Social dynamics among mule deer and how they visit various environmental areas: implications for chronic wasting disease transmission

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ABSTRACT

This dissertation analyzes sociality patterns of mule deer (*Odocoileus hemionus*) and how they visit different localized environmental areas, two epidemiologically relevant sources of variability in chronic wasting disease (CWD) transmission dynamics. This dissertation seeks to answer questions such as what are the seasonal patterns of mule deer group formation and size, what factors can predict close-distance proximity and physical contacts between individuals, and in which environmental areas are different sex and age classes of deer found throughout the year. These questions are of great relevance in the study of a disease that efficiently transmits through animal-animal contact and prion contaminated environments.

Mule deer in Antelope Creek, a CWD endemic area in Saskatchewan, Canada, have been studied since 2006. I used genetic, behavioural, camera-trap, and high-resolution and high-frequency radio-telemetry data to address these questions. First, we learned that when mule deer showed clinical signs of the disease they were less likely to be reported in groups. Second, males were more likely to be found in close-distance proximity with other males in pre-rut, and with females in rut. Also, females tended to have more stable and longer lasting relationships with any other females than with males, whereas male-male relationships tended to be ephemeral. Third, individuals grouped more often with their close relatives, but the latter were not more likely to physically contact each other within groups. Lastly, grain spills were the sites most visited by deer and where they contacted the environment most often with either their mouths and antlers, or by defecating and urinating. Hence, grain spills could be of great importance for disease control, as they can be modified to reduce mule deer congregation, environmental prion contamination and transmission.

This dissertation describes several features of mule deer social behaviour, which advances our understanding of their sociobiology. These findings provide insight into how CWD may be transmitted in wild cervids and will be useful in the further development of spatially- and behaviourally-explicit dynamic epidemiological models to guide CWD management strategies.

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DEDICATION

A mi papá y mis tres mamás, por su amor incomparable.

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AUTHOR’S NOTE

The four manuscripts included in this dissertation are all intended for publication in peer-reviewed journals. The manuscripts in Chapters 3 and 4 appear here in slightly different form than originally published, as the formatting has been adjusted for inclusion in this thesis. The rest of the manuscripts will probably have more pronounced changes after the pre-publication process.

Publications during candidature

Reiczigel J, Mejía Salazar MF, Bollinger TK, Rózsa L. Comparing radio-tracking and visual detection methods to quantify group size measures. Eur J Ecol. 2015; 1(2):1-4. doi: 10.1515/eje-2015-0011 – This manuscript is not included in the thesis.

Mejía Salazar MF, Waldner C, Stookey J, Bollinger TK. Infectious disease and grouping patterns in mule deer. PLoS ONE. 2016; 11(3): e0150830. doi: 10.1371/journal.pone.0150830 – This manuscript is included in the thesis as Chapter 3.

Mejía-Salazar MF, Goldizen AW, Menz CS, Dwyer RG, Blomberg SP, Waldner CL, Cullingham CI, Bollinger TK. Mule deer spatial association patterns and potential implications for transmission of an epizootic disease. PLoS ONE. 2017. doi: 10.1371/journal.pone.0175385. This manuscript is included in the thesis as Chapter 4.

Manuscripts included in this thesis

All co-authors consented to the manuscripts being included in this thesis.

Co-authors’ contributions to each manuscript are described at the beginning of each chapter.

Chapter 3

Chapter 3 has been published in PLoS ONE: Mejía Salazar MF, Waldner C, Stookey J, Bollinger TK. Infectious disease and grouping patterns in mule deer. PLoS ONE. 2016; 11(3): e0150830. doi: 10.1371/journal.pone.0150830

The social behaviour of the host determines, to a great extent, the infectious disease dynamics within a population. This first manuscript eestablished a baseline for better understanding the link between mule deer sociobiology and chronic wasting disease (CWD) transmission. We found that the presence of clinical signs of disease affected the probability of an individual being seen in groups, and that group size significantly differs across times of the day, habitats, and seasons. These findings demonstrate that clinical signs caused by CWD affect a deer’s ability to enter and remain in a group, and that group membership varies even within a day. These features of mule deer sociality most likely have consequences in disease transmission at the population level. The next step was to look at factors that influenced the proportion of time that deer spent in close proximity (Chapter 4).

Chapter 4

Chapter 4 has been accepted for publication in PLoS ONE: Mejía-Salazar MF, Goldizen AW, Menz CS, Dwyer RG, Blomberg SP, Waldner C, Cullingham CI, Bollinger TK. Mule deer spatial association patterns and potential implications for transmission of an epizootic disease. PLoS ONE. 2017. doi: 10.1371/journal.pone.0175385.

In this chapter, we investigated factors affecting the proportion of time that individuals spend together. As CWD can be transmitted through both animal-to-animal contact and through contaminated environments, these factors can shed light on the understanding of disease spread. Our findings included marked differences in the time that males and females spend together during the mating season, and in the stability of their relationships. We also provided empirical data on the structure of adult mule deer society based on spatial proximity. These data can potentially be used in the construction of dynamic epidemiological models. The next step was to investigate factors that predict physical contacts (or interactions) that represent a high-risk of transmission between deer (Chapter 5).

Chapter 5

Chapter 5 is currently under review in Ethology: Mejía-Salazar MF, Waldner C, Cullingham CI, and Bollinger TK. Predictors and reciprocity of high-risk interactions for disease transmission among mule deer.

In this chapter, to shed light on the importance of deer-to-deer prion transmission, we investigated the factors that predict rates of high-risk physical contacts between mule deer, and whether these interactions were reciprocal. We found that genetic relatedness was a positive predictor of association strength, that association strength was a positive predictor of high-risk contacts, and that the importance of genetic relatedness with respect to interaction rate is mediated through the strength of the association. This chapter is the last one dedicated to improving our understanding of prion transmission through social relationships. The next chapter (Chapter 6) improves our understanding of mule deer visitation to various environmental areas with the potential for environmental prion deposition and intake.

Chapter 6

Chapter 6 will be shortened for publication: Mejía-Salazar MF, Waldner C, Hwang YT, and Bollinger TK. Visitation to environmental sites by mule deer in a chronic wasting disease endemic area.

Besides prion transfer through either group membership (Chapter 4) or physical contacts (Chapter 5), prion environmental contamination plays a fundamental role in the epidemiology of CWD. Deer infected with CWD deposit prions in frequently used areas, where prions can remain bioavailable and infective for many years. By investigating the patterns of visitation of deer to various small geographical areas, such as grain spills, salt licks, carcasses and bed sites, we can rank these sites based on their relative importance with respect to prion contamination. This chapter analyses how various sex and age classes of mule deer visited eight different sites across biologically relevant seasons. Our findings suggest that control programs which reduce the access of deer to grain spills during specific times of the year could potentially result in a significant reduction in CWD transmission.

CHAPTER 1. GENERAL INTRODUCTION

1.1 BRIEF RATIONALE

Chronic wasting disease (CWD), an invariably fatal and contagious prion encephalopathy, is an important emerging global disease. CWD infects several species of cervids under natural conditions and a range of other species, including non-human primates, rodents and domestic animals, under experimental conditions (Haley and Hoover, 2015, and references therein). Although a disease with low zoonotic potential (Barria et al., 2014b), since it is a transmissibly spongiform encephalopathy, consumption of CWD-infected foods is not recommended. The risk of CWD being transmitted to traditional domestic livestock appears to be very low (Hamir et al., 2005); however, the disease is readily transmitted from farmed to wild cervids, and vice versa, resulting in devastating economic losses (e.g., Smith, 2016). CWD is a reportable disease and the Canadian Government has spent over 54 million dollars to compensate game farmers for animals culled as part of disease management programs. The effect of CWD on wild cervids and ecosystems is projected to be significant and has been driving declines in populations of wild mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) over the last three decades (Miller et al., 2008; Edmunds et al., 2016). CWD in wild cervids threatens the sport hunting industry and subsistence hunting, while the disease in farmed cervids results in an ongoing threat to game farming and the agricultural industry. The importance of CWD is challenging for the general public, hunters, deer farmers, and wildlife management agencies to understand and appreciate, because the impacts at the population level appear negligible in the early phase of an epidemic (Jolles et al., 2005; Cross et al., 2009). Decreased population growth, abundance decline, low adult survival, and eventual shift in age structure may not be evident for years or even decades (Albon et al., 2002). The geographic distribution and prevalence of CWD keeps expanding every year and has now been diagnosed in cervids in 24 states in the United States of America, three Canadian provinces, South Korea and recently Norway; the control of its spread is of the utmost importance. Because sociality has significant effects on the long-term dynamics of diseases (Vander Wal et al., 2012; Schauber et al., 2015), to accurately model CWD dynamics and suggest efficient control measures, we need to better understand social behaviour of mule deer and how they visit different types of locations. Specifically, we need to determine how they spend time in close proximity to each other, how they physically contact each other, and how they congregate and interact with the environment at specific areas that can be relevant for prion contamination.

Social animals transfer information and pathogens by means of relationships, as sociality promotes inter-individual behaviours and sharing of physical space (Alcock, 2009). In addition, relationships between social individuals are neither random nor homogeneous; that is, individual *A* is not equally likely to relate with every other individual, and does not relate with others with the same frequency, intensity and duration (Croft et al., 2008). Hence, individuals have heterogeneous risk of infection.

Disease spread through a population is determined by the transmission rate of infection (β), which can be defined in terms of the contact rate (κ) and the probability of pathogen transmission given a contact (γ), using the formula: $\beta = \kappa * \gamma$. It is challenging to estimate γ and therefore efforts are made to calculate contact networks (a "who contacts whom" matrix of contact rates between individuals) (Dobson, 2004; Craft and Caillaud, 2011) that can serve as a proxy for transmission networks (e.g., Bansal et al., 2010). However, as not all contacts lead to disease transmission, a transmission network is almost always a subset of the contact network, depending on the pathogen and its transmission modes.

Modeling is a tool that simplifies complex phenomena (Vynnycky and White, 2010). Dynamic disease models such as network analysis and agent-based models allow researchers to estimate epidemiological parameters, mimic observed patterns, and conduct "what if" scenarios by testing disease control measures without having to perform live experiments (Vynnycky and White, 2010). These learning tools are useful in the study of mule deer (*Odocoileus hemionus*) populations infected with CWD to manage sources of complexity including:

- In mule deer societies group size and composition change as environmental conditions and individual requirements change (Bowyer et al., 2001; Lingle, 2003). This strongly suggests that mule deer, as other cervids (Geist, 1998b), live in societies characterized by intermediate to high degrees of fission-fusion dynamics, in which subgroups are frequently formed (Aureli et al., 2012).

- Deer can survive for a long time (mean = 23 months) once infected (Williams and Miller, 2002), and although the point at which infected individuals become infectious is unknown, prion shedding is likely progressive through the states of infection (Williams and Miller, 2002). Moreover, as the latent period is shorter than the incubation period, both the infectious and the incubation periods overlap in time, leading to asymptomatic, but infectious individuals (Tamgüney et al., 2009). For this reason, selective removal of clinically sick deer from the population as a control measure is impractical (Gross and Miller, 2001; but see Wolfe et al., 2004; Wild et al., 2011).
- CWD prions enter the environment through deer secretions and excretions (Gough and Maddison, 2010, and the references therein), and through infected carcasses (Miller et al., 2004; Angers et al., 2006). Once in the environment, CWD prions can bind to soil particles, stabilize against degradation, and remain infective for at least 2.5 years outside of the host (Miller et al., 2004). Thus, the infectiousness of prions shed into the environment could exceed the host´s life span.
- Current control strategies have not been effective in limiting the geographic spread of CWD (e.g., Alberta Prion Research Institute et al., 2011; Arkansas Game and Fish Commission, 2016; McKenzie, 2016).

It is still uncertain which mode of transmission, animal to animal or environmental, is most important in the dynamics of CWD spread. In theoretical modeling, the relative importance of environmental transmission appears to be stronger than that of animal to animal transmission (Almberg et al., 2011; Vasilyeva et al., 2015). However, no models have specifically considered social behaviour in their formulation. Other models in which contact rates and group membership have been studied suggest that animal to animal contacts are the main driver of CWD transmission (e.g., Schauber et al., 2015); however, a “contact” in these studies was defined as a proximal association and not a physical contact per se. Importantly, social structure and behaviour of the host population have epidemiological implications in the spread of infections, as they influence both transmission pathways (Nunn et al., 2015).

At the individual level, both sick and healthy animals show a behavioural response to disease. For instance, healthy individuals can show aversion to diseased conspecifics (Loehle, 1995). A clear example of this behavioural immunity strategy includes healthy lobsters avoiding individuals infected with PaV1 virus (Butler et al., 2015), and with that, preventing an epizootic over a large seascape. Likewise, infected individuals can show sickness behaviour to conserve energy for use in immune responses (Hart, 1988). This set of physiological and behavioural changes includes lethargy, anorexia and seclusion (Hart, 1988), which can in turn limit spread of disease by decreasing contact with susceptible individuals.

In disease ecology, there are already numerous studies in humans based on empirically-based social data (e.g., Jolly and Wylie, 2002; Eubank et al., 2004), and studies in wildlife disease ecology are increasing in numbers. Some important examples include Cross et al. (2004), Vicente et al. (2007), and Drewe et al. (2010), in which the knowledge of social behaviour of the host, in these cases African buffaloes (*Syncerus caffer*), European badgers (*Meles meles*) and meerkats (*Suricata suricatta*), respectively, have helped understand the dynamics of spread of bovine tuberculosis (*Mycobacterium bovis*).

In the case of CWD, soil is an environmental reservoir for prion infectivity (Johnson et al., 2006b). Currently, it is not feasible to quantify prions in soils or in other environmental samples under non-experimental conditions. This prevents researchers from being able to rank different sites, such as antler rubs, waterholes, and grain spills, in order of transmission risk based on the concentration of prions in the environment. Alternatively, we can quantify the frequency and intensity of mule deer visitation to these environmental sites as proxies for relative risk of transmission. The results can serve to direct control measures regarding sites such as grain spills that can be modified through removal, alteration, or relocation to reduce the local potential for deer aggregation and prion contamination.

Under all these circumstances, expanding our understanding of cervid social systems is important because a disease such as CWD needs to be studied using dynamic models that are behaviourally- and spatially explicit to suggest more effective disease control strategies (Vynnycky and White, 2010). Therefore, this study of mule deer social behaviour and their patterns of visitation to specific environmental areas of interest has the potential to not only enhance epidemiologic models, but also to expand the knowledge of cervid behavioural ecology and sociobiology.

1.2 BACKGROUND

1.2.1 Ecology and behaviour of mule deer

Out of the 10 genera of New World deer, the mule deer is the most recent deer to evolve (originating 10,000 years or less ago) (Geist, 1998a). It shares with the rest the pointed hooves, the pendicular penis of the males, the presence of preorbital glands, and pedal or interdigital glands on the pelvic limbs. However, it is distinguished by having the largest metatarsal glands, the biggest ears in proportion to the head, and by a characteristic bouncing gate, known as stotting (Anderson and Wallmo, 1984).

There are seven distinct subspecies of mule deer, and their differences depend on the geographic distribution, skull anatomy, body size, fur coloration and tooth characteristics (Rees et al., 1966; Anderson and Wallmo, 1984; Bauer and Bauer, 2000). Our project focuses on the Rocky Mountain mule deer (*O. hemionus hemionus*), which has the largest distribution of the mule deer genera (from Alaska to the North of Mexico). It is the only subspecies found in Saskatchewan (Anderson and Wallmo, 1984; Mackie, 1994; Kie and Czech, 2000), where it overlaps with pronghorn (*Antilocapra americana*), white-tailed deer, elk (*Cervus canadensis*) and moose (*Alces alces*). According to the Saskatchewan Ministry of Environment, in 2006, there were an estimated 43,000 mule deer in Saskatchewan (Saskatchewan Ministry of Environment, 2008).

Mule deer are found in various habitat types, but they are more commonly found in rugged terrain and open areas (Wood, 1989; Lingle, 2003). Among the movement patterns reported in deer, including mule deer, dispersal and migration distances vary between seasons, and age and sex classes (Garrott et al., 1987; Wood, 1989), and movement patterns can also differ according to topography and hunting pressure (Long et al., 2008) in the area.

The gestation period is about 183-218 days (with a mean of 200-208 days), and about 50% of the young are born between late May and late June (Anderson and Wallmo, 1984). In our study area in southern Saskatchewan, fawning dates ranged from May 27th to July 6th from 2009 to 2011, with most fawns born in the 2nd and 3rd weeks of June (Perera, 2012). As expected in environments with well-defined seasonality, births coincide with early growth stages of plants, when they are low in fiber and toxins but high in nutrients; the peak of lactation coincides with the peak of food availability (White, 1991). Once fawns are born, mothers remain isolated with their offspring, away from other individuals (Wood, 1989; Geist, 1998a; Lingle, 2003). Females without fawns form small groups (Geist, 1998a) and the lower the fawn birth rate, the larger the female groups (Geist, 1998a, p. 261). It is not until late summer when mothers allow other members of the population to join her group.

The rut usually begins in the fall (November) as females enter estrus and males, who are polygynous, become more aggressive, with marked augmentation in the size of the neck and increased frequency of fights for dominance (Prothero, 2002). Estrus lasts about 24 hours and it is repeated every 28 days until the female becomes pregnant (Prothero, 2002). Does may be severely harassed by small bucks during the rut, so they may seek older males' attention in order to keep younger bucks away (Anderson and Wallmo, 1984; Prothero, 2002). The dominant buck visits either various female groups or one female group within his home range. A mature buck may make a large circuit as he seeks receptive does, and he usually uses the same trail to complete the circuit every one or two days; when he is not traveling the circuit, he uses a specific area called the center area (Prothero, 2002). Bucks move away from doe groups after the rut and they spend the early winter alone or with one or two cohorts (Anderson and Wallmo, 1984; Bauer and Bauer, 2000; Prothero, 2002). As winter progresses, males, females and previous year fawns congregate in large herds (Wood, 1989; Prothero, 2002; Lingle, 2003; Miller et al., 2004). As weather conditions improve late in winter, males separate from females and fawns, and form bachelor groups (Anderson and Wallmo, 1984; Bauer and Bauer, 2000; Prothero, 2002). Inbreeding is more detrimental for females than for males because the physiological costs of pregnancy and nurture of a defective offspring are only paid by the mother. Therefore, just before the new fawns are born, pregnant females encourage their current male offspring to leave the area and these males start dispersing (Geist, 1998a). This strategy reduces the chances of females copulating with close relatives. Also, the death of the doe reduces the likelihood of her male fawn dispersing as a yearling (Hölzenbein and Marchinton, 1992), which supports the previous observation.

Females and males use their territory differently, demonstrating sexual segregation, which may result in different patterns of social association (Barboza and Bowyer, 2000) and different risk of contracting disease. Two strong hypotheses likely explain this sexual segregation: the risk of predation (Lingle, 2002) and the sexual difference in body size (Barboza and Bowyer, 2000). However, they are not mutually exclusive. Females use areas that likely increase offspring survival, such as areas proximal to water, with low coyote activity, and with hiding plant cover and palatable browse resources (Main and Coblentz, 1996). On the other hand, mule deer and white-tailed deer have a small rumen (about 10% of body weight) compared to 23% in red deer and domestic sheep and cattle (Geist, 1998a). Moreover, mule deer have about half the rumen volume of a sheep: 7.8 versus 13.9 liters (Geist, 1998a). Therefore, mule deer have a high rate of rumen turnover when compared to other ruminants. Because of this, mule deer are concentrate selectors that need to select rapidly digestible food, such as forbs and browse. Moreover, mule deer have small mouths, allowing them to select specific plants and the most nutritious parts of the plant. Even within the same species, there are differences in foraging preferences between sexes and this can potentially explain the different use of habitat throughout the year (Barboza and Bowyer, 2000).

1.2.2 Chronic wasting disease

Prion diseases are characterized by spongiform degeneration of nervous tissue, as in all other transmissible spongiform encephalopathies (TSEs). They are a group of incurable, always fatal, infectious disorders caused by a self-propagating aberrant form (PrP^{TSE}) of the normal, host-encoded cellular prion protein (PrP^{C}). This makes prion diseases quite distinct from conventional pathogenic agents with a nucleic acid component.

Ruminant TSEs include: chronic wasting disease (CWD) in cervids, scrapie (SC) in sheep and goats, and bovine spongiform encephalopathy (BSE) or “mad cow” in domestic cattle (Chesebro, 2003; Beekes and McBride, 2007). The appearance of prion diseases in different species over the years seem to be the result of the contagion moving from one species to another. For example, the transmission of scrapie to cattle was most probably the origin of BSE in the 1980s (Hope et al., 1988; Bradley, 2001) and since then, BSE transmission from cattle has been confirmed in domestic and zoo felids (FSE) (Wyatt et al., 1991; Hilbe et al., 2009), zoo ungulates (Horn et al., 2001), and humans (producing vCJD in the 1990s) (Knight et al., 2001). However, the origins of the prion strain that causes CWD are unclear, and whether it was initiated by another prion type such as scrapie or if it is a native disease of North American cervids cannot currently be determined.

In contrast with other TSEs, CWD has been diagnosed in both farmed and free-ranging members of the deer family in North America, including elk, mule deer, white-tailed deer, and moose (Williams and Miller, 2002; Kreeger et al., 2006; Baeten et al., 2007; McKenzie, 2016). It is also present in captive herds of red deer (*Cervus elaphus*) and sika deer (*Cervus nippon*) in South Korea (Williams and Miller, 2002; Kim et al., 2005), and most recently, in free-ranging reindeer (*Rangifer tarandus tarandus*) and moose in Norway (McKenzie, 2016). CWD was first detected in 1967 in captive mule deer in a research facility near Fort Collins, Colorado, USA, but it was not considered a TSE until 1980 (Williams and Young, 1980). Based on historical records, CWD occurred in free-ranging cervids in Colorado since at least 1985, and it contributed to a 45% decline in deer abundance in that area over 18 years (Miller et al., 2008; Edmunds et al., 2016). CWD was first introduced into Canada as a result of the importation of mule deer from Denver Zoo to Toronto Zoo in 1974, but it was not until 1978 that it was described and characterized as a TSE (Dubé et al., 2006), soon followed by the diagnosis of the disease in captive mule deer and Rocky Mountain elk (*Cervus elaphus nelsoni*) in Wyoming (Williams and Young, 1980). CWD was introduced into the province of Saskatchewan, Canada, through imported farmed elk from the USA in the late 1980s, but was not detected until 1996 in an elk game farm (Williams and Miller, 2002). Since 1997 more than 44,000 wild cervids, including mule deer, white-tailed deer, elk and moose, have been tested for CWD in the province as part of the Saskatchewan Ministry of Environment and the Canadian Wildlife Health Cooperative surveillance program. By 2000, when CWD became a reportable disease in Canada, it was found in wild mule deer in an area south of Lloydminster, near the Alberta border, and by October of 2005 the first CWD-positive free ranging mule deer was detected in Alberta (Bollinger et al., 2004; Schwantje, 2006). From 2009 to 2011, the prevalence of CWD in all cervids combined grew 2.6 times; in mule deer, the prevalence increased 4.2 times (Canadian Cooperative Wildlife Health Centre, 2011).

1.2.2.1 CWD impacts

The fact that cervids are affected by CWD is a serious concern for wildlife management agencies as it has ecological, cultural, economic and political impacts. Wild cervids are valued in their geographic distribution range for many reasons. As large herbivores, they play a major role in control of biomass and biodiversity of plants and in nutrient cycling (Olff and Ritchie, 1998), and their carcasses enhance biodiversity in terrestrial ecosystems and are an available source of protein and calcium for scavengers and predators (Carter et al., 2007). Cervids also have an aesthetic significance for humans, not only as part of the scenery, but also as game species. Hunting of cervids is of cultural and economic importance wherever they are present. For example, the gross expenditure related to hunting of all game species in Saskatchewan, Canada, amounts to over 107 million dollars each year, with mule deer representing about 17% of cervid licenses sold in 2007 (Saskatchewan Ministry of Environment, 2008). Moreover, CWD poses a potential risk for human and livestock health as transmission to these species, although unlikely, remains a possibility (Barria et al., 2014a). As well, there is evidence that licence sales decline in response to CWD in an area (Vaske et al., 2004).

Geographic spread has continued despite control efforts, which are limited to activities focused on the reduction of the number of infected animals, including culling, regulation of big game feeding and preclusion of cervid translocation (Alberta Prion Research Institute et al., 2011). Each year, new cases in captivity and the wild are reported in the USA and Canada, and the response programs include quarantine and/or depopulation of captive populations, organized surveillance programs, and prohibition of translocation from CWD endemic areas. There are great costs related to these activities; for example, the Canadian government has spent at least $30 million CAD in indemnity for farmed elk herds alone (Chronic Wasting Disease Alliance, April 2002).

1.2.2.2 CWD epidemiology

The etiological agent of all TSEs is an infectious prion protein that propagates in the absence of nucleic acid. There is a normal, cellular isoform of the prion protein (denoted as PrP^{C}) (Prusiner, 1998), which is expressed in numerous tissues other than in the nervous system, yet its cellular role is unknown. For PrP^{TSE} to cause disease, the expression of host PRNP-encoded PrP^{C}

is required (Prusiner, 1982). PrP^{TSE} induces misfolding of the PrP^{C} α-helical structure into a re-folded β-sheet, hence the name misfolded prion protein (Pan et al., 1993). This conformational change leads to insolubility in nondenaturing detergents and resistance to proteolysis and is the reason why it can also be referred as PrP^{RES} (Meyer et al., 1986). This resistance is the reason why pathological prions accumulate in the central nervous system, causing its degeneration (Prusiner, 1998) and culminating in clinical disease. Moreover, prions form protein aggregates that can be of different types according to their structures, a phenomenon called amyloid polymorphism. Each of these different structures are called prion strains and affect the pathology and disease transmission by, for example, varying the incubation period, the distribution of brain lesions, and even the transmissibility between species (Johnson et al., 2006a; Johnson et al., 2011; for a general review see Stein and True, 2014).

There is evidence of at least two CWD strains in experiments using CWD susceptible transgenic mice, hamsters and ferrets (Raymond et al., 2007; Angers et al., 2010; Perrott et al., 2012; Duque-Velásquez et al., 2015) (for a summary of findings see Haley and Hoover, 2015). One strain produces a longer incubation period and more localized prion deposition, while the other produces a shorter incubation period and a wider distribution of prion deposition (Haley and Hoover, 2015). Polymorphisms (i.e., the primary structure) in the PRNP gene also affect how susceptible cervids are to CWD, and the length of the incubation period (Jewell et al., 2005; Johnson et al., 2006a; Johnson et al., 2011; Wolfe et al., 2014). Mule deer of all three genotypes (225SS, 225SF and 225FF) are susceptible to the disease; however, the probability of finding infected individuals with 225SS genotype is many times (from 4 to 213) greater than for individuals with 225SF genotype (Jewell et al., 2005). Genotype 225FF is extremely rare in the population. It also seems that longer incubation periods, and hence longer survival times, are more common in the 225SF genotype than in the 225SS genotype (Jewell et al., 2005).

Epidemics of CWD are sustained largely by horizontal transmission, via either close contact between individuals or via contaminated environments (Miller et al., 2006; Mathiason et al., 2009). Several sources of contagion, including urine, feces, saliva, milk, placenta, and velvet, have been identified from symptomatic and asymptomatic animals infected with PrP^{TSE}. Reported species include transgenic mice, hamsters, deer, sheep, goats, and cows, among others (for an exhaustive list of references, see Gough and Maddison, 2010). However, whether there is a high rate of horizontal transmission from animal to animal or if the transmission is mainly sustained by contaminated environments, is debatable. Nevertheless, both pathways implicate excretions and secretions as vectors of infectivity, and the most likely natural infection route of CWD is via oral intake. The role and relative importance of other transmission pathways are currently under investigation, including: sexual (Gatti et al., 2002), from mother to offspring (Nalls et al., 2013), through aerosols (Haybaeck et al., 2011; Denkers et al., 2013; Nichols et al., 2013), and through vectors or reservoirs such as coyotes (Nichols et al., 2015), birds (VerCauteren et al., 2012), and plants (Pritzkow et al., 2015). However, it is important to recognize that the long-distance geographic spread of CWD in recent decades has not been caused by wind, coyotes or birds, but by ungulate commerce and transport by humans of live infected deer or their subproducts, such as urine (e.g., Kim et al., 2005).

CWD is characterized by a long incubation period followed by a shorter symptomatic period, and by asymptomatic, but infectious individuals. The length of the states of infection and disease might be influenced by many factors, such as the route of transmission, the infectious dose of prions, the prion strain, and the age, sex and genotype of the host (Jewell et al., 2005; Miller and Conner, 2005; Dulberger et al., 2010). Table 1-1 is an attempt to summarize the published data on states of infection and disease of CWD in mule deer.

Table 1-1. Duration of states of infection and disease in mule deer infected with CWD.

Reported duration	Details	Reference
Minimum incubation period (from infection to clinical signs arousal)		
16-17 months.	The youngest animal diagnosed was 17 months of age. Did not clarify if under natural conditions.	(Williams and Miller, 2002)
15 months.	Experimental conditions, PO.	(Williams and Miller, 2002)
17-21 months.	Experimental conditions, IC.	(Williams and Young, 1992)
12-20 months.	Experimental conditions.	(Mathiason et al., 2009)
14.5-19 months (442-578 days).	Experimental conditions in 6-month old fawns, PO.	(Fox et al., 2006)
19-23 months (225SS deer), 36 months (225SF deer).	Experimental conditions, in 3 to 4-month old fawns, PO.	(Jewell et al., 2005)
Incubation plus symptomatic periods, or latent plus infectious periods (from infection to death or removal)		
Mean: 20-25 months.	Experimental conditions, PO; survival can exceed 25 months.	(Williams and Miller, 2002)
Symptomatic period (from clinical signs arousal to death or removal)		
Few days to 12 months. Most animals from few weeks to 3-4 months.	Experimental conditions, PO.	(Williams and Miller, 2002)
2 weeks – 8 months.	In captivity.	(Williams and Young, 1980)
106-289 days.	Experimental conditions in 6-month old fawns, PO.	(Fox et al., 2006)

PO = *per os*; IC = intracranial.

It is challenging to gather literature about the specific duration of the different states of the disease and infection in mule deer. The reasons for this include: 1) the information has been generated from experimental scenarios including non-natural routes of infection, such as via intracranial inoculation (e.g., Hamir et al., 2008), 2) the experimental scenarios include non-cervid species such as transgenic rodents and ferrets (Sigurdson et al., 2006; Sigurdson et al., 2008; Perrott et al., 2012), and 3) many of the published papers focused on detecting the PrP^{CWD} (the PrP^{TSE} for CWD) by immunohistochemistry (Spraker et al., 2002), but didn´t test for infectivity, so the transition between latent and infectious periods has not been defined.

On the contrary, the symptomatic period in captive deer (predominantly mule deer) has been well characterized (Williams and Young, 1980; Mathiason et al., 2009; Mathiason, 2010). Reported clinical signs include: drooping head and ears, laterally wide leg stance, repetitive and exaggerated lifting of the legs, head tossing, piloerection and consequent rough hair coat, flaccid hypotonia of facial muscles, esophageal hypotonia and dilatation with consequent difficulty in swallowing, drooling, grinding of the teeth, polydipsia and polyuria, episodes of reduced awareness (stand for several minutes with lowered head and a fix stare, and then revert to a more normal state of alertness), decreased interactions with unaffected deer in the group, hyperexcitability, and gradual weight loss with terminal anorexia (Williams and Young, 1980; Mathiason et al., 2009; Mathiason, 2010). It is possible that once infected, free-ranging deer survive longer than experimentally infected deer because doses under experimental conditions are typically far greater than doses under natural conditions, and the greater the infectious dose, the lower the survival time (Miller et al., 2012). However, if the infectious dose under experimental and natural conditions were the same, the clinical course in free-ranging cervids would probably be shorter than that of captive cervids because the former are also confronted with factors that affect their survival such as predation and finding food, water and shelter (Williams and Miller, 2002; Miller et al., 2008).

1.3 APPLICABILITY, OBJECTIVES AND HYPOTHESES

The simplest of the mathematical models for spread of infectious disease assumes that infected and susceptible hosts move and contact each other randomly within an area of fixed size, as chemical molecules in solution would do (Kermack and McKendrick, 1927). This simplification in homogeneous-mixing compartmental models may be adequate for certain host-pathogen systems in which host populations are nearly homogeneous (e.g., Anderson et al., 1992; Mollison et al., 1994; Bansal et al., 2007). However, extensions to this model, which can incorporate age-and-sex specific contact patterns, spatial structure, and even individual heterogeneities are often more useful when investigating complex disease systems, such as those in which transmission occurs through direct animal contact, and through the environment. New approaches such as agent-based and network modelling explicitly focus on capturing the heterogeneous patterns of interaction among hosts that underlie disease transmission (for examples, see Bansal et al., 2007).

In this dissertation, the main goal was to thoroughly describe epidemiologically relevant sources of variability in mule deer sociality. These include group size, spatial association indices, contact rates, and visitation to different environmental sites. Augmenting our knowledge of these host parameters will make data available for dynamic modelling designed to guide control strategies for the transmission of diseases such as CWD. Our data can specifically inform the model as estimates for parameters and as calibration targets.

The objectives and hypotheses for each study were:

1. Chapter 3 –*Infectious disease and grouping patterns in mule deer*.

Objectives:

- Examine whether factors, including presentation of CWD clinical signs, season or year, habitat, sex and age, are useful predictors of grouping behaviour (i.e., deer being grouped or alone).
- Examine if the same factors are useful predictors of group size.
- Quantify levels of sociality by calculating three measures of group size (typical, mean, and median group sizes).

Hypotheses/Predictions:

- The sickness behaviour and social immunity hypotheses predict that CWD will affect mule deer social behaviour; therefore, clinically sick individuals will be more likely to be found in solitude than clinically healthy individuals.
- Cervid group size increases with habitat openness, and varies across seasons; therefore, the largest mule deer groups will happen in winter and in open habitats.

2. Chapter 4 – *Mule deer spatial association patterns and potential implications for transmission of an epizootic disease*.

Objectives:

- Determine whether sex, age, CWD status, spatial overlap, genetic relatedness and time of the year are related to the strength of pairwise proximal associations.
- Test for sex, age and CWD status differences in the temporal stability of proximal associations.

Hypotheses/Predictions:

- Sex, CWD status, home range overlap, genetic relatedness and time of the year will be useful predictors of strength of association. The more genetically related individuals will be more strongly associated. Females and males will be more strongly associated during the mating season. Home range overlap will be positively correlated with association strength in every season, and for all sexes.
- There will be differences in the temporal stability of associations between sex and CWD status classes. The likelihood of females re-associating with other females will be greater than that of re-associating with males. The likelihood of re-association will be affected by the disease status of the associating pairs, but predicting directionality is difficult.

3. Chapter 5 – *Relatedness and group membership shape high-risk interactions for disease transmission among mule deer*.

Objectives:

- Determine whether genetic relatedness, sex, age, CWD-disease status and association strength are useful predictors of high-risk interaction rates among mule deer.
- Determine whether these interactions are reciprocal.
- Determine whether genetic relatedness, sex, age, and CWD-disease status are useful predictors of association strength as defined by group membership.

Hypotheses/Predictions:

- Genetic relatedness will not predict interaction rates, nor association strength.
- Reciprocity of contact rates will vary with sex and age of the interacting deer.

4. Chapter 6 – *Visitation to environmental sites by mule deer in a chronic wasting disease endemic area.*

Objectives:

- Describe the frequency of visits by different sex-and-age classes of mule deer to a variety of environmental site types such as grain spills, waterholes, salt licks, antler rubs, and bedding areas, throughout the year.
- Describe the intensity of mule deer visitation to these sites.
- Describe the frequency of visits in which mule deer contact the environment in a way that can transmit the disease at the previously mentioned environmental site types.

Hypotheses/Predictions:

- As grain is a highly attractive source of nutrients, we anticipate grain spills to be the most visited sites by every sex-and-age class of mule deer.
- Adult females and fawns will visit water sources, beds and browse areas more frequently during fawning season to increase offspring survival.
- Males will visit rubs more frequently during pre-rut to leave territorial marks to communicate with other deer in the same area in preparation for rut season.
- As we expect large group sizes in each picture taken at grain sources, these will be the most intensely visited sites of all, and where the highest frequencies of contacts with the environment occur.

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CHAPTER 2. GENERAL METHODS

Our animal handling protocol was approved by the University of Saskatchewan's Animal Research Ethics Board (Permit number 20050135) and adhered to the Canadian Council on Animal Care guidelines for humane animal use. Permits to capture and handle deer were obtained from Saskatchewan Ministry of Environment. Permission to conduct research on private land within the study area were obtained verbally from land owners. Permission to conduct research within the Cabri Regional Park (GPS: 50.66824 -108.26791) was obtained from The Saskatchewan Regional Parks Association.

2.1 STUDY AREA AND STUDY POPULATION

Antelope Creek (50.66°N, 108.27°W at center) is a 258 km^2 area in rural southern Saskatchewan, Canada. The north boundary adjoins the South Saskatchewan River and is characterized by several creek valleys that create a network of coulees, ravines and draws of natural vegetation surrounded by agricultural land that extends to the south (Acton et al., 1998). The most common habitat is crop (46.6%), followed by grassland (35.6%), shrub (7.8%), mixed grassland and shrub (7.6%), woodland (2%) and open water (0.3%).

This study area is located within the mixed grassland ecoregion which is characterized by a semiarid climate (Acton et al., 1998) with mean extreme minimum and maximum temperatures of -34.16°C and 35.1°C, respectively (Government of Canada, 2014). Its soil is predominantly clay and clay loam (College of Agriculture, 1923). This area has a variety of grasses, forbs, shrubs, annual crops and perennial forage crops (see Table 2-1). Very little forest is present, with only small patches of creeping juniper (*Juniperus horizontalis*), chokecherry (*Prunus virginiana*), and prickly rose (*Rosa acicularis*) (Thompson and Hansen, 2001; Silbernagel, 2010). The prevailing type of agriculture is grain farming (Saskatchewan Ministry of

Agriculture, 2012). Mixed farming practices, with diversification of crops and the keeping of livestock, have been in place for several decades in this area (College of Agriculture, 1923).

Table 2-1. Common and scientific names of common plants in Antelope Creek.

Grasses	
Wheatgrass	*Agropyron spp*
Needle-and-thread	*Stipa comata*
Blue grama grass	*Bouteloua gracilis*
June grass	*Koelaria macrantha*
Forbs	
Pasture sage	*Artemisia frigida*
Moss phlox	*Phlox hoodii*
Shrubs	
Snowberry	*Symphoricarpos*
Wolf willow	*Eleagnus commutata*
Annual crops	
Barley	*Hordeum vulgare*
Durum wheat	*Triticum durum*
Wheat	*Triticum spp*
Oats	*Avena sativa*
Canola	*Brassica napus*
Peas	*Pisum sativum*
Lentils	*Lens culinaris*
Perennial forage crops	
Alfalfa	*Medicago sativa*

The population of free ranging Rocky Mountain mule deer (*Odocoileus hemionus hemionus*) in the study area was estimated to range from 322 to 422 mule deer in 2007 and 2009 (unpublished data), and was mostly (67% of adults) non-migratory (Skelton, 2010). Their predominant predators are coyotes (*Canis latrans*) and humans (*Homo sapiens sapiens*), as black bears (*Ursus americanus*) and gray wolves (*Canis lupus*) have been extirpated from the area. Rocky Mountain mule deer and white-tailed deer (*Odocoileus virginianus*) have been recognized as infected with chronic wasting disease (CWD) in this area since 2002, and Antelope Creek is part of wildlife management zone 13. Efforts to control the spread of CWD among free-ranging cervids have included culling and preclusion of cervid translocation (Alberta Prion Research Institute et al., 2011). Despite these efforts, the prevalence of CWD in adult mule deer increased from 0.98% (20/2046) in 2004 to 6.5% (16/246) in 2009 (unpublished data).

2.2 CAPTURE

For this study, we captured ≥ 8 month old deer at various years using a helicopter and net-gun (Webb et al., 2008), or less frequently, Clover traps (Clover, 1954) (Table 2-2). Upon capture, we chemically immobilized them with an intramuscular injection of tiletamine-zolazepam (Telazol®, Zoetis) and xylazine (Rompum®, Bayer) at 2:1 ratio = 3 to 4 mg/kg of tiletamine-zolazepam at a concentration of 258 mg/ml + 1.5 to 2 mg/kg of xylazine. Fawns were hand captured at birth as part of a concurrent study (for details, see Perera, 2012) (see Table 3-1 as well).

Table 2-2. Annual numbers of ≥ 8 month old mule deer captured with helicopter and net-gun or Clover traps in Antelope Creek.

Capture year	Capture dates	Helicopter		Trap		Total captured
		New	Recaptured	New	Recaptured	
2007	March 13 and 14, May 9	52				52
2008	March 24, April 9 and 12			7		7
2009	January 20 to March 9	31	8	26	3	68
2010	February 19 to 25	63	44			107
2011	March 19 to 23	65	40			105
2012	March 20 to 23		41			41
Total		211	133	33	3	380

All these deer were captured and fitted with a new collar. Some deer were captured and collars were removed without replacement (3 in 2009, 1 in 2010, 13 in 2011, and 27 in 2012), but those are not included in this table. Helicopter = deer captured using helicopter and net-gun, Trap = deer captured using a Clover trap, New = deer captured for the first time, Recaptured = deer that were previously captured.

We took samples of saliva, feces, hair, and blood from each captured deer. We recorded body measurements, weight, sex, and age (based on the eruption and wear patterns of mandibular teeth (Jensen, 1996). We also took biopsies of ear auricle for genetic analyses, and of tonsils and in some cases rectal mucosa (only taken from males and non-pregnant females) for CWD diagnosis.

Each individual was ear-tagged and fitted with a radio collar. We tagged one ear with a metallic yellow ear tag with a unique number (Kurl-lock #2 metal tags by Ketchum, Ontario, Canada), and the other ear with a plastic ear tag with a unique number-color combination and a contact phone number (Reyflex tags by Ketchum, Ontario, Canada). Also, on each radio-collar we attached two or four plastic tags (Ritchey Livestock ID, Colorado, USA) creating a unique color-position combination for each deer (Figure 2-1). We used both GPS (global positioning system) and VHF (very high frequency) radio-collars during the study (Table 2-3 and Table 2-4). GPS collars included models 7000, 4400 and 3300 (Lotek Wireless, Ontario, Canada), and were mostly programmed to automatically take fixes every two hours (with a few deer equipped for fixes every 1 or every 4 hours). The GPS collars also had a VHF transmitter (also called VHF beacon) for directly tracking animals but it was turned off during the night to extend the battery life. When the collar has not moved for 8 hours, a switch is activated causing the VHF signal to be heard in the receiver at a markedly increased speed, or pulse rate, than a “live” signal. The collars did not have programed or remotely activated drop-off features so we needed to plan on re-capturing collared deer before the end of the batteries lifetimes (both main battery and VHF beacon battery). The GPS 4400 and 7000 collars are equipped with UHF (ultrahigh frequency), so the data could be downloaded remotely while the collar was still on the deer. The GPS 3300 stored all the data on board for the duration of deployment and data was collected at the end of the use of the collar. The VHF (Lotek Wireless, Ontario, Canada) collars need to be located directly using an antenna and receiver either on foot or by aircraft or by other vehicles appropriately equipped. These collars also transmitted a mortality signal after remaining motionless for 8 h. Yearlings (8 to 21 months old) were fitted with a VHF collar (except in very few cases in 2007) and adults (>21 months old) were fitted with either a GPS or a VHF collar. VHF collars were either expandable (with an elastic band) or rigid and not expandable, while GPS collars were all rigid and not expandable. We added an insert made of foam encased in strong fabric to rigid collars for males to allow for neck growth during the rut. When a mortality signal was heard during tracking, the location was recorded and then the collar and/or carcass were located as soon as possible.

Figure 2-1. Example of a uniquely identified individual.

This female mule deer was uniquely identified based on the type of radio-telemetry collar (GPS 7000), the position and color of the plastic tags attached to the collar (white – dark blue), the color of the ear tag (light green), and the unique radio-frequency.

After sampling, tagging and collaring each captured deer, we chemically reversed the anesthesia with an intramuscular injection of atipamezole (Antisedan®, Pfizer; 1 mg of atipamezole per 8-12 mg of zylazine used). Deer were released as close to the capture point as possible. We intensively monitored collar signaling for two weeks after capture to detect early mortalities or movements outside the study area. We did a post-mortem examination to determine cause of death.

2.3 IDENTIFYING DEER

We had various combinations of clues to uniquely identify study deer in the field. These included: radio-frequency, radio-collar type (GPS, VHF; elastic or rigid; foamy insert in male collars), combination of plastic ear tag color and position and colors of collar tags, and body size and shape (Figure 2-1). All these features could be distinguished at a distance. For future studies, it is recommended that the GPS battery surface be marked with the deer ID using a permanent marker.

Table 2-3. Captured mule deer per year, by collar type and sex and age class. Years 2007, 2008, 2009 and 2010.

Capture year	2007			2007 Total	2008		2008 Total	2009				2009 Total	2010					2010 Total
Collar type	33	44	V		44	Ve		33	44	V	Ve		33	44	70	V	Ve	
Adults	8	12	12	32	2		2	29	18	10		57	5	11	9	53		78
Females	2	4	6	12	1		1	17	10	3		30	5	8	4	23		40
Males	6	8	6	20	1		1	12	8	7		27		3	5	30		38
Yearlings	3	6	11	20		1	1			5	6	11					29	29
Females	3	3	4	10						5		5					14	14
Males		3	7	10		1	1				6	6					15	15
Total	11	18	23	52	2	1	3	29	18	15	6	68	5	11	9	53	29	107

Table 2-4. Captured mule deer per year, by collar type and sex and age class. Years 2011 and 2012.

Capture year	2011					2011 Total	2012				2012 Total	All years' total
Collar type	33	44	70	V	Ve		33	44	70	Ve		
Adults	24	28	6	16		74	9	20	4	7	40	283
Females	8	14	2	10		34	3	9	1	6	19	136
Males	16	14	4	6		40	6	11	3	1	21	147
Yearlings				8	23	31				1	1	93
Females				8	7	15				1	1	45
Males					16	16						48
Total	24	28	6	24	23	105	9	20	4	8	41	376

NOTES: Collar types were 33 (GPS 3300), 44 (GPS 4400), V (VHF), or Ve (elastic VHF). Fawns were all fitted with a Ve.

2.4 CWD DIAGNOSIS

For live animals, we used immunohistochemistry (IHC) on palatine tonsil and rectal biopsies obtained during capture to classify the CWD status of yearlings and adults into one of three categories: negative (no immunolabeling in at least 5 lymphoid follicles), positive (immunolabeling in any number of lymphoid follicles), or inconclusive (fewer than 5 lymphoid follicles in the sample) (Schreuder et al., 1998; Wild et al., 2002). A minimum of 5 lymphoid follicles in the sample were required to ensure >95% probability of an accurate test (Geremia et al., 2015). By using this criterion, we reduced the chance of misclassifying a deer as negative due to repeated sampling or increasing age (Thomsen et al., 2012; Geremia et al., 2015). When the diagnosis was inconclusive, re-cuts of the tonsil and in some cases of rectal biopsies were tested until a final diagnosis was achieved. Inconclusive deer were considered negative for all the years previous to the CWD negative result. For dead animals, IHC was performed on portions of obex, palatine tonsil and/or retropharyngeal lymph node. If samples from these could not be retrieved (e.g., carcass too autolyzed or head missing), we used spinal cord tissue for the diagnosis.

2.5 DEFINING STUDY PERIODS

A study year ran from 1 April of one year to 31 March of the next year, and was divided into 5 distinctive seasons (Table 2-5).

Table 2-5. Seasons used in the study as defined according to mule deer biology and weather of the study area.

Season name (abbreviation)	Start and end dates	Description
Late gestation (LG)	1 Apr-15 May	Males separate from females and fawns, and form bachelor groups (the largest in the year). The frequency of cold temperatures (0 to 10°C) starts to decrease.
Fawning (F)	16 May-31 Jul	Smallest groups in the year. Fawns from the previous year start separating from their mother's group. Females give birth in synchrony, isolating themselves to give birth. Ambient warm temperatures (24 to 29°C) reach their highest point of the year.
Pre-rut (PR)	1 Aug-31 Oct	Females without fawns are in small groups. Mothers with fawns start to allow other sex and age classes to join them. More frequent male-male interactions in preparation for rut. Warm ambient temperatures start to decrease.
Rut (R)	1 Nov-15 Dec	Females enter oestrus. Males become more active to gain breeding access. Freezing ambient temperatures (-10 to 0°C) are the most common.
Early gestation (EG)	16 Dec-31 Mar	The largest mixed-sex herds of the year are formed. Largest female groups of the year. Snow-fall peaks and the ambient temperatures are the lowest of the year (below -10°C).

Information obtained from Anderson et al. (1984), Silbernagel et al. (2011) and Canadian Climate Normals from 1981 to 2010 (Government of Canada).

2.6 GENETIC ANALYSIS

Genomic DNA was extracted from ear biopsies of most captured individuals. We genotyped each sample at 16 microsatellite loci following Cullingham et al. (2011). Samples with ≥3 missing loci were discarded. Pairwise relatedness measures were estimated for 211 mule deer in SPAGeDi version 1.4 (Hardy and Vekemans, 2002) using the estimator of Queller & Goodnight (1989). This genetic relatedness coefficient (range from -1 to 1) is an unbiased estimate of relatedness based on the population's allele frequencies. A positive value indicates that a pair is more related, and a negative value indicates that a pair is less related, than average for the sampled population (Queller and Goodnight, 1989).

2.7 CAMERAS

To record the presence of mule deer at different sites within the study area, we deployed a system of triggered-by-movement cameras from July 2009 to December 2012 at 8 site types: anthropogenic food sources, antler rubs, beds, browse areas, mule deer mortalities, salt licks, trails and waterholes (Table 2-6). We attached the cameras to a metallic camera tripod to place them between 90 and 110 cm above the ground, with optical axis parallel to the ground, to best capture the entire body of a mule deer. For details on how the cameras were programmed, see Chapter 6. A station was defined as a camera deployed in a unique combination of location (Cartesian coordinate of easting and northing), site type and season. We retrieved pictures, replaced batteries and memory card, and ensured the camera was functioning properly, every 7 to 14 days. Pictures were downloaded and stored in a computer, and the picture´s metadata, including dates, site type, location and number of pictures with deer, were entered in a Microsoft Access 2013 database (Microsoft Corporation).

Table 2-6. Site types monitored with trail cameras in Antelope Creek from July 2009 to December 2012.

Site type (abbreviation)	Description
Anthropogenic food source (grain source)	A highly concentrated source of food created by humans (either intended to attract wildlife or not) that is visited by deer. Can be spilled grain, bale stacks or grain piles. Available all year, but are more common during early gestation, and rare during fawning.
Anthropogenic mineral source (salt lick)	Artificial mineral (salt) block placed on the ground by ranchers for their cattle. Available all year, but the majority during pre-rut.
Anthropogenic water source (waterhole)	A body of non-running water. More than 70% of the monitored sites were dugouts (man-made); the rest were natural wetland areas. Available all year, but the majority during pre-rut and fawning.
Antler rub (rub)	Any spot where deer rub their antlers and heads on trees, branches or posts. Antler rubs carry olfactory cues and signal presence or hierarchical status (de Vos, 1967). Only available during pre-rut, rut and early gestation.
Bed	Areas that deer use for resting. Anywhere with visible signs of deer bedding activity such as small areas with depleted or patted down vegetation. Usually found in shrub areas or on open hillsides.
Browse	Anywhere a deer may browse on low or tall shrubs. Available all year.
Mortality	Found mule deer carcasses from another ongoing study involving radio-collared deer (Silbernagel et al., 2011). Available all year, but mainly in early gestation and rut.
Trail	A well-travelled path that deer frequently use. Available all year.

See Appendix 6.1 for pictures.

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CHAPTER 3. INFECTIOUS DISEASE AND GROUPING PATTERNS IN MULE DEER

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INFECTIOUS DISEASE AND GROUPING PATTERNS IN MULE DEER

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3.2 ABSTRACT

Infectious disease dynamics are determined, to a great extent, by the social structure of the host. We evaluated sociality, or the tendency to form groups, in Rocky Mountain mule deer (*Odocoileus hemionus hemionus*) from a chronic wasting disease (CWD) endemic area in Saskatchewan, Canada, to better understand factors that may affect disease transmission. Using group size data collected on 365 radio-collared mule deer (2008-2013), we built a generalized linear mixed model (GLMM) to evaluate whether factors including CWD status, season, habitat and time of day, predicted group occurrence. Then, we built another GLMM to determine factors associated with group size. Finally, we used three measures of group size (typical, mean, and median group sizes) to quantify levels of sociality. We found that mule deer showing clinical signs of CWD were less likely to be reported in groups than clinically healthy deer after accounting for time of day, habitat, and month of observation. Mule deer groups were much more likely to occur in February and March than in July. Mixed-sex groups in early gestation were larger than any other group type in any season. Groups were largest and most likely to occur at dawn and dusk, and in open habitats, such as cropland. We discuss the implication of these results with respect to sociobiology and CWD transmission dynamics.

3.3 INTRODUCTION

The rate and pattern of information and pathogen spread within a population depends on its social structure (Godfrey, 2013; Brent, 2015). Grouping patterns are useful to describe social structure (Whitehead, 2008) and have implications for disease spread (Nunn et al., 2015b). Here, we investigate whether chronic wasting disease (CWD) infection influences grouping patterns in a free-ranging mule deer (*Odocoileus hemionus hemionus*) population. We determine factors that predict group size and occurrence, and describe group size distribution to provide empirically derived parameters for CWD transmission models.

CWD is a fatal, neurodegenerative, contagious prion disease that affects mule deer, white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*) and moose (*Alces alces*) in North America. CWD has a long incubation period (about 17 months) during which infectious prions are shed in saliva, urine and feces (Mathiason et al., 2009). Transmission occurs by direct animal to animal contact or by contact with prion contaminated environments (Miller et al., 2004). Although rates of shedding are unknown, they are assumed to be greatest during the later clinical phase of disease, which lasts from a few weeks to around 4 months (Williams and Miller, 2002). Changes in social behaviour of the host (e.g., interactions with other deer) at the early stages of clinical disease are very subtle and have only been mentioned by animal handlers familiar with captive individuals (Williams and Young, 1993). These, and other behavioural changes such as stereotypic movements (repetitive head tossing and exaggerated gait movements), diminished alertness, hyperphagia and polydipsia (Mathiason et al., 2009), can probably be attributed to lesions on certain regions of the brain caused by the accumulation of disease-associated prion protein (Williams and Young, 1993).

CWD is a serious concern for wildlife management agencies for various reasons. It has caused considerable economic, ecological, and cultural impact (Bishop, 2004), and poses a potential risk for human and livestock health if it eventually crosses the species barrier (Barria et al., 2014). Moreover, control efforts to date have not been successful, resulting in continued geographic spread. As disease prevalence increases, herds of cervids infected with CWD could be extirpated (Gross and Miller, 2001). A better understanding of transmission dynamics is needed to develop well-informed epidemiologic models and effective control strategies. However, there are several sources of complexity when modeling transmission dynamics of CWD. These include seasonal movement patterns of the host, habitat selection, contamination and persistence of prions in the environment, and transmission through individual contacts governed by cervid sociality (Silbernagel et al., 2011; Potapov et al., 2012).

Since social animals transfer both information and pathogens by means of relationships, the probability of becoming infected may increase in larger groups, and risk of infection could therefore be seen as a cost of sociality (Alcock, 2009). However, empirical evidence for this feature is mixed (Hughes et al., 2002; Nunn et al., 2015b), and the relationships between epidemiological and ecological processes is intricate (Kurvers et al., 2014). For example, group size is positively correlated with increased prevalence (percentage of infected hosts) and intensity (number of parasites in each infected host) of contact-transmitted parasites in a variety of species (Côté and Poulin, 1995). Yet, infection rates may (Otterstatter and Thomson, 2007) or may not be (VanderWaal et al., 2013) explained by measures of social connectivity such as network density and clustering coefficients. Equally important is that social behaviours that restrict the spread of pathogens have evolved in animal populations and have consequences on pathogen transmission dynamics (Loehle, 1995). These behavioural strategies can be noted in both infected and healthy individuals (Hart, 1988; Bouwman and Hawley, 2010; Butler et al., 2015).

Host group size is an important factor in various measures of disease transmission. For instance, the probability of a pandemic occurring, the average number of groups infected by the initial group, and the proportion of the population infected over the course of an epidemic, depend on group size, among other factors such as the rates of mixing among groups, and length of infectious period (Cross, 2005). The long incubation period of CWD (Williams and Miller, 2002) adds complexity to epidemiological processes, as during the majority of this time deer are capable of directly transmitting the disease and shed prions into the environment (Gough and Maddison, 2010), where prions remain bioavailable for at least 2.5 years (Miller et al., 2004).

As noted by Potapov et al. (2013) and Oraby et al. (2014), validity of CWD transmission model outcomes can depend on the level of detail of the data on deer social behaviour and dynamics of prions in the environment that are used for parameter estimation. Failure to understand factors affecting social behaviour limits the applicability and introduces error into disease models designed to inform and guide control strategies. Many important aspects of mule deer social behaviour have been described (Kucera, 1978; Bowyer et al., 2001; Lingle, 2001, 2003). Mule deer are variably gregarious, showing a great frequency of solitary individuals (up to 64%) and small groups (60.8% to 78% of groups with ≤5 deer) (Kucera, 1978; Bowyer et al., 2001). Group size and distribution of mule deer vary seasonally and with habitat conditions (Bowyer et al., 2001; Lingle, 2003). Even though group size increases with habitat openness and with distance from closed habitats, it is not affected by habitat patch size, preferred forage availability, closeness to water sources, or terrain steepness and ruggedness (Bowyer et al., 2001). With respect to group stability, both cohesiveness of groups with mule deer fawns, and frequency of associations between fawns increase over winter (Lingle, 2003). It is not yet known how CWD infection affects mule deer grouping patterns after accounting for factors such as sex, age, season, time of day, and habitat. This is probably why current CWD models, no matter how complicated or simple they are, either rely on simplified dynamics of mule deer sociality (Potapov et al., 2013; Oraby et al., 2014) or do not attempt to account for social structure (Miller et al., 2000; Wasserberg et al., 2009; Almberg et al., 2011).

We hypothesized that CWD infection affects mule deer social behaviour, thereby clinically sick individuals would be more likely to occur in solitude than clinically healthy individuals. We also anticipated that group size would vary across seasons and habitats, so that the largest groups would happen in winter and in open habitats, as previously reported in other geographical locations (Kucera, 1978; Bowyer et al., 2001; Lingle, 2003). To investigate these hypotheses, we analyzed grouping patterns of a mule deer population in a CWD endemic area. Our objectives were to 1) examine whether factors, such as season, habitat, and presentation of CWD clinical signs, were associated with deer being grouped or alone, 2) examine if the same factors were associated with group size, and 3) quantify levels of sociality by calculating three measures of group size (typical, mean and median group sizes).

3.4 MATERIALS AND METHODS

3.4.1 Study population

The study was conducted at Antelope Creek (50.66°N, 108.27°W) in south Saskatchewan, Canada, between 2007 and 2013. This area includes a 258 km^2 rural area within the mixed grassland ecoregion. The most common habitat is crop (46.6%), followed by grassland (35.6%), shrub (7.8%), mixed grassland and shrub (7.6%), woodland (2%) and open water (0.3%). The study population was composed of free ranging Rocky Mountain mule deer, with 67% of adults being non-migratory (Skelton, 2010). As black bears (*Ursus americanus*) and gray wolves (*Canis lupus*) have been extirpated from the area, coyotes (*Canis latrans*) and humans (*Homo sapiens sapiens*) are mule deer's only predators. First recognized in Antelope Creek in 1996, CWD prevalence in adult mule deer increased from 0.98% (20/2046) in 2004 to 6.5% (16/246) in 2009 (Canadian Cooperative Wildlife Health Centre, 2011).

3.4.2 Data collection

This study was approved by the University of Saskatchewan's Animal Research Ethics Board (Permit number 20050135), and adhered to the Canadian Council on Animal Care guidelines for humane animal use. Permits to conduct research within private land of the study area were obtained verbally from land owners. Permit to conduct research within the Cabri

Regional Park (GPS: 50.66824 -108.26791) was obtained from The Saskatchewan Regional Parks Association.

As part of a study of CWD transmission dynamics, we captured and radio-collared mule deer during two time periods each year between 2007 and 2012. In June and July we captured fawns, and between January and April we used helicopter and net guns, or less commonly, Clover traps (Clover, 1954), to capture juveniles and adults (Table 3-1). Captured deer were fitted with either a global positioning system (GPS) or a very-high-frequency (VHF) radio-collar (Lotek Wireless, Ontario, Canada, and Advanced Telemetry Systems, Minnesota, USA). Fawns always received an expandable VHF collar.

Table 3-1. Annual numbers of newly captured (and recaptured) mule deer in Antelope Creek by age and sex class.

	Year of capture						
Age and sex class	2007	2008	2009	2010	2011	2012	Total
Adult	32	4	47 (13)	43 (35)	44 (42)	(61)	170 (151)
Female	12	1	25 (6)	15 (25)	7 (37)	(30)	60 (98)
Male	20	3	22 (7)	28 (10)	37 (5)	(31)	110 (53)
Juvenile	20	3	11	20 (10)	22 (10)	(7)	76 (27)
Female	10	0	5	10 (5)	11 (4)	(3)	36 (12)
Male	10	3	6	10 (5)	11 (6)	(4)	40 (15)
Fawn	0	1	38	41	39	0	119
Female	0	0	20	17	22	0	59
Male	0	0	18	24	17	0	60
Total	52	8	96 (13)	104 (45)	105 (52)	(68)	365 (178)

We classified captured mule deer based on age (adult, juvenile, or fawn), sex (male or female), and CWD diagnosis (positive or negative) (explained in detail in the following section). During each season, of each year, individuals were either tracked once or twice a month. Those tracked twice a month included: all CWD-positive individuals, all CWD-negative individuals of each sex and age class with less than 10 deer, and from the list of CWD-negative deer remaining (adults and juveniles only), we did a stratified by sex and age class random selection to obtain 10 individuals from each stratum (i.e., 10 adult males, 10 adult females, 10 juvenile males, and 10 juvenile females). The remaining individuals were tracked at least once a month. Fawns were tracked with their mothers. To avoid double tracking of the same collared deer, observers targeted different deer within a day (hereafter focal deer). We tracked deer every day from December 2008 to July 2009, then on weekdays until March 2012, and during three days every two weeks until December 2012. During these tracking periods, we recorded for each group encountered: date, time, habitat, number of individuals in the group, sex and age class of every individual in the group, location using a hand-held GPS, and the distance from the observer. We defined a mule deer group as a spatially cohesive and behaviourally coordinated aggregation of deer, in which every deer was within 10 body lengths of at least one other (Bowyer et al., 2001). To consider all social units relevant to the study of social organization, we also defined a solitary deer as a group of 1 (Hirth, 1977; Monteith et al., 2007). When possible, we also recorded groups in which no radio-collared deer was present. We observed deer from an average distance of 257±172 m (± SD). All observers received training on monitoring of mule deer groups and used binoculars (10x42) and spotting scopes (15-45x60).

3.4.3 Factors related to group size and group occurrence

We considered several factors with a potential to influence group size and the occurrence of social groups. These included: time of the day, habitat, year, season, month, group type, observer experience, presence of CWD-positive deer in the group, presence of deer showing clinical signs of the disease, and age, sex and CWD status of the focal deer. The focal deer in a group with more than one collared deer was the individual targeted to be tracked within that day.

We divided the 24-h day into 5 periods: dawn, before solar noon, after solar noon, dusk, and night. Dawn began with the start of civil twilight and ended 90 min after sunrise; before solar noon started with the end of dawn and ended at solar noon; after solar noon started with the solar noon and ended at the start of dusk; dusk started 90 min before sunset and ended at the end of civil twilight; night was the remaining time between two continuous days. The times for the points separating these periods were taken from Swift Current historical data (Time and Date AS, 2014).

Habitat types used by mule deer were: grassland, low shrub, tall shrub, woodland, and crop. Grasslands consisted of herbs (small tender plants lacking woody stems, such as grasses and forbs), low shrub habitat consisted of small woody perennial plants with foliage mass close to the ground and several low-branching stems, tall shrub was habitat with shrub that was significantly higher than low shrub, woodland included spaced trees with canopy coverage of 25% to 60%, and crop contained plants grown to be harvested for agricultural use (Strahler and Strahler, 1976). Any other habitat was assigned the class “other”. Observations of deer in tall shrub habitat were excluded from analysis and descriptive statistics because some deer may have been overlooked in this densely vegetated habitat. Observations of deer fleeing from a location of cover (flushed deer) were also excluded, as it is difficult to accurately count and classify all deer in a group under such circumstances.

We divided the year into 5 seasons (Silbernagel et al., 2011): early gestation (16 Dec to 31 Mar), late gestation (1 Apr to 15 May), fawning (16 May to 31 Jul), pre-rut (1 Aug to 31 Oct), and rut (1 Nov to 15 Dec) (Table 2-5 in page 36). To identify sex and age classes of mule deer within a group we used a key (Appendix 3.1). We defined fawns as individuals from 0 to ~9 months old, juveniles from 9 to ~21 months old, and adults from 21 months and older. For both sexes, juveniles were slender and had a narrower face than adults. In contrast to juvenile males, antlers in adult males were branched, had a large base girth and spread wider than the ears.

We classified groups into 7 types according to their composition: solitary male (1 adult male or juvenile male), solitary female (1 adult female or juvenile female), group of males (≥2 adult males or juvenile males; can include fawns of any sex, but not juvenile or adult females), group of females (≥2 adult females or juvenile females; can include fawns of any sex, but not juvenile or adult males), mixed-sex group (at least 1 adult female or juvenile female and 1 adult male or juvenile male; can include any other sex and age class), adult female-fawn/juvenile dyad (2 individuals, 1 is an adult female and the other is a fawn or a juvenile of any sex), and unknown (a group of no other type that contains individuals of unknown sex and/or age class). After collecting data, we classified observers as experienced or inexperienced (observer experience); experienced were those with previous training in deer behaviour.

For each animal over 9 months of age, we used tonsil and rectal biopsies from live animals sampled during capture, and brain or spinal cord tissue from dead animals, to test for CWD with an immunohistochemical method (Wild et al., 2002). We classified collared deer as positives for CWD from day 1 of the month in which the sample was taken (e.g., if a sample taken on Feb 25 was positive, that deer was considered positive since Feb 1). Deer were classified as not positive until the time that they tested positive. Groups were classified as having a known positive when at least 1 collared individual diagnosed as CWD-positive was present in the group. We also recorded that the group had evidence of clinical signs when any deer in a group was showing signs of clinical disease. Clinical signs considered indicative of CWD included drooping ears and head, laterally wide feet stance, hocks touching, and protruding ribs and sometimes ischial tuberosities (Mathiason et al., 2009).

3.4.4 Group occurrence

We built a generalized linear mixed model (GLMM) using the GLIMMIX procedure (SAS, 2006) in SAS v9.3 to identify factors associated with the occurrence of groups. We considered records of groups (the sampling unit) with at least 1 collared deer and in which the focal deer was not a fawn (n = 2173 groups), as they were never seen alone. The final dataset included 188 radio-collared mule deer. In this mixed-effects model, the outcome was group occurrence, a binary variable defined as whether deer were grouped (≥ 2 deer in the group) or alone. The predictor variables examined were: season, month, CWD diagnosis of focal deer, known positive deer in the group, clinical signs of any deer in the group, year, habitat, time of day, observer experience, and age and sex of the focal deer. We treated the person doing the observation and the focal deer as random effects. The GLMM was based on binomial distribution and logit link function. As season and month were correlated, as well as variables focal deer diagnosis, known positive deer in the group, and clinical signs of any deer in the group, we built 6 competing models starting with each possible combination of the correlated variables (Table 3-2). To identify the best set of variables for each competing model, we first tested one model with all eligible candidate variables and manually sequentially removed the variable with the highest P-value until variables with $P < 0.05$ remained (Murtaugh, 2009). We then checked for confounding of predictors that were not significant at $P < 0.05$. If reintroduction of a covariate changed the regression coefficient of other variables of interest by 20% or more, then we considered it as a confounder and kept it in the model regardless of its statistical significance (Sullivan, 2008). We assessed all biologically relevant potential two-way interactions between predictors that were significant as fixed effects in the model. The interaction term was retained in the final model if $P < 0.05$ for the type 3 likelihood ratio test. We examined diagnostic residual plots for extreme outliers to assess model fit. After the best set of predictor variables for each competing model was identified, we chose the model with the smallest AICc (2498.07) as the best model (Table 3-2). We calculated the variance partition coefficients (VPC) to describe the variance associated with each random effect (Dohoo et al., 2009, p. 583).

Table 3-2. Competing models to predict mule deer group occurrence.

Predictor	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6 (best)
Season	XX	XX	XX			
Month				XX	XX	XX
Focal deer diagnosis	XX			XX		
Known positive in the group		XXco			X	
Clinical signs in the group			XX			XX
Time of day	XX	XX	XX	XX	XX	XX
Habitat	XX	XX	XX	XX	XX	XX
Focal deer age	XXco	XXco	XXco	X	XX	X
Focal deer sex	XXco	XXco	XXco	XXco	XXco	XXco
Observer experience	X	X	X	X	X	X
Year	X	X	X	X	X	X
Two-way interactions	XX[a]	XX[b]	XX[c]	X		X
AICc	2544.82	2551.56	2528.99	2507.12	2510.37	2498.07

Predictors marked with X or XX were tested in the initial model. Predictors marked with XX remained in the final model, some as confounders (co). The AICc corresponds to the best set of predictors for that model. Superscripts indicate significant interaction terms: a = season*habitat, and focal deer diagnosis*focal deer age, b = season*habitat, c = season*habitat, and season*clinical signs.

3.4.5 Mean group size

We developed a GLMM with the GLIMMIX procedure (SAS, 2006) in SAS v9.3 using a negative binomial distribution and a log link function to assess factors that influence mean group size. All groups (the sampling unit) with at least 1 collared deer (n = 2195 groups) entered the analysis. The final dataset included 197 radio-collared mule deer. In this GLMM, the predictor variables examined were: season, month, CWD diagnosis of focal deer, clinical signs of any deer in the group, year, habitat, group type, time of day, observer experience, and age and sex of the focal deer. We treated the person doing the observation and the focal deer as random effects to account for unmeasured differences among repeated observers and focal deer. We used the same criteria to build and evaluate the models and to check for confounders and interactions as described in the GLMM for group occurrence. As season and month were correlated, as well as focal deer diagnosis and clinical signs of any deer in the group, we built 4 competing models starting with each possible combination of the correlated variables. CWD diagnosis of focal deer and clinical signs were not significant variables ($P > 0.30$). Then, two competing models remained (one with season and one with month). We chose the one with the smallest AICc (7778.73 vs 7810.58) as best model. When doing post hoc pairwise testing, we accounted for multiple comparisons using Scheffé's method. We calculated Lin's concordance correlation coefficient (Lin, 1989) between predicted and observed group sizes to assess model fit. We calculated a mean-count ratio to denote the variance associated to random effects (Rabe-Hesketh and Skrondal, 2012, p. 697).

3.4.6 Characterization of group size

Because group size was highly skewed, we used three descriptive statistics (typical, mean and median group sizes) to adequately characterize the distribution as recommended by Reiczigel and colleagues (Reiczigel et al., 2008). First, we calculated typical group size (TGS) (also called mean crowding (Reiczigel et al., 2008)) as $\sum Gi^2/\sum Gi$, where Gi is the size of the ith group (Jarman, 1974). This metric is less sensitive to the number of records of solitary animals, and it allowed us to calculate the group size as experienced by a member (any member) within a group. In contrast, we also calculated the mean group size (MGS), which provides the number of animals experienced by an outside observer, such as a predator or a researcher. TGS is generally higher, and never lower, than MGS (Whitehead, 2008). We divided the dataset (n = 2656 groups) into two parts, one considering groups with at least 1 collared deer (n = 2195 groups), and the other with groups in which none of the members were collared (n = 461 groups). In Reiczigel et al. (2015), we compared the mean, median, quartiles and frequency distribution of group size in these two parts to look at the representation of small groups and determine viability of calculating TGS. As all comparisons lead to the same conclusion that the group size distribution is the same in the two parts (data not shown), we proceeded with the calculations using all records (n = 2656 group). Group size distribution of our sample is not normally distributed. Then, as the sample TGS is a biased estimate of the population TGS (Reiczigel et al., 2005) and sample mean and sample variance are correlated, the most appropriate choice for confidence intervals (CIs) construction is a bias-corrected and accelerated (BCa) bootstrap procedure (Efron and Tibshirani, 1994). We used this method (with 5000 repetitions) to calculate CIs of TGS. We obtained the MGS and its CIs by a BCa bootstrap with 2000 repetitions (Efron and Tibshirani, 1994; Rózsa et al., 2000). And, finally, we estimated median group size (median hereafter) and calculated the CIs by a method described by Rózsa et al. 2000 (Rózsa et al., 2000; Reiczigel et al., 2008). To identify differences in TGS, mean, and median among years, seasons, months, times of the day, habitats, and group types, we used two-sample comparison tests as justified and described in Reiczigel et al. 2008 [49] (details in Appendix 3.3). All analyses were completed using the freely available software program, QPweb 3.0 (Reiczigel et al., 2013).

3.5 RESULTS

A total of 4987 groups were observed from 16 February 2008 to 28 November 2013; 2810 records remained after removal of those with deer in tall shrub habitat or that were flushed. We limited the data we used from 16 December 2008 to 15 December 2012 (n = 2656) due to small sample sizes before and after these dates. Mean group size was 3.5 (range = 1 to 39, SD = 3.7), typical group size was 7.3 (95% CI = 6.8 to 8.1), and median group size was 2 (95% CI = 2 to 2). Most groups (83.7%) had 5 or fewer individuals [1 (34.7%), 2 (19.4%), 3 (13.7%), 4 (9.8%), and 5 (6.2%)], and groups with >15 deer were rare (2.1%). Most observed groups were mixed-sex (20.1%), followed by groups of males (19.4%), solitary males (19.2%), groups of females (15.4%), solitary females (15.2%), and adult female-fawn/juvenile dyads (5.9%); we could not assign a sex and age class to every deer in the group in 4.8% of the observations.

3.5.1 Group occurrence

The best model predicting the occurrence of groups of at least two deer included these variables: month, clinical signs, time of day, habitat, and focal deer sex, the latter served as a confounder for the effect of habitat. Eight percent of the unexplained variation in group occurrence was explained by the identity of the focal deer, while 6.6% was explained by the person doing the observation. The strength of the associations between these factors and group occurrence is described in Table 3-3. Healthy looking individuals were more likely to be seen in groups than deer showing clinical signs of CWD. The odds ratios for grouping in any given month (except for June and August) were at least 2 ($P \leq 0.002$) times greater than in July. This difference with July was particularly marked in February and March. Mule deer were significantly ($P \leq 0.002$) more likely to occur in groups in the most open habitats (crop and grassland) than in the least open habitats (low shrub and woodland).

Table 3-3. Final multivariable model for the occurrence of groups of mule deer.

This model describes the strength of association with the presence of deer showing clinical signs, month, time of day, habitat, and sex of focal deer (n= 2173 groups).

Variable		OR	95% CI	P-value
Clinical signs				0.0001
Yes [a]	no*	2.8	1.66, 4.65	0.0001
Month				<.0001
July [a]	August	1.1	0.76, 1.64	0.59
	September*	2.8	1.77, 4.44	<.0001
	October*	2.0	1.28, 30.50	0.002
	November*	2.9	1.83, 4.55	<.0001
	December*	3.7	2.24, 6.26	<.0001
	January*	8.3	4.58, 15.17	<.0001
	February*	23.3	9.06, 60.14	<.0001
	March*	15.4	7.40, 32.15	<.0001
	April*	7.1	4.09, 12.44	<.0001
	May*	3.3	2.14, 5.17	<.0001
	June	1.3	0.86, 1.85	0.23
Time of day				0.01
dawn [a]	before solar noon	0.8	0.56, 1.03	0.07
	after solar noon	0.9	0.70, 1.28	0.71
	dusk	1.3	0.96, 1.73	0.09
	night	1.7	0.47, 6.50	0.41
Habitat				<.0001
grassland [a]	crop	1.2	0.79, 1.68	0.45
	other	1.1	0.57, 2.31	0.70
woodland [a]	crop*	4.7	1.80, 12.40	0.002
	grassland*	4.1	1.66, 10.10	0.002
low shrub [a]	crop*	3.0	1.91, 4.75	<.0001
	grassland*	2.6	1.92, 3.54	<.0001
Focal deer sex				0.15
male [a]	female	1.2	0.93, 1.64	0.15

OR = Odds ratio; CI = Confidence interval; a = reference category. Variables with significant ($P < 0.05$) odds ratios marked with *.

3.5.2 Mean group size

The best model predicting group size included the factors season, time of the day, habitat, sex of focal deer, age of focal deer, group type, year, and the interaction terms of season*group type, and season*year. The sex of focal deer was included in the model to adjust for confounding of the effects of habitat and time of day on mean group size. Mean-count ratios were 1.08 for focal deer and 1.1 for observers, indicating a relatively small amount of variability in mean group size among both focal deer and observers. The overall Lin´s concordance correlation coefficient between predicted and observed group sizes was 0.8 (95% CI = 0.7-0.8). Predicted mean group sizes of main effects (except for those included in the interaction terms), with their 95% confidence intervals, are presented in Table 3-4, and their pairwise comparisons with p-values in Table 3-5. Groups were larger at dusk than before (P = 0.05) and after solar noon (P = 0.001). Groups were also smaller after solar noon than at dawn (P = 0.01). Group size increased with habitat openness; groups in crop (the most open habitat) were larger than in grassland (P = 0.002) and low shrub (P = 0.0003). Groups with an adult focal deer were larger than those with a juvenile focal deer (P = 0.03).

Table 3-4. Least squares means for predicted mean group size according to time of day, habitat, sex of focal deer, and age of focal deer.

Variable (P-value)[a]	Estimated mean group size	95% CI
Time of day (P = 0.02)		
night	2.7	2.0, 3.6
dusk	2.6	2.3, 3.0
dawn	2.5	2.2, 2.9
before solar noon	2.4	2.1, 2.7
after solar noon	2.3	2.0, 2.6
Habitat (P = 0.001)		
woodland	2.8	2.1, 3.6
other	2.7	2.3, 3.3
crop	2.7	2.3, 3.1
grassland	2.3	2.0, 2.7
low shrub	2.2	1.9, 2.5
Focal deer sex (P = 0.11)		
female	2.6	2.2, 3.0
male	2.4	2.1, 2.8
Focal deer age (P = 0.02)		
adult	2.8	2.5, 3.2
juvenile	2.6	2.3, 3.0
fawn	2.2	1.6, 2.9

a = P-values for interaction terms are: season*group type $P < 0.0001$, and season*year $P = 0.0004$.

Table 3-5. Least squares means differences for predicted mean group size according to time of day, habitat, sex of focal deer, and age of focal deer.

Variable	Relationship	Variable	P-value
Time of day			
night	>	dusk	0.78
night	>	dawn	0.62
night	>	before solar noon	0.39
night	>	after solar noon	0.21
dusk	>	dawn	0.39
dusk	>	before solar noon	0.046*
dusk	>	after solar noon	0.001*
dawn	>	before solar noon	0.21
dawn	>	after solar noon	0.01*
before solar noon	>	after solar noon	0.20
Habitat			
woodland	>	other	0.93
woodland	>	crop	0.82
woodland	>	grassland	0.17
woodland	>	low shrub	0.06
other	>	crop	0.85
other	>	grassland	0.046*
other	>	low shrub	0.01*
crop	>	grassland	0.002*
crop	>	low shrub	0.0003*
grassland	>	low shrub	0.09
Focal deer sex			
female	>	male	0.11
Focal deer age			
adult	>	juvenile	0.03*
adult	>	fawn	0.052
juvenile	>	fawn	0.20

Significant (≥0.05) p-values are marked with *.

Mixed-sex groups were significantly larger than both female and male groups in every season ($P < 0.001$) (Figure 3-1). The only time in which groups of males were significantly smaller ($P = 0.001$) than groups of females was in early gestation. The size of female groups was larger in early gestation than in the rest of the seasons ($P < 0.01$), except for late gestation ($P = 0.1$). Male groups were significantly larger in late gestation than in pre-rut ($P = 0.04$) and rut (P =

0.03). Mixed-sex groups in early gestation were significantly larger than in the rest of the seasons (P < 0.002), and larger in rut than in fawning (P = 0.02). Groups in 2010 during early gestation (P > 0.001) and late gestation (P = 0.03) were smaller than in the same seasons in 2011. Groups in rut 2011 were smaller than in rut 2010 (P = 0.005) and rut 2012 (P = 0.03).

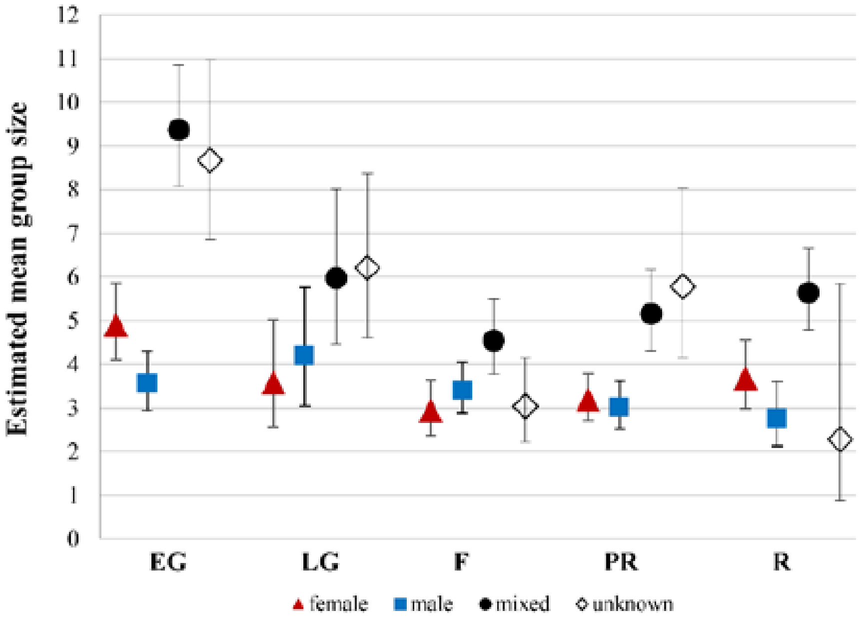

Figure 3-1. Predicted seasonal variation of mule deer mean group size by group type.

Bars are 95% confidence intervals. EG = early gestation, LG = late gestation, F = fawning, PR= pre-rut, and R = rut.

3.5.3 Characterization of group size

We further described the distribution of group sizes using TGS, mean and median (Appendix 3.2). Results from pairwise comparisons can be found in Appendix 3.3. Monthly variation of group size was substantial, and followed a seasonal pattern. Groups were smallest during June and July (fawning) and gradually increased in size until they reached the greatest sizes of the year during February and March (early gestation), then, with the start of late gestation in April, groups rapidly decreased in size (Figure 3-2). Mean and median group sizes at different times of the day followed a very similar pattern: smallest groups before solar noon and largest groups at dusk (Figure 3-3); TGS did not vary across different times of day except when before and after solar noon time periods were combined and then dusk had a significantly larger TGS than this combination (Appendix 3.2 and Appendix 3.3). Groups in low shrub were smaller than those in either crop or grassland (Figure 3-4), so size increased with habitat openness. Mixed-sex groups were larger than single-sex groups, and female groups were larger than male groups (Figure 3-5).

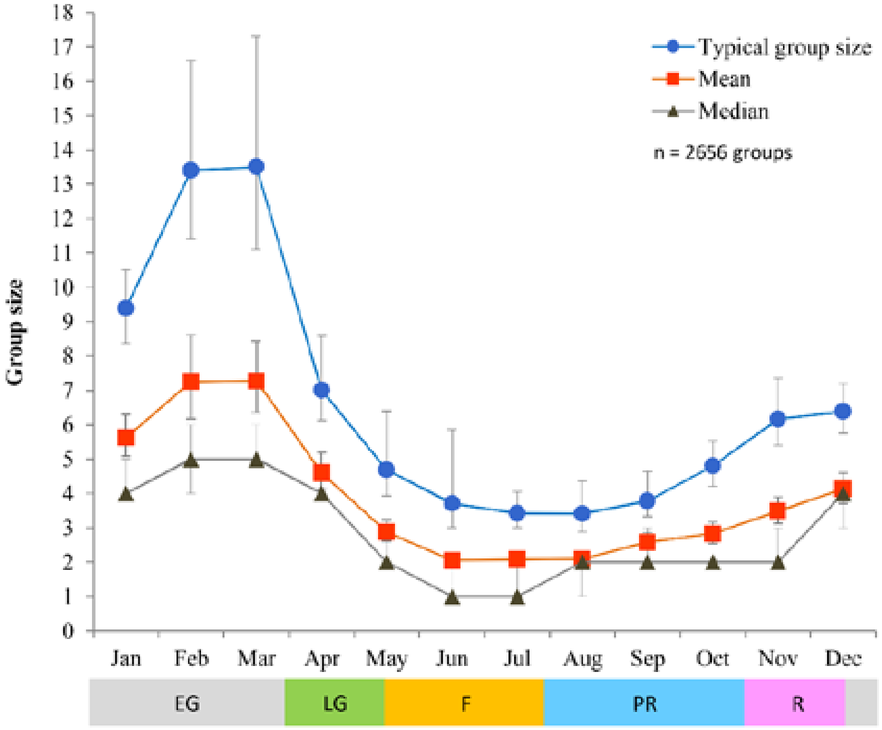

Figure 3-2. Monthly typical, mean and median mule deer group sizes.

In typical and mean group sizes, bars are 95% confidence intervals (CIs); in median group size, CIs range between 95% and 96.2%. See Appendix 3.2 for actual values. EG = early gestation, LG = late gestation, F = fawning, PR= pre-rut, and R = rut.

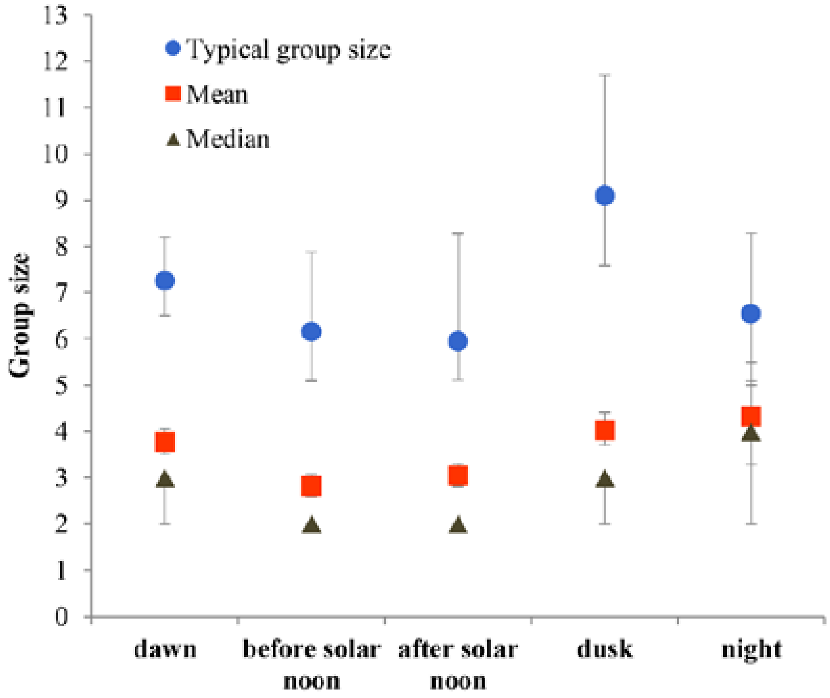

Figure 3-3. Typical, mean and median mule deer group sizes according to time of day.

In typical and mean group sizes, bars are 95% confidence intervals (CIs); in median group size, CIs range between 95% and 96.2%. See Appendix 3.2 for detail results.

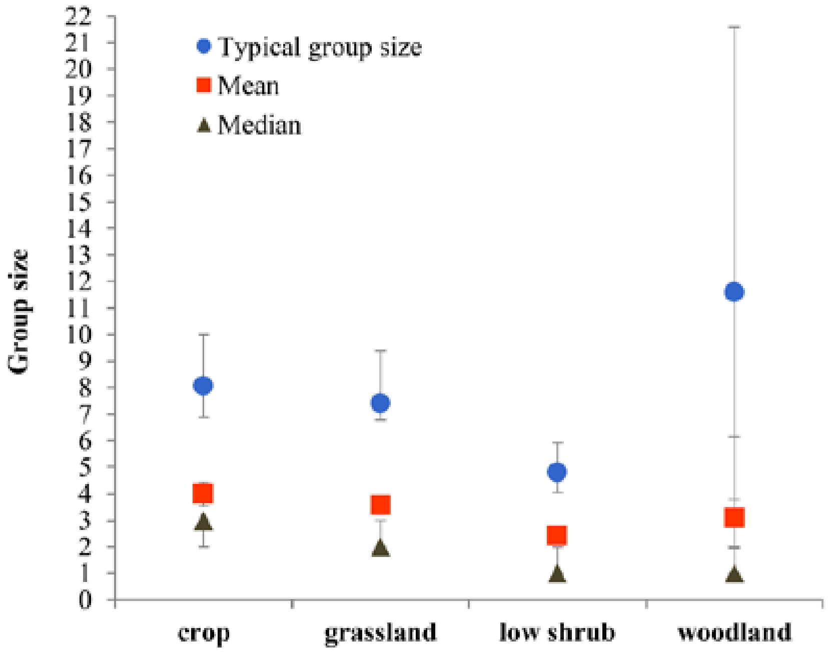

Figure 3-4. Typical, mean and median mule deer group sizes according to habitat.

In typical and mean group sizes, bars are 95% confidence intervals (CIs); in median group size, CIs range between 95% and 96.2%. See Appendix 3.2 for detail results.

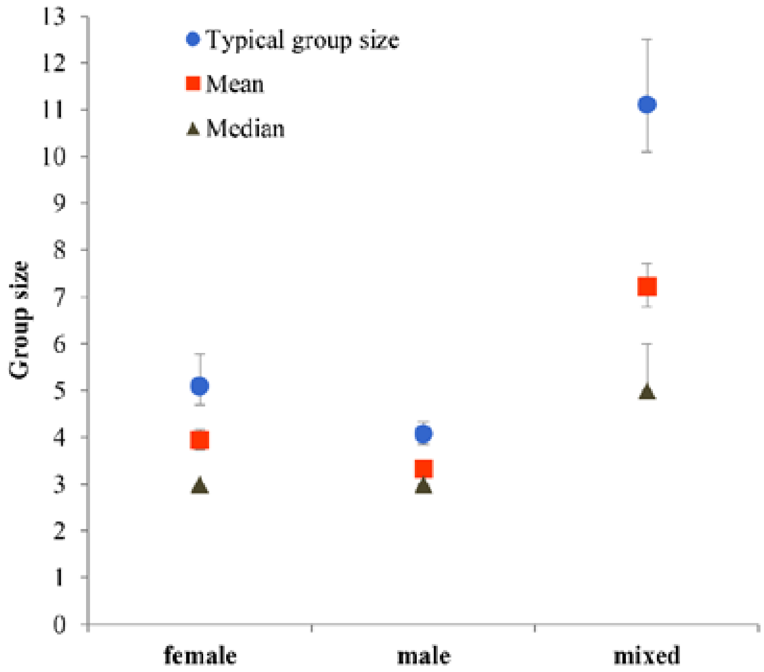

Figure 3-5. Typical, mean and median mule deer group sizes according to group type.

In typical and mean group sizes bars are 95% confidence intervals (CIs); in median group size CIs range between 95% and 96.2%. See Appendix 3.2 for detail results.

3.6 DISCUSSION

CWD, as well as other important infectious diseases in cervids such as tuberculosis and brucellosis, can be transmitted through direct contact between individual animals as well as through the environment. The importance of spatial aggregation of deer, and hence of environmental prions, has been recognized (Almberg et al., 2011), and the relative importance of environmental transmission appears to be stronger than that of direct transmission in theoretical modeling (Almberg et al., 2011; Vasilyeva et al., 2015). However, social structure and behaviour of the host population influence both transmission pathways, having epidemiological implications in the spread of infections (Nunn et al., 2015a). Therefore, disease models designed to inform CWD control strategies should also include information on social behavioural traits, such as group dynamics of mule deer (Potapov et al., 2013; Oraby et al., 2014). In this study we report exhaustive data on mule deer group sizes, and likelihood of group occurrence. In particular, we found that mule deer showing clinical signs of CWD were less likely to be reported in groups than clinically healthy deer after accounting for time of day, habitat, and month of observation.

Based on our study, we cannot identify the mechanisms behind a reduced occurrence of sick individuals in groups. However, there are probable explanations: 1) normal activities daily performed by healthy deer are challenging for sick deer, 2) healthy deer actively avoid grouping with sick deer, and 3) both strategies are occurring. Deer with CWD develop spongiform encephalopathy caused by the accumulation of disease-associated prion proteins (Spraker et al., 2002). This lesion correlates with clinical signs, such as modification of body postures, reduced awareness, and gradual weight loss with terminal anorexia (Bishop, 2004; Mathiason et al., 2009). As there is no recovery from CWD, these clinical signs affect foraging, mating, and parental care (Adelman and Martin, 2009; Monello et al., 2014), and increase the risk of death due to predation (Miller et al., 2008; Krumm et al., 2010) and vehicle collision (Krumm et al., 2005). One would expect that these signs also affect deer's ability to enter and remain in a group. Animals are capable of using behavioural immunity as a defense against contagion (Loehle, 1995), in other words, healthy individuals can show avoidance of infected animals (Behringer et al., 2006; Arakawa et al., 2010; Bouwman and Hawley, 2010). For a healthy deer, the cost of increased risk of infection might outweigh the benefits of socializing with sick individuals (Côté and Poulin, 1995). Whatever the cause, it is clear that presentation of clinical signs of CWD infection is related to mule deer probability of grouping.

It is likely that subtle behavioural changes are apparent to deer and predators, but not to humans, and we do not know at what point in the infectious period deer start showing changes in grouping behaviour. Without applying our findings in a dynamic simulation model, it is not possible to fully appreciate the implications of our findings on CWD transmission dynamics. Although CWD models published to date have not included detailed aspects of deer sociality, they recognized the advantages that this may have (Miller et al., 2006; Potapov et al., 2013). Remarkably, a study on lobsters infected with PaV1 virus has shown that the only way that empirical observations of virus prevalence over time fit simulations is when behavioural immunity is considered in the model (Butler et al., 2015). Adding the information generated by this study on differential grouping likelihoods (i.e., odds of grouping when sick) and group size to current CWD transmission dynamics models should provide new insight into this complex disease.

Similarities of our findings to reports on mule deer in geographical areas free from CWD (California, USA; and Alberta, Canada) include: 1) increase of mean group size with habitat openness (Bowyer et al., 2001), 2) comparable overall mean group size (3.5, SD = 2.1, range = 1 to 40, n = 2639 groups) (Bowyer et al., 2001), 3) smallest groups in fawning and largest in early gestation (Lingle, 2003), and 4) mixed-sex groups larger than groups of females (Bowyer et al., 2001; Lingle, 2003), and female groups larger than groups of males (Bowyer et al., 2001). These previous reports were from more natural landscapes: ranchland dominated by prairie and grassland (Lingle, 2003) and a state park comprised of upland meadows, tree and chaparral (Bowyer et al., 2001). Our findings extend these observations to agricultural lands comprised of ~50% cropland with grassland and shrub confined to a river valley and associated draws and coulees. Our study is also unique in that it describes groups from both the external observers' and the group members' viewpoints using recently developed statistical methodologies (Reiczigel et al., 2005). Comparison of results using the three measures of group size did not reveal many differences, which was expected given that mean group size tends to predict mean crowding (Reiczigel et al., 2008); however, there were differences in results. For example, any given member of a group would experience a more similar group size across different times of the day than an external observer would. Differences in significance between TGS and mean group size occurred for various months. Moreover, a disease-related variable, such as presence of deer showing clinical signs in a group, was a good predictor of group occurrence, but not of group size. These differences highlight the importance of calculating all three measures of group size, as well as investigating group occurrence, to better describe mule deer sociality.

Two trends are well-documented in cervids: group size increases with habitat openness (Lagory, 1986; Gerard and Loisel, 1995; Bender and Haufler, 1996), and group size tends to increase with population density (Putman, 1988), but not always (Semeñiuk and Merino, 2014). In our study area, mule deer have access to a patch-work of different habitat types, and we demonstrated that group size varies with habitat use. Mule deer might group more in open and flat habitats such as cropland simply because they can detect each other more easily (Gerard and Loisel, 1995; Gerard et al., 2002), and also because they are more susceptible to predation in non-rugged terrain unless they form groups to dilute the risk of predation. Groups of mule deer are known to merge with other groups and stand their ground as an anti-predator strategy (Lingle, 2001).

Female groups were estimated to be larger than male groups in early gestation. One possible explanation is that the proportion of males in mixed-sex groups is greater than that of females, which is not the case in our study (data not shown). A complementary explanation would be that contrary to the way males group, adult females are also joined by their fawns, increasing the group size more rapidly than when males form their groups. Also, female groups are larger in early gestation than in any other season; at that age, fawns are with their mother at all times, no longer needing to hide and isolate (Lingle, 2003), and are then counted as part of the group when tracking. In ungulate herds, individuals with greater nutritional requirements (e.g., pregnant and lactating females) often lead individuals to whom social cohesion is more crucial and who have larger incentives to avoid group fragmentation (e.g., juveniles and fawns) (Gueron et al., 1996; Conradt and Roper, 2003; Lingle, 2003; Conradt et al., 2009). In terms of disease transmission, as there are repeated reports of greater prevalence of CWD in adult male mule deer (Grear et al., 2006; Silbernagel, 2010), group size does not explain this observation. However, larger male home range sizes (Silbernagel, 2010), and increased direct contacts between competing males during pre-rut (Karns et al., 2009), may be major determinants in CWD transmission. We also wonder if members of groups of females are at a lower risk than members of groups of males, as in the former group type membership can be more stable even though size is larger (Lingle, 2003; Body et al., 2014). Seasonal and habitat use variation in host social behaviour, such as large group sizes in winter and in open habitats found in our study, may introduce temporal and spatial patterns in disease transmission. For example, brucellosis seroprevalence in elk is higher at feeding sites in late spring (other examples in Altizer et al., 2006; Cross et al., 2007).

Based on our field observations on mule deer, mother's hostility towards young males is evident in late gestation. Males then start migrating to new territories (Hjeljord, 2001), forming groups of males that are larger in late gestation than in pre-rut and rut. It is in pre-rut and rut when males show intense rutting activity characterized by tending of females. Typically, single older bigger males find a group of females, monitor their estral status, mate if appropriate, and then move on to the next group of females; young males spend the time alone or with 1 or 2 conspecifics sometimes awaiting for an opportunity to mate (Vos et al., 1967; Kucera, 1978). Under these circumstances, size of male groups decreases, and if male group fission rates increase, this may be a time of augmented risk of infection for males, as they would become in contact with new individuals.

From our occurrence likelihood, and TGS, mean and median calculations, we found that the largest groups happened in February and March, and the smallest in July. This is not surprising, as similar trends have been previously reported in mule deer (Lingle, 2003). Large winter aggregations are common in social cervids (Lagory, 1986; Bowyer et al., 2001), and are perhaps an accentuation of a strategy for protection against predators, particularly under conditions that would hinder escape (i.e., deep snow terrain) (Hawkins and Klimstra, 1970). Also, in July, female mule deer give birth in synchrony, isolating themselves to give birth and hide their offspring (Vos et al., 1967; Lingle, 2003).

Mule deer are known to move faster (m/min) and to use different habitats at dusk and dawn than at midday (Ager et al., 2003). This can enable deer to find conspecifics more easily, and to group as a consequence (Gerard and Loisel, 1995). In addition, mule deer are known to actively bunch together to defend against coyotes, reducing the risk of predation (Lingle, 2001). Coyote hunts occur more often at dusk, especially in summer and autumn (Lingle, 2000), and are more frequently directed at smaller groups (Lingle, 2001). All these might explain why mule deer were not only more likely to be seen in groups, but also in larger groups, at dusk than before solar noon in our study area, where coyotes are their predominant predator.

In summary, we demonstrated that the presentation of clinical signs of disease affect the probability of an individual to be seen in groups, found factors that influence group size, and described mule deer group size distribution. Our data can serve to complement future modeling and parameter estimation; we believe that well-informed spatially- and behaviourally-explicit epidemiological models can serve as important tools to inform and guide CWD management strategies.

3.7 ACKNOWLEDGMENTS

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3.8 SUPPORTING INFORMATION

Appendix 3.1 Key used to seasonally identify the sex and age classes of mule deer when doing field observations.

Appendix 3.2 Group size measures of free-ranging mule deer observed from 2008 to 2013 in Antelope Creek, Saskatchewan.

Appendix 3.3 Comparisons of typical, mean and median group sizes among month, season, habitat, time of day, group type, and year.

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3.10 APPENDIX 3.1.

Appendix 3.1. Key used to seasonally identify the sex and age classes of mule deer when doing field observations. This file was published as S1 File in Mejía Salazar MF, Waldner C, Stookey J, Bollinger TK. Infectious disease and grouping patterns in mule deer. PLoS ONE. 2016;11(3): e0150830. doi: 10.1371/journal.pone.0150830

The information in this document is based on field observations done on Rocky Mountain mule deer (*Odocoileus hemiounus hemiounus*) in southern Saskatchewan, Canada, from 2009 to 2012 and is adapted from descriptions made by Raymond F. Dasmann and Richard Taber (1956), and by Valerius Geist (1998).

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FAWNING – From May 16th to July 31st (76 days).

This is the easiest season to recognize differences among ages and sexes.

Males (adults and juveniles) have antlers.

Females are either big (adults) or small (juveniles) with no spotted fur.

Fawns are unmistakable (small and with spotted fur).

Adult males (ADM)	Adult females (ADF)
Have antlers that are: · Growing and with velvet. · Branched (≥ 2 main tines). · Base thicker than in JVM. · Spread wider than width of ears. Large body.	Are giving birth à peak around June 15th. Larger body. Mature look.

Adults are ≥2 years and 1 to 2 months old. (24, 25, 26 months; 36, 37, 38 months; and so on).

Juvenile males (JVM)	Juvenile females (JVF)
Have antlers that are: · Growing and with velvet. · Unbranched (only 2 main tines). · Straight. · Base not as thick as in ADM. · Spread narrower than width of ears. Slender and long-legged. Face smaller and narrower than in AD.	Slender and long-legged. Face smaller and narrower than in AD. Look quite young, but without white spots and antlers.

Juveniles are 1 year and 1 to 2 months old. (12, 13, 14 months).

Fawns – males and females
Newly born (peak June 15th). Coat with white spots. Cannot easily tell the difference between sexes. Fawns are 1 to 2 months old.

PRE-RUT – From August 1st to October 31st (91 days).

It is an easy season to recognize differences among ages and sexes.

Males have antlers all the time.

Be careful with ADF and JVF, they start to look similar.

Fawns in August are still unmistakable (spotted), but by September they lose their spots.

Adult males (ADM)	Adult females (ADF)
Have antlers that are: · Fully grown. · Will lose the velvet through the season (no velvet by Oct). · Branched (≥ 2 main tines). · Base thicker than in JVM. · Spread wider than width of ears. Large body.	Are nursing. Larger body. Do not look young at all. Most probably seen with fawns.

Adults are ≥2 years and 2 to 5 months old. (26, 27, 28, 29 months; 38, 39, 40, 41 months; and so on).

JVM	JVF
Have antlers that are: · Fully grown. · Will lose the velvet through the season (no velvet by Oct). · Unbranched (only 2 main tines). · Straight. · Base not as thick as in ADM. · Spread narrower than width of ears. Slender and long-legged. Face smaller and narrower than in AD.	Slender and long-legged. Face smaller and narrower than in AD.

Juveniles are 1 year and 2 to 5 months old. (14, 15, 16, 17 months).

Fawns – males and females
Spotted fur only in August. They lose their spots by September.
In October they get a winter coat that makes them look fluffy and with round faces.
Sometimes the antler buds can be seen in males.
Fawns are between 2 and 5 months old.

RUT- From November 1st to December 15th (44 days).

It is an easy season to recognize differences among sexes.

Males have antlers all the time.

Be careful with ADF and JVF, they look similar.

Fawns are easy to identify depending on the angle of the picture.

Adult males (ADM)	Adult females (ADF)
Massive neck swelling (more pronounced than in JVM). Maybe tarsal glands more stained than in JVM. Have antlers that are: · Grown with no velvet. · Branched (≥ 2 main tines). · Base diameter larger than in JVM. · Spread wider than width of ears. Large body.	Still nursing. Larger body. Most probably seen with fawns and followed by males.
Adults are ≥2 years and 5 to 6.5 months old. (29, 30 months; 41, 42 months; and so on).	

Juvenile males (JVM)	Juvenile females (JVF)
Neck swelling less pronounced than in ADM. May be tarsal glands with lighter staining than in ADM. Have antlers that are: · Grown with no velvet. · Unbranched (only 2 main tines). · Straight. · Base not as large as in ADM. · Spread narrower than width of ears. Their bodies are less slender than in pre-rut, but still more slender than ADM.	Slender and long-legged, but quite similar to ADF. Face smaller and narrower than in AD. They will also be followed by males.
Juveniles are 1 year and 5 to 6.5 months old. (17, 18 months).	

Fawns – males and females
Winter coat that makes them look fluffy and with round faces. Sometimes the antler buds can be seen in males. Fawns are between 5 and 6.5 months old.

EARLY GESTATION – From December 16th to March 31st (105 days).

Difficulties in this season:

Adult and juvenile males will lose their antlers, so they are very similar to females.

Adult and juvenile females are very similar.

Recommendation: compare deer from different pictures before making a final decision.

Adult males (ADM)	Adult females (ADF)
Most of them will lose their antlers by the end of February and beginning of March, thus they may have blood stains on frontal area of the head. If with antlers: same as in rut. Neck swelling decreases, but neck is still wider than in JVM.	Still nursing. Larger body than JV.
Adults are ≥2 years and 6.5 to 10 months old. (30, 31, 32, 33, 34 months; 42, 43, 44, 45, 46 months; and so on).	
Juvenile males (JVM)	Juvenile females (JVF)
Most of them will lose their antlers by the end of February and beginning of March, thus they may have blood stains on frontal area of the head. If with antlers: same as in rut. Neck is not as wide as in ADM.	Slender and long-legged, but quite similar to ADF. Face smaller and narrower than in AD.
Juveniles are 1 year and 6.5 to 10 months old. (18, 19, 20, 21, 22 months).	
Fawns – males and females	
Winter coat makes them look fluffy and with round faces. Sometimes the antler buds can be seen in males. Fawns are 6.5 to 10 months old.	

LATE GESTATION – From April 1st to May 15th (44 days).

This is the most difficult season to recognize differences among ages and sexes (except for fawns).

All age classes start looking similar to the next age class: fawns look quite juvenile (still with short front), juveniles are big (like small adults), adult males are slim (no big neck), and adult females look bigger than juveniles.

Antler buds in fawns look larger (in length mainly), than in juveniles and adults.

NOTE: Important is to notice that all fawns needed to be classified as juveniles, and all juveniles as adults when registering data for group sighting purposes. This is the only season in which this alteration was made.

Adult males (ADM)	Adult females (ADF)
No antlers. Neck is not swollen. No signs of antler shedding (no blood).	Large long faces. They look bigger than juveniles.
Adults are ≥2 years and 10 to 11.5 months old. (34, 35 months; 46, 47 months; and so on).	
Juvenile males (JVM)	Juvenile females (JVF)
No antlers. Just about to became adults, but smaller.	Small adults.
Juveniles are just about to become adults; they are almost 2 years old (1 year and 10 to 11.5 months old).	
Male fawns	Female fawns
Their antlers are growing and even when they are very small, they may look bigger than those in AD and JV males. Face is rounder than in JV.	Any deer that has a fawn face and has no antlers is a female fawn.
Fawns are almost 1 year old (between 10 and 11.5 months old).	

3.11 APPENDIX 3.2.

Appendix 3.2. Group size measures of free-ranging mule deer observed from 2007 to 2013 in Antelope Creek, Saskatchewan.

This file was published as S2 Table in Mejía Salazar MF, Waldner C, Stookey J, Bollinger TK. Infectious disease and grouping patterns in mule deer. PLoS ONE. 2016;11(3): e0150830. doi: 10.1371/journal.pone.0150830

Variable	Number of individuals	Number of groups	TGS	TGS lower CI[a]	TGS upper CI[a]	Mean	Mean lower CI[b]	Mean upper CI[b]	SD[c]	Median	Median lower CI	Median upper CI	Actual %CI[d]
All groups	9177	2656	7.3	6.8	8.1	3.5			3.7	2	2	2	95.0
Time of day													
Dawn	3732	727	7.3	6.5	8.2	3.8	3.5	4.1	3.6	3	2	3	95.6
Before solar noon	1781	636	6.2	5.1	7.9	2.8	2.6	3.1	3.1	2	2	2	95.4
After solar noon	1846	607	5.9	5.1	8.3	3.0	2.8	3.3	3.0	2	2	2	95.6
Dusk	2541	632	9.1	7.6	11.7	4.0	3.7	4.4	4.5	3	2	3	95.6
Night	125	29	6.5	5.1	8.3	4.3	3.3	5.5	3.2	4	2	5	96.5
Day[e]	3627	1243	6.0	5.4	7.1	2.9	2.8	3.1	3.0	2	2	2	95.0
Habitat													
Cropland	1116	282	8.1	6.9	10.0	4.0	3.5	4.4	4.0	3	2	3	95.8
Grassland	6800	1907	7.4	6.8	9.4	3.6	3.4	3.7	3.7	2	2	3	95.0
Woodland	102	33	11.6	3.8	21.6	3.1	1.9	6.1	5.2	1	1	2	96.0
Low shrub	904	373	4.8	4.1	5.9	2.4	2.2	2.7	2.4	1	1	2	95.1
Other	255	61	9.7	6.3	14.7	4.2	3.3	5.9	4.8	2	2	4	96.3
Year													
2009	1743	521	6.2	5.5	7.4	3.4	3.1	3.6	3.1	2	2	3	95.2
2010	3204	995	5.8	5.4	6.4	3.2	3.1	3.4	2.9	2	2	2	95.0
2011	3405	884	9.7	8.5	11.5	3.9	3.6	4.2	4.8	2	2	2	95.0
2012	825	256	5.8	5.1	6.7	3.2	2.9	3.6	2.9	2	2	3	95.4
Season													
Early gestation	3367	534	11.7	10.5	13.3	6.3	5.9	6.8	5.8	5	4	5	95.4
Late gestation	1019	242	6.7	6.0	8.0	4.2	3.8	4.7	3.3	4	3	4	95.9
Fawning	1797	844	3.5	3.2	4.6	2.1	2.0	2.3	1.7	2	1	2	95.4

Variable	Number of individuals	Number of groups	TGS	TGS lower CI[a]	TGS upper CI[a]	Mean	Mean lower CI[b]	Mean upper CI[b]	SD[c]	Median	Median lower CI	Median upper CI	Actual %CI[d]
Pre-rut	1710	693	4.0	3.7	4.4	2.5	2.3	2.6	2.0	2	2	2	95.3
Rut	1284	343	6.2	5.6	6.9	3.7	3.5	4.1	3.0	3	2	3	95.8
Month													
January	1096	195	9.4	8.4	10.5	5.6	5.1	6.3	4.6	4	4	5	95.8
February	908	125	13.4	11.4	16.6	7.3	6.2	8.6	6.7	5	4	6	95.1
March	1135	156	13.5	11.1	17.3	7.3	6.4	8.4	6.7	5	5	6	95.7
April	726	158	7.0	6.1	8.6	4.6	4.1	5.2	3.3	4	4	4	95.1
May	605	210	4.7	3.9	6.4	2.9	2.6	3.2	2.3	2	2	3	95.8
June	795	387	3.7	3.0	5.9	2.1	1.9	2.3	1.8	1	1	2	95.8
July	690	331	3.4	3.0	4.1	2.1	1.9	2.3	1.7	1	1	2	95.0
August	575	275	3.4	2.9	4.4	2.1	1.9	2.3	1.7	2	1	2	95.4
September	516	200	3.8	3.3	4.6	2.6	2.4	2.9	1.8	2	2	3	96.2
October	619	218	4.8	4.2	5.5	2.8	2.5	3.2	2.4	2	2	2	95.5
November	768	221	6.2	5.4	7.4	3.5	3.1	3.9	3.1	2	2	3	96.0
December	744	180	6.4	5.8	7.2	4.1	3.7	4.6	3.1	4	3	4	95.7
Group type													
Adult female-fawn/JV dyad	316	158	2.0	NA	NA	2.0	NA	NA	NA	2	NA	NA	NA
Solitary male group	510	510	1.0	NA	NA	1.0	NA	NA	NA	1	NA	NA	NA
Solitary female group	403	403	1.0	NA	NA	1.0	NA	NA	NA	1	NA	NA	NA
Group of males	1706	515	4.1	3.9	4.3	3.3	3.2	3.5	1.6	3	3	3	95.3
Group of females	1604	408	5.1	4.7	5.8	3.9	3.7	4.2	2.1	3	3	3	95.8
Mixed-sex group	3852	534	11.1	10.1	12.5	7.2	6.8	7.7	5.3	5	5	6	95.4
Group with unknowns	786	128	10.2	8.5	12.4	6.1	5.4	7.0	5.0	4	4	6	95.1

Abbreviations: TGS is typical group size; CI is confidence interval; SD is standard deviation; BCa is biased-corrected and accelerated bootstrap; JV is juvenile. [a] 95% CI for the TGS calculated by the BCa method with 5000 bootstrap replications. [b] 95% CI for the mean calculated by the Bca method with 2000 bootstrap replications. [c] SD obtained from the bootstrap 2-sample *t*-test for comparison of means. [d] When it was not possible to construct exactly 90, 95 or 99% CI, the shortest interval with at least the desired confidence level was selected, and the exact (actual) level is reported. [e] Day was a combination of before and after solar noon periods.

3.12 APPENDIX 3.3.

Appendix 3.3. Comparisons of typical, mean, and median mule deer group sizes by month, season, habitat, time of day, group type, and year. This file was published as S2 File (an Excel file) in Mejía Salazar MF, Waldner C, Stookey J, Bollinger TK. Infectious disease and grouping patterns in mule deer. PLoS ONE. 2016;11(3): e0150830. doi: 10.1371/journal.pone.0150830

Methods

1. To identify differences in typical group size (TGS) among years, seasons, months, times of the day, habitats, and group types, we used two-sample comparison tests in which two samples with nonoverlapping 97.5% CIs are significantly different at $p = 0.05$ level (Reiczigel et al. 2008).
2. To identify differences in mean group size among years, seasons, months, times of the day, habitats, and group types, we used a bootstrap two-sample t test in which a two-tailed P-value is calculated based on 1000 bootstrap replications (Rózsa et al. 2000).
3. To identify differences in median group size among years, seasons, months, times of the day, habitats, and group types, we used Mood´s median test (Reiczigel et al. 2008).

Thorough description of reasons to use these methods are found in Reiczigel et al. 2008.

All analyses were completed using the freely available software program, QPweb 3.0 (Reiczigel et al. 2013).

Results

Are shown in the sections named "year", "season", "month", "time of day", "habitat" and "group type" in this file.

For all results:

TGS = typical group size; Sig = significant difference; Non-sig = no significant difference.

Reported are the p-values resulting from the comparisons. All significant values ($p \leq 0.05$) marked in bold.

Results marked with a star (*) are those that differed in the significance among the three measures (e.g., TGS and mean were significantly different between 2009 and 2011, but not the median, hence median result marked with a star).

YEAR

Compared years		TGS	Mean	Median
2009	2010	Non-sig	0.45	0.46
	2011	Sig	0.02	0.15*
	2012	Non-sig	0.56	0.66
2010	2011	Sig	<0.0001	0.41*
	2012	Non-sig	0.99	1
2011	2012	Sig	0.01	0.58*

SEASON

Compared seasons		**TGS**	**Mean**	**Median**
Early gestation	late gestation	**Sig**	**<0.0001**	**0.01**
	pre-rut	**Sig**	**<0.0001**	**<0.0001**
	rut	**Sig**	**<0.0001**	**<0.0001**
	fawning	**Sig**	**<0.0001**	**<0.0001**
Late gestation	pre-rut	**Sig**	**<0.0001**	**<0.0001**
	rut	Non-sig	0.08	**0.03***
	fawning	**Sig**	**<0.0001**	**<0.0001**
Pre-rut	rut	**Sig**	**<0.0001**	**<0.0001**
	fawning	Non-sig*	**<0.0001**	**0.0002**
Rut	fawning	**Sig**	**<0.0001**	**<0.0001**

MONTH

Compared months		**TGS**	**Mean**	**Median**
January	February	**Sig**	**0.02**	0.63*
	March	Non-sig*	**0.01**	**0.01**
	April	Non-sig	**0.02***	0.49
	May	**Sig**	**<0.0001**	**<0.0001**
	June	**Sig**	**<0.0001**	**<0.0001**
	July	**Sig**	**<0.0001**	**<0.0001**
	August	**Sig**	**<0.0001**	**<0.0001**
	September	**Sig**	**<0.0001**	**<0.0001**
	October	**Sig**	**<0.0001**	**<0.0001**
	November	**Sig**	**<0.0001**	**<0.0001**
	December	**Sig**	**<0.0001**	0.15*
February	March	Non-sig	0.99	0.45
	April	**Sig**	**<0.0001**	0.25*
	May	**Sig**	**<0.0001**	**<0.0001**
	June	**Sig**	**<0.0001**	**<0.0001**
	July	**Sig**	**<0.0001**	**<0.0001**
	August	**Sig**	**<0.0001**	**<0.0001**
	September	**Sig**	**<0.0001**	**<0.0001**
	October	**Sig**	**<0.0001**	**<0.0001**
	November	**Sig**	**<0.0001**	**<0.0001**
	December	**Sig**	**<0.0001**	0.07*
March	April	**Sig**	**<0.0001**	**0.002**
	May	**Sig**	**<0.0001**	**<0.0001**
	June	**Sig**	**<0.0001**	**<0.0001**
	July	**Sig**	**<0.0001**	**<0.0001**
	August	**Sig**	**<0.0001**	**<0.0001**
	September	**Sig**	**<0.0001**	**<0.0001**
	October	**Sig**	**<0.0001**	**<0.0001**
	November	**Sig**	**<0.0001**	**<0.0001**
	December	**Sig**	**<0.0001**	**0.0001**
April	May	Non-sig*	**<0.0001**	**<0.0001**
	June	Non-sig*	**<0.0001**	**<0.0001**
	July	**Sig**	**<0.0001**	**<0.0001**
	August	**Sig**	**<0.0001**	**<0.0001**
	September	**Sig**	**<0.0001**	**<0.0001**
	October	**Sig**	**<0.0001**	**<0.0001**
	November	Non-sig*	**<0.0001**	**<0.0001**
	December	Non-sig	0.19	0.55
May	June	Non-sig*	**<0.0001**	**<0.0001**
	July	Non-sig*	**<0.0001**	**<0.0001**

Compared months		**TGS**	**Mean**	**Median**
	August	Non-sig*	**<0.0001**	**<0.0001**
	September	Non-sig	0.14	0.49
	October	Non-sig	0.83	0.12
	November	Non-sig	**0.03***	0.74
	December	Non-sig*	**<0.0001**	**<0.0001**
June	July	Non-sig	0.82	1
	August	Non-sig	0.77	1
	September	Non-sig*	**0.002**	**<0.0001**
	October	Non-sig*	**<0.0001**	**<0.0001**
	November	Non-sig*	**<0.0001**	**<0.0001**
	December	Non-sig*	**<0.0001**	**<0.0001**
July	August	Non-sig	0.95	1
	September	Non-sig*	**0.003**	**<0.0001**
	October	**Sig**	**<0.0001**	**0.0001**
	November	**Sig**	**<0.0001**	**<0.0001**
	December	**Sig**	**<0.0001**	**<0.0001**
August	September	Non-sig*	**0.001**	**0.0001**
	October	Non-sig*	**<0.0001**	**0.001**
	November	**Sig**	**<0.0001**	**<0.0001**
	December	**Sig**	**<0.0001**	**<0.0001**
September	October	Non-sig	0.21	0.434
	November	**Sig**	**0.001**	0.74*
	December	**Sig**	**<0.0001**	**<0.0001**
October	November	Non-sig	**0.01***	0.193
	December	**Sig**	**<0.0001**	**<0.0001**
November	December	Non-sig*	**0.03**	**0.001**

TIME OF DAY

Compared times of day		TGS	Mean	Median
after solar noon	before solar noon	Non-sig	0.18	**0.026***
	dawn	Non-sig*	**<0.0001**	**0.002**
	dusk	Non-sig*	**<0.0001**	**0.0004**
	night	Non-sig	0.06	0.067
	unknown	Non-sig*	**0.04**	**0.01**
before solar noon	dawn	Non-sig*	**<0.0001**	**<0.0001**
	dusk	Non-sig*	**<0.0001**	**<0.0001**
	night	Non-sig*	**0.02**	**0.01**
	unknown	Non-sig*	**0.04**	**0.001**
dawn	dusk	Non-sig	0.25	0.68
	night	Non-sig	0.38	0.16
	unknown	Non-sig	0.08	**0.03***
dusk	night	Non-sig	0.66	0.23
	unknown	Non-sig	0.13	0.06
night	unknown	Non-sig	0.19	1
day (before and after solar noon, together)	dawn	Non-sig*	**<0.0001**	**<0.0001**
	dusk	**Sig**	**<0.0001**	**<0.0001**
	night	Non-sig*	**0.04**	**0.03**
	unknown	Non-sig*	**0.04**	**0.001**

HABITAT

Compared habitats		TGS	Mean	Median
grassland	low shrub	**Sig**	**<0.0001**	**<0.0001**
	woodland	Non-sig	0.62	**0.003***
	crop	Non-sig	0.13	0.89
	other	Non-sig	0.36	1
low shrub	woodland	Non-sig	0.49	1
	crop	**Sig**	**<0.0001**	**<0.0001**
	other	Non-sig*	**0.02**	**0.002**
woodland	crop	Non-sig	0.39	**0.01***
	other	Non-sig	0.34	**0.02***
crop	other	Non-sig	0.75	0.88

GROUP TYPE

Compared group types		TGS	Mean	Median
female	male	**Sig**	**<0.0001**	**<0.0001**
	mixed	**Sig**	**<0.0001**	**<0.0001**
	unknown	**Sig**	**<0.0001**	0.054*
male	mixed	**Sig**	**<0.0001**	**<0.0001**
	unknown	**Sig**	**<0.0001**	**<0.0001**
mixed	unknown	Non-sig*	**0.04**	**0.03**

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CHAPTER 4. MULE DEER SPATIAL ASSOCIATION PATTERNS AND POTENTIAL IMPLICATIONS FOR TRANSMISSION OF AN EPIZOOTIC DISEASE

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4.1 PUBLICATION AND CO-AUTHORSHIP STATEMENT

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4.1.1 Author’s contributions

Conceptualization: MFMS, TKB, CLW, AWG
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Data curation: MFMS, RGD

Writing-original draft: MFMS
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MULE DEER SPATIAL ASSOCIATION PATTERNS AND POTENTIAL IMPLICATIONS FOR TRANSMISSION OF AN EPIZOOTIC DISEASE

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4.2 ABSTRACT

Animal social behaviour can have important effects on the long-term dynamics of diseases. In particular, preferential spatial relationships between individuals can lead to differences in the rates of disease spread within a population. We examined the concurrent influence of genetic relatedness, sex, age, home range overlap, time of year, and prion disease status on proximal associations of adult Rocky Mountain mule deer (*Odocoileus hemionus hemionus*) in a chronic wasting disease endemic area. We also quantified the temporal stability of these associations across different sex, age, and disease status classes. We used three years of high frequency telemetry data from 74 individuals to record encounters within 25 m of each other, and to calculate seasonal home range overlap measured by volume of intersection (VI). The strength of pairwise spatial associations between adult mule deer was independent of genetic relatedness, age and disease status. However, seasonal variation in association strength was not consistent across years, perhaps due to annual changes in weather conditions. The influence of home range overlap on association strength varied seasonally, whereby associations were strongest in pre-rut at the same level of VI. The sexes of individuals also interacted with both VI and season, such that associations were strongest between females at the same level of VI, and associations between males were the strongest in pre-rut and different-sex associations were the strongest in rut. Our findings highlight the importance of considering concurrent effects of biological and environmental factors when seeking to understand the role of social preference in behavioural ecology and disease spread. Applying this knowledge in epidemiological modelling will shed light on the dynamics of disease transmission among mule deer.

4.3 INTRODUCTION

The likelihood and duration of associations between individuals are influenced by social structure, inter-group mixing and the size and composition of social groups (Cross et al., 2010; Nunn et al., 2015). As well as influencing the transfer of information throughout a population, the properties of such associations can also affect the rate of spread of infectious diseases (Cross et al., 2004). For social species, epidemiological models that assume all hosts have equal probability of association and disease transmission, and that ignore seasonal variation, are no longer considered suitable for the study of complex diseases (Potapov et al., 2013; Schauber et al., 2015). To enhance models to guide disease control, studies are needed to quantify the extent to which individuals choose their associating partners and factors relating to these choices.

Chronic wasting disease (CWD) affects farmed and free-ranging mule deer (*Odocoileus hemionus*) and other cervids in USA and Canada, and, most recently, free-ranging reindeer (*Rangifer tarandus tarandus*) and moose (*Alces alces*) in Norway. It is a fatal, neurodegenerative, contagious prion disease that is expected to reduce mule deer population sizes (Dulberger et al., 2010; Almberg et al., 2011). Furthermore, it is providing extremely difficult to eradicate once established within wild populations (Miller et al., 2006). The complexity of this disease is due to its transmission through both direct and environmental contacts, its lengthy infectious period (>1.5 years), and the persistence of prions in the environment for at least 2.5 years (Williams et al., 2002; Miller et al., 2004). These highlight the need for detailed information on mule deer social behaviour and the dynamics of prions in the environment (Potapov et al., 2013; Oraby et al., 2014) to parameterise dynamic disease models to inform cervid population management programs. The validity of CWD transmission model outcomes is reliant on accurate parameter estimates that describe deer sociality. While there have been relevant studies done on association patterns among female white-tailed deer (Schauber et al., 2007; Schauber et al., 2015) and their home range establishment (Porter et al., 1991), it is important to collect data specific to mule deer and on both sexes.

Several factors are known to relate to how individuals socialise. When associations are defined based on two individuals being in the same area, a correlation between home range overlap and spatial association strength is expected. However, associations are not driven solely by home range overlap, but also by complex preferences and avoidances (e.g., Carter et al., 2013). Kin-biased associations in various taxa respond flexibly to changes in ecological context, such as local demography and resource abundance (Smith, 2014). This is probably why genetic relatedness sometimes correlates (e.g., Carter et al., 2013; Podgórski et al., 2014) and sometimes does not correlate (e.g., Iacolina et al., 2009; Vander Wal et al., 2016) with association patterns. Among cervids, red deer (*Cervus elaphus*) preferentially associate with kin (Albon et al., 1992), while genetic relatedness does not determine social structure of elk (*Cervus canadensis*) (Vander Wal et al., 2012).

Sex and age of the individuals, as well as time of the year, affect the number, type and duration of relationships (Whitehead, 2008; e.g., Bouwman and Hawley, 2010). Disease can also influence social relationships through strategies that restrict pathogen spread, such as behavioural immunity (Loehle, 1995) and sickness behaviour (Hart, 1988). For example, deer infected with CWD have a reduced likelihood of being found in groups (Mejía Salazar et al., 2016), probably as a result of behavioural changes caused by brain injury (e.g., diminished alertness, and ataxia) (Williams and Young, 1993). The clinical phase lasts from a few weeks to about four months under experimental conditions (Williams and Miller, 2002) and from a few months to a year based on our field observations (Mejía-Salazar, unpublished data). Studies on sociality that consider the concurrent effects of home range overlap, kinship, and seasonality, as well as life history characteristics, are therefore necessary to understand the role of preference in social organisation and in the dynamics of disease transmission.

The social life of mule deer is characterized by decisions that change dynamically over time, because ecological context, and group size and composition change on a daily basis (Mejía Salazar et al., 2016). Mule deer have a marked right-skewed distribution of group sizes (Mejía Salazar et al., 2016) with obvious seasonality driven by environmental conditions and reproductive behaviour (Vos et al., 1967; Bowyer et al., 2001; Lingle, 2003; Silbernagel et al., 2011; Mejía Salazar et al., 2016). The largest mixed-sex groups are observed in winter, while the smallest are seen during the fawning period (Bowyer et al., 2001; Mejía Salazar et al., 2016). In our study area, open flat habitat is associated with larger groups and a greater frequency of close proximity events (deer within 25 m of each other), while rugged terrain is used by many individuals in small groups (Silbernagel et al., 2011; Mejía Salazar et al., 2016).

Our first aim was to determine whether concurrent factors including sex, age, CWD status, spatial overlap, genetic relatedness and time of the year influenced the strength of pairwise associations. Our second aim was to test for sex, age and CWD status differences in the temporal stability of spatial associations. To answer these questions, we used spatial and genetic data to investigate patterns of associations among pairs of mule deer in a CWD endemic area. Our findings can serve to clarify aspects of cervid social behaviour that in turn can complement and contribute to future epidemiological modelling to guide CWD management strategies.

4.4 METHODS

4.4.1 Study population

The study was conducted between 2009 and 2012 in Antelope Creek (50.66°N, 108.27°W), a rural area within the mixed grassland ecoregion in Saskatchewan, Canada. The size of the core study area was defined by the movement of radio-collared deer and was approximately 258 km^2. The north section of the area is limited by the South Saskatchewan River and is characterized by a network of coulees with rugged terrain and natural vegetation. The climate is semiarid, with long and rigorous winters with mean extreme maximum and minimum temperatures of 35.1°C and -34.2°C, respectively (Government of Canada, 2014). The population of mule deer in the study area was estimated to range from 322 to 422 mule deer in 2007 and 2009, and was mostly (67%) non-migratory (Skelton, 2010), with a mean group size of 3.5 (SD = 3.7, range = 1 to 29) and a typical group size of 7.3 (95% CI = 6.8 to 8.1) (Mejía Salazar et al., 2016). CWD was first recognized in Antelope Creek in 1996; the prevalence in adult mule deer has substantially increased in Saskatchewan since 2004 (Canadian Wildlife Health Cooperative, 2017).

4.4.2 Data collection

Our animal handling protocol adhered to the Canadian Council on Animal Care guidelines for humane animal use and was approved by the University of Saskatchewan's Animal Research Ethics Board (Permit number 20050135). Permits to conduct research within private land of the study area were obtained verbally from land owners. A permit to conduct research within the Cabri Regional Park (GPS: 50.66824 -108.26791) was obtained from The Saskatchewan Regional Parks Association.

We captured mule deer in February or March of 2009, 2010 and 2011 using a helicopter and net-gun (Webb et al., 2008), or less frequently, using Clover traps (Clover, 1954). Upon capture, deer were chemically immobilized as described by Silbernagel et al. (2011). We collected a 5 mm ear biopsy for genetic analysis. We aged deer based on tooth wear and replacement (Jensen, 1996). Deer are usually classified as adults at 24 months of age (Dasmann and Taber, 1956); however, we classified deer as adults from 21 months old, as that was their age when we did our annual captures. Immunohistochemical (ICH) staining on palatine tonsil and sometimes rectal biopsies obtained during capture were used to classify the CWD status of live individuals into one of three categories: negative (no immunolabeling in at least 5 lymphoid follicles), positive (immunolabeling in any number of lymphoid follicles), or inconclusive (fewer than 5 lymphoid follicles in the sample (Schreuder et al., 1998; Wild et al., 2002). A minimum of 5 lymphoid follicles in the sample were assessed to provide 95% assurance of an accurate test (Geremia et al., 20158). By using this criterion, we reduced the chance of misclassifying a deer as negative due to repeated sampling or old age (Thomsen et al., 2012; Geremia et al., 2015). For an inconclusive diagnosis, re-cuts of the tonsil and in some cases rectal biopsy were tested until a final diagnosis was achieved. During this study, the diagnosis was inconclusive for 5 individuals for one year, but was negative at a later date. These deer were considered negative for all the years previous to the CWD-negative result. For dead animals, IHC was performed on portions of obex, tonsil, and /or retropharyngeal lymph node. Known positive deer were not removed from the population because data obtained from long-term intensive monitoring on both infected and healthy individuals would allow parameterization of realistic epidemiological models. Disease and population control programs based on hunting were not interrupted during the duration of this study.

We determined CWD diagnosis using tonsil and rectal biopsies tested with an immunohistochemical method; if a deer died during the study, the brain was retrieved for diagnosis (Wild et al., 2002). Adults were fitted with global positioning system (GPS) radio-collars (Lotek Wireless, Ontario, Canada) that were programmed to record position at predetermined 2 h intervals throughout the day (all at the same times). We released the deer close to their original capture location. Each year, CWD-negative deer were re-captured, re-tested, and fitted with a new collar. Deer that tested positive were not re-captured. Data from defective collars were not included in the analyses (Table 4-1).

Table 4-1. Number of adult (≥ 21 months old) mule deer fitted with GPS collars, by sex and CWD diagnosis, from 2009 to 2011.

Of 96 unique individuals (some deer were studied in more than one year), data from 74 were suitable for analyses of association strength, given available data on their genetics and locations.

	2009	2010	2011
Positive females	5	4	14
Negative females	21	14	12
Positive males	4	3	14
Negative males	12	4	20
Total	42	25	60

4.4.3 Data analysis

Defining study periods

A study year was defined by the capture period, and ran from 1 April of one year to 31 March of the next year. For this study, we included three years of data from 1 April 2009 to 31 March 2012, with each year divided into 5 seasons (Table 2-5 in page 36) (Anderson and Wallmo, 1984; Silbernagel et al., 2011; Government of Canada, 2014; Mejía Salazar et al., 2016).

4.4.3.1 Age, sex and disease status

We classified adult mule deer based on age (young adult if 1.8 to 3 years old, or old adult if >3 years old), sex (female or male), and CWD diagnosis (negative, positive, or sick). Deer were assumed to be positive for CWD from the first day of the season in which the first positive sample was taken. For example, if a sample taken on 20 Feb was positive, that deer was considered positive since 16 Dec. All deer were directly observed at least once a month (positive deer at least twice a month) and were considered sick from the moment they showed clear clinical signs of CWD, which included some or all of the following: drooping ears and head, laterally wide feet stance, hocks touching, protruding ribs and ischial tuberosities, reduced alertness, and difficulty in following a group or standing or eating (Mathiason et al., 2009). Deer were considered negative until the season in which they tested positive.

4.4.3.2 Generic relatedness

Genomic DNA was extracted from ear biopsies of most captured individuals. We genotyped each sample at 16 microsatellite loci following Cullingham et al. (2011b). Samples with ≥ 3 missing loci were discarded. Pairwise relatedness measures were estimated for 211 mule deer in the study area, including deer from other research projects, in SPAGeDi version 1.4 (Hardy and Vekemans, 2002) using the estimator of Queller & Goodnight (1989). This genetic relatedness coefficient (range from -1 to 1) is an unbiased estimate of relatedness based on the population’s allele frequencies. A negative value indicates that a pair is less related, and a positive value indicates that a pair is more related, than average for the sampled population (Queller and Goodnight, 1989).

4.4.3.3 Analysis of associations

Analysis of pairwise associations was based on radio-telemetry data from adult mule deer with GPS collars (96 different deer in the three years) (Table 4-1). Two individuals were considered associated if they were simultaneously located within 25 m from each other. We chose this threshold to account for collar error, which was 10.3 m on average (n = 16, range =

5.0 to 19.6 m) (Silbernagel et al., 2011). To obtain a list of all associated dyads per GPS-fix (i.e., every 2 hours) per season, we used the adehabitatHR package (Calenge, 2015) for R. Associations detected within the first two weeks after capture were discarded to exclude data that might be affected by behavioural changes related to capture. For the final dataset, deer were considered to be associated on a particular day if they were associated during at least one of the 2-hourly fixes. We calculated the strengths of dyadic associations among all pairs for each season using data files in linear mode and sampling periods of 1 day in SOCPROG 2.6 (Whitehead, 2009, 2014). As new deer would enter the sample with each collar deployment, while others left due to death or collar failure, we used the social affinity index as the association index because this measure helps to control for such demographic changes (Whitehead, 2008, p.98; Mann et al., 2012). We calculated social affinity indices with this formula: $x/\mathrm{Min}[(x + y_{AB} + y_A),(x + y_{AB} + y_B)]$, where x is the number of sampling days that A and B were observed together; Min stands for minimum and indicates that x will be divided by whichever of the two terms separated by the comma is smaller; y_A is the number of sampling days that A was observed without B (but B was not located), y_B is the number of sampling days that B was observed without A (but A was not located), and y_{AB} is the number of sampling days in which A and B were both observed, but not together (Whitehead, 2008, p. 98; Mann et al., 2012). The index ranges from 0 (deer never detected together within the season) to 1 (deer detected together every day of the season).

4.4.3.4 Calculation of volume of intersection (VI)

We estimated home range size for each individual for each season using the Brownian bridge kernel method (Bullard, 1991; Horne et al., 2007) in the adehabitatHR package (Calenge, 2015; Calenge et al., 2015) for R. This method estimates the utilization distribution of an animal when relocations are autocorrelated, which was the case for our data collected every 2 hours. It considers both the positions of the fixes, the path travelled by the animal, and the time dependence between successive fixes (Bullard, 1991; Horne et al., 2007). For the final calculations, we excluded fixes within the first two weeks after capture, and sequential fixes indicating a movement velocity greater than 2 km/h (an unlikely rate of movement for this species (Ager et al., 2003; Skelton, 2010)). We used an approach defined by Horne et al. (2007)

to find the maximum likelihood estimation of the parameter sig1 (a parameter to compute the variance of the position) for every deer and every season. We used a sig2 (location error) of 10 m based on collar accuracy (Silbernagel et al., 2011), and a grid resolution of 200 m. The areas of home range overlap between deer pairs per season were calculated in the adehabitatHR package (Calenge et al., 2015) for R following the volume of intersection (VI) method (Seidel, 1992). This provides a single measure of the VI between the Brownian bridge kernel home ranges of two individuals, per season. The VI ranges from 0 (when two home ranges have no overlap) to 1 (when two individuals have identical utilization distributions). As this method overcomes assumptions about random space use within a home range (Seidel, 1992), it produces more biologically meaningful results than simpler measures of areas of overlap.

4.4.3.5 Aim 1: Factors relating to association strength

To investigate the effects of multiple factors on association strength, we built a dataset of all seasonal pairwise associations, excluding records in which both the association index and VI were 0, and records with VI < 0.01 (10 of those 1272 records had association indices of 0.02 to 0.2; the rest had values of 0). We did this to exclude cases in which deer did not have the opportunity to associate. We built a set of 15 a priori linear mixed effects models using our knowledge of mule deer biology and behaviour (Table 4-2). The response variable was social affinity index, which was arcsined square-root transformed [asin (sqrt(index))]. All inferences were made on the transformed data. The predictor variables included different combinations of season, year, sex (sexes of the pair; e.g., FF for a pair of females), age (ages of the pair; e.g., YY for a pair of young adults), diagnosis (CWD diagnoses of the pair; e.g., SP for a pair of a sick and a positive deer), genetic relatedness, VI, and biologically meaningful 2-way interaction effects. For these pairwise designations, the order was considered the same (i.e., SP was the same as PS). We assigned each dyad a unique code, and treated dyad as a random effect. We used lme4 (Bates et al., 2016) and MuMIn (Barton, 2015) packages for R to fit these models. Estimates of the relative importance of each predictor variable were calculated by summing the Akaike weights across all models in the set in which the variable occurred (Burnham and Anderson, 2003, p. 167). We selected the best model based on the Akaike weights (sum is just ≥ 0.95), and the delta Δp (Δp is ≤ 5; Δp is the difference between AICc from the best model and the next model) (Burnham and Anderson, 2003, p. 168). We obtained least squares means (LSmeans) to evaluate statistical differences ($P < 0.05$) using the Bonferroni correction for multiple comparisons (Bonferroni, 1936). We report results on back-transformed association indices as predicted by the best model.

Table 4-2. Summary of 15 a priori models of mule deer association indices in Antelope Creek, Saskatchewan, Canada.

	Model ID														
Predictor variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Season	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Year	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Sex	X	X	X	X			X	X		X	X	X		X	X
Diagnosis	X		X		X			X		X	X		X		
VI	X		X			X	X	X	X		X	X	X	X	X
Age	X			X	X	X					X	X	X		
Relatedness	X						X	X		X					
Season*year	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Season*diagnosis	X		X		X			X		X	X				
Sex*relatedness	X						X	X		X					
Season*VI	X		X			X	X	X	X		X	X	X	X	X
Diagnosis*sex	X		X					X		X	X				
VI*diagnosis	X		X					X			X				
Sex*age												X			
Sex*season												X		X	X
Sex*VI															X

Predictor variables considered in each model are indicated with an X. Terms with an * are 2-way interactions. VI = volume of intersection.

4.4.3.6 Aim 2: Temporal patterns of associations

To test for sex, age and CWD status differences in temporal stability of associations among adult mule deer, we analysed lagged association rates (LARs) in SOCPROG 2.6. LARs are estimates of the probability of association *t* time units after a previous association, averaged over all associations (Whitehead, 1995, 2008). For these analyses, we only included individuals from study year 2011 because this was the year with the greatest number of deer (n = 44) for which we had continuous GPS-telemetry data for 5 continuous seasons (from April 1st 2011 to March 31st 2012). Of the 44 mule deer, there were 21 females and 23 males, 16 young adults and 28 old adults, and 12 CWD-negatives and 32 CWD-positives. CWD-positives were those that started the year with a positive diagnosis (n = 17) plus those that became positive during the year (n = 15). For this analysis, we did not classify deer as sick, as none showed clinical signs for the whole year. We investigated the between- and within-class lagged association rates (Whitehead, 2008, p. 89) for these class combinations: female-female (FF, n = 50), male-male (MM, n = 122) and female-male (FM, n = 172) pairs; old-old (OO, n= 124), young-young (YY, n = 45) and old-young (OY, n = 175) pairs; and positive-positive (PP, n = 190), negative-negative (NN, n = 20) and positive-negative (PN, n = 134) pairs. The directionality of the association (i.e., FM vs MF) can produce different results; we selected the directionality that yielded the best model fit (see below). To carry out the analyses between classes in SOCPROG 2.6, we set the sampling period as "date" (i.e., 1 day), defined associations as "grouped in sampling period", and entered the class variables (e.g., LARs from females to males). Then, a set of 7 mathematical models was fitted simultaneously to the observed LARs (Appendix 4.3, Table A4.3.3). These models were of the exponential family and were composed of all, one, or any meaningful combination of three components: rapid disassociations (associations lasting 1 day at most), casual acquaintances (associations that decay over time; their rate of decay is given by a1 and the average duration is approximated from the exponent of the exponential function, e.g., 1/a1, in days), and constant companionships (associations that do not decay or increase over time; their duration is interpreted within the context of the study period, in this case 1 year) (Whitehead, 1995). For each of the class pairs, the best fitting and most parsimonious model was indicated by the smallest quasi-Akaike information criterion (QAIC). If the difference between the QAIC of any other model and the best model, ΔQAIC, went from 0 to 2, then there was substantial support for that other model (Whitehead, 2007). The estimates of precision for the association rates and their durations were calculated with a Jackknife procedure over 3-day periods, and in some cases (MM, YY and PP) over 30, 45 and 10-day periods, respectively, to obtain better estimates (Whitehead, 2009). LARs were compared to a null association rate (NAR), the expected LAR if animals had associated randomly (Whitehead, 2007).

4.5 RESULTS

4.5.1.1 Aim 1: Factors affecting association strength

Between 2009 and 2011, 96 adult mule deer were fitted with GPS collars collecting data every 2 h, 24 h a day. Of these, data from 74 deer were suitable for analyses, given available paired data on both their genetics and locations. Association indices among these 74 deer were on average 0.12 (SE = 0.004, range 0 to 1). Most (58.7%) pairs' association indices were 0, 23.9% were 0.01 to 0.25, 15.8% were 0.26 to 0.99, and 1.6% were 1. Genetic relatedness was on average -0.003 (SE = 0.005, SD = 0.15, range -0.4 to 0.6). VI of the population was on average 0.17 (SE = 0.003, range 0.01 to 0.95). Avoidances (i.e., association indices of 0) occurred when pairs had a VI as high as 0.53, and preferences for others (i.e., association indices of 1) occurred with a VI as low as 0.05.

After fitting the a priori models predicting association strength among adult mule deer, predictor variables were ranked based on their importance (Table 4-3), and the models were ranked by AICc (Table 4-4). Based on the Akaike weights and the delta Δp, model 15 was clearly superior to the rest (delta = 0, weight = 0.9999) (Table 4-4). This model included the following statistically significant (all $P < 0.0001$) fixed effects: season, year, VI, sex, and four interactions - season*year, season*sex, season*VI and sex*VI (Appendix 4.1). The age, CWD status, and genetic relatedness of the pair were not significant ($P > 0.05$) predictors of association strength and their relative importances were very small (Table 4-3).

Table 4-3. Predictor weights used to assess the relative importance of an individual covariate within a model.

Predictor variable	Predictor weight [a]	Number of models [b]
Season	1	15
Year	1	15
Season*year	1	15
VI	1	11
Sex	1	11
Season*VI	1	11
Season*sex	1	3
Sex*VI	1	1
Age	< 0.01	7
Age*sex	< 0.01	1
Diagnosis	< 0.01	7
Diagnosis*season	< 0.01	6
Diagnosis*sex	< 0.01	5
Relatedness	< 0.01	4
Relatedness*sex	< 0.01	4
Diagnosis*VI	< 0.01	4

a = predictor weights calculated by summing the Akaike weights for all models in the a priori set in which the variable occurred. b = number of models in which the variable occurred. The larger the predictor weight, the greater the importance of that predictor.

Table 4-4. Model selection results for strength of spatial association mixed-effects model analyses.

Models are ranked according to Akaike information criterion (AICc) and presented along with the delta (Δp, the change in AICc relative to the best model), and Akaike weights. Model 15 had a 99.99% chance of being the best model.

Model ID	DF	logLik	AICc	Delta	Akaike weight
15	34	434.2	-799.7	0.0	0.9999
14	32	423.0	-781.3	18.4	1.0×10^{-4}
12	38	427.7	-778.3	21.4	2.3×10^{-5}
13	29	390.1	-721.6	78.0	1.1×10^{-17}
6	24	382.8	-717.2	82.4	1.3×10^{-18}
9	22	380.4	-716.5	83.2	8.8×10^{-19}
7	27	381.7	-708.9	90.7	2.0×10^{-20}
11	66	420.5	-706.1	93.6	4.7×10^{-21}
3	64	418.4	-706.1	93.6	4.7×10^{-21}
8	67	418.8	-700.5	99.1	3.0×10^{-22}
1	69	420.7	-700.2	99.4	2.6×10^{-22}
2	19	-523.0	1084.2	1883.9	0
4	21	-522.1	1086.6	1886.2	0
10	57	-502.3	1120.9	1920.6	0
5	44	-526.8	1143.0	1942.7	0

Here, we report multiple comparisons of LSmeans using the Bonferroni correction (Bonferroni, 1936); the association indices as predicted by the best model are back-transformed. See Appendix 4.2 for predicted values with 95% CI, and P-values, test statistics and degrees of freedom of multiple comparisons.

The interaction between season and year had a significant effect on association strength ($P < 0.0001$) (Figure 4-1 A; Appendix 4.1; Appendix 4.2, Table A4.2.2). Association indices between adult mule deer during the pre-rut were significantly different from one year to another (all $P < 0.0001$). Associations were stronger in 2010 than in 2009 in every season (all $P < 0.02$), and were also stronger in 2010 than in 2011 from fawning to early gestation (all $P < 0.045$). During 2009, association indices were significantly stronger during fawning (all $P < 0.01$) and pre-rut (all $P < 0.045$) than in the other three seasons. In 2010, associations were stronger in pre-rut than in any other season (all $P < 0.0001$) except for early gestation ($P = 0.69$), weaker in late gestation than in fawning ($P = 0.045$), and stronger in early gestation than in late gestation ($P < 0.0001$) and rut ($P = 0.02$). In 2011 associations were strongest in pre-rut (all $P < 0.01$), and stronger in fawning than in late gestation ($P = 0.002$), rut ($P < 0.0001$) or early gestation ($P = 0.002$).

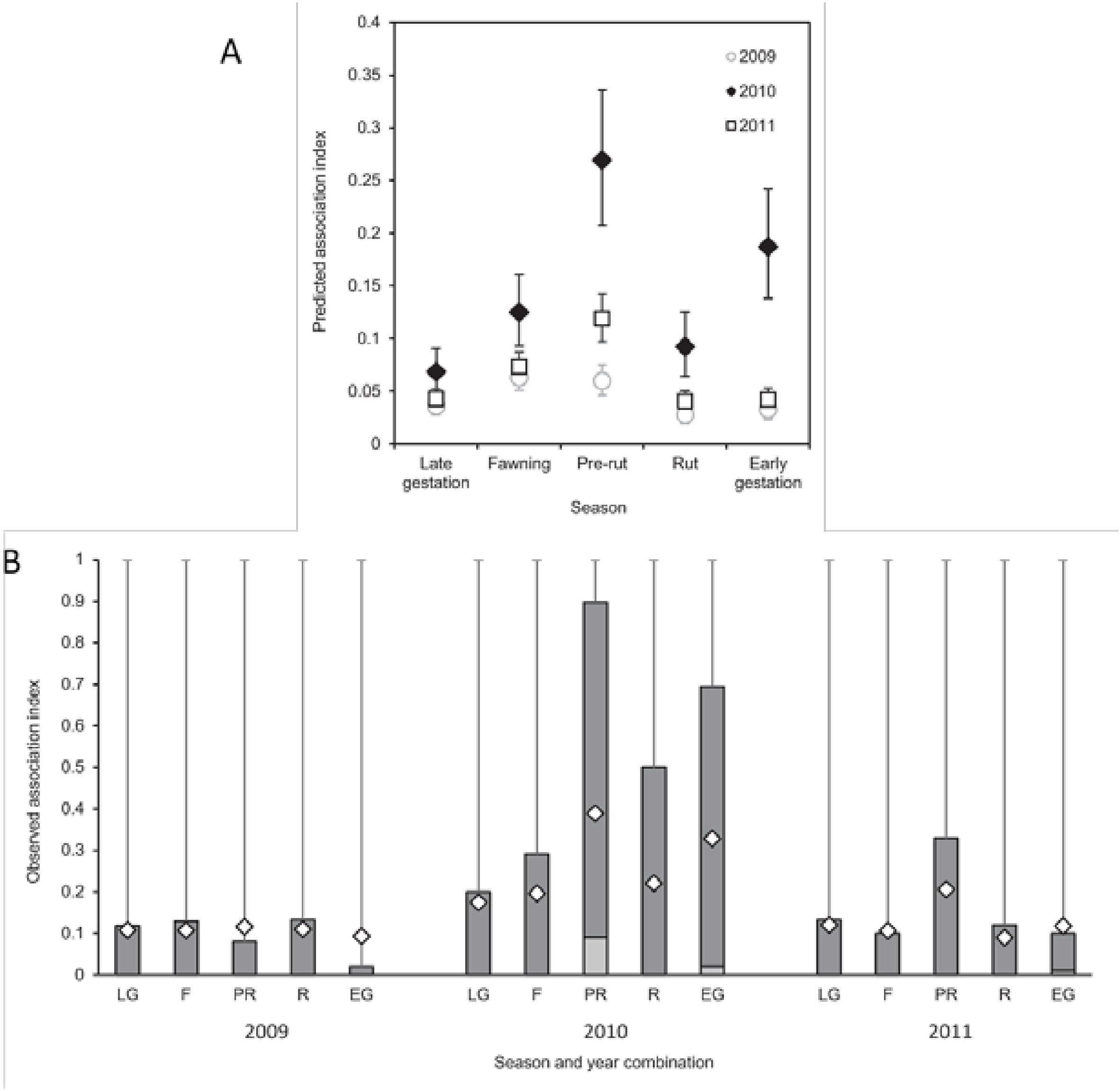

Figure 4-1. Seasonal pairwise spatial association indices by year among adult mule deer in Saskatchewan, Canada.

A. Seasonal predicted pairwise spatial association indices by year. Error bars are 95% confidence intervals (CI). For values used to generate this graph, see Appendix 4.2, Table A4.2.1. **B**. Box plots of observed association indices by season and year. LG = late gestation, F = fawning, PR = pre-rut, R = rut, and EG = early gestation. Most seasons (all except PR 2010, EG 2010 and EG 2011) had a median of 0. The maximum and minimum values in every season were 1 and 0, respectively. Interquartile 3 (in dark grey) depicts values from the median to the 75th percentile. Interquartile 2 (in light grey) depicts values from the median to the 25th percentile. Mean association index is depicted with a white rhombus.

The interaction between season and sex was also a significant predictor of association strength ($P < 0.0001$) (Figure 4-2 A; Appendix 4.1; Appendix 4.2, Table A4.2.3). Pre-rut and rut were the only seasons in which association strength differed significantly among sex classes. In pre-rut, different-sex associations were weaker than same-sex associations (all $P < 0.002$), while during the rut MM associations were significantly (all $P < 0.045$) weaker than FF and FM associations. For each pair class, the strength of the association also varied across seasons: MM associations were weakest in rut (all $P < 0.03$) and strongest in pre-rut (all $P < 0.01$), FF associations were strongest in pre-rut (all $P < 0.045$), and FM associations were weakest in late gestation (all $P < 0.01$).

Sex classes and VI also interacted significantly in their effects on association strength ($P < 0.0001$) (Figure 4-3; Appendix 4.1), as did season and VI ($P < 0.0001$) (Figure 4-4; Appendix 4.1). For all sexes (Figure 4-3) and all seasons (Figure 4-4), an increase in VI resulted in an increase in association strength. Notably, at increasing levels of VI, associations were stronger for FF pairs than for MM and FM pairs (Figure 4-3 A), and in pre-rut and fawning than in the rest of the seasons (Figure 4-4 A).

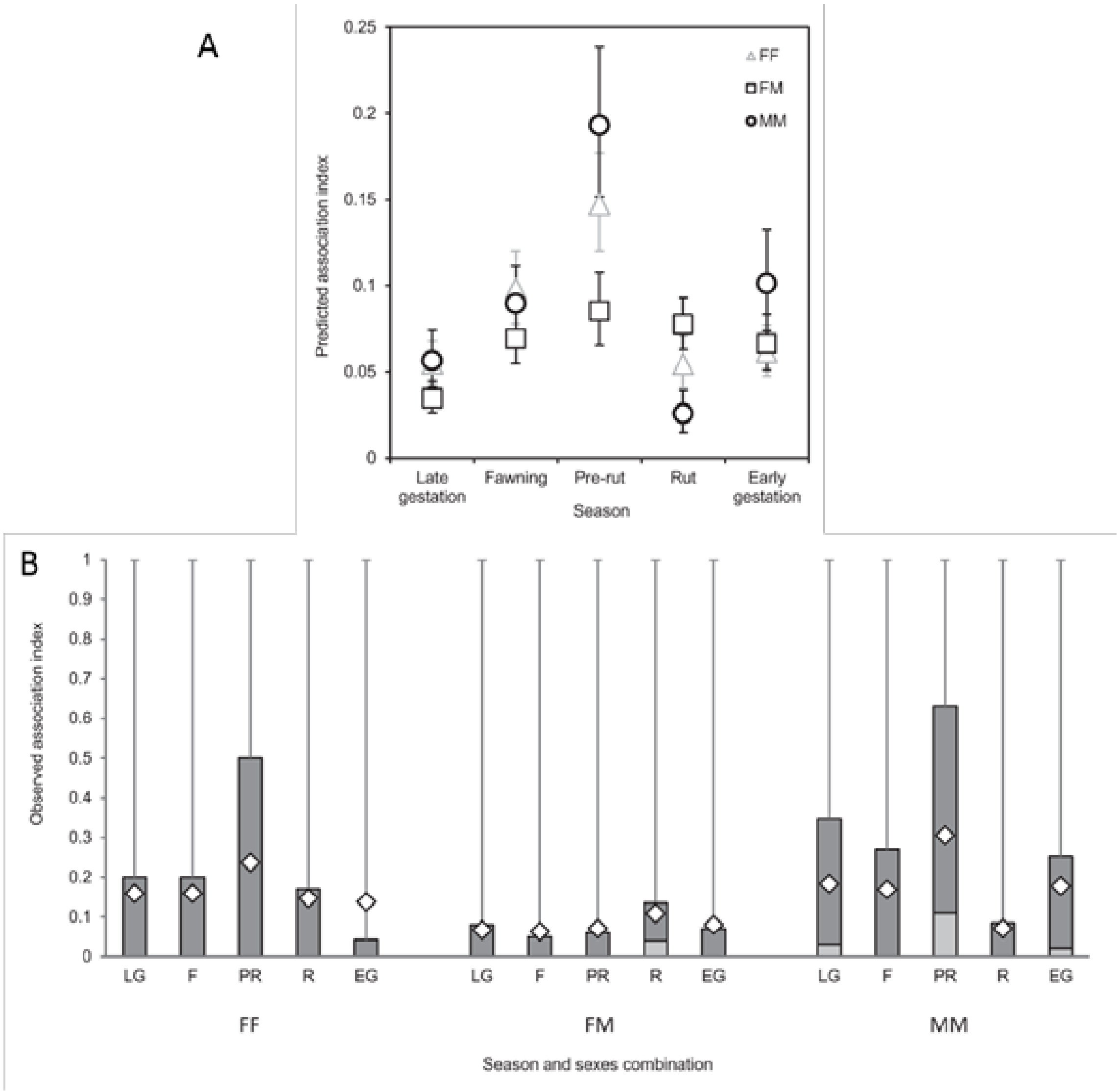

Figure 4-2. Seasonal pairwise spatial association indices by sex class among adult mule deer in Saskatchewan, Canada.

FF are pairs of females, MM are pairs of males, and FM are female-male pairs. **A.** Seasonal predicted pairwise spatial association indices by sex class. Error bars are 95% confidence intervals (CI). For values used to generate this graph, see Appendix 4.2, Table A4.2.1. **B.** Box plots of observed association indices among pairs of adult mule deer. LG = late gestation, F = fawning, PR = pre-rut, R = rut, and EG = early gestation. Most season and sex combinations (all except FM in rut, and MM in late gestation, pre-rut and early gestation) had a median of 0. The maximum and minimum values in every season and sex combinations were 1 and 0, respectively. Interquartile 3 (in dark grey) depicts values from the median to the 75th percentile. Interquartile 2 (in light grey) depicts values from the median to the 25th percentile. Mean association index is depicted with a white rhombus.

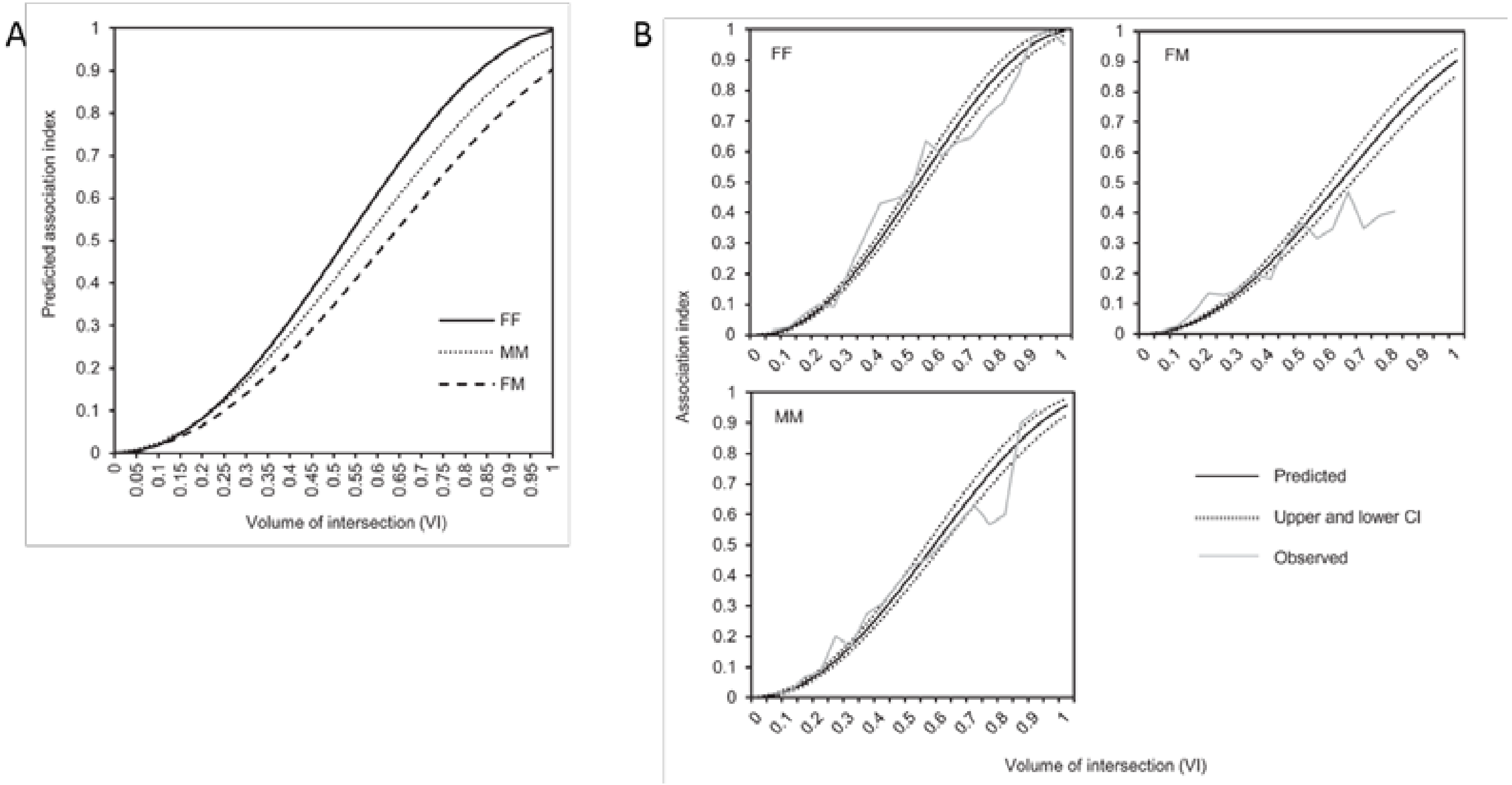

Figure 4-3. Pairwise spatial association indices by sex class at different levels of volume of intersection (VI) among adult mule deer in Saskatchewan, Canada.

FF are pairs of females, MM are pairs of males, and FM are female-male pairs. A. Predicted pairwise spatial association indices by sex class at different levels of VI. No confidence intervals (CI) are shown to facilitate graph readability. **B**. Predicted (black) and observed (grey) pairwise association indices by sex class at increasing levels of VI. 95% CI depicted in dotted lines.

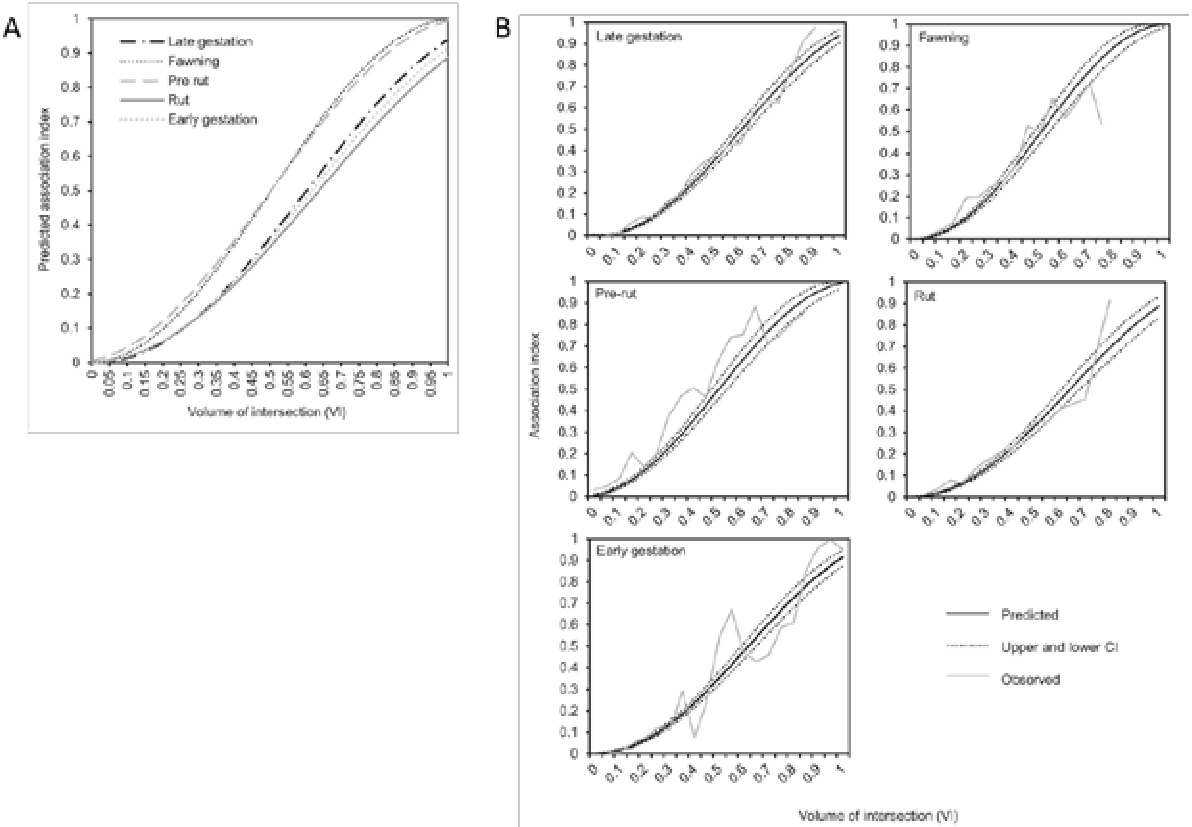

Figure 4-4. Seasonal pairwise spatial association indices at different levels of volume of intersection (VI) among adult mule deer in Saskatchewan, Canada.

A. Seasonal predicted pairwise spatial association indices at different levels of VI. No confidence intervals (CI) are shown to facilitate graph readability. **B**. Seasonal variation of predicted (black) and observed (grey) pairwise association indices at increasing levels of VI. 95% CI depicted in dotted lines.

4.5.1.2 Aim 2: Temporal patterns of associations

The class LARs among 44 adult mule deer in 2011 were best described by either one of two models: (A) a model containing rapid disassociations, constant companionships and casual acquaintances, or (B) a model containing rapid disassociations and two levels of casual acquaintances, one lasting longer than the other (Figure 4-5, Appendix 4.3, Table A4.3.3). The LARs, which decreased over time, always remained above the null association rate across all sex, age and CWD status classes (Appendix 4.3, Figures A4.3.1 to A4.3.9). Analyses between

MM and YY associations produced cyclic-like patterns in the LARs (Appendix 4.3, Figures A4.3.7 and A4.3.8), and also values of SE and duration ranges that were implausible (e.g., 96 days on average ranging from 1 to 1) (Appendix 4.3, Table A4.3.7) despite several tests with different Jackknife levels.

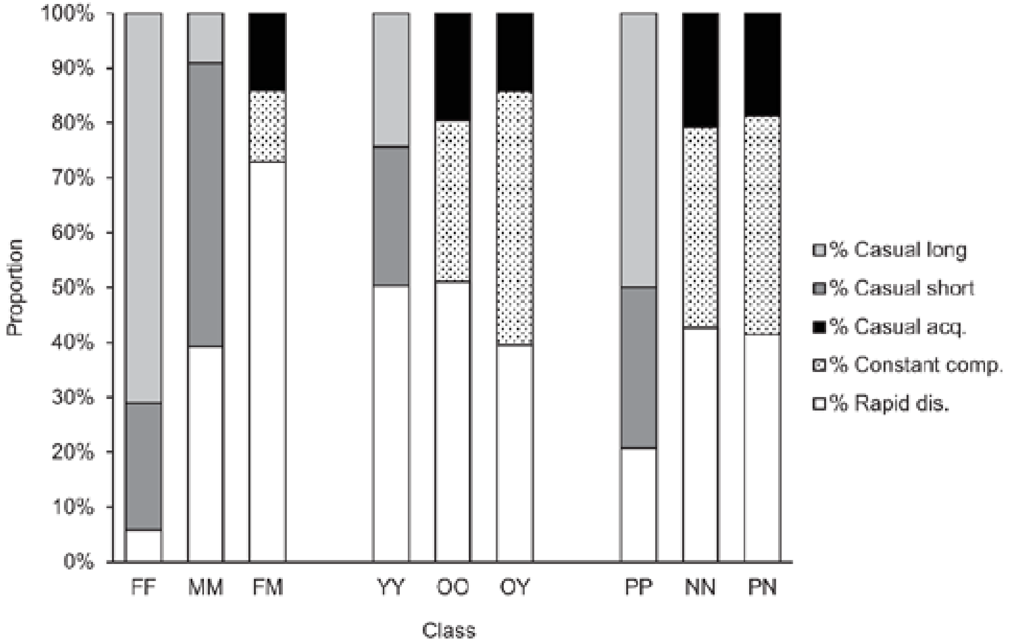

Figure 4-5. Proportions of components in lagged association rate models among 44 adult mule deer in Saskatchewan, Canada.

FF = female-female pairs, MM = male-male pairs, FM = female-male pairs; YY = young-young pairs, OO = old-old pairs, OY = old-young pairs; PP = positive-positive pairs, NN = negative-negative pairs, and PN = positive-negative pairs. Rapid disassociations were associations that lasted the sampling period (i.e., 1 day) at most. In constant companionships, the probability of re-association did not decay or increase over time within the context of the study period (i.e., 1 year). In casual acquaintances, the probability of re-association decayed over time, and their rate of decay was approximated from a1. In some cases, LARs of casual acquaintances decreased over two different time scales, one lasting longer (casual long) than the other (casual short). For formulae, and results on durations and SE, see Appendix 4.3.

With respect to sex differences in the temporal stability of class associations, the great majority (94.3%) of associations between females disassociated over two different time scales (i.e., two levels of casual acquaintances), with most (71.1%) disassociating after a longer period of association (about 980 days) and 23.2% after 1 to 3 days. Only 5.7% of FF associations lasted no more than 1 day (i.e., rapid disassociations) (Figure 4-5; Appendix 4.3, Table A4.3.7). In contrast, most (72.9%) of the FM associations lasted no more than 1 day, while the rest either decreased over time (i.e., casual acquaintances) (14.1%), lasting about 17 days, or were stable over the year (i.e., constant companionships) (13%) (Figure 4-5; Appendix 4.3, Table A4.3.5).

With respect to age differences in the temporal stability of class associations, many (51.1%) associations between older deer (OO) lasted no more than 1 day, while the rest were either stable over the year (29.4%), or decreased over time (19.6%), lasting about 39 days before disassociation occurred (Figure 4-5; Appendix 4.3, Table A4.3.5). Older and younger deer mainly (46.3%) associated at a constant rate over the year, and less commonly (14.2%) associated as casual acquaintances that were together for about 47 days before disassociating (Figure 4-5; Appendix 4.3, Table A4.3.5).

Temporal stability also differed with CWD status. Pairs of CWD-negative deer (NN), and of CWD-positive and negative deer (PN), associated similarly (Figure 4-5). Both cases had similar proportions of their elements and were better described by the model including constant companionships (model A) (Appendix 4.3, Table A4.3.5). In contrast, associations between positive deer (PP) were better described by the model without constant companionships (model B) (Appendix 4.3, Table A4.3.7). Moreover, 79.2% of PP pairs disassociated at two different time scales: 29.3% after about 2 days of association, and 49.9% after about three years (Figure 4-5; Appendix 4.3, Table A4.3.7).

4.6 DISCUSSION

Social behaviours that influence contact rates and the sharing of space in animal species are potentially important factors in information and disease spread within populations (Altizer et al., 2003; Cross et al., 2010). For example, data on association patterns have proven useful in understanding the ecology of diseases that can be transmitted though both direct and environmental contacts, such as tuberculosis in wild animals (Corner et al., 2003; Cross et al., 2004; Drewe, 2010), and CWD in female white-tailed deer (Schauber et al., 2015). We found that pairwise spatial association patterns of adult mule deer were independent of genetic relatedness, age and CWD status, but seasonal association strength varied with year, sex and home range overlap. We also found important sex and CWD status differences in the temporal stability of spatial associations. By identifying the factors related to individuals' choices of association partners, we provide empirical data to increase understanding of the possible role of social behaviour in the long-term dynamics of disease transmission among mule deer.

In this study, the strength of associations among adult mule deer varied among years, with stronger associations in 2010, and a marked peak during the winter (early gestation) of that year. The 2010 pattern may be linked to weather, as the frequency of very cold days (-34 to -10°C) was greater and the mean temperature colder in the winter of 2010 than in 2009 or 2011 (-11 vs -8 and -5°C, respectively) (Weather Spark, 2016). There was also almost twice as much snow on the ground on a daily basis during rut and early gestation in 2010 than in 2009 (means = 16 vs 9 cm) (Weather Spark, 2016). In severe winters with decreased temperatures and increased snow depth, escape from predators is hindered (Hawkins and Klimstra, 1970), forage availability declines and the energetic costs of foraging increase (Parker et al., 1984). This forces deer onto the southerly aspects of hills where solar radiation reduces snow cover, resulting in larger winter aggregations.

We also observed seasonal patterns that varied in relation to sex of the associating pair. During pre-rut, associations between males were the strongest and different-sex associations the weakest. Later, in rut, male-male association strength markedly decreased and became weaker than female-female and female-male associations. These are distinctive patterns that are likely driven by mule deer courting and mating behaviour (Vos et al., 1967). Prior to females entering oestrus, males establish their dominance using threats and intimidation displays that occur in very close proximity, when contenders circle each other, snort and lick their noses (Cowan and Geist, 1961), sometimes followed by sparring matches (Wachtel et al., 1978). These behaviours result in more male-male proximity events, and consequently in a peak in male-male spatial association indices in pre-rut. Then, in rut, the frequency and variety of male vocalizations related to courting increase (Dixon, 1934), probably to alert other males from a distance and discourage close-contact confrontations (Vos et al., 1967). Moreover, male mule deer wander more widely throughout their home ranges during rut to closely follow females to test if they are in oestrus, moving from one female group to the next (Linsdale and Tomich, 1953). Once in oestrus, females allow males to lick their genitals and copulate (Linsdale and Tomich, 1953). These behaviours increase spatial associations between adult females and males, and decrease those between males. In terms of disease, the risk of direct animal to animal transmission between adult males is likely increased in pre-rut, whereas that between adult males and females is probably increased during rut. However, these suggestions require further research, as sharing space does not necessarily translate into a greater frequency of physical contacts among deer (e.g., Castles et al., 2014; Farine, 2015), and increased spatial association may more accurately translate into increased risk of transmission through sharing contaminated environments.

Genetic relatedness was not an important predictor of spatial associations among adult mule deer in our study area, suggesting that at a very short distance (within 25 m), there is no genetic structuring among these adult mule deer. Similarly, the frequency and duration of proximity instances (within 1.4 m) were not related to genetic relatedness in elk (Vander Wal et al., 2012). At a larger spatial scale (km), a study of mule deer (Powell et al., 2013) also found low levels of genetic structure and limited genetic isolation. However, there were very few highly related adult individuals in our data set (6/982 pairs $r > 0.4$), therefore, we are unable to determine whether there would be an increased number of associations among close relatives. In contrast, spatially proximate individuals were more genetically related in studies of mule deer (Cullingham et al., 2011b), white-tailed deer (*Odocoileus virginianus*) (Grear et al., 2010; Miller et al., 2010; Cullingham et al., 2011a; Magle et al., 2013), Sitka black-tailed deer (*O. hemionus sitkensis*) (Colson et al., 2013), and non-Odocoileus cervid species (Bonnot et al., 2011; Biosa et al., 2015; Colson et al., 2016).

The strength of the correlation between kinship and spatial separation or home range overlap varies depending on the set of deer considered in the analysis, and the scale used for measuring spatial distance (Comer et al., 2005). It is not surprising that studies that limit analyses to pairs of deer in close proximity (e.g., captured within 1.5 km from each other, with known home range overlap), and that exclude dispersing males and include individuals with high genetic relatedness (e.g., does and fawns), often show a strong association between genetic and spatial distances (e.g., Magle et al., 2013). Although the spatial genetic structure of mule deer is driven by female philopatry and dispersal of males at large spatial scales (Cullingham et al., 2011b), our data suggest that even at small scales, adults tend to mix freely, suggesting that disease in adults would spread beyond family groups as it should be transmitted similarly among related and unrelated individuals (Comer et al., 2005; Vander Wal et al., 2012).

The greater the VI between pairs of mule deer, the stronger the association they exhibited, irrespective of season and sex class. These findings were expected given that two individuals must be in the same area in order to associate. However, we found cases of apparent avoidances (small association index despite large VI) and preferences (large association index despite very small VI), suggesting that mule deer do not associate at random, and that their spatial associations are only partially explained by the extent of their home range overlap, as previously reported in species with fluid fission-fusion dynamics such as eastern grey kangaroos (*Macropus giganteus*) (Carter et al., 2013; Best et al., 2014), giraffes (*Giraffa camelopardalis*) and eastern water dragons (*Intellagama lesueurii*) (Strickland et al., 2014). In our study, at the same level of VI, associations were strongest in pre-rut and fawning, emphasizing the changes in socio-spatial organisation during the mating season. Also, at the same level of VI, associations were strongest between adult females, suggesting that females are more interested in being together than are males.

Between- and within-class lagged association rate analyses indicated that classes of adult mule deer mostly disassociated, either rapidly within one day, or over long time periods. Furthermore, it also showed that in a small proportion of associations between certain classes, the probability of re-association did not increase or decrease over time, i.e., was constant. LARs always remained above the null association rate. This suggests that if deer re-associate they are more likely to do so with individuals of the same class as their previous associates than with individuals from the population at random (Whitehead, 2008).

There were marked sex differences in the temporal patterns of associations. Females mostly (71.1%) mingled with other females in long-term associations with a declining probability of re-association over time. In contrast, when females were found with males, they were mostly (72.9%) not found with males again on the next day. The long-term probability of females re-associating with other females may be explained by a greater preference for one another. The rapid splitting of female-male associations may be due to males' brief assessments of females' reproductive status, or to unintended encounters at specific focal points in the study area such as waterholes and grain spills. MM and YY were the only two models in which the

LARs appeared cyclic (i.e., decreased and then increased). A nonexponential model, such as one with a trigonometric function of the lag (e.g., cos(t)), may work best for these two cases (Whitehead, 2008, p. 206); further testing is required.

In addition to finding that age was not a significant predictor of pairwise association strengths, we observed negligible age differences in LARs among age classes. We defined an association based on proximity obtained from GPS locations, and our study only focused on adult deer as GPS collars were too heavy to be deployed in younger (< 21 months old) individuals. If we could instead define an association based on group membership from direct observations, we might find interesting age differences in association strength and in LARs, as all age classes could be included in such analyses.

We previously reported that deer showing clinical signs of CWD were less likely to be found in groups than their healthy counterparts (Mejía Salazar et al., 2016). However, in the current study, we have shown that CWD infection was not an important predictor of pairwise spatial association strengths among adult mule deer once season, year, VI, and sex of the associating pair were accounted for. As we had sufficient data on infected individuals (700/982 pairs included at least one infected deer), this finding suggests that clinical signs affect the probability of grouping, but not the proportion of time spent in association, as this was defined in our study. Fine-scale studies based on observations of direct animal to animal contacts may reveal effects of disease status on interaction rates. Interestingly though, in the LARs analysis, we found that when one or both members were CWD-negative, a proportion (~37 to 40%) of the associations had a constant probability of re-association, whereas when both members were CWD-positive, this element was not present, but rather all associations had a declining probability of re-association. The causes and consequences of these findings are unclear; however, they support the hypothesis that infected and non-infected deer relate to others in a different way.

In conclusion, we have increased understanding of the factors affecting the pairwise spatial association strengths of adult mule deer, and how individual characteristics such as sex, age and disease status alter the temporal stability of spatial associations among classes of individuals. This adds to both studies of behavioural ecology and of disease dynamics. Current epidemiological models of CWD are lacking empirical data on the structure of mule deer society.

The association matrices generated in this study can be used in network models or individual-based spatial models that require the inclusion of more realistic (i.e., heterogeneous) data on association indices to better guide and inform disease management strategies.

4.7 ACKNOWLEDGMENTS

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4.8 SUPPORTING INFORMATION

Appendix 4.1. Analysis of variance table of top model (model 15) for strength of pairwise spatial association among adult mule deer in Saskatchewan, Canada, and variance and standard deviation of random effect (i.e., dyad).

Appendix 4.2. Model output with predicted association indices and pairwise comparisons.

Appendix 4.3. Details on temporal patterns of spatial associations among mule deer in a chronic wasting disease endemic area in Saskatchewan, Canada.

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4.10 APPENDIX 4.1.

Appendix 4.1. Analysis of variance table of top model (model 15) for strength of pairwise spatial association among adult mule deer.

ANOVA table

Predictor	**SS**	**MS**	**NumDF**	**DenDF**	**F**	***P***
Season	2.0	0.5	4	2736.6	12.8	2.4E-10
Year	5.8	2.9	2	2523.9	75.6	< 2.2e-16
VI	116.5	116.5	1	1722.9	3010.0	< 2.2e-16
Sexes	0.2	0.1	2	1600.4	2.5	0.081
Season*year	1.8	0.2	8	2641.6	5.9	0.0000002
Season*VI	1.0	0.2	4	2696.5	6.1	0.0001
Season*sexes	3.5	0.4	8	2707.3	11.4	6.7E-16
VI:sexes	0.9	0.5	2	1733.1	11.7	0.00001

Random effects table

Groups	**Name**	**Variance**	**SD**
dyad	(Intercept)	0.01	0.08
Residual		0.04	0.20
Number of obs: 3044. Number of dyads: 982.			

Notes

Model 15 formula: asin(sqrt(index)) ~ season + year + VI + sexes + (1 | dyad) + season*year + season*VI + season*sexes + VI*sexes

Abbreviations: SS = the sum of the squares, MS = the mean sum of squares, DF = degrees of freedom, NumDF = DF in the numerator, DenDF = DF in the denominator, F = F-statistic, *P* = P-value, SD = standard deviation.

Satterthwaite approximation for degrees of freedom.

4.11 APPENDIX 4.2.

Appendix 4.2. Model output with predicted association indices and pairwise comparisons.

Table A.4.2.1. Least squares means for strength of pairwise spatial association mixed-effects model analysis.

Season	Year	Estimate	Lower CI	Upper CI	SE	DF	t-value
Late gestation	2009	0.191	0.166	0.215	0.013	3004	15.0
Fawning	2009	0.254	0.228	0.280	0.014	3012	18.9
Pre-rut	2009	0.247	0.217	0.278	0.015	3042	16.1
Rut	2009	0.167	0.138	0.197	0.015	3043	11.2
Early gestation	2009	0.181	0.154	0.209	0.014	3040	12.9
Late gestation	2010	0.265	0.224	0.306	0.021	2967	12.7
Fawning	2010	0.361	0.310	0.412	0.026	2944	13.9
Pre-rut	2010	0.546	0.473	0.618	0.037	2773	14.7
Rut	2010	0.309	0.256	0.362	0.027	2903	11.4
Early gestation	2010	0.447	0.381	0.514	0.034	2779	13.2
Late gestation	2011	0.208	0.186	0.231	0.012	2977	18.2
Fawning	2011	0.274	0.248	0.300	0.014	3010	20.4
Pre-rut	2011	0.352	0.317	0.387	0.018	3038	19.7
Rut	2011	0.203	0.180	0.226	0.012	2983	17.4
Early gestation	2011	0.207	0.182	0.232	0.013	3026	16.2

Season	Sexes	Estimate	Lower CI	Upper CI	SE	DF	t-value
Late gestation	FF	0.236	0.209	0.264	0.014	2578	17.0
Fawning	FF	0.319	0.283	0.354	0.018	2846	17.7
Pre-rut	FF	0.394	0.354	0.434	0.020	3004	19.3
Rut	FF	0.236	0.202	0.270	0.017	2844	13.5
Early gestation	FF	0.251	0.220	0.281	0.016	2911	15.9
Late gestation	FM	0.188	0.162	0.213	0.013	2972	14.6
Fawning	FM	0.266	0.237	0.296	0.015	2943	17.6
Pre-rut	FM	0.297	0.259	0.334	0.019	3044	15.4
Rut	FM	0.282	0.254	0.310	0.014	3019	19.8
Early gestation	FM	0.261	0.228	0.293	0.017	3036	15.7
Late gestation	MM	0.240	0.204	0.276	0.018	2998	13.2
Fawning	MM	0.304	0.267	0.341	0.019	3004	16.1
Pre-rut	MM	0.455	0.400	0.510	0.028	3026	16.3
Rut	MM	0.161	0.122	0.200	0.020	3034	8.1
Early gestation	MM	0.324	0.275	0.373	0.025	3034	12.9

Season	Estimate	Lower CI	Upper CI	SE	DF	t-value
Late gestation	0.221	0.203	0.240	0.009	2928	23.7
Fawning	0.296	0.275	0.318	0.011	3000	26.5
Pre-rut	0.382	0.352	0.412	0.015	3024	24.8
Rut	0.226	0.204	0.249	0.011	3029	19.8
Early gestation	0.278	0.252	0.305	0.014	3016	20.3

Year	Estimate	Lower CI	Upper CI	SE	DF	t-value
2009	0.208	0.194	0.222	0.007	1336	28.4
2010	0.386	0.357	0.414	0.014	2855	26.8
2011	0.249	0.235	0.263	0.007	1272	35.0

Sexes	Estimate	Lower CI	Upper CI	SE	DF	t-value
FF	0.287	0.268	0.306	0.010	812	29.9
FM	0.259	0.242	0.275	0.008	1348	31.2
MM	0.297	0.274	0.319	0.011	1533	25.9

Notes

Abbreviations: CI = confidence interval, and DF = degrees of freedom.

Sexes (the sex of the associating pair) codes: FF = pair of females, FM = female-male pair, and MM = pair of males.

Estimate' indicates the pairwise spatial association index as predicted by top model (model 15).

Model 15 formula: asin(sqrt(index)) ~ season + year + VI + sexes + (1 | dyad) + season*year + season*VI + season*sexes + VI*sexes

Table A.4.2.2 Differences of least squares means for strength of pairwise spatial association that varies by season and year (i.e., interaction term season*year).

Season codes: 1 = late gestation, 2 = fawning, 3 = pre-rut, 4 = rut, and 5 = early gestation.

Season A and Year A are season and year combination A, which has an estimate (Estimate A). Season B and Year B are season and year combination B, which has an estimate (Estimate B).

To evaluate statistical differences (at *P*<0.05) between Estimate A and Estimate B, we used the Bonferroni correction for multiple comparisons. Significant differences in bold. 'Sig' when *P < 0.0001*. Column Relationship indicates the direction of the difference between Estimate A and B. Abbreviations: R = relationship, CI = confidence interval, DF = degrees of freedom, and SE = standard error.

Order	Season A	Year A	Estimate A	R	Estimate B	Season B	Year B	P-value	P-value BC	Lower CI	Upper CI	DF	t-value	Estimate	SE
1	1	2009	0.036	<	0.063	2	2009	3E-04	**0.005**	-0.10	-0.03	2581.8	-3.64	-0.1	0.02
2	1	2009	0.036	<	0.060	3	2009	0.003	**0.05**	-0.09	-0.02	2661.8	-3.01	-0.1	0.02
3	1	2009	0.036	>	0.028	4	2009	0.22	3.29	-0.01	0.06	2734.6	1.23	0	0.02
4	1	2009	0.036	>	0.032	5	2009	0.61	9.15	-0.03	0.05	2842.7	0.51	0	0.02
5	1	2009	0.036	<	0.069	1	2010	0.001	**0.02**	-0.12	-0.03	2700.1	-3.23	-0.1	0.02
6	1	2009	0.036	<	0.125	2	2010	<2e-16	**sig**	-0.23	-0.12	2772.8	-6.04	-0.2	0.03
7	1	2009	0.036	<	0.269	3	2010	<2e-16	**sig**	-0.43	-0.28	2680.0	-9.14	-0.4	0.04
8	1	2009	0.036	<	0.092	4	2010	1E-04	**0.002**	-0.18	-0.06	2792.4	-4.01	-0.1	0.03
9	1	2009	0.036	<	0.187	5	2010	<2e-16	**sig**	-0.33	-0.19	2690.0	-7.20	-0.3	0.04
10	1	2009	0.036	<	0.043	1	2011	0.29	4.32	-0.05	0.02	3021.4	-1.06	0	0.02
11	1	2009	0.036	<	0.073	2	2011	<2e-16	**sig**	-0.12	-0.05	3033.5	-4.54	-0.1	0.02
12	1	2009	0.036	<	0.119	3	2011	<2e-16	**sig**	-0.20	-0.12	3037.8	-7.43	-0.2	0.02
13	1	2009	0.036	<	0.040	4	2011	0.48	7.16	-0.05	0.02	3034.1	-0.71	0	0.02
14	1	2009	0.036	<	0.042	5	2011	0.37	5.51	-0.05	0.02	3040.7	-0.90	0	0.02

Order	Season A	Year A	Estimate A	R	Estimate B	Season B	Year B	P-value	P-value BC	Lower CI	Upper CI	DF	t-value	Estimate	SE
15	2	2009	0.063	>	0.060	3	2009	0.74	11.06	-0.03	0.04	2553.5	0.34	0	0.02
16	2	2009	0.063	>	0.028	4	2009	<2e-16	**sig**	0.05	0.12	2733.8	4.49	0.1	0.02
17	2	2009	0.063	>	0.032	5	2009	1E-04	**0.002**	0.04	0.11	2848.6	3.89	0.1	0.02
18	2	2009	0.063	<	0.069	1	2010	0.65	9.71	-0.06	0.04	2679.7	-0.46	0	0.02
19	2	2009	0.063	<	0.125	2	2010	1E-04	**0.002**	-0.16	-0.05	2755.4	-3.81	-0.1	0.03
20	2	2009	0.063	<	0.269	3	2010	<2e-16	**sig**	-0.37	-0.22	2647.3	-7.48	-0.3	0.04
21	2	2009	0.063	<	0.092	4	2010	0.07	0.99	-0.11	0.00	2784.9	-1.84	-0.1	0.03
22	2	2009	0.063	<	0.187	5	2010	<2e-16	**sig**	-0.26	-0.12	2685.0	-5.38	-0.2	0.04
23	2	2009	0.063	>	0.043	1	2011	0.01	0.14	0.01	0.08	3027.4	2.59	0	0.02
24	2	2009	0.063	<	0.073	2	2011	0.28	4.23	-0.06	0.02	3037.2	-1.08	0	0.02
25	2	2009	0.063	<	0.119	3	2011	<2e-16	**sig**	-0.14	-0.06	3027.5	-4.44	-0.1	0.02
26	2	2009	0.063	>	0.040	4	2011	0.004	0.06	0.02	0.09	3041.1	2.91	0.1	0.02
27	2	2009	0.063	>	0.042	5	2011	0.01	0.15	0.01	0.08	3042.7	2.58	0	0.02
28	3	2009	0.060	>	0.028	4	2009	1E-04	**0.002**	0.04	0.12	2681.5	3.90	0.1	0.02
29	3	2009	0.060	>	0.032	5	2009	9E-04	**0.01**	0.03	0.11	2798.9	3.31	0.1	0.02
30	3	2009	0.060	<	0.069	1	2010	0.49	7.31	-0.07	0.03	2676.5	-0.70	0	0.03
31	3	2009	0.060	<	0.125	2	2010	1E-04	**0.002**	-0.17	-0.06	2745.4	-3.86	-0.1	0.03
32	3	2009	0.060	<	0.269	3	2010	<2e-16	**sig**	-0.37	-0.22	2635.5	-7.70	-0.3	0.04
33	3	2009	0.060	<	0.092	4	2010	0.05	0.69	-0.12	0.00	2777.0	-2.00	-0.1	0.03
34	3	2009	0.060	<	0.187	5	2010	<2e-16	**sig**	-0.27	-0.13	2670.6	-5.46	-0.2	0.04
35	3	2009	0.060	>	0.043	1	2011	0.04	0.60	0.00	0.08	3042.8	2.05	0	0.02
36	3	2009	0.060	<	0.073	2	2011	0.19	2.85	-0.07	0.01	3043.6	-1.31	0	0.02
37	3	2009	0.060	<	0.119	3	2011	<2e-16	**sig**	-0.15	-0.06	3028.4	-4.58	-0.1	0.02
38	3	2009	0.060	>	0.040	4	2011	0.02	0.29	0.01	0.08	3044.0	2.34	0	0.02
39	3	2009	0.060	>	0.042	5	2011	0.04	0.59	0.00	0.08	3042.9	2.06	0	0.02
40	4	2009	0.028	<	0.032	5	2009	0.48	7.26	-0.05	0.02	2772.6	-0.70	0	0.02
41	4	2009	0.028	<	0.069	1	2010	1E-04	**0.002**	-0.15	-0.05	2699.7	-3.91	-0.1	0.02

Order	Season A	Year A	Estimate A	R	Estimate B	Season B	Year B	P-value	P-value BC	Lower CI	Upper CI	DF	t-value	Estimate	SE
42	4	2009	0.028	<	0.125	2	2010	<2e-16	**sig**	-0.25	-0.14	2777.5	-6.60	-0.2	0.03
43	4	2009	0.028	<	0.269	3	2010	<2e-16	**sig**	-0.46	-0.30	2677.8	-9.55	-0.4	0.04
44	4	2009	0.028	<	0.092	4	2010	<2e-16	**sig**	-0.20	-0.08	2729.9	-4.77	-0.1	0.03
45	4	2009	0.028	<	0.187	5	2010	<2e-16	**sig**	-0.35	-0.21	2661.7	-7.68	-0.3	0.04
46	4	2009	0.028	<	0.043	1	2011	0.03	0.44	-0.08	0.00	3043.1	-2.18	0	0.02
47	4	2009	0.028	<	0.073	2	2011	<2e-16	**sig**	-0.15	-0.07	3044.0	-5.33	-0.1	0.02
48	4	2009	0.028	<	0.119	3	2011	<2e-16	**sig**	-0.23	-0.14	3032.4	-7.96	-0.2	0.02
49	4	2009	0.028	<	0.040	4	2011	0.06	0.93	-0.07	0.00	3044.0	-1.87	0	0.02
50	4	2009	0.028	<	0.042	5	2011	0.05	0.68	-0.08	0.00	3043.7	-2.01	0	0.02
51	5	2009	0.032	<	0.069	1	2010	6E-04	**0.01**	-0.13	-0.04	2695.0	-3.44	-0.1	0.02
52	5	2009	0.032	<	0.125	2	2010	<2e-16	**sig**	-0.24	-0.12	2759.4	-6.25	-0.2	0.03
53	5	2009	0.032	<	0.269	3	2010	<2e-16	**sig**	-0.44	-0.29	2679.1	-9.28	-0.4	0.04
54	5	2009	0.032	<	0.092	4	2010	<2e-16	**sig**	-0.19	-0.07	2772.9	-4.25	-0.1	0.03
55	5	2009	0.032	<	0.187	5	2010	<2e-16	**sig**	-0.33	-0.20	2673.3	-7.74	-0.3	0.03
56	5	2009	0.032	<	0.043	1	2011	0.13	1.97	-0.06	0.01	3040.9	-1.51	0	0.02
57	5	2009	0.032	<	0.073	2	2011	<2e-16	**sig**	-0.13	-0.05	3042.0	-4.81	-0.1	0.02
58	5	2009	0.032	<	0.119	3	2011	<2e-16	**sig**	-0.22	-0.13	3034.4	-7.57	-0.2	0.02
59	5	2009	0.032	<	0.040	4	2011	0.24	3.56	-0.06	0.01	3041.9	-1.18	0	0.02
60	5	2009	0.032	<	0.042	5	2011	0.17	2.57	-0.06	0.01	3044.0	-1.37	0	0.02
61	1	2010	0.069	<	0.125	2	2010	0.003	**0.045**	-0.16	-0.03	2539.5	-3.00	-0.1	0.03
62	1	2010	0.069	<	0.269	3	2010	<2e-16	**sig**	-0.36	-0.20	2571.4	-6.72	-0.3	0.04
63	1	2010	0.069	<	0.092	4	2010	0.19	2.82	-0.11	0.02	2648.4	-1.32	0	0.03
64	1	2010	0.069	<	0.187	5	2010	<2e-16	**sig**	-0.26	-0.11	2612.5	-4.68	-0.2	0.04
65	1	2010	0.069	>	0.043	1	2011	0.02	0.29	0.01	0.10	2978.5	2.35	0.1	0.02
66	1	2010	0.069	<	0.073	2	2011	0.71	10.70	-0.06	0.04	2983.4	-0.37	0	0.02
67	1	2010	0.069	<	0.119	3	2011	0.001	**0.02**	-0.14	-0.03	2957.2	-3.21	-0.1	0.03
68	1	2010	0.069	>	0.040	4	2011	0.01	0.14	0.02	0.11	2973.7	2.63	0.1	0.02

Order	Season A	Year A	Estimate A	R	Estimate B	Season B	Year B	P-value	P-value BC	Lower CI	Upper CI	DF	t-value	Estimate	SE
69	1	2010	0.069	>	0.042	5	2011	0.02	0.24	0.01	0.11	2975.4	2.40	0.1	0.02
70	2	2010	0.125	<	0.269	3	2010	<2e-16	**sig**	-0.27	-0.10	2531.9	-4.17	-0.2	0.04
71	2	2010	0.125	>	0.092	4	2010	0.15	2.27	-0.02	0.12	2621.4	1.43	0.1	0.04
72	2	2010	0.125	<	0.187	5	2010	0.04	0.60	-0.17	0.00	2626.8	-2.06	-0.1	0.04
73	2	2010	0.125	>	0.043	1	2011	<2e-16	**sig**	0.10	0.21	2965.9	5.41	0.2	0.03
74	2	2010	0.125	>	0.073	2	2011	0.003	**0.05**	0.03	0.14	2945.4	3.00	0.1	0.03
75	2	2010	0.125	>	0.119	3	2011	0.77	11.61	-0.05	0.07	2920.7	0.29	0	0.03
76	2	2010	0.125	>	0.040	4	2011	<2e-16	**sig**	0.10	0.21	2948.5	5.61	0.2	0.03
77	2	2010	0.125	>	0.042	5	2011	<2e-16	**sig**	0.10	0.21	2952.5	5.38	0.2	0.03
78	3	2010	0.269	>	0.092	4	2010	<2e-16	**sig**	0.15	0.32	2564.4	5.26	0.2	0.05
79	3	2010	0.269	>	0.187	5	2010	0.05	0.69	0.00	0.19	2556.2	1.99	0.1	0.05
80	3	2010	0.269	>	0.043	1	2011	<2e-16	**sig**	0.26	0.41	2796.8	8.69	0.3	0.04
81	3	2010	0.269	>	0.073	2	2011	<2e-16	**sig**	0.19	0.35	2775.4	6.91	0.3	0.04
82	3	2010	0.269	>	0.119	3	2011	<2e-16	**sig**	0.11	0.27	2771.5	4.74	0.2	0.04
83	3	2010	0.269	>	0.040	4	2011	<2e-16	**sig**	0.27	0.42	2782.3	8.84	0.3	0.04
84	3	2010	0.269	>	0.042	5	2011	<2e-16	**sig**	0.26	0.42	2789.6	8.65	0.3	0.04
85	4	2010	0.092	<	0.187	5	2010	0.001	**0.02**	-0.22	-0.06	2608.9	-3.26	-0.1	0.04
86	4	2010	0.092	>	0.043	1	2011	6E-04	**0.01**	0.04	0.16	2922.5	3.42	0.1	0.03
87	4	2010	0.092	>	0.073	2	2011	0.25	3.71	-0.02	0.09	2910.7	1.16	0	0.03
88	4	2010	0.092	<	0.119	3	2011	0.18	2.69	-0.11	0.02	2906.8	-1.34	0	0.03
89	4	2010	0.092	>	0.040	4	2011	4E-04	**0.01**	0.05	0.16	2895.5	3.56	0.1	0.03
90	4	2010	0.092	>	0.042	5	2011	6E-04	**0.01**	0.04	0.16	2911.2	3.43	0.1	0.03
91	5	2010	0.187	>	0.043	1	2011	<2e-16	**sig**	0.17	0.31	2791.5	6.72	0.2	0.04
92	5	2010	0.187	>	0.073	2	2011	<2e-16	**sig**	0.10	0.24	2791.6	4.79	0.2	0.04
93	5	2010	0.187	>	0.119	3	2011	0.01	0.20	0.02	0.17	2792.3	2.50	0.1	0.04
94	5	2010	0.187	>	0.040	4	2011	<2e-16	**sig**	0.18	0.31	2771.2	6.88	0.2	0.04
95	5	2010	0.187	>	0.042	5	2011	<2e-16	**sig**	0.17	0.31	2775.7	6.70	0.2	0.04

Order	Season A	Year A	Estimate A	R	Estimate B	Season B	Year B	P-value	P-value BC	Lower CI	Upper CI	DF	t-value	Estimate	SE
96	1	2011	0.043	<	0.073	2	2011	1E-04	**0.002**	-0.10	-0.03	2653.0	-3.93	-0.1	0.02
97	1	2011	0.043	<	0.119	3	2011	<2e-16	**sig**	-0.18	-0.10	2766.3	-7.03	-0.1	0.02
98	1	2011	0.043	>	0.040	4	2011	0.71	10.68	-0.02	0.04	2662.3	0.37	0	0.02
99	1	2011	0.043	>	0.042	5	2011	0.91	13.71	-0.03	0.03	2674.4	0.11	0	0.02
100	2	2011	0.073	<	0.119	3	2011	3E-04	**0.005**	-0.12	-0.04	2677.0	-3.65	-0.1	0.02
101	2	2011	0.073	>	0.040	4	2011	<2e-16	**sig**	0.04	0.10	2693.7	4.22	0.1	0.02
102	2	2011	0.073	>	0.042	5	2011	1E-04	**0.002**	0.03	0.10	2726.9	3.82	0.1	0.02
103	3	2011	0.119	>	0.040	4	2011	<2e-16	**sig**	0.11	0.19	2742.3	7.28	0.1	0.02
104	3	2011	0.119	>	0.042	5	2011	<2e-16	**sig**	0.10	0.19	2764.8	6.87	0.1	0.02
105	4	2011	0.040	<	0.042	5	2011	0.81	12.12	-0.04	0.03	2575.9	-0.24	0	0.02

Table A.4.2.3. Differences of least squares means for strength of pairwise spatial association that varies by season and the sex of the associating pair (i.e., interaction term season*sexes).

Order	Season A	Sexes A	Estimate A	R	Estimate B	Season B	Sexes B	P-value	P-value BC	Lower CI	Upper CI	DF	t-value	Estimate	SE
1	1	FF	0.055	<	0.098	2	FF	1E-04	**0.002**	-0.12	-0.04	2640.7	-3.93	-0.1	0.02
2	1	FF	0.055	<	0.147	3	FF	<2e-16	**sig**	-0.20	-0.11	2639.8	-6.83	-0.2	0.02
3	1	FF	0.055	>	0.054	4	FF	0.98	14.66	-0.04	0.04	2612.5	0.03	0	0.02
4	1	FF	0.055	<	0.062	5	FF	0.46	6.86	-0.05	0.02	2659.7	-0.74	0	0.02
5	1	FF	0.055	>	0.035	1	MF	0.01	0.14	0.01	0.09	2802.2	2.59	0	0.02
6	1	FF	0.055	<	0.069	2	MF	0.14	2.15	-0.07	0.01	2806.3	-1.47	0	0.02
7	1	FF	0.055	<	0.085	3	MF	0.01	0.17	-0.11	-0.01	2978.0	-2.55	-0.1	0.02
8	1	FF	0.055	<	0.078	4	MF	0.02	0.30	-0.09	-0.01	2869.4	-2.32	0	0.02
9	1	FF	0.055	<	0.066	5	MF	0.26	3.84	-0.07	0.02	3006.9	-1.14	0	0.02
10	1	FF	0.055	<	0.057	1	MM	0.87	13.02	-0.05	0.04	2896.3	-0.17	0	0.02

Order	Season A	Sexes A	Estimate A	R	Estimate B	Season B	Sexes B	P-value	P-value BC	Lower CI	Upper CI	DF	t-value	Estimate	SE
11	1	FF	0.055	<	0.090	2	MM	0.004	0.06	-0.11	-0.02	2906.1	-2.89	-0.1	0.02
12	1	FF	0.055	<	0.193	3	MM	<2e-16	**sig**	-0.28	-0.16	3043.2	-7.00	-0.2	0.03
13	1	FF	0.055	>	0.026	4	MM	0.002	**0.03**	0.03	0.12	2957.1	3.10	0.1	0.02
14	1	FF	0.055	<	0.101	5	MM	0.002	**0.03**	-0.14	-0.03	3037.6	-3.05	-0.1	0.03
15	2	FF	0.098	<	0.147	3	FF	0.003	**0.05**	-0.12	-0.03	2532.1	-2.96	-0.1	0.03
16	2	FF	0.098	>	0.054	4	FF	3E-04	**0.005**	0.04	0.13	2559.3	3.60	0.1	0.02
17	2	FF	0.098	>	0.062	5	FF	0.002	**0.03**	0.02	0.11	2742.7	3.04	0.1	0.02
18	2	FF	0.098	>	0.035	1	MF	<2e-16	**sig**	0.09	0.17	2929.2	5.97	0.1	0.02
19	2	FF	0.098	>	0.069	2	MF	0.02	0.27	0.01	0.10	2825.7	2.37	0.1	0.02
20	2	FF	0.098	>	0.085	3	MF	0.40	5.96	-0.03	0.07	3016.0	0.85	0	0.03
21	2	FF	0.098	>	0.078	4	MF	0.11	1.65	-0.01	0.08	2959.2	1.60	0	0.02
22	2	FF	0.098	>	0.066	5	MF	0.02	0.26	0.01	0.11	3030.5	2.40	0.1	0.02
23	2	FF	0.098	>	0.057	1	MM	0.002	**0.03**	0.03	0.13	2957.8	3.10	0.1	0.03
24	2	FF	0.098	>	0.090	2	MM	0.55	**8.28**	-0.03	0.06	2895.6	0.60	0	0.02
25	2	FF	0.098	<	0.193	3	MM	<2e-16	**sig**	-0.20	-0.07	3043.9	-4.11	-0.1	0.03
26	2	FF	0.098	>	0.026	4	MM	<2e-16	**sig**	0.11	0.21	2993.3	5.90	0.2	0.03
27	2	FF	0.098	<	0.101	5	MM	0.88	13.14	-0.07	0.06	3041.4	-0.16	0	0.03
28	3	FF	0.147	>	0.054	4	FF	<2e-16	**sig**	0.11	0.21	2519.4	6.36	0.2	0.02
29	3	FF	0.147	>	0.062	5	FF	<2e-16	**sig**	0.10	0.19	2698.5	5.90	0.1	0.02
30	3	FF	0.147	>	0.035	1	MF	<2e-16	**sig**	0.16	0.25	3015.8	8.61	0.2	0.02
31	3	FF	0.147	>	0.069	2	MF	<2e-16	**sig**	0.08	0.18	3007.1	5.06	0.1	0.03
32	3	FF	0.147	>	0.085	3	MF	1E-04	**0.002**	0.05	0.15	2972.4	3.88	0.1	0.03
33	3	FF	0.147	>	0.078	4	MF	<2e-16	**sig**	0.06	0.16	3024.1	4.51	0.1	0.02
34	3	FF	0.147	>	0.066	5	MF	<2e-16	**sig**	0.08	0.18	3043.9	5.12	0.1	0.03
35	3	FF	0.147	>	0.057	1	MM	<2e-16	**sig**	0.10	0.21	3018.0	5.65	0.2	0.03
36	3	FF	0.147	>	0.090	2	MM	0.001	**0.02**	0.04	0.14	3015.0	3.24	0.1	0.03
37	3	FF	0.147	<	0.193	3	MM	0.05	0.81	-0.12	0.00	3038.4	-1.93	-0.1	0.03
38	3	FF	0.147	>	0.026	4	MM	<2e-16	**sig**	0.18	0.29	3028.6	8.20	0.2	0.03

Order	Season A	Sexes A	Estimate A	R	Estimate B	Season B	Sexes B	P-value	P-value BC	Lower CI	Upper CI	DF	t-value	Estimate	SE
39	3	FF	0.147	>	0.101	5	MM	0.03	0.44	0.01	0.13	3043.1	2.18	0.1	0.03
40	4	FF	0.054	<	0.062	5	FF	0.49	7.34	-0.06	0.03	2643.9	-0.69	0	0.02
41	4	FF	0.054	>	0.035	1	MF	0.03	0.38	0.01	0.09	2923.0	2.23	0	0.02
42	4	FF	0.054	<	0.069	2	MF	0.18	2.72	-0.08	0.01	2911.2	-1.34	0	0.02
43	4	FF	0.054	<	0.085	3	MF	0.02	0.27	-0.11	-0.01	3010.9	-2.36	-0.1	0.03
44	4	FF	0.054	<	0.078	4	MF	0.03	0.44	-0.09	0.00	2894.3	-2.18	0	0.02
45	4	FF	0.054	<	0.066	5	MF	0.29	4.40	-0.07	0.02	3029.4	-1.05	0	0.02
46	4	FF	0.054	<	0.057	1	MM	0.86	12.93	-0.05	0.04	2954.6	-0.17	0	0.03
47	4	FF	0.054	<	0.090	2	MM	0.01	0.12	-0.12	-0.02	2953.5	-2.67	-0.1	0.03
48	4	FF	0.054	<	0.193	3	MM	<2e-16	**sig**	-0.28	-0.15	3044.0	-6.68	-0.2	0.03
49	4	FF	0.054	>	0.026	4	MM	0.003	**0.05**	0.02	0.12	2954.8	2.94	0.1	0.03
50	4	FF	0.054	<	0.101	5	MM	0.004	0.06	-0.15	-0.03	3040.7	-2.89	-0.1	0.03
51	5	FF	0.062	>	0.035	1	MF	0.002	**0.03**	0.02	0.10	2960.2	3.12	0.1	0.02
52	5	FF	0.062	<	0.069	2	MF	0.47	7.10	-0.06	0.03	2940.5	-0.72	0	0.02
53	5	FF	0.062	<	0.085	3	MF	0.06	0.95	-0.09	0.00	3029.3	-1.86	0	0.02
54	5	FF	0.062	<	0.078	4	MF	0.14	2.03	-0.07	0.01	2984.0	-1.50	0	0.02
55	5	FF	0.062	<	0.066	5	MF	0.61	9.14	-0.05	0.03	2916.3	-0.51	0	0.02
56	5	FF	0.062	>	0.057	1	MM	0.66	9.84	-0.04	0.06	2975.8	0.44	0	0.02
57	5	FF	0.062	<	0.090	2	MM	0.03	0.45	-0.10	-0.01	2973.3	-2.17	-0.1	0.02
58	5	FF	0.062	<	0.193	3	MM	<2e-16	**sig**	-0.27	-0.14	3043.3	-6.37	-0.2	0.03
59	5	FF	0.062	>	0.026	4	MM	4E-04	**0.01**	0.04	0.14	3005.1	3.54	0.1	0.03
60	5	FF	0.062	<	0.101	5	MM	0.01	0.11	-0.13	-0.02	3022.8	-2.69	-0.1	0.03
61	1	MF	0.035	<	0.069	2	MF	<2e-16	**sig**	-0.12	-0.04	2622.7	-4.26	-0.1	0.02
62	1	MF	0.035	<	0.085	3	MF	<2e-16	**sig**	-0.15	-0.07	2710.9	-4.95	-0.1	0.02
63	1	MF	0.035	<	0.078	4	MF	<2e-16	**sig**	-0.13	-0.06	2716.9	-5.21	-0.1	0.02
64	1	MF	0.035	<	0.066	5	MF	3E-04	**0.005**	-0.11	-0.03	2731.6	-3.63	-0.1	0.02
65	1	MF	0.035	<	0.057	1	MM	0.01	0.14	-0.09	-0.01	2955.3	-2.60	-0.1	0.02
66	1	MF	0.035	<	0.090	2	MM	<2e-16	**sig**	-0.16	-0.07	3028.5	-5.15	-0.1	0.02

Order	Season A	Sexes A	Estimate A	R	Estimate B	Season B	Sexes B	P-value	P-value BC	Lower CI	Upper CI	DF	t-value	Estimate	SE
67	1	MF	0.035	<	0.193	3	MM	<2e-16	**sig**	-0.33	-0.21	3030.0	-8.74	-0.3	0.03
68	1	MF	0.035	>	0.026	4	MM	0.26	3.89	-0.02	0.07	3038.4	1.13	0	0.02
69	1	MF	0.035	<	0.101	5	MM	<2e-16	**sig**	-0.19	-0.08	3039.2	-4.86	-0.1	0.03
70	2	MF	0.069	<	0.085	3	MF	0.18	2.76	-0.08	0.01	2603.3	-1.33	0	0.02
71	2	MF	0.069	<	0.078	4	MF	0.42	6.24	-0.05	0.02	2812.4	-0.81	0	0.02
72	2	MF	0.069	>	0.066	5	MF	0.79	11.91	-0.04	0.05	2813.0	0.26	0	0.02
73	2	MF	0.069	>	0.057	1	MM	0.26	3.87	-0.02	0.07	3019.4	1.13	0	0.02
74	2	MF	0.069	<	0.090	2	MM	0.09	1.34	-0.08	0.01	2937.0	-1.70	0	0.02
75	2	MF	0.069	<	0.193	3	MM	<2e-16	**sig**	-0.25	-0.13	3035.0	-5.99	-0.2	0.03
76	2	MF	0.069	>	0.026	4	MM	<2e-16	**sig**	0.06	0.15	3034.5	4.25	0.1	0.02
77	2	MF	0.069	<	0.101	5	MM	0.05	0.72	-0.11	0.00	3042.7	-1.98	-0.1	0.03
78	3	MF	0.085	>	0.078	4	MF	0.53	7.97	-0.03	0.06	2735.5	0.63	0	0.02
79	3	MF	0.085	>	0.066	5	MF	0.14	2.12	-0.01	0.08	2764.7	1.47	0	0.02
80	3	MF	0.085	>	0.057	1	MM	0.03	0.47	0.01	0.11	3043.3	2.16	0.1	0.03
81	3	MF	0.085	<	0.090	2	MM	0.78	11.75	-0.06	0.05	3042.8	-0.28	0	0.03
82	3	MF	0.085	<	0.193	3	MM	<2e-16	**sig**	-0.22	-0.10	3043.8	-5.17	-0.2	0.03
83	3	MF	0.085	>	0.026	4	MM	<2e-16	**sig**	0.08	0.19	3042.9	4.95	0.1	0.03
84	3	MF	0.085	<	0.101	5	MM	0.39	5.84	-0.09	0.03	3030.2	-0.86	0	0.03
85	4	MF	0.078	>	0.066	5	MF	0.30	4.52	-0.02	0.06	2677.5	1.04	0	0.02
86	4	MF	0.078	>	0.057	1	MM	0.07	0.98	0.00	0.09	3030.8	1.85	0	0.02
87	4	MF	0.078	<	0.090	2	MM	0.36	5.33	-0.07	0.02	3034.3	-0.92	0	0.02
88	4	MF	0.078	<	0.193	3	MM	<2e-16	**sig**	-0.23	-0.11	3025.6	-5.54	-0.2	0.03
89	4	MF	0.078	>	0.026	4	MM	<2e-16	**sig**	0.08	0.16	2978.6	5.79	0.1	0.02
90	4	MF	0.078	<	0.101	5	MM	0.15	2.25	-0.10	0.01	3037.1	-1.44	0	0.03
91	5	MF	0.066	>	0.057	1	MM	0.40	5.97	-0.03	0.07	3043.9	0.85	0	0.02
92	5	MF	0.066	<	0.090	2	MM	0.08	1.25	-0.09	0.01	3044.0	-1.74	0	0.03
93	5	MF	0.066	<	0.193	3	MM	<2e-16	**sig**	-0.26	-0.13	3012.4	-6.01	-0.2	0.03
94	5	MF	0.066	>	0.026	4	MM	1E-04	**0.002**	0.05	0.15	3042.9	3.87	0.1	0.03

Order	Season A	Sexes A	Estimate A	R	Estimate B	Season B	Sexes B	P-value	P-value BC	Lower CI	Upper CI	DF	t-value	Estimate	SE
95	5	MF	0.066	<	0.101	5	MM	0.01	0.20	-0.11	-0.01	3039.4	-2.47	-0.1	0.03
96	1	MM	0.057	<	0.090	2	MM	0.01	0.15	-0.11	-0.02	2622.5	-2.59	-0.1	0.02
97	1	MM	0.057	<	0.193	3	MM	<2e-16	**sig**	-0.28	-0.15	2730.8	-6.71	-0.2	0.03
98	1	MM	0.057	>	0.026	4	MM	0.002	**0.03**	0.03	0.13	2751.8	3.07	0.1	0.03
99	1	MM	0.057	<	0.101	5	MM	0.005	0.08	-0.14	-0.03	2694.7	-2.82	-0.1	0.03
100	2	MM	0.090	<	0.193	3	MM	<2e-16	**sig**	-0.21	-0.09	2624.5	-4.67	-0.2	0.03
101	2	MM	0.090	>	0.026	4	MM	<2e-16	**sig**	0.09	0.19	2777.9	5.44	0.1	0.03
102	2	MM	0.090	<	0.101	5	MM	0.52	7.73	-0.08	0.04	2717.3	-0.65	0	0.03
103	3	MM	0.193	>	0.026	4	MM	<2e-16	**sig**	0.23	0.36	2808.1	8.81	0.3	0.03
104	3	MM	0.193	>	0.101	5	MM	3E-04	**0.005**	0.06	0.20	2723.3	3.61	0.1	0.04
105	4	MM	0.026	<	0.101	5	MM	<2e-16	**sig**	-0.22	-0.10	2655.5	-5.30	-0.2	0.03

4.12 APPENDIX 4.3.

Appendix 4.3. Details on temporal patterns of spatial associations among mule deer in a chronic wasting disease endemic area in Saskatchewan, Canada.

To test for sex, age and chronic wasting disease (CWD) status differences in temporal stability of associations among adult Rocky Mountain mule deer (*Odocoileus hemionus hemionus*) we analysed lagged association rates (LARs) in SOCPROG 2.6 (Whitehead, 1995, 2008, 2009, 2014).

We included 44 individuals with every-2-hour GPS-telemetry data from April 1st 2011 to March 31st 2012 (i.e., study year 2011), and that survived the whole year.

These are the number of individuals for each sex, age and CWD status class, as well as the number of records and unique pairs in the dataset:

Table A.4.3.1. Number of mule deer individuals from different classes in the study.

Class	Number of individuals in the study
Female (F)	21
Male (M)	23
Older (O)	28
Younger (Y)	16
CWD-positive (P)	32
CWD-negative (N)	12

Table A.4.3.2. Number of records and unique pairs for different types of pairs in the study.

Class	Pair class	Record count	Unique pairs count
Sex	FF	1656	50
	FM	1365	172
	MM	2589	122
Age	OO	1645	124
	OY	3182	175
	YY	783	45
CWD diagnosis	PP	2770	190
	PN	2290	134
	NN	550	20
Total	All	5610	344

To calculate LARs in SOCPROG 2.6, we:

1. Set the sampling period as “date” (i.e., 1 day).
2. Defined associations as grouped in sampling period.
3. Simultaneously fitted a set of 7 mathematical models to the observed LARs. These models were of the exponential family and were composed of all, one, or any meaningful combination of three components: rapid disassociations (associations lasting 1 day at most), casual acquaintances (associations that decay over time; their average duration is approximated from the exponent of the exponential function, e.g., 1/a1, in days), and preferred/constant companionships (associations that do not decay or increase over time; their duration is interpreted within the context of the study period, in this case 1 year) (Whitehead, 1995).

Table A.4.3.3. Seven mathematical models fitted to the observed lagged association rates.

Model ID	Model type [a]	Quick model explanation [b]
1	a1	Rapid dis. + pref. comps
2	exp(-a1*td)	Casual acqs
3	a2*exp(-a1*td)	Rapid dis. + casual acqs
A	a2+a3*exp(-a1*td)	Rapid dis. + pref. comps + casual acqs
5	a2+(1-a2)*exp(-a1*td)	Pref. comps + casual acqs
B	a3*exp(-a1*td)+a4*exp(-a2*td)	Rapid dis. + two levels of casual acqs
7	a3*exp(-a1*td)+(1-a3)*exp(-a2*td)	Two levels of casual acqs

a The time lag is represented by 'td' and the parameters of the models by 'a1', 'a2', 'a3' and 'a4'. Representation of the parameters vary among models (e.g., a2 in model A is not the same as a2 in model B).

b Model explanations should not be taken literally without some thought as different types of social systems can produce similar patterns of lagged association rates which fit the same mathematical model (Whitehead, 2008).

1. Identified, for each of the pair classes, the best fitting and most parsimonious model by the smallest quasi-Akaike information criterion (QAIC) (Whitehead, 2007).
2. Estimated error around the association rates and their durations with a jackknife procedure over 3-day periods, and in some cases (MM, YY and PP) over 30-, 45- and 10-day periods, respectively, to obtain better estimates (Whitehead, 1995).
3. Compared LARs to a null association rate (NAR), the expected LAR if animals had associated randomly, given the daily number of associations of each individual (Whitehead, 2008).

Results

The between-class LARs were best described by either one of two models: (A) a model containing rapid disassociations, constant companionships and casual acquaintances, or (B) a model containing rapid disassociations and two levels of casual acquaintances, one lasting longer than the other.

Table A.4.3.4. Parameters (and their standard errors) as obtained from best fitting model A.

Class	Jackknife	a1	a1 SE	a2	a2 SE	a3	a3 SE
FM	3	0.057	0.029	0.130	0.012	0.141	0.033
OO	3	0.026	0.014	0.294	0.024	0.196	0.033
OY	3	0.021	0.011	0.463	0.020	0.142	0.025
NN	3	0.024	0.011	0.367	0.038	0.207	0.046
PN	3	0.031	0.008	0.399	0.020	0.187	0.028

Model A is given by the formula

a2+a3*exp(-a1*td)

where a2 is the proportion of preferred/constant companionships, a3 is the proportion of casual acquaintances, and a1 is the rate of decay of a3 (to approximate the average duration of a3, use 1/a1). The proportion of rapid disassociation is calculated by 1-a2-a3.

Table A.4.3.5. Proportions and temporal characteristics of associations as obtained from best fitting model A.

Class	% Rapid dis.	% Constant comp. (SE)	Casual acquaintances	
			% (SE)	Duration in days (range)
FM	72.9	13.0 (1.2)	14.1 (3.3)	17 (12 to 36)
OO	51.1	29.4 (2.4)	19.6 (3.3)	39 (25 to 88)
OY	39.5	46.3 (2.0)	14.2 (2.5)	47 (32 to 95)
NN	42.6	36.7 (3.8)	20.7 (4.6)	43 (29 to 79)
PN	41.4	39.9 (2.0)	18.7 (2.8)	32 (26 to 43)

Table A.4.3.6 Parameters (and their standard errors) as obtained from best fitting model B.

Class	Jackknife	a1	a1 SE	a2	a2 SE	a3	a3 SE	a4	a4 SE
FF	100	0.895	0.532	0.001	0.000	0.232	0.141	0.711	0.052
MM	30	0.010	0.721	-0.005	0.010	0.516	0.068	0.091	0.505
YY	45	0.027	0.621	-0.002	0.004	0.254	0.224	0.243	0.182
PP	10	0.538	0.397	0.001	0.000	0.293	0.169	0.499	0.046

Model B is given by the formula

$$a3*\exp(-a1*td)+a4*\exp(-a2*td)$$

where a3 is the proportion of casual acquaintances of shorter duration, a4 is the proportion of casual acquaintances of longer duration, a1 is the rate of decay of a3 (to approximate the average duration of a3, use 1/a1), and a2 is the rate of decay of a4 (to approximate the average duration of a4, use 1/a2). The proportion of rapid disassociation is calculated by 1-a3-a4.

Table A.4.3.7. Proportions and temporal characteristics of associations as obtained from best fitting model B.

		Shorter casual acquaintances		Longer casual acquaintances	
Class	% Rapid dis.	% (SE)	Duration in days (range)	% (SE)	Duration in days (range)
FF	5.7	23.2 (14.1)	1 (1 to 3)	71.1 (5.2)	980 (662 to 1889)
MM	39.3	51.6 (6.8)	96 (1 to 1)	9.1 (50.5)	182 (67 to 248)
YY	50.3	25.4 (22.4)	37 (2 to 2)	24.3 (18.2)	514 (173 to 530)
PP	20.8	29.3 (16.9)	2 (1 to 7)	49.9 (4.6)	1062 (721 to 2121)

Figures A.4.3.1 to A.4.3.5. Lagged association rate (in blue), null association rate (in red) and the best fit model (in green), for different sex, age and CWD classes of adult mule deer (*Odocoileus hemionus hemionus*) monitored from 1 April 2011 to 31 March 2012, in Antelope Creek, Saskatchewan, Canada. The best fit model included rapid disassociations, constant companions and casual acquaintances. Bars are jackknifed standard errors.

Figure A.4.3.1. Female-male

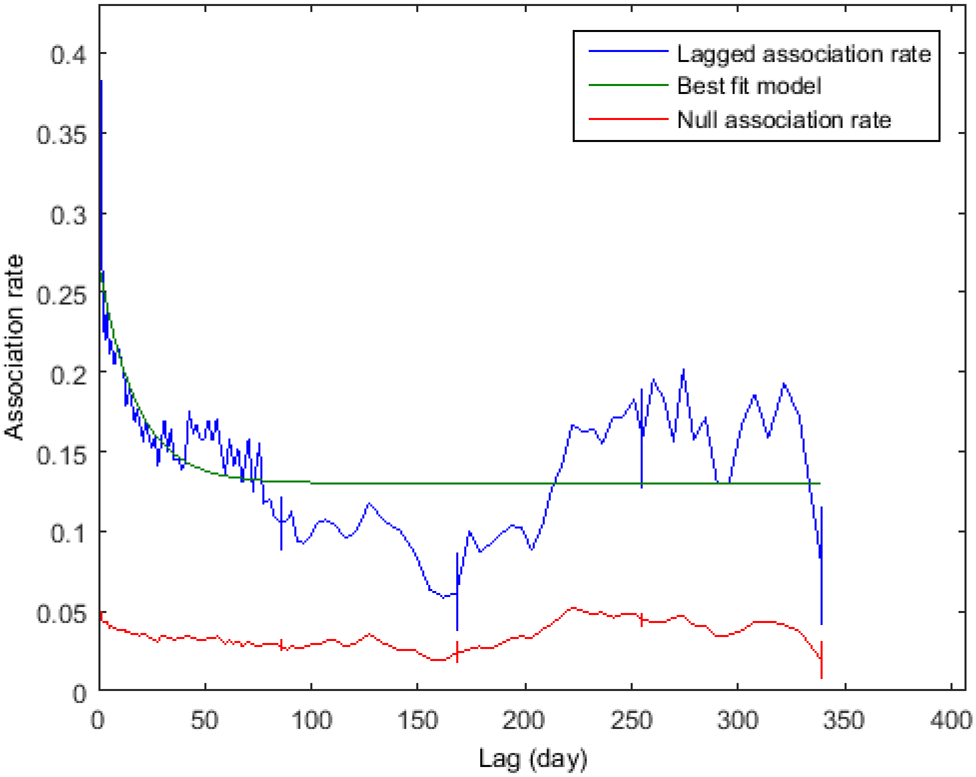


Figure A.4.3.2. Old-old.

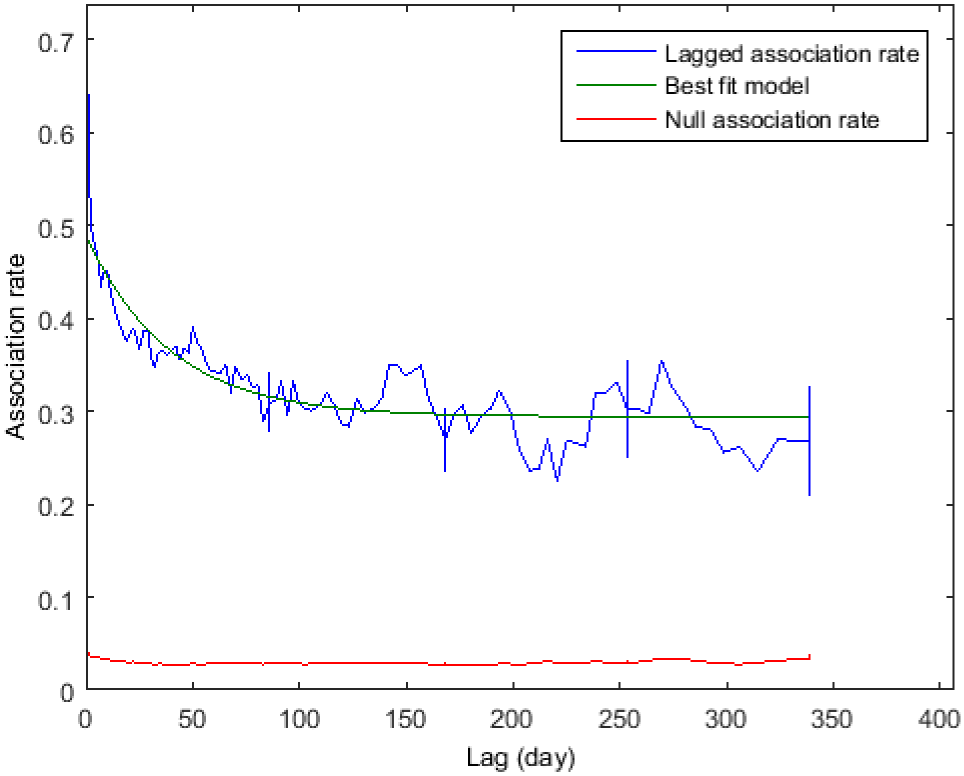


Figure A.4.3.3. Old-young.

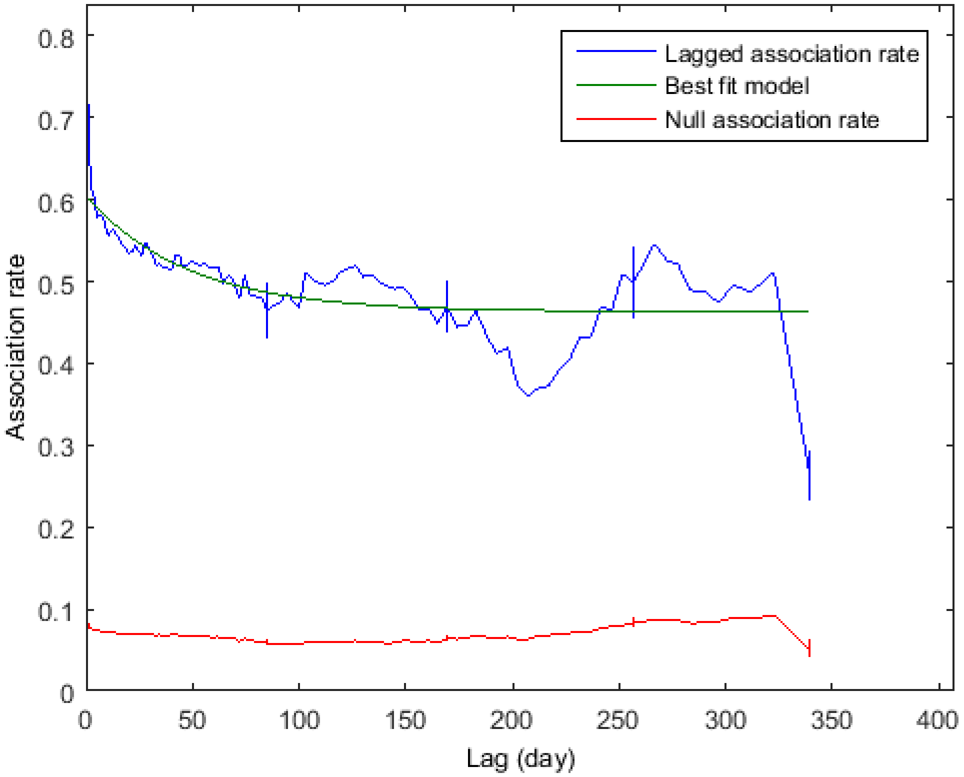


Figure A.4.3.4. Negative-negative.

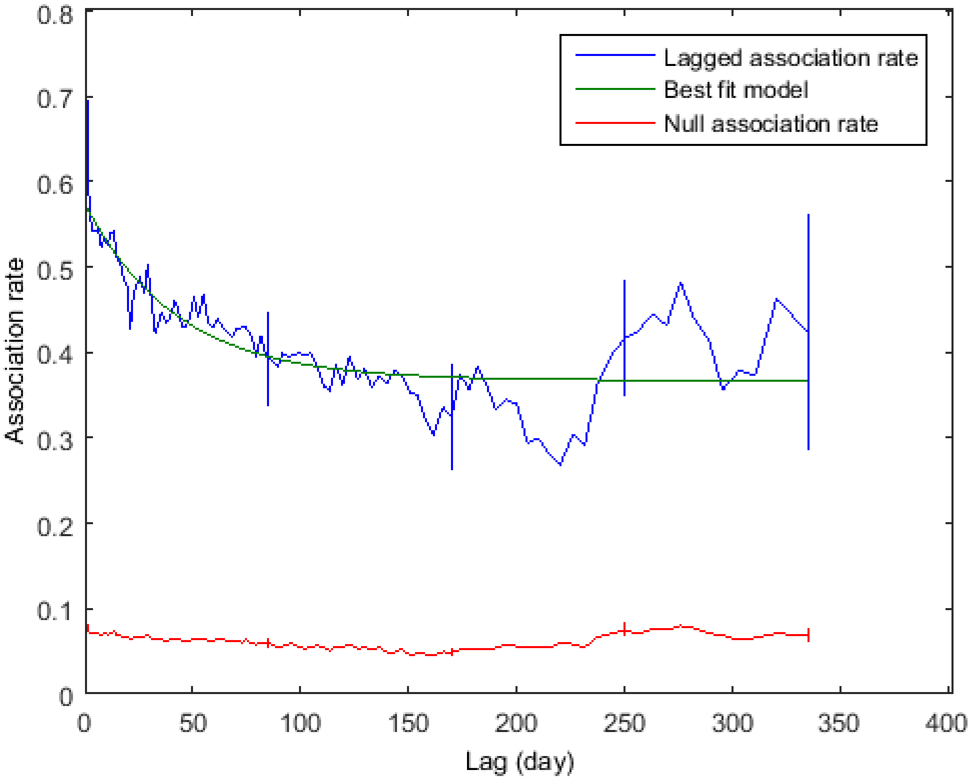
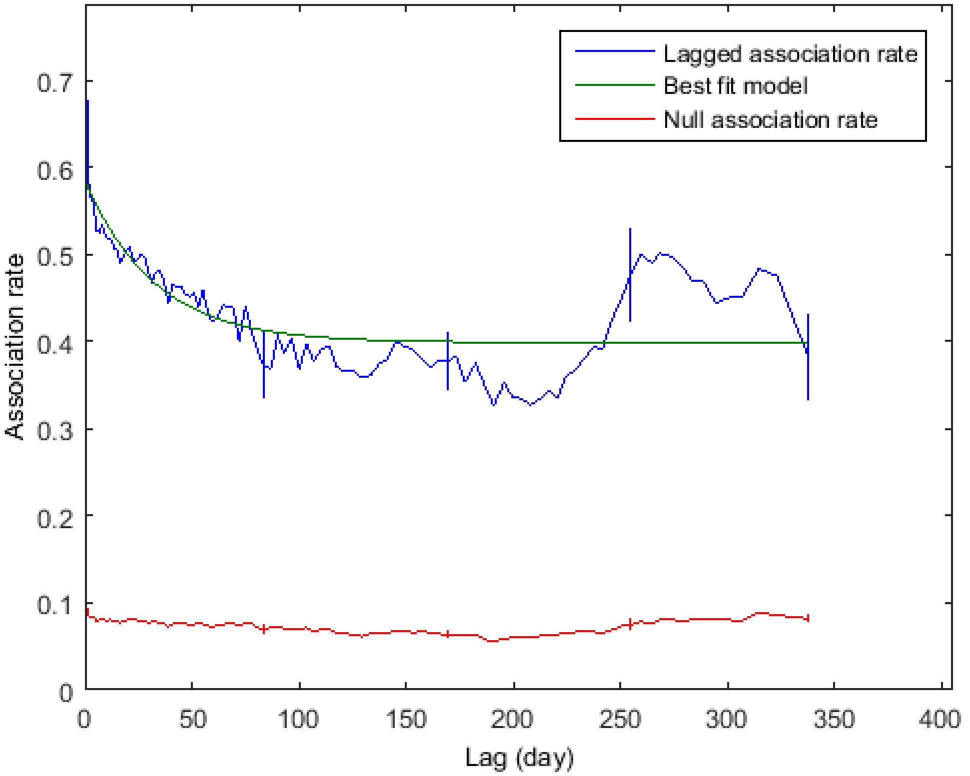


Figure A.4.3.5. Positive-negative.



Figures A.4.3.6 to A.4.3.9. Lagged association rate (in blue), null association rate (in red) and the best fit model (in green), for different sex, age and CWD classes of adult mule deer (*Odocoileus hemionus hemionus*) monitored from 1 April 2011 to 31 March 2012, in Antelope Creek, Saskatchewan, Canada. The best fit model included rapid disassociations, and two levels of casual acquaintances. Bars are jackknifed standard errors.

Figure A.4.3.6. Female-female

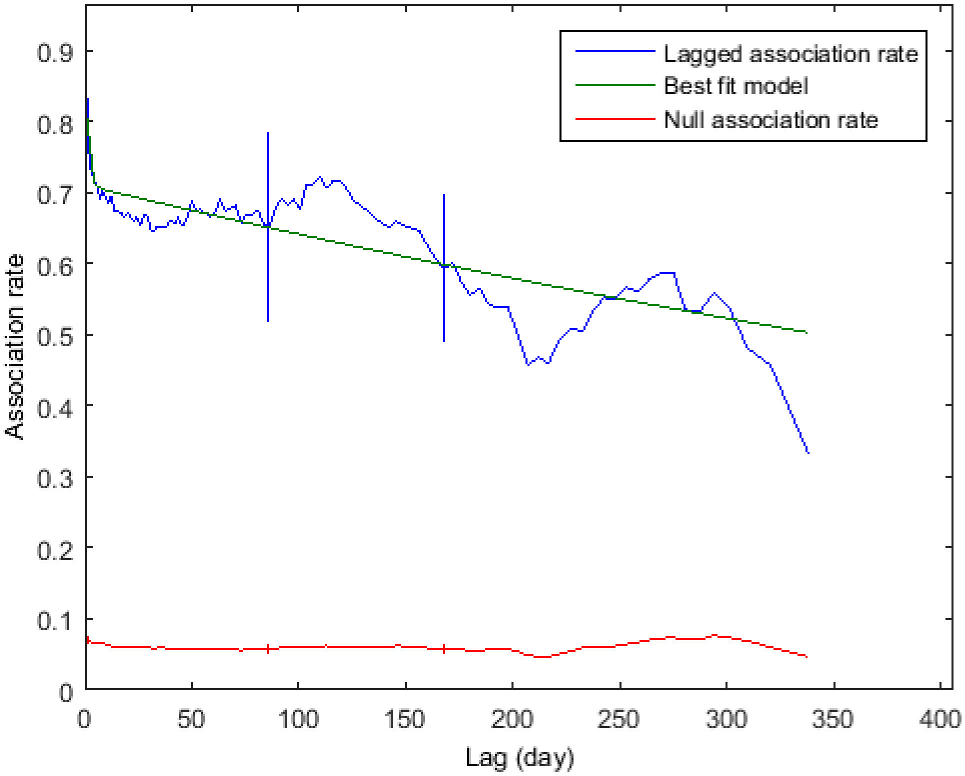


Figure A.4.3.7. Male-male.

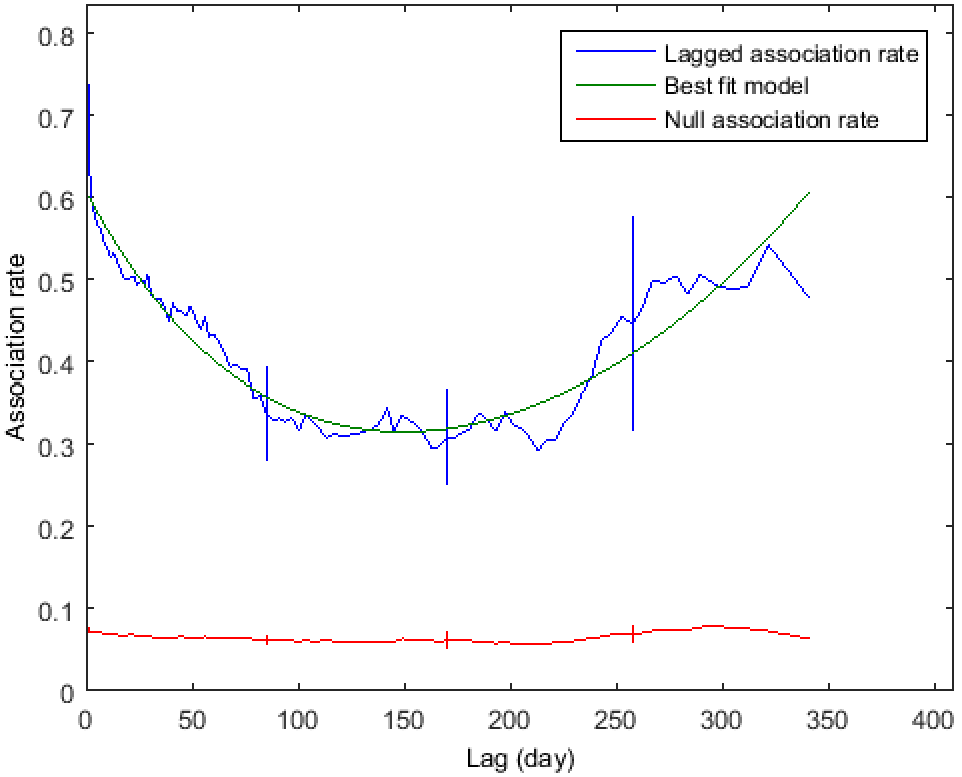


Figure A.4.3.8. Young-young.

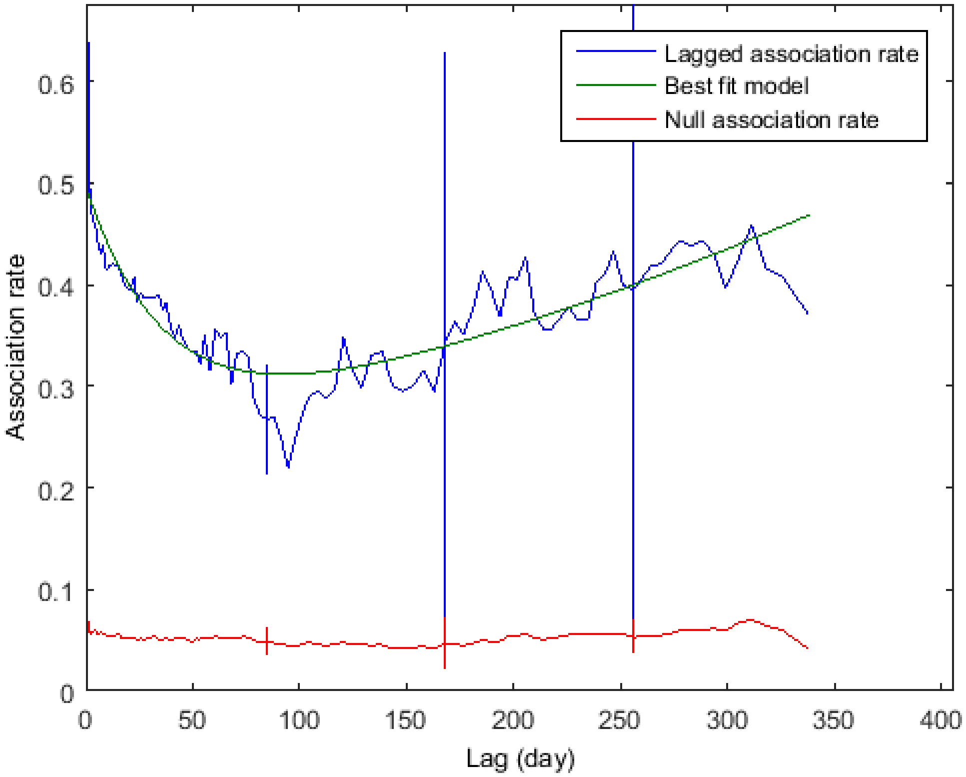
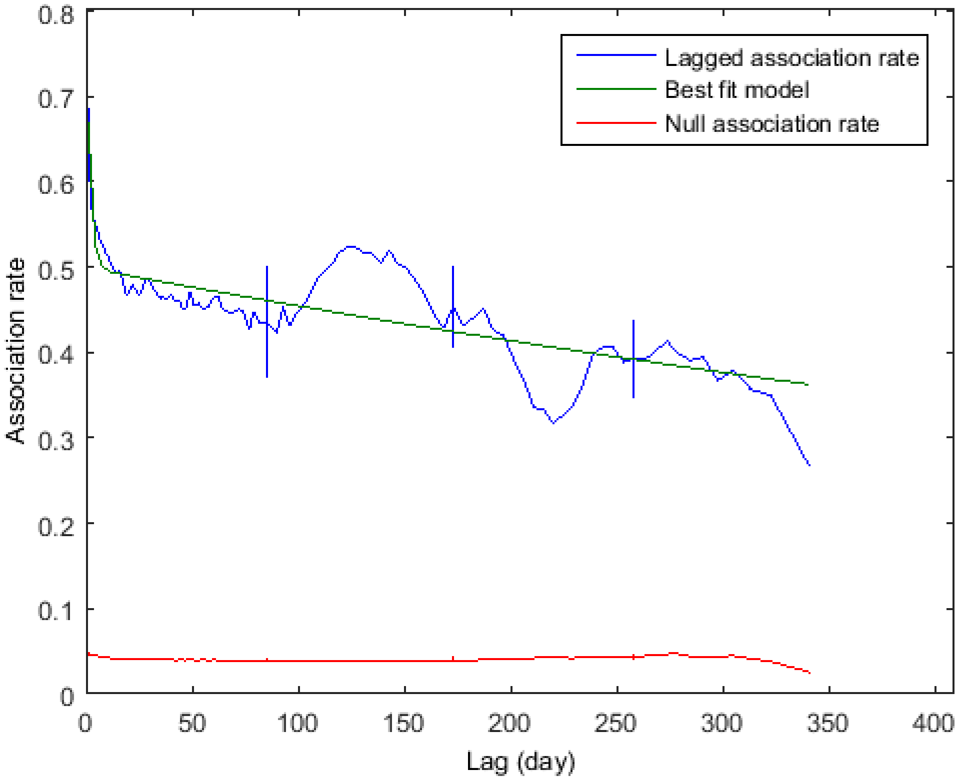


Figure A.4.3.9. Positive-positive.



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CHAPTER 5. PREDICTORS AND RECIPROCITY OF HIGH-RISK INTERACTIONS FOR DISEASE TRANSMISSION AMONG MULE DEER

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5.1 PUBLICATION AND CO-AUTHORSHIP STATEMENT

The content of the following chapter is exactly as submitted to Ethology on March 14th, 2017; however, the document has been reformatted for inclusion in this thesis. The contents will most probably change in various degrees from the published version after peer review.

5.1.1 Author’s contributions

Conceptualization: MFMS
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Formal analysis: MFMS CW TKB
Investigation: MFMS
Data curation: MFMS CC
Writing-original draft: MFMS
Writing-review: MFMS CW TKB CC
Visualization: MFMS
Supervision: TKB CW
Project administration: TKB
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PREDICTORS AND RECIPROCITY OF HIGH-RISK INTERACTIONS FOR DISEASE TRANSMISSION AMONG MULE DEER

Contact rates among mule deer

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5.2 ABSTRACT

As social structure is often a key determinant of patterns of disease spread within a population, and is built upon the non-random interactions among its members, the study of physical contacts between pairs of individuals can shed light on patterns of disease spread. North American cervids are affected by chronic wasting disease (CWD), which can be transmitted through direct physical contact. Using repeated focal observations of uniquely identified individuals, and genetic determination of relatedness, we investigated the factors that predict rates of high-risk physical contacts between mule deer (*Odocoileus hemionus*), and whether these interactions were reciprocal. High-risk contacts were those with the greatest potential to be relevant for disease transmission through physical interaction, and included sparring, Flehmen close, licking face, nose-genitalia, and nose-nose. Also, we used group membership to define the strength of associations between pairs of deer, and determine the factors that predict these associations. High-risk interactions were infrequent (1 physical contact every 238 hours of observation, on average). Association strength predicted the high-risk interaction rate. These interactions were reciprocal across all age, CWD status and sex classes. Genetic relatedness and sex homophily (i.e., pertaining to the same sex) predicted association strength. Same-sex associations were stronger than different-sex associations. Neither age nor CWD diagnosis homophily predicted interaction rates or association strength. We offer empirical data on non-random mixing between individual mule deer that can complement future epidemiological models. We discuss the implication of our results with respect to CWD transmission dynamics.

Keywords: association index, chronic wasting disease, cervid, physical contact rates, interaction rate, reciprocity, disease transmission.

5.3 INTRODUCTION

Social behaviour has important effects on the long-term dynamics of diseases (e.g., McDonald et al., 2008; Schauber et al., 2015; Vander Wal et al., 2012). In epidemiological studies, a contact network (a “who contacts whom” matrix between individuals) (Craft & Caillaud, 2011) can serve as a proxy for the disease transmission network (e.g., Bansal et al., 2010). This, in turn, helps predict how disease will spread through a population. As not all contacts lead to infection, a transmission network is almost always a subset of the contact network, depending on the pathogen and its transmission modes. In this study, we generated a contact network based on assumed high-risk interactions for the transmission of chronic wasting disease (CWD) among Rocky Mountain mule deer (*Odocoileus hemionus hemionus*) to identify host attributes that may be important in the spread of this disease.

Over the last three decades, populations of mule deer and white-tailed deer (*Odocoileus virginianus*) in North America have been affected by CWD (Edmunds et al., 2016; M. W. Miller et al., 2008), an always fatal and contagious prion encephalopathy. To date, its spread has not been controlled by management measures, and its geographic distribution continues to expand (e.g., Haley, 2016; McKenzie, 2016). CWD can be acquired as a result of exposure to both infected individuals and prion-contaminated environments. The most likely route of animal-to-animal transmission is through oral and nasal intake of prion-contaminated saliva, urine, and feces from infectious individuals (Haley, Mathiason, et al., 2009; Haley, Seelig, et al., 2009; Mathiason et al., 2009; Mathiason et al., 2006; Tamgüney et al., 2009). This is why social interactions that involve physical contact between mouths, noses and genitalia are of higher risk of transmitting disease than other types of interactions. Elucidating the factors affecting high-risk interaction rates can shed light on the transmission patterns of CWD among mule deer.

Although thorough studies on the social behaviour of *Odocoileus* spp exist (e.g., Bowyer et al., 2001; Lingle, 2003; Mejía-Salazar et al., 2017; Mejía Salazar et al., 2016), investigations of the factors related to physical contacts in the wild are missing, most likely due to the practical difficulties inherent to the collection of data of this type. Mule deer live in a society in which individuals frequently join and leave groups of varying size and composition. Most groups (83.7%) are small (≤5 individuals), but group sizes range from 1 to 39 deer (mean = 3.5, typical group size = 7.3, median = 2) (Mejía Salazar et al., 2016). The sexes have very different social lives, possibly due to differences in selection pressures. Males remain alone or in more ephemeral male groups in the nonbreeding season, increase their proximity to other males before rut and then move between groups of females during rut (Mejía-Salazar et al., 2017; Vos et al., 1967). In contrast, females prefer to associate with other females in more stable relationships; however, these do not necessarily occur between specific pairs of individuals (Mejía-Salazar et al., 2017).

The concurrent effects of kinship, sex, age, and disease status on rates of interactions that can specifically lead to transmission of disease through social contacts are unknown. Although kinship has repeatedly been reported to be a predictor of social relationships, and of affiliative and agonistic behaviours in various species (e.g., Bercovitch & Berry, 2013; Chapais et al., 1997; Hirsch et al., 2012), results from studies on free-ranging cervids are quite variable. This is likely because direct observation of their physical contacts is in most cases very difficult, and therefore the definition of a "contact" relies on proximity events such as individuals being within a certain distance of one another (it bears mention that distance ranges from m to km). For example, kinship was not related to patterns of proximity instances in elk (*Cervus canadensis*) (Vander Wal et al., 2012), but kin of mule deer (Cullingham et al., 2011), white-tailed deer (B. F. Miller et al., 2010), and non-*Odocoileus* cervid species (Biosa et al., 2015; Bonnot et al., 2011; Colson et al., 2016) were more spatially proximate than non-kin.

Disease can also shape the social network of the host (e.g., Behringer et al., 2006; Bouwman & Hawley, 2010). CWD affects the central nervous system producing behavioural changes such as lethargy, ataxia and reduced alertness (Mathiason et al., 2009). We have previously demonstrated that individuals showing clinical signs of CWD are less likely than healthy deer to be found in groups (Mejía Salazar et al., 2016).

To better understand pathogen transmission through high-risk social contacts within a mule deer population affected by CWD, we used repeated focal observations of uniquely identified individuals to calculate dyadic high-risk interaction rates and association strength. The former is the number of interactions per observation-minute per dyad, and the latter is an index indicating the proportion of times that a pair was seen in the same group out of the total times they could have been seen together. Our aims were to investigate: 1) whether genetic relatedness, sex, age, disease status and association strength are useful predictors of high-risk interaction rates among mule deer, 2) whether these interactions are reciprocal, and 3) whether genetic relatedness, sex, age, and disease status are useful predictors of association strength as defined by group membership.

5.4 METHODS

Rocky Mountain mule deer (*O. hemionus hemionus*) at Antelope Creek (a ~258 km^2 area in Saskatchewan, Canada; 50.66°N, 108.27°W) have been studied since 2006 (Silbernagel, 2010). Our animal handling protocol was conducted in accordance to the guidelines for humane animal use established by the Canadian Council on Animal Care, and was approved by the University of Saskatchewan's Animal Research Ethics Board (permit number 20050135). Permits to capture and handle deer were also obtained from the Saskatchewan Ministry of Environment.

Each June from 2008 to 2012, fawns were hand-captured, ear tagged and radio-collared (M4210, Advanced Telemetry Systems, MN, USA) (Perera, 2012). Eight-month-old and older individuals were captured every year in February or March using either Clover traps (Clover, 1954), or a helicopter and net-gun (Webb et al., 2008). Upon capture, they were chemically immobilized (see Silbernagel et al., 2011), aged based on tooth wear (Jensen, 1996), sampled for CWD testing (immunohistochemistry on tonsil and/or rectal biopsies) (Wild et al., 2002), sampled for genetic analysis (5 mm ear biopsy), individually marked with 2 ear tags, and fitted with either a GPS or a VHF radio-collar (3300L, 4400M, 7000SU, LMRT-4, Lotek Wireless, ON, Canada). Collars also had unique combinations of multi-coloured tags. Deer were chemically reversed from the anesthesia and released close to their original capture location. CWD-negative deer were re-captured, re-tested and re-collared in the following years. Each captured individual was classified based on sex (F = female, M = male), CWD diagnosis (N = negative, P = positive) and age (Fa = fawns: 1 to 365 days, J = juveniles: 366 to 730 days, and A = adults: ≥731 days; all were considered born on May 16th).

Ear biopsies were used to extract genomic DNA from most captured individuals. Each sample was genotyped at 16 microsatellite loci (see Cullingham et al., 2011). Samples with ≥ 3 missing loci were discarded. Using the estimator of Queller & Goodnight (Queller & Goodnight, 1989) in SPAGeDi version 1.4 (Hardy & Vekemans, 2002), we calculated pairwise relatedness measures for 211 mule deer in the study area. This relatedness coefficient ranges from -1 to 1; a positive value indicates that a pair is more related, and a negative value indicates that a pair is less related, than a random pair drawn from the sampled population (Queller & Goodnight, 1989).

5.4.1 Measuring behaviour

The population of mule deer in Antelope Creek was estimated to be about 420 in 2009. From a total of 240 collared deer that were present for part or all of the study period (10 May 2010 to 1 September 2011), we did focal individual follows on 133 (F = 51, M = 82; N = 83, P = 50; J = 36, A = 97) to obtain interaction rates and association indices. All collared deer in this study were individually recognizable due to their unique radio-frequencies, and combinations of sex, age, collar type, and ear and collar tags. Only collared mule deer were used for estimating interaction rates and association indices because sex, age, CWD diagnosis and genetic relatedness of those individuals was known. The list of deer to be observed, hereafter focal deer, included all CWD-positive individuals (from 15 to 34 at any point in time) and a stratified random sample of the CWD-negative individuals (up to 11 adult females, 11 adult males, 11 juvenile females and 11 juvenile males; the count ranged from 39 to 44). This list was created at the beginning of each study period: 1 August, 1 November, 15 December, 1 April, and 16 May (Mejía Salazar et al., 2016). We were able to observe between 21 and 25 focal deer every week. To avoid observing the same individual twice during the same week, each observer targeted different deer within a week. Times of observations were balanced across all focal deer within a season. The focal deer in a group with more than one collared deer was the individual targeted to be tracked within that day.

Behaviour was recorded when deer are most active based on rate of movement (Eberhardt et al., 1984) - during dawn and dusk (30 min before to 1.5 h after sunrise, and 1.5 h before to 30 min after sunset). As deer are not habituated to the presence of human observers, we used hunter’s tactics to approach deer without being seen, heard or smelled. The observer was equipped with binoculars (10 x 42), a spotting scope (20-60 x 82 mm), voice recorder, watch, range finder, and compass. The observer was an average of 256 m (from 40 to 800, n = 856 measurements) away from the deer.

5.4.1.1 Estimation of interaction rates

We used focal animal sampling and continuous recording (Altmann, 1974; Martin & Bateson, 2007) to register all interactions in which a physical contact between two different radio-collared deer occurred. The deer initiating the contact and the one receiving it were entered as D1 and D2, respectively. For the definition of all behaviours and a detailed description of behavioural sampling, see the Appendix 5.1. Those interactions with the greatest potential to be relevant for disease transmission through physical contact were merged into a composite class (high-risk interactions), and included: sparring, Flehmen close, licking face, nose-genitalia, and nose-nose. During these types of interactions, there is contact of the mouth and/or nose with saliva, urine and feces, which are proven to be CWD infectious (Gough & Maddison, 2010, and references therein). Certainly, a physical contact, or even close proximity, does not guarantee disease transmission, but the probability of contagion should intuitively increase as the frequency of high-risk interactions increases. Other behavioural events such as Flehmen from far away and striking, were also recorded, but were not considered of high risk for prion disease transmission, and were not included in our analyses.

In parallel, we registered the duration of the observation session to calculate the total amount of time that each pair could have been observed interacting. At the end of the sampling period, from 973 observation sessions on 133 focal individuals totaling 53,872 observation-minutes, only 150 interaction instances were observed among 88 collared deer. From those, 47 deer were involved in 66 high-risk interaction instances with the potential for CWD disease transmission. In addition to recording the observed interactions, we added null interactions (i.e., the instances in which a pair in the group, involving the focal deer, could have interacted, but didn't) (Whitehead, 2008, p. 75). Moreover, as not all individuals were observed all the time, interaction rates were then calculated in Microsoft Excel 2013 by dividing the total number of contacts per dyad by the total time in which either of the two individuals was focal (Whitehead, 2008, p. 95-97). These data were stored in an interaction rates matrix spreadsheet in which rows represent givers (D1) and the columns the receivers (D2) of physical contacts. This matrix included 158 different deer (F = 60, M = 98; N = 98, P = 60; A = 130, J = 27, Fa = 1). The mean (±SD) dyadic rate of high-risk interactions per observation-minute was 0.00007 ± 0.0003 (i.e., 1 high-risk interaction every 238 ± 67 hours of observation, on average).

5.4.1.2 Estimation of association strength

We also obtained association indices among radio-collared mule deer. We recorded the group membership of the focal individuals in 1,092 groups from 10 May 2010 to 1 September 2011. A group was defined as a spatially cohesive and behaviourally coordinated aggregation of deer in which every deer was within 10 body lengths of at least one other deer (Mejía Salazar et al., 2016); groups were clearly apparent visually (Bowyer et al., 2001). Observations of deer fleeing from a location of cover, or those in tall shrub habitat, were excluded, as it is difficult to accurately count and classify all deer in a group under such circumstances (Mejía Salazar et al., 2016).

We calculated association indices based on group membership (i.e., if 2 deer were seen in the same group, they were deemed to be associating) using the social affinity index in SOCPROG 2.7 (Whitehead, 2009). Individuals not associated with any other (i.e., in groups of 1) were also considered in the dataset to properly calculate association indices (Whitehead, 2008, p. 76). Social affinity is a measure of the strength of association between individuals during the study period, and is defined by: $x/\mathrm{Min}[(x + y_{AB} + y_A),(x + y_{AB} + y_B)]$, where x is the number of sampling days that A and B were observed together; Min stands for minimum and indicates that x will be divided by whichever of the 2 terms separated by the comma is smaller; y_A is the number of sampling days that A was observed without B (but B was not located), y_B is the number of sampling days that B was observed without A (but A was not located), and y_{AB} is the number of sampling days in which A and B were both observed, but not together (Mann et al., 2012; Mejía-Salazar et al., 2017; Whitehead, 2008, p. 98). Social affinity ranges from 0 (deer never seen together) to 1 (deer seen together every time), and helps to control for demographic changes (i.e., deer entering and leaving the study area or the study sample) (Whitehead, 2008, p. 98). These data were stored in an association strength matrix spreadsheet. This matrix included 167 different deer (F = 64, M = 103; N = 64, P = 103; A = 136, J = 29, Fa = 2).

5.4.2 Statistical analyses

We used the multiple regression quadratic assignment procedure (MRQAP) with a double-semi-partialing technique to determine what factors were useful to predict interaction rates and association strength among mule deer. The MRQAP builds on the Mantel test to examine for a relationship between a dependent matrix and an independent matrix while controlling for multiple independent matrices, all of which are dyadic variables (Dekker et al., 2007). In network regression methods such as MRQAP, the p-value and the directionality of the coefficient are the statistics of primary interest (Gibbons, 2004), while the size of the correlation coefficient offers little reliability as a predictor of the relationship strength between variables (Krackhardt, 1988).

We used SOCPROG 2.7 to conduct separate MRQAP tests with 1000 permutations: one with interaction rate as the dependent matrix, and genetic relatedness, association strength, and sex, age and diagnosis homophily as the independent matrices (MRQAP 1 in Table 1); the other with association strength as the dependent matrix, and genetic relatedness, and sex, age and diagnosis homophily as the independent matrices (MRQAP 2 in Table 3). The three homophily matrices were built in SOCPROG 2.7 by assigning a value of 0 to dissimilar dyads, and 1 to similar dyads. Of the 158 deer in the original interaction rates matrix, MRQAP 1 was performed on 137 individuals (those with both relatedness coefficients and interaction rates). While of the 167 deer in the original association strength matrix, MRQAP 2 was performed on 146 individuals (those with both relatedness coefficients and association indices). The number of deer in these matrices is greater than the number of focal deer because these matrices include all individuals with whom focal deer grouped at some point in the study period. Genetic relatedness among the 137 individuals was -0.003 on average (SD = 0.16, range -0.53 to 0.76), and -0.001 on average (SD = 0.16, range -0.53 to 0.76) for the 146 individuals.

To evaluate reciprocity of interactions between classes, we used the reciprocity vs. unidirectionality Hemelrijk Rr-test with 1000 random permutations in SOCPROG 2.7. This method is a Mantel test variant that ranks values within rows to take individual variation into account, and tests the null hypothesis that asymmetric interactions are unidirectional (i.e., not reciprocal) (Hemelrijk, 1990). If the degree of interaction of deer A to deer B is correlated with that of B to A, the interaction is considered to be reciprocal, otherwise it is considered unidirectional. These tests were performed on all individuals in the high-risk interaction matrix (n = 158).

All mixing matrices (i.e., interaction rates matrix and association strength matrix), genetic relatedness matrix, homophily matrices and the list of attributes for all deer in the statistical analyses are available in Data set S1 in the published manuscript.

5.5 RESULTS

Association strength was the only significant (at $P < 0.05$) positive predictor of high-risk interaction rates among mule deer in a model containing genetic relatedness, and sex, age and diagnosis homophily (MRQAP 1 in Table 5-1).

Table 5-1. MRQAP 1 results relating multiple independent variables to interaction rates among 137 mule deer.

Variable	Partial correlation coefficient	P-value
Interaction rate (dependent variable)		
Association strength*	0.11	<0.0001*
Genetic relatedness	0.01	0.17
Sex homophily	-0.002	0.76
Age homophily	-0.01	0.20
Diagnosis homophily	0.01	0.30

MRQAP 1 was run to determine if genetic relatedness, association strength, and sex, age and diagnosis homophily were useful predictors of interaction rates. Significant ($P < 0.05$) predictor variable marked with *.

With respect to interaction reciprocity between classes (Table 5-2), pairwise interactions were correlated with each other for all sex and diagnosis classes, indicating that interactions between these classes were reciprocal. Physical contacts between adults, and adults and juveniles, were also reciprocal.

Table 5-2. Hemelrijk Rr-test results to test for interaction reciprocity among 158 mule deer.

Pair	Correlation coefficient	P-value	Sample size	Conclusion
F-F	0.42	<0.0001	60	Reciprocal
F-M	0.17	0.01	60 F, 98 M	Reciprocal
M-M	0.22	<0.0001	98	Reciprocal
A-A	0.28	<0.0001	130	Reciprocal
A-J	0.23	0.03	130 A, 27 J	Reciprocal
N-N	0.19	<0.0001	98	Reciprocal
N-P	0.38	<0.0001	98 N, 60 P	Reciprocal
P-P	0.18	0.001	60	Reciprocal

F = female, M = male, A = adult, J = juvenile, N = CWD-negative, P = CWD-positive. We did not have enough pairs of fawns interacting with other fawns, adults and juveniles, and of juveniles interacting with other juveniles, so the Rr-test could not be run on those pairs. All correlations were significant ($P < 0.05$).

Genetic relatedness and sex homophily were significant (at $P < 0.05$) positive predictors of association strength among mule deer, while age and diagnosis homophily were not (MRQAP 2 in Table 5-3). Based on these results, we ran a Mantel test with 1000 permutations on 167 individuals to determine if association strengths within and between sexes were similar (Schnell et al., 1985; Whitehead, 2014). Mean (±SD) associations within same-sex classes (0.020 ± 0.017) were significantly stronger than those between different sex classes (0.011 ± 0.012) (two-sided Mantel test, $t = 5.03$, $r = 0.04$, $P < 0.0001$). The strongest associations occurred between females (0.030 ± 0.021), followed by those between males (0.015 ± 0.011), and those between females and males (0.011 ± 0.008).

Table 5-3. MRQAP 2 results relating multiple independent variables to association strength among 146 mule deer.

Variable	Partial correlation coefficient	*P* value
Association strength (dependent variable)		
Genetic relatedness*	0.07	<0.0001*
Sex homophily*	0.07	<0.0001*
Age homophily	0.01	0.46
Diagnosis homophily	-0.01	0.27

MRQAP 2 was run to determine if genetic relatedness, and sex, age and diagnosis homophily were useful predictors of association strength. Significant ($P < 0.05$) predictor variables marked with *.

Given that genetic relatedness and sex homophily were significant predictors of association strength, and association strength was a significant predictor of high-risk interaction rates, we repeated the MRQAP with 1000 permutations with interaction rate as the dependent matrix and all other independent matrices except for association strength. This allowed us to explore whether the effect of association strength was mediating the relationship between relatedness or sex homophily after accounting for the other independent variables, and high-risk interaction rate. Genetic relatedness was a significant positive predictor of interaction rate ($P = 0.02$, partial correlation coefficient = 0.02), in the model without association strength, while sex, age and diagnosis homophily were not significant (all $P > 0.2$).

5.6 DISCUSSION

Pathogen transmission through social relationships among animals can be better understood by studying the factors that determine how they group and physically contact each other (e.g., Bouwman & Hawley, 2010; Butler et al., 2015). It has been previously recognized that the level of detail of deer social behaviour can determine the validity of CWD transmission model outcomes (Oraby et al., 2014; Potapov et al., 2013). However, such data were not available in the literature. In this study, we offer fine-scale empirical data on non-random mixing between individual mule deer that can complement social network-informed epidemiological analyses. Moreover, we gained knowledge about the factors that predict mule deer social relationships, and recognized important aspects to consider when suggesting CWD management control strategies.

Our findings indicate that interactions among mule deer are particularly infrequent. In our study, about a quarter of the observed deer participated in high-risk physical contacts, and after close to 900 observation-hours, only 84 non-high-risk interactions and 66 high-risk interactions were observed, even during periods of the day in which deer are reported to be most active (Eberhardt et al., 1984) and most likely to form groups (Chapter 3: Mejía Salazar et al., 2016). This suggests that unless CWD transmission is highly efficient during these interactions, direct animal-to-animal spread is likely to be slow. This assumes that high-risk interactions do not occur at a higher rate during non-crepuscular time periods and that our observations reflect peak interaction rates. If true, it adds to the debate as to which mode of transmission (animal-to-animal or through contaminated environments) drives CWD spread dynamics (Schauber et al., 2015; Vasilyeva et al., 2015).

We found that 1) genetic relatedness is a positive predictor of association strength, 2) association strength is a positive predictor of high-risk interaction rate, and 3) the importance of genetic relatedness with respect to interaction rate is mediated through the strength of the association. Consequently, given that close related individuals were more likely to be part of the same group, and as individuals more frequently seen in the same group were more likely to physically interact with each other, genetically related individuals were also more likely to interact and increase the risk of CWD transmission through direct contact. Indirect transmission of CWD can also occur among group members due to common contact with the environment (M. W. Miller et al., 2004).

In a previous study on the same mule deer population (Chapter 4: Mejía-Salazar et al., 2017), spatial association strength was independent of genetic relatedness. That study included only adult individuals with GPS collars from which we had genetic relatedness data, and a pair was considered to be associated if found in close proximity based on GPS locations recorded every 2 h. In contrast, in the present study we included deer of any age with either GPS or VHF collars from which we had genetic relatedness data, and we defined an association instance if seen in the same group during dawn and dusk. In this case, we report that relatedness was a predictor of association strength as defined by group membership. However, how useful this predictor is (i.e., how strong the correlation is between these two variables) is yet to be investigated. Previous studies on white-tailed deer found a strong relationship between degree of spatial overlap, or geographical distances, and genetic relatedness; however, their analysis only included adult females or highly related individuals (i.e., females and fawns) (Magle et al., 2013; B. F. Miller et al., 2010) (but see Comer et al., 2005).

Age and CWD status were not predictors of either high-risk interaction rates or association strength. Similarly, we have previously found that pairwise spatial association patterns were independent of age and CWD status (Chapter 4: Mejía-Salazar et al., 2017) and in a separate analysis (Chapter 3: Mejía Salazar et al., 2016), that mule deer showing clinical signs of CWD were 3 times more likely to be found alone than in a group. The high-risk interactions were reciprocal regardless of the CWD diagnosis status of the interacting deer, suggesting that CWD-infected deer are not receiving more contacts than the ones they give, or vice versa, so high-risk interaction rate reciprocity is diagnosis-independent. All these findings together suggest that being in the clinical phase of CWD affects the probability of grouping, but in the preclinical phase or once in a group, CWD does not affect the probability of grouping again with the same individuals, nor the probability of reciprocating physical contacts.

The high-risk interactions between sexes were reciprocal, indicating that the risk of receiving or transferring CWD prions through physical contact is sex-independent. However, reciprocity between age classes could only be tested between adults and between adults and juveniles because fawns were not observed directing high-risk contacts to anyone, and juveniles only directed those towards adults. It is likely that a larger sample size of young deer, or observations at a different time of the day, are needed to observe young deer interacting with others in a high-risk manner. Even though reciprocity between adults and fawns could not be tested in this analysis, it is important to note that all high-risk interactions, as defined in this study, occurred in a single direction, from adults to fawns, and that this can have implications for prion exposure through direct contact at a very young age. Our findings on reciprocity do not help to explain why the prevalence of CWD in various geographic areas is often higher for adult males than for females (Grear et al., 2006), or vice versa (Edmunds et al., 2016). More complex analyses comparing interaction rates among sex, age and diagnosis classes at specific seasonal, diurnal or site-specific periods might help predict the effect of high-risk contacts on overall CWD dynamics (Appendix 5.2).

Associations between members of the same sex were stronger than those between different sexes, indicating that mule deer in this population exhibit sex-based homophily in which individuals preferentially group with conspecifics of the same sex. This supports previous findings indicating that adult female-female spatial associations tend to be stronger than male-male and female-male association regardless of the proportion of home range overlap between them (Chapter 4: Mejía-Salazar et al., 2017).

It is difficult to use intuition alone to understand CWD transmission patterns; however, incorporation of the non-random mixing between individuals obtained in this study can enhance the applicability of dynamic models which consider both routes of prion transmission (Potapov et al., 2013; Vynnycky & White, 2010).

5.7 DATA ACCESSIBILITY

Analyses reported in this article can be reproduced using the data provided as supplementary material when this study gets published.

5.8 SUPPLEMENTARY MATERIAL

Appendix 5.1. Protocol for recording mule deer behavioural events.

Appendix 5.2. Data exploration for future research questions relevant to high-risk contact patterns for the transmission of chronic wasting disease among mule deer.

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5.12 APPENDIX 5.1.

Appendix 5.1. Protocol for recording mule deer behavioural events.

All these behaviours are classified in independent and as rigorously as possible defined categories that describe the behaviour in sufficient detail:

Behaviour	Definition
Allogrooming (A)	One deer grooms another deer, or two deer simultaneously groom one another.
Copulation (C)	Two deer attempt to mate, or mate.
Flehmen close (Fc)*	The upper lip is everted exposing the upper gums while drawing air inside the mouth in order to facilitate the detection of pheromones by the vomeronasal organ. The event occurs is in a close distance (less or equal than 1 body length).
Licking body (LB)	One deer licks the body (any part of the body except the face or the genitalia) of another deer.
Licking face (LF)*	One deer licks the face (i.e., eyes, nose and mouth) of another deer.
Genital sniff (GS)*	One deer touches another deer’s genitalia with its nose, and/or licks it.
Nose-nose (NN)*	One deer touches another deer’s nose with its nose.
Rubbing (R)	Moving along in contact with a surface while applying pressure on the body or the antlers.
Selfgroom (SG)	Nibbling, biting or licking a part of the body with their own mouth.
Sparring (AL)*	Two males fight or spar with their antlers.
Stotting (ST)	An antipredator behaviour. Vertical leaping with all 4 limbs leaving the ground simultaneously with the limbs held stiff and straight while the deer is airborne.
Strike (Str)	One deer hits another deer with a sharp movement forward of one hand. The attempt is also written down as a strike.
Suckling (Su)	A fawn suckles from an adult female.

Touch (T)	One deer comes in contact with another so as to feel or push. Should not include: nose-nose, nose-genitalia, nose-body, nose-face, allogroom, antler fighting, copulation, licking, strike or suckling.
Trot (Tr)	Quadrupedal asymmetrical movement forward that is quicker than walking, but is not yet stotting.
Urination and/or defecation (U/D)	Expel of urine through the urethra and/or expel feces through the anus. From the distance, sometimes is difficult to determine if the deer is urinating or defecating; in these cases, writing down the incertitude is adequate.

(*) These physical contacts or interactions are considered relevant for the transmission of chronic wasting disease through direct animal-to-animal contact. All these were merged into a composite class (high-risk interactions), and are the ones included in the analyses in this study.

Sampling and recording

Focal animal sampling and continuous recording.- concentrate on just one deer (the focal deer) and record what it does for up to 120 minutes. No record is taken of what any other animals in the same or different group are doing, except when an interaction occurs between the focal deer and another collared deer in the group.

Registering

Direct observations are placed during dawn and dusk (from 30 min before to 1.5 h after sunrise, and from 1.5 h before to 30 min after sunset, respectively). The night previous to the observation session, the times for sunrise and sunset are investigated. Then the next observation is scheduled.

The observer, equipped with a voice recorder, timer, binoculars, scope, receiver, antenna, range finder and compass, practices a series of different techniques in order to be able to observe focal deer without being smelled, seen and heard.

The times when the deer is found and the observation session starts and ends are recorded. If the deer is out of sight due to any reason, the time will be registered and the observer should try to find the deer again in order to continue with the observation session. All instances of physical contact are recorded using a voice recorder.

The group composition is recorded at the beginning, every time it changes and at the end of the observation session. A location point is registered at the beginning, whenever the observed has to change position in order to find the deer again and at the end of the observation session. The habitat is registered all the time.

Once the observation session is saved in the voice recorder, the information is entered in the computer using this form:

Hour	Interactions				Group ID	Location ID	Remarks
	D1	D2	C	H			
6:50	335	137	Fc	2	1	1	

The contacts (C) are represented by their abbreviation. For the interactions, there are D1, D2 and Habitat columns. For the asymmetric interactions, as in Flehmen close (deer A will direct a Flehmen towards deer B), the first individual listed is considered the actor (D1) and the second the receiver (D2).

The habitat (H) is coded: 1-grassland, 2-low shrub, 3-tall shrub, 4-woodland, 5-crop, 6-hay, 7-wetland, 8-unvegetated, 9-other (please specify).

The group compositions and the locations are listed in progressive numbers along the time (the first one is 1, the second one is 2, and so on).

Group composition was entered in a table:

Group ID	Total size	Adult			Juvenile			Fawn			Unknown		Unk	Collared deer
		M	F	Unk	M	F	Unk	M	F	Unk	M	F		
1	4	2	1			1								2

M = male; F = female; Unk = unknown

Other data are also collected during an observation session:

Observer: the name of the person recording the behavioural data.

Ambient temperature: we used the thermometer from the vehicles and from the weather station at home.

Observer tool: binoculars (10 x 42) and spotting scopes (15-45 x 60).

Focal deer attributes: Species (mule deer), age class (adult, juvenile, fawn and unknown), sex (male, female and unknown), and chronic wasting disease diagnosis at the moment (positive and negative).

Focal deer body condition and clinical signs arousal evaluation:

Are the ribs?	1-not visible; 2-slightly visible; 3-protruding
Are the ears?	1-one perked up; 2-both perked up; 3-both drooping flat to the side of the head
Is there excessive saliva?	1-no; 2-yes
Is the coat?	1-normal for the season; 2-abnormal (dull, rough, patchy)
Hocks touching?	1-no; 2-yes
Is the collar?	1-tight; 2-loose; 3-snug against neck
Neck cuts due to collar?	1-no; 2-yes, 3-not sure
Is the gait?	1-regular; 2- slower than others; 3-irregular; 4-other (write down)

Data

Each observation session is recorded in the computer. The observation session is divided in partial observation sessions according to the combination of habitat, group composition and location. The total number of minutes of the observation session and the frequency of the interactions are summarized to obtain interaction rates.

5.13 APPENDIX 5.2.

Appendix 5.2. Data exploration for future research questions relevant to high-risk contact patterns for the transmission of chronic wasting disease among mule deer.

Based on the following data, it would be interesting to investigate which factors (e.g., genetic relatedness, sex, age and CWD diagnosis) predict whether deer contact or not contact each other (i.e., a binary response variable) in a high-risk manner for CWD transmission.

<u>Mean high-risk contacts per mule deer dyad per observation-minute</u>

Table 5.2.1. Mean and standard deviation of high-risk contacts per dyad per observation-minute, by class, as reported by SOCPROG after running Mantel tests for within vs between contact rates.

Class	Contact directionality[1]	Pairs[2]	Mean	SD
Sex	F-F	3600	0.00003	0.00015
	F-M	5880	0.00006	0.00027
	M-F	5880	0.00003	0.00013
	M-M	9604	0.00011	0.00044
Age	AD-AD	16900	0.00007	0.00030
	AD-Fa	130	0.00064	0.00000
	AD-JV	3510	0.00005	0.00041
	JV-AD	3510	0.00007	0.00026
	JV-Fa	27	0	0
	JV-JV	729	0	0
	Fa-AD	130	0	0
	Fa-Fa	1	0	0
	Fa-JV	27	0	0
CWD diagnosis	N-N	9604	0.00005	0.00021
	N-P	5880	0.00004	0.00028
	P-N	5880	0.00006	0.00033
	P-P	3600	0.00018	0.00077
	Overall	24964	0.00007	0.00025

[1] Contact directionality refers to "who contacts whom". F = female, M = male; AD = adult, Fa = fawn, JV = juvenile; N = negative and P = positive to CWD; SD = standard deviation. [2] Pairs are the number of different pairs of mule deer (or dyads) in the analyses. The graphic representation of this table is found in Figure 5.2.1.

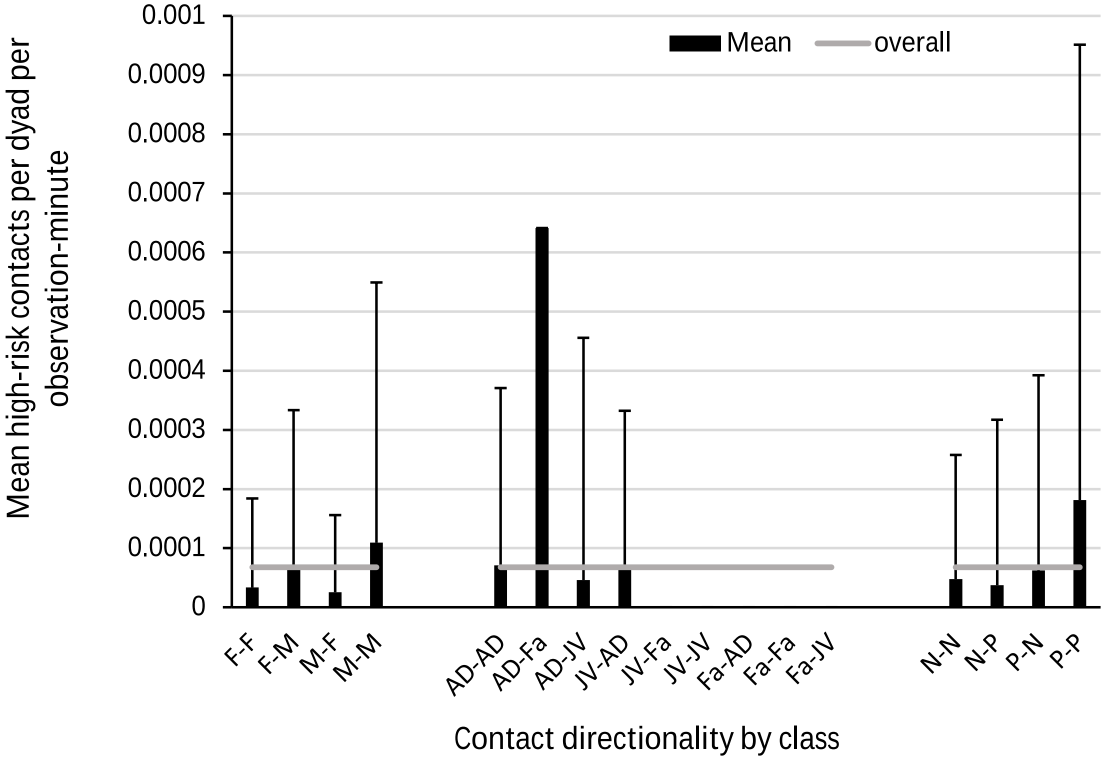

Figure 5.2.1 High-risk contact rates among mule deer. Note the apparent difference in magnitudes in all classes. Contacts from males to males appear to be much more frequent than those between the other sex combinations. Also, high-risk contacts appear to be much more frequent from adults to fawns, than from adults to adults and to juveniles. In the same way, there is an increase in the frequency of high-risk contacts between CWD-positive individuals, when compared to any other CWD diagnosis combination.

Genetic relatedness of interacting vs non-interacting mule deer

Table 5.2.2. Descriptive statistics of genetic relatedness coefficients of pairs of deer that interacted (i.e., physically contacted each other) versus those of pairs of deer that did not interact.

	Interacting	Non-interacting
Mean	0.18	-0.003
Standard Deviation	0.36	0.16
Median	0.28	-0.01
Minimum	-0.35	-0.53
Maximum	0.61	0.76
Count of pairs	13	9290
Count of deer	23	114
% of highly related pairs (r > 0.4)	46.2	1.5

Table 5.2.3. Relatedness coefficients and dyadic high-risk contacts per observation-minute of deer that interacted.

D1[1]	D2[1]	Relatedness coefficient	Contact rate
137	335	0.3	0.004
329	788	0.5	0.004
375	721	0.5	0.004
657	673	-0.1	0.004
334	723	0.6	0.005
461	472	-0.3	0.007
472	474	-0.3	0.009
543	655	-0.2	0.012
289	736	0.4	0.015
329	455	0.6	0.018
394	655	-0.1	0.019
104	639	-0.1	0.023
437	819	0.5	0.083

[1] D1 and D2 represent the deer identifications of the deer in the pair. D1 directed the interaction to D2.

Although mean ±SD genetic relatedness among pairs of deer that were involved in high-risk contact events notoriously differed from that of deer that did not contact each other (0.18 ± 0.36 vs -0.003 ± 0.16, respectively) (Table 5.2.2), note there is no correlation between contact rate and genetic relatedness (Table 5.2.3) (i.e., a high contact rate is not predicted by a high relatedness coefficient). This supports our findings from MRQAP 1, in which we determined that genetic relatedness is not a good predictor of high-risk contact rates. Moreover, the distribution of genetic relatedness among interacting pairs is quite irregular, and our sample had pairs with high and low relatedness coefficients (Figure 5.2.2). The distribution of genetic relatedness among non-interacting pairs (9303 pairs) is close to normal (Figure 5.2.3). By looking at the number of pairs that interacted (13 pairs), one wonders if a greater sample size would yield a different result. However, from our study, we concluded that high-frequency contacts among mule deer are extremely rare, and the effort to observe more high-risk contacts in the field would be tremendous and most probably practically unfeasible. On the other hand, we observed more than 9 thousand pairs of deer that did not interact. We believe that we have an adequate overall sample size.

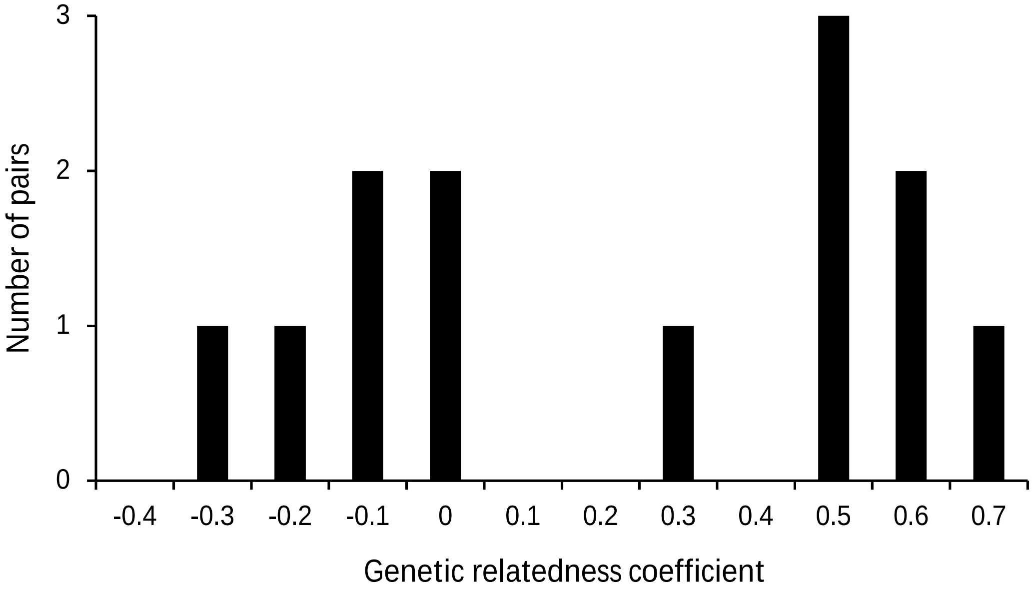

Figure 5.2.2. Histogram of genetic relatedness among interacting mule deer.

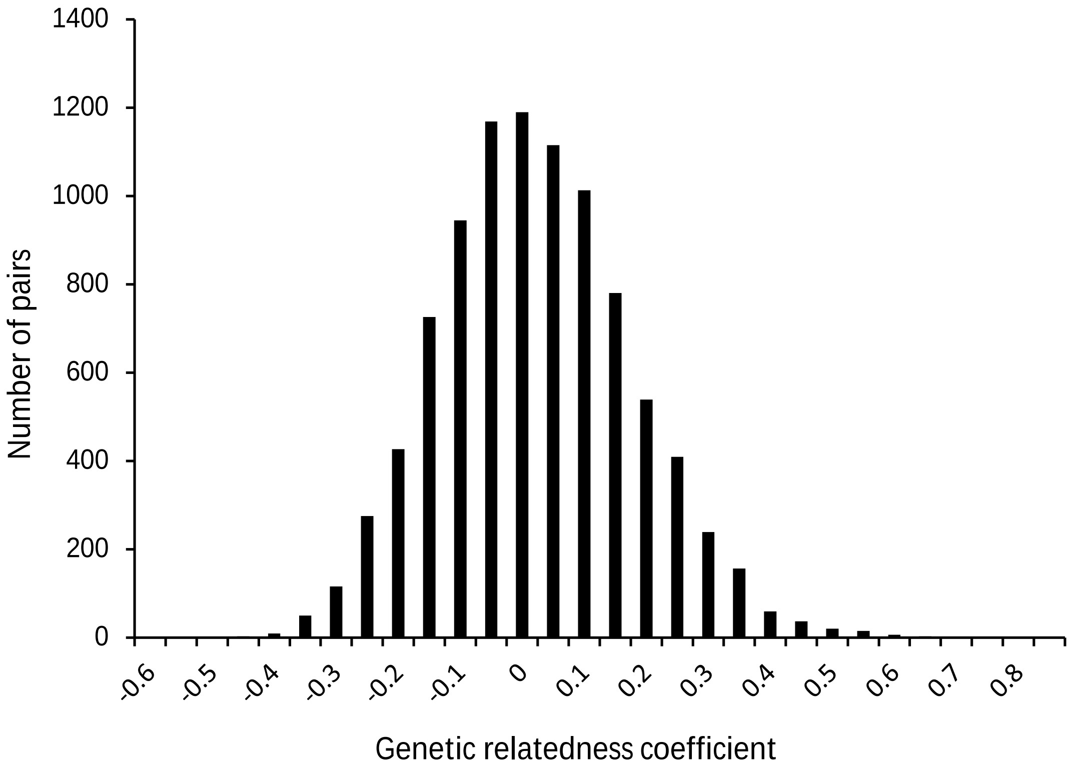

Figure 5.2.3. Histogram of genetic relatedness among non-interacting mule deer.

CHAPTER 6. VISITATION TO ENVIRONMENTAL SITES BY MULE DEER IN A CHRONIC WASTING DISEASE ENDEMIC AREA

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6.1 PUBLICATION AND CO-AUTHORSHIP STATEMENT

The following chapter will be formatted and adjusted for submission to Ecological Applications. Only a subset of models will be included in the version for publication.

6.1.1 Author's contributions

Conceptualization: MFMS, TKB, CW, YTW

Methodology: MFMS, TKB, CW

Collection of field data: MFMS

Formal analysis: MFMS, CW, TKB

Investigation: MFMS

Data curation: MFMS

Writing-original draft: MFMS

Writing-review: MFMS, TKB, CW, YTW

Visualization: MFMS

Supervision: TKB, CW

Project administration: TKB

Funding Acquisition: TKB, MFMS

VISITATION TO ENVIRONMENTAL SITES BY MULE DEER IN A CHRONIC WASTING DISEASE ENDEMIC AREA

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6.2 ABSTRACT

Prions that cause chronic wasting disease (CWD) in cervids can bind to soil particles, stabilize against degradation, and remain infective for years outside of the host. Infectious cervids shed prions in secretions and excretions for a long period of time, consequently depositing the agent in frequently used areas, which are important in the epidemiology of CWD. Unfortunately, effective tools for quantifying CWD prions in soil, water and other environmental sources are currently not available. Our goal was to investigate patterns of visitation by mule deer (*Odocoileus hemionus hemionus*) and their contact with the environment as a proxy for environmental prion contamination and potential transmission. For this, we deployed a system of triggered-by-movement cameras at 8 different environmental site types. We assessed whether the relative frequency of visitation of distinct sex-and-age classes, and the rates of behaviours with a high risk for environmental transmission, differed by season and site type. We found that anthropogenic sites, such as grain sources and salt licks, were visited more frequently by several sex-and-age classes of mule deer than non-anthropogenic sites such as browse sites. We also determined that mule deer more often contacted the environment at the former type of sites. We discuss the potential risk of certain environmental sites in the transmission of CWD among mule deer.

Keywords: *mule deer; chronic wasting disease; anthropogenic; visitation rates; environmental prion contamination; camera; modeling wildlife diseases;* Odocoileus hemionus.

6.3 INTRODUCTION

Although chronic wasting disease (CWD) is transmitted directly via animal to animal contact and through the environment (Miller and Williams, 2003; Miller et al., 2004), recent research suggests that environmental persistence can be the main driver in host population decline and prevalence increase (Almberg et al., 2011). Because current laboratory techniques do not allow quantification of prions in environmental samples, alternative techniques to estimate the relative importance of small geographic areas with respect to prion contamination are needed. Here we use mule deer visitation to frequently used areas as a proxy for environmental prion contamination and potential transmission.

CWD is a contagious prion disease affecting free-ranging North American cervids (Williams and Miller, 2002). So far, control efforts have been unsuccessful, resulting in continued geographic spread and increased prevalence in endemic zones. CWD has a long incubation period (Williams and Miller, 2002) during the majority of which deer are capable of directly transmitting the disease and contributing to environmental contamination (Gough and Maddison, 2010) via shedding prions in saliva (Mathiason et al., 2006), urine (Haley et al., 2009), feces (Tamgüney et al., 2009), and velvet (Angers et al., 2009), and through infected carcasses (Miller et al., 2004; Angers et al., 2006).

Simulation models, using data from CWD epidemics in the United States of America (USA), suggest that disease prevalence, severity of cervid population declines, and projected recurrent epidemics are driven by duration of prion infectiousness in the environment (Almberg et al., 2011; Sharp and Pastor, 2011). This appears to be related to the capacity of prions to bind to soil particles, in specific to montmorillonite clays (Walter et al., 2011), which enhances their oral and intranasal infectivity (Johnson et al., 2007; Nichols et al., 2013), and stabilizes them to resist thermal degradation and microbial decomposition (Johnson et al., 2007; Wiggins, 2009). Previous investigations suggest that CWD prions can remain available and infectious for at least 2.5 years (Miller et al., 2004), and scrapie prions for at least 16 years (Georgsson et al., 2006). This suggests that for CWD, similar to scrapie, the infectiousness of the environment could exceed the host´s life span. Therefore, despite a low epidemic growth rate, the basic reproductive number (R_0) might be larger than expected when only considering animal-animal transmission pathways (Almberg et al., 2011).

Although soil is an environmental reservoir for prion infectivity (Johnson et al., 2006), currently there are no cost-effective, validated techniques for quantifying prions in soils or other environmental samples under natural conditions. There are unknown prion-soil interactions and adsorption dynamics, which limit prion detection sensitivity and estimation of prion adsorption capacity of soil (Saunders et al., 2008; Wyckoff et al., 2013). Prion detection in soil has only been successful under experimental conditions in laboratories (Cooke et al., 2007; Genovesi et al., 2007; Maluquer de Motes et al., 2008; Wyckoff et al., 2013). Lacking detection methods forces us and other researchers to find alternatives for estimating risk of environmental exposure to prions.

CWD researchers have used various radio-telemetry techniques to evaluate how factors such as habitat, age, sex, and season, affect proximity rates of cervids, and to understand common use of space and therefore disease transmission (Conner and Miller, 2004; Schauber et al., 2007; Kjær et al., 2008; Silbernagel et al., 2011; Vander Wal et al., 2012). This knowledge has been used to help understand changes in prevalence and geographic spread at a broad spatial scale, but don’t capture the heterogeneity in fine spatial scale contact.

Cervids don’t randomly move in their environment, but instead commonly use networks of trails to travel among bed sites, feeding areas, watering holes, salt licks, and chemical sign points such as antler rubs and scrapes. Consequently, these relatively small geographic areas where deer congregate could accumulate large quantities of prions and become important in the epidemiology of CWD. For example, supplemental feed sources such as grain and hay, either unintentionally or intentionally made available to deer, can alter deer behaviour, causing them to spend more time at these site types than at natural feed sources (Thompson et al., 2008) and are a known risk factor for disease transmission (Miller and Williams, 2003; Sorensen et al., 2014). Mineral licks are similar attractants (Weeks, 1978). Such sites have the potential to be modified or removed to reduce prion contamination and transmission, if our speculations are correct.

However, very little is known about how season and site type influence mule deer visitation rates. Current knowledge of these factors consists of selection and use of beds and water developments in Arizona, USA, by desert mule deer (*O.h. eremicus*) (Hazam and Krausman, 1988; Tull et al., 2001; Germaine et al., 2004; Krausman et al., 2006), and the effect of the sex and age of white-tailed deer on visitation to mineral licks and their behaviour at feeding sites in various places in USA (Ozoga, 1972; Weeks, 1978; Wiles and Weeks, 1986; Schultz and Johnson, 1992; Atwood and Weeks Jr, 2002, 2003; Thompson et al., 2008). To our knowledge, the only published research done in Canada concluded that diurnal use of natural mineral licks by mule deer was greater in June and early July than in May and August in Jasper National Park (Carbyn, 1975). For this reason, and as an alternative to sampling soil and other environmental sources, we propose to describe the relative frequency of visits by mule deer to different environmental site types as an indicator of site importance for prion accumulation and potential CWD transmission.

Other researchers looking at wildlife activity patterns have used a variety of methods arguing that these allow for their observations to be independent. These include defining single visitation events, programming the cameras to take pictures at large time intervals, and allocating the cameras at certain distances from one another so the same individual does not visit 2 or more cameras within a day. For example, some defined a single visitation event as multiple pictures taken within 60 min at the same site of the same identifiable individual, or of animals they could not identify as different (Bridges et al., 2004). Others programmed the cameras to take 1 picture every 30 min assuming animals do not spend more than 30 min at each site (Atwood and Weeks Jr, 2003). We find problems with these methods as to subjectively establish a bout length (e.g., pictures within 60 min) or an inter-bout length (30 min delay between pictures) can lead to biases that would directly affect the visitation rates (number of visits per camera-day). Moreover, these bouts would vary by site and season, – a reason why it will be wrong to set a unique interval for all site and season combinations in our study.

It is feasible to classify a series of pictures as a single event when the targeted species allows for individual identification, such as tigers that are individually distinguished based on their stripes, or when using marked (e.g., ear tagged) individuals, or when the observations are done in short and specific times of the year (e.g., adult male white-tailed deer with fully calcified antlers). In the case of mule deer, we are unable to tell for certain the difference between one individual and another throughout all seasons; therefore, we cannot confidently establish the start and the end of a “visitation event”, and we cannot calculate the duration of visits and the number of different individuals visiting each site.

To handle the inherent challenges of studying mule deer using remote photography, we looked at the relative visitation rates occurring at different environmental sites by counting number of pictures per camera day as a measure of opportunity for site contamination. We aimed to estimate differences in the relative frequency of visits by different sex-and-age classes of Rocky Mountain mule deer (*O. h. hemionus*) to a variety of environmental site types in southern Saskatchewan throughout the year. We also aimed to estimate the differences in the rate of behaviours with a high risk for environmental transmission. These measurements can serve as a relative indicator of site importance for prion accumulation and potential CWD transmission.

We anticipate patterns of visitation by mule deer to environmental sites to be influenced by the life histories of mule deer. In particular, we anticipate that every sex-and-age class will visit grain sources the most, as grain is a highly attractive source of nutrients (Gordon and Prins, 2008). Furthermore, adult females and fawns will visit water sources, and bed and browse areas more often during fawning season, as female mule deer use areas likely to increase offspring survival, such as areas close to water, with low coyote activity (slopes >10°), and with hiding plant cover and palatable browse resources (Hazam and Krausman, 1988; Main and Coblentz, 1996). Finally, we also anticipate juvenile and adult males to visit rubs more frequently during pre-rut than in any other season because that is when it is more important to leave territorial marks to communicate with other deer in the same area in preparation for rut season (Kile and Marchinton, 1977; Alexy et al., 2001).

6.4 METHODS

6.4.1 Study site

Antelope Creek (50.66°N, 108.27°W) is a 258 km^2 area in rural southern Saskatchewan, Canada. The north boundary adjoins the South Saskatchewan River and is characterized by a network of coulees of natural vegetation surrounded by cultivated agricultural land that extends to the south (Acton et al., 1998). It is located within the mixed grassland ecoregion (Acton et al., 1998) and its soil is rich in clay and loam (College of Agriculture, 1923). It is a CWD endemic zone in which Rocky Mountain mule deer and white-tailed deer have been recognized as infected since 1996. Efforts to control the spread of CWD among free-ranging cervids have included culling and preclusion of cervid translocation (Alberta Prion Research Institute et al., 2011).

Despite these efforts, the prevalence of CWD in adult mule deer in Saskatchewan has significantly increased since 2009 (Canadian Wildlife Health Cooperative, 2017).

6.4.2 Sampling design

From 8 July 2009 to 13 December 2012, 28 triggered-by-movement cameras from three manufacturers (22 MI Scouting, 3 Bushnell Sentry and 3 LTL Acorn) were used to record the presence of Rocky Mountain mule deer at sites in the study area. MI Scouting cameras were deployed in 93.2% (345/370) of the camera stations used in the analysis, whilst Bushnells in 4.1% (15/370) and LTL Acorns in 2.7% (10/370); the two latter models were only used when we temporally ran out of the primary model cameras. All these cameras had a control board with a passive infrared sensor which detects a combination of infrared radiation change and movement (Skelton, 2008). These devices take a picture in less than a second once movement is sensed, and we programmed them to keep taking single pictures every 5 seconds (0.083 min) while the camera still sensed movement, and to stop taking pictures after 30 seconds of inactivity, 24 h a day. Either flash or infrared light were used to capture pictures in low-light conditions. The MI Scouting cameras had an image delay default setting of 5 sec, image delay in the Bushnell was 30 sec and was not indicated for the LTL Acorn, but we achieved an average delay of 20 sec. Cameras were attached to a metallic camera tripod that allowed placement of the cameras between 90 and 110 cm above the ground, with an optical axis parallel to the ground, to better capture whole mule deer bodies.

We monitored 8 different site types based on earlier field observations of mule deer behaviour. These site types included: anthropogenic food sources, which were primarily grain spills or storage piles (grain sources), antler rubs, beds, browse areas, mule deer mortalities, salt licks, trails and waterholes (Table 2-6 in page 38; and Figures A1 to A8 in Appendix 6.1). Through the duration of the study, the area was intensely monitored by a field crew. Cameras were set at all known sites that had evidence of frequent visitation.

Consistent with a previously published study of mule deer in the same area (Silbernagel et al., 2011), we divided the year into 5 seasons, which were: fawning, pre-rut, rut, early gestation and late gestation (Table 2-5 in page 36). A station was defined as a camera deployed in a unique combination of location (Cartesian coordinate of easting and northing), site type and season. Cameras were deployed anywhere from 1 to 145 days (mean=37.7 days, mode=14 days) and were removed if the station became unsuitable, such as when pictures with deer were not taken for 6 continuous days, the camera stopped working, or weather conditions affected access to the station. We inspected the stations every 7 to 14 days to retrieve pictures, to replace batteries and memory card, and to ensure the camera was functioning properly (we triggered the sensor and took pictures of ourselves when arriving and when leaving the site; these pictures with humans were not considered in the final counts). Pictures were then downloaded and stored in a computer, and then the picture´s metadata, including dates, site type, location and number of pictures with deer, were entered in a Microsoft Access database (Microsoft Corporation). The final dataset included 30,377 pictures with mule deer from 370 stations in 11,587 camera-days (CD), defined as the number of days in which a camera was active, that is, in which a camera had the potential to take pictures, no matter if it took pictures with deer or not. In cases where batteries failed, storage was exceeded, camera malfunction was suspected or noted, or something affected the capacity of the camera to adequately take pictures (such as when the camera was knocked down, or the sensor was obstructed with condensation) the data was excluded from the counts and the number of CD was adjusted to the last reliable record. Slight underestimation of count data on bed sites may occur due to the reduced movement of deer in comparison to other sites such as salt licks, in which deer are standing up and vigilant. There was only one day in which bedded deer triggered the camera every 17 min on average. As this represented 0.01% (1/11,587) of the CD and 0.3% (1/370) of the stations it had no substantive effect on the results.

6.4.3 Calculation of frequency of visitation and frequency of environmental contacts

We estimated frequency of visitation for each sex-and-age class from the number of pictures that captured mule deer at different sites. We also estimated the frequency with which mule deer contacted the environment from the number of pictures that captured mule deer contacting the environment (as in Fig. A1, A2 and A3). Site visitation served as a proxy for environmental prion contamination and potential transmission.

To ensure maximum observer agreement when extracting data from the pictures, AC_1 coefficients, the agreement coefficients in which only total agreement between the raters is considered as an agreement (Gwet, 2012), were calculated for 6 raters as proposed by Gwet

(Gwet, 2008, 2012) using AgreeStat2011.2/3 (Advanced Analytics, 2010). The two raters with the highest coefficient of agreement ($AC_1 = 0.55$, SE=0.06, 95% confidence interval [CI] = 0.43 to 0.67, $p<0.001$) looked at and classified all of the collected pictures with deer (total = 33,263 pictures). They used a key (S1 File in Mejía Salazar et al., 2016) to differentiate mule deer in the photos into the following 10 sex-and-age classes: adult male (ADM), adult female (ADF), juvenile male (JVM), juvenile female (JVF), fawn of any sex (Fawn), adult of unknown sex (ADUnk), juvenile of unknown sex (JVUnk), male of unknown age (MUnk), female of unknown age (FUnk), and unknown sex and age (Unk). Deer were considered to be fawns from 0 to 1 year of age, juveniles from 1 to 2 years of age, and adults from 2 years of age and older. Mule deer aged every May 16th, which represents the first day of fawning season. In addition, we counted the number of pictures where at least one mule deer touched the environment with any part of its mouth, nose or antlers, or scraped the ground with the hooves of its fingers (2012), or defecated or urinated. We considered these types of contacts to be of moderate to high risk for transmission of diseases such as chronic wasting disease. We did not distinguish between those behaviours likely to result in prion deposition, or uptake, or both. We also counted the number of pictures in which at least two deer were contacting each other (e.g., grooming each other).

Finally, for each station, we summarized: 1) counts of pictures with deer (AllDeerPics, the sum of all pictures with mule deer), 2) pictures with males (MalePics, sum of pics with JVM, ADM and MUnk), 3) pictures with females (FemalePics, sum of pics with JVF, ADF and FUnk), 4) pictures with fawns (FawnPics, sum of pics with fawns of any sex), 5) pictures of deer contacting the environment (ContactEnvironmentPics) (Table 6-1), and 6) pictures of deer contacting each other. For the latter, only 0.5% (55/11,587) of the pictures with ≥2 deer captured deer contacting each other, we were unable to investigate differences among season and site type with these limited numbers. The total number of camera-days was calculated for each station.

Table 6-1. Values used to build final models of the effect of season and site type (covariates) in the number of pictures with different sex-and-age classes of mule deer (response variable).

Response variable (outcome)	Description of the response variable	Significance of season (at $p \leq 0.2$)	Significance of site (at $p \leq 0.2$)	Significance of season and site (at $p \leq 0.05$)	Significance of interaction term (at $p \leq 0.05$)	Final model structure
AllDeerPics	Pics with mule deer	**0.002**	**<0.0001**	**0.01, 0.0001**	**0.02**	$\beta_0 + \beta_{season} + \beta_{site} + \beta_{season*site} + \varepsilon$
ADMpics	Pics with ADM	**<0.0001**	**0.001**	**0.001, 0.035**	0.46	$\beta_0 + \beta_{season} + \beta_{site} + \varepsilon$
ADFpics	Pics with ADF	0.26	**0.14**	0.23, 0.1561	N/A	N/A
AdultPics	Sum of pics with ADM + ADF + adults of unknown sex	**0.001**	**0.0002**	**0.01, 0.002**	**0.03**	$\beta_0 + \beta_{season} + \beta_{site} + \beta_{season*site} + \varepsilon$
JVMPics	Pics with JVM	0.47	0.98	N/A	N/A	N/A
JVFPics	Pics with JVF	0.22	**0.01**	***0.17*, 0.01**	0.85	$\beta_0 + \beta_{season} + \beta_{site} + \varepsilon$
JuvenilePics	Sum of pics with JVM + JVF + juveniles of unknown sex	**0.19**	**0.11**	0.2, 0.12	N/A	N/A
FawnPics	Sum of pics with fawns of any sex	**<0.0001**	**<0.0001**	**<0.0001, <0.0001**	**<0.0001**	$\beta_0 + \beta_{season} + \beta_{site} + \beta_{season*site} + \varepsilon$
MalePics	Sum of pics with ADM + JVM + males of unknown age	**<0.0001**	**0.02**	**0.001, *0.14***	0.11	$\beta_0 + \beta_{season} + \beta_{site} + \varepsilon$
FemalePics	Sum of pics with ADF + JVF + females of unknown age	0.32	0.02	***0.27*, 0.02**	0.5	$\beta_0 + \beta_{season} + \beta_{site} + \varepsilon$
ContactEnvironmentPics	Pics with mule deer contacting the environment	**0.0004**	**<0.0001**	**0.02, <0.0001**	**0.04**	$\beta_0 + \beta_{season} + \beta_{site} + \beta_{season*site} + \varepsilon$

Notes: Significant values in **bold**. Confounder values in *italics*. Abbreviations: pics, pictures; ADM, adult male; ADF, adult female; JVM, juvenile male; JVF, juvenile female; N/A, not applicable because no predictors were identified.

6.4.4 Statistical analysis

To assess the effect of season and site type on the frequency of visitation of mule deer, we fitted several generalized linear mixed models using the GLIMMIX procedure in SAS v9.3, with one model per outcome (Table 6-1, page 193). The outcome variables were the frequency with which each sex-and-age class visited sites (measured as the number of pictures taken), and the frequency of environmental contact (measured as the number of pictures with mule deer contacting the environment). The data were explored initially using a Poisson distribution and log link function. However, the negative binomial distribution was used in the final data analysis because we identified overdispersion after accounting for the structure of the data. The negative binomial model also returned smaller AICs, indicating better fit than the Poisson (Dohoo et al., 2009; IDRE - Institute for Digital Research and Education, 2013). The natural log of camera days was used as the offset, and the random intercept was camera station to account for unmeasured differences among sites. Model building was completed using manual forward selection, with a screening criterion of $p \leq 0.2$ for the initial bivariate or unadjusted analysis, and $p<0.05$ as the criterion for significance in the multivariable analysis. When building the multivariable model for each outcome, if one covariate changed the regression coefficient or effect size of the other by 10% or more, then we considered it as a confounder and kept it in the model (Sullivan, 2008; Dohoo et al., 2009). We examined the potential for interaction between season and site type where both were significant as fixed effects in the model. The interaction term was retained in the final model if $p<0.05$.

6.5 RESULTS

Table 6-2, Table 6-3 and Table 6-4 summarize site visitation measures collected over the course of this study. Mule deer in 30,377 pictures from 370 stations were classified by sex-and-age class (Table 6-2). The mean number of pictures with mule deer per camera-day (pics/CD) per station was 2.6 (SD = 5.9, SE = 0.3, range from 0.02 to 61) (Table 6-2). Over the study period, as many as 50.5% (187/370) of the stations captured one picture with mule deer per CD. Most (80.3%, 297/370) of the stations had fewer than three pictures with mule deer per CD. Grain sources had the greatest visitation mean; mortality sites had the lowest (Table 6-3). More pictures per CD were captured in late gestation than in any other season, and the fewest pictures were obtained during fawning (Table 6-4). Contact with the environment was captured in 14.1% of the pictures with mule deer (4,298/30,377) in 63.8% of the stations (236/370), with a mean of 0.34 pics/CD per station (SD = 1.27, SE = 0.07, median = 0.05, mode = 0, range from 0 to 16.9). Most stations (60.8%, 225/370) had either 1 or 2 pictures with mule deer contacting the environment per CD.

Table 6-2. Descriptive values of counts of pictures with mule deer by sex-and-age class. July 2009 to December 2012.

Sex-and-age class	Total picture counts[1]	Mean	SD	SE	Min	Max
Adult male	6464	0.53	1.64	0.08	0	16.26
Adult female	6957	0.53	2.10	0.11	0	30.46
Juvenile male	2690	0.26	0.71	0.04	0	7.00
Juvenile female	2816	0.28	1.05	0.05	0	13.62
Adult	13930	1.01	3.09	0.16	0	31.32
Juvenile	5688	0.55	1.51	0.08	0	15.62
Fawn	4988	0.41	1.23	0.06	0	11.11
Male	9296	0.81	2.05	0.11	0	19.00
Female	10639	0.88	2.89	0.15	0	40.25
Environmental[2]	4298	0.34	1.27	0.07	0	16.89
All deer	30377	2.61	5.86	0.31	0.02	61.11

Notes: [1] Total picture counts: number of pictures in which the corresponding sex-and-age class was seen.

[2] Environmental: pictures in which at least one deer was observed contacting the environment.

Mean: mean number of pictures per camera-day.

Abbreviations: SD, standard deviation; SE, standard error; Min and Max, minimum and maximum number of pictures per CD.

Table 6-3. Descriptive values of counts of pictures with mule deer by site type. July 2009 to December 2012.

Type of site	Total picture counts[1]	CD	pics/CD	Mean	SD	ST	SE
Grain sources	12280	1102	11.14	10.73	15.80	33	2.75
Bed	6612	1962	3.37	3.13	4.19	65	0.52
Salt lick	1546	685	2.26	2.10	3.39	17	0.82
Trail	3554	2057	1.73	1.73	1.86	70	0.22
Rub	1108	704	1.57	2.06	2.49	26	0.49
Browse	2800	2095	1.34	1.42	1.66	77	0.19
Waterhole	1165	921	1.26	1.58	2.21	29	0.41
Mortality	1312	2061	0.64	0.78	1.16	53	0.16
Total	30377	11587	2.62	2.61	5.86	370	0.30

Notes: [1] Total picture counts: number of pictures with mule deer taken in each site type.

Mean: Mean number of pictures per camera-day.

Abbreviations: CD, camera-days; pics/CD, number of pictures per CD; SD, standard deviation; ST, number of stations; SE, standard error calculated as $\boldsymbol{SD}/\sqrt{\boldsymbol{ST}}$.

Table 6-4. Descriptive values of counts of pictures with mule deer by season. July 2009 to December 2012.

Season	Total picture counts[1]	CD	pics/CD	Mean	SD	ST	SE
Fawning	5205	2282	2.28	2.04	3.10	72	0.36
Pre-rut	7999	3345	2.39	2.48	6.67	104	0.65
Rut	4285	1566	2.74	2.54	4.23	72	0.50
Early gestation	8660	3406	2.54	2.77	6.16	91	0.65
Late gestation	4228	988	4.28	4.01	9.41	31	1.69
Total	30377	11587	2.62	2.61	5.86	370	0.30

Notes: [1] Total picture counts: number of pictures with mule deer taken in each site type.

Mean: Mean number of pictures per camera-day in that season.

Abbreviations: CD, camera-days; pics/CD, number of pictures per CD; SD, standard deviation; ST, number of stations; SE, standard error calculated as $\boldsymbol{SD}/\sqrt{\boldsymbol{ST}}$.

Statistical analysis revealed that there was no difference in the count of pictures with ADF, JVM, and juveniles among seasons or site types (Table 6-1, page 193). However, the counts of pictures of ADM, JVF, males and females did vary by season and site type (Table 6-1, page 193). There was also a significant interaction between site type and season in some models, such that the extent of differences between site types in the counts of pictures with adults, fawns, all deer, and contact with the environment varied by season (Table 6-1, page 193). Season was a confounder of the association between site type and female and JVF visitations, and site was a confounder of the association between season and the number of visits by male deer (Table 6-1, page 193).

6.5.1 Frequency of visitation of adult males

Adult males were more likely to visit grain sources than waterholes (p=0.02), beds (p=0.02), browse sites (p=0.02), trails (p=0.001) and mortality sites (p=0.0008). They were also more likely pictured during early gestation than in fawning (p=0.03) or pre-rut (p<0.001) (Appendix 6.2).

6.5.2 Frequency of visitation of males

Males were more likely to visit grain sources than trails (p=0.01) and mortality sites (p=0.01). They were also more likely pictured during early gestation than in any other season (p<0.04), and less likely in pre-rut than in either rut (p=0.02) or fawning (p=0.03) (Appendix 6.2).

6.5.3 Frequency of visitation of juvenile females

Juvenile females were photographed more frequently at beds than any other sites (p<0.04) except for grain sources (p=0.38) and saltlicks (p=0.12). They also visited grain sources more than waterholes (p=0.02) or browse (p=0.04) sites. They appeared in pictures more often during pre-rut (p=0.05) and early gestation (p=0.02) than in fawning (Appendix 6.2).

6.5.4 Frequency of visitation of females

The number of pictures with females did not vary by season (p>0.09); however, it did by site. Females visited bed sites more than any other site (p<0.04), except for grain sources (p=0.89) and saltlicks (p=0.06). They also visited grain sources more than waterholes (p=0.02) and browse sites (p=0.02) (Appendix 6.2).

6.5.5 Frequency of visitation of adults

Adults visited grain sources in early gestation more than: every site type in pre-rut (p<0.01) and rut (p<0.01); every other site in early gestation (p<0.01); beds, browses and mortalities in late gestation (p<0.005) and fawning (p<0.03); and waterholes and trails during fawning (p<0.0001) (Appendix 6.3). The second, third and fourth greatest rates of visitation during the year occurred in late gestation; at waterholes, grain sources and saltlicks, respectively.

Adults were more likely found at waterholes during late gestation than in any other season ($p<0.04$), except in rut ($p=0.06$).

During fawning, adults visited beds and salt licks more frequently than waterholes and browse sites ($p<0.05$). In pre-rut, adults visited bed sites more frequently than waterholes ($p=0.05$) and the least frequently visited stations were mortality sites ($p<0.021$). In rut, there was no difference in adult visitation to the site types ($p>0.05$). By early gestation, adult visits to grain sources reached their annual peak, and were substantially more frequent ($p<0.015$) than to any other site within the same season. The average frequency of visitation to salt licks changed abruptly from early gestation (0.38 pics/CD; 95% CI 0.04 to 3.2) to late gestation (7.1 pics/CD; 95% CI 1.1 to 48) ($p=0.04$). Finally, in late gestation, visits to waterholes (the most visited sites in that season) were more frequent than to beds (the least visited sites of the season) ($p=0.05$) (Appendix 6.3).

6.5.6 Frequency of visitation of fawns

The most frequently visited sites by fawns were salt licks during late gestation (13 pics/CD; 95% CI 0.38 to 444) (Appendix 6.4). This visitation was greater than to salt licks in pre-rut ($p=0.047$), and every site during fawning ($p<0.04$), except for bed sites ($p=0.19$). Fawns visited waterholes less in fawning than in pre-rut ($p=0.01$), early gestation ($p=0.026$), and late gestation ($p=0.001$).

When first born, fawns were more likely to be observed in bed sites than at waterholes ($p=0.003$), browse sites ($p<0.0001$), grain sources ($p<0.0001$), trails ($p=0.001$), or mortality sites ($p<0.0001$). In pre-rut, following similar preferences, fawns were pictured in beds more than in any other site ($p<0.01$), with the exception of grain sources ($p=0.51$). In rut season, fawns aged 5-6 months visited grain sources more than browse sites ($p=0.018$). By early gestation, when fawns were between 6 and 10 months old, they again preferred beds over rubs ($p=0.02$), browse sites ($p=0.01$) and trails ($p=0.04$). In late gestation, when they were 10 or 11 months old, only beds were visited more than browse sites ($p=0.02$) (Appendix 6.4).

6.5.7 Frequency of visitation of all mule deer

Grain sources were the most visited sites across all seasons except in fawning, when they were the least visited sites (Appendix 6.5). Cameras at grain sources in late gestation captured the largest number of pictures with deer per CD (29 pics/CD; 95% CI 11 to 77). This was greater than almost all other site and season combinations ($p<0.05$), with the exception of grain sources from pre-rut ($p=0.52$), rut ($p=0.08$), and early gestation ($p=0.45$); bed sites in pre-rut ($p=0.18$); rubs in early gestation ($p=0.22$); and salt licks ($p=0.4$), waterholes ($p=0.22$) and beds ($p=0.67$) in late gestation.

During fawning, beds were visited more than any other site ($p<0.02$), except for salt licks ($p=0.21$). Then in pre-rut, both grain sources ($p<0.05$) and beds ($p<0.01$) were visited more than all other sites, with the exception of beds compared to rubs ($p=0.07$) and grain sources ($p=0.44$). In rut, salt licks, the least visited sites, were visited less frequently than rubs ($p=0.01$), beds ($p=0.02$), browses ($p=0.03$) and grain sources ($p=0.01$). By early gestation, grain sources were visited more than all other sites ($p<0.03$), except for rubs ($p=0.36$). In late gestation, grain sources and beds were visited more than browses ($p=0.01$ and 0.001), trails ($p=0.004$ and 0.002) and mortality sites ($p=0.01$ and 0.001) (Appendix 6.5).

6.5.8 Frequency of contact with the environment

Environmental contacts were identified most frequently in early gestation and late gestation, and at grain sources, waterholes and saltlicks (Appendix 6.6). During fawning, deer contacted the environment more at salt licks and beds than in waterholes ($p=0.02$ and 0.01), browses ($p=0.04$ and 0.02) and mortality sites ($p=0.03$ and 0.03). In pre-rut, contacts in beds occurred more frequently than in any other sites ($p<0.02$) except for grain sources ($p=0.75$) and salt licks ($p=0.09$). There was no difference in contacts at rubs among the seasons ($p>0.13$). In rut, environmental contacts occurred more often at grain sources than at browse sites ($p=0.04$). From rut to early gestation, there was a marked increase in environmental contacts at grain sources (from 1.3 in rut to 6.2 pics/CD, $p=0.01$). Environmental contacts at grain sources in early gestation were greater than for any other site and season combination during the year ($p<0.05$), except for grain sources in fawning ($p=0.56$). Finally, in late gestation, contacts at waterholes were significantly greater than at browse sites (p=0.01), trails (p=0.01) and mortality sites (p=0.005) (Appendix 6.6).

6.6 DISCUSSION

In this study, we measured mule deer frequency of visitation to different types of sites across multiple seasons. We found that not only did many of the investigated sex-and-age classes most frequently visited anthropogenic sites, but also that contacts with the environment were more frequent at these sites (especially at grain sources in early gestation).

There are circumstances that may increase the risk of CWD transmission among deer at anthropogenic sites, both via animal-animal contacts and contaminated environments. First, aggregation associated with artificial feeding and water provision has been shown to increase disease risk to deer (Dunkley and Cattet, 2003; Miller et al., 2007; Vicente et al., 2007). Weeks (Weeks, 1978), found that white-tailed deer visiting salt licks in Indiana, USA, would congregate in a 30 m^2 area and compete for resources with an interaction frequency much greater than previously reported. Likewise, aggregations of approximately 200 white-tailed deer were recorded at winter cuttings in Michigan (Ozoga, 1972). Also, in some areas, the creation of an artificial attraction site with grain, water or minerals leads to unnaturally high deer densities (Valone, 1989; Brits et al., 2002; Kilpatrick and Stober, 2002; Campbell et al., 2006; Cooper et al., 2006; Peterson and Messmer, 2011) and gives rise to a space-use pattern resembling central-place foraging, as recently reported in moose (van Beest et al., 2010).

Anthropogenic sites have the potential to be manipulated to attempt to control CWD. Previously, a reduction in grain sources and water supplies has proven to be important in reducing the prevalence of other diseases in deer. For example, in Michigan (Hickling, 2002) and Minnesota (Carstensen and DonCarlos, 2011) bovine tuberculosis prevalence was reduced by banning food supplementation (Hickling, 2002) and reducing white-tailed deer densities (Carstensen and DonCarlos, 2011). However, we recommend being thoughtful and considering effects of these practices when constructing epidemiological models of CWD transmission and when developing strategies to control CWD spread, as they may produce diverse results. For example, adult female mule deer (Hervert and Krausman, 1986) and white-tailed deer (Kilpatrick and Stober, 2002; Campbell et al., 2006) moved outside their home range to find new sources of water when access to it was denied (Hervert and Krausman, 1986) and shifted their core areas of activity due to the presence of introduced bait sites (Kilpatrick and Stober, 2002; Campbell et al., 2006). In short, culling of deer and the removal or re-distribution of attractive sources of grain, water and salt can impact the movement patterns and distances traveled by deer (Hervert and Krausman, 1986; Ordway and Krausman, 1986; Wiles and Weeks, 1986; Farnsworth et al., 2005; Mysterud, 2010; Lendrum et al., 2013; Lintott et al., 2013), which may either support or prevent the geographic expansion of CWD, depending on how they are implemented.

6.6.1 Anthropogenic grain sources

Our results revealed that grain sources play an important role in site visitation in each sex-and-age class as they were the most visited sites in some point of the year in every case. Grain is an attractive source of nutrients to mule deer and other mammals, as it is highly digestible, palatable, available and accessible (Gordon and Prins, 2008), thus its widespread use among hunters. This accentuates in mule deer, as they naturally select to browse highly digestible food in response to their high rate of rumen turnover compared to other ruminants (Geist, 1998).

We expected females to visit beds, waterholes and browses more frequently, however, even when indeed they preferred beds over the rest of the sites, it was not significantly more than grain and salt licks; and they even preferred grain sources over the natural source of nutrients, browse. In another study (Peterson and Messmer, 2011), when adult female mule deer activities were compared in two areas with and without a winter-feeding program, it was clear that deer traveled further from bed to feed sites, using the same trails, and once there, congregated in higher numbers, and spent more time in the proximity, increasing their densities in bedding areas next to food stations.

We also reported that fawns were more likely found at bed sites from 0 to 5 months of age (which is expected for concealment and survival purposes) and from 6 to 10 months of age, when they follow their mothers more closely. However, they were more frequently pictured at grain sources during the 1.5 month long rut season, when they are 5-6 months old. We cannot think of a sound explanation for this behaviour, but along with reported observations of adult females and adult males dominating over fawns at feeding stations (Ozoga, 1972; Peterson and

Messmer, 2011), this may make rut a time of increased environmental and animal-animal prion exposure for fawns.

The effects of artificially feeding cervids, either intentionally or unintentionally, are widely documented (Thompson et al., 2008; van Beest et al., 2010; Peterson and Messmer, 2011; Oro et al., 2013; Sorensen et al., 2014). Artificial feeding restricts movements, alters seasonal migration and local distribution patterns, moves habitat selection in the direction of central place foraging, changes the mating system, produces small-scale changes in spatial genetic structure, increases intraspecific competition, alters plant community structure and species composition, and moves wild evolutionary traits closer to those linked to a semi-domestic state (for a list of references see Mysterud, 2010; Peterson and Messmer, 2011; Oro et al., 2013). The great danger of artificial feeding sources relies on the fact that in these sites deer congregate and interact more frequently and for longer periods with other deer and with the environment, thus enhancing the probability of disease transmission (Miller et al., 2007; Vicente et al., 2007; Thompson et al., 2008).

Our findings reinforce existing beliefs that anthropogenic grain sources are important because of both their potential for CWD transmission and as sites that can be regulated for disease given that they are the result of human activity. This is why several states of the USA (Colorado, Wisconsin, Illinois, Maryland, Minnesota, Missouri, Montana, New York, North Dakota, Virginia and West Virginia) in which CWD has been diagnosed in the wild, have implemented a ban on baiting and feeding wild cervids, at least in affected areas (Chronic Wasting Disease Alliance). CWD is present in wild cervids in the Canadian provinces of Alberta and Saskatchewan. In Alberta, baiting for the purpose of hunting cervids is unlawful (Province of Alberta, 2000; Alberta Government, 2013); however, feeding cervids is not banned or regulated. In Saskatchewan, baiting for hunting is allowed from August 1st to the end of hunting season in any year, whereas feeding wild ungulates between January 1st and July 31st in any year is prohibited since 2002 (Government of Saskatchewan, 1981). Currently, there is no formal initiative to address the unintentional creation of attractive feeding sources for deer, such as grain spills.

6.6.2 Salt licks

With respect to salt licks, another anthropogenic site, the frequency of visitation and contact with the environment of all deer, and specifically of adults and fawns, peaked during late gestation (April 1st to May 15th). These findings are similar to those of white-tailed deer in the USA. In Louisiana, lick use was greater in spring, summer and autumn than during winter (Schultz and Johnson, 1992). In Indiana, lick use was greater in early spring and null in winter (Weeks, 1978), and adult females visited mineral licks more than yearling females, and adult and yearling males (Atwood and Weeks Jr, 2002). These salt lick use patterns have been hypothesized to be due to an increased need for sodium associated with gestation and lactation (Weeks and Kirkpatrick, 1976), to antlerogenesis needs (Weeks and Kirkpatrick, 1976), and to the so-called "sodium drive". The latter results from the high dietary potassium in early spring forage, which decreases absorption of fecal water, increasing sodium and water loss, and producing a negative sodium balance that drives deer to seek auxiliary sodium supplies (Weeks and Kirkpatrick, 1976). Specifically in our study population, the overall mean birth date is June 12th (Perera, 2012), and with an average pregnancy length of 203 days (188 - 218 days), reproductive females in late gestation season are in their last third of pregnancy. Also, antler growth begins in May, peaks in September, when the antler skin starts to be shed, and calcification is completed by November (field observations, unpublished data). In general, therefore, it seems that the previously mentioned hypotheses might also help to partially explain the observed peak of visitation to salt licks in late gestation in our study.

6.6.3 Waterholes

Building water catchments (man-made or modified water sources) for wildlife has been a common practice in other parts of the world, such as in arid western USA and some parts of Africa (Krausman et al., 2006). In the 1990s, various negative impacts caused by these facilities were proposed, prompting research on desert mule deer, but not on Rocky Mountain mule deer in prairie habitats.

The water sources we monitored were mainly (>70%) man-made dugouts, which are commonly used as water sources for livestock. The dugouts fill through catchment of runoff water from melting snow and rain. Our results indicate that late gestation is an important season for visitation and contact with the environment at waterholes, as mule deer preferred waterholes over any other site during this season.

These findings contrasted with previous research in Rocky Mountain mule deer and Columbian black-tailed deer (*O.h. columbianus*) in California (Boroski and Mossman, 1998), and in desert mule deer (Hervert and Krausman, 1986) and mule deer (Rosenstock et al., 2004) in Arizona. Both Hervert et al. (Hervert and Krausman, 1986) and Rosenstock et al. (Rosenstock et al., 2004) found that water developments are used year round, with peak visitation by mule deer and other species (Rosenstock et al., 2004) occurring when average ambient temperature reaches its maximum annual levels and the average relative humidity decreases, which in Arizona occurs during May, June and July. Female desert mule deer in Arizona drink more water during late summer than do males, which supports the hypothesis of greater use of water sources due to high energetic demands, such as lactation (Hazam and Krausman, 1988). In contrast, in our study area, from May to the end of July (fawning season), when the ambient temperatures are the highest of the year (Government of Canada, 2014), mule deer were more likely pictured at beds than at waterholes. A possible explanation is that as mule deer in our area have access to beds on the hill sides and in shrub areas, which offer cool wind and shade, and to plants rich in water content, they might use these as a strategy to cool their body temperature and obtain water sources during the hottest months of the year, unlike desert mule deer, which do not have access to these resources.

Moreover, the peaks of visitation to waterholes in our study area occurred in April and the first half of May in late gestation season. This period corresponds to spring, when the snow melts as the proportion of the day in freezing (-10°C to 0°C) and frigid (below -10°C) ambient temperatures decreases (Government of Canada, 2014). These changes allow a significant increase in availability of fresh running water that fills dugouts, probably a feature that attracts mule deer.

More importantly, CWD prions have been detected in water in a CWD endemic zone at a time of increased water runoff from melting snow during summer (Nichols et al., 2009). Nichols et al. (2009) speculated that prion levels in water increased to detectable levels as water from runoffs has a greater concentration of inorganic components such as minerals and clays (Nichols et al., 2009), to which prions strongly bind (Johnson et al., 2006; Wyckoff et al., 2013). Those findings, in addition to coincidental high visitation rate to these sites in our study area, could greatly potentiate the frequency and the probability of mule deer encountering CWD prions in the environment.

6.6.4 Antler rubs

Both antler rubs and scrapes likely serve as territorial markers to communicate with other deer in the area. Antler rubs are branches or trees on which deer rub their antlers and facial glands; these sites are used throughout the whole breeding season (Moore and Marchinton, 1971; Kile and Marchinton, 1977). Scrapes are areas of bare ground beneath overhanging tree branches that are marked with body glands secretions, feces, urine, or saliva, and that are intensely used only just before or during the peak of breeding season (Kile and Marchinton, 1977; Alexy et al., 2001). Since their use by white-tailed deer was first described in 1954 (Pruitt, 1954), it is clear that they intensely use scrapes and antler rubs, and their frequency of visitation and behaviour at these sites have been widely studied (Alexy et al., 2001; Kinsell, 2010). Specifically, Kinsell (2010) have described behaviours that have the potential to spread CWD among adult males in the population, and he also commented on scrape-related activities of adult male white-tailed deer which puts them at a greater risk of depositing, and getting exposed to, prions in the environment.

In our study, we monitored frequency of visitation of mule deer to antler rubs. Contrary to studies in white-tailed deer (Alexy et al., 2001; Kinsell, 2010), we did not investigate the effect of sex and age on site visitation, rather, we investigated how various sex-and-age classes visited these sites during pre-rut, rut and early gestation, as they are not available during late gestation and fawning. We found that mule deer did not visit rubs more in one season than another; we suspect these results have stemmed from the sampling distribution rather than from a true lack of difference, as antler rubs are so abundant and widely distributed. We recommend monitoring specific types of antler rubs that are preferentially used by deer as described by Kile (Kile and Marchinton, 1977). On the other hand, our findings do not contradict the known importance of these sites with respect to indirect deer communication and the potentially increased risk for CWD transmission (Kinsell, 2010).

6.6.5 Future work

With our data, further research might explore the effect of sex and age class in the rates of visitation by site type. It will also be useful to more closely analyze the risk behaviours in grain sources to differentiate the contacts between prion deposition and prion acquisition, by sex and age class. Furthermore, the number of different male mule deer visiting various site types can be calculated during those months in which their antlers are fully grown. In addition, as more than 100 mule deer were radio-collared in this same study area, radio-telemetry techniques could be applied to calculate the probability of deer presence given a distance to specific sites, such as water, grain sources and saltlicks, or to calculate distances traveled to find attractants such as salt, grain and water, and how the presence and abundance of these sites can affect home range sizes.

6.6.6 Conclusions

Prion accumulation in CWD endemic areas is most likely at environmental sites that are used frequently and intensively by large numbers of deer (Miller et al., 2004; Georgsson et al., 2006; Mathiason et al., 2009; Gough and Maddison, 2010). The potential for CWD transmission both from prions in the environment and directly from infected deer is, therefore, also relatively higher at these locations. We demonstrated that mule deer in our study area preferentially visited anthropogenic sources of grain, and that they were most likely to be seen contacting the environment (i.e., defecating, urinating and eating from the ground) at such sites. Our findings suggest that anthropogenic sites, and in specific grain sources, could potentially play a central role in CWD transmission, as they represent a small area for focal and repeated exposure to prions. Until such time that analytical techniques are developed to detect concentrations of CWD prions in the environment, our results can be used to rank the relative importance of various environmental sources of CWD prions in disease transmission models for this region.

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6.8 SUPPLEMENTAL MATERIAL

Appendix 6.1. Photographs and description of the site types monitored in Antelope Creek, Saskatchewan, Canada, from July 2009 to December 2012.

Appendix 6.2. Summary of results of generalized linear mixed models in which the number of pictures with mule deer varied by season and site type.

Appendix 6.3. Summary of results from the model with counts of pictures with adult mule deer.

Appendix 6.4. Summary of results from the model with counts of pictures with mule deer fawns.

Appendix 6.5. Summary of results from the model with counts of pictures with mule deer.

Appendix 6.6. Summary of results from the model with counts of pictures with mule deer contacting the environment.

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6.10 APPENDIX 6.1.

Appendix 6.1. Photographs and description of the site types monitored in Antelope Creek from July 2009 to December 2012.

Fig. A6.1.1. Mule deer at a salt lick. Antelope Creek, Saskatchewan, Canada. 19 April 2010. Mule deer lick salt blocks placed on the ground by ranchers for their cattle. These blocks were available all year, and mainly during pre-rut. The fawn on the left hand side of the picture was wearing a VHF (very high frequency) radio telemetry collar and an orange ear tag as part of another ongoing study (Silbernagel et al., 2011).

Fig. A6.1.2. Mule deer at a waterhole. Antelope Creek, Saskatchewan, Canada. 14 May 2010. Mule deer visit waterholes and drink water, sometimes in groups, as depicted here. These bodies of stagnant water were available all year, but froze during winter. More than 70% of the waterholes in which trail cameras were deployed, were dugouts (made by humans).

Fig. A6.1.3. An adult male mule deer at an antler rub site. Antelope Creek, Saskatchewan, Canada. 29 November 2009. Mule deer visit antler rubs. Males, sometimes rub their antlers and heads on trees, branches or posts, leaving noticeable abrasions, like the ones that are evident on this picture. These sites carry olfactory cues and signal presence or hierarchical status, and are available from August to the end of March. The adult male on this picture carried a GPS (global positioning system) collar as part of another ongoing study (Silbernagel et al., 2011).

Fig. A6.1.4. Male mule deer bedded at a bed site. Antelope Creek, Saskatchewan, Canada. 22 August 2010. Mule deer lie down to ruminate, rest, assist thermoregulation and avoid predation. They lie down at bed sites, either on the side of a hill (as in this picture) or within shrub areas; in both, the ground was devoid of vegetation and scratch marks were evident.

Fig. A6.1.5. Male mule deer feeding at a browse site. Antelope Creek, Saskatchewan, Canada. 13 September 2010. Browse sites were defined as areas where deer were seen selecting their food.

Fig. A6.1.6. Several mule deer visiting a grain source. Antelope Creek, Saskatchewan, Canada. 27 February 2012. Mule deer visited highly concentrated sources of grain created by humans, as in this grain spill from the bins used for storage. Many times they were in groups, as shown in this picture. The deer with a VHF (very high frequency) collar and green tags was a 1 year and 8 months old male diagnosed as chronic wasting disease positive in April of 2012.

Fig. A6.1.7. Mule deer traversing a trail. Antelope Creek, Saskatchewan, Canada. 10 March 2012. Mule deer frequently use the same trails to move within their home range. These trails are noticeable for their lack of vegetation (bare soil is left) and their narrow width. When more than one deer use the trail at the same time, they typically follow one another, as shown in this picture.

Fig. A6.1.8. Mule deer visiting a mortality site. Antelope Creek, Saskatchewan, Canada. 21 April 2011. We deployed cameras where mule deer carcasses from another ongoing studies involving radio-collared deer were found (Reiczigel et al., 2015; Mejía Salazar et al., 2016). As depicted by this photograph, mule deer tend to investigate the carcass by walking towards the remains and sniffing them. In this case, the carcass was from an adult female mule deer that died of chronic wasting disease.

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6.11 APPENDIX 6.2.

Appendix 6.2. Summary of results of generalized linear mixed models in which the number of pictures with mule deer varied by season and site type.

To assess the effect of season and site type on the frequency of visitation of mule deer we fit a generalized linear mixed model using the GLIMMIX procedure in SAS v9.3. The outcome variable was the frequency with which different sex-and-age classes visited sites measured as the number of pictures taken, the offset was the camera-days, the distribution was negative binomial, the link function was log, and the random intercept was station. Model building was completed using manual forward selection, with a screening criterion of $p \leq 0.2$ for the initial univariable analysis, and $p < 0.05$ as the criterion for significance in the multivariable analysis. When building the multivariable model for each outcome, if one covariate changed the regression coefficient or effect of the other by 10% or more, then we considered it as a confounder and kept it in the model. We examined the potential for interaction between season and site type where both were significant as fixed effects in the model. The interaction term was retained in the final model if $p<0.05$.

The counts of pictures with adult males (ADM), juvenile females (JVF), males (sum of pictures of ADM, JVM and males of unknown age category) and females (sum of pictures of ADF, JVF and females of unknown age category) vary by season and site type.

The results of these 4 models are presented in this document. The summary of results includes:

- Table with the solutions of fixed effects in which fawning and mortality were the reference season and site, respectively.
- Least squares means (LSM) table. It indicates the estimated number of pictures per camera day, by season, and the lower and upper 95% confidence levels.
- Table with the significant pair-wise comparisons of the differences between the seasons. The sign < helps to identify which values (as shown in the LSM table) were significantly greater than others.
- Least squares means table. It indicates the estimated number of pictures per camera day, by site type, and the lower and upper levels.

- Table with the significant pair-wise comparisons of the differences between the site types. The signs < and > help to identify which values (as shown in the LSM table) were significantly greater than others.
- Graph of the predicted visitation rates (number of pictures per camera-day) during each season.
- Graph of the predicted visitation rates (number of pictures per camera-day) to each site type.

Results from the model predicting number of pictures with adult male mule deer.

Table A6.2.1. Solutions for fixed effects – Adult male model

Effect	Season	Site	Estimate[1]	SE	DF	P	Lower[1,2]	Upper[1,2]
Intercept			-0.79	0.41	277	0.05	-1.59	0.01
season	pre-rut		-0.67	0.30	81	0.03	-1.26	-0.07
season	rut		0.23	0.33	81	0.49	-0.43	0.88
season	early gestation		0.71	0.32	81	0.03	0.07	1.35
season	late gestation		0.10	0.37	81	0.79	-0.64	0.83
season[3]	fawning		0[3]	.	.	.	.	.
site		salt lick	0.79	0.64	81	0.22	-0.48	2.06
site		waterhole	0.30	0.53	81	0.57	-0.75	1.35
site		rub	0.91	0.54	81	0.10	-0.17	1.99
site		bed	0.55	0.42	81	0.20	-0.30	1.39
site		browse	0.57	0.40	81	0.16	-0.23	1.36
site		grain source	1.68	0.48	81	0.001	0.72	2.64
site		trail	0.13	0.41	81	0.75	-0.69	0.96
site[3]		mortality	0[3]	.	.	.	.	.

SE = standard error; DF = degrees of freedom. [1] Units are log(pics/CD) where pics/CD is the number of pictures per camera-day. [2]Lower and Upper are the confidence limits on the mean (probability) scale. [3] The reference category.

Table A6.2.2. Season least squares means – Adult male model

Season	Estimate[1]	Lower[1]	Upper[1]
pre-rut	0.43	0.25	0.73
rut	1.06	0.65	1.71
early gestation	1.71	1.07	2.75
late gestation	0.93	0.46	1.87
fawning	0.84	0.49	1.46

[1] In pics/CD.

Table A6.2.3. Differences of significant season least square means – Adult male model

Season	Relationship	Season	P
fawning	<	early gestation	0.03
pre-rut	<	early gestation	<.0001
pre-rut	<	rut	0.004
pre-rut	<	fawning	0.03

< = the estimate of the season on the left is smaller than the estimate of the season on the right.

Table A6.2.4. Site least squares means – Adult male model

Site	Estimate[1]	Lower[1]	Upper[1]
salt lick	1.08	0.36	3.27
waterhole	0.66	0.28	1.53
rub	1.21	0.49	3.00
bed	0.85	0.46	1.56
browse	0.86	0.51	1.46
grain source	2.63	1.17	5.90
trail	0.56	0.31	1.00
mortality	0.49	0.25	0.95

[1] In pics/CD.

Table A6.2.5. Differences of significant site least square means – Adult male model

Site	Relationship	Site	P
waterhole	<	grain source	0.02
bed	<	grain source	0.02
browse	<	grain source	0.02
trail	<	grain source	0.001
mortality	<	grain source	0.001

< = the estimate of the site on the left is smaller than the estimate of the site on the right.

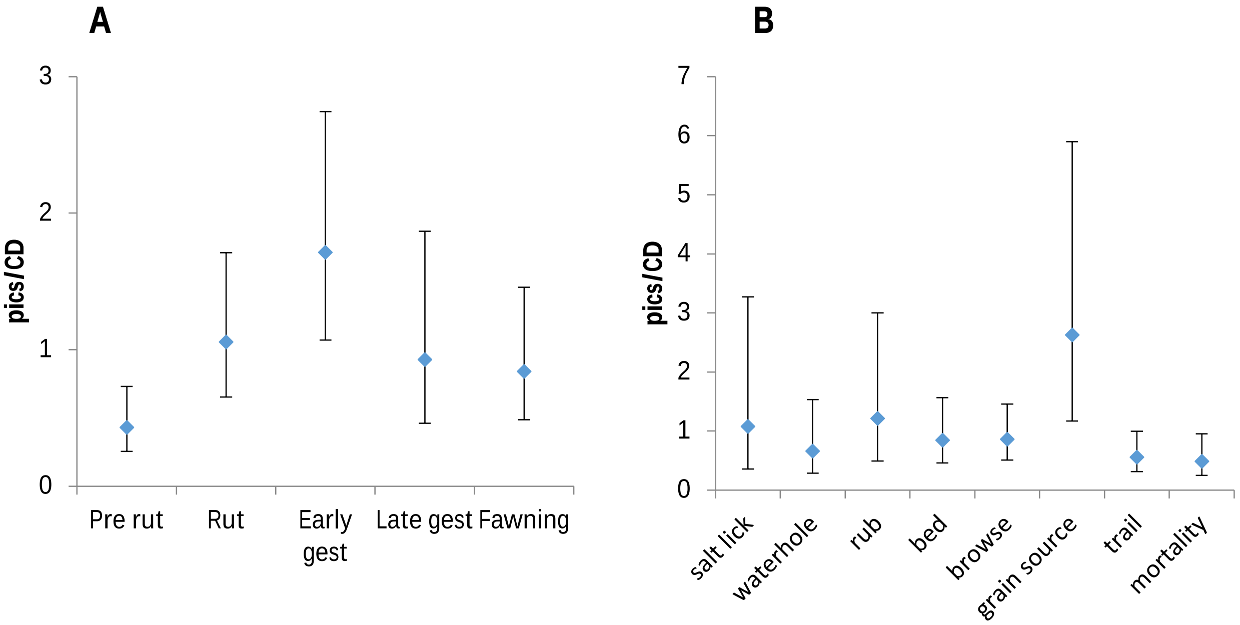


Fig. A6.2.1. Predicted visitation by adult males by season (A) and by site type (B). Pics/CD = number of pictures per camera-day. Error bars indicate lower and upper limits.

Results from the model predicting number of pictures with juvenile female mule deer.

Table A6.2.6. Solutions for fixed effects – Juvenile female model

Effect	Season	Site	Estimate[1]	SE	DF	P|	Lower[1,2]	Upper[1,2]
Intercept			-1.43	0.46	277	0.002	-2.33	-0.52
season	pre-rut		0.74	0.37	81	0.05	0.01	1.46
season	rut		0.69	0.40	81	0.09	-0.11	1.49
season	early gestation		1.00	0.40	81	0.02	0.20	1.81
season	late gestation		0.67	0.52	81	0.20	-0.36	1.71
season[3]	fawning		0[3]	.	.	.	.	.
site		salt lick	0.08	0.69	81	0.91	-1.30	1.45
site		waterhole	-0.89	0.61	81	0.15	-2.10	0.32
site		rub	-0.10	0.63	81	0.88	-1.35	1.16
site		bed	1.13	0.47	81	0.02	0.20	2.07
site		browse	-0.46	0.45	81	0.31	-1.36	0.44
site		grain source	0.66	0.55	81	0.24	-0.44	1.75
site		trail	0.21	0.46	81	0.65	-0.71	1.12
site[3]		mortality	0[3]	.	.	.	.	.

SE = standard error; DF = degrees of freedom. [1] Units are log(pics/CD) where pics/CD is the number of pictures per camera-day. [2]Lower and Upper are the confidence limits on the mean (probability) scale. [3] The reference category.

Table A6.2.7. Season least squares means – Juvenile female model

Season	Estimate[1]	Lower[1]	Upper[1]
pre-rut	0.54	0.29	1.00
rut	0.52	0.28	0.96
early gestation	0.71	0.36	1.41
late gestation	0.51	0.18	1.46
fawning	0.26	0.13	0.50

[1] In pics/CD.

Table A6.2.8. Differences of significant season least square means – Juvenile female model

Season	Relationship	Season	P
fawning	<	early gestation	0.02
fawning	<	pre-rut	0.05

< = the estimate of the season on the left is smaller than the estimate of the season on the right.

Table A6.2.9. Site least squares means – Juvenile female model

Site	Estimate[1]	Lower[1]	Upper[1]
salt lick	0.48	0.15	1.56
waterhole	0.18	0.06	0.54
rub	0.41	0.13	1.30
bed	1.39	0.65	2.97
browse	0.28	0.14	0.56
grain source	0.86	0.31	2.37
trail	0.55	0.27	1.11
mortality	0.45	0.20	0.98

[1] In pics/CD.

Table A6.2.10. Differences of significant site least square means – Juvenile female model

Site	Relationship	Site	P
waterhole	<	bed	0.001
rub	<	bed	0.04
browse	<	bed	0.0003
trail	<	bed	0.03
mortality	<	bed	0.02
waterhole	<	grain source	0.02
browse	<	grain source	0.04

< = the estimate of the site on the left is smaller than the estimate of the site on the right.

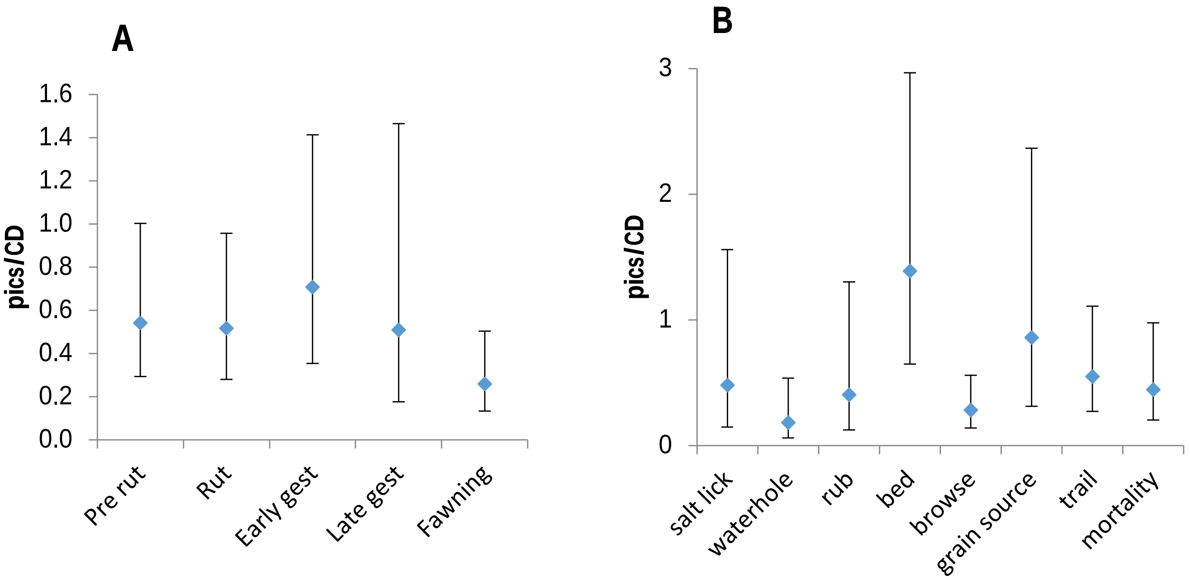


Fig. A6.2.2. Predicted visitation by juvenile females by season (A) and by site type (B). Pics/CD = number of pictures per camera-day. Error bars indicate lower and upper 95% CI

Results from the model predicting number of pictures with male mule deer.

Table A6.2.11. Solutions for fixed effects - Male model

Effect	Season	Site	E[1]	SE	DF	P	Lower[1,2]	Upper[1,2]
Intercept			0.05	0.31	277	0.88	-0.56	0.65
season	pre-rut		-0.50	0.23	81	0.03	-0.95	-0.04
season	rut		0.04	0.26	81	0.86	-0.47	0.55
season	early gestation		0.56	0.25	81	0.03	0.06	1.06
season	late gestation		-0.06	0.28	81	0.83	-0.62	0.50
season[3]	fawning		0[3]	.	.	.	.	.
site		salt lick	0.35	0.52	81	0.50	-0.68	1.38
site		waterhole	0.35	0.41	81	0.40	-0.47	1.17
site		rub	0.66	0.44	81	0.13	-0.20	1.53
site		bed	0.60	0.34	81	0.08	-0.07	1.28
site		browse	0.50	0.32	81	0.12	-0.13	1.14
site		grain source	1.12	0.39	81	0.006	0.34	1.90
site		trail	0.13	0.33	81	0.70	-0.53	0.78
site[3]		mortality	0[3]	.	.	.	.	.

E= Estimate; SE = standard error; DF = degrees of freedom. [1] Units are log(pics/CD) where pics/CD is the number of pictures per camera-day. [2]Lower and Upper are the confidence limits on the mean (probability) scale. [3]The reference category.

Table A6.2.12. Season least squares means – Male model

Season	Estimate[1]	Lower[1]	Upper[1]
pre-rut	1.01	0.70	1.47
rut	1.74	1.19	2.55
early gestation	2.91	2.01	4.22
late gestation	1.57	0.91	2.71
fawning	1.67	1.11	2.50

[1] In pics/CD.

Table A6.2.13. Differences of significant season least square means – Male model

Season	Relationship	Season	P
pre-rut	<	early gestation	<.0001
rut	<	early gestation	0.03
late gestation	<	early gestation	0.04
fawning	<	early gestation	0.03
pre-rut	<	rut	0.02
pre-rut	<	fawning	0.03

< = the estimate of the season on the left is smaller than the estimate of the season on the right.

Table A6.2.14. Site least squares means – Male model

Site	Estimate[1]	Lower[1]	Upper[1]
salt lick	1.50	0.61	3.69
waterhole	1.50	0.79	2.87
rub	2.05	1.00	4.20
bed	1.93	1.20	3.10
browse	1.75	1.16	2.64
grain source	3.22	1.67	6.21
trail	1.20	0.77	1.87
mortality	1.06	0.63	1.77

[1] In pics/CD.

Table A6.2.15. Differences of significant site least square means – Male model

Site	Relationship	Site	P
trail	<	grain source	0.01
mortality	<	grain source	0.01

< = the estimate of the site on the left is smaller than the estimate of the site on the right.

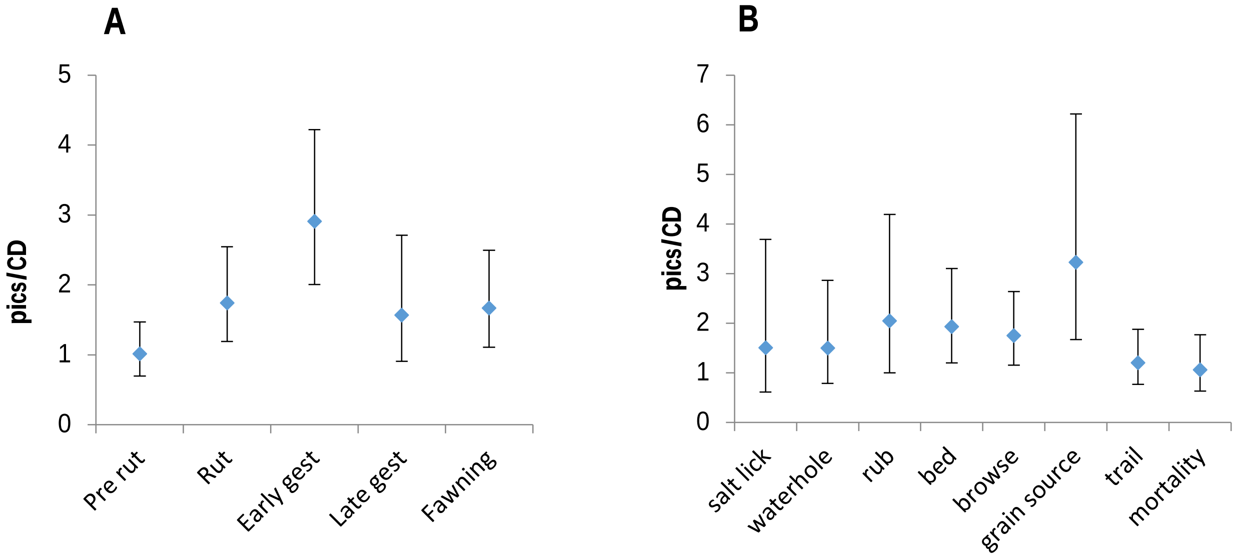


Fig. A6.2.3. Predicted visitation by males by season (A) and by site type (B). Pics/CD = number of pictures per camera-day. Error bars indicate 95% CI.

Results from the model predicting number of pictures with female mule deer.

Table A6.2.16. Solutions for fixed effects – Female model

Effect	Season	Site	Estimate[1]	SE	DF	P	Lower[1,2]	Upper[1,2]
Intercept			0.53	0.30	277	0.08	-0.06	1.12
season	pre-rut		0.27	0.23	81	0.25	-0.19	0.74
season	rut		0.01	0.27	81	0.97	-0.52	0.54
season	early gestation		0.38	0.26	81	0.15	-0.14	0.91
season	late gestation		0.54	0.32	81	0.09	-0.09	1.18
season[3]	fawning		0[3]	.	.	.	.	.
site		salt lick	-0.17	0.47	81	0.72	-1.10	0.76
site		waterhole	-0.44	0.39	81	0.26	-1.23	0.34
site		rub	-0.22	0.42	81	0.60	-1.04	0.61
site		bed	0.67	0.32	81	0.04	0.04	1.30
site		browse	-0.26	0.30	81	0.38	-0.86	0.33
site		grain source	0.62	0.38	81	0.11	-0.14	1.39
site		trail	0.07	0.31	81	0.83	-0.55	0.68
site[3]		mortality	0[3]	.	.	.	.	.

SE = standard error; DF = degrees of freedom. [1] Units are log(pics/CD) where pics/CD is the number of pictures per camera-day. [2]Lower and Upper are the confidence limits on the mean (probability) scale. [3] The reference category.

Table A6.2.17. Season least squares means – Female model

Season	Estimate[1]	Lower[1]	Upper[1]
pre-rut	2.30	1.62	3.26
rut	1.77	1.20	2.60
early gestation	2.57	1.71	3.85
late gestation	3.01	1.67	5.41
fawning	1.75	1.17	2.61

[1] In pics/CD.

There were no significant differences of season least square means in the female model.

Table A6.2.18. Site least squares means – Female model

Site	Estimate[1]	Lower[1]	Upper[1]
salt lick	1.82	0.82	4.05
waterhole	1.38	0.73	2.60
rub	1.73	0.87	3.48
bed	4.22	2.69	6.64
browse	1.66	1.14	2.41
grain source	4.01	2.03	7.93
trail	2.30	1.54	3.44
mortality	2.15	1.33	3.49

[1] In pics/CD.

Table A6.2.19. Differences of significant site least square means – Female model

Site	Relationship	Site	P
waterhole	<	bed	0.003
rub	<	bed	0.03
browse	<	bed	0.001
trail	<	bed	0.04
mortality	<	bed	0.04
waterhole	<	grain source	0.02
browse	<	grain source	0.02

< = the estimate of the site on the left is smaller than the estimate of the site on the right.

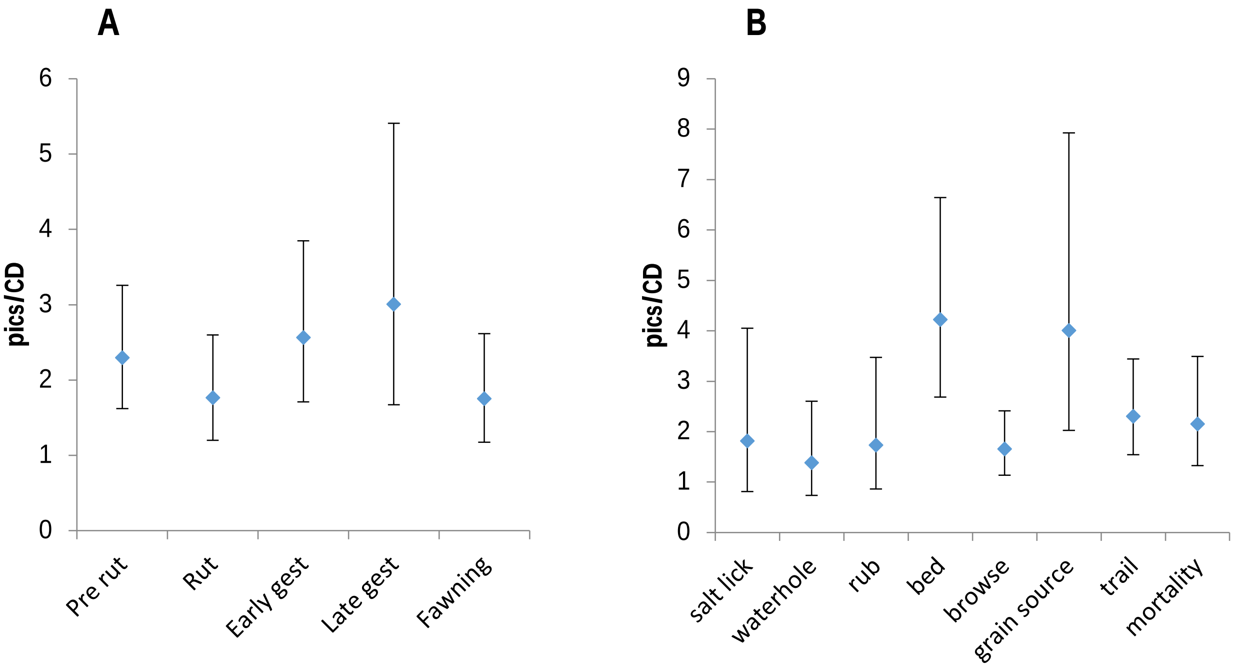


Fig. A6.2.4. Predicted visitation by females by season (A) and by site type (B). Pics/CD = number of pictures per camera-day. Error bars indicate lower and upper 95% CI.

6.12 APPENDIX 6.3.

Appendix 6.3. Summary of results from the model with counts of pictures with adult mule deer.

To assess the effect of season and site type on the frequency of visitation of mule deer we fit a generalized linear mixed model using the GLIMMIX procedure in SAS v9.3. The outcome variable was the frequency with which different sex-and-age classes visited sites measured as the number of pictures taken, the offset was the camera-days, the distribution was negative binomial, the link function was log, and the random intercept was station. Model building was completed using manual forward selection, with a screening criterion of $p \leq 0.2$ for the initial univariable analysis, and $p < 0.05$ as the criterion for significance in the multivariable analysis. When building the multivariable model for each outcome, if one covariate changed the regression coefficient or effect of the other by 10% or more, then we considered it as a confounder and kept it in the model. We examined the potential for interaction between season and site type where both were significant as fixed effects in the model. The interaction term was retained in the final model if $p<0.05$.

There was a significant interaction between site type and season in this model, such that the extent of differences between site types in the counts of pictures with adult mule deer varied by season.

The results of this model include:

- Table with the solutions of fixed effects.
- Least squares means (LSM) table. It indicates the estimated number of pictures per camera day, by season and site combinations, and their lower and upper 95% confidence levels.
- Table with the significant pair-wise comparisons of the differences between seasons and site type combinations. The signs < and > help to identify which values (as shown in the LSM table) were significantly greater than others.
- Table and graph of the predicted number of pictures with adult mule deer per camera-day for each season and site type combination.

Table A6.3.1. Solutions for Fixed Effects - Adult model

Effect	Season	Site	Estimate[1]	SE	DF	P	Lower[1]	Upper[1]
Intercept			0.33	0.43	276	0.45	-0.52	1.18
season	pre-rut		-1.71	0.77	56	0.03	-3.24	-0.18
season	rut		0.26	0.58	56	0.66	-0.91	1.42
season	early gest		0.98	0.49	56	0.05	-0.01	1.97
season	late gest		0.78	0.51	56	0.13	-0.24	1.80
season	fawning		0	.	.	.	.	.
site		salt lick	1.28	0.67	56	0.06	-0.07	2.62
site		waterhole	-0.39	0.62	56	0.54	-1.64	0.87
site		rub	-0.53	0.69	56	0.45	-1.91	0.86
site		bed	1.32	0.51	56	0.01	0.29	2.35
site		browse	0.10	0.52	56	0.85	-0.94	1.14
site		grain source	-7.63	13.23	56	0.57	-34.13	18.87
site		trail	0.54	0.51	56	0.30	-0.49	1.57
site		mortality	0	.	.	.	.	.
season*site	pre-rut	salt lick	0.79	0.97	56	0.42	-1.15	2.72
season*site	pre-rut	waterhole	2.07	0.93	56	0.03	0.21	3.93
season*site	pre-rut	rub	2.77	1.01	56	0.01	0.74	4.79
season*site	pre-rut	bed	1.22	0.86	56	0.16	-0.50	2.94
season*site	pre-rut	browse	1.82	0.85	56	0.04	0.11	3.52
season*site	pre-rut	grain source	9.84	13.26	56	0.46	-16.72	36.39
season*site	pre-rut	trail	1.46	0.84	56	0.09	-0.22	3.15
season*site	pre-rut	mortality	0	.	.	.	.	.
season*site	rut	salt lick	-1.97	1.06	56	0.07	-4.09	0.15
season*site	rut	waterhole	0.22	0.96	56	0.82	-1.69	2.14
season*site	rut	rub	0.82	0.77	56	0.29	-0.73	2.37
season*site	rut	bed	-1.37	0.74	56	0.07	-2.85	0.11
season*site	rut	browse	-0.08	0.73	56	0.91	-1.54	1.38
season*site	rut	grain source	8.26	13.24	56	0.54	-18.26	34.79
season*site	rut	trail	-0.64	0.73	56	0.38	-2.09	0.82
season*site	rut	mortality	0	.	.	.	.	.
season*site	early gest	salt lick	-3.57	1.28	56	0.01	-6.13	-1.00
season*site	early gest	waterhole	-1.22	1.18	56	0.31	-3.57	1.14
season*site	early gest	rub	0	.	.	.	.	.
season*site	early gest	bed	-1.69	0.65	56	0.01	-3.00	-0.39
season*site	early gest	browse	-0.25	0.65	56	0.70	-1.54	1.04

Effect	Season	Site	Estimate[1]	SE	DF	P	Lower[1]	Upper[1]
season*site	early gest	grain source	8.85	13.23	56	0.51	-17.66	35.35
season*site	early gest	trail	-1.03	0.65	56	0.12	-2.33	0.27
season*site	early gest	mortality	0	.	.	.	.	.
season*site	late gest	salt lick	-0.43	1.03	56	0.68	-2.49	1.64
season*site	late gest	waterhole	1.69	1.03	56	0.11	-0.38	3.75
season*site	late gest	bed	-1.93	0.69	56	0.01	-3.32	-0.55
season*site	late gest	browse	-0.20	0.68	56	0.77	-1.57	1.16
season*site	late gest	grain source	8.52	13.24	56	0.52	-18.00	35.04
season*site	late gest	trail	-0.25	0.81	56	0.76	-1.86	1.37
season*site	late gest	mortality	0	.	.	.	.	.
season*site	fawning	salt lick	0	.	.	.	.	.
season*site	fawning	waterhole	0	.	.	.	.	.
season*site	fawning	bed	0	.	.	.	.	.
season*site	fawning	browse	0	.	.	.	.	.
season*site	fawning	grain source	0	.	.	.	.	.
season*site	fawning	trail	0	.	.	.	.	.
season*site	fawning	mortality	0	.	.	.	.	.

SE = standard error; DF = degrees of freedom; early gest = early gestation; late gest = late gestation. [1]Units are log(pics/CD) where pics/CD is the number of pictures per camera-day; Lower and Upper are the confidence limits on the mean (probability) scale.

Table A6.3.2. Season*site least squares means - Adult model

Season	Site	Estimate[1]	Lower[1]	Upper[1]
pre-rut	salt lick	1.98	0.79	4.95
pre-rut	waterhole	1.35	0.71	2.57
pre-rut	rub	2.36	1.05	5.32
pre-rut	bed	3.19	1.76	5.77
pre-rut	browse	1.71	0.99	2.95
pre-rut	grain source	2.29	0.76	6.95
pre-rut	trail	1.87	1.14	3.07
pre-rut	mortality	0.25	0.07	0.90
rut	salt lick	0.90	0.21	3.92
rut	waterhole	1.53	0.44	5.27
rut	rub	2.41	1.26	4.64
rut	bed	1.70	0.81	3.59
rut	browse	1.83	0.94	3.58
rut	grain source	3.39	1.47	7.82
rut	trail	1.63	0.83	3.22
rut	mortality	1.80	0.82	3.97

Season	Site	Estimate[1]	Lower[1]	Upper[1]
early gest	salt lick	0.38	0.04	3.19
early gest	waterhole	0.75	0.11	5.15
early gest	rub	2.19	0.59	8.10
early gest	bed	2.55	1.32	4.91
early gest	browse	3.19	1.71	5.95
early gest	grain source	12.50	7.20	21.70
early gest	trail	2.27	1.19	4.34
early gest	mortality	3.71	2.18	6.30
late gest	salt lick	7.11	1.06	47.68
late gest	waterhole	11.15	2.11	58.93
late gest	bed	1.64	0.66	4.08
late gest	browse	2.74	1.26	5.99
late gest	grain source	7.42	1.98	27.83
late gest	trail	4.07	1.18	14.03
late gest	mortality	3.04	1.38	6.71
fawning	salt lick	4.99	1.78	13.96
fawning	waterhole	0.95	0.38	2.35
fawning	bed	5.20	2.97	9.11
fawning	browse	1.54	0.87	2.72
fawning	grain source	0.00	0.00	2×10^8
fawning	trail	2.39	1.36	4.20
fawning	mortality	1.39	0.58	3.32

early gest = early gestation; late gest = late gestation. [1]Units are pics/CD, the number of pictures per camera-day; Lower and Upper are the confidence limits on the mean (probability) scale.

Table A6.3.3. Differences of significant season*site least squares means - Adult model

Season	Site	Relationship[1]	Season	Site	P[2]
pre rut	saltlick	>	pre rut	mortality	0.01
pre rut	saltlick	<	early gest	grain source	0.001
pre rut	waterhole	<	pre rut	bed	0.05
pre rut	waterhole	>	pre rut	mortality	0.02
pre rut	waterhole	<	early gest	grain source	<.0001
pre rut	waterhole	<	early gest	mortality	0.02
pre rut	waterhole	<	late gest	waterhole	0.02
pre rut	waterhole	<	late gest	grain source	0.02
pre rut	waterhole	<	fawning	saltlick	0.03
pre rut	waterhole	<	fawning	bed	0.002
pre rut	rub	>	pre rut	mortality	0.004
pre rut	rub	<	early gest	grain source	0.001
pre rut	bed	>	pre rut	mortality	0.001
pre rut	bed	<	early gest	grain source	0.001

Season	Site	Relationship[1]	Season	Site	P[2]
pre rut	bed	>	fawning	waterhole	0.03
pre rut	browse	>	pre rut	mortality	0.01
pre rut	browse	<	early gest	grain source	<.0001
pre rut	browse	<	early gest	mortality	0.04
pre rut	browse	<	late gest	waterhole	0.04
pre rut	browse	<	late gest	grain source	0.04
pre rut	browse	<	fawning	bed	0.01
pre rut	grain source	>	pre rut	mortality	0.01
pre rut	grain source	<	early gest	grain source	0.01
pre rut	trail	>	pre rut	mortality	0.005
pre rut	trail	<	early gest	grain source	<.0001
pre rut	trail	<	late gest	waterhole	0.04
pre rut	trail	<	fawning	bed	0.01
pre rut	mortality	<	rut	waterhole	0.05
pre rut	mortality	<	rut	rub	0.002
pre rut	mortality	<	rut	bed	0.01
pre rut	mortality	<	rut	browse	0.01
pre rut	mortality	<	rut	grain source	0.001
pre rut	mortality	<	rut	trail	0.01
pre rut	mortality	<	rut	mortality	0.01
pre rut	mortality	<	early gest	rub	0.02
pre rut	mortality	<	early gest	bed	0.002
pre rut	mortality	<	early gest	browse	0.001
pre rut	mortality	<	early gest	grain source	<.0001
pre rut	mortality	<	early gest	trail	0.003
pre rut	mortality	<	early gest	mortality	0.0002
pre rut	mortality	<	late gest	saltlick	0.005
pre rut	mortality	<	late gest	waterhole	0.001
pre rut	mortality	<	late gest	bed	0.02
pre rut	mortality	<	late gest	browse	0.002
pre rut	mortality	<	late gest	grain source	0.0005
pre rut	mortality	<	late gest	trail	0.003
pre rut	mortality	<	late gest	mortality	0.002
pre rut	mortality	<	fawning	saltlick	0.0005
pre rut	mortality	<	fawning	bed	<.0001
pre rut	mortality	<	fawning	browse	0.01
pre rut	mortality	<	fawning	trail	0.002
pre rut	mortality	<	fawning	mortality	0.03
rut	saltlick	<	early gest	grain source	0.001
rut	saltlick	<	late gest	waterhole	0.03
rut	saltlick	<	late gest	grain source	0.04
rut	saltlick	<	fawning	bed	0.03

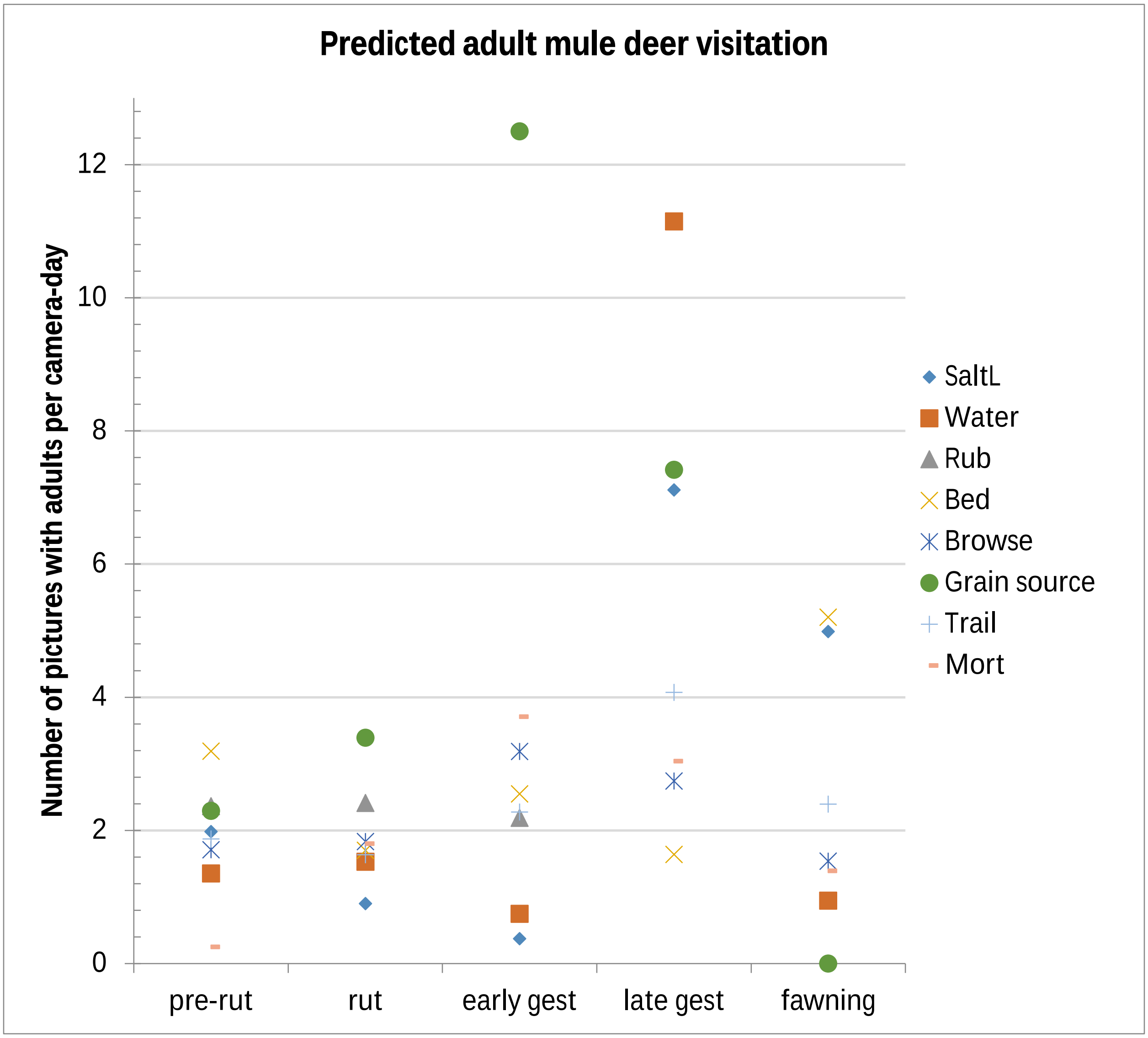
Season	**Site**	**Relationship**[1]	**Season**	**Site**	**P**[2]
rut	waterhole	<	early gest	grain source	0.003
rut	rub	<	early gest	grain source	0.0002
rut	bed	<	early gest	grain source	<.0001
rut	bed	<	late gest	waterhole	0.04
rut	bed	<	fawning	bed	0.02
rut	browse	<	early gest	grain source	<.0001
rut	browse	<	late gest	waterhole	0.05
rut	browse	<	fawning	bed	0.02
rut	grain source	<	early gest	grain source	0.01
rut	grain source	>	fawning	waterhole	0.04
rut	trail	<	early gest	grain source	<.0001
rut	trail	<	late gest	waterhole	0.04
rut	trail	<	late gest	grain source	0.04
rut	trail	<	fawning	bed	0.01
rut	mortality	<	early gest	grain source	0.0001
rut	mortality	<	fawning	bed	0.03
early gest	saltlick	<	early gest	grain source	0.002
early gest	saltlick	<	early gest	mortality	0.04
early gest	saltlick	<	late gest	saltlick	0.04
early gest	saltlick	<	late gest	waterhole	0.02
early gest	saltlick	<	late gest	grain source	0.02
early gest	saltlick	<	fawning	saltlick	0.03
early gest	saltlick	<	fawning	bed	0.02
early gest	waterhole	<	early gest	grain source	0.01
early gest	waterhole	<	late gest	waterhole	0.04
early gest	rub	<	early gest	grain source	0.01
early gest	bed	<	early gest	grain source	0.0004
early gest	browse	<	early gest	grain source	0.001
early gest	browse	>	fawning	waterhole	0.03
early gest	grain source	>	early gest	trail	0.0001
early gest	grain source	>	early gest	mortality	0.002
early gest	grain source	>	late gest	bed	0.0003
early gest	grain source	>	late gest	browse	0.002
early gest	grain source	>	late gest	mortality	0.005
early gest	grain source	>	fawning	waterhole	<.0001
early gest	grain source	>	fawning	bed	0.03
early gest	grain source	>	fawning	browse	<.0001
early gest	grain source	>	fawning	trail	<.0001
early gest	grain source	>	fawning	mortality	<.0001
early gest	mortality	>	fawning	waterhole	0.01
early gest	mortality	>	fawning	browse	0.03
late gest	waterhole	>	late gest	bed	0.05

Season	**Site**	**Relationship[1]**	**Season**	**Site**	**P[2]**
late gest	waterhole	>	fawning	waterhole	0.01
late gest	waterhole	>	fawning	browse	0.03
late gest	waterhole	>	fawning	mortality	0.03
late gest	bed	<	fawning	bed	0.02
late gest	grain source	>	fawning	waterhole	0.01
late gest	grain source	>	fawning	browse	0.03
late gest	grain source	>	fawning	mortality	0.04
fawning	saltlick	>	fawning	waterhole	0.02
fawning	saltlick	>	fawning	browse	0.05
fawning	waterhole	<	fawning	bed	0.002
fawning	bed	>	fawning	browse	0.003
fawning	bed	>	fawning	mortality	0.01

[1] Relationship indicates that the predicted number of pictures with adult mule deer in the season and site combination from the left is larger (>) or smaller (<) than the one in the season and site combination from the right. [2] Significant difference was considered if $p<0.05$.

Table A6.3.4 and Fig. A6.3.1. Predicted number of pictures with adult mule deer per camera-day for each season and site type combination.

Site type	Pre-rut	Rut	Early gestation	Late gestation	Fawning
Salt lick	1.98	0.90	0.38	7.11	4.99
Waterhole	1.35	1.53	0.75	11.15	0.95
Rub	2.36	2.41	2.19		
Bed	3.19	1.70	2.55	1.64	5.20
Browse	1.71	1.83	3.19	2.74	1.54
Grain source	2.29	3.39	12.50	7.42	7×10^{-4}
Trail	1.87	1.63	2.27	4.07	2.39
Mortality	0.25	1.80	3.71	3.04	1.39



6.13 APPENDIX 6.4.

Appendix 6.4. Summary of results from the model with counts of pictures with mule deer fawns.

To assess the effect of season and site type on the frequency of visitation of mule deer we fit a generalized linear mixed model using the GLIMMIX procedure in SAS v9.3. The outcome variable was the frequency with which different sex-and-age classes visited sites measured as the number of pictures taken, the offset was the camera-days, the distribution was negative binomial, the link function was log, and the random intercept was station. Model building was completed using manual forward selection, with a screening criterion of $p \leq 0.2$ for the initial univariable analysis, and $p < 0.05$ as the criterion for significance in the multivariable analysis. When building the multivariable model for each outcome, if one covariate changed the regression coefficient or effect of the other by 10% or more, then we considered it as a confounder and kept it in the model. We examined the potential for interaction between season and site type where both were significant as fixed effects in the model. The interaction term was retained in the final model if $p<0.05$.

There was a significant interaction between site type and season in this model, such that the extent of differences between site types in the counts of pictures with mule deer fawns varied by season.

The results of this model include:

- Table with the solutions of fixed effects.
- Least squares means (LSM) table. It indicates the estimated number of pictures per camera day, by season and site combinations, and their lower and upper 95% confidence levels.
- Table with the significant pair-wise comparisons of the differences between seasons and site type combinations. The signs < and > help to identify which values (as shown in the LSM table) were significantly greater than others.
- Table and graph of the predicted number of pictures with mule deer fawns per camera-day for each season and site type combination.

Table A.6.4.1. Solutions for Fixed Effects - Fawn model

Effect	Season	Site	Estimate[1]	SE	DF	P	Lower[1]	Upper[1]
Intercept			-27.22	1.37	276	<.0001	-29.92	-24.52
season	pre-rut		26.70	1.58	56	<.0001	23.54	29.86
season	rut		27.38	1.51	56	<.0001	24.35	30.42
season	early gest		28.62	1.43	56	<.0001	25.75	31.48
season	late gest		27.68	1.21	56	<.0001	25.26	30.10
season	fawning		0	.	.	.	.	.
site		salt lick	25.40	1.75	56	<.0001	21.90	28.90
site		waterhole	23.28	1.85	56	<.0001	19.57	26.99
site		rub	-2.81	1.20	56	0.02	-5.21	-0.41
site		bed	27.40	1.44	56	<.0001	24.51	30.29
site		browse	23.17	1.63	56	<.0001	19.91	26.42
site		grain source	-3.81	1.43	56	0.01	-6.67	-0.95
site		trail	25.06	1.28	56	<.0001	22.51	27.62
site		mortality	0	.	.	.	.	.
season*site	pre-rut	salt lick	-26.23	2.06	56	<.0001	-30.36	-22.09
season*site	pre-rut	waterhole	-23.10	2.07	56	<.0001	-27.24	-18.96
season*site	pre-rut	rub	3.22	1.57	56	0.05	0.07	6.37
season*site	pre-rut	bed	-24.98	1.69	56	<.0001	-28.37	-21.58
season*site	pre-rut	browse	-23.58	1.85	56	<.0001	-27.29	-19.87
season*site	pre-rut	grain source	5.57	1.86	56	0.004	1.85	9.29
season*site	pre-rut	trail	-24.26	1.54	56	<.0001	-27.35	-21.17
season*site	pre-rut	mortality	0	.	.	.	.	.
season*site	rut	salt lick	-26.49	2.19	56	<.0001	-30.88	-22.11
season*site	rut	waterhole	-24.35	2.23	56	<.0001	-28.81	-19.88
season*site	rut	rub	3.54	1.45	56	0.02	0.64	6.44
season*site	rut	bed	-26.60	1.67	56	<.0001	-29.95	-23.26
season*site	rut	browse	-24.08	1.83	56	<.0001	-27.74	-20.42
season*site	rut	grain source	5.00	1.71	56	0.005	1.58	8.42
season*site	rut	trail	-25.04	1.52	56	<.0001	-28.09	-21.98
season*site	rut	mortality	0	.	.	.	.	.
season*site	early gest	salt lick	-34.25	18.79	56	0.07	-71.88	3.38
season*site	early gest	waterhole	-24.45	2.32	56	<.0001	-29.09	-19.81
season*site	early gest	rub	0	.	.	.	.	.
season*site	early gest	bed	-27.14	1.58	56	<.0001	-30.31	-23.98
season*site	early gest	browse	-24.73	1.74	56	<.0001	-28.21	-21.26
season*site	early gest	grain source	3.71	1.54	56	0.02	0.63	6.79

Effect	Season	Site	Estimate[1]	SE	DF	P	Lower[1]	Upper[1]
season*site	early gest	trail	-26.26	1.42	56	<.0001	-29.11	-23.41
season*site	early gest	mortality	0	.	.	.	.	.
season*site	late gest	salt lick	-23.29	2.39	56	<.0001	-28.09	-18.50
season*site	late gest	waterhole	-21.67	2.16	56	<.0001	-25.99	-17.34
season*site	late gest	bed	-25.88	1.48	56	<.0001	-28.85	-22.92
season*site	late gest	browse	-23.97	1.62	56	<.0001	-27.21	-20.73
season*site	late gest	grain source	5.04	0	56	<.0001	.	.
season*site	late gest	trail	-26.17	0	56	<.0001	.	.
season*site	late gest	mortality	0	.	.	.	.	.
season*site	fawning	salt lick	0	.	.	.	.	.
season*site	fawning	waterhole	0	.	.	.	.	.
season*site	fawning	bed	0	.	.	.	.	.
season*site	fawning	browse	0	.	.	.	.	.
season*site	fawning	grain source	0	.	.	.	.	.
season*site	fawning	trail	0	.	.	.	.	.
season*site	fawning	mortality	0	.	.	.	.	.

SE = standard error; DF = degrees of freedom; early gest = early gestation; late gest = late gestation. [1]Units are log(pics/CD) where pics/CD is the number of pictures per camera-day; Lower and Upper are the confidence limits on the mean (probability) scale.

Table A.6.4.2. Season*site least squares means - Fawn model

Season	Site	Estimate[1]	Lower[1]	Upper[1]
pre-rut	salt lick	0.26	0.06	1.22
pre-rut	waterhole	0.71	0.27	1.89
pre-rut	rub	0.89	0.24	3.32
pre-rut	bed	6.70	2.87	15.66
pre-rut	browse	0.39	0.17	0.92
pre-rut	grain source	3.45	0.57	20.80
pre-rut	trail	1.32	0.63	2.80
pre-rut	mortality	0.59	0.12	2.84
rut	salt lick	0.39	0.04	3.94
rut	waterhole	0.40	0.05	3.40
rut	rub	2.43	0.90	6.59
rut	bed	2.60	0.87	7.79
rut	browse	0.47	0.16	1.36
rut	grain source	3.87	0.98	15.28
rut	trail	1.21	0.41	3.53
rut	mortality	1.17	0.32	4.25
early gest	salt lick	$6x10^{-4}$	$3x10^{-20}$	$1x10^{13}$

Season	Site	Estimate[1]	Lower[1]	Upper[1]
early gest	waterhole	1.26	0.09	18.17
early gest	rub	0.24	0.03	2.32
early gest	bed	5.19	1.90	14.16
early gest	browse	0.84	0.33	2.11
early gest	grain source	3.66	1.60	8.36
early gest	trail	1.22	0.46	3.23
early gest	mortality	4.02	1.81	8.95
late gest	salt lick	13.00	0.38	443.63
late gest	waterhole	7.95	0.61	104.60
late gest	bed	7.19	1.65	31.25
late gest	browse	0.71	0.20	2.53
late gest	grain source	5.43	0.43	68.89
late gest	trail	0.52	0.06	4.67
late gest	mortality	1.58	0.43	5.83
fawning	salt lick	0.16	0.02	1.42
fawning	waterhole	0.02	0.00	0.24
fawning	bed	1.19	0.49	2.91
fawning	browse	0.02	3E-03	0.10
fawning	grain source	$3x10^{-14}$	$1x10^{-15}$	$1x10^{-12}$
fawning	trail	0.12	0.04	0.32
fawning	mortality	$2x10^{-12}$	$1x10^{-13}$	$2x10^{-11}$

early gest = early gestation; late gest = late gestation. [1]Units are pics/CD, the number of pictures per camera-day; Lower and Upper are the confidence limits on the mean (probability) scale.

Table A.6.4.3. Differences of significant season*site least squares means - Fawn model

Season	Site	Relationship[1]	Season	Site	P[2]
pre-rut	salt lick	<	pre-rut	bed	0.001
pre-rut	salt lick	<	pre-rut	grain source	0.03
pre-rut	salt lick	<	rut	rub	0.02
pre-rut	salt lick	<	rut	bed	0.02
pre-rut	salt lick	<	rut	grain source	0.01
pre-rut	salt lick	<	early gest	bed	0.002
pre-rut	salt lick	<	early gest	grain source	0.004
pre-rut	salt lick	<	early gest	mortality	0.003
pre-rut	salt lick	<	late gest	salt lick	0.05
pre-rut	salt lick	<	late gest	waterhole	0.03
pre-rut	salt lick	<	late gest	bed	0.003
pre-rut	salt lick	<	late gest	grain source	0.05
pre-rut	salt lick	>	fawning	browse	0.02
pre-rut	salt lick	>	fawning	grain source	<.0001
pre-rut	salt lick	>	fawning	mortality	<.0001

Season	Site	Relationship[1]	Season	Site	P[2]
pre-rut	waterhole	<	pre-rut	bed	0.001
pre-rut	waterhole	<	rut	grain source	0.05
pre-rut	waterhole	<	early gest	bed	0.01
pre-rut	waterhole	<	early gest	grain source	0.01
pre-rut	waterhole	<	early gest	mortality	0.01
pre-rut	waterhole	<	late gest	bed	0.01
pre-rut	waterhole	>	fawning	waterhole	0.01
pre-rut	waterhole	>	fawning	browse	0.0004
pre-rut	waterhole	>	fawning	grain source	<.0001
pre-rut	waterhole	>	fawning	trail	0.01
pre-rut	waterhole	>	fawning	mortality	<.0001
pre-rut	rub	<	pre-rut	bed	0.01
pre-rut	rub	<	early gest	bed	0.04
pre-rut	rub	<	late gest	bed	0.04
pre-rut	rub	>	fawning	waterhole	0.01
pre-rut	rub	>	fawning	browse	0.001
pre-rut	rub	>	fawning	grain source	<.0001
pre-rut	rub	>	fawning	trail	0.02
pre-rut	rub	>	fawning	mortality	<.0001
pre-rut	bed	>	pre-rut	browse	<.0001
pre-rut	bed	>	pre-rut	trail	0.01
pre-rut	bed	>	pre-rut	mortality	0.01
pre-rut	bed	>	rut	salt lick	0.02
pre-rut	bed	>	rut	waterhole	0.02
pre-rut	bed	>	rut	browse	0.0003
pre-rut	bed	>	rut	trail	0.02
pre-rut	bed	>	rut	mortality	0.03
pre-rut	bed	>	early gest	rub	0.01
pre-rut	bed	>	early gest	browse	0.002
pre-rut	bed	>	early gest	trail	0.01
pre-rut	bed	>	late gest	browse	0.00
pre-rut	bed	>	late gest	trail	0.03
pre-rut	bed	>	fawning	salt lick	0.002
pre-rut	bed	>	fawning	waterhole	<.0001
pre-rut	bed	>	fawning	bed	0.01
pre-rut	bed	>	fawning	browse	<.0001
pre-rut	bed	>	fawning	grain source	<.0001
pre-rut	bed	>	fawning	trail	<.0001
pre-rut	bed	>	fawning	mortality	<.0001
pre-rut	browse	<	pre-rut	grain source	0.03
pre-rut	browse	<	pre-rut	trail	0.04
pre-rut	browse	<	rut	rub	0.01

Season	**Site**	**Relationship**[1]	**Season**	**Site**	**P**[2]
pre-rut	browse	<	rut	bed	0.01
pre-rut	browse	<	rut	grain source	0.01
pre-rut	browse	<	early gest	bed	0.0002
pre-rut	browse	<	early gest	grain source	0.0004
pre-rut	browse	<	early gest	mortality	0.0002
pre-rut	browse	<	late gest	waterhole	0.03
pre-rut	browse	<	late gest	bed	0.001
pre-rut	browse	>	fawning	waterhole	0.03
pre-rut	browse	>	fawning	browse	0.002
pre-rut	browse	>	fawning	grain source	<.0001
pre-rut	browse	>	fawning	mortality	<.0001
pre-rut	grain source	>	fawning	salt lick	0.03
pre-rut	grain source	>	fawning	waterhole	0.001
pre-rut	grain source	>	fawning	browse	<.0001
pre-rut	grain source	>	fawning	grain source	<.0001
pre-rut	grain source	>	fawning	trail	0.002
pre-rut	grain source	>	fawning	mortality	<.0001
pre-rut	trail	<	early gest	bed	0.03
pre-rut	trail	<	early gest	mortality	0.05
pre-rut	trail	<	late gest	bed	0.04
pre-rut	trail	>	fawning	waterhole	0.002
pre-rut	trail	>	fawning	browse	<.0001
pre-rut	trail	>	fawning	grain source	<.0001
pre-rut	trail	>	fawning	trail	0.0003
pre-rut	trail	>	fawning	mortality	<.0001
pre-rut	mortality	<	early gest	bed	0.02
pre-rut	mortality	<	early gest	grain source	0.04
pre-rut	mortality	<	early gest	mortality	0.03
pre-rut	mortality	<	late gest	bed	0.02
pre-rut	mortality	>	fawning	waterhole	0.02
pre-rut	mortality	>	fawning	browse	0.004
pre-rut	mortality	>	fawning	grain source	<.0001
pre-rut	mortality	>	fawning	mortality	<.0001
rut	salt lick	<	early gest	bed	0.04
rut	salt lick	<	late gest	bed	0.04
rut	salt lick	>	fawning	browse	0.03
rut	salt lick	>	fawning	grain source	<.0001
rut	salt lick	>	fawning	mortality	<.0001
rut	waterhole	<	early gest	bed	0.03
rut	waterhole	<	early gest	mortality	0.05
rut	waterhole	<	late gest	bed	0.03
rut	waterhole	>	fawning	browse	0.03

Season	Site	Relationship[1]	Season	Site	P[2]
rut	waterhole	>	fawning	grain source	<.0001
rut	waterhole	>	fawning	mortality	<.0001
rut	rub	>	rut	browse	0.03
rut	rub	>	fawning	salt lick	0.03
rut	rub	>	fawning	waterhole	0.001
rut	rub	>	fawning	browse	<.0001
rut	rub	>	fawning	grain source	<.0001
rut	rub	>	fawning	trail	<.0001
rut	rub	>	fawning	mortality	<.0001
rut	bed	>	rut	browse	0.03
rut	bed	>	fawning	salt lick	0.03
rut	bed	>	fawning	waterhole	0.001
rut	bed	>	fawning	browse	<.0001
rut	bed	>	fawning	grain source	<.0001
rut	bed	>	fawning	trail	0.0001
rut	bed	>	fawning	mortality	<.0001
rut	browse	<	rut	grain source	0.02
rut	browse	<	early gest	bed	0.002
rut	browse	<	early gest	grain source	0.004
rut	browse	<	early gest	mortality	0.002
rut	browse	<	late gest	waterhole	0.05
rut	browse	<	late gest	bed	0.004
rut	browse	>	fawning	waterhole	0.02
rut	browse	>	fawning	browse	0.002
rut	browse	>	fawning	grain source	<.0001
rut	browse	>	fawning	mortality	<.0001
rut	grain source	>	early gest	rub	0.04
rut	grain source	>	fawning	salt lick	0.02
rut	grain source	>	fawning	waterhole	0.001
rut	grain source	>	fawning	browse	<.0001
rut	grain source	>	fawning	grain source	<.0001
rut	grain source	>	fawning	trail	0.0001
rut	grain source	>	fawning	mortality	<.0001
rut	trail	>	fawning	waterhole	0.004
rut	trail	>	fawning	browse	0.0001
rut	trail	>	fawning	grain source	<.0001
rut	trail	>	fawning	trail	0.002
rut	trail	>	fawning	mortality	<.0001
rut	mortality	>	fawning	waterhole	0.005
rut	mortality	>	fawning	browse	0.0003
rut	mortality	>	fawning	grain source	<.0001
rut	mortality	>	fawning	trail	0.006

Season	Site	Relationship[1]	Season	Site	P[2]
rut	mortality	>	fawning	mortality	<.0001
early gest	waterhole	>	fawning	waterhole	0.03
early gest	waterhole	>	fawning	browse	0.01
early gest	waterhole	>	fawning	grain source	<.0001
early gest	waterhole	>	fawning	mortality	<.0001
early gest	rub	<	early gest	bed	0.02
early gest	rub	<	early gest	grain source	0.03
early gest	rub	<	early gest	mortality	0.02
early gest	rub	<	late gest	waterhole	0.05
early gest	rub	<	late gest	bed	0.01
early gest	rub	>	fawning	grain source	<.0001
early gest	rub	>	fawning	mortality	<.0001
early gest	bed	>	early gest	browse	0.01
early gest	bed	>	early gest	trail	0.04
early gest	bed	>	late gest	browse	0.02
early gest	bed	>	fawning	salt lick	0.01
early gest	bed	>	fawning	waterhole	0.0001
early gest	bed	>	fawning	bed	0.03
early gest	bed	>	fawning	browse	<.0001
early gest	bed	>	fawning	grain source	<.0001
early gest	bed	>	fawning	trail	<.0001
early gest	bed	>	fawning	mortality	<.0001
early gest	browse	<	early gest	grain source	0.02
early gest	browse	<	early gest	mortality	0.01
early gest	browse	<	late gest	bed	0.02
early gest	browse	>	fawning	waterhole	0.01
early gest	browse	>	fawning	browse	0.0002
early gest	browse	>	fawning	grain source	<.0001
early gest	browse	>	fawning	trail	0.01
early gest	browse	>	fawning	mortality	<.0001
early gest	grain source	>	late gest	browse	0.03
early gest	grain source	>	fawning	salt lick	0.01
early gest	grain source	>	fawning	waterhole	0.0002
early gest	grain source	>	fawning	browse	<.0001
early gest	grain source	>	fawning	grain source	<.0001
early gest	grain source	>	fawning	trail	<.0001
early gest	grain source	>	fawning	mortality	<.0001
early gest	trail	<	late gest	bed	0.05
early gest	trail	>	fawning	waterhole	0.003
early gest	trail	>	fawning	browse	<.0001
early gest	trail	>	fawning	grain source	<.0001
early gest	trail	>	fawning	trail	0.001

Season	Site	Relationship[1]	Season	Site	P[2]
early gest	trail	>	fawning	mortality	<.0001
early gest	mortality	>	late gest	browse	0.02
early gest	mortality	>	fawning	salt lick	0.01
early gest	mortality	>	fawning	waterhole	0.0001
early gest	mortality	>	fawning	bed	0.05
early gest	mortality	>	fawning	browse	<.0001
early gest	mortality	>	fawning	grain source	<.0001
early gest	mortality	>	fawning	trail	<.0001
early gest	mortality	>	fawning	mortality	<.0001
late gest	salt lick	>	fawning	salt lick	0.04
late gest	salt lick	>	fawning	waterhole	0.004
late gest	salt lick	>	fawning	browse	0.001
late gest	salt lick	>	fawning	grain source	<.0001
late gest	salt lick	>	fawning	trail	0.01
late gest	salt lick	>	fawning	mortality	<.0001
late gest	waterhole	>	fawning	salt lick	0.02
late gest	waterhole	>	fawning	waterhole	0.001
late gest	waterhole	>	fawning	browse	0.0002
late gest	waterhole	>	fawning	grain source	<.0001
late gest	waterhole	>	fawning	trail	0.003
late gest	waterhole	>	fawning	mortality	<.0001
late gest	bed	>	late gest	browse	0.02
late gest	bed	>	fawning	salt lick	0.01
late gest	bed	>	fawning	waterhole	0.0001
late gest	bed	>	fawning	bed	0.04
late gest	bed	>	fawning	browse	<.0001
late gest	bed	>	fawning	grain source	<.0001
late gest	bed	>	fawning	trail	<.0001
late gest	bed	>	fawning	mortality	<.0001
late gest	browse	>	fawning	waterhole	0.01
late gest	browse	>	fawning	browse	0.001
late gest	browse	>	fawning	grain source	<.0001
late gest	browse	>	fawning	trail	0.03
late gest	browse	>	fawning	mortality	<.0001
late gest	grain source	>	fawning	salt lick	0.04
late gest	grain source	>	fawning	waterhole	0.003
late gest	grain source	>	fawning	browse	0.0004
late gest	grain source	>	fawning	grain source	<.0001
late gest	grain source	>	fawning	trail	0.01
late gest	grain source	>	fawning	mortality	<.0001
late gest	trail	>	fawning	browse	0.02
late gest	trail	>	fawning	grain source	<.0001

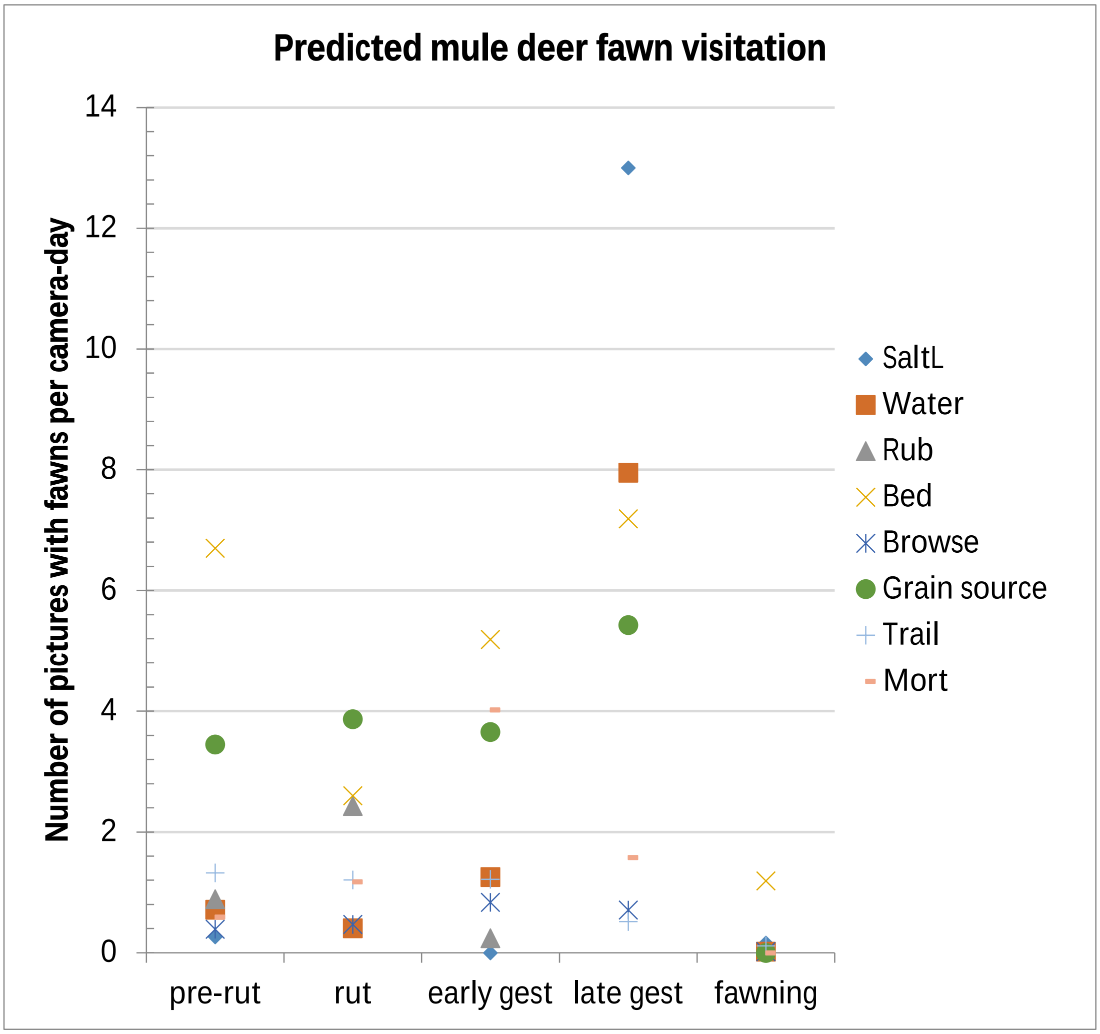
Season	**Site**	**Relationship**[1]	**Season**	**Site**	**P**[2]
late gest	trail	>	fawning	mortality	<.0001
late gest	mortality	>	fawning	waterhole	0.003
late gest	mortality	>	fawning	browse	0.0001
late gest	mortality	>	fawning	grain source	<.0001
late gest	mortality	>	fawning	trail	0.003
late gest	mortality	>	fawning	mortality	<.0001
fawning	salt lick	>	fawning	grain source	<.0001
fawning	salt lick	>	fawning	mortality	<.0001
fawning	waterhole	<	fawning	bed	0.003
fawning	waterhole	>	fawning	grain source	<.0001
fawning	waterhole	>	fawning	mortality	<.0001
fawning	bed	>	fawning	browse	<.0001
fawning	bed	>	fawning	grain source	<.0001
fawning	bed	>	fawning	trail	0.001
fawning	bed	>	fawning	mortality	<.0001
fawning	browse	>	fawning	grain source	<.0001
fawning	browse	>	fawning	mortality	<.0001
fawning	grain source	<	fawning	trail	<.0001
fawning	grain source	<	fawning	mortality	0.01
fawning	trail	>	fawning	mortality	<.0001

[1] Relationship indicates that the predicted number of pictures with mule deer fawns in the season and site combination from the left is larger (>) or smaller (<) than the one in the right.

[2] Significant difference was considered if $p<0.05$.

Table A.6.4.4 and Fig. A.6.4.1. Predicted number of pictures with mule deer fawns per camera-day for each season and site type combination.

Site type	Pre-rut	Rut	Early gestation	Late gestation	Fawning
Salt lick	0.26	0.39	0.00	13.00	0.16
Waterhole	0.71	0.40	1.26	7.95	0.02
Rub	0.89	2.43	0.24		
Bed	6.70	2.60	5.19	7.19	1.19
Browse	0.39	0.47	0.84	0.71	0.02
Grain source	3.45	3.87	3.66	5.43	$3x10^{-14}$
Trail	1.32	1.21	1.22	0.52	0.12
Mortality	0.59	1.17	4.02	1.58	$1.5x10^{-12}$



6.14 APPENDIX 6.5.

Appendix 6.5. Summary of results from the model with counts of pictures with mule deer.

To assess the effect of season and site type on the frequency of visitation of mule deer we fit a generalized linear mixed model using the GLIMMIX procedure in SAS v9.3. The outcome variable was the frequency with which different sex-and-age classes visited sites measured as the number of pictures taken, the offset was the camera-days, the distribution was negative binomial, the link function was log, and the random intercept was station. Model building was completed using manual forward selection, with a screening criterion of $p \leq 0.2$ for the initial univariable analysis, and $p < 0.05$ as the criterion for significance in the multivariable analysis. When building the multivariable model for each outcome, if one covariate changed the regression coefficient or effect of the other by 10% or more, then we considered it as a confounder and kept it in the model. We examined the potential for interaction between season and site type where both were significant as fixed effects in the model. The interaction term was retained in the final model if $p<0.05$.

There was a significant interaction between site type and season in this model, such that the extent of differences between site types in the counts of pictures with mule deer varied by season.

The results of this model include:

- Table with the solutions of fixed effects.
- Least squares means (LSM) table. It indicates the estimated number of pictures per camera day, by season and site combinations, and their lower and upper 95% confidence levels.
- Table with the significant pair-wise comparisons of the differences between seasons and site type combinations. The signs < and > help to identify which values (as shown in the LSM table) were significantly greater than others.
- Table and graph of the predicted number of pictures with mule deer per camera-day for each season and site type combination.

Table A6.5.1. Solutions for Fixed Effects - All mule deer model

Effect	Season	Site	Estimate[1]	SE	DF	P	Lower[1]	Upper[1]
Intercept			1.45	0.29	276	<.0001	0.88	2.02
season	pre-rut		-0.18	0.43	56	0.67	-1.05	0.68
season	rut		0.32	0.39	56	0.41	-0.46	1.10
season	early gest		0.80	0.33	56	0.02	0.13	1.47
season	late gest		0.30	0.37	56	0.41	-0.43	1.04
season	fawning		0	.	.	.	.	.
site		salt lick	0.53	0.46	56	0.25	-0.38	1.45
site		waterhole	-0.40	0.42	56	0.34	-1.23	0.43
site		rub	0.33	0.46	56	0.47	-0.59	1.25
site		bed	1.04	0.34	56	0.004	0.35	1.73
site		browse	0.03	0.35	56	0.93	-0.66	0.73
site		grain source	-0.67	0.76	56	0.38	-2.20	0.86
site		trail	0.31	0.35	56	0.37	-0.38	1.01
site		mortality	0	.	.	.	.	.
season*site	pre-rut	salt lick	-0.11	0.60	56	0.85	-1.31	1.09
season*site	pre-rut	waterhole	0.88	0.56	56	0.12	-0.24	2.00
season*site	pre-rut	rub	0.47	0.61	56	0.44	-0.76	1.70
season*site	pre-rut	bed	0.36	0.50	56	0.48	-0.64	1.35
season*site	pre-rut	browse	0.23	0.50	56	0.65	-0.77	1.22
season*site	pre-rut	grain source	2.38	0.90	56	0.01	0.58	4.19
season*site	pre-rut	trail	0.23	0.49	56	0.64	-0.75	1.21
season*site	pre-rut	mortality	0	.	.	.	.	.
season*site	rut	salt lick	-1.58	0.72	56	0.03	-3.03	-0.14
season*site	rut	waterhole	0.56	0.63	56	0.38	-0.71	1.83
season*site	rut	rub	-0.01	0.52	56	0.99	-1.05	1.04
season*site	rut	bed	-0.78	0.49	56	0.12	-1.76	0.20
season*site	rut	browse	0.15	0.48	56	0.75	-0.82	1.12
season*site	rut	grain source	1.27	0.85	56	0.14	-0.44	2.98
season*site	rut	trail	-0.39	0.49	56	0.42	-1.36	0.58
season*site	rut	mortality	0	.	.	.	.	.
season*site	early gest	salt lick	-1.14	0.76	56	0.14	-2.67	0.38
season*site	early gest	waterhole	-0.41	0.73	56	0.58	-1.88	1.06
season*site	early gest	rub	0	.	.	.	.	.

Effect	Season	Site	Estimate^1	SE	DF	P	Lower^1	Upper^1
season*site	early gest	bed	-1.08	0.44	56	0.02	-1.96	-0.21
season*site	early gest	browse	0.01	0.43	56	0.98	-0.85	0.87
season*site	early gest	grain source	1.42	0.77	56	0.07	-0.13	2.97
season*site	early gest	trail	-0.93	0.44	56	0.04	-1.80	-0.05
season*site	early gest	mortality	0	.	.	.	.	.
season*site	late gest	salt lick	0.39	0.76	56	0.62	-1.14	1.92
season*site	late gest	waterhole	1.12	0.66	56	0.10	-0.21	2.45
season*site	late gest	bed	0.34	0.48	56	0.48	-0.62	1.29
season*site	late gest	browse	0.04	0.48	56	0.93	-0.92	1.00
season*site	late gest	grain source	2.29	0.88	56	0.01	0.53	4.04
season*site	late gest	trail	-0.65	0.58	56	0.26	-1.80	0.50
season*site	late gest	mortality	0	.	.	.	.	.
season*site	fawning	salt lick	0	.	.	.	.	.
season*site	fawning	waterhole	0	.	.	.	.	.
season*site	fawning	bed	0	.	.	.	.	.
season*site	fawning	browse	0	.	.	.	.	.
season*site	fawning	grain source	0	.	.	.	.	.
season*site	fawning	trail	0	.	.	.	.	.
season*site	fawning	mortality	0	.	.	.	.	.

SE = standard error; DF = degrees of freedom; early gest = early gestation; late gest = late gestation. ^{1}Units are log(pics/CD) where pics/CD is the number of pictures per camera-day; Lower and Upper are the confidence limits on the mean (probability) scale.

Table A6.5.2. Season*site least squares means - All mule deer model

Season	Site	Estimate^1	Lower^1	Upper^1
pre-rut	salt lick	5.40	2.91	10.03
pre-rut	waterhole	5.72	3.81	8.60
pre-rut	rub	7.91	4.68	13.38
pre-rut	bed	14.25	9.84	20.65
pre-rut	browse	4.59	3.21	6.56

Season	Site	Estimate[1]	Lower[1]	Upper[1]
pre-rut	grain source	19.65	9.42	40.98
pre-rut	trail	6.11	4.45	8.40
pre-rut	mortality	3.54	1.86	6.73
rut	salt lick	2.06	0.75	5.60
rut	waterhole	6.87	3.04	15.52
rut	rub	8.12	5.33	12.38
rut	bed	7.60	4.73	12.20
rut	browse	7.05	4.57	10.88
rut	grain source	10.70	6.07	18.85
rut	trail	5.42	3.46	8.49
rut	mortality	5.87	3.49	9.86
early gest	salt lick	5.14	1.57	16.79
early gest	waterhole	4.22	1.32	13.44
early gest	rub	13.18	5.61	31.00
early gest	bed	9.03	5.81	14.02
early gest	browse	9.86	6.68	14.55
early gest	grain source	20.14	14.01	28.94
early gest	trail	5.14	3.35	7.88
early gest	mortality	9.46	6.67	13.42
late gest	salt lick	14.45	3.68	56.64
late gest	waterhole	11.87	4.13	34.10
late gest	bed	22.73	12.84	40.25
late gest	browse	6.20	3.69	10.41
late gest	grain source	28.98	10.85	77.42
late gest	trail	4.11	1.72	9.80
late gest	mortality	5.76	3.32	9.98
fawning	salt lick	7.25	3.57	14.75
fawning	waterhole	2.85	1.57	5.20
fawning	bed	11.99	8.27	17.40
fawning	browse	4.39	3.00	6.43
fawning	grain source	2.18	0.53	8.96
fawning	trail	5.82	3.99	8.50
fawning	mortality	4.25	2.38	7.60

early gest = early gestation; late gest = late gestation. [1]Units are pics/CD, the number of pictures per camera-day; Lower and Upper are the confidence limits on the mean (probability) scale.

Table A6.5.3. Differences of significant season*site least squares means - All mule deer model

Season	Site	Relationship[1]	Season	Site	P[2]
pre-rut	salt lick	<	pre-rut	bed	0.01
pre-rut	salt lick	<	pre-rut	grain source	0.01
pre-rut	salt lick	<	early gest	grain source	0.001
pre-rut	salt lick	<	late gest	bed	0.001
pre-rut	salt lick	<	late gest	grain source	0.01
pre-rut	salt lick	<	fawning	bed	0.03
pre-rut	waterhole	<	pre-rut	bed	0.002
pre-rut	waterhole	<	pre-rut	grain source	0.005
pre-rut	waterhole	<	early gest	grain source	<.0001
pre-rut	waterhole	<	late gest	bed	0.0002
pre-rut	waterhole	<	late gest	grain source	0.003
pre-rut	waterhole	<	fawning	bed	0.01
pre-rut	rub	<	pre-rut	grain source	0.05
pre-rut	rub	>	rut	salt lick	0.02
pre-rut	rub	<	early gest	grain source	0.005
pre-rut	rub	<	late gest	bed	0.01
pre-rut	rub	<	late gest	grain source	0.02
pre-rut	rub	>	fawning	waterhole	0.01
pre-rut	bed	>	pre-rut	browse	<.0001
pre-rut	bed	>	pre-rut	trail	0.001
pre-rut	bed	>	pre-rut	mortality	0.0004
pre-rut	bed	>	rut	salt lick	0.001
pre-rut	bed	>	rut	rub	0.05
pre-rut	bed	>	rut	bed	0.04
pre-rut	bed	>	rut	browse	0.02
pre-rut	bed	>	rut	trail	0.002
pre-rut	bed	>	rut	mortality	0.01
pre-rut	bed	>	early gest	waterhole	0.05
pre-rut	bed	>	early gest	trail	0.001
pre-rut	bed	>	late gest	browse	0.01
pre-rut	bed	>	late gest	trail	0.01
pre-rut	bed	>	late gest	mortality	0.01
pre-rut	bed	>	fawning	waterhole	<.0001
pre-rut	bed	>	fawning	browse	<.0001
pre-rut	bed	>	fawning	grain source	0.01
pre-rut	bed	>	fawning	trail	0.001
pre-rut	bed	>	fawning	mortality	0.001
pre-rut	browse	<	pre-rut	grain source	0.001
pre-rut	browse	<	rut	rub	0.04

Season	Site	Relationship[1]	Season	Site	P[2]
pre-rut	browse	<	rut	grain source	0.01
pre-rut	browse	<	early gest	rub	0.03
pre-rut	browse	<	early gest	bed	0.02
pre-rut	browse	<	early gest	browse	0.005
pre-rut	browse	<	early gest	grain source	<.0001
pre-rut	browse	<	early gest	mortality	0.005
pre-rut	browse	<	late gest	bed	<.0001
pre-rut	browse	<	late gest	grain source	0.001
pre-rut	browse	<	fawning	bed	0.0004
pre-rut	grain source	>	pre-rut	trail	0.005
pre-rut	grain source	>	pre-rut	mortality	0.001
pre-rut	grain source	>	rut	salt lick	0.001
pre-rut	grain source	>	rut	rub	0.04
pre-rut	grain source	>	rut	bed	0.03
pre-rut	grain source	>	rut	browse	0.02
pre-rut	grain source	>	rut	trail	0.004
pre-rut	grain source	>	rut	mortality	0.01
pre-rut	grain source	>	early gest	waterhole	0.03
pre-rut	grain source	>	early gest	trail	0.002
pre-rut	grain source	>	late gest	browse	0.01
pre-rut	grain source	>	late gest	trail	0.01
pre-rut	grain source	>	late gest	mortality	0.01
pre-rut	grain source	>	fawning	waterhole	0.0001
pre-rut	grain source	>	fawning	browse	0.001
pre-rut	grain source	>	fawning	grain source	0.01
pre-rut	grain source	>	fawning	trail	0.005
pre-rut	grain source	>	fawning	mortality	0.002
pre-rut	trail	>	rut	salt lick	0.04
pre-rut	trail	<	early gest	grain source	<.0001
pre-rut	trail	<	late gest	bed	0.0002
pre-rut	trail	<	late gest	grain source	0.004
pre-rut	trail	>	fawning	waterhole	0.03
pre-rut	trail	<	fawning	bed	0.01
pre-rut	mortality	<	rut	rub	0.03
pre-rut	mortality	<	rut	grain source	0.01
pre-rut	mortality	<	early gest	rub	0.02
pre-rut	mortality	<	early gest	bed	0.02
pre-rut	mortality	<	early gest	browse	0.01
pre-rut	mortality	<	early gest	grain source	<.0001
pre-rut	mortality	<	early gest	mortality	0.01
pre-rut	mortality	<	late gest	bed	<.0001
pre-rut	mortality	<	late gest	grain source	0.001

Season	**Site**	**Relationship[1]**	**Season**	**Site**	**P[2]**
pre-rut	mortality	<	fawning	bed	0.002
rut	salt lick	<	rut	rub	0.01
rut	salt lick	<	rut	bed	0.02
rut	salt lick	<	rut	browse	0.03
rut	salt lick	<	rut	grain source	0.01
rut	salt lick	<	early gest	rub	0.01
rut	salt lick	<	early gest	bed	0.01
rut	salt lick	<	early gest	browse	0.01
rut	salt lick	<	early gest	grain source	<.0001
rut	salt lick	<	early gest	mortality	0.01
rut	salt lick	<	late gest	salt lick	0.02
rut	salt lick	<	late gest	waterhole	0.02
rut	salt lick	<	late gest	bed	0.0001
rut	salt lick	<	late gest	grain source	0.0004
rut	salt lick	<	fawning	salt lick	0.04
rut	salt lick	<	fawning	bed	0.002
rut	waterhole	<	early gest	grain source	0.02
rut	waterhole	<	late gest	bed	0.02
rut	waterhole	<	late gest	grain source	0.03
rut	rub	<	early gest	grain source	0.002
rut	rub	<	late gest	bed	0.005
rut	rub	<	late gest	grain source	0.02
rut	rub	>	fawning	waterhole	0.01
rut	rub	>	fawning	browse	0.03
rut	bed	<	early gest	grain source	0.002
rut	bed	<	late gest	bed	0.004
rut	bed	<	late gest	grain source	0.02
rut	bed	>	fawning	waterhole	0.01
rut	browse	<	early gest	grain source	0.001
rut	browse	<	late gest	bed	0.002
rut	browse	<	late gest	grain source	0.01
rut	browse	>	fawning	waterhole	0.02
rut	grain source	>	early gest	trail	0.04
rut	grain source	>	fawning	waterhole	0.002
rut	grain source	>	fawning	browse	0.01
rut	grain source	>	fawning	grain source	0.04
rut	grain source	>	fawning	mortality	0.03
rut	trail	<	early gest	browse	0.05
rut	trail	<	early gest	grain source	<.0001
rut	trail	<	late gest	bed	0.000
rut	trail	<	late gest	grain source	0.003
rut	trail	<	fawning	bed	0.01

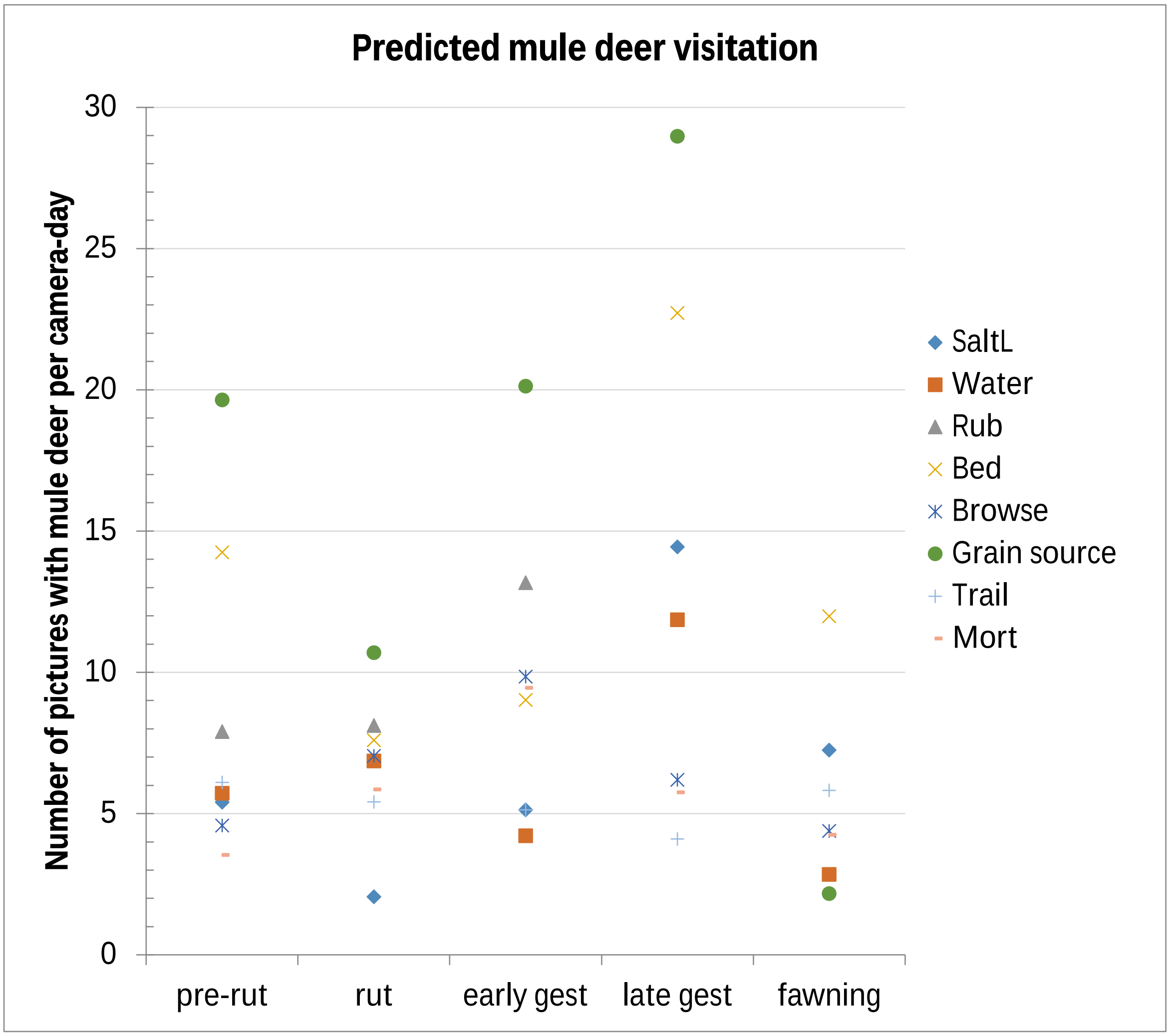
Season	Site	Relationship[1]	Season	Site	P[2]
rut	mortality	<	early gest	grain source	0.000
rut	mortality	<	late gest	bed	0.001
rut	mortality	<	late gest	grain source	0.01
rut	mortality	<	fawning	bed	0.03
early gest	salt lick	<	early gest	grain source	0.03
early gest	salt lick	<	late gest	bed	0.03
early gest	salt lick	<	late gest	grain source	0.03
early gest	waterhole	<	early gest	grain source	0.01
early gest	waterhole	<	late gest	bed	0.01
early gest	waterhole	<	late gest	grain source	0.01
early gest	rub	>	fawning	waterhole	0.005
early gest	rub	>	fawning	browse	0.02
early gest	rub	>	fawning	grain source	0.03
early gest	rub	>	fawning	mortality	0.03
early gest	bed	<	early gest	grain source	0.01
early gest	bed	<	late gest	bed	0.01
early gest	bed	<	late gest	grain source	0.03
early gest	bed	>	fawning	waterhole	0.003
early gest	bed	>	fawning	browse	0.02
early gest	bed	>	fawning	mortality	0.04
early gest	browse	<	early gest	grain source	0.01
early gest	browse	>	early gest	trail	0.03
early gest	browse	<	late gest	bed	0.02
early gest	browse	<	late gest	grain source	0.04
early gest	browse	>	fawning	waterhole	0.001
early gest	browse	>	fawning	browse	0.004
early gest	browse	>	fawning	grain source	0.04
early gest	browse	>	fawning	mortality	0.02
early gest	grain source	>	early gest	trail	<.0001
early gest	grain source	>	early gest	mortality	0.004
early gest	grain source	>	late gest	browse	0.001
early gest	grain source	>	late gest	trail	0.001
early gest	grain source	>	late gest	mortality	0.0004
early gest	grain source	>	fawning	salt lick	0.01
early gest	grain source	>	fawning	waterhole	<.0001
early gest	grain source	>	fawning	bed	0.05
early gest	grain source	>	fawning	browse	<.0001
early gest	grain source	>	fawning	grain source	0.002
early gest	grain source	>	fawning	trail	<.0001
early gest	grain source	>	fawning	mortality	<.0001
early gest	trail	<	early gest	mortality	0.03
early gest	trail	<	late gest	bed	0.0001

Season	Site	Relationship[1]	Season	Site	P[2]
early gest	trail	<	late gest	grain source	0.002
early gest	trail	<	fawning	bed	0.004
early gest	mortality	<	late gest	bed	0.01
early gest	mortality	<	late gest	grain source	0.03
early gest	mortality	>	fawning	waterhole	0.001
early gest	mortality	>	fawning	browse	0.004
early gest	mortality	>	fawning	grain source	0.05
early gest	mortality	>	fawning	mortality	0.02
late gest	salt lick	>	fawning	waterhole	0.03
late gest	waterhole	>	fawning	waterhole	0.01
late gest	bed	>	late gest	browse	0.001
late gest	bed	>	late gest	trail	0.002
late gest	bed	>	late gest	mortality	0.001
late gest	bed	>	fawning	salt lick	0.01
late gest	bed	>	fawning	waterhole	<.0001
late gest	bed	>	fawning	bed	0.04
late gest	bed	>	fawning	browse	<.0001
late gest	bed	>	fawning	grain source	0.003
late gest	bed	>	fawning	trail	0.0002
late gest	bed	>	fawning	mortality	0.0001
late gest	browse	<	late gest	grain source	0.007
late gest	browse	<	fawning	bed	0.04
late gest	grain source	>	late gest	trail	0.004
late gest	grain source	>	late gest	mortality	0.01
late gest	grain source	>	fawning	salt lick	0.02
late gest	grain source	>	fawning	waterhole	0.0001
late gest	grain source	>	fawning	browse	0.001
late gest	grain source	>	fawning	grain source	0.002
late gest	grain source	>	fawning	trail	0.003
late gest	grain source	>	fawning	mortality	0.001
late gest	trail	<	fawning	bed	0.03
late gest	mortality	<	fawning	bed	0.03
fawning	salt lick	>	fawning	waterhole	0.05
fawning	waterhole	<	fawning	bed	0.0001
fawning	waterhole	<	fawning	trail	0.05
fawning	bed	>	fawning	browse	0.0004
fawning	bed	>	fawning	grain source	0.02
fawning	bed	>	fawning	trail	0.01
fawning	bed	>	fawning	mortality	0.004

[1] Relationship indicates that the predicted number of pictures with mule deer in the season and site combination from the left is larger (>) or smaller (<) than the one in the season and site combination from the right. [2] Significant difference was considered if $p<0.05$.

Table A6.5.4 and Fig. A6.5.1. Predicted number of pictures with mule deer per camera-day for each season and site type combination.

Site type	pre-rut	rut	early gestation	late gestation	fawning
Salt lick	5.40	2.06	5.14	14.45	7.25
Waterhole	5.72	6.87	4.22	11.87	2.85
Rub	7.91	8.12	13.18		
Bed	14.25	7.60	9.03	22.73	11.99
Browse	4.59	7.05	9.86	6.20	4.39
Grain source	19.65	10.70	20.14	28.98	2.18
Trail	6.11	5.42	5.14	4.11	5.82
Mortality	3.54	5.87	9.46	5.76	4.25



6.15 APPENDIX 6.6.

Appendix 6.6. Summary of results from the model with counts of pictures with mule deer contacting the environment.

To assess the effect of season and site type on the frequency of visitation of mule deer we fit a generalized linear mixed model using the GLIMMIX procedure in SAS v9.3. The outcome variable was the frequency with which different sex-and-age classes visited sites measured as the number of pictures taken, the offset was the camera-days, the distribution was negative binomial, the link function was log, and the random intercept was station. Model building was completed using manual forward selection, with a screening criterion of $p \leq 0.2$ for the initial univariable analysis, and $p < 0.05$ as the criterion for significance in the multivariable analysis. When building the multivariable model for each outcome, if one covariate changed the regression coefficient or effect of the other by 10% or more, then we considered it as a confounder and kept it in the model. We examined the potential for interaction between season and site type where both were significant as fixed effects in the model. The interaction term was retained in the final model if $p<0.05$.

There was a significant interaction between site type and season in this model, such that the extent of differences between site types in the counts of pictures with mule deer contacting the environment varied by season.

The results of this model include:

- Table with the solutions of fixed effects.
- Least squares means (LSM) table. It indicates the estimated number of pictures per camera day, by season and site combinations, and their lower and upper 95% confidence levels.
- Table with the significant pair-wise comparisons of the differences between seasons and site type combinations. The signs < and > help to identify which values (as shown in the LSM table) were significantly greater than others.
- Table and graph of the predicted number of pictures with mule deer contacting the environment per camera-day for each season and site type combination.

Table A.6.6.1. Solutions for Fixed Effects - Environmental contacts model

Effect	Season	Site	Estimate[1]	SE	DF	P	Lower[1]	Upper[1]
Intercept			-1.60	0.61	276	0.01	-2.79	-0.40
season	pre-rut		-0.77	1.07	56	0.48	-2.91	1.37
season	rut		0.53	0.79	56	0.50	-1.05	2.11
season	early gest		1.59	0.67	56	0.02	0.25	2.94
season	late gest		0.26	0.72	56	0.72	-1.19	1.71
season	fawning		0	.	.	.	.	.
site		salt lick	1.85	0.85	56	0.03	0.14	3.56
site		waterhole	-0.21	0.85	56	0.80	-1.91	1.49
site		rub	-1.13	0.91	56	0.22	-2.96	0.70
site		bed	1.52	0.68	56	0.03	0.15	2.89
site		browse	0.33	0.70	56	0.64	-1.08	1.74
site		grain source	-5.90	16.12	56	0.72	-38.21	26.40
site		trail	0.57	0.70	56	0.42	-0.83	1.97
site		mortality	0	.	.	.	.	.
season*site	pre-rut	salt lick	-0.24	1.28	56	0.85	-2.81	2.32
season*site	pre-rut	waterhole	1.12	1.28	56	0.38	-1.44	3.68
season*site	pre-rut	rub	2.23	1.40	56	0.12	-0.57	5.03
season*site	pre-rut	bed	1.16	1.15	56	0.32	-1.15	3.46
season*site	pre-rut	browse	0.87	1.18	56	0.46	-1.49	3.23
season*site	pre-rut	grain source	8.36	16.16	56	0.61	-24.02	40.73
season*site	pre-rut	trail	0.49	1.16	56	0.67	-1.83	2.82
season*site	pre-rut	mortality	0	.	.	.	.	.
season*site	rut	salt lick	-2.98	1.60	56	0.07	-6.19	0.23
season*site	rut	waterhole	-0.32	1.30	56	0.80	-2.92	2.27
season*site	rut	rub	1.98	1.04	56	0.06	-0.10	4.06
season*site	rut	bed	-1.14	0.96	56	0.24	-3.07	0.78
season*site	rut	browse	-0.37	0.98	56	0.71	-2.33	1.59
season*site	rut	grain source	7.24	16.14	56	0.66	-25.09	39.57
season*site	rut	trail	-0.42	0.97	56	0.67	-2.36	1.52
season*site	rut	mortality	0	.	.	.	.	.
season*site	early gest	salt lick	-2.33	1.43	56	0.11	-5.20	0.55
season*site	early gest	waterhole	-1.63	1.66	56	0.33	-4.95	1.69
season*site	early gest	rub	0	.	.	.	.	.
season*site	early gest	bed	-1.76	0.84	56	0.04	-3.45	-0.07
season*site	early gest	browse	-0.63	0.85	56	0.46	-2.33	1.07
season*site	early gest	grain source	7.73	16.13	56	0.63	-24.57	40.04
season*site	early gest	trail	-2.18	0.88	56	0.02	-3.95	-0.40
season*site	early gest	mortality	0	.	.	.	.	.

Effect	Season	Site	Estimate[1]	SE	DF	P	Lower[1]	Upper[1]
season*site	late gest	salt lick	1.07	1.24	56	0.39	-1.40	3.55
season*site	late gest	waterhole	3.44	1.29	56	0.01	0.86	6.03
season*site	late gest	bed	0.15	0.90	56	0.86	-1.64	1.95
season*site	late gest	browse	0.22	0.94	56	0.81	-1.65	2.10
season*site	late gest	grain source	8.52	16.14	56	0.60	-23.81	40.84
season*site	late gest	trail	-1.43	1.28	56	0.27	-4.00	1.13
season*site	late gest	mortality	0	.	.	.	.	.
season*site	fawning	salt lick	0	.	.	.	.	.
season*site	fawning	waterhole	0	.	.	.	.	.
season*site	fawning	bed	0	.	.	.	.	.
season*site	fawning	browse	0	.	.	.	.	.
season*site	fawning	grain source	0	.	.	.	.	.
season*site	fawning	trail	0	.	.	.	.	.
season*site	fawning	mortality	0	.	.	.	.	.

SE = standard error; DF = degrees of freedom; early gest = early gestation; late gest = late gestation. [1]Units are log(pics/CD) where pics/CD is the number of pictures per camera-day; Lower and Upper are the confidence limits on the mean (probability) scale.

Table A.6.6.2. Season*site least squares means - Environmental contacts model

Season	Site	Estimate[1]	Lower[1]	Upper[1]
pre-rut	salt lick	0.47	0.16	1.40
pre-rut	waterhole	0.23	0.10	0.53
pre-rut	rub	0.28	0.09	0.93
pre-rut	bed	1.37	0.73	2.59
pre-rut	browse	0.31	0.15	0.64
pre-rut	grain source	1.09	0.30	3.95
pre-rut	trail	0.27	0.15	0.51
pre-rut	mortality	0.09	0.02	0.56
rut	salt lick	0.11	0.01	1.40
rut	waterhole	0.20	0.04	1.07
rut	rub	0.81	0.37	1.76
rut	bed	0.50	0.20	1.26
rut	browse	0.33	0.14	0.82
rut	grain source	1.31	0.49	3.53
rut	trail	0.40	0.16	0.98
rut	mortality	0.35	0.12	0.97
early gest	salt lick	0.62	0.07	5.72
early gest	waterhole	0.16	0.01	2.58
early gest	rub	0.32	0.06	1.82
early gest	bed	0.78	0.34	1.79
early gest	browse	0.74	0.35	1.59
early gest	grain source	6.23	3.41	11.39
early gest	trail	0.20	0.08	0.51
early gest	mortality	1.00	0.52	1.92
late gest	salt lick	4.90	0.56	42.92
late gest	waterhole	6.67	1.01	44.16
late gest	bed	1.40	0.51	3.85
late gest	browse	0.46	0.17	1.24
late gest	grain source	3.59	0.81	15.98
late gest	trail	0.11	0.01	0.89
late gest	mortality	0.26	0.09	0.82
fawning	salt lick	1.29	0.37	4.44
fawning	waterhole	0.16	0.05	0.56
fawning	bed	0.93	0.49	1.76
fawning	browse	0.28	0.13	0.59
fawning	grain source	$6\text{x}10^{-4}$	$5\text{x}10^{-18}$	$6\text{x}10^{10}$
fawning	trail	0.36	0.17	0.75
fawning	mortality	0.20	0.06	0.68

early gest = early gestation; late gest = late gestation. [1]Units are pics/CD, the number of pictures per camera-day; Lower and Upper are the confidence limits on the mean (probability) scale.

Table A.6.6.3. Differences of significant season*site least squares means - Environmental contacts model.

Season	Site	Relationship[1]	Season	Site	P[2]
pre-rut	salt lick	<	early gestation	grain source	<.0001
pre-rut	salt lick	<	late gestation	salt lick	0.05
pre-rut	salt lick	<	late gestation	waterhole	0.02
pre-rut	salt lick	<	late gestation	grain source	0.03
pre-rut	waterhole	<	pre-rut	bed	0.001
pre-rut	waterhole	<	pre-rut	grain source	0.04
pre-rut	waterhole	<	rut	rub	0.03
pre-rut	waterhole	<	rut	grain source	0.01
pre-rut	waterhole	<	early gestation	bed	0.04
pre-rut	waterhole	<	early gestation	browse	0.04
pre-rut	waterhole	<	early gestation	grain source	<.0001
pre-rut	waterhole	<	early gestation	mortality	0.01
pre-rut	waterhole	<	late gestation	salt lick	0.01
pre-rut	waterhole	<	late gestation	waterhole	0.002
pre-rut	waterhole	<	late gestation	bed	0.01
pre-rut	waterhole	<	late gestation	grain source	0.002
pre-rut	waterhole	<	fawning	salt lick	0.02
pre-rut	waterhole	<	fawning	bed	0.01
pre-rut	rub	<	pre-rut	bed	0.02
pre-rut	rub	<	early gestation	grain source	<.0001
pre-rut	rub	<	late gestation	salt lick	0.02
pre-rut	rub	<	late gestation	waterhole	0.01
pre-rut	rub	<	late gestation	bed	0.04
pre-rut	rub	<	late gestation	grain source	0.01
pre-rut	bed	>	pre-rut	browse	0.003
pre-rut	bed	>	pre-rut	trail	0.001
pre-rut	bed	>	pre-rut	mortality	0.01
pre-rut	bed	>	rut	waterhole	0.04
pre-rut	bed	>	rut	browse	0.01
pre-rut	bed	>	rut	trail	0.03
pre-rut	bed	>	rut	mortality	0.03
pre-rut	bed	<	early gestation	grain source	0.001
pre-rut	bed	>	early gestation	trail	0.001
pre-rut	bed	>	late gestation	trail	0.02
pre-rut	bed	>	late gestation	mortality	0.01
pre-rut	bed	>	fawning	waterhole	0.003
pre-rut	bed	>	fawning	browse	0.002
pre-rut	bed	>	fawning	trail	0.01
pre-rut	bed	>	fawning	mortality	0.01
pre-rut	browse	<	rut	grain source	0.02

Season	Site	Relationship[1]	Season	Site	P[2]
pre-rut	browse	<	early gestation	grain source	<.0001
pre-rut	browse	<	early gestation	mortality	0.02
pre-rut	browse	<	late gestation	salt lick	0.02
pre-rut	browse	<	late gestation	waterhole	0.004
pre-rut	browse	<	late gestation	bed	0.02
pre-rut	browse	<	late gestation	grain source	0.004
pre-rut	browse	<	fawning	salt lick	0.05
pre-rut	browse	<	fawning	bed	0.03
pre-rut	grain source	>	pre-rut	mortality	0.03
pre-rut	grain source	<	early gestation	grain source	0.01
pre-rut	grain source	>	early gestation	trail	0.03
pre-rut	grain source	>	fawning	waterhole	0.03
pre-rut	trail	<	rut	rub	0.03
pre-rut	trail	<	rut	grain source	0.01
pre-rut	trail	<	early gestation	bed	0.04
pre-rut	trail	<	early gestation	browse	0.04
pre-rut	trail	<	early gestation	grain source	<.0001
pre-rut	trail	<	early gestation	mortality	0.004
pre-rut	trail	<	late gestation	salt lick	0.01
pre-rut	trail	<	late gestation	waterhole	0.002
pre-rut	trail	<	late gestation	bed	0.01
pre-rut	trail	<	late gestation	grain source	0.002
pre-rut	trail	<	fawning	salt lick	0.03
pre-rut	trail	<	fawning	bed	0.01
pre-rut	mortality	<	rut	rub	0.03
pre-rut	mortality	<	rut	grain source	0.01
pre-rut	mortality	<	early gestation	bed	0.03
pre-rut	mortality	<	early gestation	browse	0.03
pre-rut	mortality	<	early gestation	grain source	<.0001
pre-rut	mortality	<	early gestation	mortality	0.01
pre-rut	mortality	<	late gestation	salt lick	0.01
pre-rut	mortality	<	late gestation	waterhole	0.002
pre-rut	mortality	<	late gestation	bed	0.01
pre-rut	mortality	<	late gestation	grain source	0.002
pre-rut	mortality	<	fawning	salt lick	0.02
pre-rut	mortality	<	fawning	bed	0.02
rut	salt lick	<	early gestation	grain source	0.003
rut	salt lick	<	late gestation	salt lick	0.03
rut	salt lick	<	late gestation	waterhole	0.01
rut	salt lick	<	late gestation	grain source	0.02
rut	waterhole	<	early gestation	grain source	0.000
rut	waterhole	<	late gestation	salt lick	0.02

Season	**Site**	**Relationship**[1]	**Season**	**Site**	**P**[2]
rut	waterhole	<	late gestation	waterhole	0.01
rut	waterhole	<	late gestation	grain source	0.01
rut	rub	<	early gestation	grain source	<.0001
rut	rub	>	early gestation	trail	0.02
rut	rub	<	late gestation	waterhole	0.04
rut	rub	>	fawning	waterhole	0.03
rut	bed	<	early gestation	grain source	<.0001
rut	bed	<	late gestation	waterhole	0.02
rut	bed	<	late gestation	grain source	0.03
rut	browse	<	rut	grain source	0.04
rut	browse	<	early gestation	grain source	<.0001
rut	browse	<	late gestation	salt lick	0.02
rut	browse	<	late gestation	waterhole	0.01
rut	browse	<	late gestation	bed	0.04
rut	browse	<	late gestation	grain source	0.01
rut	grain source	<	early gestation	grain source	0.01
rut	grain source	>	early gestation	trail	0.01
rut	grain source	>	late gestation	trail	0.04
rut	grain source	>	late gestation	mortality	0.03
rut	grain source	>	fawning	waterhole	0.01
rut	grain source	>	fawning	browse	0.02
rut	grain source	>	fawning	trail	0.04
rut	grain source	>	fawning	mortality	0.02
rut	trail	<	early gestation	grain source	<.0001
rut	trail	<	late gestation	salt lick	0.04
rut	trail	<	late gestation	waterhole	0.01
rut	trail	<	late gestation	grain source	0.01
rut	mortality	<	early gestation	grain source	<.0001
rut	mortality	<	late gestation	salt lick	0.03
rut	mortality	<	late gestation	waterhole	0.01
rut	mortality	<	late gestation	grain source	0.01
early gestation	salt lick	<	early gestation	grain source	0.05
early gestation	waterhole	<	early gestation	grain source	0.01
early gestation	waterhole	<	late gestation	waterhole	0.03
early gestation	rub	<	early gestation	grain source	0.002
early gestation	rub	<	late gestation	waterhole	0.02
early gestation	rub	<	late gestation	grain source	0.04
early gestation	bed	<	early gestation	grain source	<.0001
early gestation	bed	>	early gestation	trail	0.03
early gestation	bed	<	late gestation	waterhole	0.04
early gestation	bed	>	fawning	waterhole	0.03
early gestation	browse	<	early gestation	grain source	<.0001

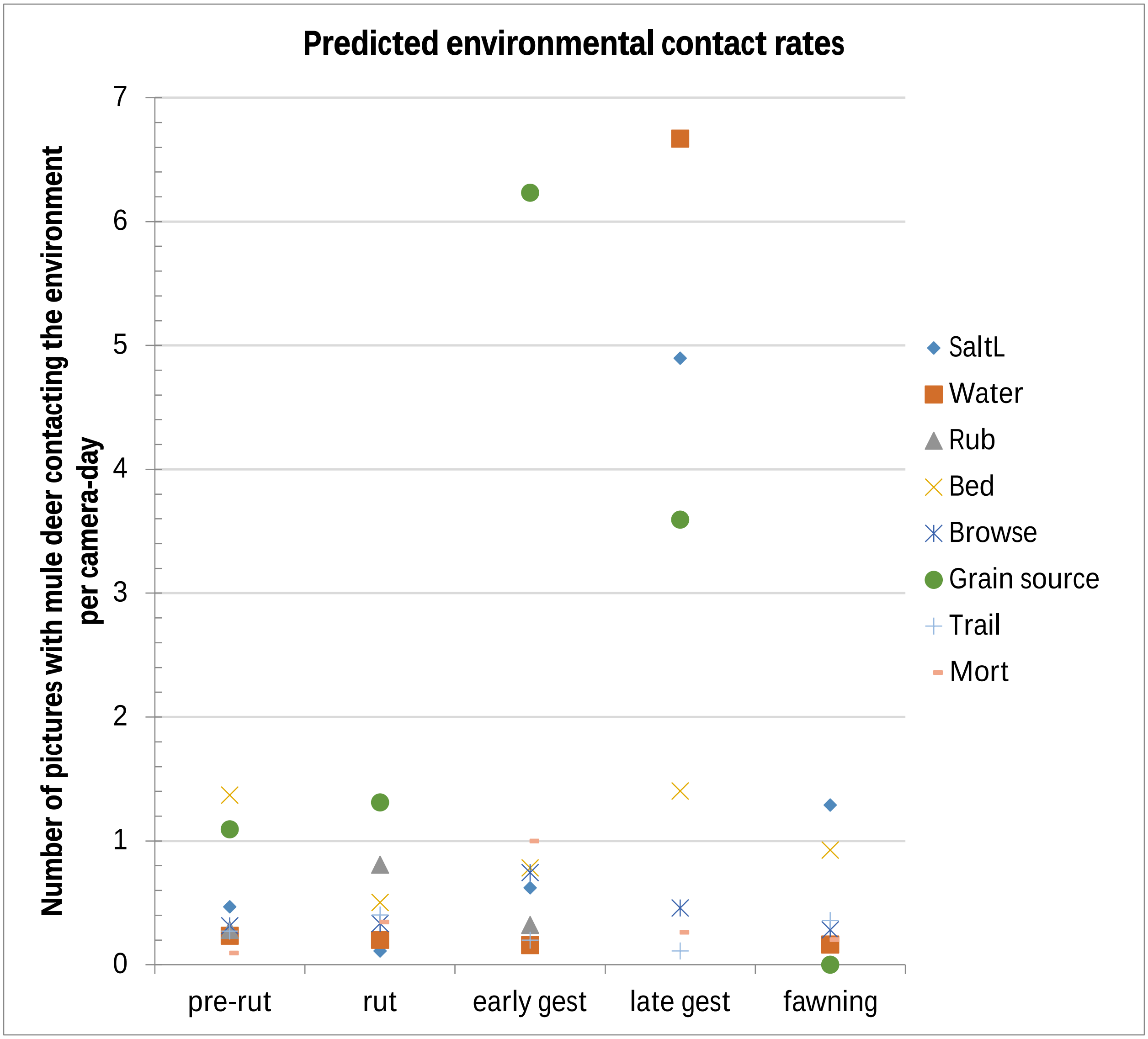
Season	Site	Relationship[1]	Season	Site	P[2]
early gestation	browse	>	early gestation	trail	0.03
early gestation	browse	<	late gestation	waterhole	0.03
early gestation	browse	>	fawning	waterhole	0.03
early gestation	grain source	>	early gestation	trail	<.0001
early gestation	grain source	>	early gestation	mortality	<.0001
early gestation	grain source	>	late gestation	bed	0.01
early gestation	grain source	>	late gestation	browse	<.0001
early gestation	grain source	>	late gestation	trail	0.0004
early gestation	grain source	>	late gestation	mortality	<.0001
early gestation	grain source	>	fawning	salt lick	0.02
early gestation	grain source	>	fawning	waterhole	<.0001
early gestation	grain source	>	fawning	bed	<.0001
early gestation	grain source	>	fawning	browse	<.0001
early gestation	grain source	>	fawning	trail	<.0001
early gestation	grain source	>	fawning	mortality	<.0001
early gestation	trail	<	early gestation	mortality	0.01
early gestation	trail	<	late gestation	salt lick	0.01
early gestation	trail	<	late gestation	waterhole	0.002
early gestation	trail	<	late gestation	bed	0.01
early gestation	trail	<	late gestation	grain source	0.001
early gestation	trail	<	fawning	salt lick	0.02
early gestation	trail	<	fawning	bed	0.01
early gestation	mortality	>	late gestation	trail	0.05
early gestation	mortality	>	late gestation	mortality	0.03
early gestation	mortality	>	fawning	waterhole	0.01
early gestation	mortality	>	fawning	browse	0.01
early gestation	mortality	>	fawning	trail	0.03
early gestation	mortality	>	fawning	mortality	0.02
late gestation	salt lick	>	late gestation	browse	0.05
late gestation	salt lick	>	late gestation	trail	0.01
late gestation	salt lick	>	late gestation	mortality	0.02
late gestation	salt lick	>	fawning	waterhole	0.01
late gestation	salt lick	>	fawning	browse	0.02
late gestation	salt lick	>	fawning	trail	0.02
late gestation	salt lick	>	fawning	mortality	0.01
late gestation	waterhole	>	late gestation	browse	0.01
late gestation	waterhole	>	late gestation	trail	0.01
late gestation	waterhole	>	late gestation	mortality	0.005
late gestation	waterhole	>	fawning	waterhole	0.001
late gestation	waterhole	>	fawning	browse	0.003
late gestation	waterhole	>	fawning	trail	0.01
late gestation	waterhole	>	fawning	mortality	0.003

Season	**Site**	**Relationship**[1]	**Season**	**Site**	**P**[2]
late gestation	bed	>	late gestation	trail	0.03
late gestation	bed	>	late gestation	mortality	0.03
late gestation	bed	>	fawning	waterhole	0.01
late gestation	bed	>	fawning	browse	0.01
late gestation	bed	>	fawning	trail	0.03
late gestation	bed	>	fawning	mortality	0.02
late gestation	browse	<	late gestation	grain source	0.02
late gestation	grain source	>	late gestation	trail	0.01
late gestation	grain source	>	late gestation	mortality	0.01
late gestation	grain source	>	fawning	waterhole	0.002
late gestation	grain source	>	fawning	browse	0.003
late gestation	grain source	>	fawning	trail	0.01
late gestation	grain source	>	fawning	mortality	0.004
late gestation	trail	<	fawning	salt lick	0.05
fawning	salt lick	>	fawning	waterhole	0.02
fawning	salt lick	>	fawning	browse	0.04
fawning	salt lick	>	fawning	mortality	0.03
fawning	waterhole	<	fawning	bed	0.01
fawning	bed	>	fawning	browse	0.02
fawning	bed	>	fawning	mortality	0.03

[1] Relationship indicates that the predicted number of pictures with mule deer contacting the environment in the season and site combination from the left is larger (>) or smaller (<) than the one in the season and site combination from the right. [2] Significant difference was considered if $p<0.05$.

Table A.6.6.4 and Fig A.6.6.1. Predicted number of pictures with mule deer contacting the environment per camera-day for each season and site type combination.

Site type	Pre-rut	Rut	Early gestation	Late gestation	Fawning
Salt lick	0.47	0.11	0.62	4.90	1.29
Waterhole	0.23	0.20	0.16	6.67	0.16
Rub	0.28	0.81	0.32		
Bed	1.37	0.50	0.78	1.40	0.93
Browse	0.31	0.33	0.74	0.46	0.28
Grain source	1.09	1.31	6.23	3.59	$6\text{x}10^{-4}$
Trail	0.27	0.40	0.20	0.11	0.36
Mortality	0.09	0.35	1.00	0.26	0.20



CHAPTER 7. GENERAL DISCUSSION

7.1 SUMMARY OF THE OBJECTIVES AND RESULTS

Our main objectives were to (1) explore epidemiologically relevant factors shaping mule deer social behaviour, and their visitation to environmental sites, and (2) to do so in a manner that expands our understanding of prion transmission among mule deer in a prairie habitat. Specifically, we examined intrinsic (e.g., sex and chronic wasting disease diagnosis) and extrinsic (e.g., habitat and time of year) factors that may relate to grouping patterns (Chapter 3), the proportion of time that pairs of deer spend in spatial proximity (Chapter 4), the rate of high-risk physical contacts between individuals (Chapter 5), and the frequency of visitation to various environmental sites (Chapter 6).

For our first manuscript (Chapter 3), we used group size data collected from 2008 to 2013 on 365 radio-collared mule deer to build two separate generalized linear mixed models (GLMM) in SAS v9.3 to evaluate whether factors such as CWD status, sex, age, season, habitat and time of day, predicted group occurrence, and to determine if these factors were associated with group size. Group occurrence was defined as whether deer were grouped ($\geq$ 2 deer in the group) or alone. Then, because group size was highly skewed, we used three descriptive statistics (typical, mean and median group sizes) to adequately characterize the distribution of mule deer group size (Reiczigel et al., 2008). We demonstrated that clinically healthy individuals were ~3 times more likely to be seen in groups than deer showing clinical signs of CWD, and that the size of the groups strongly varied with habitat, time of the day, season, and the group members' sex and age classes. We made a unique and detailed description of mule deer group size distribution across times of day, habitats, years, seasons, months, and group types (Appendices 3.2 and 3.3).

In our second manuscript (Chapter 4), we used three years of GPS location data collected every 2 hours from >70 adult mule deer to create a linear mixed-effects model analysis in R to assess whether sex, age, CWD diagnosis, home range overlap (HRO), genetic relatedness and time of the year influenced the proportion of time deer spent within 25 m of each other (i.e., strength of pairwise spatial associations). We also analysed lagged association rates in SOCPROG 2.6 to test for sex, age and CWD status differences in the temporal stability of spatial associations of >40 adults. We found that the strength of spatial associations was independent of genetic relatedness, age and disease diagnosis. However, the seasonal strengths varied with HRO and sex. Associations were strongest in pre-rut at the same level of HRO. Male-male associations were the strongest in pre-rut, and different-sex associations were the strongest in rut. We also found marked sex differences in the temporal patterns of associations. Females mostly mingled with other females in long-term associations, but related to males in a much more ephemeral fashion.

For our third manuscript (Chapter 5), we did direct observations of behaviour on 133 individuals of all ages during 2 years to record their group membership and quantify pairwise interactions with the potential to be relevant for CWD transmission through direct physical contact. Using SOCPRPG 2.7, we ran two separate MRQAP (multiple regression quadratic assignment procedure) tests to determine whether genetic relatedness, and sex, age and disease status homophilies were useful predictors of association strength as defined by group membership, and whether the same factors and association strength were useful predictors of high-risk interaction rates among mule deer. We then ran a Hemelrijk Rr-test in SOCPROG to determine whether these interactions were reciprocal or unidirectional. We found that high-risk contacts are rare events among mule deer, even at times of the day when they are most active, and that contact rates are predicted by group membership, but not kinship. These interactions were reciprocal across all age, CWD status and sex classes. Relatedness and sex homophily predicted association strength, with same-sex associations being stronger than different-sex associations.

Finally, in our fourth manuscript (Chapter 6), we assigned sex and age classes to mule deer in >30,000 pictures from 370 triggered-by-movement camera stations deployed from 2009 to 2012 at eight different small geographic area types that deer frequently and repeatedly visited. Site types examined included grain spills, waterholes, salt blocks, and browse and bed sites. With these data, we fitted several GLMMs in SAS v9.3, one per sex-and-age class of deer, to assess the effect of season and site type on the frequency of visitation of mule deer, measured as the number of pictures taken, and the frequency of environmental contact, measured as the number of pictures with mule deer contacting the environment. These analyses allowed us to rank these site types according to their relative importance with respect to prion contamination, and to identify in which site type specific sex-and-age classes of deer can be more frequently found at various times of the year. We demonstrated that not only did most sex-and-age classes of deer preferentially visit grain spills, but also that contact with the environment occurs more often at these places. Supplemental feed sources such as grain and hay, either unintentionally or intentionally made available to deer, may play a central role in the potential for CWD transmission and its control.

7.2 RELEVANCE OF THIS WORK

Social behaviour has important effects on the long-term dynamics of diseases (Vander Wal et al., 2012; Schauber et al., 2015). This dissertation is a timely and valuable addition to the scientific literature as it will promote a greater understanding of cervid sociobiology, and of the relationship between mule deer sociality and disease transmission, a field growing in importance in several other taxa (e.g., Cross et al., 2004; Hamede et al., 2009; Bouwman and Hawley, 2010; Ryan et al., 2013; Fairbanks et al., 2014; Butler et al., 2015).

In particular, populations of mule deer and white-tailed deer in North America have been affected by CWD over the last three decades (Miller et al., 2008; Edmunds et al., 2016). During this time, CWD's spread has not been controlled by management measures, and its geographic distribution expands every year. Wildlife managers are in pressing need of information that can help them guide deer population management programs. Likewise, detailed data describing mule deer social behaviour and visitation to environmental areas to better parameterize epidemiological models are urgently needed by disease modellers (Potapov et al., 2013).

Even though others have described various aspects of mule deer sociality (Dixon, 1934; Linsdale and Tomich, 1953; Goldberg and Haas, 1978; Kucera, 1978; Wachtel et al., 1978; Geist, 1981; Koutnik, 1981; Bowyer, 1984; Halford et al., 1987; Relyea and Demarais, 1994; Geist, 1998; Bowyer et al., 2001; Lingle, 2001; Prothero, 2002; Lingle, 2003; Lingle et al., 2007), investigations of factors related to individuals' choices of associating and interacting partners were missing. The results of this dissertation include: 1) a description of group size distribution across several habitats, times of the day and seasons by calculating not one, but three measures of group size, 2) reported patterns of proximal associations among more than 70 adult deer based on an every-2 h GPS locations data set that spans three years, 3) defined matrices of high-risk contact rates obtained from direct observation of individuals, and 4) reported seasonal visitation patterns of several age and sex classes of mule deer to many environmental sites with the risk of prion deposition and intake. This information can be used for future model building to guide disease control and population management.

7.2.1 Social relationships among mule deer are not random

We found evidence that mule deer in Antelope Creek live in a fluid fission-fusion social system. Such system can work to ameliorate the negative aspects (e.g., competition for resources) and to increase the benefits (e.g., reduce predation risk) of grouping (Krause and Ruxton, 2002). Mule deer society is dynamic, with highly variable group sizes forming and being broken across different times of the day, different seasons, and in different habitats, and that vary depending on the sex of the members (Chapter 3). For example, the largest groups occurred at dusk, in open habitats, and in winter, when the risk of predation by coyotes (*Canis latrans*) significantly increases (Lingle, 2000, 2001), and most deer were seen alone during the midday and in closed habitats, when the benefit of grouping is reduced. These features of mule deer sociality most likely have consequences in disease transmission at the population level (Craft, 2015), as pathogens are expected to transmit faster and more widely in populations with a high variation in group size (Caillaud et al., 2013) and with more mixing of individuals between groups (Keeling and Eames, 2005).

More evidence that social relationships among mule deer do not occur at random came from studying spatial associations patterns (Chapter 4). As some individuals had a small association index despite a large proportion of home range overlap, whereas others had a large association index despite a very small home range overlap, we demonstrated that some individuals actively avoid or prefer others. This indicates that their spatial associations are only partially explained by the extent of their home range overlap, as previously reported in other species with fluid fission-fusion dynamics (Carter et al., 2013; Best et al., 2014; Strickland et al., 2014).

7.2.2 Significance of sex and genetic relatedness

We found marked sex differences in social relationships owing to individuals preferentially grouping with conspecifics of the same sex (Chapters 3 to 5). These sex-based homophily features can explain, at least in part, the greater prevalence of CWD in adult male mule deer (Grear et al., 2006; Jennelle et al., 2014): (1) male groups were smaller than female groups (Chapter 3), (2) spatial associations between males were weaker and less stable than those between females regardless of the proportion of home range overlap (Chapter 4), (3) group membership between males was weaker than between females (Chapter 5), and (4) high-risk physical contact rates between males appear to be greater than those between females (preliminary results; Appendix 5.2, Fig. 5.2.1). It seems that males are more likely to mix more often with new individuals, consequently putting themselves at a greater risk of eventually associating with infected deer. Other possible explanations for greater male CWD prevalence include larger male home ranges (Silbernagel, 2010).

Moreover, clear seasonal patterns of spatial proximity strength varied according to sex; strength of association between males was markedly greater in pre-rut, while that between sexes was greater in rut (Chapter 4). This, together with previous reports of increased direct contacts between competing males during pre-rut (Karns et al., 2009), show that pre-rut may be a time of augmented risk of infection between males, whereas rut may be for infection between males and females.

Genetic relatedness was not an important predictor of spatial associations based on spatial proximity among adults (Chapter 4). However, at a finer scale (Chapter 5), related individuals of any age class were more likely to be seen in the same group over time. That study included only adult individuals with GPS collars from which we had genetic relatedness data, and a pair was considered to be associated if found in close proximity based on GPS locations recorded every 2 h. In contrast, in Chapter 5, we included deer of any age with either GPS or VHF collars from which we had genetic relatedness data, and we defined an association instance if seen in the same group during dawn and dusk. In that case, we report that relatedness was a predictor of association strength as defined by group membership. It is relevant to keep in mind that transmission through sharing small geographical spaces, such as when feeding while in a group, should be more likely among genetically related individuals, as these are more likely to be found in the same groups over time (Chapter 5).

7.2.3 Effect of CWD on social behaviour

Behavioural responses to CWD are evident at the individual level and during the clinical phase of the disease. Brain lesions produced by the accumulation of CWD prions cause lethargy, ataxia, reduced alertness, and difficulty in finding shelter and food, and trouble avoiding predators and vehicles (Krumm et al., 2005; Miller et al., 2008; Mathiason et al., 2009; Krumm et al., 2010). One wonders if less obvious changes in individual behaviour during the pre-clinical phase affects social behaviour, such as grouping and physically contacting others, and how this might affect the risk of disease transmission. We found that mule deer showing clinical signs of CWD were less likely to be observed in groups than clinically healthy deer, after accounting for time of day, habitat, and month of observation (Chapter 3). In contrast, clinical signs did not affect the proportion of time adult deer spent in close proximity after accounting for season, year, VI, and sex of the associating pair (Chapter 4). These findings suggest that clinical signs affect the probability of grouping, but that once in a group, do not affect the frequency of close proximity events, at least among adults.

We could not test for the effect of obvious clinical signs on either group membership strength or high-risk contact rates because we did not have enough deer at this stage of disease for the duration of that study (Chapter 5). However, disease status (i.e., being positive or negative) did not affect the proportion of times deer were seen in the same group after accounting for sex, age and genetic relatedness, nor the rate at which high-risk contacts occurred after accounting for sex, age, genetic relatedness and group membership (Chapter 5).

7.2.4 Relevance of the two transmission modes of CWD

The discussion on which mode of transmission (animal-to-animal or through contaminated environments) drives CWD spread dynamics is an ongoing debate (Almberg et al., 2011; Schauber et al., 2015; Vasilyeva et al., 2015; Samuel and Storm, 2016). In theoretical modeling, the relative importance of environmental transmission appears to be stronger than that of direct transmission (Almberg et al., 2011; Vasilyeva et al., 2015). However, most models have not considered the heterogeneous contact structure of the host, which has shown to influence both transmission pathways (Nunn et al., 2015a). This has epidemiological implications in the spread of other infectious diseases that can also be transmitted through both pathways, such as tuberculosis (Corner et al., 2003; Cross et al., 2004; Drewe, 2010).

In our study, high-risk physical contacts were rare and occurred only among a quarter of the observed deer (Chapter 5). This suggests that unless CWD transmission is highly efficient during these contacts, direct animal-to-animal spread is likely to be slow. Although proximity does not necessarily translate into a greater frequency of physical contacts among individuals (e.g., Castles et al., 2014; Farine, 2015), we found that the strength of group membership predicts high-risk contact rates (Chapter 5). This finding needs to be interpreted with caution. As MRQAP tests cannot determine the strength of the correlation between these two variables, we can only determine that group membership is a predictor of interactions, but not how useful it is. Nevertheless, group membership may accurately translate into increased risk of transmission through not only sharing of contaminated environments, but also through animal-to-animal contact (Alcock, 2009). Therefore, it is likely that an individual that spends time with a group containing at least one infected deer would be at a high risk of contagion regardless if they physically contact each other or not because of common contact with the environment.

Some researchers have argued that “direct contacts” (Schauber et al., 2015), and not environmental reservoirs (Samuel and Storm, 2016), are the primary mechanism of CWD transmission (at least among female and juvenile white-tailed deer). However, their models do not estimate parameters specific to animal-to-animal mode of transmission: 1) Schauber et al. (2015) definition of a “contact” is not in fact a physical contact, but rather a proximity event (being within 10 m of each other), and 2) Samuel et al. (2016) data relied on assumptions about the infectious contact structure between and among sexes that combine both transmission mechanisms (Jennelle et al., 2014). Moreover, several researchers (e.g., Silbernagel et al., 2011; Schauber et al., 2015; Tosa et al., 2015) have defined a contact as either home range overlap or as simultaneous locations within a defined distance. I postulate that these are instances in which a physical contact is possible, but should not be used as proxies for direct animal-to-animal disease transmission. Even though we demonstrated that group membership strength (i.e., the proportion of times being seen within the same group) is a significant predictor of high-risk contact rates, and that kinship influences to some degree stronger group memberships, there is no evidence that kinship predicts the rate at which deer interact in a high-risk manner for CWD transmission (Chapter 5). For this reason, it would be reasonable to consider a high-risk contact matrix as an accurate sub-matrix of a direct transmission matrix. Due to the financial and logistic constrains of observing high-risk contacts among mule deer, using the group membership strength matrix as a proxy for a high-risk contact matrix may be adequate, as group membership is a predictor of contact rate.

It would be advisable for researchers investigating CWD epidemics to use accurate terms that allow for clear differentiation between a physical interaction (i.e., a direct contact) and a situation in which a contact may or may not occur (i.e., an association measure, e.g., simultaneous GPS locations within certain meters apart, group membership, home range overlap, or distance at death or capture). What is more, the use of imprecise definitions of what constitutes a social group, or of untested surrogates for contact rates, will most probably lead to erroneous model assumptions or recommendations on control strategies, such as culling complete “social groups” (Tosa et al., 2016) and reducing the number of large “social groups” (Magle et al., 2013).

7.2.5 Prion environmental contamination

Relatively small geographic areas that are more intensely used by deer, or where deer congregate in greater numbers, or both, can accumulate large quantities of prions and become important in the epidemiology of CWD. By looking at the relative visitation rates by various sex and age classes of mule deer to several small geographic areas, our goal was to estimate their relative importance with respect to prion contamination (Chapter 6). We found that grain sources were the most frequently visited sites by all sex and age classes in all seasons except for fawning, when these sites are scarce. There is strong evidence that artificial food supplies impact several aspects of deer behaviour (Thompson et al., 2008; van Beest et al., 2010; Peterson and Messmer, 2011; Oro et al., 2013; Sorensen et al., 2014), often enhancing the probability of disease transmission (Miller et al., 2007; Vicente et al., 2007; Thompson et al., 2008). On the other hand, there is also evidence that prevalence of other cervid diseases can be efficiently reduced by limiting access to grain supplies (e.g., Hickling, 2002). According to our results, a significant reduction in CWD transmission could be achieved by reducing deer access to grain spills during pre-rut (August, September and October), and early and late gestation (mid-December to mid-May) (Chapter 6). This would reduce deer congregations at such areas and the subsequent interactions among deer and of deer with the environment. It would also significantly reduce exposure to, and deposition of, large quantities of prions in the environment. CWD control programs that do not consider implementing a ban on baiting and feeding wild cervids, and the reduction of unintentional grain spills or similar point sources of feed, are not likely to be successful.

7.3 LIMITATIONS OF THE STUDIES

Contrary to other species such as tigers and giraffes, physical characteristics of mule deer are not distinctive enough to allow for individual identification. For this reason, in order to identify one individual from another, artificial identification given by radio-collars, and ear and collar tags, is required to analyse mule deer sociality. Moreover, in Chapter 6, our inability to distinguish individuals from one another beyond their sex and age class on pictures prevented us from classifying a series of pictures as a single visitation event and quantifying the number of different individuals visiting each site. Attempting to recognize unmarked individual deer throughout the year would hamper the repeatability of the study. However, we proposed to use the number of pictures per camera day as a measure of opportunity for site contamination. We also evaluated the differences in the rate of behaviours with a high risk for environmental transmission. These two measurements can serve as a relative indicator of site importance for prion accumulation and potential CWD transmission.

Collection of radio-telemetry data using GPS-collars was limited to only adult (>21 months old) individuals due to the weight of the collars. Hence, the conclusions in Chapter 4 are limited to only adult mule deer.

We could only obtain genetic relatedness coefficients from 57.8% (211 out of 365) of the radio-collared mule deer. For this reason, in Chapter 4 and 5, individuals without genetic relatedness data could not enter certain analysis.

In Chapter 5, focal animal follows were done during dawn and dusk only. This may limit our ability to make conclusions on mule deer behaviour at other times of the day. However, based on a radio-telemetry study looking at movement and activity patterns of radio-collared mule deer during the day and night in Washington, USA, this species had clearly defined crepuscular activity patterns (Eberhardt et al., 1984). Moreover, in Chapter 3, we found that mule deer were more likely to group during dawn, and that the groups were largest at dusk (Mejía Salazar et al., 2016). Our observations on physical contacts should be reflecting the periods of times in which individuals are more likely to interact.

7.4 AREAS FOR FUTURE RESEARCH

Additional research can be done using the detailed behavioural data collected in this thesis. For instance, one could examine whether high-risk contact rates vary with group size and composition (i.e., is the contact rate greater in large groups, and is the contact rate different in male, female or mixed-sex groups?). Moreover, as disease prevalence is predicted to decrease as the population is increasingly subdivided into defined social groups (Nunn et al., 2015b), future research looking at group stability (i.e., how often groups form and break, and what factors determine the rate of group fusion and fission) and modularity degree (i.e., is the population divided into many subgroups?), can help better understand disease dynamics within this mule deer population. Also, both association (determined by either spatial proximity or group memberships) and contact data could be used to construct social networks to identify individuals that play a key role for disease transmission, and if they share specific characteristic, such as sex, age, or even home range size.

Also, the extensive radio-telemetry dataset collected in this study allows for various relevant questions to be answered. For instance, if combined with geographical locations of points of interest, such as grain spills, waterholes and saltlicks, one could look at pairs that were frequently found <25 m apart despite having a very small home range overlap, and see if these attractive sites are points of congregation. Other studies combining genetic data could serve to answer 1) whether female mule deer population expands as a series of overlapping home ranges, 2) whether related deer, both males and females of any age, have greater spatial overlap than unrelated deer, and 3) whether home range overlap is greater in females than in males at various times of the year.

7.5 CONCLUDING REMARKS

Our findings strongly suggest that not considering the heterogeneities in mule deer social behaviour and the visitation by mule deer to small geographic areas across all sex and age classes can severely hamper our ability to understand CWD epidemics, evaluate its impact, and propose effective control strategies.

It is hard to use intuition alone to understand CWD transmission dynamics across several seasons and habitats, and among different sex and age classes; however, the findings and data generated during this dissertation can improve future epidemic dynamic models (Vynnycky and White, 2010; Potapov et al., 2013). Appropriately parameterized spatially- and behaviourally-explicit epidemiological models will be valuable tools to guide CWD management strategies.

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