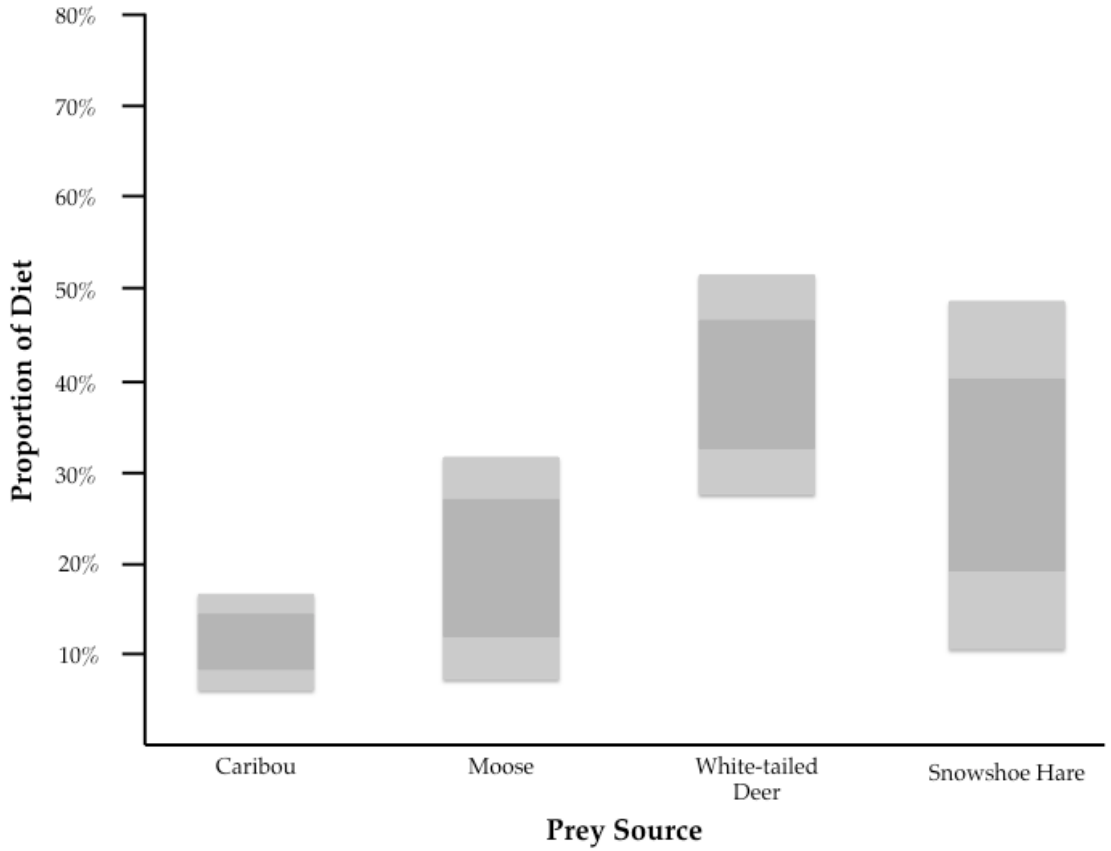

Figure 4.2. Estimates of the proportion of gray wolf (*Canis lupus*) diet (75% (lighter grey) and 50% (darker grey) confidence intervals) using four prey sources (caribou (*Rangifer tarandus caribou*, n= 13), moose (*Alces americanus*, n= 12), snowshoe hare (*Lepus americanus*, n= 13) and white-tailed deer (*Odocoileus virginianus*, n= 23)). Estimates were made using nitrogen and carbon stable isotope ratios of muscle tissue samples in the SIAR package in R (Parnell and Jackson, 2011; R Core Development Team, 2011).

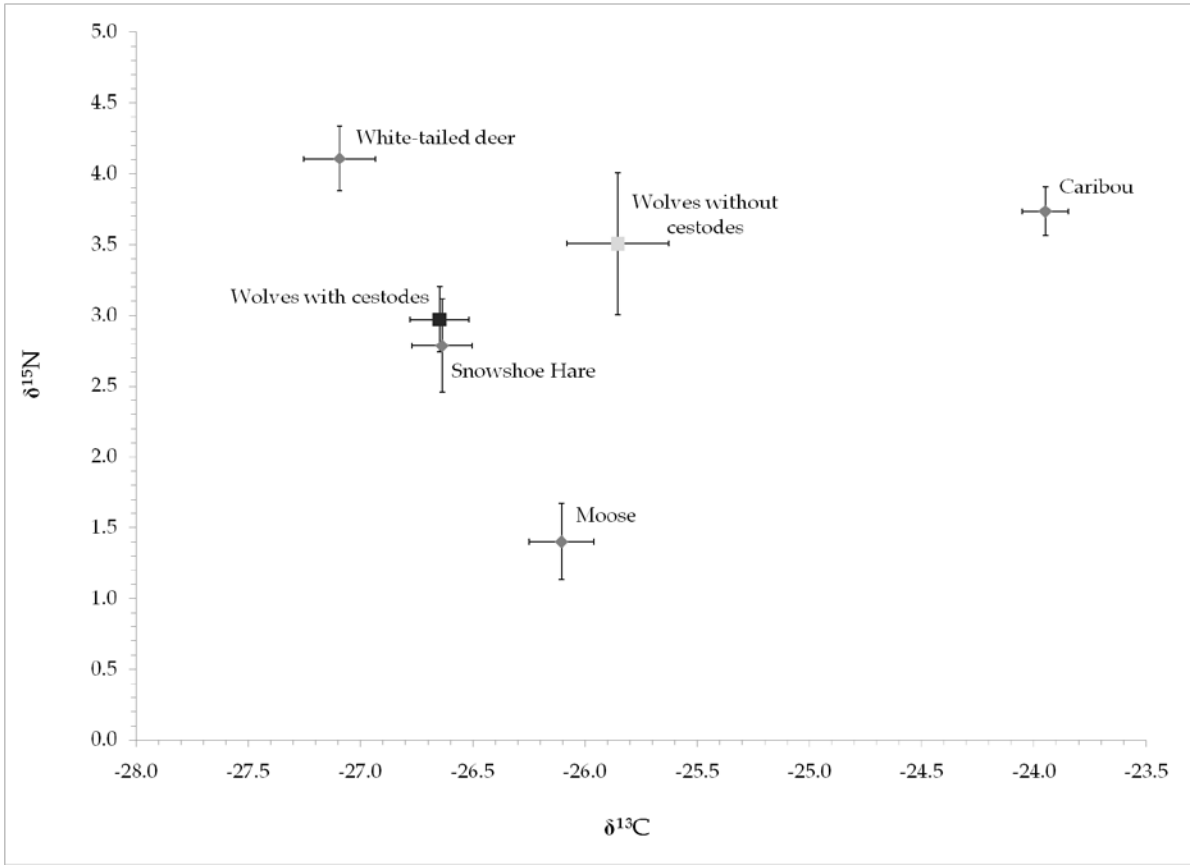

Figure 4.3. Stable isotope biplot of the ratios of eastern Manitoba gray wolves (*Canis lupus*, mean ±SE, corrected for trophic discrimination (Roth and Hobson 2000)) with means and standard errors of common prey, caribou (*Rangifer tarandus caribou*, n= 13), moose (*Alces americanus,* n= 12), snowshoe hare (*Lepus americanus*, n= 13) and white-tailed deer (*Odocoileus virginianus*, n= 23) from muscle tissue. Snowshoe hare values were from Roth et al. (2007) and adjusted for temporal shifts in atmospheric carbon (Long et al. 2005).

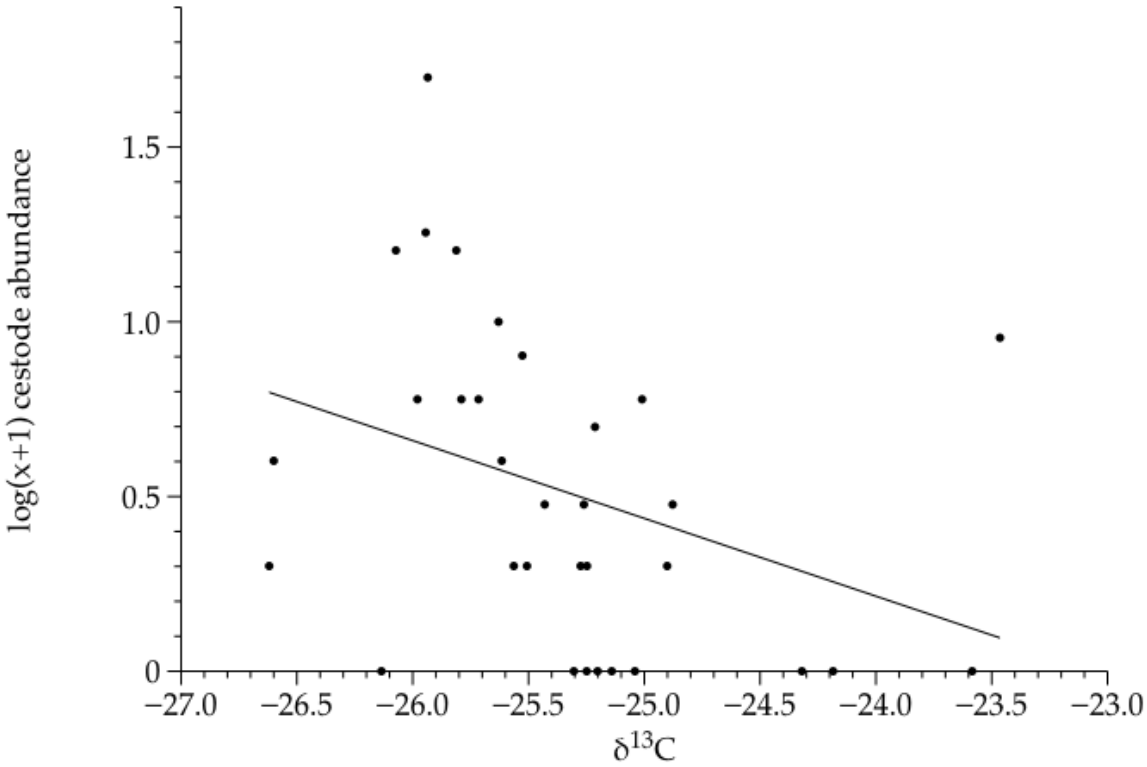

Figure 4.4. Relationship between cestode abundance (number of cestodes per gray wolf (*Canis lupus*)) and $\delta^{13}C$ of muscle tissue from gray wolves harvested in 2011 and 2012 in southeastern Manitoba, Canada.

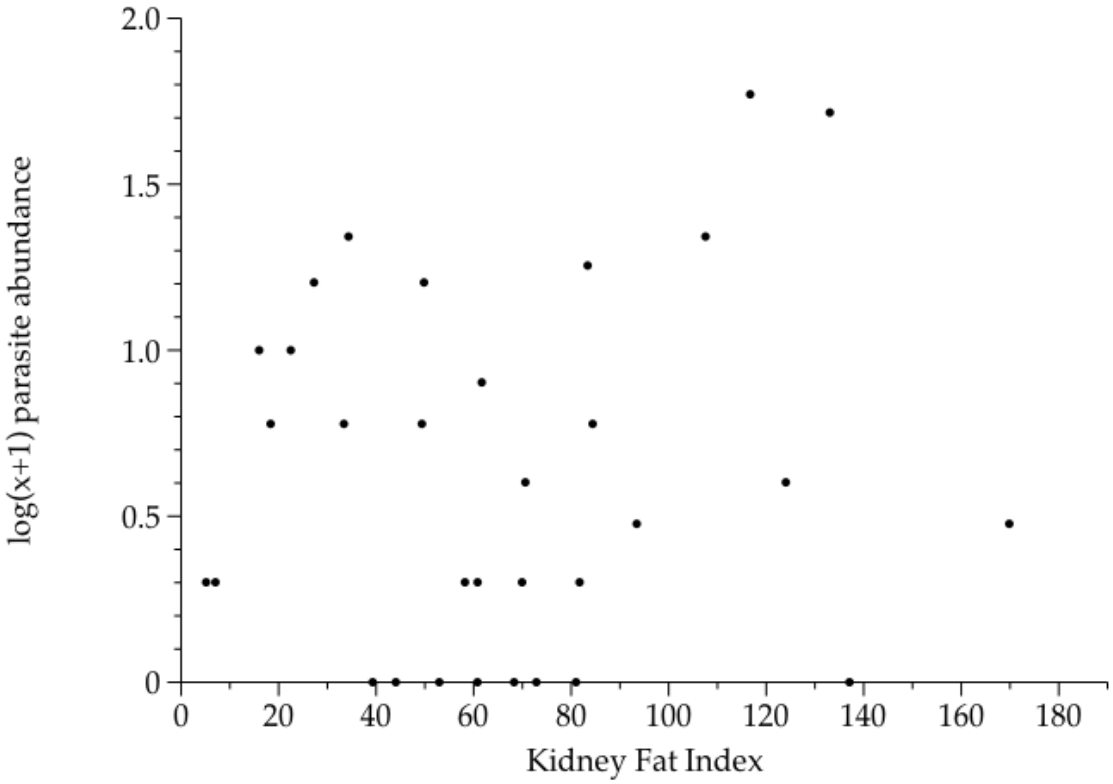

Figure 4.5. Kidney fat index and total parasite abundance in gray wolves (*Canis lupus*) from 2011 and 2012 from southeastern Manitoba, Canada.

Chapter 5: Thesis Conclusion

Parasites have the potential to modify the ecology and evolution of all interactions (Price et al. 1986; Lafferty et al. 2008). Parasites strongly influence ecosystem dynamics by impacting energy flow, population dynamics, interspecific competition, overall biodiversity, and the structuring of many food webs (Hudson et al. 2006). Biotic differences, such as host behavior, sex, age, morphology, physiology, condition, diet, as well as variation in parasite specificity and genetics can cause variation in parasite infection and ultimately determine host exposure. The parasite communities of wolves (*Canis lupus*), arctic foxes (*Vulpes lagopus*) and red foxes (*Vulpes vulpes*) were influenced by diet, behavior, age, sex, and life history.

In Chapter 2, we illustrated sex-biased parasitism in the arctic fox, with males having more cestodes. However, males and females had the same prevalence and number of nematodes. As male and female arctic foxes have similar home ranges and morphology (Eide et al. 2004), these differences are likely due to either differences in diet or physiology (e.g. testosterone). The 'immunocompetence handicap hypothesis' (ICHH) proposes that testosterone should inhibit immunity and males with higher testosterone should be more vulnerable to parasite infection (Jacobs and Zuk 2012). However, if differences in immunity were driving the male bias in parasite loads there should be differences in both cestodes and nematodes (Hepworth et al. 2012; Jacobs and Zuk 2012).

We found differences in diet between male and female arctic foxes. Males had lower mean $\delta^{15}N$ values than females, suggesting a diet including more small mammals.

As small mammals are an important intermediate host for many species of cestodes, a diet with a higher proportion of these food sources should explain this higher cestode load (Raoul et al. 2010). Differences in diet between sexes of arctic fox have not been documented previously (Angerbjorn et al. 2004). As both male and female foxes are the same size, it is still uncertain what is driving these differences in diet.

Age-related differences in parasites were found in arctic fox, with juveniles having a higher abundance and intensity of nematodes compared to adults. Recruitment of new naïve hosts during the breeding season may be contributing to these differences (Lutermann et al. 2012). Many nematodes can be horizontally transferred through the indirect contamination of a food source or area with parasite eggs; this transmission is related to host density (Arneberg et al. 1998; Schmidt and Roberts 2009). Juveniles have not been exposed to these parasites and remain at the den in high densities during the first months of their lives (Macpherson 1969). Arctic fox populations are cyclic and contain a large number of juveniles when food availability is high (Macpherson 1969). Changes in age structure over time due to these cyclic populations have the potential to impact the population's parasites. Cyclic population dynamics exhibited by lemmings are often reflected in arctic fox population dynamics and affect the use of alternative food sources by foxes (Chesemore 1968; Roth 2002). Collared lemming populations cycles are dampening in many areas of the Arctic, with lower peak densities, reducing food availability and potentially reducing recruitment during boom years (Loos-Frank 2000; Ims et al. 2008). As juveniles seem to be an important host for nematodes, changes in

recruitment due to climate change have the potential to largely impact parasite populations over time.

In Chapter 3, we showed that arctic fox had more parasites than red foxes. These differences are likely caused by differences in behavior, life history, and diet. Feces were commonly encountered at arctic fox dens whereas very few red fox dens had feces present. Defecating at another location, away from the den, reduces the probability of a fox becoming infected with many species of nematodes (Ezenwa 2004). Further, arctic foxes have larger litters than red foxes (Macpherson 1969; Saunders 1988). Differences in life history may explain the higher nematode numbers in arctic fox, as host density is strongly linked to the prevalence and intensity of parasites (Arneberg et al. 1998).

Diet differences between the species may be driving arctic foxes to have more cestodes. The $\delta^{13}C$ values were related to cestode abundance in both arctic and red fox, suggesting a fox with a diet containing a greater proportion of small mammals will also have a higher infection rate for cestodes.

The large differences in parasites between arctic and red foxes suggests that other factors may be at work. Differences in life history and subsequent investment in immunity can play an important role in the parasite prevalence and load of a host (Ricklefs and Wikelski 2002; Schmid-Hempel and Ebert 2003; Johnson et al. 2012). Hosts have to make trade-offs between investment in immunity and other activities, such as reproduction and growth (Ricklefs and Wikelski 2002; Schmid-Hempel and Ebert 2003; Johnson et al. 2012). Species that are considered to be ‘slow-lived’ (longer life span and slower growth rates) are suggested to invest more resources in their immune

responses as their parasite exposure increases (Schmid-Hempel and Ebert 2003). Arctic fox have a shorter life and a higher average litter size than the red fox (Macpherson 1969; Saunders 1988). High investment in reproduction by arctic fox, especially in years where food is abundant, could lead to preferential investment in reproduction at the cost of immunity. However, red fox live longer and may have to invest more in immunity over time to ensure that they survive to continue to reproduce throughout their life (Saunders 1988).

Arctic and red fox have been interacting in this region for a long time. Recent changes in climate may allow the red fox to move farther north, impacting these interactions (Parmesan 2006). Red fox abundance may continue to increase in this region if temperatures continue to increase. Furthermore, red fox parasites that have been limited by their free-living stages or intermediate hosts could start to move into this northern region (Kutz et al. 2009). As their definitive host is already present, these parasites could expand rapidly. Arctic fox may be naive to these parasites and the effects on their immune system, reproduction, and survival are still unknown (Kutz et al. 2005).

Finally, in Chapter 4, we showed that wolves' diet affected their gastrointestinal parasite community. Wolves that consumed a higher proportion of white-tailed deer (*Odocoileus virginianus*) had more cestodes than wolves that consumed a higher proportion of moose (*Alces americanus*). This is the first time that we have been able to link the long-term diet of an individual wolf to its parasite community. Interactions between predators that compete for scarce resources may be exacerbated through parasite transmission. Moose populations are declining in this region while white-tailed deer

populations are increasing; these interactions may have long term effects on both populations. Moose may undergo increased predation pressure as wolf populations increase in response to the additional white-tailed deer in the area. If white-tailed deer are acting as reservoirs of infection and maintaining higher parasite prevalence and intensity (Samuel et al. 1976), an increase in parasite populations in the ecosystem could also affect moose, making them more vulnerable to predation, and further reducing their populations.

As with any method, there are limitations to using stable isotopes as a proxy for diet. Differences in life history or behavioral traits, such as ontogeny, growth, migration, and fasting can all affect stable isotopes. Starvation can increase the $\delta^{15}N$ value of an animal (Olive et al. 2003); therefore, it is important to know whether any of the canids in this analysis were emaciated, as their $\delta^{15}N$ values could be elevated and thus might not accurately represent that individual's diet. Similarly, because wolves tend to prey on younger or weaker members of their prey species, it would be useful to know the age and sex of the prey animals sampled which is a limitation of the prey samples used in Chapter 4 (Kunkel et al. 1999, Peterson and Ciucci, 2003). The stable isotope ratios of samples from hunter-harvested white-tailed deer and moose may not represent the animals killed and fed on by wolves. For example, a weaker individual may be starving, or a younger animal's muscle tissue may reflect its diet while nursing; either of these situations will result in an animal that is both more likely to be part of a wolf's diet and would have a higher $\delta^{15}N$ value than those killed by hunters. Variation in the lipid or protein content of food sources can also influence the $\delta^{13}C$values found in the predator (Stephenson et al.

1986; Ambrose and Norr 1993). Further, little experimental data has been collected to determine tissue turnover rates, (e.g., muscle) and most of the research has been performed on small mammals or birds (e.g., Tieszen et al. 1983; Hobson 1993). Although we attempted to estimate turnover time from the few studies that have been done, we can only make educated estimates of the approximate turnover rate of muscle in the canids.

A few main relationships were apparent in the three research chapters. Body condition was not related to the parasite abundance or intensity in wolves, arctic and red foxes. The lack of relationship was unexpected as parasites are expected to be stressors on their host and detrimental to the condition of their host (Marcogliese and Pietrock 2011). The intensity of infection in the canids may have been too low for any symptoms of the infection to be present, as there may be a threshold before any symptoms appears (Schmidt and Roberts 2009). The wolves from southeastern Manitoba were in good condition with lots of subcutaneous and visceral fat present during necropsy. Other stressors, such as food availability, could have been driving the variation in body condition in arctic and red foxes. We were not able to quantify microparasites, including protozoan, bacteria, and viruses, in either foxes or wolves. These microparasites can reproduce rapidly in their hosts, reaching high numbers in a short period of time (Schmidt and Roberts 2009). Microparasites can be a large stressor on their host and detrimental to their overall condition (Schmidt and Roberts 2009). The exclusion of these parasites may explain the lack of relationship between body condition and the helminthes in my study. Although body condition was unrelated to parasite numbers, impacts on reproductive output and stress are yet to be understood and may vary as the population cycles.

Parasite communities were related to the diet of the host. Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$), as a proxy for diet, were related to the parasite prevalence and abundance in all three canid species. In the arctic fox, differences in diet influenced the sex-biased parasite loads. In arctic and red fox, diet was also related to higher cestode prevalence and abundance in arctic fox, which had a diet that included more small mammals. Interestingly, nematode abundance was related to nitrogen stable isotopes in the red fox, suggesting that paratenic hosts may be playing a role in the transmission of nematodes in this species of fox. Finally, wolves that consumed a higher proportion of white-tailed deer had a greater prevalence and abundance of parasites compared to wolves that relied mainly on moose. Overall, changing climatic conditions as well as other anthropogenic impacts continue to affect prey availability and diversity; these changes may have repercussions on parasite communities and overall population dynamics.

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Appendices:

Appendix I: **Fecal float results**

Table 6.1. Prevalence (%P) and mean intensity (I) of parasites in arctic and red fox fecal samples from Wapusk National Park in May-June 2010 (26 arctic fox dens, 3 red fox dens), April 2011 (45 arctic fox dens, 2 red fox dens), and April 2012 (46 arctic fox dens, 10 red fox dens). Fecal floats were performed as described below.

Parasite Taxa	**2010**				**2011**				**2012**			
	Arctic Fox		Red Fox		Arctic Fox		Red Fox		Arctic Fox		Red Fox	
	%P	I	%P	I	%P	I	%P	I	%P	I	%P	I
Helminths												
Alaria sp.	7.7	2	33	1	-	-	-	-	-	-	-	-
Taeniidae	3.8	1	-	-	-	-	-	-	-	-	-	-
Toxocara leonine	3.8	4	-	-	40	3.1	-	-	3.8	4	-	-
Toxocara canis	12	1	-	-	11	0.85	-	-	12	1	-	-
Spirocerca sp.	3.8	1	-	-	-	-	-	-	-	-	-	-
Ancylostomidae	12	1.6	-	-	4.4	0.67	-	-	-	-	-	-
Protozoa												
Eimeriidae	3.8	2	-	-	-	-	-	-	-	-	-	-

Appendix II: **Endoparasite Feces Protocol** (Quantitative endoparasite feces count based on McCurin and Bassert 2002)

1. Take the mass of each feces sample (using a scale which measures to 0.01g)

2. Take subsample (0.5g) of feces and place into glass test tube. Add flotation solution (magnesium sulphide*) to fill half of the test tube. The remaining sample will be used for dietary reconstruction.

3. Using a metal spatula, mix sample well breaking up large chunks (to strain large pieces of debris, which if applicable can be used for dietary reconstruction)

4. Seal top of test tube and mix vigorously for 30 seconds.

5. Add more solution until the tube is completely full. Carefully place cover slide on top of the test tube.

6. Wait for 5 minutes to allow flotation process to finish.

7. Remove cover slip vertically and place on a slide.

8. Examine slide under compound microscope. Count eggs, oocysts, and other parasites using grid as a landmark (moving right using a top-left decision (on the grid if an organism occurs on the grid line count those which occur on the top or left line and ignore those on the bottom or right lines to ensure no double counting)).

9. Take a picture (create a digital image) of each individual egg, oocyst, or parasite (for identification at a later date).

10. Total parasite eggs per gram of feces can be determined based upon known mass of sample. Divide total count by mass of subsample to yield parasites (or eggs/oocysts, etc.) per gram.

11. Clean every surface with 70% ethanol and all wastes will be sent through the autoclave before being disposed.

*Magnesium sulphide preparation

1. Mix salt (Epsom salt) with distilled water until no more salt dissolves (and excess settles on the bottom).

2. Stand overnight to ensure solution is fully saturated (sediment still remaining on bottom of container)

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Appendix III: Comparison of stomach and intestine contents of necropsied arctic (*Vulpes lagopus*) and red fox (*Vulpes vulpes*) collected from December 2011-February 2012

	Frequency of occurrence			
	Arctic Fox (n=49)		Red Fox (n=18)	
Food Items	**No.**	**%**	**No.**	**%**
Mammals (total)	3	8.2	1	-
Caribou hair	1	2.0	-	-
Other ungulates	1	2.0	-	-
Small mammal	-	-	1	
Bone	1	2.0	-	-
Insect	1	2.0	-	-
Bird Feathers	2	4.1	1	
Vegetation	2	4.1	-	-
Non-food items (total)	4	8.2	1	-
Garbage	2	4.1	1	
Plastic	2	4.1	-	-