University of Alberta

WEST NILE VIRUS AND PARASITES IN GREATER SAGE-GROUSE (*CENTROCERCUS UROPHASIANUS*) POPULATIONS.

by

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This work is dedicated to Cody, Amber, Shayna and Becca for stopping by the field house and reminding me that a lumpy rock, a crushed egg, a garter snake and a pile of dirt are all marvelous things.

ABSTRACT

Given the importance of disease in species management, I investigated the role of West Nile virus (WNv) and selected parasites on Greater Sage-Grouse (*Centrocercus urophasianus*; sage-grouse) populations. WNv can reduce late summer survival of sage-grouse. In southeastern Alberta an experimental mitigation program was successful in reducing a WNv vector (*Culex tarsalis*). However, the development of *C. tarsalis* and WNv transmission appears dependent on weather and local conditions. Second, I examined the relationship between specific parasites, male sexual traits and mating success on Alberta and Wyoming leks. Between Alberta and Wyoming, there was variation in parasite prevalence and the important sexual traits in models for male mate success. While WNv demonstrates that disease has the potential to reduce survival, the role of parasites in sexual selection demonstrates that through other mechanisms, parasites and disease can be important to population dynamics.

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Chapter 1

General Introduction

The Greater Sage-Grouse (*Centrocercus urophasianus*, hereafter sage-grouse) is an endangered species in Canada (COSEWIC 2004). While declines in sage-grouse populations are largely linked with changing habitats (Aldridge and Brigham 2003, Schroeder et al. 2004), small populations are now vulnerable to other stressors. For example, stochastic disease events can shrink species range and extirpate local populations (see review Smith et al. 2006). In 2003 West Nile virus (WNv) spread into the eastern range of sage-grouse, highlighting disease as a management issue. Naugle et al. (2004) then reported a 25 % reduction in late-summer survival when compared to pre-WNv survival in Alberta, Montana and Wyoming and also when compared to survival in an unaffected population. Uncertainty regarding how WNv would continue to manifest in Alberta, and the high mortality of sage-grouse demanded an immediate management response. The Alberta Greater Sage-Grouse Recovery Action Group (2005) identified the epidemiology of WNv as a research priority.

The role of disease in species endangerment and extinction is generally linked to high mortality. Altizer et al. (2007) listed examples of infectious disease causing high mortality in threatened species: canine-distemper in black-footed ferrets (*Mustela nigripes*; Dobson and Lyles 2000); rabies in African wild dogs (*Lycaon pictus*; Kat et al. 1995); withering disease in black abalone (*Haliotis cracherodii*; Altstatt et al. 1996) and Ebola virus in chimpanzees (*Pan troglodytes*) and gorillas (*Gorilla gorilla*; Walsh et al. 2003, Leroy et al. 2004).

Two theoretical mechanisms for disease-induced extinction, despite low host density, include non-density-dependent transmission and the presence of disease reservoirs (de Castro and Bolker 2005). Factors relating to small populations are, however, a third mechanism for disease-induced extinction through a variety of predisposing factors, including inbreeding and Allee effects (de Castro and Bolker 2005). In diseases with density-dependent transmission, prevalence of disease and

parasites should theoretically decline with decreasing host population size and density (Anderson and May 1979). Thus, threatened hosts may actually have fewer parasites (Lyles and Dobson 1993). There is evidence from primates of reduced parasite richness relative to increased threat status of the host (Altizer 2007). However, an overall reduction in parasite prevalence and richness, could have implications for sage-grouse genetic diversity.

In the spring, male sage-grouse display together on lek sites to attract mates; however, relatively few males obtain the majority of copulations (Wiley 1973, Hartzler and Jenni 1988). However, the strong directional selection of such a highly skewed mating system could be predicted to erode genetic variation. In this 'lek paradox,' there is reduced potential for the maintenance of strong female choice (Borgia 1979, Taylor and Williams 1982). Fluctuations in the relationship between parasite and host could operate as a mechanism for resolving the 'lek paradox' and maintaining genetic diversity in a lek species (Hamilton and Zuk 1982, Boyce 1990). There is evidence from captive and wild populations of sage-grouse, that parasites reduce the quality of secondary sexual characteristics, and thus the mating success, of male sage-grouse (Johnson and Boyce 1991, Spurrier et al. 1991). Through population isolation and a reduction in host density, loss of parasites with density-dependent transmission could disrupt this potentially important mechanism. Therefore, the loss of parasite-mediated sexual selection could present an example of de Castro and Bolker's (2005) third mechanism of disease-induced extinction where a disease-related risk is more subtle than through dramatic mortality events.

In sage-grouse there is some limited evidence that small isolated populations may have fewer parasites. In a relatively isolated California population, Gibson (1990) reported a lower diversity and a lower prevalence of blood parasites than Johnson and Boyce (1991) reported in a large Wyoming population. Furthermore, there was no evidence that rare blood parasites were related to sexual selection in California (Gibson 1990).

To contribute directly to research needs identified by the Alberta Greater Sage-Grouse Recovery Plan (2005), Chapter 2 examines WNv in southern Alberta and in Alberta's sage-grouse and (1) investigates *Culex tarsalis* mosquito populations on the prairie landscape relative to WNv transmission and weather patterns and (2) evaluates a larvicide mitigation program intended to reduce sage-grouse exposure to WNv.

Behavioural factors contributing to the decline of grouse populations are poorly understood (Peterson 2004). Chapter 3 examines the role of parasites in sage-grouse sexual selection and uses (1) correlative evidence to identify parasite-mediated sexual characteristics and (2) an information-theoretic approach to investigate the importance of these characteristics in mate choice relative to parasite prevalence and population size.

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Chapter 2

Effective microbial larvicide application for mosquito vectors of West Nile virus in southern Alberta.

1. Chapter Abstract

West Nile virus (WNv) first spread into Alberta in 2003, resulting in human, livestock and wildlife risks, including 25% mortality of an endangered species, the Greater Sage-Grouse. Adult *Culex tarsalis*, a vector of WNv, was monitored at mosquito-trap sites across Alberta's grasslands from 2003 to 2006. To mitigate health and conservation risks caused by WNv, we implemented a microbial mosquito larvicide treatment program from 2004 to 2006 in a portion of sage-grouse range in southern Alberta. To test the effectiveness of this strategy, we monitored larval development, adult mosquito numbers, and hen sage-grouse survival within a treatment and control area. Adult mosquito trapping indicates *C. tarsalis* population development and activity was closely tied to temperatures. During 2004 to 2006, cool temperatures and lack of moisture limited development of *C. tarsalis* in the larvicide treatment area. Although high WNv-associated mortality did not occur in sage-grouse subsequent to the outbreak in 2003, the mitigation treatment was effective in reducing both larval and adult mosquitoes at the intended scale. Thus, appropriately applied microbial larvicide can substantially reduce exposure of human and wildlife populations to mosquito vectors in southern Alberta. As the pattern of WNv transmission in Alberta continues to emerge, monitoring of annual mosquito development and virus activity will be essential for guiding the location and extent of microbial larvicide programs.

2. INTRODUCTION

West Nile virus (WNv) was detected in New York City in 1999 and has spread widely across North America (McLean 2006). Since 2003, when the virus first reached Alberta (Pybus 2003), there have been risks to human health, livestock and wildlife. Three hundred and twenty-two clinical human cases of WNv have been reported in Alberta (Centre for Infectious Disease Prevention and Control 2007). In livestock, 186 confirmed clinical cases have been reported in horses and 35 % were euthanized or died from WNv (Morin 2007). Furthermore, some surviving horses have suffered permanent neurological disorders (Ollis 2007). In wildlife populations, disease-related extinctions and extirpations are mainly a concern where there are other causes of population decline (McCallum and Dobson 1995, Smith et al. 2006). Conservationists predicted that WNv could contribute to extirpations of vulnerable species in North America (Male 2003, Peterson et al. 2004).

Greater Sage-Grouse (*Centrocercus urophasianus*, hereafter sage-grouse) is an endangered species in Canada (COSEWIC 2004) and is highly susceptible to WNv. Naugle et al. (2004) reported a 25 % reduction in late-summer survival compared to pre-WNv survival in Alberta, Montana and Wyoming and when compared to an unaffected population. Severe mortalities also have been recorded for American crows (*Corvus brachyrhynchos*); however, population impacts on native wild birds are largely unknown (Yaremych et al. 2004, Caffrey et al. 2005, McLean 2006). The Centers for Disease Control and Prevention (CDC) currently report 233 North American bird species with known WNv-associated mortality (CDC 2007). This list includes 30 species with a status of *sensitive* and five with a status of *at-risk* for extinction or extirpation in Alberta (Alberta Sustainable Resource Development 2005).

A strategy for limiting WNv in the Alberta sage-grouse range was initiated in 2004 and framed in an adaptive management context where the goals of the project were both conservation action and scientific evaluation through an experimental approach. There are rarely opportunities for scientific controls in wildlife disease management efforts, however, they can be extremely beneficial to future management efforts

(McCallum and Dobson 1995). Because provincial monitoring in 2003 determined that *Culex tarsalis* was the primary vector of WNv in Alberta (Alberta Environment, unpublished data), the project included a mitigation program targeting larval *C. tarsalis* mosquitoes. Research goals of the project were:

(1) Developing an understanding of factors that contribute to WNv transmission in southeast Alberta.

(2) Assessing the effectiveness of the mitigation program in reducing *C. tarsalis* populations at the local scale.

3. Study Areas

(1) General WNv monitoring was performed at a large-scale across Alberta's grasslands ecoregion as part of the Alberta provincial government's West Nile virus response plan. Mosquito trapping stations were spaced throughout the grasslands in 2003 to 2006. Station locations were not constant among years (Figure 2-1, Table 2-1).

(2) The mitigation experiment took place at the local scale in the Manyberries area of Alberta's grasslands. Alberta's sage-grouse are located in ~4000 km^2 in the southeastern corner of the province and our study was located in an 1100 km^2 core-use area identified by Aldridge (2007) in the Manyberries area (Figure 2-1). This area is composed of dry mixed-grass prairie, with silver sagebrush (*Artemisia cana*) (Aldridge and Brigham 2003). In July and August, 25 year mean daily summer temperature averaged 19.1 °C and precipitation averaged ~70 mm (AAFC-AAC 2006). Surface water in the area consisted mainly of: (1) small dugouts created for livestock watering; (2) shallow ephemeral pools of water in depressions and ditches; (3) creek beds that contain small pools of standing water but flow with spring melt water and heavy rain showers and (4) a few permanent shallow wetlands.

4. METHODS

4.1 Mosquito Monitoring Protocol

Under the Alberta West Nile virus response plan, mosquitoes were monitored on a weekly basis between June and September at trap stations across southeastern Alberta by local municipalities and Alberta Environment. Trap stations captured host-seeking mosquitoes using standard CDC mosquito surveillance traps (Model #2836BQ, BioQuip Products, Inc., Rancho Dominguez, California). Trapping in Manyberries began in 2004 and a total of four traps were operated in each year. Two CDC mosquito surveillance traps were placed approximately 500 m east and 500 m west of the area centre in each of a control and treatment area. At other grasslands stations, between one and six traps were located outside municipal larvicide treatment areas. All traps were baited with CO_2 gas released from either dry ice or compressed gas cylinders and run without lights to reduce the attraction of non-host seeking mosquitoes and non-target species. Traps were activated in the early evening and mosquitoes were collected after sunrise the following morning. Trapping efficacy increased after 2003 because station locations were optimized and trapping protocols were adjusted to allow trapping at remote locations through the use of CO_2 cylinders. Trapping began in week 25 (week of June 20) and ran until week 36 (week of September 5) in 2004, 2005 and 2006; however in 2003, trapping did not start until week 28. Only half of stations operated in week 36. In 2003, there was no trapping station in Manyberries (Table 2-1).

Captured mosquitoes were frozen and sorted by species into pools of <50. These pools were tested for WNv at the Provincial Laboratory in Calgary, Alberta, Canada using Nucleic Acid Sequence Based Amplification and positive tests were confirmed by TaqMan Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) (Lanciotti et al. 2000). Only *C. tarsalis* pools had positive tests. For each week, a minimum infection rate was calculated as the number of *C. tarsalis* pools to test positive for WNv divided by the total number of mosquitoes tested and scaled by 1000. Minimum infection rates were not calculated past week 35 (week

of August 28) because the reduced trapping effort from that week probably would have influenced the measurement.

For each trapping station, a weekly capture of *Culex* per trap night (CT) was calculated as the total catch of *C. tarsalis* for that week divided by the number of trap nights. A single trap running for a full overnight period equalled one trap night. Standardized *Culex* per trap night (SCT) was calculated for the grasslands as the average of the CT, weighted equally for each trapping station running that week. For Manyberries, CT was calculated separately for the control and treatment areas.

4.2 Larvicide Program – Overview

To test the effectiveness of microbial larvicide to control mosquitoes, two similar areas were selected in each year. One area was an experimental control that received no larvicide treatment. The other received the experimental treatment of larvicide. Adult and larval *C. tarsalis* monitoring was used to determine the effectiveness of this larvicide treatment in reducing *C. tarsalis* populations at the local scale. Samples of marked sage-grouse were monitored in each area to quantify WNv-associated mortality.

4.3 Larvicide Program – Selecting Sample Areas

C. tarsalis disperse to seek hosts (Reisen et al. 1991). To limit the impact of mosquito dispersal into the treatment area and to encompass the sample of marked sage-grouse hens, the treatment and control areas were circular areas of 6 km radius (113 km^2). Due to this large scale, the treatment was limited to only one area. Therefore to allow comparison, it was extremely important to be certain that the treatment and control area were as similar as possible. To ensure this, we identified areas with both: (1) similar standing water and thus, larval habitat and (2) similar available sage-grouse habitat, based on brood habitat models from Aldridge (2007). Because this approach effectively focused the treatment area on critical source brood rearing habitat as identified by Aldridge (2007), in each year the treatment area included ~24% of Alberta's critical source brood-rearing habitat.

A moisture index was estimated to identify standing water and referred to as the compound topographic index (CTI; Moore et al. 1993, Geissler et al. 1995). An ArcInfo algorithm (Rho 2002) and a digital elevation model were used to calculate CTI. To anchor the CTI index, existing standing water bodies (Alberta provincial base features data; 1:20 000 scale) were used to determine which CTI values identified known standing water. The average CTI value at water bodies was 13.4 with a standard deviation of 2.7. To be conservative, CTI moisture index values ≥10.0 were considered to indicate a potential standing water source indicative of suitable mosquito breeding habitat.

Sage-grouse habitats were identified with the resource selection function (RSF) model from Aldridge (2007) for sage-grouse brood rearing habitat. RSF Bins 9 and 10 were considered a conservative cut off to indicate a high probability of sage-grouse use. By combining CTI values ≥10.0 with RSF bins ≥9, the co-occurrence of sage-grouse and mosquitoes was identified for each 30 m pixel within the study area.

The co-occurrence model for selecting sampling areas was based on a moving window with a radius equivalent to the sample area. The proportion of the 113 km^2 area around each 10 m pixel on the landscape that contained the co-occurrence of sage-grouse and mosquito habitat was calculated. The highest proportion of this co-occurrence was 44 %. However, most cells were below 10 %. Only sample areas that had at least 10 % co-occurrence were considered suitable sites for centring the mosquito sampling areas.

The final consideration for circle placement was the location of marked hens. In order to test the effectiveness of the treatment on sage-grouse survival, the treatment areas had to correspond to the brood locations of marked hens. Radio collars permitted the detection of dead and/or dying hens (see 4.6). Using the co-occurrence model, circle locations in each year were selected to include as many of the marked hens as possible (Figure 2-2).

4.4 Larvicide Program – Treatment Protocol

In each year, one of the two sample areas was chosen randomly and treated with larvicide granules. A combination of *Bacillus thuringiensis* ssp. *Israelensis* (*Bti*) (VectoBac® PCP #18158) and *B. sphaericus* (VectoLex CG® PCP #28008) were used (Valent BioSciences Canada Ltd.; Toronto ON). These products were chosen because they have relatively limited or no impact on non-target species and are biodegradable: they do not persist for the long-term in the environment (Lacey and Mulla 1989, Brown et al. 2004, Merritt et al. 2005). Because VectoLex CG® has a longer duration of effectiveness but is also more expensive, it was used preferentially in locations with limited access and only for water-bodies expected to persist for more than a few days. Water removal is not a viable management option because although wet areas provide larval *C. tarsalis* habitats, mesic habitats are used by hens with broods and may be important in productivity (Aldridge 2007).

In 2004, we used only VectoBac® and intended to treat all shallow standing water within the 113 km^2 treated sample area. The first treatment coincided with the initial mid-June mosquito hatch and the subsequent treatments occurred two, four, six and eight weeks later in early and mid-July and again in early and mid-August. In total, 5 rounds of VectoBac® were applied.

In 2005 and 2006, VectoBac® was again used for the first and fifth round. The protocol was, however, adapted to include VectoLex CG®. This product has an increased duration of larvicide activity against *C. tarsalis*, allowing more efficient coverage of the entire 6 km radius treatment area.

Both larvicides were applied using handheld fertilizer applicators (Scotts® Handy Green II® Hand-Held Spreader) set at "2" which provided an even distribution of the larvicide within the recommended application rates. Estimated application was typically ~0.6 to 1.0 g/m^2 with a slightly higher application of VectoLex CG® (~1.5 g/m^2) if water was particularly deep (>1 m) or murky due to suspended solids.

To determine the effect of the treatment on mosquito populations, adult mosquito captures from the treatment and control area were modelled using negative-binomial regression. The fit of the model distribution was evaluated following Drukker (2000). To adjust for the weekly trapping events, standard errors were corrected by clustering for individual trap (Rogers 1993). The variable *year* was included to account for the difference in overall *C. tarsalis* populations between years. The variable *week effect* was included to account for the difference in overall *C. tarsalis* populations between weeks. *Week effect* was calculated as the absolute value of the number of weeks from the peak catch. This analysis was conducted with Stata 9 software (StataCorp 2005).

4.5 Larvicide Program – Dipping Protocol

Following O'Malley (1995), larval dipping was performed using a white plastic dipper. Dipping was used to regulate the exact timing and location of treatment application within rounds. In 2006, repeated dipping of predetermined sites was used to: (1) determine the effectiveness of the treatment; (2) measure the duration of effectiveness of VectoLex® and (3) characterize important water types. Potential dipping sites were classified into one of 5 different water types: dugout; ditch; overflow; tall-banked creek and short-banked creek (Table 2-2). Five of each water type were randomly selected within each circle for repeated sampling. Prior to each treatment round, all 50 sites were sampled for the presence or absence of larval *C. tarsalis*, although many sites dried up through the summer. Characteristics at each site were recorded including the amount of shade, type of vegetation, height of vegetation, presence of aquatic predators and the surface area and depth of the water body.

Because *C. tarsalis* tend to develop around the edges of water bodies, water depth was measured 1 m from the edge. However, if the water was <2 m across, depth was measured in the centre. Depths <30 cm were classified as shallow, 30 to 50 cm as medium and >50 cm as deep. Shade was classified as present if it covered >50% of the water source at noon. Vegetation type was classified by the predominant vegetation within a 1 m square plot at the primary dipping site. Vegetation classes were: (1) bare dirt; (2) willow (*Salix* spp.); (3) thick shrubs (*Rosa* spp. and *Artemisia cana*); (4)

wetland vegetation (*Carex* spp. and *Scirpus* spp.) and 5) flooded upland vegetation (various grasses and forbs). Vegetation height was the average height of the three tallest individuals predominant in the plot. Water body size was an estimate of the surface area of the water body.

The occurrence of larval *C. tarsalis* was modelled using logistic regression. Because the nature of standing water changed drastically through the summer, dipping from the pretreatment dipping and each treatment round were modelled separately. The variable treatment area was included in all models to test the effect of treatment in larval reduction. Hosmer and Lemeshow (2000) model building procedures were used to create a full model containing the important predictor variables for each dipping round. Final models were validated using the area under a receiver operating characteristic curve (ROC). This analysis was conducted with Stata 9 software (StataCorp 2005).

4.6 Sage-Grouse Monitoring

During breeding season (March through May), sage-grouse were captured from six of nine active leks in southeastern Alberta using walk-in traps (Schroeder and Braun 1991) and spotlighting (Connolly et al. 2003). Captured females were fitted with 14 g necklace-style radiotransmitters (RI-2BM transmitters; Holohil Systems Ltd; Carp, ON Canada). These transmitters were equipped with a motion triggered mortality switch that allowed assessment of hen survival status without flushing the birds.

Hens were located with a 3 or 5-element Yagi antenna and an R-1000 scanning telemetry receiver (Communications Specialists, Inc. Orange, California). When signals could not be located, the study area was searched using a fixed-wing aircraft. Hens were not flushed while nesting or brooding young chicks, but were located from a distance of >40 m. Once hens were off nests for 3 weeks, they were flushed on a weekly basis to determine their breeding status or flock size. During the period of potential exposure to WNv (mid-June to mid-September), hens were located on a 1 or 2 day schedule that allowed collection of mortalities before carcasses were scavenged.

All sage-grouse carcasses underwent complete necropsies and microscopic examination of routine tissues by histopathology at the Fish and Wildlife Disease Laboratory in Edmonton. Birds were tested for WNv at the Canadian Cooperative Wildlife Health Centre in Saskatoon, Saskatchewan using real-time RT-PCR (Shi 2001).

Naugle et al. (2004, 2005) reported no evidence of resistance to WNv in sage-grouse from 363 birds tested for antibodies. Furthermore, a 2003 experiment with artificially infected sage-grouse found high susceptibility (Clark et al. 2006). To continue searching for resistance or evidence of infection survival in the Alberta population, serum from 49 live-sampled male and female sage-grouse, captured in 2004 to 2006, was tested for WNv antibodies at the Wyoming State Veterinary Lab in Laramie, Wyoming, USA using plaque reduction neutralization assays (Weingartl et al. 2003).

4.7 Weather Data

Temperature has been linked to the development of *C. tarsalis* adults and larvae (Brust 1990, 1991) and directly to WNv amplification and transmission (Reisen et al. 2006). Weather data for the grasslands was estimated using Brooks weather data provided by Environment Canada (2007, Climate ID: 3030QLP). Long-term weather data for the Manyberries area was provided by the Onefour Agriculture and Agri-food Canada Research Station (AAFC-AAC 2004, unpublished weather data) and in 2003 to 2006 by Environment Canada (2007, Climate ID: 3044923).

5. Results

5.1 Grasslands Adult Mosquito Capture

Grassland Standardized *Culex* per trap night (SCT), an index of the weekly catch of *C. tarsalis* adult mosquitoes, varied between years (Figure 2-3). In 2003, 2005 and 2006, captures of *C. tarsalis* increased with warm temperatures and then dropped off as temperatures fell. In 2004 however, temperatures remained cool and catches were low throughout the summer (Figure 2-3). Manyberries mosquito captures

followed the general pattern in 2005 but not in 2006 (Figure 2-4). Cumulative degree days (base 18) indicate that 2003 and 2006 were steadily warm while in 2005, mean temperatures stayed below 18 °C in week 31 (early August, Figure 2-5). In 2003 to 2006, total precipitation between week 25 (week of June 20) and week 36 (week of September 5) was 32.2, 103.8, 72.2, 18.4 respectively (Figure 2-6).

WNv was not detected in 2004 and 2005 in adult mosquitoes in the grasslands region, although it was detected in 27 of 175 mosquito pools in 2003 and 110 of 712 mosquito pools in 2006. The difference in the total capture of *C. tarsalis* and the number of pools tested was partially due to an increase in trapping effort and efficacy of trapping after 2003. Minimum infection rates calculated for weeks 28 to 35 were similar in 2003 and 2006, increasing from 0 to 30 (Figure 2-7).

5.2 Microbial Larvicide Treatment

In 2004, captures of *C. tarsalis* were extremely low. In Manyberries, only 24 *C. tarsalis* were captured in 60 trap nights (4 traps running for 15 nights over 12 weeks). Because average CT was <0.1 and too minimal to detect a meaningful difference between the treatment and control areas, 2004 was excluded from further analysis. In 2005, 2265 *C. tarsalis* were caught in 64 trap nights (4 traps running for 16 nights over 11 weeks). Average CT in the treatment area and control area was 42.2 and 16.5, respectively (Table 2-3). In 2006, a total of 317 *C. tarsalis* were caught in 52 trap nights (4 traps running for 13 nights over 11 weeks). Average CT in the treatment area and control area was 12.05 and 1.65, respectively (Table 2-3). Using a negative binomial regression, there was a significant difference ($p=0.008$) in catches of adult *C. tarsalis* between the treatment and control area (Table 2-4). There was also a strong effect of year ($p=0.036$) and trapping week ($p<0.0001$). In 2004 to 2006, no *C. tarsalis* mosquito pool tested positive for WNv from the Manyberries trapping station.

To determine if larval presence was similar between the two areas before the experiment began, a dipping round prior to the first treatment application was conducted. Treatment area had no significant effect in this logistic regression ($p=0.787$). The logistic regression models for subsequent dipping rounds all included

treatment area as a significant effect ($p<0.05$, Table 2-5). Of the potential predictor variables, final models included only water depth (Table 2-5). Larval *C. tarsalis* were found in shallower water prior to the treatment and in rounds 1 and 2. By round 3 and 4, water depth dropped out of the models as larval *C. tarsalis* were found in all depths of water. Many of the dipping sites dried up through the summer with less than half of the original locations still containing water by round 4 (n=22).

5.3 Sage-Grouse Survival

In 2004, 19 sage-grouse were regularly radiotracked, 26 in 2005 and 30 in 2006. During the period of potential WNv exposure in late-June to mid-September, five birds died. All five carcasses were collected within 24 hours of death and tested for WNv. Three of the carcasses were fresh raptor kill sites and two birds were completely intact. One bird died from WNv infection. This brood-rearing hen was found in the control area, completely intact on 9 August 2005 with her head twisted back under her wing. She was observed with a drooping, shaky head and uncoordinated movements six hours before her death. These symptoms are similar to those recorded for sage-grouse and other avian species (Marra et al. 2004, Walker et al. 2004). No antibodies for WNv were detected in captured birds.

6. Discussion

6.1 WNv in Alberta's Grasslands

In Alberta, the annual development of *C. tarsalis* populations were closely tied to temperature. While SCT appeared to be lower in 2003 than 2006, direct comparison was difficult due to improvements in trapping station locations and methodology. Nevertheless, in the years with consistently high temperatures (2003 and 2006, Figure 2-6), *C. tarsalis* populations and minimum infection rates follow the same pattern and increase through the summer (Figure 2-3, Figure 2-7). By contrast, in years where cool temperatures disrupted development (2004 and 2005), *C. tarsalis* populations were limited and infection rates were lower than could be detected through mosquito trapping. Furthermore, this pattern is consistent with the timing of wild bird mortality. The Alberta wild bird surveillance program conducted by the Fish and Wildlife

Division of Alberta Sustainable Resource Development tested dead birds submitted by the public. Initial WNv-associated mortality in 2003 and 2006 preceded that of 2004 and 2005 (Pybus 2007).

In 2004 and 2005, the Manyberries adult *C. tarsalis* trap data followed the same general pattern as the grasslands SCT. There were almost no *C. tarsalis* captured in 2004 in any trapping stations including Manyberries. In 2005 there was a steady increase that dropped off with cool temperatures in late July and early August (week 32, Figure 2-4). In 2006, however, Manyberries *C. tarsalis* capture did not follow the general grasslands trend of increasing through the summer (Figure 2-4). Populations may have been limited by a low availability of water suitable for larval development. Cumulative precipitation for Manyberries indicates that 2006 was much drier than 2003 to 2005 inclusive (Figure 2-6). Furthermore, there is a plateau in the cumulative precipitation, indicating a period with almost no additive precipitation. Larval *C. tarsalis* are capable of surviving in very small depressions and puddles (Brust 1990), so even small additions of water through the summer, as in 2005, would likely have allowed increased development of *C. tarsalis* populations through this period. In most other grassland trap sites, lack of moisture was likely not a limiting factor due to an initially greater amount of standing water because of: (1) higher annual precipitation; (2) the presence of irrigation and (3) the proximity of many municipalities and their trap stations to water sources and river systems.

Temperature has emerged as a key component of WNv infectivity and transmission in North America (Reisen et al. 2006). Warm temperatures allow faster virus amplification and increase infectivity within blood-fed mosquitoes (Reisen et al. 2006). In southern Alberta, this effect may be compounded because larval development and host-seeking activity of *C. tarsalis,* the primary Alberta vector of WNv, appears limited by periods of cool temperatures (Figure 2-3). Furthermore, in Alberta *C. tarsalis* can develop either three or four generations each year. Thus, environmental conditions that slow development of early mosquito generations may also contribute to a reduction of populations in the late-summer by limiting the number

of generations produced that year. Thus in Alberta, warm temperatures have the potential to increase both vector populations and virus infectivity.

The spread of WNv into new areas has been linked to years with above-normal temperatures (Reisen et al. 2006). The Alberta distribution of WNv infection in birds is primarily of the southeastern, grasslands region of the province (Pybus 2007). Since temperature is important in vector development and virus activity, there is potential that climate change leading to concurrent warm summers will push the virus northward into new wildlife and human populations. Within the Manyberries area, there was little standing water and low summer precipitation. The divergence in mosquito capture from the grasslands pattern in 2006 (Figure 2-4) indicates that in the Manyberries area, moisture may be a limiting factor for *C. tarsalis* development (see summary, Table 2-6). From these results, high-risk years for WNv are predicted to be those with: (1) a warm springs that facilitate both early virus activity and the rapid development of early generations of *C. tarsalis*; (2) warm summer temperatures and (3) rainfall, even if of limited amount, in July and August that supplies shallow pools of water in creek beds and ditches.

6.2 Does larvicide treatment reduce *C. tarsalis* populations?

Larval dipping indicates that both VectoLex CG® and VectoBac® effectively killed *C. tarsalis* larval in shallow prairie water (Table 2-5). VectoLex CG® required only one application within each treatment round, whereas VectoBac® sometimes needed reapplication within just a few days. Larval dipping also indicated that *C. tarsalis* are opportunistic in the kind of water they use. In pretreatment and rounds 1 and 2, larval *C. tarsalis* were present in the shallowest water that was typically found in overflow areas around dugouts and ditches but not in deeper water. By late summer (at the end of rounds 3 and 4), hot conditions meant that almost any remaining standing water became suitable larval habitat including even deep water (>0.5 m), such as livestock dugouts and the few deep pools remaining in creek beds.

Testing the effectiveness of the larvicide program in reducing adult *C. tarsalis* at the local scale is useful for both future wildlife management and for Alberta

municipalities. While the effectiveness of *Bacillus* larvicides in killing larval mosquitoes is generally well tested, there have been few scientific tests of how this translates into reductions of local adult mosquito populations in Alberta's grasslands. Although Alberta municipalities widely use microbial larvicide treatment, there is little research into its effectiveness at the intended scale because municipalities cannot risk human exposure by creating realistic experimental controls that receive no treatment. The possibility that migrating adult mosquitoes might counteract the effectiveness of a larvicide program is a serious concern in treatment programs. Furthermore, municipalities can be under pressure to extend their WNv treatment programs beyond *Bacillus* larvicides to include non-biodegradable chemicals and adulticiding. Our results however, indicate that persistent and local application of *Bacillus* based larvicides, within the current legal application rates of <1 g/m^2 and <1.68 g/m^2 for VectoBac® and VectoLex CG® can result in a local reduction of adult *C. tarsalis* on a prairie landscape.

There may be a future need for treatment programs to protect vulnerable grassland wildlife populations. The lack of antibodies in captured sage-grouse, is an indicator that they remain susceptible to WNv in high risk years. Impacts of West Nile virus on other sensitive prairie species remain largely unknown although mortalities in loggerhead shrike (*Lanius ludovicianus*) and swainson's hawk (*Buteo swainsoni*) have been recorded in Alberta (Pybus 2003). Anthropogenic activities that generate larval habitat near human or sensitive wildlife populations, such as coal bed methane discharge ponds, may require effective vector mitigation programs (Zou et al. 2006).

7. Conclusions

The successful reduction of adult and larval *C. tarsalis* indicates that appropriately applied microbial larvicide can substantially reduce exposure of human and wildlife populations to mosquito vectors in southern Alberta. Because development of *C. tarsalis* appears closely tied to temperature and moisture regimes, local differences in temperature and moisture will influence the annual risk presented by WNv. As the pattern of WNv transmission in Alberta continues to emerge,

monitoring of mosquito development and virus activity will be essential in determining the need and guiding the location and extent of microbial larvicide programs.

8. Recommendations

If managers wish to employ a microbial larvicide program, the following recommendations are made:

1. Moisture and temperature conditions through the summer should regulate when larvicide treatment is required. As determined appropriate, treatment might be cancelled or suspended during low-risk years and in low-risk areas. Cooperation with regional WNv monitoring programs is essential to identify high-risk years. Early virus activity, detected from wild bird surveillance and mosquito monitoring, coupled with local moisture and temperature conditions are critical indicators of WNv risk.

2. Vectolex CG® was highly effective for full 2 week periods in the study area. Manufacturers recommended VectoLex CG® be rotated with other larvicides to prevent development of larval resistance. It is advised that VectoLex CG® is used only during the peak period of *C. tarsalis* development and alternatives such as VectoBac® for late treatment rounds. To save money, VectoLex CG® use was restricted to either locations that have limited access and take time to revisit or those water-bodies expected to persist for more than a few days.

3. The high efficacy of VectoLex CG® means that delaying the first treatment round until July may be possible without losing treatment effectiveness. This approach would allow more leeway in identifying a high-risk year but should be evaluated through adult mosquito surveillance.

4. To lower costs and human effort, treatment should be targeted for the best larval *C. tarsalis* habitat. Because *C. tarsalis* are opportunistic, in any given year extensive larval dipping following O'Malley (1995) should be used to limit the treatment to appropriate water types.

5. It is recommended that any further treatment effort for Alberta's sage-grouse continues to focus on brood-rearing habitat. These hens are likely the most at risk for WNv because they move to areas with moisture to provide good forage to their chicks. In this study, the treatment area of 113 km^2 included ~24 % of Alberta's critical source brood-rearing habitat. One summer-student technician could effectively treat this area at two-week intervals. The amount of larvicide used each round varied greatly. A maximum of 35 kg of VectoLex CG® or VectoBac® was sufficient for a treatment round covering ~113 km^2 land area.

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Table 2-1. Locations of trapping stations across the Alberta grasslands. In 2003, fewer traps were located in the most southeastern part of the province and trapping activity began later. In 2004 to 2006, trapping occurred between week 25 (week of June 20) and week 36 (week of September 5), although some stations did not participate in week 36. See Figure 2-1 for map locations.

2003	2004	2005	2006
Brooks[1]	Bow Island	Bow Island	Bow Island
Calgary[1]	Brooks	Brooks	Brooks
Drumheller[1]	Calgary	Calgary	Calgary
Lethbridge[1]	Cardston	Cardston	Cardston
Magrath[1]	Claresholm	Claresholm	Claresholm
Medicine Hat[1]	Coaldale	Coaldale	Coaldale
Oyen[1]	Drumheller	Consort	Consort
Raymond[1]	Hanna	Drumheller	Drumheller
Strathmore[2]	High River	Hanna	Foremost
Vulcan[2]	Lethbridge	High River	Hanna
High River[3]	Magrath	Manyberries	Magrath
Coaldale[4]	Manyberries	Medicine Hat	Manyberries
	Medicine Hat	Milk River	Medicine Hat
	Milk River	Oyen	Milk River
	Oyen	Picture Butte	Oyen
Trapping began:	Strathmore	Raymond	Picture Butte
[1]week 28	Suffield	Strathmore	Raymond
[2]week 31	Taber	Suffield	Rolling Hills
[3]week 32	Vulcan	Taber	Strathmore
[4]week 33		Tilley	Suffield
[5]week 35		Vulcan	Taber
			Tilley
			Vulcan

Table 2-2. Classification of water types used in the water dipping protocol. Five sites from each classification were selected for repeated dipping in the experimental treatment and control areas.

Water Type	Classification	Description
Permanent	Dugout	Open water used for livestock watering with >25 m^2 surface area.
Ephemeral	Ditch	Water accumulates in the low areas beside roads and old rail lines.
Ephemeral	Overflow	Areas with upland vegetation where water flooded in from another source, such as a dugout or creek.
Typically consist of only small pools of water. Creeks do however, flow with spring melt water and during heavy rain showers.	Tall-banked creek	Part of a creek system with a bank cut-down of >1 m.
	Short-banked creek	Part of a creek system with a bank cut-down of <1 m.

Table 2-3. Average CT (*Culex* per trap night) in the Manyberries treatment and control area for each year. CT was extremely low in 2004 preventing meaningful comparison. In 2005 and 2006 CT was higher in the control area than in the larvicide treated area.

	Control Area	Treatment Area
2004	<0.01	<0.01
2005	42.2	16.5
2006	12.1	1.65

Table 2-4. Estimated coefficients (β), robust standard error (RSE), p-values (p) and model fit parameters (Wald statistic, p-value and log pseudolikelihood) for negative binomial regression describing the influence of larvicide treatment on *C. tarsalis* capture. Regression was estimated using weekly CT at four traps in 2005 and 2006. Standard errors are adjusted by clustering on individual traps to account for the repeated weekly trapping events.

	β	RSE	p
Constant	3.96	0.52	<0.0001
Treatment	-1.39	0.52	0.008
Week effect	-0.7498	0.061	<0.0001
Year	1.16	0.55	0.036
Wald χ^2	199.54	Observations	80
p-value	<0.0001	Number of traps	8
Log pseudolikelihood	-235.26087		

Table 2-5. Logistic regression models for the occurrence of larval *C. tarsalis* in each dipping round. Of several potential predictor variables, final models included only water depth. In rounds 3 and 4, however, water depth dropped out of the model. Treatment area was not significant in the pretreatment dipping round indicating there was no difference in larval occurrence before the treatment began. Model constants and the predictor variables water depth and treatment area are presented as estimated coefficients with standard errors in brackets. P-values for the variable treatment area (p-treatment) and model validation as the area under an ROC curve (ROC) also are presented. Significant coefficients are in bold (<0.05).

Round	Number of Sites (n)	Constant	Water depth	Treatment Area	p-treatment	ROC
0-pretreatment (week 24)	50	**4.13(1.29)**	**-2.37(0.62)**	0.22 (0.80)	0.787	0.88
1 (week 26)	50	1.48(0.86)	**-0.95 (0.43)**	**-1.37 (0.69)**	0.047	0.77
2 (week 28)	30	1.61(0.99)	**-0.99(0.49)**	**-3.20 (1.14)**	0.005	0.77
3 (week 30)	28	0.93(0.52)	Not included	**-2.77 (1.16)**	0.017	0.78
4 (week 32)	22	-0.13(0.52)	Not included	**-2.35 (1.16)**	0.043	0.74

Table 2-6. Summary of *C. tarsalis* and WNv activity in Alberta based on best available information. WNv was first detected in Alberta's grassland region in 2003.

Year	Weather Trends	Result
2003	Warm temperatures and some limited rainfall	Across the grasslands, infected *C. tarsalis* populations built up and the WNv minimum infection rate increased through the summer. High mortality recorded in Alberta sage-grouse (Naugle et al. 2004).
2004	Cool spring and summer Plenty of available water	*C. tarsalis* populations declined. Low virus activity and WNv were not detected in grasslands mosquitoes. No mortality recorded in Alberta sage-grouse.
2005	Warm spring and early summer but a cool August Plenty of available water	Infected *C. tarsalis* populations built up but quickly declined. WNv was not detected in grasslands mosquitoes. Low mortality recorded in Alberta sage-grouse (1 hen confirmed).
2006	Warm spring and summer and restricted rainfall Standing water declines in dry areas	Across the grasslands, infected *C. tarsalis* populations build up and the WNv minimum infection rate increases through the summer. In Manyberries, *C. tarsalis* populations were likely limited by scarce standing water and no mortality was recorded in sage-grouse.

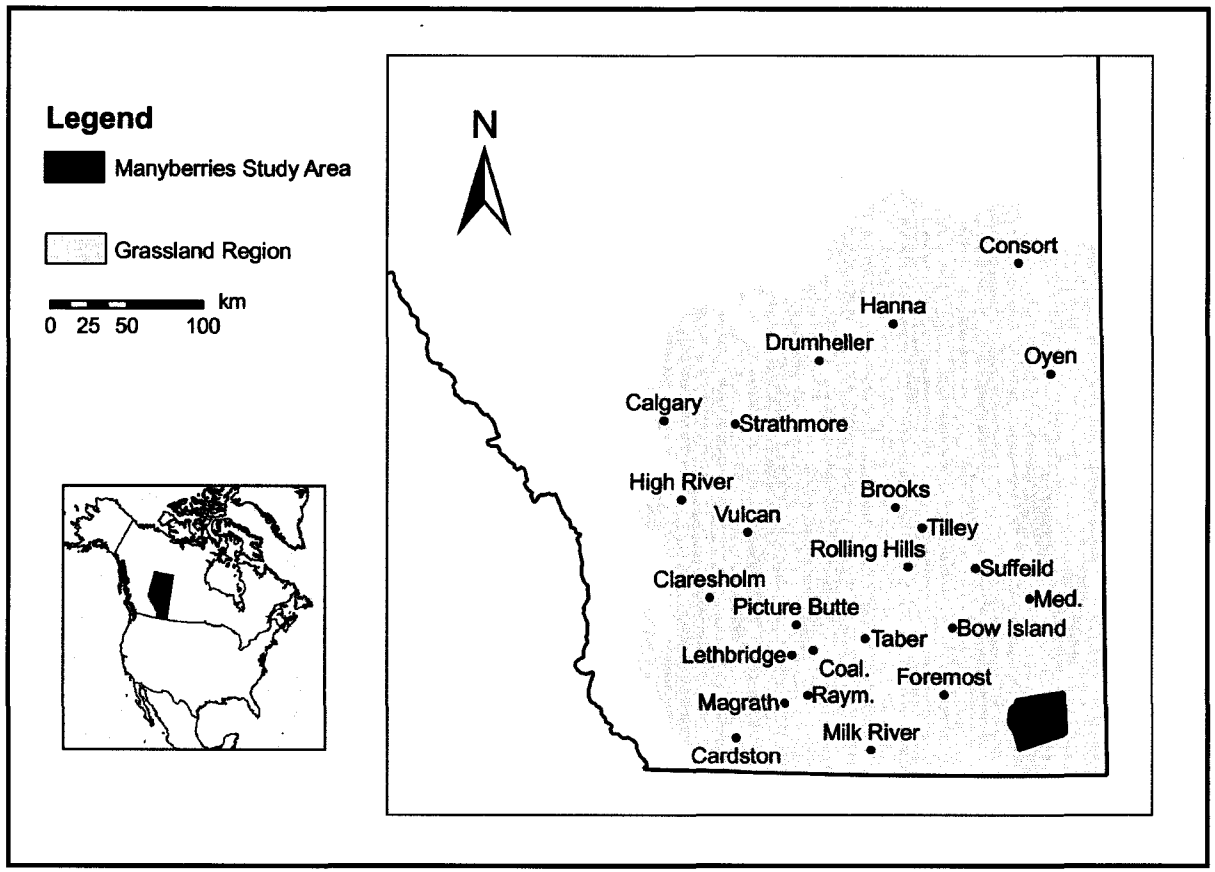

Figure 2-1. Map of the study areas in southeastern Alberta, Canada. Each marked location ran a mosquito trapping station that operated between one and six CDC mosquito traps. Table 2-1 lists which of these stations operated in each year. The Manyberries study area where the larvicide experiment took place, is highlighted in dark grey. Abbreviated location names are used for Medicine Hat (Med.), Raymond (Ram.) and Coaldale (Coal.).

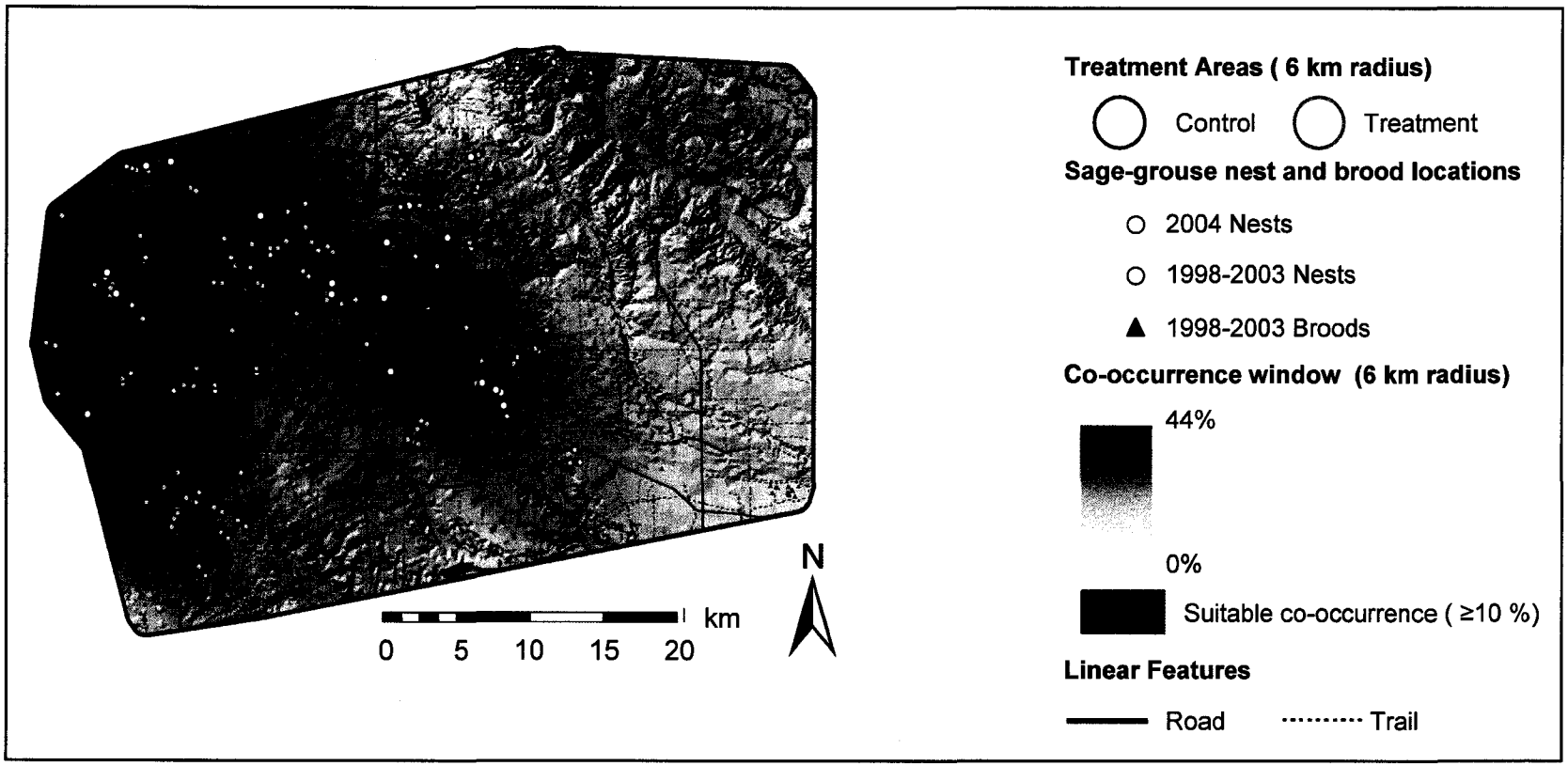

Figure 2-2. The treatment and control areas within the Manyberries study area. The current (2004) nest locations are indicated by white dots. Yellow dots and blue triangles indicate known sage-grouse nest and brood locations prior to this study (Aldridge 2007). The moving window for the percent co-occurrence of larval and sage-grouse habitat is indicated in shades of yellow. Darker shades are applied to pixels with a greater percent of co-occurrence in the surrounding 113 km^2. Red shading indicates pixels with at least 10 % co-occurrence.

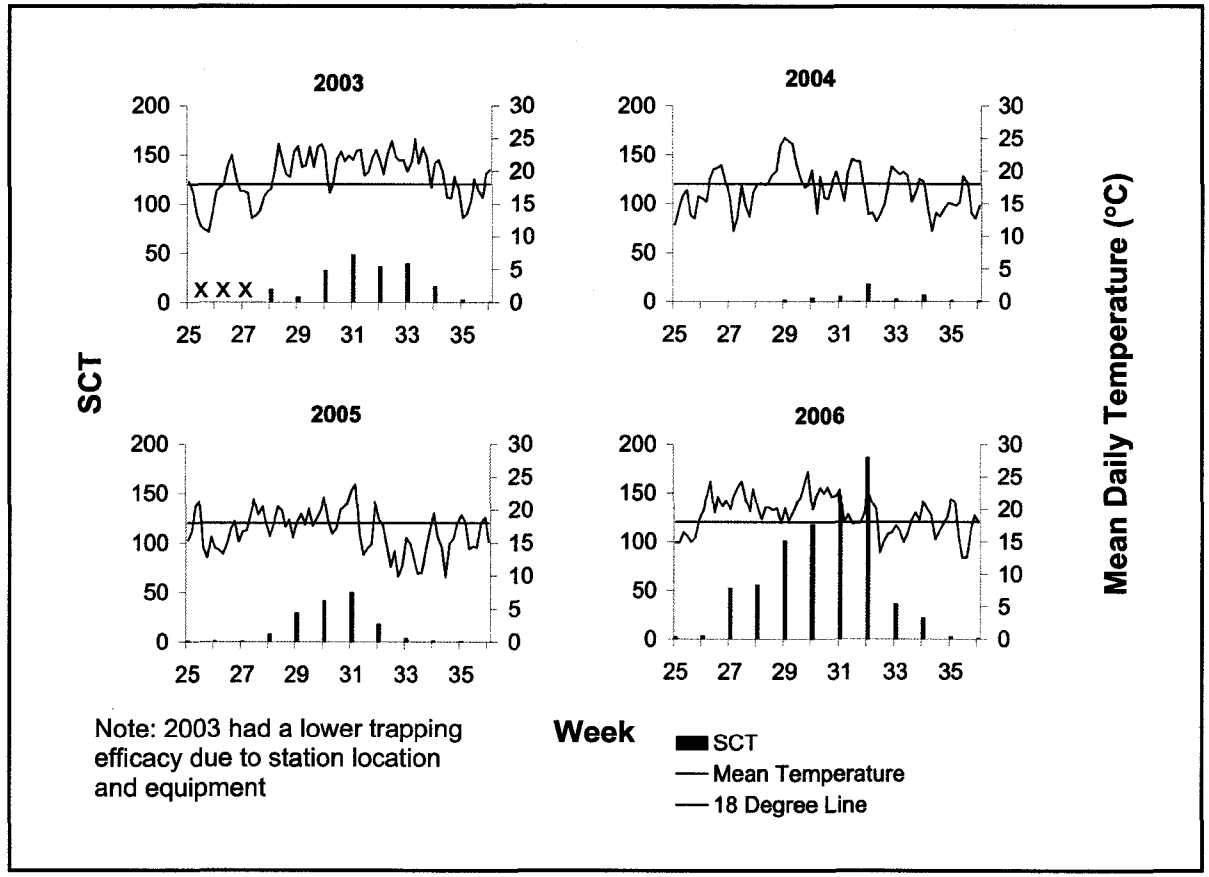

Figure 2-3. Daily mean temperature in relation to capture of adult *C. tarsalis* at grasslands trapping stations (SCT). Note that capture efficacy was lower in 2003. Adult *C. tarsalis* are captured in greater numbers during warm weather. Temperature data is from Brooks (Environment Canada 2007, Climate ID: 3030QLP).

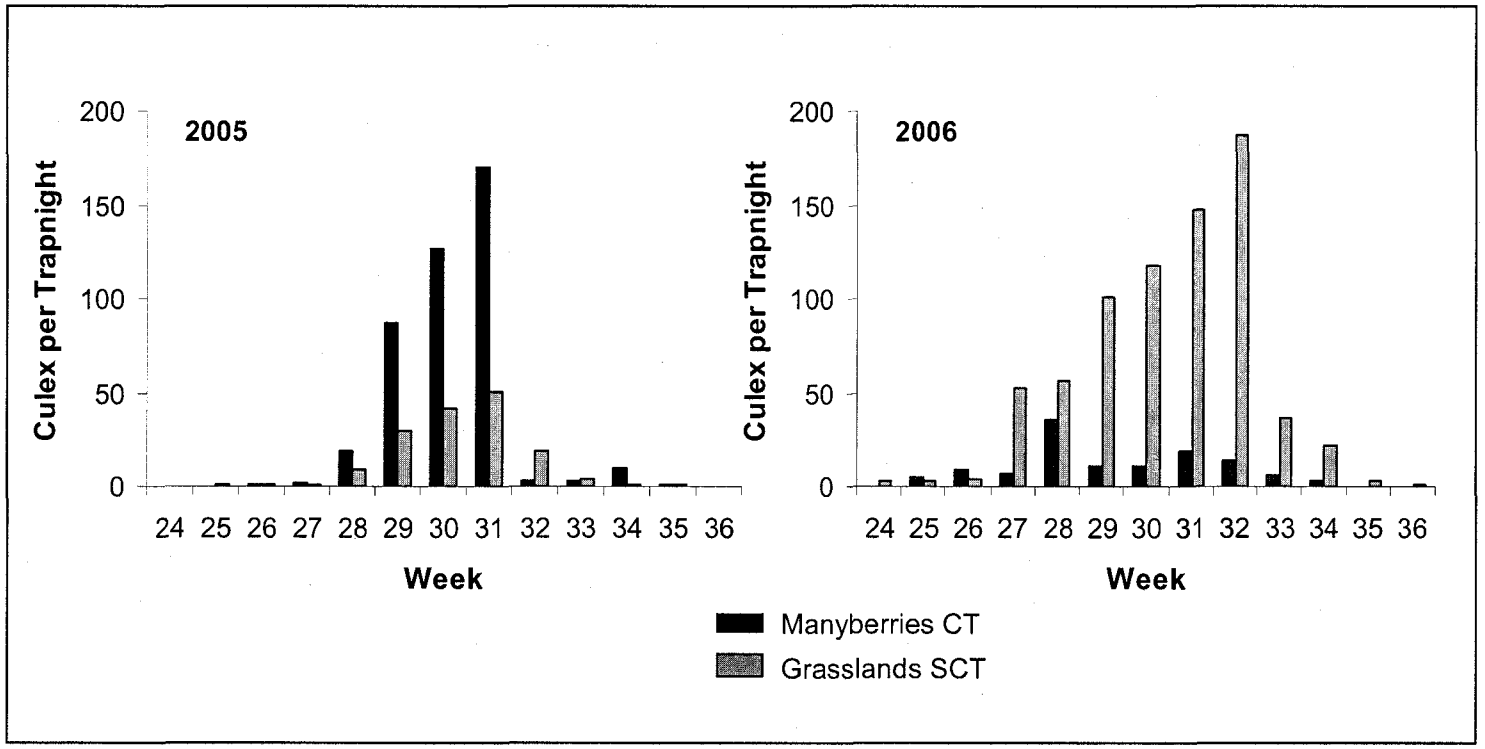

Figure 2-4. Comparison of grasslands SCT and the CT from the control area in Manyberries. In 2005, the Manyberries station captured higher CT than the grasslands SCT; however the same pattern is apparent in both measures. *C. tarsalis* captures rose through weeks 28 to 31 (week of July 11 to week of August 1) and then dropped off in week 32 (week of August 8). In 2006, the Manyberries station did not follow the same trend as the grasslands. While the grasslands SCT rises through to week 32 (week of August 8) and then dropped off in week 33 (week of August 15), the Manyberries SCT peaks in week 28 (week of July 11) and then remains low through the rest of the summer.

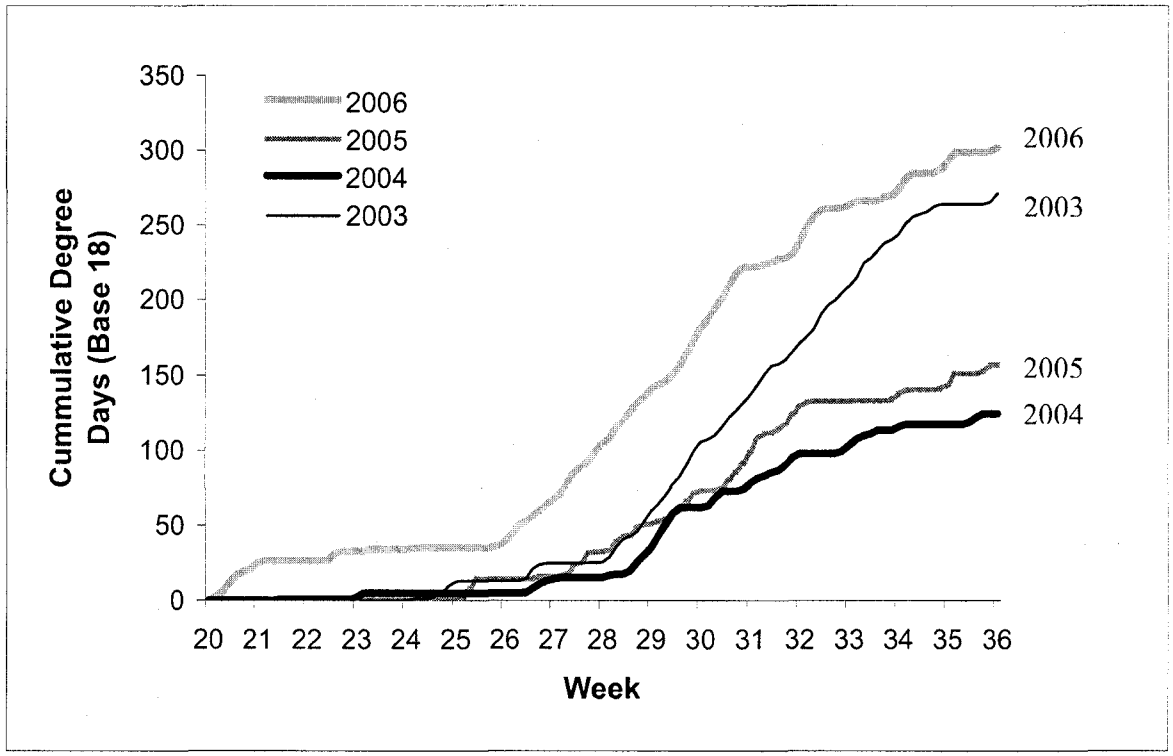

Figure 2-5. Cumulative degree days at Onefour (Environment Canada 2007, Climate ID: 3044923) from week 20 (week of May 23) through week 36 (week of September 5). Degree days were calculated as the additive number of degrees above 18 °C. Week 20 was the first date a temperature exceeded 18 °C. 2003 and 2006 had higher degree day accumulation than 2005 or 2004.

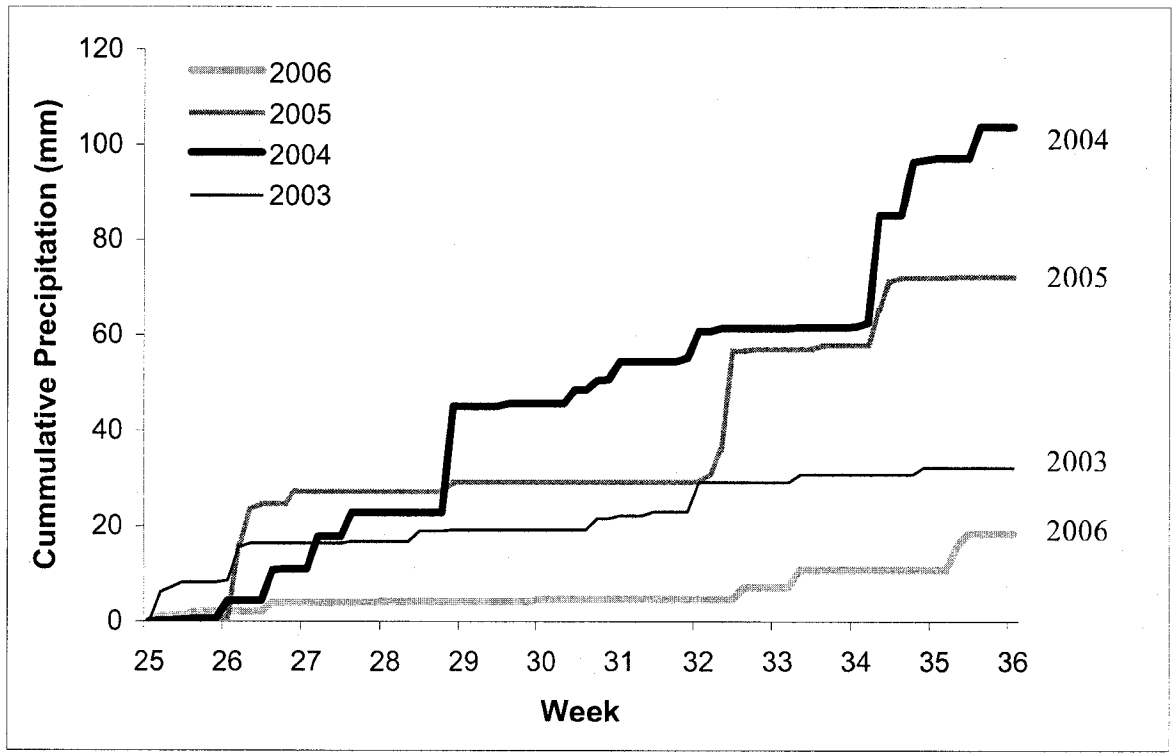

Figure 2-6. Cumulative precipitation (mm) at Onefour (Environment Canada 2007, Climate ID: 3044923) during mosquito trapping activity from week 25 (week of May 20) through week 36 (week of September 5). 2006 had lower precipitation than in 2003, 2004 or 2005. Whilst 2003 also had lower precipitation than 2004 and 2005, in 2006 there was small additive precipitation between weeks 27 to 32 which are critical for development of *C. tarsalis* populations.

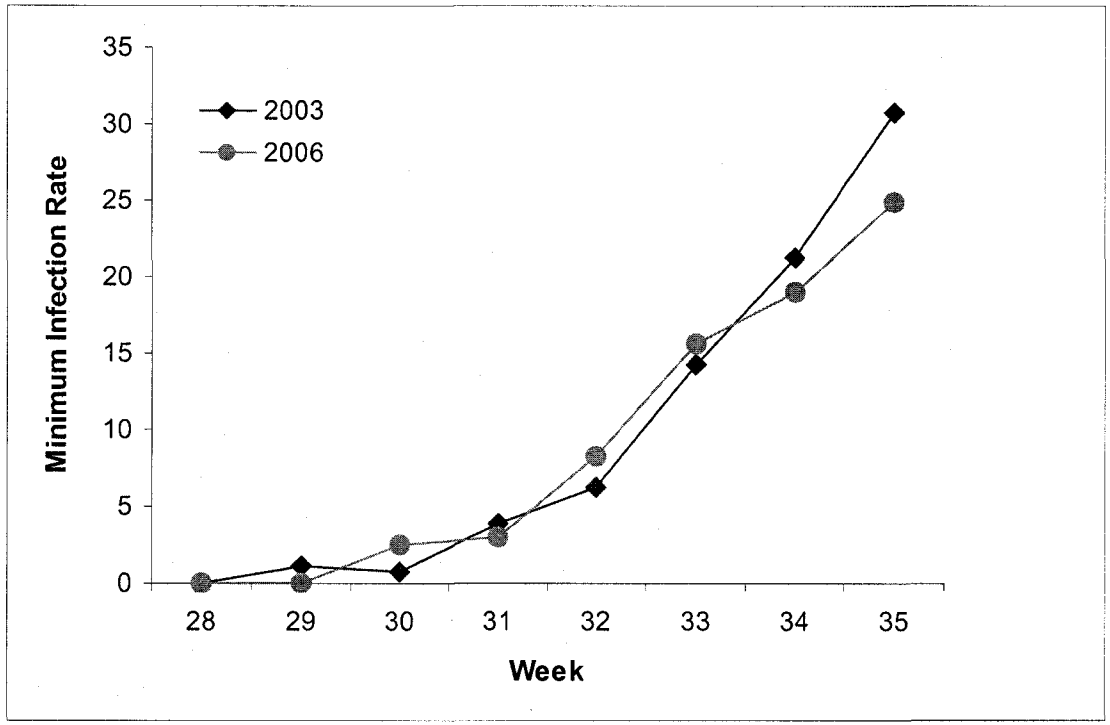

Figure 2-7. Minimum weekly infection rate was calculated for each week as the number of mosquito pools to test positive for WNv out of the total number of mosquitoes tested across the grasslands region. The trend in minimum weekly infection rate of WNv was similar in 2003 and 2006, rising through the summer. There was no detectable infection of mosquitoes at grasslands trapping stations in 2004 and 2005. There was no detectable infection of mosquitoes from the Manyberries trapping station in 2004 to 2006. Despite comparable WNv infection rates, no sage-grouse died from WNv in 2006 unlike the high mortality reported in 2003.

Chapter 3

Evidence of fluctuating sexual selection from leks of Alberta and Wyoming Greater Sage-Grouse.

1. Chapter Abstract

Adaptive mate choice depends on processes that maintain heritable variation in traits that signal the genetic quality of prospective mates. The 'lek paradox' however, is that in a lek-breeding species, strong directional selection could be predicted to reduce heritable variation. Through fluctuating parasite-host interactions that prevent directional selection, the Hamilton-Zuk (1982) hypothesis provides one resolution for this 'lek paradox'. Low density populations however, could lose an interaction between parasites and sexual selection. This study uses a correlative approach to investigate parasite-mediated sexual selection (PMSS) in both a small and a large sage-grouse population. Measurements of sexual traits and behaviours were made of males from Alberta and Wyoming leks. There is evidence that lek attendance, ornament colour and air sac hematomas were parasite-mediated aspects of sexual display. Furthermore, Alberta's endangered sage-grouse are not isolated from parasites or PMSS. Supporting the hypothesis that PMSS fluctuates, there was variation in: i) parasite prevalence and the quality of parasite-mediated sexual characteristics and ii) the relative importance of sexually selected characters in models for male mate success. Therefore, strong fluctuating sexual selection could potentially provide a mechanism for resolving the lek paradox and the maintenance of genetic diversity in small declining populations of lek species.

2. INTRODUCTION

Host-parasite coevolution is an important driver of many life history adaptations (Haldane 1949, Hamilton and Zuk 1982, May and Andersson 1983, Lindstrøm et al. 2004, Møller et al. 2004). Host-parasite coevolution may have an important role in mate choice and sexual reproduction. The Hamilton-Zuk hypothesis (1982) proposed that cyclic coevolution of parasites and heritable host resistance will allow maintenance of genetic variation in sexually selected characteristics. Studies with invertebrates, fish, birds, lizards and mammals have linked parasites to sexual traits and performance (see review Møller 1990, Pagel and Bodmer 2003, see review Cotton et al. 2004, Cox and John-Alder 2007). Furthermore, the interaction between parasites and hosts may be fundamental to the evolution and maintenance of sex itself (Hamilton 1980, Lively 1987, Ebert and Hamilton 1996). Sex can enable the spread or creation of advantageous traits, such as parasite resistance, and choice for mates with resistant genes could outweigh the high cost of sexual reproduction (Hurst and Peck 1996).

Kirkpatrick and Ryan (1991) applied two broad classes of hypotheses for the evolution of female choice for indirect benefits: (1) Fisher's (1930) model for runaway selection and (2) "good genes" models. Under good genes models, females choose males based on genetic benefits for their offspring. To obtain these genetic benefits, females must be able to discriminate the genetic quality of different males (Zahavi 1975). Female choice then promotes evolution of exaggerated traits that signal genetic quality. Condition-dependent secondary sexual signals are those that honestly signal male condition and male condition is influenced by a combination of genetic and environmental variation (see review Westneat and Birkhead 1998). Condition-dependent signals could express genetic qualities, such as heterozygosity or reflect a male's genetic resistance to environmental stressors, such as nutritional limitations or parasite infection (see review Cotton et al. 2004).

In a highly skewed mating system, extreme directional sexual selection could be expected to drive beneficial alleles to fixation. In this 'lek paradox,' there is seemingly little potential for the maintenance of strong female choice (Taylor and Williams 1982).

Potential resolutions to the lek paradox have been proposed. These include "good genes" approaches such as the Hamilton and Zuk (1982) host parasite coevolutionary cycle model and the Rowe and Houle (1996) genic-capture model. Under the genic-capture model, directional selection is maintained if condition-dependent sexual traits 'capture' genetic variation across many loci relating to condition (see review Tomkins et al. 2004). Under the host-parasite model, fluctuating selection results from condition-dependent traits reflecting genetic parasitic resistance (Hamilton and Zuk 1982). The optimal male is then variable through time because of a 'Red Queen' interaction where parasites and hosts evolve together (see review Tomkins et al. 2004). Because parasite-mediated sexual traits are potentially condition-dependent (condition depends on both environmental factors and genetic state of the host), parasite-mediated sexual selection (PMSS) could have an important role in both the parasite coevolutionary cycle model and the genic-capture model.

The Greater Sage-Grouse (*Centrocercus urophasianus*, hereafter sage-grouse) is a classic example of a highly polygynous lek-breeding bird (Höglund and Alatalo 1995). In the spring, male sage-grouse display together on traditional lek sites to attract mates; however, relatively few males obtain the majority of copulations (Wiley 1973, Gibson and Bradbury 1985, Hartzler and Jenni 1988). Sage-grouse are an interesting model species for studies of sexual selection because: (1) direct benefits to females of mate choice are limited due to lack of male paternal care and (2) aggregation on lek sites facilitates both bird capture and the field observation of sexual display and female choice.

Consistent with PMSS, research conducted with lek-breeding black grouse (*Tetrao tetrix*) and both captive and wild sage-grouse indicates females preferentially choose males with low parasite loads (Boyce 1990, Johnson and Boyce 1991, Spurrier et al. 1991, Höglund et al. 1992). Gibson (1990) however, found that a comparatively isolated California population of sage-grouse had low blood parasite diversity and prevalence, and was unable to demonstrate that parasite load was an indicator of mating success. This dichotomy suggests that through local extirpation of parasites, small populations could potentially lose an interaction between parasites and sexual signals

that maintains genetic diversity (Boyce 1990). Understanding the influence of population isolation on the relationship between parasites and lek behaviour will benefit management efforts for small, isolated populations of sage-grouse.

In Canada, sage-grouse have experienced drastic population declines and 75 % of known Alberta lek sites are now inactive (Alberta Fish and Wildlife 2007). Canadian provincial and federal governments recognize sage-grouse as an endangered species (COSEWIC 2004, Aldridge and Brigham 2003). Population declines across sage-grouse range are largely attributed to factors of habitat loss, degradation and fragmentation (Aldridge and Brigham 2003, Schroeder et al. 2004). However, in small isolated populations, disease has the potential to exacerbate these issues (Naugle et al. 2004, Peterson 2004). Using data collected in 1987 to 1990 from a large Wyoming population and from Alberta's small population in 2005 and 2006, this paper uses a correlative and information-theoretic approach to examines the role of condition-dependent sexual traits and parasite-mediated sexual selection on sage-grouse leks.

3. Study Areas

This study took place in both Alberta, Canada and Wyoming, USA (Figure 3-1). In 2005 and 2006, field studies in southeastern Alberta were made at four leks located 4 to 60 km south and east of Manyberries. In 1987 to 1990, field studies in southeastern Wyoming were made on 11 leks located 25 to 100 km north of Laramie, as described by Johnson and Boyce (1991). In Wyoming, the surrounding rangeland is characterized by big sage-brush (*Artemisia tridentata*), whilst in Alberta the surrounding rangeland is characterized by silver sage-brush (*A. cana*).

4. Field Methods

4.1 Bird Captures

Captures took place between March 15 and May 15. In Wyoming, sage-grouse were captured by night-lighting and rocket netting as described by Giesen et al. (1982). In Alberta, birds were captured by night-lighting and a drop-net (Connolly et al. 2003, Bush *in progress*). Wyoming males were fitted with unique colour combinations of

patagial tags; Alberta males were fitted with unique legband colour combinations. Body mass, bill depth, tarsometatarsus length, middle toe length, keel length, tail length and wing chord were measured. Ornament colour was scored by comparison of eye combs with standardized colour chips that varied in hue and saturation. Scores of 1 were a saturated yellow and scores of 8 were a dull grey-green. Because hematomas and lice are visible on male air sacs and have been linked to mate choice in previous studies (Boyce 1990, Spurrier et al. 1991), the number of lice and hematomas on air sacs were counted. Fecal samples were collected passively as birds defecated during handling. Cecal droppings were not collected. Bird cloacae and fecal samples were examined visually for evidence of tapeworms (Class: Cestoda). Fecal samples were suspended in saline solution and parasites were lifted onto microscope slides with coverslips and examined under 100 × magnification to identify coccidia oocysts (*Eimera* spp.).

Protozoan and helminth parasites of sage-grouse have been reported in Colorado (Stabler et al. 1977), Wyoming (Boyce, 1990), California (Gibson, 1990) and Washington (Clark et al., 1968). Stabler et al. (1977) identified *Plasmodium pedioecetii*, *Hemoproteus canachites*, *Leucocytozoon bonasae* (= *L. lovati*), *Trypanosoma avium*, and microfilariae. To detect blood parasites, a blood sample was taken from either a brachial vein or a clipped hallux nail. Blood smears were fixed and stained with Giemsa or Giemsa-like stain (Protocol Hema 3 stain set; Fisher Diagnostics Middletown VA, USA). Slides were examined under 1000× oil immersion to identify blood parasites (*Plasmodium* spp., *Hemoproteus* spp. and *Leucocytozoon* spp.).

4.2 Lek Observation

Between March 25 and May 15 in each year, lek observations began when birds first became identifiable, typically about half an hour before sunrise, and concluded when the lek was finished for the morning. Lek observations consisted of: (1) continuous monitoring for copulations; (2) periodic scan observations of the entire lek and (3) five-minute focal observations of individual males.

Leks were scanned at first visibility and when observers judged that a change in lek activity had occurred. Such occurrences included: copulations or hen solicitation behaviour; predators disrupting lek activity; males sitting down or birds leaving the lek. During a lek scan, observers noted the number of males and females in attendance. Because the Alberta leks were small, it also was possible to count the number of males that were active during each scan. A male was defined as active if he was standing or engaged in contact fighting but not if he was foraging, preening, sitting or in a non-contact face-off with another male. On Wyoming leks, activity scans were typically not possible due to the greater size of the leks.

Focal observations of individual males lasted for a five minute period. Any male that could be uniquely identified through a leg band, patagial tag or unique plumage markings was preferentially observed. On Alberta leks, non-uniquely identifiable males also were chosen at random for observation. During each observation, the number of full strut-sequences (struts), as described by Wiley (1978), were counted. Struts involve the tossing of the body, lifting and dropping of air sacs and are accompanied by popping vocalizations. At the start of each focal observation, the proximity of the nearest hen to the focal male was estimated.

5. Methods – Analysis

All analysis excluded juvenile birds (first breeding season). While hens may copulate with juvenile sage-grouse, physical characteristics and lek behaviours are different from adults, preventing a functional comparison. Some aspects of sage-grouse lek behaviour, such as the acoustic component of display and male-male interactions were outside the scope of this study. All analysis was done using Stata 9 software (StataCorp 2005).

5.1 Male Success

Male birds were scored as successful if they were either observed to copulate or be solicited by a hen. During solicitation the hen stands close to a displaying male, crouching low to the ground and lifting her tail in the air. Although some solicitated

males were not observed to copulate, because they had clearly been 'selected' by hens, they were considered successful.

5.2 Body Condition Index

Biometric measurements were analyzed separately for Wyoming and Alberta. While there are problems with all methods of generating a body condition index (Cotton et al. 2004), a principal components approach can include correlated characteristics and reflects a broad range of body size variables (Kirk and Gosler 1994). Biometric measurements, excluding weight, were condensed using a principal component analysis of the correlation matrix (PCA) (Rabe-Sesketh and Everitt 2003). Coefficients were scored for principal components with an eigenvalue greater than one. Sage-grouse strut displays are energetically demanding and males are known to lose weight during the lek season (Beck and Braun 1978). Residuals were scored from a linear regression of mass on capture date and the coefficients from the PCA. If required, dummy variables for year accounted for variation in weight between years. To be conservative, an alpha of 0.10 was applied in variable inclusion for this analysis. The scored residuals of this regression are an index of body condition representing the proportion of body mass that is independent of overall bird size.

5.3 Mean Strut Rate

Because previous work has demonstrated that males strut vigorously when a hen is nearby (Gibson and Bradbury 1985), distance to the nearest hen was included when calculating strut rates. Males also strut less vigorously and less often in high winds, with predator disturbance and through the morning; although the exact relationship to time since sunrise varied through the lek season and with moon phase. To correct strut rates for these factors is not a straightforward task. Predator disturbances can occur before dawn and be difficult to record; the influence of factors like wind or moon phase can interact with hen visitation and date. For simplicity, the proxy of lek activity was used to describe the overall strutting intensity of the lek at the beginning of each focal observation. Lek activity was calculated differently in Alberta and Wyoming due to differences in lek attributes and the data collected during scans. In Alberta, lek activity was calculated as the percentage of males active out of the highest count of males at that

lek. For Wyoming, lek activity was calculated as the percentage of males present out of the highest count of males at that lek.

To generate a corrected strut rate for each focal observation, we modified the regression procedure described by Gibson and Bradbury (1985) to include lek activity. With lek activity in the model, there was an inverse linear relationship between distances to hen and strut rate until 35 m on Wyoming leks and 45 m on Alberta leks where the relationship ceased to be linear and plateaued. At these distances, male sage-grouse are far enough from the hen that there is no additive influence of distance on strut rate. Thus, for strut counts when no hen was present or hens were at distances greater than 35 and 45 m respectively, this maximum distance was applied to maintain the linear relationship between variables. A zero-inflated negative binomial linear regression was used to model the relationship of strut counts from focal observations to distance to hen and lek activity. A corrected strut rate was then estimated as the deviation from the predicted strut rate by scoring the partial residuals. Because the regression is intended as a correction, individual strut counts were considered independent and focal observations of both marked and unmarked males were included.

Using the scored partial residuals, all captured males were assigned a "mean strut rate" calculated as the mean of their corrected strut rates. Because male sage-grouse might vary in quality between years, calculations of mean strut rate were restricted to include only the focal observations of a male made during the year of his capture.

5.4 Lek Attendance

Following the method of Johnson and Boyce (1991), lek attendance was calculated for each male as the percentage of days a male was sighted on the lek out of the potential maximum number of days that male could have been individually identified.

5.5 Condition-dependence

Overall prevalence of each parasite group was calculated as the percentage of captured males detected to be infected with each parasite. Spearman's rho for ordinal data was used to test for significant correlations between parasites and sexual display traits. From previous research, we expected the presence of ectoparasites to increase hematomas, and blood parasites to reduce lek attendance (Johnson and Boyce 1991). We also predicted that: (1) blood parasites could reduce ornament colour through increased demand for carotenoids by immune function (Lozano 1994) and (2) endoparasites could reduce ornament colour by disrupting absorption of carotenoids through damage to the gut wall (Zhao et al. 2006). A linear regression was used to test for relationships between the body condition index, mean strut rate and the presence of parasites.

5.6 Female Mate Choice

A set of candidate logistic regression models for male success was generated. To test for PMSS, we included display variables correlated with parasite prevalence including lek attendance (*attendance*), ornament colour (*colour*) and air sac hematomas (*hematomas*). We included mean strut rate (*strut rate*), based on previous studies of male success (Gibson and Bradbury 1985). The variables body condition index (*body condition*) and a body condition strut rate interaction (*strut*body condition*) were included because of evidence that strut rate is energetically costly (Vehrencamp et al. 1978). Through inclusion of multiple traits and by using the same candidate models in different spatial locations, we tested if sexual characteristics demonstrate a heightened condition dependency reflective of the currently prevalent parasites. Candidate model performance was compared using an information-theoretic approach. Akaike information criteria (AIC) and small sample Akaike information criteria (AIC_c) was used to accept the most parsimonious models (Burnham and Anderson 2002). Relative to the 'best' model, models with a change in AIC score of <2 were accepted. Model variables were weighted according to the number of counts made for each male.

To visualize the interaction between mean strut rate and the body condition index males were defined as in good or poor condition if they were greater than one

standard deviation above or below a centred mean body condition. Using these classifications, the probability of mate success against strut rate could be graphed (UCLA ATS 2007).

6. RESULTS

6.1 Male Characteristics

Thirty-nine males were captured in Alberta and 211 males in Wyoming. Lek size varied considerably with a mean of 14 and 51males on Alberta and Wyoming leks respectively. Alberta leks ranged between an 9 and 23 for average male attendance. Wyoming leks ranged between 8 and 70 average male attendance. Of the 39 captured Alberta males, 13 were classified as successful. Of the 211 captured Wyoming males, 35 were classified as successful.

From a principal components analysis of biometric measurements, loadings of univariate measures were substantial for principal component one and two (PC1 and PC2, Table 3-1). To generate a body condition index, size components (PC1 and PC2) and date of capture were all significant in calculating the body condition index ($p<0.05$). In Wyoming, year effects also were significant ($p<0.10$).

A total of 1153 and 738 focal strut observations were made of males on Wyoming and Alberta leks, respectively. Of the Alberta strut counts, 271 counts were of unmarked males. To correct these strut counts, distance to hen and lek activity were significant ($p<0.01$) in both Wyoming and Alberta (Table 3-3). The mean of the number of focal observations made of marked males was 12.2 ± 3.48 in Alberta. In Wyoming, the respective mean and standard deviation were 5.13 ± 5.31.

6.2 Parasite Prevalence

Common parasites were classified into three groups: (1) ectoparasites including chewing lice (*Lagopoecus gibsoni* and *Goniodes centrocerci*); (2) endoparasites including coccidia (*Eimera* spp.) and tapeworms (class: Cestoda) and (3) blood parasites including avian malaria (*Plasmodium* spp.) and other hematozoa (*Leucocytozoon* sp. and *Hemoproteus* sp.). Because of low prevalence of

Leucocytozoon and *Hemoproteus*, only malaria was considered in the analysis of blood parasites.

Prevalence varied by study area (Table 3-4). The kind of endoparasite also varied between years in Alberta. In 2005, coccidia was prevalent (52.9 %) but tapeworms were not (11.8 %). In 2006 however, coccidia was not found (0 %) but tapeworms were prevalent (50 %).

In both study areas, the presence of lice was significantly correlated with the presence of hematomas ($p<0.05$, Table 3-5). Malaria and lek attendance were significantly negatively correlated in Wyoming ($p<0.05$, Table 3-5). Furthermore, lek attendance was more variable in Wyoming and only parasitized males had lek attendance of less than 15 % (Figure 3–2). Avian malaria was not correlated with eye comb colour ($p<0.05$, Table 3-5). Endoparasites were significantly correlated with eye comb colour in Alberta ($p<0.05$, Table 3-5). Eye comb colour scores of 1 were the most yellow in hue and had the strongest saturation. Eye comb colour scores of 8 contained the least yellow hue but more green-grey hue and a weaker saturation. Eye comb colour scores ranged from 1 to 8 in Alberta, while in Wyoming they only ranged from 1 to 4. The colour range of non-parasitized males was similar in both Alberta and Wyoming (Figure 3-3).

Parasites were not consistent predictors of the body condition index (Table 3-6). Malaria was a significant predictor variable ($p<0.05$) of the body condition index in Wyoming. Only in Alberta were ectoparasites a significant predictor variable ($p<0.005$) for the body condition index.

6.3 Female Mate Choice

All variables included in candidate models (*strut rate, body condition, strut rate*body condition, colour, hematomas and attendance*) were included in a top model for both Wyoming and Alberta. Two Alberta models had a change in small sample AIC score of less than 2. These models included the variables *strut rate, body condition, strut rate*body condition, colour* and *hematomas* (Table 3-7). Two Wyoming models

had a change in AIC score of less than 2. These models included the variables *strut rate, body condition, strut rate*body condition, hematomas* and *attendance* (Table 3-8). Colour was not in the top model for Wyoming where there was low prevalence of intestinal parasites. Lek attendance was not in the top models for Alberta, where there was low prevalence of malaria (Table 3-9). The interaction term *strut*body condition* from these models indicates that the probability of mating has a steeper slope for males in good condition than for those in average or poor condition with increasing strut rate (Figure 3-4).

7. DISCUSSION

7.1 Condition-dependent?

Cotton et al. (2004) reinforce the importance of testing sexual traits for heightened condition-dependency. Through inclusion of multiple traits, there was evidence that sexual traits have heightened sensitivity to particular states of condition, but otherwise convey little information. For example, while ornament colour was correlated with endoparasite infection, it was a poor indicator of ectoparasite and malaria infection (Table 3-5). Similarly, hematomas and lek attendance were significantly correlated with only one characteristic each: lice infestation and malaria infection, respectively.

Sage-grouse strut displays are energetically demanding (Beck and Braun 1978, Vehrencamp 1989). Mean strut rate however, was not a significant predictor of body condition (Table 3-6). Nevertheless, the inclusion of the interaction term between body condition and mean strut rate in the top models for both Wyoming and Alberta indicates that strut displays are likely condition-dependent. With increasing male strut rate, the probability of mating increases at a steeper slope when males are in good condition (Figure 3-4). The interaction term may represent differences in duration or consistency between good and poor quality displays.

Carotenoid pigments are derived solely from a bird's diet and are responsible for the yellow, red and orange colours used for avian sexual traits (Gray 1996).

Carotenoids have other biological functions, including roles in immune function (Lozano 1994). Lozano (1994) proposed that carotenoid-dependent colour could signal not only access to carotenoids but overall individual immune system status. Fitze et al. (2007) determined that the carotenoids used in plumage colouration of great tits (*Parus major*) were different from those used in cellular immune response. Thus carotenoids could vary in their function and be signals for any combination of (1) immune resistance in the past, present or future; (2) ability to access carotenoids and (3) ability to absorb carotenoids.

In sage-grouse, there is no evidence that colour score related to immediate immune demands. There was no correlation between colour and avian malaria, which would be expected to generate a heightened immune response. The similarity in eye comb colour between the unparasitized Wyoming birds and the unparasitized Alberta birds (Figure 3–3) also provides evidence that access to carotenoids was similar for birds in both populations. Endoparasite prevalence however, was significantly correlated with air sac colour (Table 3-5). Coccidia are well known to reduce absorption of carotenoids in domestic poultry through damaging mucosa or hemorrhage of the gut wall (Ruff et al. 1975, Zhao et al. 2006). Treatment for coccidiosis improved colour scores of the skin in Three-Yellow-Chickens (*Gallus gallus* var. *domesticus*), a strain of domestic chicken noted for the particular orange-yellow colour of its skin (Zhao et al. 2006). Coccidia infection is known to reduce the size of red ornaments on wild turkeys (*Meleagris gallopavo*; Bucholz 1995) and influence the feather plumage of birds (Hõrak et al. 2004, Costa and Macedo 2005). Other groups of endoparasites also have been documented to reduce ornament quality. For example, intestinal nematodes reduced the colour saturation of combs in red jungle fowl (*Gallus gallus*; Zuk et al. 1990) and experimentally-lowered nematode infection increased both the plasma carotenoid concentration and comb redness of male red grouse (*Lagopus lagopus*; Martínez-Padilla et al. 2007).

Colour was included in the AIC-selected models for male success in Alberta. Furthermore, all models without colour had weightings < 1 (Table 3-7). On Wyoming leks, where endoparasite infection was not prevalent, colour was not included in either

of the two top models. Therefore, the reduction of colour scores with endoparasite infection in sage-grouse, and the subsequent importance of colour in male choice indicates ornament colour may signal endoparasite-mediated ability to absorb carotenoids (Table 3-9).

Consistent with previous research, ectoparasites were correlated with the presence of hematomas on air sacs (Johnson and Boyce 1991, Spurrier et al. 1991, Table 3-5). Ectoparasites were a significant predictor of body condition in Alberta but not in Wyoming (Table 3-6). This may reflect different over-winter thermoregulatory costs of ectoparasites between the two study areas. Malaria infection in sage-grouse reduced lek attendance of birds in Wyoming. Infected erythrocytes erupt in the morning, causing illness during the display period (Stabler and Kitzmiller 1976). Malaria infection was also a significant predictor of body condition (Table 3-6). Thus, malaria may mediate lek attendance directly through morning illness, and indirectly through condition.

The relative importance to male success of the sexual signal varied with parasite prevalence. While ornament colour was not in the selected models for Wyoming, it was in the top-ranked model for Alberta, where endoparasites were at higher prevalence (Table 3-9). Similarly, lek attendance was not included in the selected models for Alberta, where there was low prevalence of avian malaria (Table 3-9). Finally, hematomas were included in top models for both locations and lice were present in both locations (Table 3-9). Møller and Pomiankowski (1993) concluded that the multiple ornaments in birds do not signal the quality of an individual but are likely Fishererian traits. Our results however, provide support for their alternative multiple-message hypothesis where each sexual signal reveals a somewhat separate aspect of male quality (Møller and Pomiankowski 1993). Furthermore, in Iwasa and Pomiankowki's (1994) model for the evolution of multiple traits, if the cost of choice for multiple signals is similar to the cost of choice for a single signal (as in lek attending females), condition-dependence can be maintained across multiple ornaments.

7.2 Evidence of the Red Queen?

The Red Queen in *Through the Looking-Glass* (Lewis Carroll 1872), tells Alice in the Garden of Live Flowers: "Now, *here*, you see, it takes all the running *you* can do to keep in the same place." Van Valen (1973) adopted the Red Queen as a term to describe coevolution where species must evolve (run) to remain extant (in the same place). The Red Queen hypothesis describes processes of coevolution where evolutionary change by one species promotes evolution in another (Van Valen 1973). Thus, coevolution of a parasite and host can be dynamic and impose selection, without causing directional change, in the parasite or the host (Hamilton and Zuk 1982). Variation in the functionality of sexual traits in Alberta and Wyoming provides evidence for such fluctuating sexual selection on sage-grouse leks. Three requirements of parasite-mediated fluctuating selection are met: (1) some male secondary traits are parasite-mediated (Table 3-5); (2) changes in parasite prevalence can be reflected in mate choice (Table 3-9) and (3) parasite prevalence is variable on sage-grouse leks.

On Alberta leks, the predominant endoparasite infection changed between 2005 and 2006 from coccidia to tapeworm. Evidence from Colorado, Oregon, California, Wyoming and Alberta indicates blood parasite prevalence can vary between locations and year. Between 1991 and 2001 in Nevada and Colorado, Dunbar et al. (2003) reported 11 % prevalence of blood parasites in hen sage-grouse (*Leucocytozoon*: 10 %, *Plasmodium*: 0.5 %, microfilariae 1 %). At the Nevada study area, breeding season prevalence varied from 0 to 39 % between years. Between 1974 and 1976 in Colorado, Stabler et al. (1977) reported 50 % prevalence of blood parasites (*Leucocytozoon*: 45 %, *Trypanosoma:* 8 %, *Plasmodium:* 1 %, microfilariae: 3%). In California, Gibson (1990) found only one kind of blood parasite (*Hemoproteus:* 37.5 %). In this study, prevalence of blood parasites was extremely low in Alberta with none found (0 %) while Wyoming leks had an overall blood parasite prevalence of 30 % (*Plasmodium:* 27 %, *Leucocytozoon* and *Hemoproteus:* 11 %) (Table 3-4).

However, these year to year and lek-variable changes in parasite prevalence however, may not imply strict coadaptational cycles as envisaged by Hamilton and Zuk (1982) and the Red Queen hypothesis. Non-genetic environmental factors that

influence parasite-host interactions, such as vector abundance, flock sizes, soil moisture and temperature regimes also could act to cause fluctuations in PMSS.

7.3 Implications

Westneat (2006) suggested that an understanding of ecological factors that vary in space and time is necessary because long-term evolutionary lags in the functionality and expression of sexual signals may obscure the interpretation of mate choice studies. PMSS in sage-grouse provides an example that on a shorter time-scale, ecological and environmental factors also must be considered. Parasite prevalence could drastically influence the interpreted result of a sexual selection study or mask effects if researchers are unaware of an environmental factor that induces fluctuation. For example, if this study had occurred only in Alberta, there would have been no evidence of the link between attendance and mate choice that has been observed in Wyoming and California (Gibson and Bradbury 1985, Johnson and Boyce 1991, this study). Conversely, if the study had looked only at Wyoming, we would have concluded no evidence for the functionality in male ornament colour found in Alberta. While captive populations allow manipulative experiments, Cotton et al. (2004) asserted there is also a role for long-term concurrent studies of sexual selection theory with wild, realistic populations. Our findings reinforce this need: long-term studies of wild populations in multiple locations will benefit our understanding of the natural patterns and processes of sexual selection.

A correlation-based approach was used to determine if Alberta's endangered sage-grouse had become isolated from a potential mechanism of sexual selection. The diversity of parasites and evidence for condition-dependent and parasite-mediated sexual selection in Alberta reveals that such isolation has not occurred. Furthermore, the potential for fluctuating sexual selection that reflects rapidly changing prevalence of parasites may have implications for genetic diversity in Alberta. Despite population declines, substantial genetic variation remains in Alberta's sage-grouse (Bush *in progress* (b)). Selection processes within small populations may be important mechanisms in preventing genetic drift (Kaeuffer et al 2007). For example, natural selection in mouflon sheep (*Ovi aries*) may have maintained heterozygosity in an

insular population (Kaeuffer et al. 2007). Contrary to the 'lek paradox,' the strong selection arising from the lek breeding system of sage-grouse may actually work to maintain genetic diversity, even in declining populations.

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Table 3-1. Loadings of biometric variables into the first two Principal Components (PC1 and PC2) for each study area. Components one and two had eigenvalues greater than one.

	Wyoming		Alberta	
	PC1	PC2	PC1	PC2
Tail length	0.4531	0.3690	0.5452	-0.4225
Wing length	0.4697	0.3225	0.5722	-0.1610
Keel length	0.5191	0.1751	0.1271	0.4306
Tarsus length	-0.3667	0.5788	0.4559	0.5010
Toe length	-0.3436	0.6235	0.2896	0.4696
Bill depth	0.2284	0.0742	0.2597	-0.3723

Table 3-2. Beta coefficients (β), standard errors (SE), sample size (n) and R-squared values for the body condition index models used to correct body mass for body size and capture date. Separate models were generated for Wyoming and Alberta. Variables included: the scores for principal component one and two (size-PC1 and size-PC2) and the date of capture within each year (captureday). For the Wyoming analysis, dummy variables for year (year88, year89, year90) accounted for variation between years. No significant year effects were detected in Alberta, so year was not included in that model.

	Wyoming		Alberta	
	β	SE	β	SE
constant	2975	24.50**	3132	60.00**
size-PC1	19.78	11.51 **	46.16	20.82**
size-PC2	40.22	11.01**	62.13	23.34**
captureday	-4.07	1.107**	-5.52	2.450**
year88	129.84	31.52**	/	/
year89	-99.48	41.10**	/	/
year90	-75.51	42.19 *	/	/
R-squared	0.16		0.35	
n	326		39	

Note: (*) denotes coefficients significant at the $p<0.10$ level and (**) denotes significance at the $p<0.05$ level.

Table 3-3. Beta coefficients (β), standard errors (SE) and log likelihoods (LL) for the zero-inflated negative binomial regressions correcting individual male strut rate for distance to nearest hen (hendist) and lek activity (lekact). The inflation parameter was distance to hen. The overdispersion parameter coefficient is alpha. Strut counts of unmarked birds were included and counts were considered independent for correction purposes. Beta coefficients and standard errors are reported at 100 times their original values.

	Wyoming		Alberta	
	β	SE	β	SE
constant	319.9	3.627	304.8	8.623
hendist	-2.724	0.1545	-1.845	0.1316
lekact	28.58	6.825	41.60	8.754
inflation coefficient	5.528	0.6511	5.917	0.9354
inflation constant	-270.3	16.03	-405.7	37.50
alpha	28.69	1.698	-13.90	7.345
LL and sample size	-4087	n =1153	-2684	n =738

Note: All coefficients significant ($p<0.01$).

Table 3-4. The prevalence of parasites in male sage-grouse captured in Wyoming and Alberta. Prevalence is reported as the percentage infected out of total captured.

	Alberta	Wyoming
Malaria	0 %	27.3 %
Other Blood Parasites	0 %	11 %
Endoparasites	56.4 %	4.1 %
Ectoparasites	74.4 %	23.2 %
Sample Size (n)	39	211

Table 3-5. Spearman rho correlation coefficients between parasites and male attributes of captured birds from Alberta and Wyoming. Sample size is indicated by (n). Significant correlations are in bold ($p<0.05$ with a Bonferroni correction for multiple comparisons). Parasites included the presence or absence of ectoparasites (lice), endoparasites including coccidia and tapeworms (endo) and avian malaria (malaria). No malaria was found in Alberta males and almost no endoparasites were found in Wyoming males during the study period. Attributes of males included the ornament colour score (colour), the presence of hematomas on air sacs (hema) and lek attendance (attend). Not present is indicated by (n.p.).

Alberta					
n=39	endo	colour	lice	hema	attend
colour	**0.8723**				
lice	-0.0419	0.1365			
hema	0.0890	0.1691	**0.7676**		
attend	0.2127	0.1584	-0.0633	0.1207	
malaria	n.p	n.p	n.p	n.p	n.p

Wyoming					
n=203	endo	colour	lice	hema	attend
colour	n.p				
lice	n.p	-0.0249			
hema	n.p	-0.0328	**0.2327**		
attend	n.p	-0.0388	0.0328	-0.1083	
malaria	n.p	0.0968	-0.0852	-0.0539	**-0.3234**

Table 3-6. Estimated coefficients (β), p-values (p) and model fit parameters (F statistic, p-value (p) and degrees of freedom (d.f.)) for regression describing the relationship of parasites and mean strut rate to the body condition index. Potential parasites included malaria, ectoparasites and endoparasites. Endoparasites were excluded from the Wyoming model and malaria was excluded from the Alberta model because of low prevalence (n.p.).

	Alberta		Wyoming	
	β	p	β	p
malaria	n.p.	n.p.	**-62.80**	**0.03**
ectoparasites	**-160.66**	**0.005**	1.59	0.953
endoparasites	24.08	0.654	n.p.	n.p.
strut	4.40	0.50	9.65	0.443
constant	83.83	0.07	14.02	0.554
F statistic	3.20		1.84	
p	0.035		0.47	
d.f.	35		205	
Adjusted R^2	0.14.8		0.012	

Table 3-7. AIC selected models for breeding success of 39 males on Alberta leks. Model log likelihood (LL), number of model parameters (K_i), small sample AIC (AIC_c), change in AIC_c (Δ_i) from lowest model, Akaike weights (w_i), cumulative AIC weight (Σw_i) and model validation through ROC areas (ROC) are reported. Accepted models (Δ_i <2) are in bold. Variables in models include mean strut rate (strut), hematoma count (hema), lek attendance (attend), ornament colour score (colour) and the calculated body condition index (bci). Models are weighted by the number of strut counts made of each male.

Model	Model structure	LL	K_i	AIC_c	Δ_i	w_i	(Σw_i)	ROC
1	**strut + colour + hema**	**-5.08**	**4**	**19.33**	**0**	**0.47**	**0.47**	**0.99**
2	**strut + bci + strut*bci + colour**	**-4.65**	**5**	**21.12**	**1.79**	**0.19**	**0.66**	**0.99**
3	strut + colour	-7.34	3	21.37	2.04	0.17	0.83	0.97
4	strut + colour + attend	-6.50	4	22.18	2.85	0.11	0.94	0.97
5	colour	-9.78	2	23.89	4.57	0.05	0.99	0.95
6	strut + bci + strut*bci + hema	-15.01	5	41.84	22.51	<0.01	1.00	0.87
7	strut + bci + strut*bci	-17.25	4	43.68	24.35	<0.01	1.00	0.83
8	hema	-19.21	3	45.11	25.78	<0.01	1.00	0.62
9	strut + bci + strut*bci + attend	-16.81	5	45.44	26.11	<0.01	1.00	0.84
10	strut	-20.70	2	45.73	26.41	<0.01	1.00	0.76
11	strut + attend	-20.54	3	47.77	44.77	<0.01	1.00	0.77
12	strut + hema + attend	-19.35	4	47.88	28.55	<0.01	1.00	0.81
13	strut + hema	-23.41	2	51.15	31.83	<0.01	1.00	0.82
null	null	-25.12	1	52.35	33.02	<0.01	1.00	0.50
14	attend	-25.04	2	54.41	35.09	<0.01	1.00	0.53

Table 3-8. AIC selected models for breeding success of 110 males on Wyoming leks. Model log likelihood (LL), number of model parameters (K_i), AIC (AIC), change in AIC (Δ_i) from lowest model, Akaike weights (w_i), cumulative AIC weight (Σw_i) and validation through ROC areas (ROC) are reported. Accepted models (Δ_i <2) are in bold. Variables in models include mean strut rate (strut), presence or absence of hematomas (hema), lek attendance (attend), ornament colour score (colour) and the calculated body condition index (bci). Models are weighted by the number of strut counts made of each male.

Model	Model structure	LL	K_i	AIC	Δ_i	w_i	(Σw_i)	ROC
1	**strut + hema + attend**	**-102.75**	**4**	**213.50**	**0**	**0.52**	**0.52**	**0.74**
2	**strut + bci + strut*bci + attend**	**-101.91**	**5**	**213.82**	**0.32**	**0.44**	**0.96**	**0.77**
3	strut + attend + colour	-105.61	4	219.22	5.72	0.03	0.97	0.72
4	attend	-110.89	2	225.78	12.28	<0.01	1.00	0.73
5	strut + colour + hema	-109.20	4	226.40	12.90	<0.01	1.00	0.67
6	strut + attend	-110.61	3	227.22	13.72	<0.01	1.00	0.73
7	strut + bci + strut*bci + hema	-108.82	5	227.64	14.14	<0.01	1.00	0.73
8	hema	-113.02	2	230.04	16.54	<0.01	1.00	0.65
9	strut + hema	-112.54	3	231.08	17.58	<0.01	1.00	0.67
10	colour	-116.53	2	237.06	23.56	<0.01	1.00	0.56
11	strut +bci + strut*bci + colour	-113.68	5	237.36	23.86	<0.01	1.00	0.65
12	strut + colour	-115.77	3	237.54	24.04	<0.01	1.00	0.57
13	strut + bci + strut*bci	-117.59	4	243.18	29.68	<0.01	1.00	0.67
null	null	-121.56	1	245.12	31.62	<0.01	1.00	0.50
14	strut	-120.88	2	245.76	32.26	<0.01	1.00	0.53

Table 3-9. Parasite prevalence, correlation of parasites to sexual display traits and the inclusion of these display traits in the top-ranked models for Alberta and Wyoming. Not present is indicated by (n.p.).

Parasite Type		Alberta	Wyoming
Ectoparasites	Prevalent?	yes	yes
Correlated Trait	Hematomas	yes	yes
	Hematomas in top models?	yes	yes
Blood Parasites	Prevalent?	no	yes
Correlated Trait	Lek Attendance	n.p.	yes
	Attendance in top models?	no	yes
Endoparasites	Prevalent?	yes	no
Correlated Trait	Ornament colour score	yes	n.p.
	Colour in top models?	yes	no

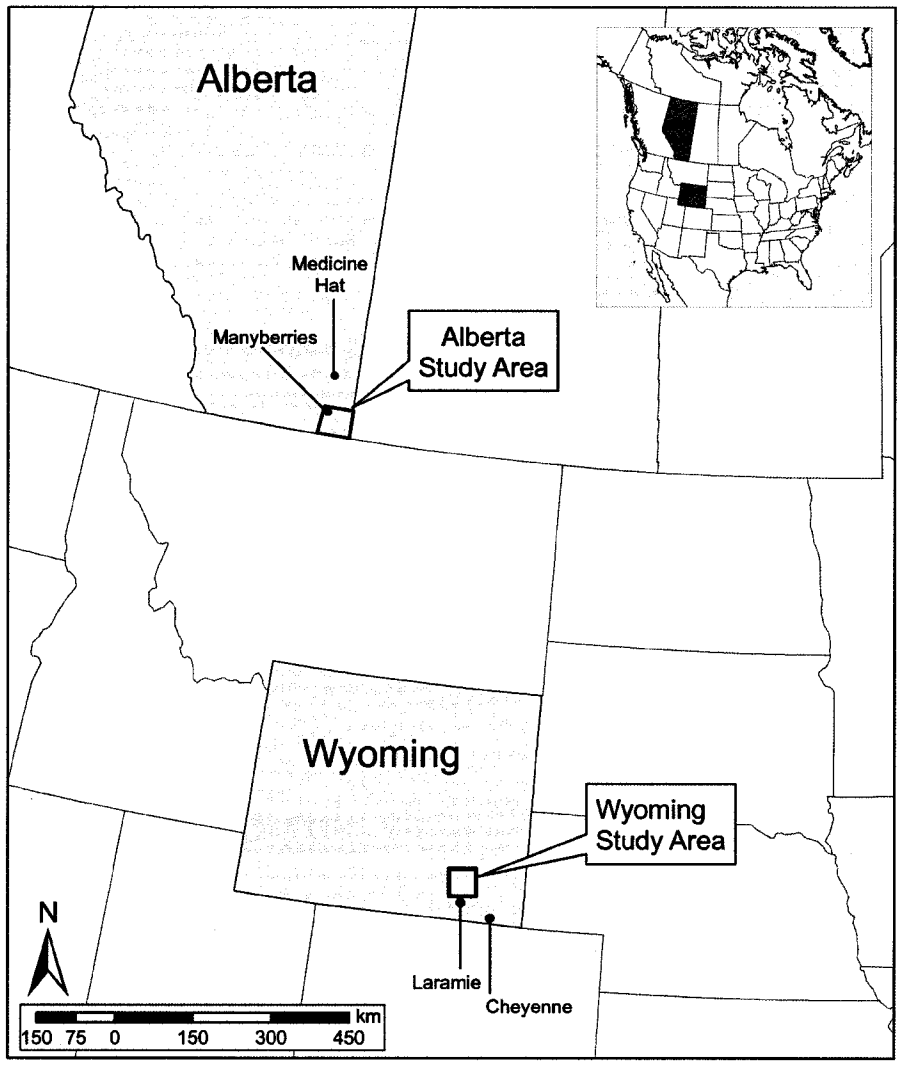

Figure 3-1. Map of the two study areas in North America. Alberta leks were between 4 and 60 km south and east of Manyberries. Wyoming leks were between 25 and 100 km north of Laramie.

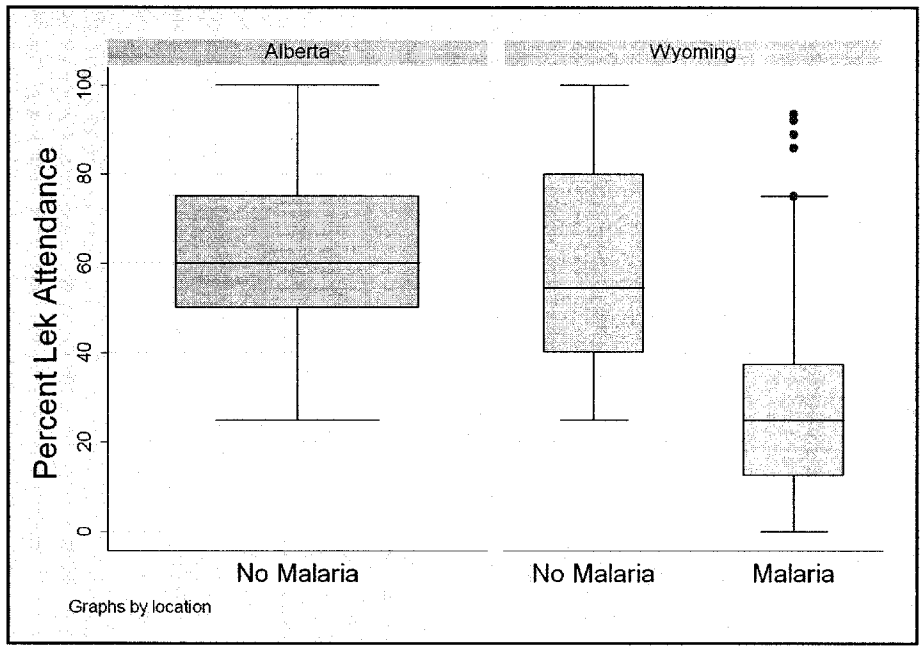

Figure 3-2. Box plot of male lek attendance by malaria status and study area. In Wyoming, lek attendance was higher among healthy males (middle) than males with malaria (bottom right). Lek attendance by healthy Wyoming males was similar to that of all Alberta males.

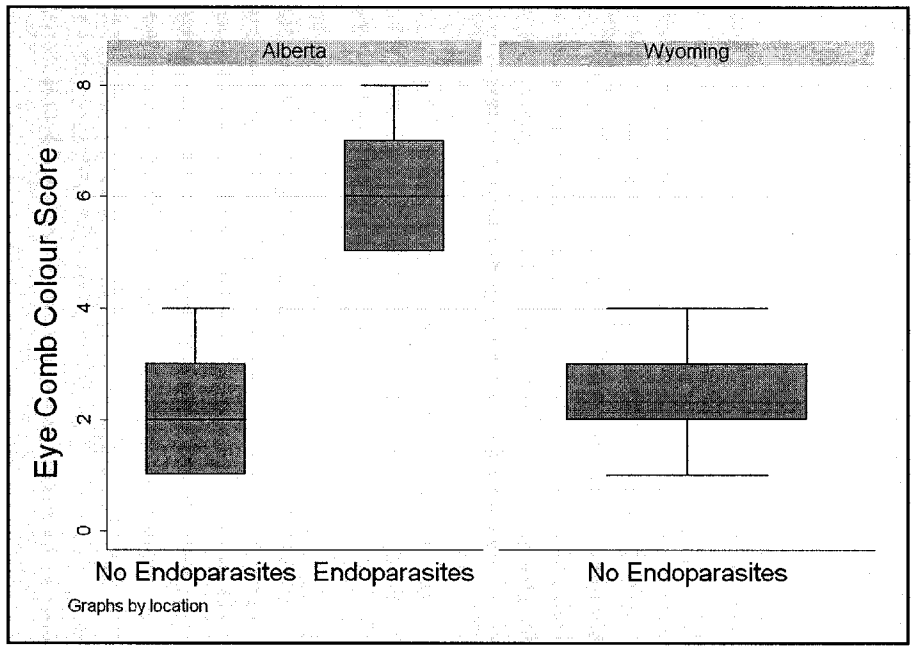

Figure 3-3. Box plot of male ornament colour score (eye comb colour score) by endoparasite status and study area. Eye comb colour scores ranged between 1 (saturated yellow) to 8 (dull grey-green). Colour scores among healthy Alberta males (bottom left) and Wyoming males (right) were similar. Parasitized males in Alberta however (middle), had higher scores for eye comb colour.

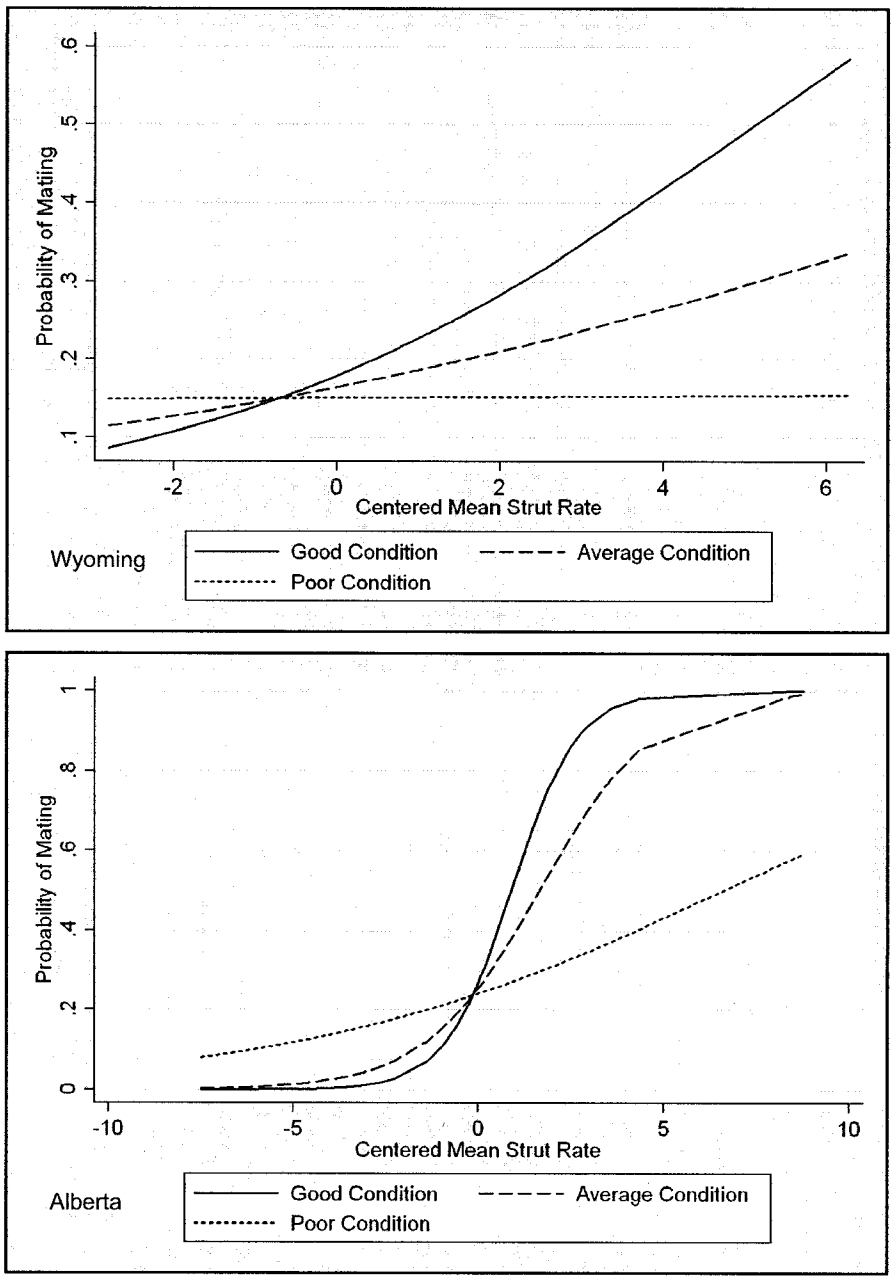

Figure 3-4. The interaction term for the body condition index and mean strut rate indicates body condition and quality of strut display are likely related. The probability of mating has a steeper slope for males in good condition than for those in average or poor condition.

Chapter 4

General Conclusion

Disease is highly cited as an important issue in endangered species management; however, evidence-based understanding of the role of disease in species decline is limited (Smith et al. 2006). The previous chapters examine two very different influences of parasites and disease on Greater Sage-Grouse (*Centrocercus urophasianus*, hereafter sage-grouse). This chapter discusses West Nile virus (WNv) and lek behaviour in relation to sage-grouse management and future research needs.

1. West Nile virus and Sage-Grouse Management

It remains difficult to predict the long-term impact of West Nile virus on sage-grouse population persistence in Alberta. The low virus activity reported in Manyberries in 2004 to 2006 (Chapter 2) may not represent long-term risks. There remains potential for a 'perfect storm' of (1) warm temperatures and (2) additive mid- and late-summer moisture that leads to high virus activity. Even occasional high mortality in Alberta's sage-grouse could disrupt recovery efforts. Furthermore, although the lack of antibodies in Alberta birds is at least partially because of low exposure, Clark et al. (2006) provided experimental evidence of a limited ability to develop resistance, even when exposed to low viremia.

Because of its ability to use a broad range of reservoir and vector species, WNv is now well-established in almost all North American avian communities (McLean 2006). Nonetheless, WNv is still a relatively new disease in North America, reaching Alberta only in 2003 and is still not found in British Columbia. Through time, the risks of WNv infection to sage-grouse may be reduced because less virulent strains are more likely to be maintained in reservoir populations of other species. The virus also may adapt to colder climates and become more consistent in its annual prevalence in Alberta. For example, the South African strain of WNv (H442) has had a long evolutionary history with its mosquito vectors and avian hosts. This strain of virus replicated well in vector mosquitoes and transmitted in relatively cool temperatures (Cornel et al. 1993).

The interaction between host susceptibility, reservoir competence, vector populations and local environmental conditions remain important in efforts to understand WNv ecology (Marra et al. 2004).

The WNv research program was created as an immediate strategy to (1) mitigate a potential crisis for an endangered species and (2) examine several unknowns regarding WNv in Alberta. Interdisciplinary cooperation among several agencies was critical in getting this strategy running quickly and effectively. Furthermore, project results benefit WNv management efforts for both humans and wildlife.

2. Behaviour and Sage-Grouse Management

Peterson (2004) called for ecologically-based studies that address not only the prevalence of parasites but also (1) their relation to lek behaviour and (2) their significance to sensitive populations of prairie grouse (of the genus *Tympanuchus* spp.). Allee effects resulting from the interaction of lek behaviour with population isolation, small population size or low density could be important in the viability of lek species. Chapter 3 identified evidence of parasite-mediated sexual selection (PMSS) on both Alberta and Wyoming leks, indicating a mechanism of sexual selection had not been lost in Alberta through reduced density or population isolation. Furthermore, the variation in parasite prevalence and the difference in top selected models between locations provides evidence that PMSS could result in fluctuation of the optimal male.

Further research to understand other aspects of sage-grouse lek behaviour also may be important in management efforts for small populations. In *Tympanuchus* spp., when leks reach fewer than 5 males, male behaviour can become erratic and leks generally disappear (Peterson 2004). One effect of low population density may already be evident in Alberta. Sage-grouse and Sharp-Tail Grouse (*Tympanuchus phasianellus*; hereafter sharp-tail) hybrids could be an example of an Allee effect, where female mate choice is directly influenced by a reduction in lek number, size or quality. In Alberta, there have been several reports of sage-grouse hybridizing with sharp-tail (Aldridge et al. 2001, Alberta Fish and Wildlife 2007), and both sage-grouse hens and males have

been observed visiting sharp-tail leks (Alberta Fish and Wildlife 2007). Genetic research is currently in progress to determine the extent of hybridization in Alberta's small population of sage-grouse (Bush *in progress*). While there might be predation benefits from formations of multi-species leks (Gibson et al. 2002), the hybridization itself suggests that for some reason a male from a sharp-tail lek may appear to be a better choice than a male sage-grouse. Alternatively, if female lek behaviour includes copying (Gibson et al. 1991, Gibson 1996), hens might simply copy the choice of heterospecific hens. Sage-grouse might attend sharp-tail leks because their own species is at a low density on the landscape (Aldridge et al. 2001) and vestigial sensory biases could attract birds to leks of the wrong species (Phelps et al. 2001, Gibson et al. 2002). Research to identify the specific behavioural mechanisms that lead to hybrids, may be important in directing conservation action for low density populations and small leks.

The role of parasites in mediating sage-grouse sexual characteristics exemplifies that while infections like WNv can reduce survival, parasites and diseases are also components of biological diversity in and of themselves. Considering the complex evolutionary relationship between host and infection is necessary to understand the implications of parasites and diseases in regulating endangered populations.

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