THE ECOLOGY AND FUTURE DISTRIBUTION OF WEST NILE VIRUS IN THE CANADIAN PRAIRIE PROVINCES

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By

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Abstract

This thesis describes aspects of the ecology of West Nile virus (WNV) including factors contributing to the distribution of WNV, possible future distribution, and effects of WNV on host abundance in the Canadian prairies provinces: Alberta, Saskatchewan, and Manitoba.

Using mosquito surveillance data collected between 2005 and 2008, models integrating abiotic and biotic factors were constructed to predict the weekly and monthly scales of WNV infection rate in *Culex tarsalis*, which is the primary vector of WNV in the Canadian prairies.

At the weekly scale, the WNV infection rate in *Cx. tarsalis* increased with increasing *Cx. tarsalis* abundance and mean temperature lagged from 1 to 8 weeks, but decreased with an increasing mean precipitation lagged from 2 to 6 weeks. Furthermore, precipitation was a 'distorter variable' which altered the association between *Cx. tarsalis* abundance and the WNV infection rate.

Study at the monthly scale showed that higher mean temperature and time lagged mean temperature elevated were associated with increased numbers of *Cx. tarsalis* and higher WNV infection rates. However, increasing precipitation was associated with higher abundance of *Cx. tarsalis* and lower WNV infection rate. In addition, this study found that increased temperature fluctuation and wetland land cover were associated with decreased WNV infection rate in *Cx. tarsalis*.

Climate change could drive dramatic alterations in the spatial and temporal distribution and overall incidence of vector-borne diseases. The constructed models and biological thresholds were used to predict the distribution of *Cx. tarsalis* and WNV infection rate in the prairie provinces under a range of potential future climate and habitat conditions. In the current endemic regions, the projected WNV infection rate under the median outcome

scenario in 2050 was 18 times higher than under current climate conditions. Seasonal occurence of *Cx. tarsalis* infected with WNV extended from June to August to include May and September. Moreover, models predicted northward range expansion for *Cx. tarsalis* and WNV.

The declines of susceptible bird abundance caused by WNV may further influence the bird community composition and, in turn, affect the incidence of WNV through a dilution effect. The North American Breeding Bird Survey data was used to evaluate the effect of WNV on the abundance of selected birds in the Canadian prairies, as well as the effects of bird community composition on the WNV risk. There was no significant decline in bird abundances of selected birds following the emergence of WNV. These findings suggest that the effect of WNV on selected bird abundance and bird community composition is insignificant. In addition, there is no evidence to support the association between bird community composition and WNV infection rate in *Cx.tarsalis* in the Canadian prairies.

Lastly, findings in this thesis and current knowledge were integrated to create a decision making flowchart for the prevention of WNV infection in the prairie provinces.

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Dedication

I would like to dedicate this thesis to my family, especially…

To Mom and Dad for always be there for me

To old sister for your support, especially the financial part

僅將此論文獻給我的家庭

爸媽，謝謝您們永遠的關心與愛

老姐，謝謝妳的支持，尤其是當我經濟拮据時

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AB	Alberta
ADD	Accumulative degree days
AIC	Akaike information criterion
AICc	AIC value corrected for small sample size
RM-ANOVA	Repeated measures analysis of variance
Arbovirus	Arthropod-borne viruses
AVHRR	Advanced Very High Resolution Radiometer
B	Mean bias
BBS	North America Breeding Bird Survey
BCR	Bird Conservation Region
CBC	Christmas Bird Count
CCWHC	Canadian Cooperative Wildlife Health Centre
CFIA	Canadian Food Inspection Agency
CI	Confidence interval
Cx.	*Culex*
D	Vapour pressure deficit
DF	Degree of freedom
DIC	Deviance information criterion
EIP	Extrinsic incubation period
ENSO	EI Niño/Southern Oscillation
GCMs	General circulation models
GIS	Geographic information system
GLM	Generalized linear model

GLMM	Generalized linear mixed model
GPS	Global positioning system
IPCC	Intergovernmental Panel on Climate Change
IPCC-TGICA	Intergovernmental Panel on Climate Change Task Group on Data and Scenario Support for Impact and Climate Assessment
MB	Manitoba
MCMC	Markov Chain Monte Carlo
MIR	Minimum infection rate
ML-IR	Maximum Likelihood infection rate
NAO	North Atlantic Oscillation
NSC	WNV National Steering Committee
PET	Potential evapotranspiration
PHAC	Public health agency of Canada
R_0	Basic reproductive rate
RMSE	Root mean square error
RT-PCR	Reverse transcription polymerase chain reaction
SK	Saskatchewan
SLE	Saint Louis encephalitis
SRES	Special Report on Emissions Scenarios
WCVM	Western College of Veterinary Medicine
WEE	Western equine encephalitis
WNV	West Nile Virus

Chapter 1 Introduction

1.1 Background

West Nile virus (WNV) is a member of the genus *Flavivirus* classified in the family of Flaviviridae. Other viruses in the same genus include dengue virus, Japanese encephalitis virus, St. Louis encephalitis, yellow fever virus, and tick-borne encephalitis virus. Most *Flaviviruses* are transmitted by mosquitoes or ticks (Kuno et al. 1998). The virus was first isolated from a febrile woman in Uganda in 1937 (Smithburn et al. 1940). After the first identification of WNV, sporadic outbreaks were recorded in Africa, Eurasia, Australia, and the Middle East (Dauphin et al. 2004, Kramer et al. 2008).

The first outbreak of WNV in the Western Hemisphere occurred in the New York City area in 1999. WNV was isolated from human cases of encephalitis, mosquitoes, and multiple bird species in late summer (Lanciotti et al. 1999, Nash et al. 2001). Afterwards, WNV rapidly dispersed across most of North America, Mexico, Central America, the Caribbean, and South America (Komar and Clark 2006, Morales et al. 2006, Bosch et al. 2007, Artsob et al. 2009). Presently, WNV occurs in all continents except Antarctica.

In Canada, the first evidence of WNV activity was identified in an infected bird in Ontario in 2001; since then, the virus has spread across southern Canada (Drebot et al. 2003, Artsob et al. 2009, Public Health Agency of Canada 2013). The Canadian prairies provinces of Alberta,

Saskatchewan, and Manitoba generally have the highest number of human cases per capita in Canada since the incursion of WNV in 2002 into Manitoba and Saskatchewan and 2003 into Alberta; particularly important in 2003 and 2007, when explosive outbreaks of WNV occurred. Of the 2,315 reported human cases in Canada in 2007, more than 99% occurred in the prairie provinces (1285 from Saskatchewan, 578 from Manitoba, and 318 from Alberta) (Public Health Agency of Canada 2007).

A predictive risk model, clarifying the effects of abiotic and biotic factors on vector abundance and WNV risk, could be an important tool for risk prediction, as a foundation for public health intervention and wildlife disease management. In addition, models using environmental and climate factors as primary explanatory variables could be used to evaluate and predict the potential effects of climate change on the distribution of WNV in the future. However, due to large landscape differences in North America, the effects of abiotic and biotic factors on WNV may not be similar in different ecological regions and models developed in one region are not necessarily applicable for all of Canada or the North American continent. For example, the primary mosquito vector involved in the WNV transmission cycle in the Canadian prairies is *Culex tarsalis*, and in the eastern Canadian provinces it is *Cx. pipiens* (Curry 2004, Hongoh et al. 2012). Therefore, studies specifically focused on the prairie provinces, a highly endemic region, are needed to address regional concerns.

1.2 Investigative approach

In Canada, WNV surveillance programs including mosquito collection and screening for WNV were initiated as early as 2000 (Drebot et al. 2003). Mosquito infection rates in primary

vectors are commonly used to predict the risk of mosquito-borne diseases (Eldridge 1987) and have been demonstrated to be a more sensitive indicator for WNV risk than surveillance of dead or infected birds (Brownstein et al. 2004). The mosquito species *Culex tarsalis* Coquillett is considered to be the primary vector of WNV in the Canadian prairies.

The studies in this thesis were based on the investigation of WNV infection rate in *Cx. tarsalis* in three prairie provinces from 2005 to 2008, a study period that includes the largest outbreak year (2007) in North America to date. The objectives of this thesis were to explore the following aspects of the ecology of WNV transmission in the prairie provinces: i) the effects of environmental and biotic factors on the risk of WNV and to construct statistical models for predicting the spatial and temporal distribution of WNV; ii) the effect of WNV on the abundance of birds; iii) the potential effects of future climate change on the risk of WNV.

In this thesis, chapter 2 reviews the relevant literature for WNV distribution, disease ecology, vector ecology, and factors affecting the distribution of vector and vector-borne diseases. Chapter 3 describes the weekly variation of WNV infection in *Cx. tarsalis*. The study used data on the weekly variation of WNV infection rate to construct predictive models and generate risk maps of WNV in the Canadian prairies. Chapter 4 describes the monthly changes of *Cx. tarsalis* abundance, WNV infection rate in *Cx. tarsalis*, and various environmental variables contributing to the changes. The objectives of this study were to construct models for predicting the abundance and WNV infection rate of *Cx. tarsalis*. In Chapter 5, the monthly models were applied to evaluate the potential effects of climate change on the spatial and temporal distribution of *Cx. tarsalis* and WNV. Chapter 5 combines the models of *Cx. tarsalis* abundance, and WNV infection rate with projected future climates to predict the spatial and temporal distribution of *Cx.*

tarsalis and WNV infection rate in the prairie provinces. This study evaluated the possible effects of future climate conditions on the occurrence of WNV and *Cx. tarsalis*. Chapter 6 focused on the study of the relationship between bird host abundance/community and WNV transmission intensity in the Canadian prairies. This chapter evaluated the influence of WNV on the abundance and community composition of selected bird species, as well as the effects of bird community composition on WNV transmission intensity in its enzootic cycle. Chapter 7 provides the major conclusions from the studies in this thesis with possible future implications and research directions.

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Chapter 2 Literature review

2.1 Introduction

The presence of West Nile virus (WNV) in the Old World has had a long history (Smithburn et al. 1940), but it had never been considered as a significant pathogen for humans and animals until its incursion into the New York region in 1999 (Hayes 2001, Zeller and Schuffenecker 2004, Gerhardt 2006). Since the introduction of WNV into the Western Hemisphere, this virus has become a major public health, wildlife conservation and veterinary concern. It has caused unexpected cases of neurological disease and mortality in humans, horses, and birds (Hayes 2001) and rapidly spread across the Americas (Artsob et al. 2009). Understanding the risk factors that influence the distribution of WNV is important for minimizing the disease risks and implementing public health interventions, particularly in highly endemic regions such as the Canadian prairies.

The first part of this review presents the historical and current distribution of WNV in the Old and New World. The second part reviews the hosts and vectors involved in disease transmission cycle with an emphasis on the Canadian prairie region. The third part discusses the possible abiotic and biotic factors that may affect the distribution of arthropod vectors and WNV. Because climate can dramatically influence the distribution of vector and vector-borne diseases, this review also examines the potential effects of climate change on the distribution of vector-borne disease and WNV. In addition, the impact of WNV on the abundance of hosts and possible effects of biodiversity on the

distribution of vector-borne disease are discussed in this section. Finally, this review evaluates different surveillance components, spatial analyses and statistical models as tools for predicting the risks of WNV infection.

2.2 Historical and current distribution

WNV is currently the most widespread arthropod-borne virus (arbovirus) in the world (Kramer et al. 2008). This virus was first isolated in the blood of a febrile woman in Uganda in 1937 (Smithburn et al. 1940). Since then, sporadic cases and major outbreaks of WNV in humans and horses have been recorded in Africa, Europe, Asia, Australia (Kunjin virus, subtype of WNV), and the Middle East (Scherret et al. 2001, Dauphin et al. 2004, Kramer et al. 2008). Serological surveys after the isolation of WNV in Africa revealed exposure to the virus was widely distributed in native human populations of Uganda, Kenya, the Belgian Congo (former title of Democratic Republic of the Congo), Sudan, and Egypt (Smithburn and Jacobs 1942, Melnick et al. 1951, Smithburn 1952).

Initially, WNV infection was considered as a non-symptomatic or mild disease linked to mild flu-like symptoms in humans (Zeller and Schuffenecker 2004). A few outbreaks of WNV-induced encephalitis affecting humans and horses were recorded in Israel in the 1950s, and then in France from 1962 to 1963 (Murgue et al. 2001a, Weinberger et al. 2001). However, more frequent outbreaks of WNV and increasing numbers of cases with severe encephalitis and neurological symptoms, especially in the Mediterranean Basin, have been reported since the 1990s (Murgue et al. 2001a, Kramer et al. 2008). Major outbreaks of human WNV infection in the Old World included the epidemics of Algeria in 1994 (Le Guenno et al. 1996), Romania in 1996 (Tsai et al. 1998), Tunisia in 1997 (Triki et al. 2001), Russia in 1999 (Platonov et al. 2001), and Israel in 2000 (Siegel-Itzkovich

2000). Outbreaks of WNV in horses were also reported in Morocco in 1996 (Tber 1996), Italy in 1998 (Autorino et al. 2002), France in 2000 (Murgue et al. 2001b), and Israel in 2000 (Steinman et al. 2002). Currently, WNV continues to circulate in endemic regions, but has also expanded into new geographic regions (Danis et al. 2011, Reusken et al. 2011).

The first isolation of WNV in the Western Hemisphere was from an outbreak of human encephalitis in the New York City area in late summer of 1999 (Lanciotti et al. 1999, Nash et al. 2001). Since then, WNV rapidly dispersed across most of North America, Central America, the Caribbean, and South America (Komar and Clark 2006, Morales et al. 2006, Bosch et al. 2007, Artsob et al. 2009). By the end of 2000, WNV had dispersed southward from the New York City and distributed over a large area of the Atlantic coastal region. Moreover, it continued spreading southward into the Caribbean and Central America (Drebot et al. 2003, Fernández-Salas et al. 2003, Ulloa et al. 2003, Komar and Clark 2006). In 2001, this virus spread westward and northward from the Atlantic and Gulf coastal regions to the Mississippi in the United States and the Great Lakes region in Canada. In 2002, WNV dispersed explosively across most of North America, southern Mexico and the Caribbean islands of Jamaica, Hispaniola and Guadeloupe (Dupuis et al. 2005, Komar et al. 2005b, Lefrancois et al. 2006). In 2004, seropositive equines were reported in northern Columbia, which was the first evidence of WNV activity in South America (Mattar et al. 2005). Later, WNV activity was detected in Venezuela (Bosch et al. 2007), Brazil (Melandri et al. 2012), and Argentina (Morales et al. 2006).

In Canada, WNV surveillance programs were initiated in some regions as early as 2000, after the outbreak in New York City. The first evidence of WNV activity in Canada was identified in an infected bird found dead in Ontario in 2001. Subsequently, the virus

spread westward to the provinces of Manitoba, and Saskatchewan in 2002, Alberta in 2003, and British Columbia in 2009. It also spread eastward to the provinces of Quebec and Nova Scotia in 2002, and New Brunswick in 2003 (Artsob et al. 2009, Roth et al. 2010, Public Health Agency of Canada 2013). By 2010, WNV had been documented in mosquitoes, birds, humans, and other mammals across southern Canada (Drebot et al. 2003, Artsob et al. 2009, Public Health Agency of Canada 2013). The Canadian prairies, which is primarily a grassland ecozone located in the southern part of provinces of Saskatchewan, Manitoba and Alberta (Figure 2.1), generally has had the highest human incidence of WNV in Canada since the incursion of WNV, sepcifically in 2003 and 2007 (Table 2.1).

Phylogenetic studies indicated that WNV strains can be divided into two major lineages and other additional minor lineages (Pesko and Ebel 2012). Lineage 1 is the most widely distributed worldwide and can be further divided into 3 clades: 1a, 1b, and 1c (May et al. 2011). Clade 1a is distributed in Africa, Europe, the Middle East, Russia, and the Americas. Clade 1b is a subset of WNV named Kunjin virus, which is distributed in Australia, while the virus in India is classified as Clade 1c (Scherret et al. 2001, May et al. 2011). Lineage 2 was originally distributed in sub-Saharan Africa and Madagascar. However, in 2004, WNV lineage 2 was isolated from birds with neurological disease in Hungary (Erdélyi et al. 2007). This was the first record of lineage 2 WNV inducing neuroinvasive disease in birds (Erdélyi et al. 2007). Since then, outbreaks in humans and birds associated with lineage 2 have been found in many countries in Europe (Bakonyi et al. 2006, Vázquez et al. 2010, Papa et al. 2011).

Lineage 1 were previously considered to have higher virulence than Lineage 2 because the highly virulent strains isolated from North Africa, Europe, Asia, and North

America were all members of lineage 1(Burt et al. 2002, Venter and Swanepoel 2010). However, the number of neuroinvasive disease cases in humans, horses and birds caused by lineage 2 WNV has recently been found to be increasing in both Africa and Europe (Burt et al. 2002, Bakonyi et al. 2006, Erdélyi et al. 2007, Vázquez et al. 2010, Venter and Swanepoel 2010, Papa et al. 2011) (Figure 2.2). Phylogenetic studies also indicate that the pathogenicity of WNV is genotype specific but not related to lineage or geographic distribution (Beasley et al. 2002, Burt et al. 2002).

2.3 Mechanisms contributing to the spread of WNV in the Western Hemisphere

Several mechanisms have been postulated to contribute to the rapid spread of WNV throughout the Western Hemisphere. These mechanisms include: 1) sufficient abundance of competent vectors and hosts; 2) changes in the WNV genome leading to transmissibility; 3) spread by bird migration.

In the enzootic cycle of WNV, virus primarily circulates between local avian fauna and ornithophilic mosquito vectors (Work et al. 1955, Taylor et al. 1956). Many bird species in the order of Passeriformes, such as house finches, American crows, black-billed magpies, house sparrows, and American robins, are highly competent for WNV amplification and transmission (Komar et al. 2003, Komar et al. 2005a). Most of these competent bird species are relatively abundant and widely distributed (Dunn and Alderfer 2011). Similarly, many mosquito species, especially the species of *Culex*, have been found to be highly capable of amplifying and transmiting WNV in the different regions of the Western Hemisphere (Turell et al. 2001, Turell et al. 2002, Turell et al. 2005). The wide selection and abundance of competent hosts and vectors for WNV allow the localized establishment of WNV when introduced to a new region.

The 1999 North American WNV strain (strain named NY99) most closely resembles a strain from a domestic goose isolated in Israel in 1998 (99.8% identical) (Lanciotti et al. 1999). These strains were highly virulent to birds and caused unexpected neuroinvasive disease resulting in high mortality (Lanciotti et al. 1999, Malkinson et al. 2002). In 2001, a new subtype of WNV emerged in the North America, namely WN02 (Ebel et al. 2004, Davis et al. 2005). This newly emerged genotype rapidly displaced the NY99 strain in the New World. It showed a shorter extrinsic incubation period in vectors that led to more cycles of amplification and thus to a higher infection rate in avian competent hosts. Laboratory experiments also indicated that the infection and transmission rates in *Cx. pipiens* (main vector in eastern North America) and *Cx. tarsalis* (main vector in western North America) were higher for WN02 than for NY99 (Kilpatrick et al. 2008). These changes in genotype may have promoted rapid spread of the WN02 strain across the continent (Ebel et al. 2004, Moudy et al. 2007, Snapinn et al. 2007).

There are more than 300 bird species migrating between North and Central or South America (Reed et al. 2003). Experimental infection of migratory birds has shown that WNV infection does not inhibit migratory behavior; in turn, migratory status does not affect WNV titres in infected birds (Owen et al. 2006). Therefore, migratory birds might be a vehicle to spread WNV (Malkinson et al. 2002). The dispersion pattern of WNV in the Western Hemisphere was consistent with an elliptical migration route of many songbirds along the Atlantic coastal region during the fall migration and a more inland route during the spring migration (Rappole et al. 2000, Reed et al. 2003). Other endemic or short distance dispersing bird species might also contribute to the expansion. For example, the common grackle, which is a highly gregarious and mobile species, migrates between the Rocky Mountains and the east coast. It has been suggested as a principal species which

contributed to the westward dispersion of WNV across North American during 2002 (Artsob et al. 2009).

2.4 Ecology of WNV transmission

Philip and Smadel (1943) first demonstrated experimentally that the virus can be transmitted by a mosquito species namely *Aedes albopictus*. Other competent mosquito species, including *Culex* (*Cx*.) *pipiens* var. *pallens* and *Cx. tritaeniorhynchus*, were later identifed (Kitaoka 1950). Subsequently, studies conducted in Egypt from 1952 to 1954 clarified that WNV is primarily transmitted and amplified in the enzootic cycle between birds and ornithophilic mosquitoes, with occasional spillover into mammals through blood feeding of mosquitoes (Figure 2.3) (Work et al. 1955, Taylor et al. 1956).

Mammals, such as horses and humans, are considered dead-end and incidental hosts that cannot amplify WNV to a sufficient viral titre to maintain the transmission cycle. However, WNV can induce viremia and result in clinical signs in certain mammals (Bunning et al. 2002, Abutarbush et al. 2004, Ratterree et al. 2004). Other transmission routes for WNV in humans include intrauterine transmission (Hayes and O'Leary 2004), breast milk (Hayes and O'Leary 2004), blood transfusion, organ transplantation, percutaneous inoculation, and aerosol exposure (Nir et al. 1965, Hayes and O'Leary 2004, Hayes et al. 2005). In addition, vertical transmission in mosquitoes and horizontal transmission in amplifying birds occur and are postulated as potential overwintering mechanisms for WNV in temperate regions (McLean et al. 2001, Nasci et al. 2001, Banet-Noach et al. 2003, Goddard et al. 2003, Reisen et al. 2006a).

WNV has been isolated from many mosquito species worldwide, nevertheless only a small number are considered to be competent vectors (Hayes et al. 2005). *Culex*

mosquitoes are the primary vectors of WNV, such as *Cx. pipiens* and *Cx. modestus* in Europe (Hubàlek and Halouzka 1999, Balenghien et al. 2006), *Cx. univittatus* in Africa and the Middle East, and *Cx. vishnui* in Asia (Hubàlek and Halouzka 1999). There have been at least 59 WNV infected mosquito species identified in North America (Hayes et al. 2005, Turell et al. 2005). The primary vectors in the northeastern United States are *Cx. pipiens* and *Cx. restuans* (Andreadis et al. 2001, Hayes et al. 2005, Kramer et al. 2008) and *Cx. tarsalis* and *Cx. quinquefasciatus* in the southwestern United States (Bell et al. 2005, Hayes et al. 2005).

In Canada, mosquito surveillance programs have found ten species of mosquitoes infected with WNV in Ontario, Quebec and Manitoba between 1999 and 2002, with over 80% of these being *Cx. pipiens*, *Cx. restuans* or a mixture of both (Drebot et al. 2003). In the Canadian prairies, *Cx. tarsalis* is the primary vector for WNV (Curry 2004, Yiannakoulias et al. 2006) (Figure 2.4). Grassland and farm land are the habitats for *Cx. tarsalis* (Jenkins 1950, Chuang et al. 2011). The boreal forest transition zone is identified as the northernmost limit of *Cx. tarsalis* and WNV distribution in western North America (Curry 2004). Besides WNV, *Cx. tarsalis* is the primary competent vector for St. Louis encephalitis and western equine encephalitis in western Canada (Artsob 2000, Curry 2004, Hongoh et al. 2009).

Culex tarsalis is one of the most efficient WNV vectors evaluated in laboratory studies (Turell et al. 2005) and the predominant vector in the Canadian prairies during the summer WNV season (Curry 2004). Several biological features of *Cx. tarsalis* facilitate the transmission of WNV in enzootic cycles. *Culex tarsalis* can vertically transmit WNV to its offspring (Goddard et al. 2003). It takes several blood meals and produces multiple generations per season in southern Canada (Curry 2004). Furthermore, it is known to feed

on both avian and mammalian hosts and plays the role of the "bridge vector" for spillover of WNV out of its enzootic cycle and into humans and other mammalian species (Tempelis et al. 1965, Kent et al. 2009).

Birds are the primary vertebrate hosts of WNV. A variety of bird species, including species in the orders Passeriformes (song birds), Charadriiformes (shorebirds), Strigiformes (owls), and Falconiformes (hawks), develop a sufficient WNV viremia to infect feeding mosquitoes (Komar et al. 2003). However, species of Columbiformes (pigeons), Piciformes (woodpeckers), and Anseriformes (ducks) do not amplify WNV well (Komar et al. 2003). Other vertebrate species considered to be suitable competent hosts are alligators (*Alligator mississippiensis*) and lake frogs (*Rana ridibunda*) (Kostiukov et al. 1985, Klenk et al. 2004).

Although birds are hosts for WNV, impacts of WNV on abundance of birds have also been observed, especially for Corvids, which are highly susceptible to WNV infection (Komar et al. 2003, Marra et al. 2004). Bird mortality due to WNV infection was first identified in wild migrating white storks and a flock of domestic geese in 1998 in Eilat, Israel (Malkinson et al. 2002). Although, serological evidence had shown the presence of antibodies in wild birds in many countries of Europe, Africa, and Asia, this was the first record of WNV induced mortality in wild birds (Savage et al. 1999, Malkinson et al. 2002). At least 326 bird species have been reported with WNV infection (Centers for Disease Control and Prevention; available from http://www.cdc.gov/ncidod/dvbid/westnile/birdspecies.htm, accessed 28 August 2012), as well as other mammals, reptiles, and amphibian species (Marra et al. 2004).

WNV has caused declines in abundance of birds (Marra et al. 2004, Naugle et al. 2004, LaDeau et al. 2007), such as American crow (*Corvus brachyrhynchos*), greater sage-

grouse (*Centrocercus urophasianus*), and American white pelican (*Pelecanus erythrorhynchos*) (Caffrey et al. 2003, Marra et al. 2004, Naugle et al. 2004, Yaremych et al. 2004, Sovada et al. 2008). Specifically, studies of the effects of WNV on bird abundance using long term survey data, such as North American Breeding Bird Survey (BBS) or Christmas Bird Count (CBC), have revealed significant declines abundance of several bird species (LaDeau et al. 2007, Foppa et al. 2011). Corvids, including American crow, black-billed magpie, blue jay, and common raven, were the main bird species submitted for WNV screening in the dead bird surveillance program in Canada (Zimmer 2005). In this surveillance program, the American crow accounted for 70% of the positive dead birds submitted from 2003 to 2005. Moreover, raptor species were also commonly found to be affected by WNV in Canada (Zimmer 2005).

2.5 Factors influencing arthropod vectors and WNV distribution

Abiotic factors, such as temperature and precipitation, and biotic factor, such as habitat type, are known to be important factors which determine the distribution of both vectors and vector-borne diseases in the environment (Reisen 1995, Reisen et al. 2006b, Reisen et al. 2008, Chuang et al. 2011) (Figure 2.5). In addition, these ecological factors affect the seasonal and spatial overlap among key hosts in the sylvatic amplification cycles. For instance, drought-induced concentration of mosquitoes and birds on shrinking wetland habitats may enhance the transmission of WNV (Wang et al. 2010). Climate contributes to the distribution of vector-borne diseases in the following three ways: a) reproduction, maturity and survival of vectors, which in turn drive the distribution and abundance of vectors; b) blood seeking activity of arthropod vectors; and c) rates of

pathogen amplification (development and/or multiplication) within vectors (Gubler et al. 2001, Hunter 2003).

Increasing environmental temperature shortens the maturation time required for both the vector and the extrinsic incubation period of virus within the vector (Reisen 1995, Reisen et al. 2006b). Furthermore, increasing temperature accelerates the gonotrophic cycle of vector and decreases mosquito survival rate. These mechanisms collectively affect WNV and vector-borne diseases transmission by influencing the contact rate between hosts and vectors and viral transmission rate per contact (Reisen et al. 2006b, Zou et al. 2007).

Climate changes are expected to influence the distribution of both vectors and vector borne pathogens, and contribute to the expansion or shifting of endemic regions (Brownstein et al. 2005, Lafferty 2009, Ostfeld 2009a). In addition, it is possible that climate change affects vector-borne diseases indirectly through effects on habitat, vegetation and host community composition (Lafferty 2009, Ostfeld 2009a) (Figure 2.5).

The effect of precipitation on the WNV enzootic cycle is paradoxical. Increased precipitation creates standing water suitable for mosquito breeding and thereby increases mosquito abundance. In contradiction, other studies have demonstrated that preceding droughts can increase the incidence of WNV in the western United States (Landesman et al. 2007) and the Canadian prairies (Epp et al. 2009). Possible explanations of drought-increased WNV incidence include the decrease of mosquito competitors or predators and the congregation of avian hosts and mosquito vectors on wetland habitat, as well as changes in the composition of the avian host community (Chase and Knight 2003, Shaman et al. 2005, Wang et al. 2010).

Ezenwa et al. (2007) found a negative association between WNV infection rate of

Culex mosquitoes and wetland coverage. Wetland coverage was positively associated with bird diversity and thus it was possible that this represents an example of the "dilution effect", in which increased bird diversity led to overall decreases in the mosquito infection rate (Ezenwa et al. 2006, Ezenwa et al. 2007). Biodiversity has been hypothesized to affect vector-borne diseases, including WNV, through a "dilution effect" (Ostfeld and Keesing 2000a, Keesing et al. 2006, Ostfeld 2009b). The mechanisms by which increasing biodiversity decreases the vector-borne disease transmission include reducing the contact rate between competent host and vectors, reducing the probability of transmission per contact, regulating the population density of competent hosts, increasing the death rates of infected individuals, facilitating the recovery from infection, and reducing vector density (Ostfeld and Keesing 2000b, Schmidt and Ostfeld 2001, Keesing et al. 2006).

Contact between infected competent vectors and hosts is essential for maintaining the enzootic cycle of an arbovirus in a geographic area; in other words, a minimum threshold of vector and host interaction is needed to allow for virus transmission (Reeves 1965). Therefore, abundance of primary competent vectors is usually an indicator for the risks of vector-borne diseases (Reeves 1965, Saugstad et al. 1972, Murray 1995). A positive association between vector abundance and vector-borne disease has been demonstrated for other arboviruses such as Japanese encephalitis, western equine encephalitis and St. Louis encephalitis (Reeves 1965, Pant 1972, Wegbreit and Reisen 2000, Arunachalam et al. 2009). Furthermore, vector abundance of *Cx. tarsalis*, *Cx. p. quinquefasciatus*, *Cx. pipiens* and *Cx. restuans* has been used as an indicator to predict human WNV risks in different regions (Kilpatrick et al. 2005, Reisen et al. 2009, Kwan et al. 2010).

Vector community composition also plays an important role in the transmission of vector-borne diseases. For example, *Cx. restuans* is the most abundant *Culex* species in June and July in the Connecticut region of the United States, in contrast to *Cx. pipiens*,

which has higher abundance in August and September (Andreadis et al. 2001, Kunkel et al. 2006). In this region, WNV infected *Cx. restuans* may play an important role in the transmission of WNV among wild birds in early summer and *Cx. pipiens* may amplify the virus later in the summer (Andreadis et al. 2001). Feeding preference of vector species is also an important factor which determines the contact among birds and vectors and affects WNV transmission (Molaei et al. 2006). Ornithophilic vectors are enzootic vectors; whereas, generalists that feed on a higher proportion of mammals are considered bridge vectors, which spread WNV from its enzootic cycle to dead-end hosts such as humans and horses (Kilpatrick et al. 2005, Molaei et al. 2006). However, for many vector species, host selection is not fixed and could be changed in different season. A shift in feeding preference was found in *Culex tarsalis* and *Culex nigripalpus*, from primary ornithophilic in the early transmission season to general feeder (mammals and birds) in the late summer (Tempelis et al. 1965, Edman and Taylor 1968). Such a change of blood-feeding behavior may contribute to WNV spillover into mammals (Kent et al. 2009).

2.6 WNV surveillance and risk prediction

The objective of disease surveillance for public health purposes is to monitor the existing epidemiological situation and possibly predict the likelihood of human disease outbreaks, thus permitting interventions for such outbreaks (Eldridge 1987). Many surveillance components have been developed as indicators for arbovirus activity and risk prediction, such as weather data, vector density, infection rate in vectors, seroconversion of sentinel hosts, and human cases (Eldridge 1987). These components were also commonly adopted for WNV surveillance (Eidson et al. 2001, Drebot et al. 2003, Corrigan et al. 2006, Chuang et al. 2011, Chen et al. 2012, Chuang et al. 2012). Many of these components provided periods of time prior to the outbreaks of human cases which allowed

the intervention measures to be implemented prior to the outbreaks (Eldridge 1987, Nielsen et al. 2008). However, the sensitivity for each component as a predictor of diseases risks was different. In order to produce accurate forecasts of disease risks, a combination of predictors is recommended (Eldridge 1987). As compared to WNV infection in dead birds, WNV infection rate in vectors has been demonstrated to be a better predictor of WNV epidemics in people (Brownstein et al. 2004). However, WNV-infected birds can be used as an early warning for WNV activity in a region (Eidson et al. 2001).

Following the introduction of WNV into the Western Hemisphere in 1999, Health Canada established the WNV National Steering Committee (NSC) whose primary mandate was to develop national guidelines, coordinate the WNV National Surveillance Program and respond to possible introduction of WNV into Canada (Drebot et al. 2003). NSC was comprised of individuals from various organizations and disciplines, including representatives from numerous branches or centers in Health Canada, other federal agencies such as the Canadian Food Inspection Agency (CFIA), Environment Canada, and provincial ministries of health. In addition, nongovernmental organizations such as the Canadian Cooperative Wildlife Health Centre (CCWHC), Canadian Blood Services, and individuals from academia were recruited to serve on the Committee. Surveillance focused on birds, mosquitoes, horses and humans in order to monitor WNV activity in Canada. Through the various surveillance programs, the incursion and spread of WNV in Canada was under constant monitoring. In addition, these surveillance programs, especially for the abundance and WNV infection rate of mosquitoes, could be used to forecast the risks of WNV (Drebot et al. 2003). Data collected in the surveillance programs provided further opportunities to explore the relationships between various abiotic and biotic drivers of the ecology of WNV.

Various spatial tools and analyses are useful in detecting patterns of vector-borne

diseases distribution and predicting disease risks. These tools include geographic information system (GIS), global positioning system (GPS), remote sensing, and spatial statistics (Kitron 1998). The distribution of vector-borne diseases usually presents strong spatial patterns which are associated with or affected by various ecological processes, such as weather or habitat. Mapping of the spatiotemporal dynamics of disease facilitates the identification of factors that govern the spatial pattern and the rate of disease spread (Ostfeld et al. 2005). GIS is a technique to store, retrieve, manipulate, analyze, and output data collected from various sources with spatial attributes, such as GPS. In addition, GIS can be used to predict the risks of vector-borne diseases in unsampled regions or future time periods by incorporating geostatistical methods (Bunnell et al. 2003, Ostfeld et al. 2005).

Remote sensing techniques are used to obtain information of objects without making physical contact with the object (Campbell 2011). Although remote sensing techniques cannot measure the vector or vector-borne diseases directly, many techniques have been used to characterize the environment or habitat for vectors (Kalluri et al. 2007). Environmental factors related to vectors or vector-borne diseases such as land use, vegetation, and climate can be measured from space and used for predictive model construction (Rogers 2006). By utilizing GIS and remote sensing data, epidemiologists can examine the spatial and/or temporal clusters (distribution pattern), identify environmental risk factors, and predict disease risks (Rogers and Randolph 2003). These techniques have been widely implemented in studies of vector-borne diseases such as malaria, Lyme disease, eastern equine encephalitis, and WNV to predict the distribution vector-borne diseases (Kitron and Kazmierczak 1997, Kleinschmidt et al. 2000, Moncayo et al. 2000, Ostfeld et al. 2005, Chuang et al. 2012).

2.7 Conclusions and research needs

This literature review has summarized many aspects of vector-borne diseases with an emphasis on WNV distribution, transmission cycle, surveillance programs, and the tools to predict the risks for human and animal health. However, there are still many epidemiological and ecological questions regarding WNV and its mosquito vectors in the Canadian prairies. What is the spatial and temporal distribution of WNV in this grassland ecozone region? What kind of factors may influence this distribution? What kind of factors may influence the distribution and abundance of the primary vector, specifically *Cx. tarsalis*? Does WNV decrease the abundance of its hosts? Do the effects of WNV on the abundance of susceptible bird species change the bird species diversity or community composition? Does bird diversity or community composition influence the WNV incidence? Lastly, will anthropogenic environmental alterations, such as climate and landscape change, influence the distribution of WNV? Although many statistical models have been constructed for predicting disease risks of WNV in North America since its incursion, these models usually cannot be applied to predict risks in other regions, particularly when those regions have different ecological dynamics or primary vector species (Brooker et al. 2002).

To address these questions, the objectives of this thesis are to evaluate the effects of abiotic and biotic factors on the distribution of WNV and *Cx. tarsalis*, as well as the relationship between WNV and its bird hosts. Understanding the effects of these factors could further facilitate predictive model construction to identify areas with high risk of WNV. In addition, predictive models can be used as a tool to evaluate the effects of climate change. Those are critical information for disease intervention and mitigating the impacts of WNV on humans, wildlife, and environment in the most highly endemic region in Canada in the current and future era.

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Table 2.1 Total human total cases and incidence of West Nile virus in each province and territory of Canada from 2003 to 2010.

Provinces and territorries	2003		2004		2005		2006		2007		2008		2009		2010	
	Cases	Incid.[C]	Cases	Incid.	Cases	Incid.	Cases	Incid.	Cases	Incid.	Cases	Incid.	Cases	Incid.	Cases	Incid.
Newfoundland and Labrador	0	0.00	0	0.00	0	0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Prince Edward Island	0	0.00	0	0.00	1[b]	0	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Nova Scotia	2[b]	0.21	0	0.00	1[b]	0.11	0	0.00	1[b]	0.11	0	0.00	0	0.00	0	0.00
New Brunswick	1[b]	0.13	0	0.00	1[b]	0.13	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Quebec	17	0.23	3[a]	0.04	4	0.05	1	0.01	2[b]	0.03	2	0.03	1	0.01	0	0.00
Ontario	893[a]	7.35	13[a]	0.11	95[a]	0.76	42[a]	0.33	12[a]	0.09	3	0.02	4	0.03	1	0.01
Manitoba	142[a]	12.26	3	0.26	55	4.68	50	4.24	578	48.70	12	1.00	2	0.17	0	0.00
Saskatchewan	937[a]	94.05	5[a]	0.50	58[a]	5.82	19[a]	1.91	1285[a]	129.26	17[a]	1.69	1	0.10	2	0.19
Alberta	272[a]	8.62	1[b]	0.03	10[a]	0.31	39[a]	1.16	318[a]	9.16	1[b]	0.03	2	0.06	1	0.03
British	20[b]	0.49	0	0.00	0	0.00	0	0.00	19[b]	0.44	1[b]	0.02	3	0.07	1	0.02

Columbia															
Yukon	1[b]	3.28	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Northwest Territories	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Nunavut	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00

a: Total cases include some cases related to travel outside the province/ territory; b:All related to travel outside the province/territory; c: Incid.: Human incidence (cases per 100,000 individuals).

Human incidence of each year is estimated by dividing total cases by population. Population data of each province/ territory is accessed from Statistics Canada (http://www5.statcan.gc.ca/bsolc/olc-cel/olc-cel?catno=91-209-XWE&lang=eng#formatdisp; accessed on February, 2013).

WNV cases data is accessed from Public Health Agency of Canada (http://www.phac-aspc.gc.ca/wnv-vwn/; accessed on February, 2013).

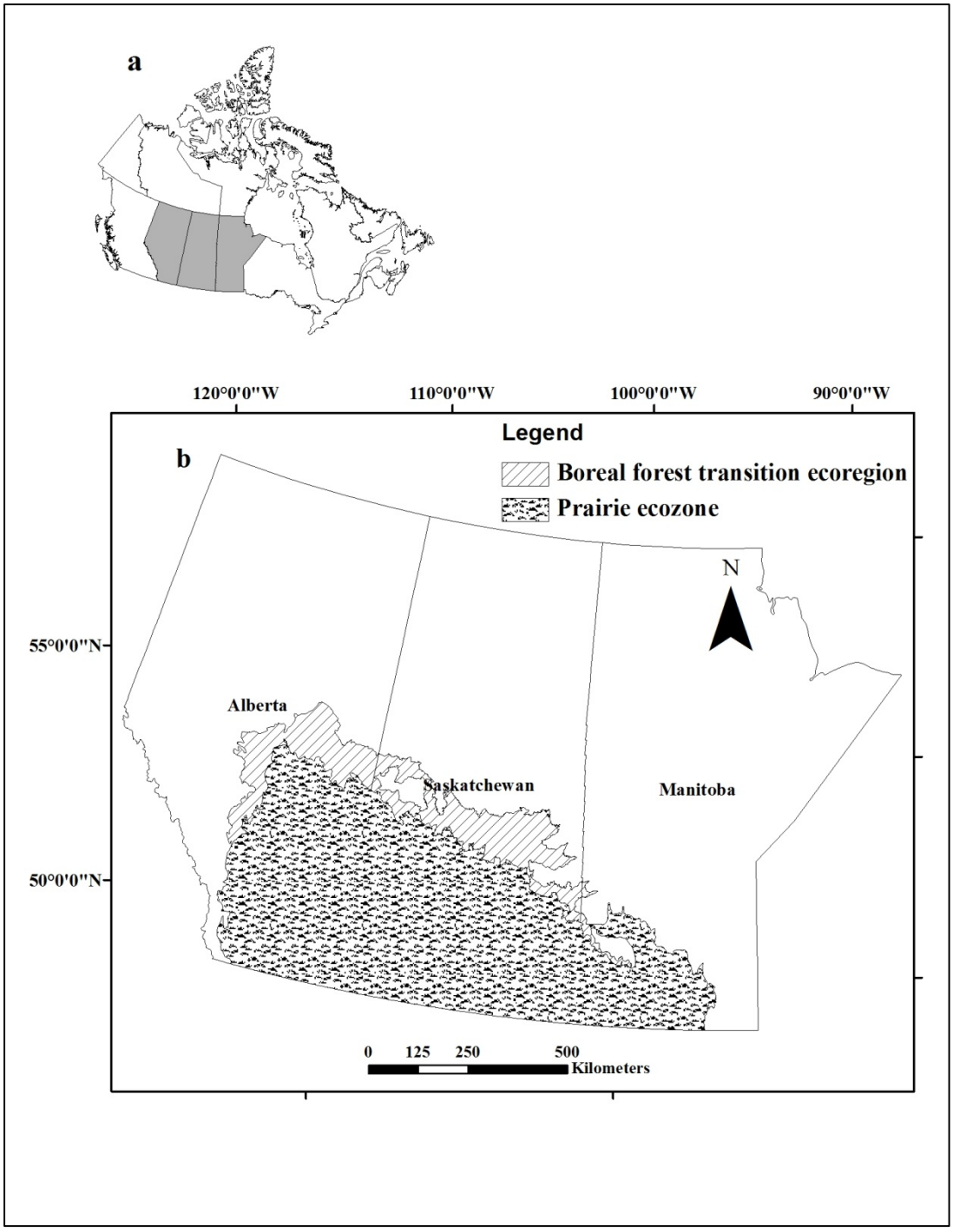

Figure 2.1 The distribution of prairie ecozone in the prairie provinces (provinces of Alberta, Saskatchewan, and Manitoba), Generally, this region has the highest incidence of West Nile virus infection since its incursion into Canada. a.Grey color indicates location of prairie provinces within in Canada; b. Enlargement of Prairie provinces showing distribution of prairie ecozone.

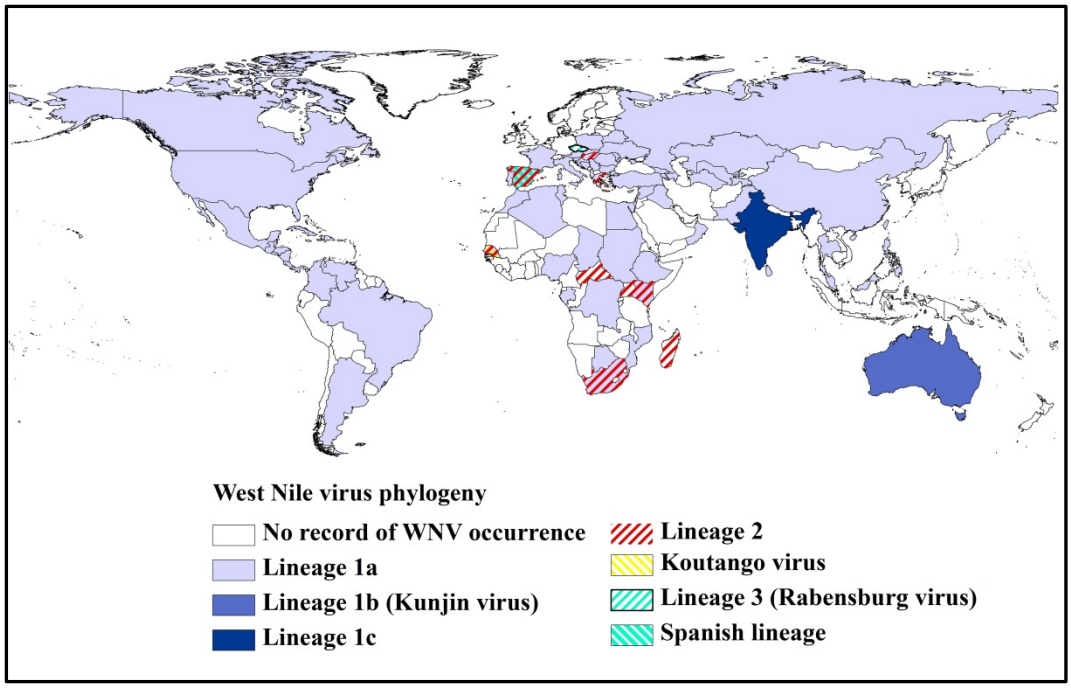

Figure 2.2 Worldwide distribution (January 2013) of different West Nile virus lineages. The distribution of different WNV lineages is based on May et al. 2001; Peso and Ebel 2012 ; Hubàlek and Halouzka 1999; European Centre for Disease Prevention and Control; and literature cited in the text.

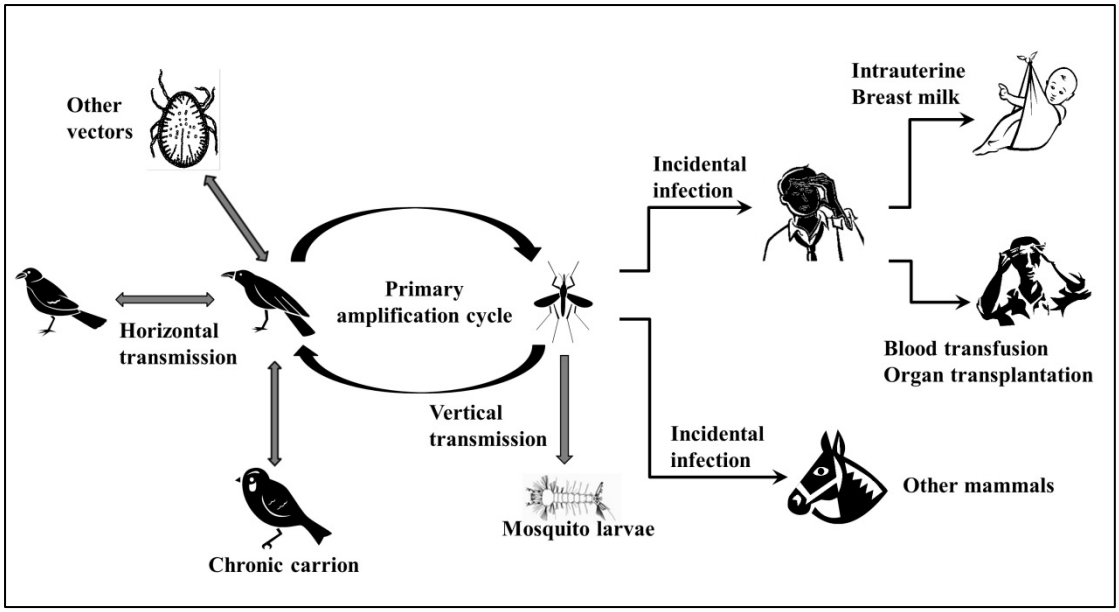

Figure 2.3 The transmission cycle of West Nile virus.

Figure 2.4 Approximation of the geographical distribution of *Culex tarsalis* in North America (Darsie Jr and Ward 2005). AB (Alberta), SK (Saskatchewan), and MB (Manitoba) indicate the three prairie provinces in Canada.

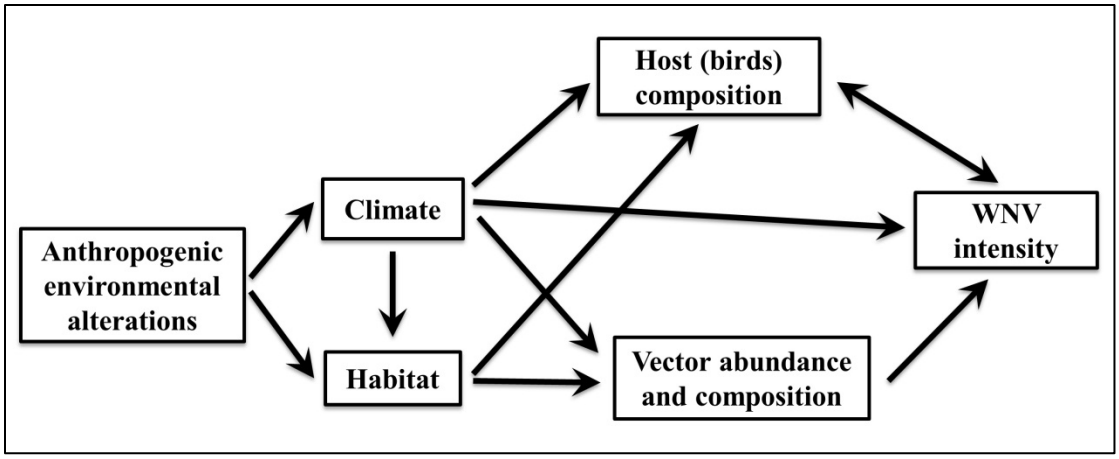

Figure 2.5 Diagram of possible causal relationships between abiotic and biotic factors, and vector-borne diseases, with WNV as an example.

Chapter 3 Predicting weekly variation of *Culex tarsalis* (Diptera: Culicidae) infected with West Nile virus in a newly endemic region, the Canadian prairies[1]

Preface

This chapter describes the weekly variation of WNV infection in *Cx. tarsalis*. The study used data on the weekly variation of WNV infection rate to construct predictive models and generate risk maps of WNV in the Canadian prairies.

3.1 Introduction

The first outbreak of West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) in the Western Hemisphere was in New York City in 1999 (Nash et al. 2001). WNV then rapidly dispersed across most of North America (Artsob et al. 2009). In Canada, WNV surveillance programs were initiated soon after the outbreak in New York City, and included surveillance of wild birds, sentinel chicken flocks, mosquitoes, horses, and humans. The first evidence of WNV in Canada was an infected bird found in Ontario in 2001, after which the virus continued to spread westward. By 2010, WNV had been documented in mosquitoes, birds, humans, and other mammals across southern Canada (Drebot et al. 2003, Yiannakoulias et al. 2006, Artsob et al. 2009). The Canadian prairies (provinces of Saskatchewan, Manitoba and Alberta) had the highest human disease incidence in Canada, especially during 2007. During

[1] Chen, C.-C., T. Epp, E. Jenkins, C. Waldner, P. S. Curry, and C. Soos. 2012. Predicting weekly variation of *Culex tarsalis* (Diptera: Culicidae) West Nile virus infection in a newly endemic region, the Canadian prairies. J. Med. Entomol. 49: 1144-1153.

this epidemic year, a total of 2,215 WNV cases were reported in prairie provinces, including 1,285 from Saskatchewan, 578 from Manitoba, and 318 from Alberta (Public Health Agency of Canada 2007).

WNV is spread and amplified between competent bird species and ornithophilic mosquito vectors, with occasional spillover into the mammals (Work et al. 1955, Taylor et al. 1956). WNV has been isolated from at least 59 mosquito species in North America (Hayes et al. 2005, Turell et al. 2005); however, mosquitoes of the genus *Culex* are considered the primary vectors of WNV worldwide (Hubàlek and Halouzka 1999, Kramer et al. 2008). In the Canadian prairies, *Culex tarsalis* Coquillett is considered to be the main vector of WNV based on several biological and ecological features which facilitate efficient transmission (Curry 2004, Yiannakoulias et al. 2006). This species is capable of transmitting WNV vertically to its progeny (Goddard et al. 2003), and feeds on both avian and mammalian hosts (Tempelis et al. 1965). In addition to high vector competence (Goddard et al. 2002, Turell et al. 2002), *Cx. tarsalis* is the most abundant species in the Canadian prairies during the summer transmission period (Curry 2004). The Canadian prairies, located on the north end of the Great Plains, are an ecosystem with unique climate, habitat, fauna, flora, and land use. Furthermore, based on temperature limitations, the Canadian prairies are also the northernmost limit of WNV distribution in the North America. Temperature also limits the northern distribution of other vectors, such as *Culex pipiens* complex, in this region (Mattingly et al. 1951, Hongoh et al. 2012). Consequently, the pattern of WNV occurrence in this area might differ from the southern parts of the Great Plains or more urbanized ecosystems. This research focused on the relationship between WNV occurrence and environmental variables in the prairie ecosystem, where there is a critical need for risk prediction and understanding the potential effects of

climate change on this newly emerging vector borne disease.

Environmental variables including temperature, precipitation, avian species diversity, and land cover are key predictors of the WNV mosquito infection rate (Ezenwa et al. 2006, Ezenwa et al. 2007, Ruiz et al. 2010). In turn, the mosquito infection rate is a good predictor of epidemics of mosquito borne disease and can provide advance warning for the initiation of public health interventions (Eldridge 1987, Bernard et al. 2001, Brownstein et al. 2004, Mendez et al. 2006, Nielsen et al. 2008). This has been demonstrated using *Cx. tarsalis* and *Cx. pipiens quinquefasciatus* Say in California (Reisen et al. 2009, Kwan et al. 2010). The objectives of this study were to understand the environmental factors that affect the *Cx. tarsalis* infection rate and to develop models that predict the spatial and temporal distribution of WNV infected *Cx. tarsalis* in the Canadian prairies, using temperature, precipitation, land cover, and *Cx. tarsalis* abundance. We adapted the generalized linear mixed model to construct a predictive model and then used it to create predictive maps of *Cx. tarsalis* infection rate within a Geographic Information System (GIS) framework.

3.2 Materials and methods

3.2.1 Mosquito data

Data on mosquito trap sites locations, abundance as the number of *Cx. tarsalis* per trap night, and infection from test results of pooled female *Cx. tarsalis* (using reverse transcription polymerase chain reaction (RT-PCR) from across the prairie provinces were obtained from June to September for 2005 to 2008 from the Public Health Agency of Canada (original data from Alberta Environment, Manitoba Public Health and Healthy Living, and Saskatchewan Ministry of Health) (Lanciotti et al. 2000). Mosquito sampling sites were distributed within

the different health regions across the southern half of the prairie provinces (Figure 3.1). The Center for Disease Control miniature light traps (with CO_2 bait) were used for mosquito sampling. The mosquito collection period was generally started in late May and lasted until the end of August (in Manitoba, the collection period ended the first week of September). During each week, the trap was set for one night at each of the mosquito collection sites in Alberta and Manitoba, but for one to four nights per week at Saskatchewan collection sites.

Counts of *Cx. tarsalis* per trap site per night were transformed by ln(y+1) to normalize the data distribution prior to analysis (Trawinski and Mackay 2009). *Culex tarsalis* infection rate per 1000 was computed using PooledInfRate (version 3.0), A Microsoft® Excel plug-in (Biggerstaff 2006) by Maximum Likelihood (ML-IR) and minimum infection rate (MIR) methods (Chiang and Reeves 1962, Biggerstaff 2006). The infection rate of WNV in female *Cx. tarsalis* was treated as the dependent variable in the model and therefore the accuracy of infection rate estimation was important for model development. Nonetheless, because infection rates in the mosquito population were usually low, especially early in the transmission season, 0 estimates of arbovirus in mosquitoes were often seen. Therefore, to achieve reasonable detection probability, larger mosquito sample sizes for screening was needed (Gu et al. 2004, Gu and Novak 2004). Gu and Novak (2004) suggested that for a medium detection probability of 0.5 per 1,000, 693 mosquitoes are required. In addition, we also found extremely high infection rates in some records where a positive result was from only a few mosquitoes included in the pool. Excluding observations from the dataset with low *Cx. tarsalis* sample size late in the season (usually in the early September) also removed records with high infection rates; however, most female *Cx. tarsalis* are usually preparing for hibernation during this period and do not take a blood meal (Curry 2004). Therefore, the risk

of WNV transmission was considered to be low. Based on these findings, we excluded samples with <100 female *Cx. tarsalis* per site per week from the analysis to prevent potential outliers and incorrect estimation of *Cx. tarsalis* infection rate resulting from small sample size (Gu et al. 2004, Ellis 2005).

3.2.2 Land cover

The land cover dataset was derived from the Advanced Very High Resolution Radiometer (AVHRR) sensor operating on board the United States National Oceanic and Atmospheric Administration satellites. AVHRR Land Cover Digital Data was downloaded from Natural Resources Canada. Satellite image taken in 1995.The land use in this area was considered to have remained stable after imaging (Huffman et al. 2006). Data for the prairie provinces were extracted and converted to a single 1 km^2 GIS raster layer. Eleven different types of land cover categories were included in the original dataset. According to habitat utilization by *Cx. tarsalis* (Jenkins 1950), land cover was simplified into forest (including deciduous, transitional coniferous and mixed forests), water, barren land, agricultural land (including cropland and rangeland), and urbanized area.

3.2.3 Climate data

Climate datasets were downloaded from the National Climate Data and Information Archive, Environment Canada. The daily weather datasets were used to create weekly mean temperature (unit 0.1 °C; WMT) and weekly mean total precipitation per day (unit 0.1 mm; WMP). To understand the time series effects of temperature and precipitation prior to the occurrence of WNV, we also created mean temperature and precipitation variables lagged

from one to eight weeks (16 variables). These time lagged variables represented weather occurring prior to mosquito sampling. Weather variables from each station were used for interpolation by the inverse distance weighted method to create prairie-wide climate layers in ArcGIS (Environmental System Research Institute, California).

3.2.4 Data analysis

All statistical analyses were performed with the SAS statistical software package, version 9.2 (Statistical Analysis System, Cary, NC).

3.2.4.1 Principal component analysis

The Pearson correlation test was used to test for multi-collinearity between independent variables. The criterion of Pearson correlation test was set at 0.8 of any pair of independent variable to indicate the multi-collinearity (Dohoo et al. 2009). If multi-collinearity existed among variables, principal component analysis was conducted to create a subset of uncorrelated predictor variables (components), and these uncorrelated predictor variables were used for model construction (Dohoo et al. 1997). Components selected were based on eigenvalues and the cumulative percentage of variance accounted by the components. The value of each record's principal component score was estimated by summing the principal component coefficients (for each independent variable) multiplied by the standardized values of the independent variables for the specific record (Lafi and Kaneene 1992).

3.2.4.2 Model construction

The frequency distribution of the *Cx. tarsalis* infection rate did not fit a Gaussian distribution; therefore, we adopted the generalized linear mixed model (GLMM), with a log link function for the model construction of the *Cx. tarsalis* infection rate (PROC GLIMMIX, SAS Institute 2008).

The Pearson correlation analysis of independent variables revealed that multi-collinearity existed between mean weekly temperature and mean temperature lagged from one to eight weeks. Results of principal component analysis for weekly mean temperature found that three components had eigenvalues larger than 1 and explained 69% of variation. Each component score was generated by summing the principal component coefficients (Table 3.1) multiplied by the standardized value of specific record of each variable in the component. According to the factor loadings (Table 3.1), two to eight weeks lagged mean temperature were found to load on component 1, one week lagged mean temperature had major loading on component 2, and component 3 was mainly loaded by weekly mean temperature.

Explanatory variables assessed as fixed effects were *Cx. tarsalis* abundance, land cover, variables generated by principal component analysis, weekly mean precipitation, and mean precipitation lagged from one to eight weeks. Health regions where traps were located were considered as a random effect. The negative binomial distribution was chosen in the model according to (a) a preliminary analysis that showed the formula 'Pearson Chi-Square divided by degrees of freedom (DF)' value was close to one and (b) the lowest Akaike information criterion (AIC) value. The parameters of GLMM model were estimated by Gauss-Hermite quadrature method (Bolker et al. 2009). Explanatory variables selected in the GLMM final models were based on the Wald test with a significant p value threshold set at 0.05. The

significance and coefficients of explanatory variables were compared between variables tested alone in the GLMM, full and final fitted model. The AICc values and AIC weights were computed for each model and used to assess the fit of models (Burnham and Anderson 2002, Bolker et al. 2009). Four-fifths of the records were randomly selected from the dataset for model construction and the remaining one-fifth of the records were used for model validation. Standardized Pearson residuals were estimated for the validation of the dataset and a scatter plot of standardized against predicted *Cx. tarsalis* infection rates used for assessment of the model prediction ability. Once the final model was fitted, Moran's *I* test was used to test the spatial autocorrelation of model residuals. The final fitted GLMM model was applied to create the prediction maps of WNV infection rate in *Cx. tarsalis* in each 1 by 1 KM grid using ArcGIS 10 (Environmental System Research Institute, California). Maps of human WNV incidence (cases per 100,000 individuals) for the entire transmission season (May – September) were created for each health region in the prairie provinces for 2005 to 2008 to compare with predicted *Cx. tarsalis* infection rate. Human WNV case data was retrieved from prairie provincial governmental websites (Government of Alberta 2005-2008, Government of Manitoba 2005-2008, Government of Saskatchewan 2005-2008).

3.3 Results

Out of 309 mosquito-sampling sites, 96 were located in Alberta, 38 in Saskatchewan and 175 in Manitoba (Figure 3.1). In general, the highest *Cx. tarsalis* abundance in the Canadian prairies was during weeks 30 to 32 (late July to early August), except in 2008 when peak abundance was occurred in week 34 (Figure 3.2). The highest observed mean WNV infection rate of pooled *Cx. tarsalis* for each week of all mosquito sampling sites was at week 34 in

2005 to 2007. In 2008, there was a lower mean *Cx. tarsalis* infection rate overall when compared with other years, and no significant peak in infection rate was observed (Figure 3.2).

Weekly mean temperatures were highest at week 29 and 30 in all three prairie provinces from 2005 to 2008. No obvious trend was observed for weekly precipitation (Figure 3.2).

The final fitted model based on the training dataset (four-fifths of total observations) had the lowest AIC value and AIC weight of 0.67 (Table 3.2). Based on the model, *Cx. tarsalis* infection rate increased with greater *Cx. tarsalis* abundance and time lagged mean temperature (component 1 and 2), while it decreased with greater time lagged precipitation (Table 3.3). In addition, we found that *Cx. tarsalis* abundance had a significant positive association only when time lagged precipitation was controlled for, which indicated that time lagged precipitation was exhibiting distorter variable effects on the relationship between *Cx. tarsalis* abundance and the infection rate. We also found a positive correlation between *Cx. tarsalis* abundance and time lagged precipitation. Pearson correlation coefficients of 5 weeks lagged and 6 weeks lagged precipitation were 0.115 ($p < 0.0001$) and 0.06 ($p = 0.0315$), respectively. There was no significant spatial autocorrelation detected in the model residuals by Moran's *I* test ($p = 0.688$).

This study used the final fitted GLMM model to predict the *Cx. tarsalis* infection rate in the validation dataset and estimate the residuals. The standardized Pearson residuals plotted against predicted *Cx. tarsalis* infection rate revealed that the fitted model could generally predict the validation dataset effectively (Figure 3.3). Maps of the predicted *Cx. tarsalis* infection rate for weeks 33 and 34 (late August, which had the highest *Cx. tarsalis* infection rate) and human WNV incidence in each health region of prairie provinces from 2005 to 2008 were created (Figure 3.4). We used the *Cx. tarsalis* infection rate of 20 per 1000 as a criterion

to represent high risk area in the predictive maps based on the mean mosquito infection rate in August, 2007. In the predictive maps, the southern part of the Canadian prairies is generally at higher risk, especially in the southeast Alberta, southwest to southeast of Saskatchewan and Southwest of Manitoba.

3.4 Discussion

In the present model, the *Cx. tarsalis* infection rate increased as *Cx. tarsalis* abundance and components of time lagged mean temperature increased, but decreased with increasing precipitation values lagged 2 – 6 weeks.

Multi-collinearity was observed between mean temperature and lagged temperature. When multi-collinearity exists, it results in an unstable estimation of the regression coefficients and an incorrect estimation of the variance for the coefficients in a regression model. Using principal component analysis to create a new set of uncorrelated predictor variables from the original variable set was used to deal with this statistical problem (Dohoo et al. 1997, Graham 2003). Factor loadings (Table 3.1) estimated by principal component analysis showed that Component 1 was loaded by temperatures lagged for 2 to 8 weeks and component 2 appeared to represent mostly temperatures lagged for one week. Both components were significantly associated with *Cx. tarsalis* infection rate, but not component 3 which was mainly loaded by weekly mean temperature. This finding indicates the positive effect of lagged temperature on *Cx. tarsalis* infection rate. Increasing environmental temperature shortens the maturation time requirement for both *Cx. tarsalis* and the virus (the extrinsic incubation period; EIP). In addition, it shortens the gonotrophic cycle and affects mosquito survival. In combination, these relationships influence virus transmission by

increasing the contact rate between mosquito and host (Reisen 1995, Reisen et al. 2006, Zou et al. 2007).

Extrinsic incubation period refers to the time from when the mosquito ingests an infectious blood meal to when it becomes capable of transmitting an acquired arbovirus infection and is an important component of vectorial capacity and the force of virus transmission (Reisen 1989). The estimated temperature threshold for the amplification of WNV in *Cx. tarsalis* is 14.3 ºC and the EIP required for median virus transmission is 109 degree-days (Reisen et al. 2006). In the fitted model, both component 1 and 2, which are composed of lagged temperature time points, has positive effects on the *Cx. tarsalis* infection rate. Weekly mean temperature was usually highest at weeks 29 to 30 (generally the last 2 weeks of July), followed by the peak *Cx. tarsalis* infection rate at week 34 (generally the 3rd week of August) in the prairie area. The time requirement for mosquito maturation and EIP would explain why the lagged weekly temperature had significant effects on the mosquito infection rate (Ruiz et al. 2010).

A significant association between mosquito abundance and the WNV infection rate is not surprising as the contact rate between infectious vectors and susceptible hosts is essential for amplifying virus within the enzootic cycle. In other words, a minimum threshold of vector and host abundance is needed to allow for virus transmission (Reeves 1965). Therefore, abundance of competent vectors is usually an indicator or predictor for the prevalence of pathogen occurrence and is linearly related to the force of transmission in the vectorial capacity equation (Reeves 1965, Saugstad et al. 1972, Murray 1995). This positive association has been demonstrated for other arboviruses such as Japanese encephalitis, western equine encephalitis and St. Louis encephalitis (Reeves 1965, Pant 1972, Wegbreit and Reisen 2000,

Arunachalam et al. 2009). Furthermore, human WNV risk has also been predicted by the abundance of *Cx. tarsalis, Cx. p. quinquefasciatus, Cx. pipiens* and *Cx. restuans* (Kilpatrick et al. 2005, Reisen et al. 2009, Kwan et al. 2010).

In the analysis, time lagged precipitation distorted the association between *Cx. tarsalis* abundance and the infection rate. This result indicated that increasing *Cx. tarsalis* abundance alone is not sufficient to predict WNV infection rate in *Cx. tarsalis*. Other factors, such as precipitation or variables influenced by precipitation, must exist in the habitat to alter the association.

In contrast to temperature, the influence of precipitation on the enzootic cycle of WNV was highly variable. In the model, increased precipitation was associated with decreases in the *Cx. tarsalis* infection rate 2-6 weeks later. Increased precipitation is generally perceived to increase mosquito abundance due to the creation of sites suitable for oviposition and larval development and therefore should increase the risk of WNV infection (Landesman et al. 2007, Wang et al. 2010). Alternatively, other researches also demonstrate that preceding droughts can increase the incidence of WNV in humans in the western United States (Landesman et al. 2007) and the Canadian prairies (Epp et al. 2009), which might correlate with the primary vector, *Cx. tarsalis*, of these regions. In addition, a unimodal relationship between precipitation and human incidence was found in the northern Great Plains of the United States (Wimberly et al. 2008). Reasons for this may include drought-induced decreases in mosquito competitors or predators, and increased densities of avian hosts and mosquito vectors on dwindling wetland habitat (Chase and Knight 2003, Shaman et al. 2005, Wang et al. 2010). However, positive associations between time lagged precipitation and *Cx. tarsalis* abundance have been found in California and the northern Great Plain habitat in South Dakota as well as

in this study (Reisen et al. 2008, Chuang et al. 2011). The positive correlation between preceded precipitation and *Cx. tarsalis* abundance could rule out the possibility of effects of predators and competitors on *Cx. tarsalis* abundance in these regions. Therefore, other underlying factors, such as the aggregation of competent susceptible hosts and *Cx. tarsalis* (Shaman et al. 2005, Ezenwa et al. 2007), and composition of avian community might influence the effects of precipitation on *Cx. tarsalis* infection rate and distort the association between vector abundance and infection rate. In this model, the association between agriculture irrigation and mosquito abundance was not tested. Irrigation might create suitable larvae habitat for *Cx. tarsalis* and increase the *Cx. tarsalis* abundance. However, farms with irrigation comprise only one, two and eight percent of the total farms in Manitoba, Saskatchewan and Alberta, respectively (Poirier 2009). Yiannakoulias et al. (2006) found that there was no association between irrigation areas and WNV human incidence in Alberta. Another study in South Dakota (part of the northern Great Plain) also did not find a statistically significant association between *Cx. tarsalis* abundance and distance to irrigation operation (Chuang et al. 2011). The effects of agriculture irrigation on *Cx. tarsalis* abundance in the Canadian prairies could be mild in the present day; however, the intensity and area covered by irrigation might be altered in the future due to climate change, thereby changing its importance in influencing mosquito abundance.

This research revealed the associations between weather variables and *Cx. tarsalis* infection rate of WNV in the Canadian prairies. The significant effects of time lagged weather variables on the *Cx. tarsalis* infection rate predicted WNV occurrence and provided time for the implementation of intervention methods implementation, especially mosquito control. In addition, warmer conditions and increased variability in precipitation are already being

observed and are projected to accelerate in the Canadian prairies (Sauchyn et al. 2009). Predictive models for WNV in this area are critical tools for public health and wildlife management in a future of rapid climate change.

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Table 3.1 Standardized coefficients of three components and factor loading of weekly mean temperature and time lagged mean temperature variables on three components.

	Component 1		Component 2		Component 3	
	Factor loading	Coefficients	Factor loading	Coefficients	Factor loading	Coefficients
WMT	-0.08	-0.065	0.15	0.07	**0.93**	0.789
WMT_1LAG	0.12	-0.171	**0.92**	0.793	0.12	0.027
WMT_2LAG	**0.61**	0.167	0.14	0.001	-0.11	-0.112
WMT_3LAG	**0.75**	0.271	-0.13	-0.321	0.43	0.381
WMT_4LAG	**0.66**	0.112	0.45	0.252	0.05	0.002
WMT_5LAG	**0.80**	0.225	0.16	-0.02	-0.20	-0.186
WMT_6LAG	**0.82**	0.266	0	-0.201	0.19	0.155
WMT_7LAG	**0.66**	0.117	0.43	0.239	0	-0.045
WMT_8LAG	**0.77**	0.209	0.20	0.016	-0.13	-0.137

WMT denotes weekly mean temperature and WMT_1LAG to WMT_8LAG represent weekly mean temperature lagged from one to eight weeks. Bold type indicates the primary loading (> 0.6) of each temperature variables on components.

Table 3.2 Comparison of Akaike information criterions and Akaike weights for WNV *Cx. tarsalis* infection rate models in the Canadian prairies.

Model	Variables included	AIC	AICc	ΔAIC	Akaike weight
GLMM	**Fitted variables[a]**	**4538.6**	**4538.9**	**0**	**0.67**
GLMM	Fitted variables Land cover	4541.3	4541.7	2.7	0.17
GLMM	Full variables[b]	4549.4	4550.2	10.8	0.003
GLM	Fitted variables	4542.8	4543	4.2	0.08
GLM	Full variables	4552	4552.7	13.4	0.0008

Bold text indicates the final fitted model.

AICc denotes AIC value corrected for small sample size; ΔAIC, difference in AIC value from final fitted model; GLMM, Generalized linear mixed model; GLM, Generalized linear model.

[a] Fitted model included Mosquito abundance, component 1, component 2, and 2 to 6 weeks lagged mean weekly precipitation variables.

[b] Full model included Mosquito abundance, land cover, component 1, component 2, component 3, mean weekly precipitation, and one to eight weeks lagged mean weekly precipitation variables.

Table 3.3 Explanatory variables statistics of each variable tested alone and final fitted variables in the generalized linear mixed model.

	Variable test alone					Final fitted model				
				95% CI					95% CI	
Variables	Coefficient	SE	P value	Lower	Upper	Coefficient	SE	P value	Lower	Upper
Intercept						1.1545	0.287	0.0007	0.557	1.752
MA	0.082	0.053	0.122	-0.022	0.187	0.278	0.05	<0.0001	0.18	0.376
Component 1	0.541	0.094	<0.0001	0.356	0.726	0.453	0.103	<0.0001	0.251	0.655
Component 2	0.279	0.091	0.0023	0.1	0.458	0.341	0.13	0.009	0.085	0.597
WMP_2LAG	-0.021	0.002	<0.0001	-0.025	-0.017	-0.013	0.003	<0.0001	-0.018	-0.008
WMP_3LAG	-0.014	0.002	<0.0001	-0.019	-0.009	-0.01	0.002	<0.0001	-0.014	-0.005
WMP_4LAG	-0.013	0.001	<0.0001	-0.016	-0.011	-0.007	0.002	<0.0001	-0.011	-0.004
WMP_5LAG	-0.011	0.002	<0.0001	-0.014	-0.007	-0.009	0.003	0.0005	-0.014	-0.004
WMP_6LAG	-0.004	0.002	0.0446	-0.007	-0.0001	-0.006	0.002	0.001	-0.009	-0.002

MA, Mosquito abundance; SE, standard error; WMP_2LAG to WMP_6LAG, two to six weeks lagged mean total precipitation.

Component 1is mainly loaded by two to eight weeks lagged temperature; component 2 is loaded by one week lagged temperature.

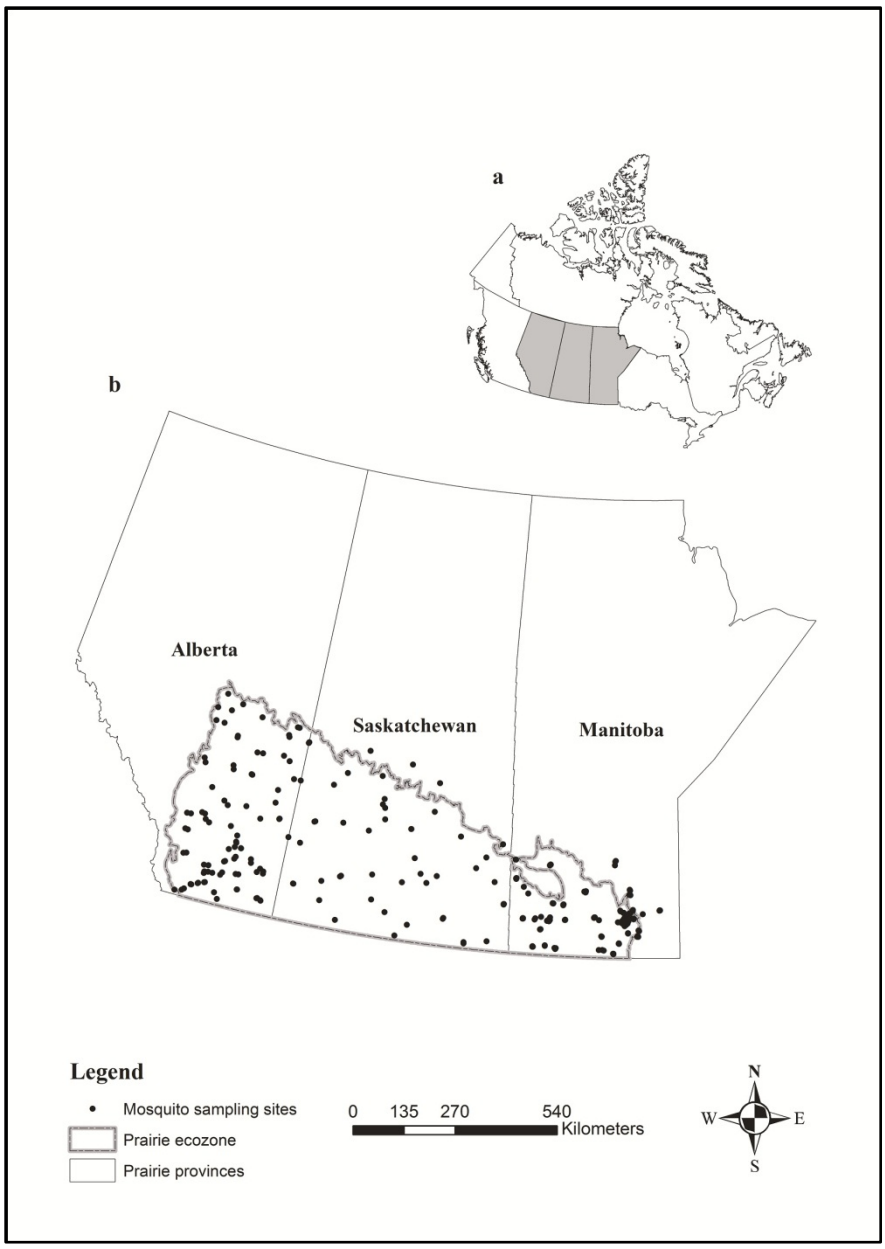

Figure 3.1 Distribution of mosquito sampling sites in the Canadian prairies (provinces of Alberta, Saskatchewan, and Manitoba) for the period from 2005 to 2008. a. Location of prairie provinces (grey spot) in Canada; b. the distribution of the Canadian prairies ecozone.

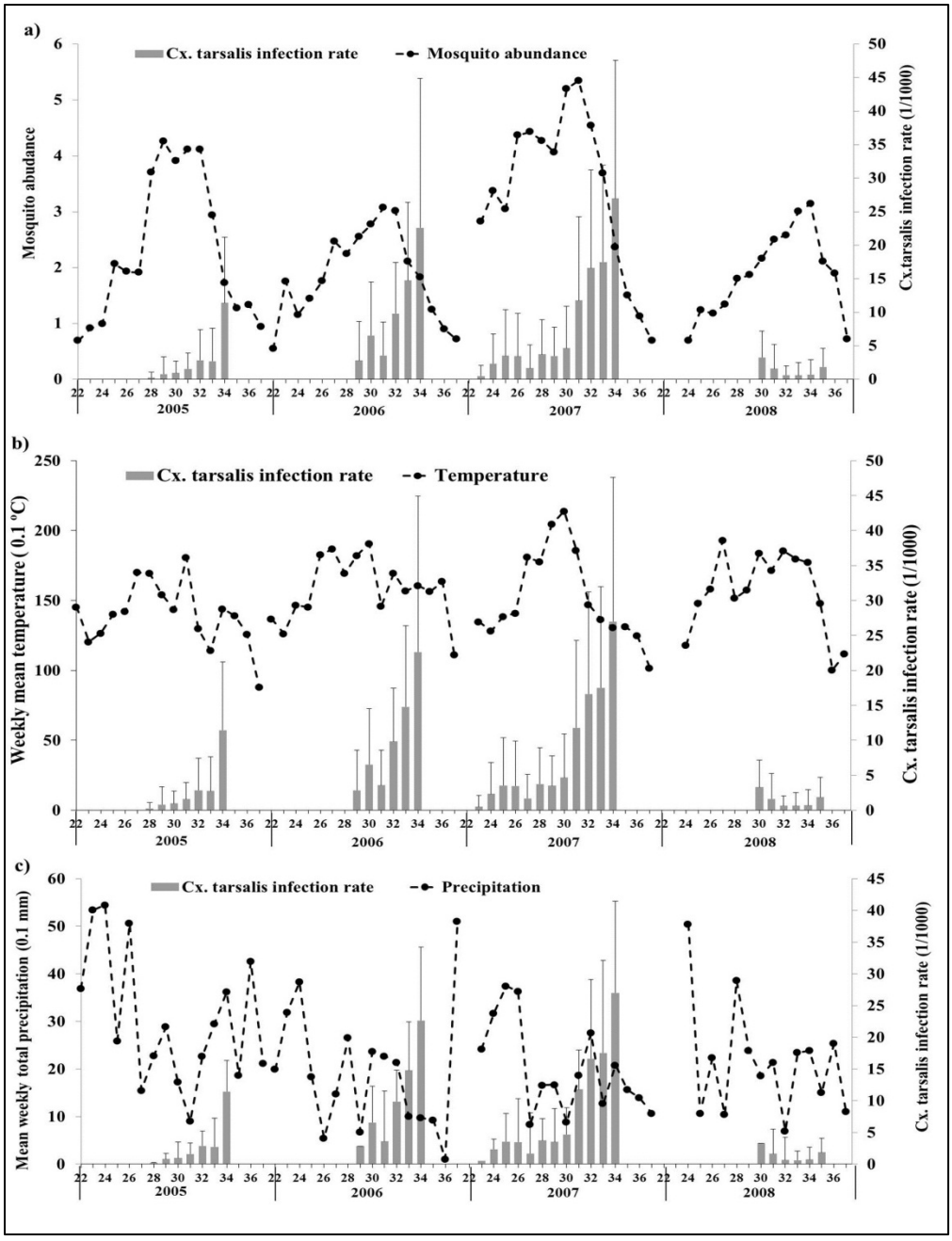

Figure 3.2 Temporal patterns of weekly *Cx. tarsalis* abundance (a), weekly mean temperature (unit 0.1ºC) (b), and weekly mean total precipitation per day (unit 0.1 mm) (c) compared to mean WNV infection rate in female *Cx. tarsalis* in the Canadian prairies. Error bar indicates the standard deviation of mean *Cx. tarsalis* infection rate of all trap sites at each week.

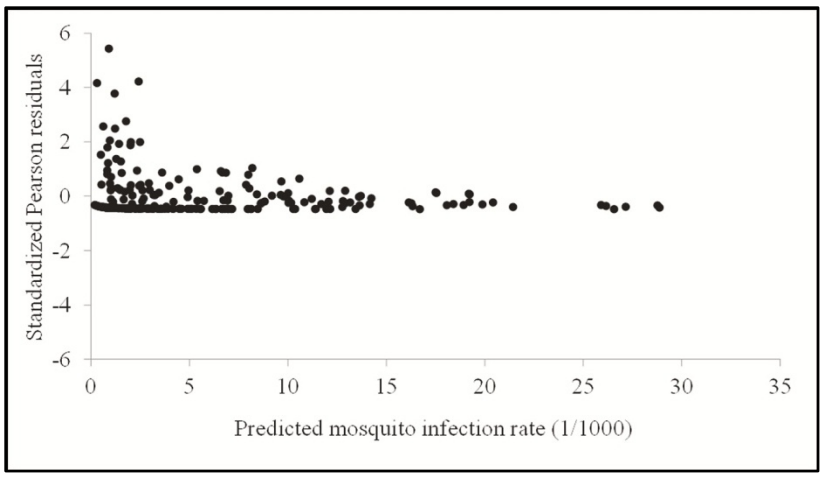

Figure 3.3 Plot of standardized Pearson residuals against predicted mosquito infection rate of validation dataset.

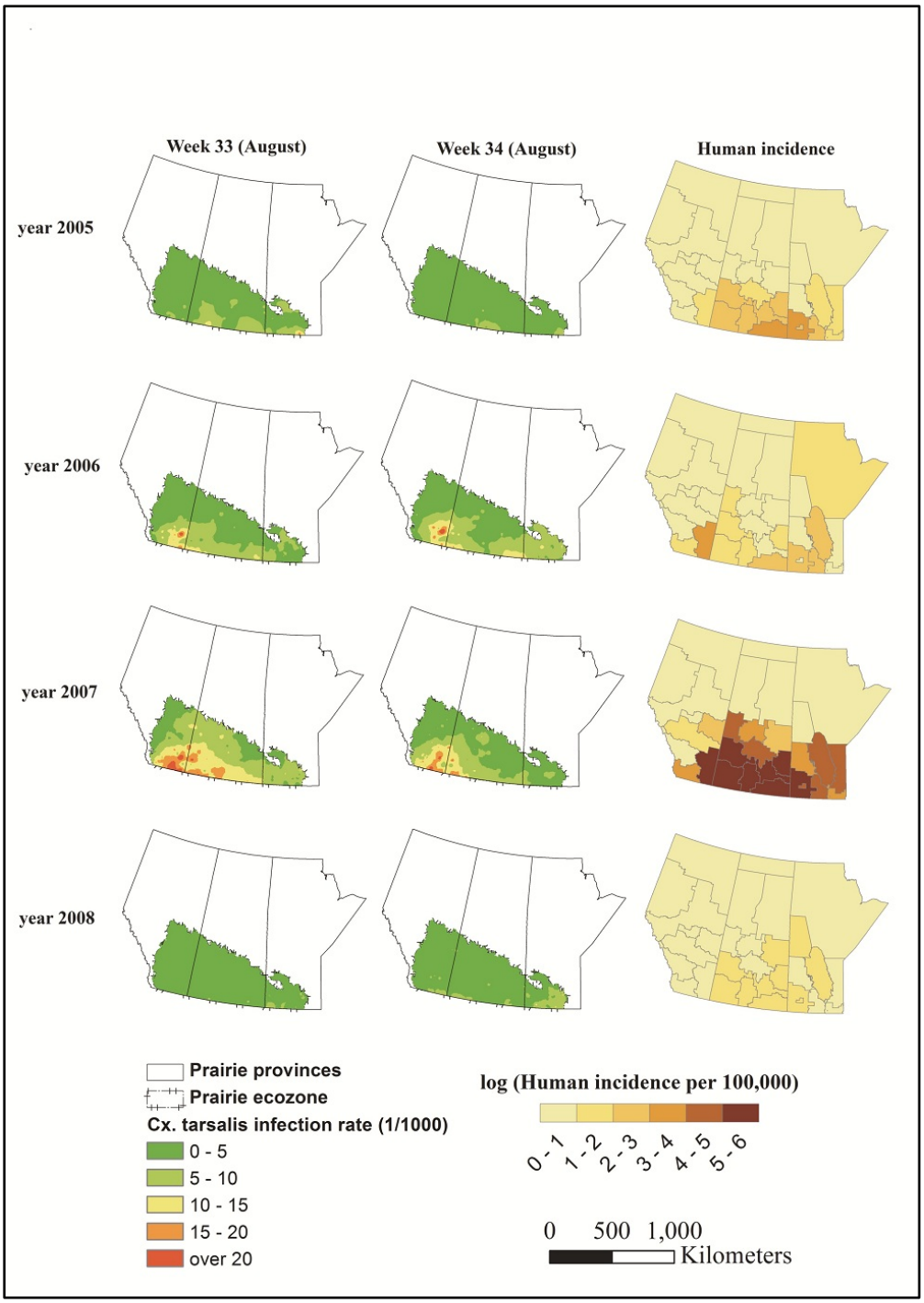

Figure 3.4 Maps of predicted WNV infection rate in female *Cx. tarsalis* per 1,000 at week 33 and 34, 2005-2008 in the Canadian prairies and log transformed human incidence (cases per 100,000 individuals) for each health region in the entire WNV transmission season.

Chapter 4 Modeling monthly variation of *Culex tarsalis* (Diptera: Culicidae) abundance and West Nile virus infection rate in the Canadian prairies

Preface

This chapter describes the monthly changes of *Cx. tarsalis* abundance, WNV infection rate in *Cx. tarsalis*, and various environmental variables contributing to the changes. The objectives of this study were to construct models for predicting the abundance and WNV infection rate of *Cx. tarsalis*. The monthly model was constructed in order to compare the resulting variables of importance with the previous weekly model. In addition, due to the limitation of predicted future climate datasets, the monthly model was the finest temporal scale to be applied to predict the effects of future climate change on WNV.

4.1 Introduction

Since the introduction of West Nile virus (WNV) into eastern North America in 1999 (Nash et al. 2001), WNV has become an endemic disease in the most of southern Canada, especially in the Canadian prairie provinces of Alberta, Saskatchewan, and Manitoba which have had the highest human infection rate in Canada. Of the 2,315 cases in Canada in 2007, more than 98% occurred in the three prairie provinces (Public Health Agency of Canada 2007). Saskatchewan alone had over half the human cases reported in Canada. Research focusing on the drivers of WNV occurrence in the prairie ecosystem is needed to inform WNV control and public health interventions.

West Nile Virus is primarily transmitted and amplified among local avian fauna and ornithophilic mosquito vectors, with occasional spillover into mammalian through

mosquito blood feeding (Epstein 2001, Curry 2004, Hayes et al. 2005, Turell et al. 2005). Therefore, the WNV infection rate in mosquito vectors is commonly utilized as an indicator of pathogen transmission intensity (Eldridge 1987, Gu et al. 2006) and has been demonstrated to be a better indicator for WNV activity than surveillance of dead or infected birds (Brownstein et al. 2004). Surveillance of mosquito infection rate also provides sufficient lead time for intervention and management of mosquito borne diseases (Eldridge 1987, Brownstein et al. 2004).

Although WNV has been isolated from at least 59 mosquito species in North America, only a small portion including mosquitoes belonging to the genera *Culex* (Diptera: Culicidae) have been shown to be competent vectors (Hayes et al. 2005). *Culex tarsalis* Coquillett is considered to be the main vector of WNV in the Canadian prairies (Curry 2004, Yiannakoulias et al. 2006). It is one of the most efficient WNV vectors evaluated in the laboratory studies (Turell et al. 2005) and the predominant species in the Canadian prairies during the summer WNV season (Curry 2004). Several biological features of *Cx. tarsalis* also facilitate the transmission of WNV in the enzootic cycles. *Culex tarsalis* can vertically transmit WNV to its offspring (Goddard et al. 2003), it takes several blood meals, and it produces multiple generations per season (Curry 2004). Furthermore, it is known to feed on both avian and mammalian hosts and plays the role of the "bridge vector" which transmits WNV out of its enzootic cycle to humans and other mammalian species (Tempelis et al. 1965).

Environmental variables such as temperature, precipitation and habitat type influence both *Cx. tarsalis* abundance and its WNV infection rate (Reisen 1995, Reisen et al. 2006, Reisen et al. 2008b, Chuang et al. 2011). In addition, environmental factors may influence seasonal and spatial overlap among key hosts in the sylvatic amplification cycles. For instance, drought-induced congregation of mosquitoes and birds on shrinking wetland

habitats may enhance the transmission of WNV (Wang et al. 2010). Therefore, understanding how environmental and biotic factors influence abundance of mosquito and WNV infection rate in mosquitoes is important in predicting the risks of WNV.

The Canadian prairies are at the northern limit of WNV distribution in the Western hemisphere. Climate and habitat suitability for *Cx. tarsalis* determine the distribution of this mosquito and WNV in the prairie provinces (Jenkins 1950). Future alterations in climate or habitat might expand the current spatial and temporal distribution of *Cx. tarsalis* and WNV (Lafferty 2009, Hongoh et al. 2012). A predictive model using environmental factors as the primary explanatory variables could be applied to evaluate the potential effects of climate change.

Many studies have been conducted to clarify the effects of environmental and biotic factors on the risk of WNV and predict the distribution of WNV in North America since its incursion. However these models usually cannot be applied to predict risks in other regions, particularly when those regions have different ecological dynamics or primary vector species (Brooker et al. 2002). Previous work had evaluated the effects of climate factors on the distribution of WNV by constructing a weekly model to predict the weekly variation of WNV infection rate in the Canadian prairies (Chen et al. 2012). The objectives of this present study were to clarify how environmental and biotic factors affect the abundance and WNV infection rate of *Cx.tarsalis* on a monthly scale and compare this to the weekly model. In addition, due to the limitation of time scale of the climate change dataset, it was necessary to construct a monthly model fitted with the monthly dataset, the finest time scale for evaluating the effects of climate change.

4.2 Materials and Methods

4.2.1 Mosquito Data

WNV infection of pooled female *Cx. tarsalis* was determined using reverse transcription polymerase chain reaction (RT-PCR) (Lanciotti et al. 2000, Drebot et al. 2003). Data on mosquito trap locations, abundance as the number of *Cx. tarsalis* per trap night, and WNV infection from across the prairie provinces were obtained from May to September for 2005 to 2008 from the Public Health Agency of Canada. The original data were supplied to PHAC from Alberta Environment, Manitoba Public Health and Healthy Living, and Saskatchewan Ministry of Health.

Mosquito sampling sites were distributed within the different health regions across the southern half of the prairie provinces (Figure 4.1). The new standard miniature light traps with photocell controlled CO_2 release (Model 1012-CO2; John W. Hock Company, Florida) were used for mosquito sampling. The mosquito collection period generally began in late May and lasted until the end of August (in Manitoba, the collection period ended the first week of September). During each week, the trap was operated for one night at each of the mosquito collection sites in Alberta and Manitoba, but for one to four nights per week at Saskatchewan collection sites. Monthly mean of *Cx. tarsalis* abundance was estimated for each collection site.

WNV infection rate (per 1000 *Cx. tarsalis*) was computed using PooledInfRate (version 3.0), a Microsoft® Excel plug-in (Biggerstaff 2006) by Maximum Likelihood (ML-IR) and minimum infection rate (MIR) methods (Chiang and Reeves 1962, Biggerstaff 2006). WNV infection rates in the mosquito were usually low, especially in the early transmission season. In this period, estimations of arbovirus infection rate in mosquitoes with value of zero were commonly recorded. Therefore, to achieve reasonable detection probability, large mosquito sample size for screening was required (Bernard et al.

2001, Gu et al. 2004, Gu and Novak 2004). Gu and Novak (2004) suggested that for a medium detection probability of 0.5, 693 mosquitoes are required.

In addition, this study also found extremely high infection rates in some records where a positive result was obtained from pooled test comprised of only a few mosquitoes. Excluding observations from the dataset with low *Cx. tarsalis* sample sizes in the late season of WNV (usually in the early September) might also remove the records with high infection rate; however, female *Cx. tarsalis* are usually inseminated and preparing for hibernation in this period in which they do not take a blood meal (Curry 2004) and the risk of WNV transmission is considered to be low. Based on these findings, we excluded observations with samples less than 100 female *Cx. tarsalis* per site per week from the analysis to prevent potential outliers and incorrect estimation of WNV infection rate resulting from small sample size (Gu and Novak 2004). The monthly mean WNV infection rate in *Cx. tarsalis* was calculated for each collection sites.

4.2.2 Land Cover

The land cover dataset was derived from the Advanced Very High Resolution Radiometer (AVHRR) sensor operating on board the United States National Oceanic and Atmospheric Administration satellites. AVHRR Land Cover Digital Data was downloaded from Natural Resources Canada. Although the satellite image was taken in 1995, the land use in this area was considered to have remained relatively stable until the start of the study period (Huffman et al. 2006).

Data for the prairie provinces were extracted and converted to a single 1 km^2 GIS raster layer. Eleven different types of land cover categories were included in the original dataset. According to habitat utilization by *Cx. tarsalis* (Jenkins 1950), land cover was simplified into forest including deciduous, transitional coniferous and mixed forests, water,

barren land, agricultural land including cropland and rangeland, and urbanized area (Figure 4.3). To analyze the association between land cover, *Cx. tarsalis* abundance, and infection rate, we used 20 km radius buffer zones around collection sites, defined according to the flight distance of *Cx. tarsalis* (Reisen and Lothrop 1995, Yiannakoulias et al. 2006) to determine percentage of the primary composition of land cover type within the buffer zones.

4.2.3 Climate Data

Daily mean temperature, daily maximum temperature, daily minimum temperature, and daily total precipitation were downloaded from the National Climate Data and Information Archive, Environment Canada. The daily weather datasets were used to create various predicting variables (Table 4.1). Daily maximum and minimum temperature were used to calculate the accumulative degree days (Zou et al. 2007) by the single sine method (Allen 1976) for current month, and two and three months accumulative degree days (Table 4.1). We also created the monthly mean degree days predictors by using monthly mean maximum and minimum temperature (Schrag et al. 2011). For estimating the monthly mean degree days, the low temperature threshold for WNV amplification in *Cx. tarsalis* was set as 14.3°C (Reisen et al. 2006). Finally, to determinate possible nonlinear effects of weather variables on *Cx. tarsalis* abundance and WNV infection rate, this study also created second order polynomial variables using the centered value of temperature and precipitation. Weather predictors of each climate station were interpolated by the inverse distance weighted method to create prairie-wide climate raster layers in ArcGIS. There was a total of 473 climate stations located in the Canadian prairies which were evenly distributed across the study area.

4.2.4 Data Analysis

Counts of *Cx. tarsalis* per trap site per night were transformed by ln(y+1) to normalize the data distribution prior to analysis. Models to predict *Cx. tarsalis* abundance using environmental factors were constructed using linear mixed model (PROC MIXED, SAS ver. 9.2, SAS Institute, Cary, N.C.).

A generalized linear mixed model with a log link function (PROC GLIMMIX, SAS ver. 9.2, SAS Institute, Cary, N.C.) was then used to develop a model for *Cx. tarsalis* infection rate prediction. A negative binomial distribution was chosen in the *Cx. tarsalis* infection rate model according to a preliminary analysis where the formula 'Pearson Chi-Square divided by degrees of freedom (DF)' value was close to one and the lowest Akaike information criterion (AIC) value. These values indicated a better data fit of the negative binomial distribution compared to other distributions in the generalized linear model family.

The Pearson correlation test was used to test for multi-collinearity between explanatory variables. If the correlation between any pair of variables was larger than 0.8, the more significant variable (lower -2 log likelihood) was chosen for further model construction (Dohoo et al. 2009b). We also conducted principal component analysis for determining the relationship between explanatory variables and compared the results with those of the Pearson correlation. Components selected were based on eigenvalues (> than 1) and the cumulative percentage of variance accounted for by the components (> than 80%). Factor loading larger than 0.6 of each variables was considered to be correlated with the component (Guadagnoli and Velicer 1988). Health regions where mosquito collection traps were located were used as a random effect for both *Cx. tarsalis* abundance and infection rate models. The variable parameters were estimated by the restricted maximum likelihood and Gauss-Hermite quadrature method for *Cx. tarsalis* abundance and infection

rate models, respectively (Bolker et al. 2009).

4.2.5 Model Selection and Validation

Explanatory variables selected in the models of *Cx. tarsalis* abundance and WNV infection rate were based on the Wald test with p threshold value set at 0.05 (Bolker et al. 2009, Dohoo et al. 2009a). The AICc (AIC value corrected for finite sample sizes) values and AICc weights were used to assess the models fit and select the best fitted model (Burnham and Anderson 2002, Bolker et al. 2009, Dohoo et al. 2009a). Four-fifths of the records were randomly selected from the dataset for model construction and the remaining one-fifth of the records were used for model validation. Standardized residuals and standardized Pearson residuals were estimated for the validation dataset of *Cx.tarsalis* abundance and infection rate models, respectively. Values of Root Mean Square Error (RMSE) were calculated for training and validation datasets to validate and compare the predictability of the model on each dataset. Moran's *I* test was used to test the spatial autocorrelation of residuals for the final models.

The final model of WNV infection rate in *Cx. tarsalis* was applied to create maps of the predicted WNV infection rate in the Canadian prairies in each 1 by 1 KM grid using ArcGIS 10 (Environmental System Research Institute, California). To compare the predicted *Cx. tarsalis* infection rate and actual human WNV incidence in the prairie provinces, maps of human WNV incidence (cases per 100,000 individuals) were created for the entire transmission season (May – September) for each health region for 2005 to 2008. Human WNV case data was retrieved from prairie provincial governmental websites (Government of Manitoba-Manitoba Health. 2005-2008, Government of Alberta-Health and Wellness. 2005 -2008, Government of Saskatchewan-Public Health. 2005 -2008).

4.2.6 Comparing predictability between monthly and weekly models

In this thesis, monthly and weekly models were constructed to predict the WNV infection rate in *Cx. tarsalis*. In order to compare the predictability of monthly and weekly models, the values of root mean square error (RMSE) and mean bias (B) were calculated for training and validation datasets of both models.

Equations for estimating the RMSE and B are as followed:

Root mean square error (RMSE):

$$\text{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(\hat{u} - u)^2}$$

Mean bias (B):

$$\text{B} = \frac{1}{n}\sum_{i=1}^{n}(\hat{u} - u)$$

Here,

n is the sample size.

û is the linear prediction of WNV infection in *Cx. tarsalis*.

u is the log transformed observed WNV infection rate in *Cx. tarsalis*.

The two parameters, RMSE and B, aimed to compare the differences between predicted and observed WNV infection rate. Values closer to zero of both parameters represented a better predictability when comparing monthly and weekly WNV infection rate models.

4.3 Results

4.3.1 Descriptive Statistics

Out of 309 mosquito collection sites, 96 were in Alberta, 38 in Saskatchewan and 175 in Manitoba (Figure 4.1). The highest observed mean abundance of *Cx. tarsalis* was in July (for 2005 to 2007) or August (for 2008) (Figure 4.2). The highest observed mean *Cx. tarsalis* infection rates were in August for 2005 to 2007; there was no obvious trend observed in 2008 (Figure 4.2).

July was the warmest month with temperature around 20 ºC. The mean temperature in July, 2007 was higher than other years, ranging from one to three Celsius degree, and 8.6% higher than mean temperature in July of study period. The range of monthly temperature fluctuation was from 6.3 to 20 ºC in the Canadian prairie ecozone. In 2007, total precipitation in May was highest compared to other years with a mean temperature closer to 2006 but higher than 2005 and 2008 in the Canadian prairies (Figure 4.2). Agriculture land cover predominated the buffered zones of mosquito collection sites; 94.3% of sampling sites had agriculture land as the primary land cover type. Mean percentage of agriculture land was 86.1% in each buffer zone. The other two primary land cover types identified were forest (2.21%) and water (3.52%) (Figure 4.3).

4.3.2 Constructed Models

Based on the results of Pearson correlation and principal component analysis, explanatory variables could be grouped into four highly correlated components (Table 4.1). The cumulative variances explained by these four components of principal component analysis were 84.6% and 86.1% for *Cx. tarsalis* abundance and infection rate models, respectively. In the final *Cx. tarsalis* abundance model, increased mean monthly temperature, 1 month lagged mean temperature, total precipitation, and temporal lags of precipitation from 1 to 2 months were significantly associated with increased *Cx. tarsalis* abundance, while an inverse association between forest land cover and *Cx. tarsalis* abundance was observed (Table 4.2).

In the final WNV infection rate model, we found that increasing *Cx. tarsalis* abundance, and 1 month lagged temperature were associated with increased WNV infection rate, while one month lagged mean precipitation, 3 months total precipitation, and water land cover were inverse associated with decreased WNV infection rate (Table 4.3).

In addition, we found the inverse association between 3 months total of monthly mean degree days and WNV infection rate when the lagged mean temperature was controlled. Time lagged mean temperature was the distorter variable which distorted the coefficient of 3 months total of monthly mean degree days from positive when this variable was tested alone to negative.

Second-order polynomial variables of temperature and precipitation were not significantly associated with *Cx. tarsalis* abundance and infection rate (Table 4.2 and 4.3). There was no significant spatial autocorrelation detected for residuals of *Cx. tarsalis* infection rate by Moran's *I* test ($p = 0.65$). The RMSE of training and validation dataset was 0.97 and 1.0, respectively. Both RMSE values were close to one and close to each

other which indicated the accuracy and precision of model predictability.

We used the *Cx. tarsalis* infection rate of 20 per 1000 as a criterion to represent the high risk area in the predictive maps based on the mean mosquito infection rate in August, 2007, a major epidemic period of WNV in the Canadian prairies. In the predictive maps, the southern part of the Canadian prairies was generally at higher risk, especially in southeast Alberta, southwest to southeast Saskatchewan and southwest Manitoba (Figure 4.4).

4.3.3 Comparing the predictability of weekly and monthly models

Results showed that values of RMSE and B of the monthly model were generally closer to zero than weekly model (Table 4.4 and 4.5). These findings indicated a better predictability of monthly model than weekly model.

4.4 Discussion

This study analyzed the effects of environmental drivers on *Cx. tarsalis* abundance and infection rate of WNV from 2005 to 2008. Most of the geographic regions at highest risk based on predicted WNV infection rate in *Cx. tarsalis* were consistent with the distribution of human WNV cases between 2005 and 2008. The slight differences between the distribution of mosquito infection rate and human incidence were expected, and can potentially be explained by social and economic factors, population density, risk perception, mosquito control programs, and the intensity of human case detection in different provinces. However, mosquito infection rate as an indicator for early season forecasting or predicting of WNV human incidence has been previously demonstrated (Brownstein et al. 2004). Mosquito infection rate was also influenced by variation in climate factors. Of particular interest here, understanding the relationship between climate

factors and mosquito infection on monthly scale could be adopted to predict WNV activity under different climate change scenarios.

In this study, more explanatory variables were evaluated compared with the previously published weekly model (Chen et al. 2012). The variables associated significantly with WNV infection rate in this study were similar to the weekly model; however, on the monthly time scale, we found an inverse relationship between mean degree days and WNV infection rate when time lagged temperature was controlled. In addition, we demonstrated the effects of land cover composition in a 20 km radius buffer zone on the abundance of *Cx. tarsalis* and WNV infection rate. We also found a better predictability of monthly model than weekly model based on the values of Root Mean Square Error (data not shown).

In both *Cx. tarsalis* abundance and WNV infection rate models on the monthly time scale, increasing temperature and lagged temperature significantly increased *Cx. tarsalis* abundance and infection rate. The mean temperature in May and June, 2007 was higher than 2005 and 2008, with the highest mean temperature in July during study period. High environmental temperature with the antecedent highest precipitation in May and *Cx. tarsalis* abundance in June might have contributed to the outbreak of WNV in 2007. Increasing environmental temperature shortens the maturation time required for *Cx. tarsalis* and the extrinsic incubation period of virus. Furthermore, it also accelerates the gonotrophic cycle and affects mosquito survival. In combination, these relationships influence virus transmission by increasing the contact rate between mosquito and host (Reisen et al. 2006, Zou et al. 2007).

This study found the distorted effect of time lagged mean temperature on the 3 months total of monthly mean degree days for the WNV infection rate model. Increasing 3 months total of monthly mean degree days will decrease the WNV infection rate in *Cx.*

tarsalis when variable of time lagged mean temperature is controlled. The Pearson correlation and principal component analysis revealed that mean temperature fluctuations, monthly mean degree days, and 2 and 3 months total of monthly mean degree days were highly correlated. These variables were all estimated based on the maximum and minimum temperature and indicated the degree of temperature fluctuation. These findings indicated that increasing temperature fluctuation could decrease the *Cx. tarsalis* infection rate in the environment with similar time lagged mean temperature. The effect of high temperature fluctuation has been proposed to limit the midgut infection of flaviviruses in mosquitoes by preventing the virus from entering the midgut epithelial cells or limiting initial replication of virus in these midgut cells (Kramer and Ebel 2003). Adverse effect of high temperature fluctuations have also been observed in the transmission of dengue virus by *Aedes aegypti* (Lambrechts et al. 2011) and western equine encephalomyelitis by *Cx. tarsalis* (Kramer et al. 1983, Reisen et al. 1993).

The probable contact rate between an infected competent vector and a susceptible host is essential for maintaining the enzootic cycle of an arbovirus in a geographic area, although herd immunity of host population might dampen the transmission of WNV. A minimum threshold of vector and susceptible host interaction is needed to allow for virus transmission (Reeves 1965). Therefore, abundance of suitable competent vectors is usually an indicator of the prevalence of pathogen occurrence (Reeves 1965, Saugstad et al. 1972, Murray 1995). A positive association has been demonstrated for other arboviruses such as Japanese encephalitis, western equine encephalitis and St. Louis encephalitis (Reeves 1965, Wegbreit and Reisen 2000, Arunachalam et al. 2009). Furthermore, abundance of *Cx. tarsalis, Cx. p. quinquefasciatus, Cx. pipiens* and *Cx. restuans* has also been used as an indicator to predict the human WNV risk in different regions (Kilpatrick et al. 2005, Reisen et al. 2009, Kwan et al. 2010).

In contrast to temperature, the influence of precipitation on the enzootic cycle of WNV is paradoxical. Increased precipitation is generally believed to create standing water suitable for mosquito breeding and increase mosquito abundance. Positive association between time lagged precipitation and *Cx.tarsalis* abundance were also observed in this study. In 2007, total precipitation in May was highest compared to other years with the mean temperature closer to 2006 but higher than 2005 and 2008 in the Canadian prairies. The high precipitation might have contributed to the explosive occurrence of *Cx. tarsalis* in June. Other researchers have demonstrated that preceding droughts can increase the incidence of WNV in humans in the western United States (Landesman et al. 2007) and the Canadian prairies (Epp et al. 2009). Potential explanations for the effects of precipitation on WNV incidence include drought induced decreases in mosquito competitors or predators and increase the abundance of Cx. tarsalis,, increased congregation of susceptible avian hosts and mosquito vector on dwindling wetland habitat, and changes in the composition of the avian host community (Chase and Knight 2003, Shaman et al. 2005, Wang et al. 2010). In this study, precipitation was positively associated with *Cx. tarsalis* abundance, similar to studies in California and the northern Great Plain habitat in South Dakota (Reisen et al. 2008a, Chuang et al. 2011) but an inverse association between precipitation and WNV infection rate in *Cx. tarsalis* was observed. These findings suggest that other factors, such as the aggregation of competent hosts and *Cx. tarsalis* (Shaman et al. 2005), or alterations to composition of the avian community might better explain the effects of precipitation on WNV infection rate in *Cx. tarsalis* in the Canadian prairies (Ezenwa et al. 2007).

Nonlinear relationships between climate factors and arthropod vectors are commonly observed. For instance, Reisen et al.(2008a) found that spring *Cx. tarsalis* abundance was positively correlated with temperature in winter and spring, whereas summer abundance

was correlated negatively with spring temperature and not correlated with summer temperature in California. A unimodal relationship between precipitation and WNV incidence was demonstrated in the northern Great Plains and the optimal total precipitation from May to July was approximately 200 mm (Wimberly et al. 2008). In this study, all second-order polynomial variables were not associated with *Cx. tarsalis* abundance or infection rate. Total precipitation in May to July of 2005 to 2008 ranged from 79 to 332 mm and observed high risk areas had the lowest precipitation values. The warmest summer mean temperature was around 20 °C in July (Figure 4.2), which was much lower than the mean temperature of 30 °C for the same month in California (Reisen et al. 2009). Low summer environmental temperatures across the Canadian prairies could be the main reason for findings in the study. In this temperature range, the development rate of *Cx. tarsalis* and the replication rate of WNV in the *Cx. tarsalis* are linearly related with environmental temperature (Reisen 1995, Reisen et al. 2006).

Ezenwa et al. (2007) found a negative association between WNV infection rate among *Culex* mosquitoes and wetland coverage. Wetland area has been positively associated with bird species diversity and thus it is possible that this represents an example of the dilution effect, in which increased bird diversity led to overall decreases in the mosquito infection rate (Ezenwa et al. 2006, Ezenwa et al. 2007). In the fitted model, *Cx. tarsalis* abundance was not significantly different between wetland and agriculture as the primary composition of land cover types; however, a significant negative association was found between wetland and *Cx. tarsalis* infection rate. This result reflects the effect of possible underlying factors, such as the differences of bird species composition between different land cover types and also indicates that vector abundance alone was not sufficient to predict the intensity of WNV occurrence.

This study clarifies the relationship between environmental factors and the

abundance of *Cx. tarsalis* and infection rate of WNV in the Canadian prairies. The observed association between environmental temperature and WNV infection rate could provide sufficient time to predict WNV occurrence and initiate disease control and public health interventions. In addition, warmer temperature and increased variability in precipitation are already being observed and are projected to accelerate in the Canadian prairies (Shepherd and McGinn 2003). Predictive monthly models for vector-borne diseases are critical tools for public health and wildlife management in a future of rapid climate change. These models are adopted to assess the effects of changing climate conditions on WNV distribution in the Canadian prairie provinces in the next chapter.

4.5 References

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Table 4.1 Descriptions of variables used in both *Cx. tarsalis* abundance and infection rate models and relationships between explanatory variables based on the Pearson correlation and principal component analysis. Variables with the same arabic number indicated that the Pearson correlations are larger than 0.8 or have factor loading larger than 0.6 in each component of principal component analysis.

Variables	**Correlation (>0.8)**		**PCA component (Factor loading >0.6)**		**Variables description**
	LMM	**GLMM**	**LMM**	**GLMM**	
Monthly mean temperature	1	1	1	1	Monthly mean temperature of the month of mosquito data collection
1 month lagged mean temperature	2	2	2	2	1 month lagged mean monthly temperature
2 month lagged mean temperature	2	2	2	2	2 months lagged mean monthly temperature
3 months mean temperature	2	2	2	2	Including mosquito collection month, and previous 1 and 2 months
Winter mean temperature		4	3	3	From December to February

Monthly mean degree days	3	3	3	3	Monthly mean degree day of the mosquito data collection month
2 months total of monthly mean degree days	3	3	3	3	Created by summing the monthly mean degree days of the month of mosquito data collection and previous month
3 months total of monthly mean degree days	-	3	-	3	Created by summing the monthly mean degree days of the month of mosquito data collection, previous one and two months. Not applied in the LMM
Mean temperature fluctuations	3	3, 4	3	3	Monthly mean maximum temperature minus monthly mean minimum temperature
1 months accumulative degree days	1	1	1	1	The accumulative degree days of data collection month
2 months accumulative degree days	2	2	2	2	The accumulative degree days of data collection month and previous months
3 months accumulative	-	2	-	2	The accumulative degree days of data collection

degree days					month and previous one and two months. Not used in the LMM
1 month lagged mean precipitation	-	4	-	4	1 month lagged monthly mean daily total precipitation.
Monthly total precipitation					Monthly total precipitation
1 month lagged total precipitation		4	4	4	1 month lagged monthly total precipitation
2 month lagged total precipitation			2	2	2 month lagged monthly total precipitation
Total precipitation of previous year			4	4	Annual total precipitation of previous year
3 months total precipitation			4	4	The total precipitation of mosquito collection month, and previous one and two months

LMM: Linear mixed model for predicting *Cx. tarsalis* abundance; GLMM: Generalized linear mixed model for predicting WNV infection rate in *Cx. tarsalis*; "-": variable is not used in the model construction;

Table 4.2 Estimated coefficients of explanatory variables and comparison of corrected Akaike information criterions (AICc) and Akaike weights for models of *Cx. tarsalis* abundance. Single variable represents the explanatory variables assessed individually. Final model indicates the final fitted model with the lowest AICc value. Polynomial model is model fitted with second order polynomial variables. Full model is model fitted with all created explanatory variables.

Variables	**Single variable**		**Final model**		**Polynomial model**		**Full model**	
	Coef.	**95% CI**	**Coef.**	**95% CI**	**Coef.**	**95% CI**	**Coef.**	**95% CI**
Intercept			-3.48*	-4.05 to -2.91	0.62*	0.23 to 1.02	-3.93*	-4.6 to -3.25
Weather								
Monthly mean temperature	0.25*	0.22 to 0.26	0.22*	0.2 to 0.25	0.23*	0.2 to 0.25	0.22*	0.19 to 0.25
1 month lagged temperature	0.08*	0.07 to 0.1	0.07*	0.05 to 0.09	0.07*	0.05 to 0.09	0.06*	0.04 to 0.09
Winter mean temperature	-0.04*	-0.06 to -0.01					-0.03*	-0.06 to -0.01
Monthly mean degree days	0.28*	0.23 to 0.33					0.032	-0.04 to 0.1
Monthly total precipitation	-0.003*	-.004 to -.001	0.0033*	0.002 to 0.005	0.005*	0.003 to 0.006	.0032*	0.002 to 0.005
1 month lagged precipitation	0.006*	.005 to .007	0.0042*	0.003 to 0.005	0.004*	0.003 to 0.005	.0037*	0.002 to 0.004
2 month lagged precipitation	0.005*	.004 to .006	0.0033*	.002 to .004	0.003*	.002 to .004	.003*	0.002 to 0.005

Land cover								
Forest	-0.48*	-0.91 to -0.04	-0.54*	-0.9 to -0.17	-0.51*	-0.87 to -0.14	-0.59*	-0.95 to -0.22
Water	-0.11*	-0.47 to -0.26					0.03	-0.28 to 0.34
Polynomial								
Square of monthly temperature					0.006	-0.0003 to 0.01		
Square of monthly precipitation					0	-4E-05 to 8E-6		
AICc			4524		4557.4		4529.3	
AICc weight			0.92		5.16E-08		0.076	

Coef.: estimated variable coefficient; *: $P < 0.05$.

Table 4.3 Estimated coefficients of explanatory variables and comparison of corrected Akaike information criterions (AICc) and Akaike weights for models of WNV infection rate. Single variable represents the explanatory variables assessed individually. Final model indicates the final fitted model with the lowest AICc value. Polynomial model is model fitted with second order polynomial variables. Full model is model fitted with all created explanatory variables.

Variables	**Single variable**		**Final model**		**Polynomial model**		**Full model**	
	Coef.	**95% CI**	**Coef.**	**95% CI**	**Coef.**	**95% CI**	**Coef.**	**95% CI**
Intercept			-2.26*	-4.47 to -0.05	1.25	-1.71 to 4.21	-1.64	-5.64 to 2.37
Cx. tarsalis abundance	0.16*	0.03 to 0.30	0.55*	0.31 to 0.79	0.57*	0.29 to 0.85	0.58*	0.28 to 0.87
Weather								
Monthly mean temperature	-0.14*	-0.2 to -0.08					-0.04	-0.18 to 0.10
1 month lagged temperature	0.25*	0.21 to 0.29	0.32*	0.22 to 0.41	0.29*	0.21 to 0.38	0.32*	0.21 to 0.42
Winter mean temperature	0.23*	0.06 to 0.40					0.01*	-0.13 to 0.15
3 months total of monthly mean degree days	0.20*	0.16 to 0.24	-0.10*	-0.2 to -0.01	-0.06	-0.15 to 0.03	-1.10	-0.21 to 0.002
Monthly total precipitation	-	-0.02 to -0.01					-0.01	-0.02 to 0.003

	0.015 *							
1 month lagged mean precipitation	-0.48*	-0.56 to -0.39	-0.27*	-0.36 to -0.18	-0.25*	-0.37 to -0.13	-0.43*	-0.62 to -0.24
2 month lagged total precipitation	0.003	-.001 to .01					-0.01	-0.02 to 0.003
3 months total precipitation	-0.085	-0.11 to 0.06	-0.05*	-0.08 to -0.02	-0.04*	-0.07 to -0.02	0.013	-.06 to .08
Land cover								
Forest	-1.3*	-1.84 to -0.76					-0.43	-1.27 to 0.41
Water	-1.31*	-2.8 to -0.182	-1.52*	-2.56 to -0.47	-1.33*	-2.46 to -0.19	-1.61*	-2.85 to -0.38
Polynomial								
Square of 1 month lagged mean temperature					0.01	-.007 to 0.04		
Square of 1 month lagged mean precipitation					0.01	-0.02 to 0.04		
AICc			2911		2913.13		2915.49	
AICc weight			0.717		0.207		0.076	

Coef.: estimated variable coefficient; *: $P < 0.05$

Table 4.4 Root mean square error of training dataset and validation dataset of monthly and weekly models for WNV infection rate in *Cx. tarsalis*.

Models	Datasets	
	Training	Validation
Monthly	0.965	1.009
Weekly	1.114	1.143

Table 4.5 Mean bias of training dataset and validation dataset of monthly and weekly models for WNV infection rate in *Cx. tarsalis*.

Models	Datasets	
	Training	Validation
Monthly model	-0.09	-0.17
Weekly model	0.181	0.18

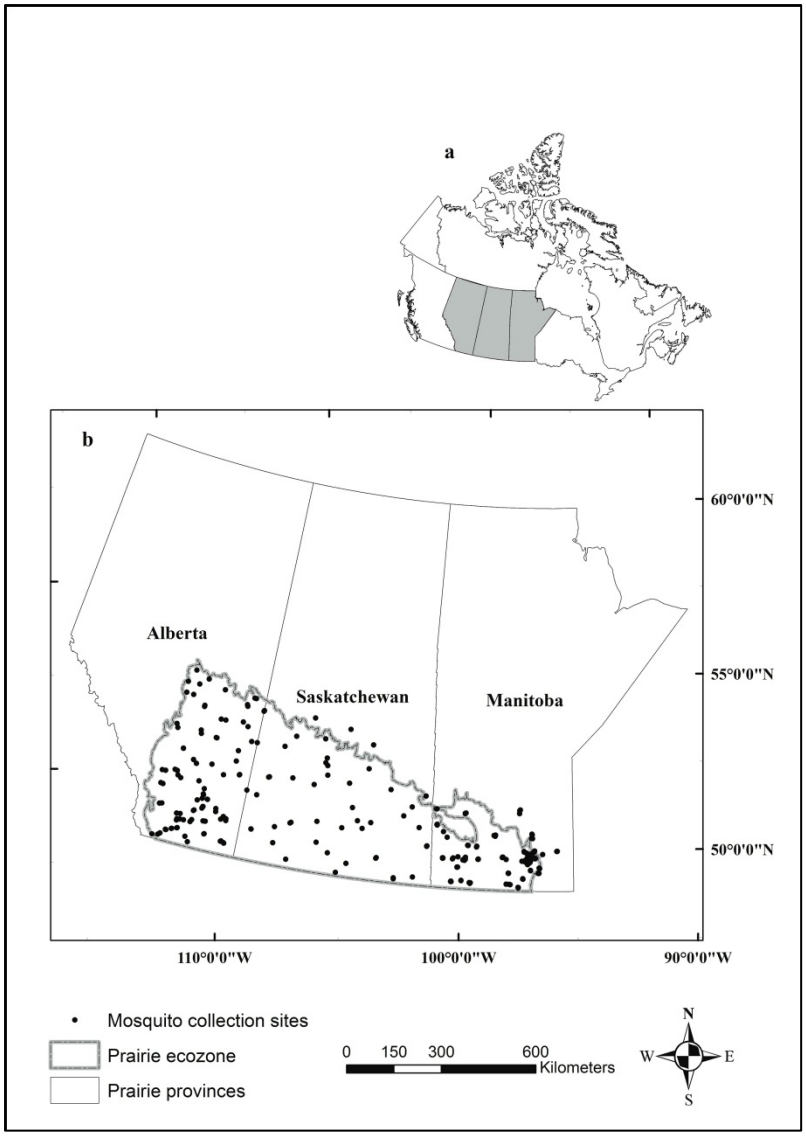

Figure 4.1 Distribution of mosquito sampling sites in the Canadian prairies provinces of Alberta, Saskatchewan, and Manitoba for the period from 2005 to 2008. a. Location of prairie provinces (grey spot) in Canada. b. The distribution of sampling sites across the Canadian prairies ecozone.

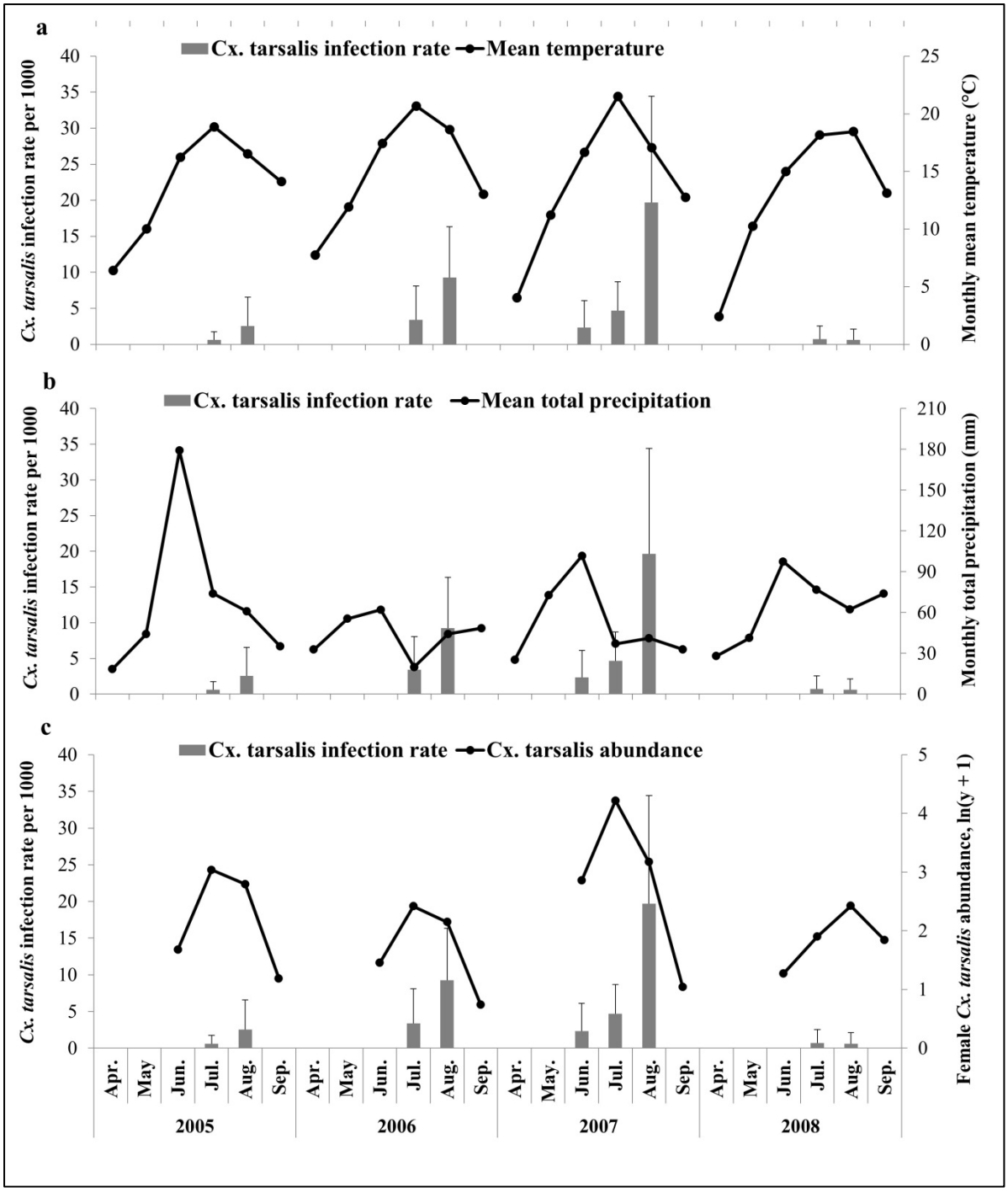

Figure 4.2 Temporal trends of (a) Monthly mean temperature (unit 1ºC). (b) Monthly total precipitation (unit 1 mm). (c) Monthly mean abundance of *Cx. tarsalis*, ln(y+1) transformed, compared to monthly mean WNV infection rate in female *Cx. tarsalis* in the Canadian prairies. Error bar indicates the standard deviation of mean *Cx. tarsalis* infection rate.

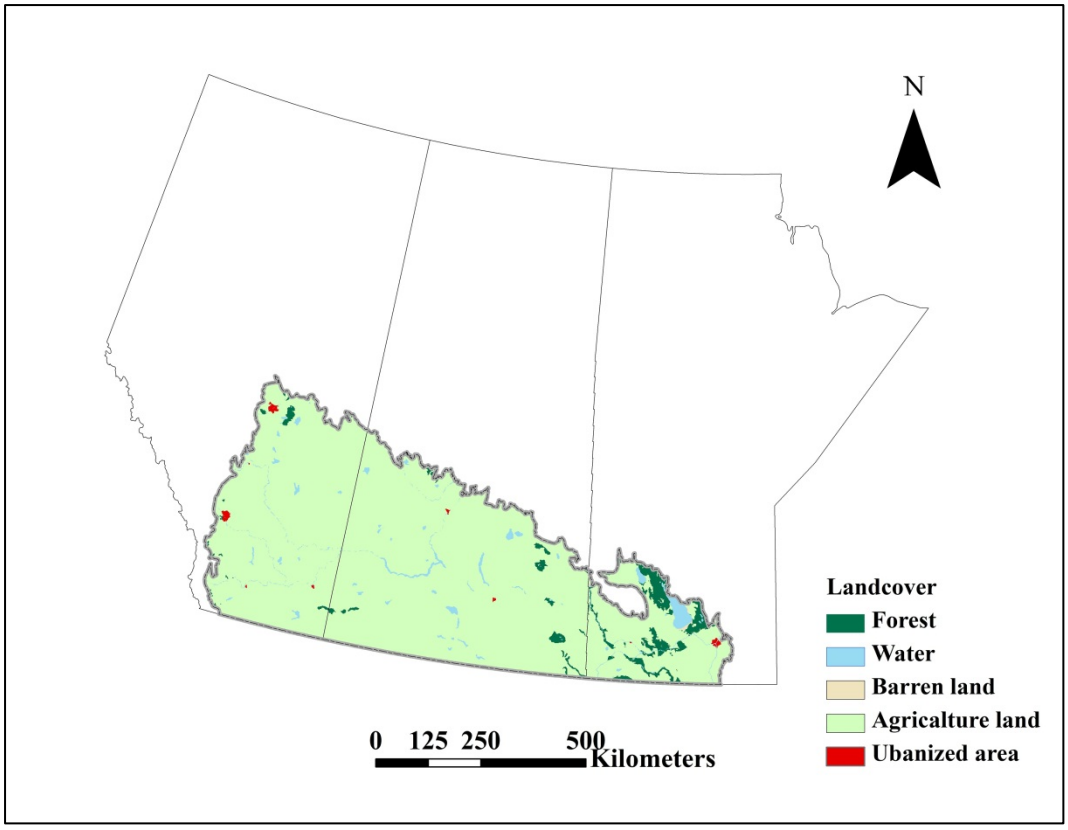

Figure 4.3 Distribution of land cover types in the Canadian prairies.

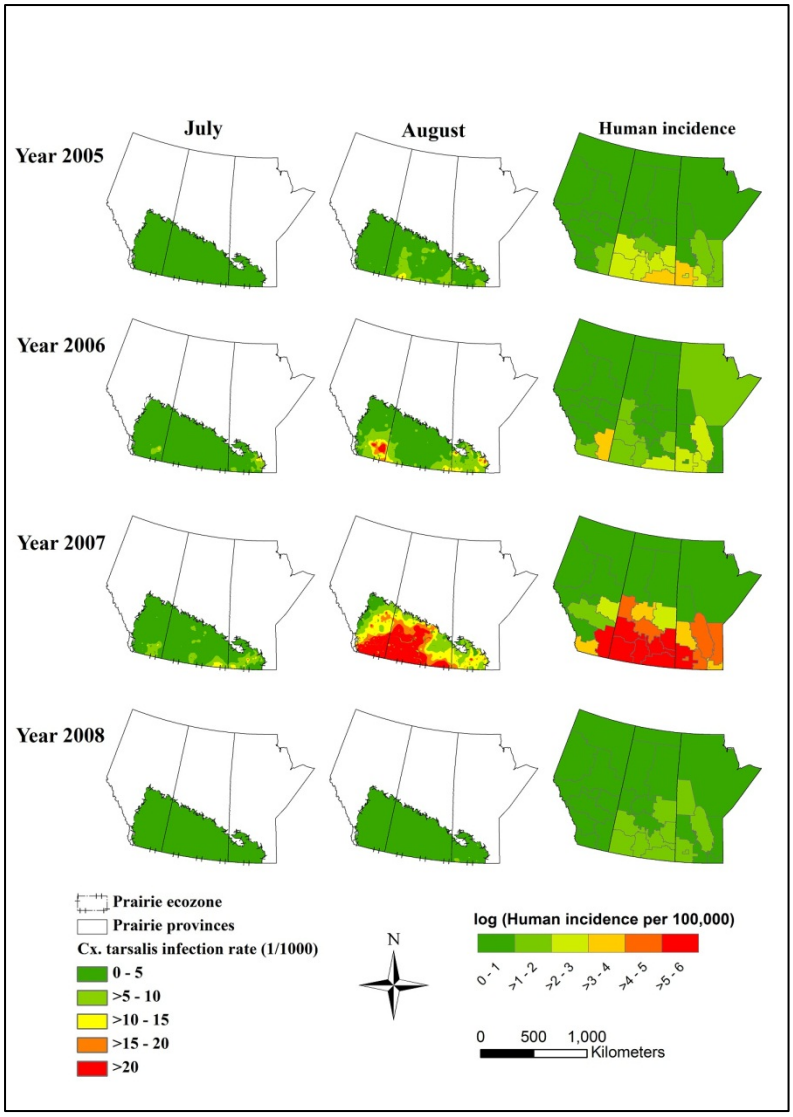

Figure 4.4 Maps of predicted WNV infection rate in female *Cx. tarsalis* per 1,000 in July and August, 2005-2008 in the Canadian prairies and log transformed human incidence (cases per 100,000 individuals) for each health region in the entire WNV transmission season.

Chapter 5 Climate Change and West Nile Virus in a Highly Endemic Region of North America[2]

Preface

In this chapter, the monthly models were applied to evaluate the potential effects of climate change on the spatial and temporal distribution of *Cx. tarsalis* and WNV. Chapter 5 combines the models of *Cx. tarsalis* abundance, and WNV infection rate with projected future climates to predict the spatial and temporal distribution of *Cx. tarsalis* and WNV infection rate in the prairie provinces. This study evaluated the possible effects of future climate conditions on the occurrence of WNV and *Cx. tarsalis*. Dr. Elaine Barrow contributed a contracted (internal, unpublished) report that provided the framework for the approach used to project climate change in the Canadian prairie provinces.

5.1 Introduction

Climate conditions, such as temperature and precipitation, are among many important factors that determine the spatial and temporal distribution of vectors and vector-borne diseases. Changes in climate influence the occurrence of vector-borne diseases in the following three major ways: a) reproduction, development, and survival of vectors, which in turn drive the distribution and abundance of vectors; b) blood seeking activity of vectors; and c) rates of pathogen amplification, through development, multiplication, and survival within

[2] Chen, C. C., E. Jenkins, T. Epp, C. Waldner, P. S. Curry and C. Soos. 2013. Climate change and West Nile Virus in a highly endemic region of North America. International Journal of Environmental Research and Public Health 10:

vectors (Gubler et al. 2001, Hunter 2003). In addition, climate conditions may affect the distribution, abundance, behavior, phenology of reproduction, and migration of vertebrate hosts (Kovats et al. 2001, Greer et al. 2008). Therefore, climate change will drive dramatic alterations in the spatial and temporal distribution and overall incidence of vector-borne diseases. Besides these direct effects of climate change on vector-borne diseases, climate change can also lead to substantial alterations in landscape, which in turn influence the distribution and abundance of hosts, vectors, and vector-borne pathogens (Chan et al. 1999, Gubler et al. 2001). Without taking these ecological effects of climate change and their interactions into consideration, projections of the potential effects of climate change on vector-borne diseases will remain inaccurate (Chan et al. 1999, Gubler et al. 2001).

West Nile virus (WNV) from the family Flaviviridae, genus Flavivirus was introduced into the Western Hemisphere in 1999 (Nash et al. 2001). Since that time the Canadian prairies, grassland ecozone in the southern parts of the provinces of Manitoba, Saskatchewan, and Alberta (Figure 5.1), have generally had the highest human incidence of clinical cases of WNV infection in Canada. During the 2007 epidemic season, a total of 2,215 confirmed clinical cases of WNV infection were reported in Canadians, of which 98% occurred in the prairie provinces, including 1,285 in Saskatchewan, 578 in Manitoba, and 318 in Alberta (Public Health Agency of Canada 2007). As a newly introduced vector-borne disease affecting a wide range of vertebrate hosts, WNV remains a significant concern for public health and wildlife conservation in the Canadian prairies.

In the Canadian prairies, the mosquito species *Culex tarsalis* Coquillett is the principal vector for WNV (Curry 2004, Yiannakoulias et al. 2006) This mosquito species is one of the most competent WNV vectors evaluated to date in laboratory studies (Turell et al. 2005) and is the predominant potential vector species in the Canadian prairies during the summer WNV

season (Curry 2004). The southern boundary of the boreal forest transition zone is identified as the northernmost limit of WNV distribution in western North America (Curry 2004), although *Cx. tarsalis* has been recorded further north (Waeckerlin et al. 2012). Climate, particularly temperature and precipitation, and habitat preference determine the distribution of *Cx. tarsalis* in western North America (Jenkins 1950). Grassland and agriculture areas are the preferred land cover type for *Cx. tarsalis* in the Canadian prairies (Curry 2004, Yiannakoulias et al. 2006, Epp et al. 2009) and other regions of the Great Plains (Jenkins 1950, Chuang et al. 2011). Stagnant water bodies with high organic content are favored sites for oviposition by *Cx. tarsalis* (Beehler and Mulla 1995, Curry 2004). In the Canadian prairies, larvae of *Cx. tarsalis* are commonly found in many temporary water bodies, such as artificial containers, water-filled hoof prints, and weedy roadside ditches (Jenkins 1950, Curry 2004). Furthermore, large water bodies and running water are not suitable for larval development due to the disturbance and lower nutrition concentration (Zou et al. 2006). Studies have found that the percentage of wetland is not associated with the abundance of *Cx. tarsalis* and WNV risk in the Canadian prairies and northern Great Plains (Wimberly et al. 2008, Epp et al. 2009, Chuang et al. 2011, Chuang et al. 2012). Several biological features of *Cx. tarsalis* facilitate the transmission of WNV including its capacity to: vertically transmit WNV to its offspring (Goddard et al. 2003); produce multiple generations per season; and take multiple blood meals during each generation (Curry 2004). Because it feeds on both avian and mammalian hosts, *Cx. tarsalis* plays the role of a "bridging vector" transmitting WNV from its enzootic cycle to humans and other mammalian species (Lee et al. 2002, Kent et al. 2009, Thiemann et al. 2012). Besides WNV, *Cx. tarsalis* is the primary competent vector for the St. Louis and Western Equine Encephalitis viruses in North America (Artsob 2000, Curry 2004, Hongoh et al. 2009).

Changes in future climate will not only influence the distribution of vectors and

pathogens, but also the habitat suitability for vectors (Lafferty 2009). Therefore, the assessment of possible effects of climate change on grassland distribution is critical for predicting the occurrence of *Cx. tarsalis* and WNV under future climate change. In a warmer and possibly drier future climate, current grassland habitat in the Canadian prairie ecozone might be replaced by the grassland flora found in the United States, and the boreal forest in the northern prairie provinces might be replaced by aspen parkland and grassland (Rizzo and Wiken 1992, Hogg and Hurdle 1995, Thorpe 2011). The southern boundary of the boreal forest fits very closely with the zero isoline of the annual climate moisture index, estimated by mean annual precipitation minus potential evapotranspiration (PET) (Hogg 1994).

In the present study, we integrated empirically derived, biologically-relevant temperature thresholds for *Cx. tarsalis* survival and WNV development, and statistical models in order to predict the effects of climate change on the distribution and abundance of *Cx. tarsalis* and WNV in the Canadian prairies, one of the most highly endemic regions in North America. Furthermore, we took into account potential changes in landscape as a result of climate change, including predicting the distribution of grassland habitat under future climate changes (Hogg and Hurdle 1995, Thorpe 2011). The objectives were to assess and predict the potential effects of climate change on the abundance of *Cx. tarsalis* and infection rate of WNV in *Cx. tarsalis* in the Canadian prairie ecozone. In addition, we explored the possibility of northward expansion of *Cx. tarsalis* and WNV out of their current distribution area in the Canadian prairie ecozone. Due to lacking of information on the responses of competent bird distribution under climate change, the assumption was made that competent bird for WNV amplification would continue to present in the study region under climate change. This assumption was made based on the two reasons. First, many competent bird species, such as American crow, black-billed magpie, American robin, common grackle, house finch and house sparrow, etc. are wide distributed in the either boreal forest and

grassland ecozones in the North America (Komar et al. 2003, Dunn and Alderfer 2011). The current distribution of these competent birds indicated the ability of adoption to various habitat and climate conditions. Second, the most likely effect of climate change on the spatial distribution of birds is the northward shifting of distribution (Devictor et al. 2008, Walther 2010). Under this condition, distribution of birds in the Great Plain, a highly WNV endemic region in the United States, might shift northward into the Canadian prairie provinces. Therefore, it is reasonable to assume the persistent present of competent birds under climate change in the Canadian prairies.

5.2 Materials and methods

5.2.1 Selection of three climate change outcome scenarios

Monthly climatology data between 1961and 1990 were downloaded from the CRU Global climate data set (IPCC Data Distribution Centre; http://www.ipcc-data.org/; accessed in December, 2012) to represent the 30-year baseline climate conditions of the prairie provinces (IPCC-TGICA. 2007). For creating outcome scenarios representing a wide array of future climate conditions in the prairie provinces, we considered a total of 142 experiments in three future emission scenarios (SRA2, SRA1B, SRB1) constructed using 24 general circulation models (GCMs) (Randall et al. 2007) (Table 5.1). These were selected on criteria of plausibility and best international standards of practice at the time. Experiments of each emission scenario represent results of simulations that assume a forcing of 1% per year in equivalent CO_2 concentration, radiative forcing and variably include aerosol effects. Future emission scenarios of greenhouse gases and aerosols in the atmosphere depend on factors such as population and economic growth and energy use (IPCC-TGICA. 2007).

Average changes with respect to 1961-1990 were calculated for the 30 year periods

centered on the 2020s (2010-2039), 2050s (2040-2069) and 2080s (2070-2099) for each of the 142 experiments. In order to select three outcome scenarios, representing cool and wet, median, and warm and dry climate conditions in the prairie provinces, scatter plots of changes in mean temperature and precipitation associated with 142 experiments were created for the study area. The 2050s time slice was used to select the three outcome scenarios; by the 2080s, the magnitude of uncertainty in results increased substantially. The selection of representative outcome scenarios (median and range) followed the guidelines put forward by the Intergovernmental Panel on Climate Change Task Group on Data and Scenario Support for Impact and Climate Assessment (IPCC-TGICA. 2007).

Future climate for each variable, such as monthly mean temperature and monthly total precipitation, was computed by combining baseline climate condition and predicted changes for each of the three outcome scenarios and the three time slices.

5.2.2 Models for *Cx. tarsalis* abundance and WNV infection rate

Recorded data on abundance of *Cx. tarsalis* and WNV infection rate were obtained from mosquito trapping in the prairie provinces from May to September for 2005 to 2008 from the Public Health Agency of Canada, Alberta Environment, Manitoba Public Health and Healthy Living, and Saskatchewan Ministry of Health. Counts of *Cx. tarsalis* per trap site per night were transformed by ln(y+1) to normalize the data distribution prior to analysis (Trawinski and Mackay 2009). *Culex tarsalis* infection rate (defined as the number of mosquitoes infected with WNV in 1000 pooled mosquitoes) was computed using PooledInfRate (version 3.0), a Microsoft® Excel plug-in (Biggerstaff 2006) by Maximum Likelihood (ML-IR) and minimum infection rate (MIR) methods (Chiang and Reeves 1962, Biggerstaff 2006).

Two models using temperature and precipitation as the primary explanatory variables were constructed to predict the abundance of *Cx. tarsalis* and WNV infection rate in *Cx.*

tarsalis in the Canadian prairies (descriptions of the models are in Chapter 4). Briefly, a linear mixed model was used to predict abundance of *Cx. tarsalis*. Parameters were estimated by the restricted maximum likelihood method. We then adopted a generalized linear mixed model, with a log link function for prediction of *Cx. tarsalis* infection rate. A negative binomial distribution was chosen in the *Cx. tarsalis* infection rate model based on a preliminary analysis where the formula 'Pearson Chi-Square divided by degrees of freedom (DF)' value was close to one and the lowest Akaike information criterion (AIC) value. A summary of the final *Cx. tarsalis* abundance model and the WNV infection rate model, as well as parameter coefficients, are shown in Table 5.2.

5.2.3 Modeling grassland distribution

We used models constructed by Hogg (1997) and simplified Penman-Monteith method to predict the future boundaries of the boreal forest and grassland in the prairie provinces (Hogg 1997). The predicted distribution of grassland habitat was used as a criterion for the occurrence of *Cx. tarsalis* under the three selected future outcome scenarios. To estimate the PET, a series of conditions and corresponding equations simplified from the Penman-Monteith method (Hogg 1997) were derived as follows:

For Tmean > 10 ºC: PET = 93 D exp(A/9300)

For 10 ºC ≥ Tmean > -5 ºC: PET = (6.2Tmean + 31) D exp(A/9300)

For Tmean ≤ -5 ºC: PET = 0

Tmean = mean monthly temperature (unit: ºC)

D = vapour pressure deficit (unit: KPa)

A = altitude (unit: meter)

The vapour pressure deficit (D) was calculated using this formula:

$$D = 0.5\ (e_{Tmax} + e_{Tmin}) - e_{Tdew}$$

eTmax = saturated vapor pressure at the maximum monthly mean temperature

eTmin = saturated vapor pressure at the minimum monthly mean temperature

eTdew = saturated vapor pressure at the dew point temperature.

The eTdew was set as equal to the saturation vapour pressure at a temperature of 2.5 °C lower than mean minimum temperature (Hogg 1997).

5.2.4 Maps of current and future distribution

Using these models and the baseline and projected climate conditions in the prairie provinces, ArcGIS version 10 (Environmental System Research Institute, Redlands, California) was used to create maps of current and future abundance of *Cx. tarsalis* and WNV infection rate.

5.2.4.1 Maps of *Cx. tarsalis* abundance

According to the thermal tolerance limits and habitat requirements for *Cx. tarsalis*, the occurrence of *Cx. tarsalis* was set as zero when the monthly mean temperature was lower than 14 °C or higher than 35 °C (Reisen 1995), or the habitat was not grassland. The constructed *Cx. tarsalis* abundance model (Table 5.2) was then populated with current and future climate conditions under the three outcome scenarios to map the monthly abundance and distribution of *Cx. tarsalis* in the Canadian prairie provinces.

5.2.4.2 Maps of WNV infection rate in *Cx. tarsalis*

In the WNV infection rate model, WNV transmission was considered possible when the following criteria were met: a) the primary vector, *Cx. tarsalis*, was present; b) the temperature was higher than 14.3 ºC, the minimum temperature for WNV amplification to occur in *Cx. tarsalis* (Reisen et al. 2006); and c) at least 82 degree days were accumulated in a 12 day feeding period of the life cycle for a female *Cx. tarsalis.* This represents the minimum amount of warming needed to complete the extrinsic incubation period of the virus in the mosquito (Reisen et al. 1993, Schrag et al. 2011). The WNV infection rate model was then populated with current and future climate conditions under the three outcome scenarios to predict distribution and rates of WNV infection in *Cx. tarsalis* in the study region.

5.3 Results

5.3.1 Selection of three climate change outcome scenarios

Based on predicted changes in mean temperature and precipitation in the 2050s, three outcome scenarios representing three GCMs and two emissions scenarios were selected to represent future of cool and wet, median, and warm and dry climate conditions in the

Canadian prairie provinces (Table 5.3). Average temperature was predicted to increase by 1-7 °C for all months in all selected outcome scenarios and time slices (Table 5.3). Although average annual total precipitation was projected to increase (by 21-46 mm) in all selected outcome scenarios and time slices, changes in precipitation varied among different months and areas in the prairie provinces. Under the warm and dry outcome scenario, the largest decreases in monthly precipitation occurred in July and August, with decreases by over 50% in some areas.

5.3.2 Grassland distribution

Prediction of grassland distribution showed two main areas where climate would be appropriate for grassland under current and proximal future climate scenarios. Under current conditions, the principal area of grassland habitat was the Canadian prairie ecozone, and a second, smaller patch was located in northern Alberta (Figure 5.1). Under the cool and wet future outcome scenario, the overall area of grassland habitat decreased by 23,004 $KM^2$and 7,762 KM^2 in the 2020s and 2050s, respectively, with an expansion of 57,132 KM^2 in the 2080s (Table 5.4, Figure 5.2). In the median and warm, dry scenarios, grassland habitat expanded in all time slices (298,683 KM^2 expansion for the median scenario in 2050s, range 17,765 – 842,110 KM^2), and furthermore, the two regions of grassland habitats merged into a large grassland by the 2050s (Table 5.4, Figure 5.2).

5.3.3 *Culex tarsalis* abundance and distribution

Increases in the following factors were associated with an increase in abundance of *Cx. tarsalis* in the model: mean temperature, one month lagged mean temperature, monthly total

precipitation, and one and two months lagged total precipitation. The predicted warmest mean temperature for all of the selected outcome scenarios and time slices was lower than the upper threshold (35 °C) for survival of adult female *Cx. tarsalis*. The temporal distribution of *Cx. tarsalis* under current climate conditions was restricted to a period from June to August, with highest abundance in July and August (Table 5.5). Under future climate conditions, the temporal occurrence of *Cx. tarsalis* was extended between May and September for all selected outcome scenarios by the 2080s, and for all but the cool wet scenario by the 2050s. Furthermore, abundance of *Cx. tarsalis* was predicted to increase 1.4 times under the median outcome scenario in the 2050s (1.1 times under the cool, wet scenario in the 2020s, and 2 times under the warm, dry scenario in the 2080s) in the Canadian prairie ecozone compared to baseline climate conditions (Table 5.5, Figure 5.2).

Projected spatial distribution showed that the highest abundance of *Cx. tarsalis* occurred in the southern part of the Canadian prairies under baseline climate conditions and all selected future outcome scenarios (Figure 5.2). Except for the cool and wet scenario in the 2020s, the distribution of *Cx. tarsalis* expanded northward under the future outcome scenarios and time slices. The expansion of geographical distribution of *Cx. tarsalis* was 33,195 KM^2 (1.60 fold increase) under the median scenario in the 2050s (no change under the cool, wet scenario in the 2020s and a 3 fold increase under the warm, dry outcome scenario in the 2080s) (Table 5.4, Figure 5.2). Climate conditions in the northern parts of the prairie provinces (up to 60°N latitude, currently parkland and boreal forest habitats) were suitable for *Cx tarsalis* under current and future climate scenarios and therefore, the northward expansion of *Cx. tarsalis* will be primarily restricted by the absence of suitable grassland habitat (Figure 5.2).

5.3.4 WNV distribution and infection rate in *Cx. tarsalis*

Under baseline climate conditions, the current temporal distribution and transmission season of WNV was limited to a period between June and August in the Canadian prairies. August was the month with the highest mean WNV infection rate of *Cx. tarsalis* (Table 5.6). The temporal occurrence of WNV in the Canadian prairie ecozone was extended from the current months of June to August to include May and September in all selected future outcome scenarios by the 2080s, and all but the cool and wet outcome scenario in the 2050s (Table 5.6). Compared to baseline, the August infection rate for the median scenario in the 2050s increased 18 fold (1.3 fold change under the cool, wet scenario in the 2020s and 27 fold change under the warm, dry scenario in the 2080s) (Table 5.6).

The projected future WNV distribution showed a decrease in distribution area of 23,258 KM^2 in the cool and wet outcome scenario in the 2020s, due to decreased area of grassland habitat. However, in all other future outcome scenarios and time slices, the northward expansion of WNV was projected. The expansion of WNV under the median scenario in the 2050s was 332,460 KM^2, representing a 1.6 fold increase from baseline conditions (no change under the cool, wet scenario in the 2020s, and a two fold increase under the warm, dry outcome scenario in the 2080s) (Table 5.4, Figure 5.3). WNV infection rate in the southern half of the Canadian prairies was generally higher than that in the north. Furthermore, most predicted areas of high WNV activity were located in the predicted grassland habitat for all selected outcome scenarios and time periods (Figure 5.3).

5.4 Discussion

Climate change is expected to influence the distribution of both vectors and vector borne pathogens, and contribute to the expansion or shifting of endemic regions (Brownstein et al.

2005, Lafferty 2009, Ostfeld 2009). This study demonstrates the potential for substantial expansion of the transmission season and geographic distribution of a recently introduced vector-borne disease in a highly endemic region of North America as a result of rapid climate and landscape change.

We constructed models for predicting abundance of the primary mosquito vector *Cx. tarsalis* and WNV infection rate, and populated these with data from baseline and selected future climate scenarios to assess the effects of climate and landscape change on WNV in the Canadian prairie ecozone. Under even the most optimistic of scenarios, WNV will undergo northern range expansion and extension of the transmission season by the 2050s. Based on a middle-of-the-road scenario, approximately half to two/thirds of the northern portion of the prairie provinces will have a climate newly suitable for WNV transmission by the 2050s. Under the most extreme warming conditions, peak mosquito infection rates could be 30 times that of baseline, representing a substantial increase in infection pressure for people and animals alike.

Although higher temperatures may lead to increased mosquito mortality and thus represent a natural check on viral amplification, results suggest that mean monthly temperatures will not exceed the upper threshold for survival of adult female *Cx. tarsalis*. In addition, mosquitoes may select cooler microhabitats if temperatures exceed tolerances. Therefore, the observed temporal and spatial distribution of WNV in the Canadian prairies will remain primarily determined by the lower temperature limitation for WNV amplification in *Cx. tarsalis* (estimated to be 14.3 °C) (Reisen et al. 2006). Laboratory experiments demonstrate that the temperature threshold for survival of *Cx. tarsalis* is generally between 14°C and 35°C, and within this range, temperature is positively correlated with development rate of vector (Reisen 1995, Henn et al. 2008). Therefore, climate change will lead to higher

development rates for vector without a compensatory increase in mosquito mortality. Moreover, increased temperatures will also increase the infection rate of WNV in *Cx. tarsalis*, especially in the southern part of the Canadian prairies.

Many factors besides climate are important determinants of the distribution and incidence of vector borne diseases, such as habitat suitability for competent vectors (Reeves 1965). Changes in future climate could also induce shifts in habitat distribution and affect habitat suitability for vectors (Lafferty 2009, Ostfeld 2009). In the current study, we used the constructed model to predict the distribution of grassland habitat under current and selected future outcome scenarios. Northward expansion of grassland has been predicted in Western Canada in a future of climate change, with boreal forest replaced by aspen parkland and grassland, and current Canadian grassland types replaced by those found in the U. S. Great Plains (Rizzo and Wiken 1992, Hogg and Hurdle 1995, Thorpe 2011). These latitudinal shifts in vegetation zones will create more suitable habitat for *Cx. tarsalis* in the northern part of the prairie provinces, while maintaining suitable habitat in the current Canadian prairie ecozone. However, the spatial expansion of *Cx. tarsalis* and WNV distribution in the prairie provinces will lag behind the shifts of vegetation zones.

The predicted distribution of grassland revealed another smaller area located in northern Alberta where the climate is appropriate for grassland habitat (Figures 1 and 2). Isolated grasslands resembling mixed prairie communities of the northern Great Plains are observed in this area (Schwarz et al. 1986, Schwarz and Wein 1992, Schwarz and Wein 1997). In addition, recent studies have also revealed *Cx. tarsalis* in the region of these grassland remnants, which extend into the southern Northwest Territories, although no WNV was detected (Waeckerlin et al. 2012). These empirical observations validated the prediction that if grassland habitat is available, *Cx. tarsalis* can already establish in the northern regions of

the prairie provinces under current and projected future climate conditions; however, the activity of WNV remains low or nonexistent in these regions under current climate conditions. Moreover, the Canadian prairie represents the northernmost edge of WNV distribution in the western hemisphere. As *Cx. tarsalis*, WNV, and other arboviruses expand northward out of their current endemic area into regions where humans, domestic livestock, and wildlife lack immunity, these vector-borne diseases may emerge in these newly vulnerable populations (Patz and Reisen 2001, Dobson 2009).

Temperature increases and the ecological impact of climate change are predicted to be greater in temperate and polar regions than in tropical regions (Meehl et al. 2007, Dobson 2009, Rohr et al. 2011). Increasing environmental temperature shortens the maturation time required for *Cx. tarsalis* and the extrinsic incubation period of West Nile virus. Furthermore, it also accelerates the mosquito gonadotrophic cycle and affects mosquito survival. Although beyond the scope of the current study, these relationships will influence virus transmission by increasing the contact rate between *Cx. tarsalis* and competent vertebrate hosts (Reisen et al. 2006, Zou et al. 2007).

Although we have demonstrated that changing climate and habitat will drastically alter the current distribution and abundance of a newly-introduced vector-borne disease, a number of other factors will also affect the ecology of WNV, and will in turn be affected by climate conditions. These factors include ability of hosts to migrate, disperse and adapt to changing local environments, host resistance to disease, biotic interactions, evolutionary change, other anthropogenic alternations of environment, and efforts of disease control (Pearson and Dawson 2003, Lafferty 2009). Future models addressing how these factors will affect the ecology of WNV are critically needed. In addition, climate change predictions are themselves subject to uncertainty in terms of the magnitude and scale of physical and socioeconomic

drivers, which will need to be addressed to more accurately predict changes in the ecology of vectors and vector-borne diseases. Finally, the precise lag time of habitat change (i.e. from boreal forest to grassland) and subsequent dispersal of vectors and hosts to newly suitable habitat remain unclear. Therefore, in order to validate the predictions and improve the predictive ability of these models, further monitoring of distribution and abundance of *Cx. tarsalis* and WNV is recommended, especially in regions that we have identified as vulnerable to range expansion and enhanced endemic amplification within the next 20-100 years.

The present study evaluated the potential effects of future climate and landscape change on the increased distribution and abundance of a newly-introduced vector-borne disease (WNV) and its primary vector (*Cx. tarsalis*) in a highly endemic region of North America. Studies like this one that use predictive models based on recorded data, known biological thresholds, and the best available climate scenarios covering the full range of outcomes provide vital information for public health professionals and policy makers to set priorities for mitigation and adaptation in the near future.

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Table 5.1 Experiments considered in the current study under given General Circulation Models and emissions scenarios to select future median and extreme climate scenarios for the Canadian Prairies. (source: Elaine Barrow, contracted internal unpublished report)

General circulation models	SRA2	SRA1B	SRB1	Resolution Latitude (°)	Resolution Longitude (°)
BCCR-BCM2.0	1	1	1	1.9	1.9
CGCM3.1_T47	5	5	5	2.8	2.8
CGCM3.1_T63	1	1	1	1.9	1.9
CNRM-CM3	1	1	1	1.9	1.9
CSIROMk3.0	1	1	1	1.9	1.9
CSIROMk3.5	1	1	1	1.9	1.9
ECHAM5	3	4	3	1.9	1.9
ECHO-G	3	3	3	3.9	3.9
FGOALS		3	3	2.8	2.8
GFDL-CM2.0	1	1	1	2.0	2.5
GFDL-CM2.1	1	1	1	2.0	2.5
GISS-AOM		2	2	3.0	4.0
GISS-EH		3		4.0	5.0
GISS-ER (run number)[1]	1 (1)	2 (2, 4)	1 (1)	4.0	5.0
INGV-SXG	1	1		1.1	1.1
INM-CM3.0	1	1	1	4.0	5.0
IPSL-CM4	1	1	1	2.5	3.75

MIROC3.2-hires		1	1	1.1	1.1
MIROC3.2-medres	3	3	**3 (run 2)**	2.8	2.8
CGCM2.3.2	5	5	5	2.8	2.8
NCAR-CCSM (run numbers)	4 (1-4)	7 (1-3, 5-7, 9)	8 (1-7, 9)	1.4	1.4
NCAR-PCM	4	4	**3 (run 2)**	2.8	2.8
UKMO-HadCM3	1	1	1	2.5	3.75
UKMO-HadGEM1	1	**1**		1.3	1.9
Total experiments	40	54	48		

[1] Run number of the experiments used in the current study.

Bold text indicates the experiments selected as median and extreme climate outcome scenarios in the current study

Table 5.2 Coefficients of variables in the final models of *Cx. tarsalis* abundance and WNV infection rate.

Variables (unit)	***Cx. tarsalis* abundance**		**WNV infection rate**	
	Coefficient	**95% CI**	**Coefficient**	**95% CI**
Intercept	-3.48	-4.05 to -2.91	-2.26	-4.47 to -0.05
Cx. tarsalis abundance (log(y+1))			0.55	0.31 to 0.79
Climate variables				
Monthly mean temperature (1 °C)	0.22	0.2 to 0.25		
1 month lagged temperature (1 °C)	0.07	0.05 to 0.09	0.32	0.22 to 0.41
3 months total of monthly mean degree days (dd)			-0.10	-0.2 to -0.01
Monthly total precipitation (1 mm)	0.0033	0.002 to 0.005		
1 month lagged precipitation (1 mm)	0.0042	0.003 to 0.005	-0.27	-0.36 to -0.18
2 month lagged precipitation (1 mm)	0.0033	0.002 to 0.004		
3 months total precipitation (1 mm)			-0.05	-0.08 to -0.02

CI: confidence interval

Table 5.3 Outcome scenarios selected to represent the range of effects of future climate change in the Canadian prairie provinces and their associated changes in mean annual temperature and precipitation in 3 time slices, compared to baseline climate conditions (1961-1990). Acknowledgement of the source of this table is an internal report prepared by Elaine Barrows as part of contracted work performed for the research project.

Experiments and time slices	**Emissions Scenarios**	**Outcome Scenarios**	**Change in annual total precipitation (SD); mm**	**Change in mean annual temperature (SD); ºC**
2010-2039 (2020s)				
NCAR-PCM run 2	B1	Cool, wet	22.4 (8.9)	1.14 (0.27)
MIMR	B1	Median	25.8 (16.3)	1.63 (0.18)
UKMO-HadGEM1 run 1	A2	Warm, dry	44.2 (16.9)	1.65 (0.40)
2040-2069 (2050s)				
NCAR-PCM run 2	B1	Cool, wet	41.1 (12.8)	1.77 (0.28)
MIMR	B1	Median	37.9 (28.1)	3.04 (0.11)
UKMO-HadGEM1 run 1	A2	Warm, dry	21.3 (22.9)	4.03 (0.39)

2070-2099 (2080s)				
NCAR-PCM run 2	B1	Cool, wet	52.3 (12.6)	2.42 (0.33)
MIMR	B1	Median	46.4 (31.2)	4.24 (0.19)
UKMO-HadGEM1 run 1	A2	Warm, dry	29.4 (31.4)	6.80 (0.50)

SD: standard deviation

Table 5.4 Predicted distribution and range expansion of grassland habitat, the mosquito vector *Cx. tarsalis*, and WNV in the prairie provinces under current and future climate conditions. The predicted distribution of *Cx. tarsalis* and WNV was assumed to be limited by the availability of grassland habitat.

Outcome scenarios	**Distribution area[2]**			**Area expansion[3]**			**Fold change[4]**		
and time slices	**Grassland**	***Cx.tarsalis***	**WNV**	**Grassland**	***Cx.tarsalis***	**WNV**	**Grassland**	***Cx.tarsalis***	**WNV**
Current[1]	607,018	566,506	539,877						
2010-2039									
Cool, wet	543,502	536,042	516,619	-23,004	-30,464	-23,258	0.90	0.95	0.96
Median	727,509	727,029	711,578	120,491	160,523	171,701	1.20	1.28	1.32
Warm, dry	624,783	617,767	586,466	17,765	51,261	46,589	1.03	1.09	1.09
2040-2069									
Cool, wet	599,256	599,256	582,998	-7762	32,750	43,121	0.99	1.06	1.08
Median	905,701	905,701	872,337	298,683	339,195	332,460	1.49	1.60	1.62

Warm, dry	1,198,242	1,198,242	1,151,876	591,224	631,736	611,999	1.97	2.12	2.13
2070-2099									
Cool, wet	664,150	664,150	657,321	57,132	97,644	117,444	1.09	1.17	1.22
Median	1,082,641	1,082,641	1,036,084	475,623	516,135	496,207	1.78	1.91	1.92
Warm, dry	1,449,128	1,449,128	1,263,070	842110	882,622	723,193	2.39	2.56	2.34

[1]The current distribution area of *Cx. tarsalis* and WNV are in the Canadian prairie ecozone based on the 1961-1990 climate condition.

[2]To estimate the distribution area of outcome scenarios, the availability of grassland habitat is set as a criterion for *Cx. tarsalis* in the prairie provinces.

[3]Area expansion = future distribution area (based on outcome scenarios) minus current distribution area.

[4]Fold change = future distribution area (based on outcome scenarios) divided by the current distribution area.

Table 5.5 Temporal distribution, mean abundance, log(y+1) transformed, and fold change of *Cx. tarsalis* abundance in the Canadian prairie ecozone for current and three future periods of selected outcome scenarios.

Outcome	**May**	**June**		**July**		**August**		**September**
scenarios	**Abun (SD)**	**Abun (SD)**	**Fold change[1]**	**Abun (SD)**	**Fold change**	**Abun (SD)**	**Fold change**	**Abun (SD)**
Current	None	1.22 (0.33)		2.26 (0.32)		2.20 (0.32)		None
2010-2039								
Cool, wet	None	1.48 (0.32)	1.21	2.56 (0.29)	1.13	2.43 (0.31)	1.10	None
Median	None	1.57 (0.31)	1.29	2.68 (0.32)	1.19	2.75 (0.30)	1.25	0.28 (0.60)
Warm, dry	None	1.71 (0.31)	1.40	2.62 (0.28)	1.16	2.65 (0.30)	1.20	0.08 (0.33)
2040-2069								
Cool, wet	None	1.57 (0.29)	1.29	2.66 (0.26)	1.18	2.54 (0.31)	1.15	0.05 (0.28)
Median	0.05 (0.16)	1.97 (0.26)	1.61	3.03 (0.29)	1.34	3.02 (0.27)	1.37	1.11 (0.76)
Warm, dry	0.37 (0.39)	2.30 (0.32)	1.89	3.34 (0.34)	1.48	3.49 (0.32)	1.59	1.90 (0.50)

2070-2099								
Cool, wet	0.02 (0.09)	1.72 (0.33)	1.41	2.75 (0.28)	1.22	2.69 (0.30)	1.22	0.28 (0.63)
Median	0.49 (0.36)	2.26 (0.27)	1.85	3.32 (0.32)	1.47	3.39 (0.33)	1.54	1.83 (0.36)
Warm, dry	1.18 (0.27)	3.04 (0.31)	2.49	4.27 (0.32)	1.89	4.33 (0.31)	1.97	2.87 (0.26)

Abun: abundance of *Cx. tarsalis*; SD: standard deviation; None: no occurrence of *Cx. tarsalis*.

[1]Fold change = future abundance (based on outcome scenarios) divided by the current abundance of *Cx. tarsalis*.

Table 5.6 Temporal distribution and fold change of WNV infection rate (number of infected mosquitos per 1000 mosquitoes) in *Cx. tarsalis* mosquitoes in the Canadian prairie ecozone for current and three future periods of selected outcome scenarios.

Outcome	**May**	**June**		**July**		**August**		**September**
scenarios	**IR(SD)**	**IR (SD)**	**Fold change[1]**	**IR (SD)**	**Fold change**	**IR (SD)**	**Fold change**	**IR (SD)**
Current	None	0.54 (0.25)		1.33 (0.45)		2.23 (0.94)		None
2010-2039								
Cool, wet	None	0.51 (0.16)	0.94	1.14 (0.42)	0.86	2.88 (1.27)	1.29	None
Median	None	0.86 (0.25)	1.59	2.02 (0.96)	1.52	5.17 (2.64)	2.32	0.44 (1.32)
Warm, dry	None	0.67 (0.22)	1.24	1.67 (0.71)	1.26	2.84 (1.42)	1.27	0.40 (0.99)
2040-2069								
Cool, wet	None	0.72 (0.23)	1.33	1.62 (0.66)	1.22	3.20 (1.47)	1.43	0.06 (0.41)
Median	0.02 (0.09)	1.91 (0.71)	3.54	4.87 (1.91)	3.66	17.91 (10.0)	8.03	4.32 (4.63)
Warm, dry	0.17 (0.21)	1.37 (0.46)	2.54	5.91 (3.08)	4.44	18.08 (9.98)	8.11	10.18 (6.93)

2070-2099								
Cool, wet	0.004 (0.03)	1.01 (0.31)	1.87	1.91 (0.87)	1.44	3.74 (1.76)	1.68	0.28 (0.87)
Median	0.001 (0.001)	1.79 (0.64)	3.31	5.53 (2.57)	4.16	19.95 (11.81)	8.95	9.44 (7.06)
Warm, dry	0.61 (0.22)	2.70 (0.93)	5.00	17.55 (6.70)	13.20	61.21 (27.78)	27.45	30.89 (11.66)

IR: WNV infection rate in *Cx. tarsalis*; SD: standard deviation; None: absence of WNV in the *Cx. tarsalis*.

[1]Fold change = future WNV infection rate (based on outcome scenarios) divided by the current infection rate in *Cx. tarsalis*.

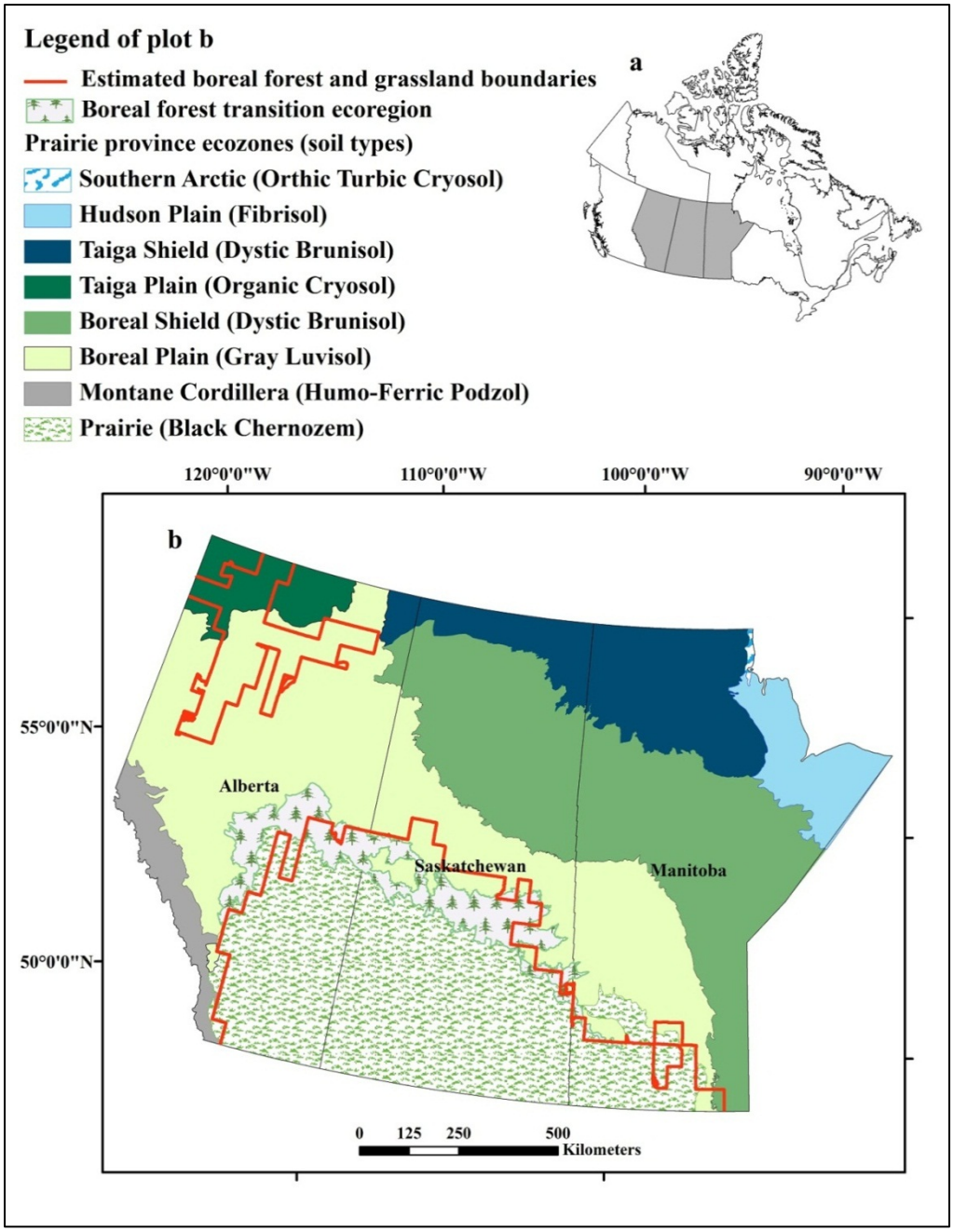

Figure 5.1 Distribution of ecozones and soil types in the prairie provinces of Alberta, Saskatchewan, and Manitoba, Canada and the boreal forest and prairie boundaries estimated using baseline climate conditions. (a) Location of prairie provinces (grey shading) in Canada. (b) Enlargement of Prairie provinces and distribution of ecozones.

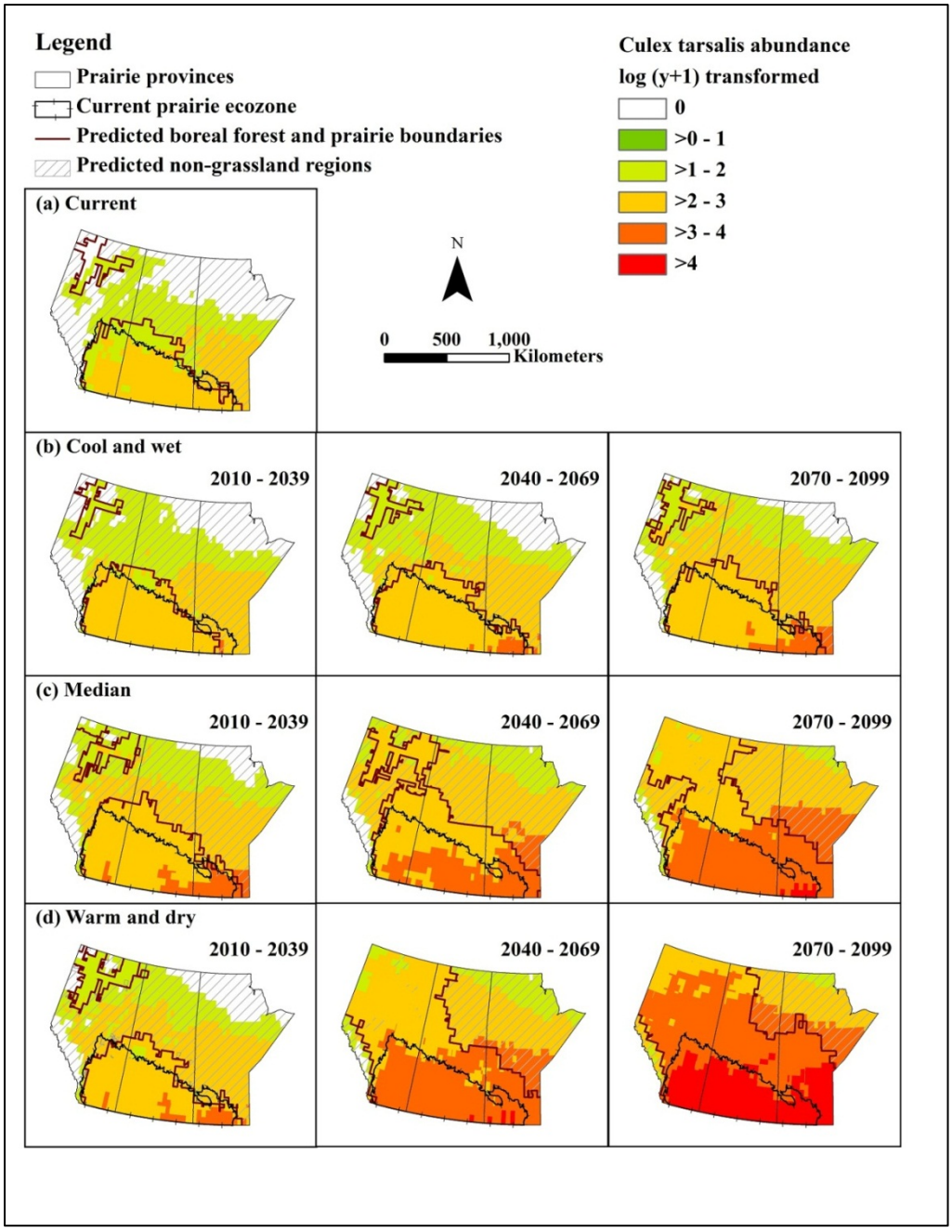

Figure 5.2 Projected spatial distribution and abundance, log (y+1) transformed, of *Cx. tarsalis* in August in the prairie provinces under current and selected outcome scenarios in three future time slices. The possible spatial distribution of *Cx. tarsalis* restricted by the predicted grassland distribution is indicated by the solid red line.

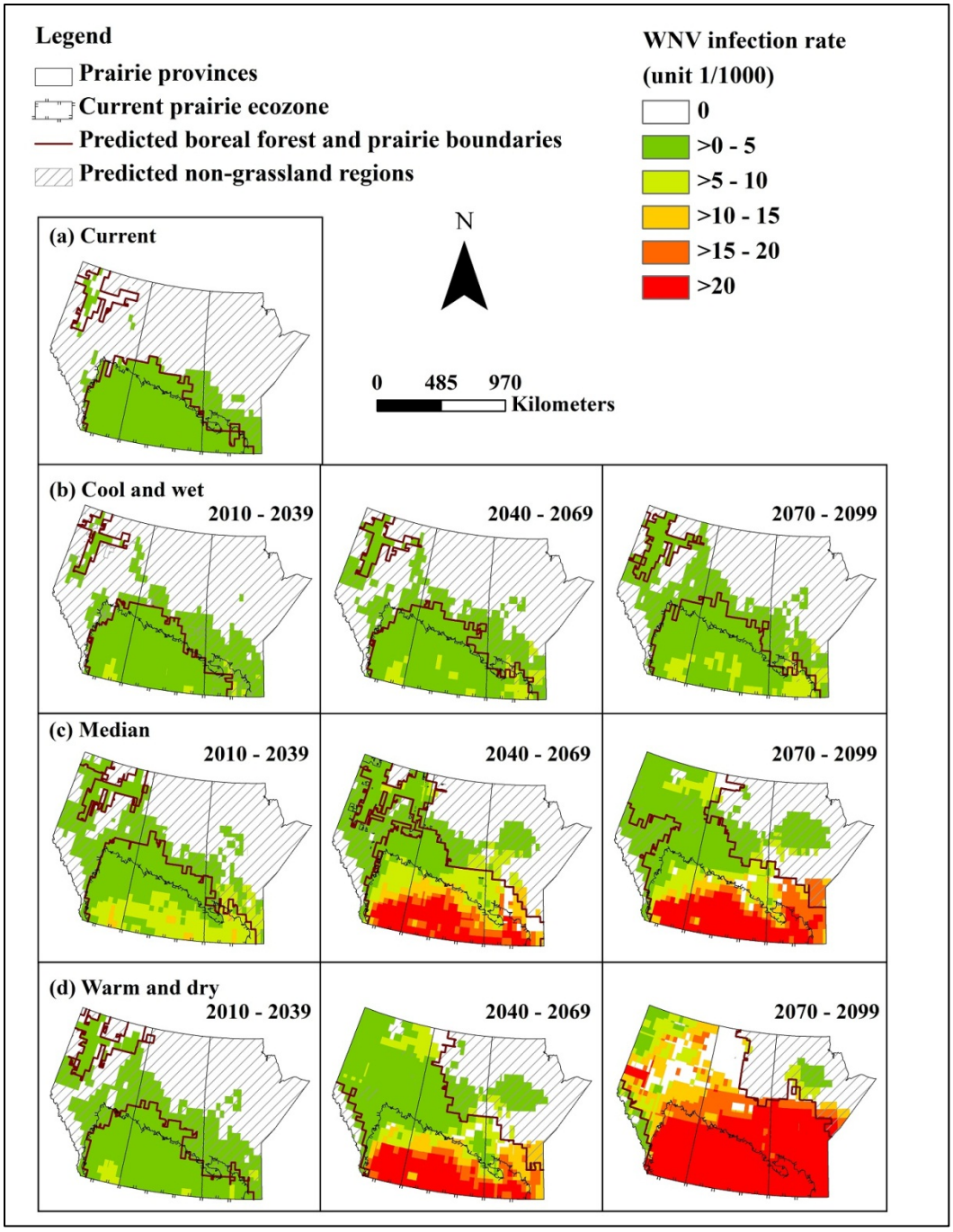

Figure 5.3 Projected WNV infection rate in *Cx. tarsalis* in August in the prairie provinces under the current (1961-1990) and selected outcome scenarios in three future time periods. The possible spatial distribution of WNV restricted by the predicted grassland distribution is indicated by the solid red line.

Chapter 6 The relationship between West Nile virus, bird abundance and communities in the Canadian prairies

Preface

This chapter focused on the relationship between bird host abundance/community and WNV transmission intensity in the Canadian prairies. This chapter evaluated the influence of WNV on the abundance and community composition of selected bird species, as well as the effects of bird community composition on WNV transmission intensity in its enzootic cycle.

6.1 Introduction

Since the incursion of West Nile Virus (family Flaviviridae, genus Flavivirus; WNV) into the New York region in 1999 and its subsequent spread across North America, studies have revealed declines of abundance in some intensively monitored bird populations, such as American crow (*Corvus brachyrhynchos*), greater sage-grouse (*Centrocercus urophasianus*), and American white pelican (*Pelecanus erythrorhynchos*) (Caffrey et al. 2003, Marra et al. 2004, Naugle et al. 2004, Yaremych et al. 2004, Sovada et al. 2008). In addition, studies focused on long term trends of population abundance using North American Breeding Bird Survey (BBS) data revealed significant declines of the abundance of several bird species in the northeastern United States after the incursion of WNV (LaDeau et al. 2007, Wheeler et al. 2009, Foppa et al. 2011). West Nile Virus causes neuroinvasive disease and high mortality in susceptible bird species, particularly corvids which are highly susceptible to the WNV strain circulating in the Western Hemisphere (Komar et al. 2003, Marra et al. 2004, McLean 2006). WNV has been reported in 326 bird

species (Centers for Disease Control and Prevention; available from http://www.cdc.gov/ncidod/dvbid/westnile/birdspecies.htm, accessed 28 August 2012), as well as in mammalian, reptilian, and amphibian species (Marra et al. 2004).

The Canadian prairies are considered highly endemic for WNV, with the majority of human cases in Canada reported in this region; however, the most important avian hosts for amplification of WNV remain unknown in this region. This region is also one of the most important regions for breeding and migration for many North American birds, including species of high concern for conservation. In Canada, corvids and raptors are the major species found to be affected clinically by WNV (Drebot et al. 2003, Zimmer 2005). Although dead bird surveillance is an effective method to detect WNV activity (Eidson et al. 2001), it is difficult to evaluate the effects of WNV on specific bird populations, particularly when the majority of specimens submitted for screening are corvids (Drebot et al. 2003). Furthermore, these surveillance programs often lack background population data and have variable sample collection efforts between urban and rural regions. There is a need to determine whether WNV has had, or has the potential to have, impacts on the abundance of these important wildlife species. Studies demonstrating the effects of disease on free-living populations of wildlife are rare, and a robust investigative approach based on observed data versus projected models is critically needed.

In addition, it is important to determine if the recent introduction of WNV has led to changes in bird community composition that may in turn result in enhanced or decreased WNV transmission. If the most competent hosts are also the most susceptible to WNV-induced mortality, then population declines in these hosts could lead to decreased transmission and allow recovery of wild bird populations (Komar et al. 2003, Kilpatrick et al. 2007, LaDeau et al. 2007). Transmission of WNV would then recur following the recovery

of competent bird populations. WNV infection rate in *Cx. tarsalis* and human cases varied greatly between 2005 to 2008, with 2007 representing a record peak in the Canadian prairies (Chen et al. 2012). Years with higher WNV infection rate, such as 2007, may have higher impact on susceptible bird abundance and diminish susceptible bird population in the following year (2008). Therefore, we predict that we should observe annual variation in avian community composition indices (such as species richness and Shannon-Weiner species diversity) in this time period.

Finally, we also predict that changes in bird community composition and biodiversity may in turn affect transmission of WNV, as measured by infection rate in *Cx tarsalis* (Ezenwa et al. 2006, Swaddle and Calos 2008). Biodiversity and the composition of host communities have been hypothesized to affect transmission of various vector-borne diseases through a "dilution effect" (Ostfeld and Keesing 2000b, Keesing et al. 2006, Ostfeld 2009). The dilution effect depicts an inverse relationship between host diversity and prevalence of vector-borne pathogens (as an indicator of transmission); i.e., increasing the diversity of a host community can lead to an increase in the proportion of vectors which feed on non-competent host species, resulting in a lower infection prevalence in the vector population and dampening pathogen transmission (Ostfeld and Keesing 2000a). There are four criteria which are required for the dilution effect to operate for transmission of a vector-borne pathogen: a) the host selection of primary vector is generalized; b) the pathogen is mainly transmitted by contact between host and vector; c) competence varies among hosts; d) a positive correlation exists between host competence and dominance (Ostfeld and Keesing 2000b). Other studies have revealed that *Cx. tarsalis* prefers to feed on specific bird species (Lee et al. 2002, Kent et al. 2009, Thiemann et al. 2012) and is able to transmit WNV vertically to its progeny (Goddard et al. 2003); therefore, the first two criteria may not apply to WNV transmission in the Canadian prairies. However, Komar et al. (2003) demonstrated

variation in competence index among bird hosts, suggesting that the third criterion is met. This study will evaluate whether the last criterion might apply – namely, whether the most dominant avian species were those most competent for WNV as demonstrated by correlation between competence and relative abundance of selected bird species.

To assess the relationships among transmission intensity of WNV, bird populations, and community composition, we used observed (BBS data) and projected bird abundance using constructed models, as well as observed WNV infection rate in *Culex tarsalis* in the Canadian prairies. WNV infection rate in the *Cx. tarsalis* population can be used as an indicator of transmission intensity, and is a good predictor of epidemic risk (Brownstein et al. 2004). In this study, we evaluated the relationships between WNV and birds by investigating (i) the effect of WNV on population abundance of selected bird species; (ii) changes in bird community composition following the introduction of WNV; (iii) the influence of bird community composition on WNV infection rate in *Cx. tarsalis* (iv) correlation between bird competence and relative abundance.

6.2 Materials and methods

6.2.1 Study area

The Canadian prairies (figure 6.1), located on the north end of the Great Plains and Bird Conservation Region 11 (BCR11), is an ecosystem with grassland as a principal land cover type, followed by aspen parkland or wetland (Canadian Prairie Partners in Flight 2004). This area has been recognized as an important habitat for a large diversity of bird species. It is estimated that 228 species of land birds use BCR11 for breeding and wintering regularly and six species of landbirds are found exclusively in this area (Mengel 1970, Canadian Prairie Partners in Flight 2004). In addition, the Canadian prairies generally have had the highest

prevalences of WNV in Canada, particularly in 2003 and 2007.

The mosquito species, *Cx. tarsalis*, is the primary vector of WNV in the Canadian prairies (Curry 2004, Yiannakoulias et al. 2006). This arthropod vector is capable of transmitting WNV vertically to its progeny (Goddard et al. 2003), and it mainly feeds on avian hosts in the early transmission season (Tempelis et al. 1965, Kent et al. 2009). In addition to the high competence for WNV transmission (Goddard et al. 2002, Turell et al. 2002), it is the most abundant species in the Canadian prairies during the summer transmission period (Curry 2004).

6.2.2 Impact of WNV on selected bird population abundance

6.2.2.1 Breeding bird survey data

We used BBS data from 1986 to 2010 to estimate population abundance of selected avian species in the Canadian prairies, prior to and following the introduction of WNV in this region in 2002/2003. The BBS routes are 39.43 km in length, each of which contains 50 evenly distributed sampling points. A skilled observer conducts a 3 minute sampling point count during which all birds seen and/or heard are recorded. The sum of counts from the 50 points in a route survey is used as an index of abundance along that route for that year. The BBS is usually conducted once a year in June, at the beginning of the WNV season in the Canadian prairies. Generally, the same observer surveys a route for a series of years. Most routes, but not all, are surveyed every year; routes in remote areas tend to be surveyed less consistently (Link and Sauer 2002). A BBS route was included in the study if there were surveys conducted for at least 10 years between 1986 to 2010 as well as a maximum of three missing years of data after WNV emergence (2002 to 2010) (Figure 6.1).

6.2.2.2 Bird species selection

Bird species selected in order to evaluate the effects of WNV on population abundance included susceptible species in which WNV is known to cause mortality or have population impacts (Komar et al. 2003, Zimmer 2005, LaDeau et al. 2007, Wheeler et al. 2009, Foppa et al. 2011), species of conservation priority in the Canadian prairies (Canadian Prairie Partners in Flight 2004), and species which are thought to be relatively resistant to WNV (Sullivan et al. 2006, Wheeler et al. 2009). We selected seven susceptible species that experience high mortality rates, including American crow, blue jay (*Cyanocitta cristata*), black-billed magpie (*Pica hudsonia*), American robin (*Turdus migratorius*), common grackle (*Quiscalus quiscula*), house sparrow (*Passer domesticus*), and house wren (*Troglodytes aedon*). We selected Baird's sparrow (*Ammodramus bairdii*), Swainson's hawk (*Buteo swainsoni*), Ferruginous hawk (*Buteo regalis*), and loggerhead shrike (*Lanius ludovicianus*) as species for which there are active conservation priorities in the prairie ecozone (Canadian Prairie Partners in Flight 2004). For resistant species, we selected four Icteridae, including red winged blackbird (*Agelaius phoeniceus*), brewer's blackbird (*Euphagus cyanocephalus*), yellow-headed blackbird (*Xanthocephalus xanthocephalus*), and brown-headed cowbird (*Molothrus ater*) (Reisen and Hahn 2007).

6.2.2.3 Climate variables

EI Niño/Southern Oscillation (ENSO)- and North Atlantic Oscillation (NAO)-induced global inter-annual climate variation have been demonstrated to markedly affect bird populations in the Western Hemisphere (Jaksic 2001, Nott et al. 2002). To control for the effects of climate on bird population abundance, variables of ENSO and NAO were included in the construction of models of trends in abundance of bird species. The Multivariate EI Nino/Southern Oscillation (ENSO) indices over winter and spring in each year were

downloaded from http://www.cdc.noaa.gov/~kew/MEI/ (accessed on December 10, 2012). North Atlantic Oscillation (NAO) indices of winter and spring were downloaded from http://www.cgd.ucar.edu/cas/jhurrell/indices.html (accessed on December 10, 2012). Spearman rank correlation was applied to compare the correlation between four indices and bird population abundance of selected bird species from 1986 through 2001 (prior to the emergence of WNV). The indices of ENSO and NAO that correlated with the abundance of major portion of selected species was then applied as an explanatory variable for constructing the predictive models of bird population abundance (LaDeau et al. 2007). The criterion value of Spearman rank correlation was set as $P< 0.10$ (LaDeau et al. 2007).

6.2.2.4 Hierarchical model for bird population abundance prediction

Bayesian hierarchical regression was used to construct models for predicting bird abundance with the annual BBS data. We first fit the model using 25 years of data, from 1986 to 2010, adjusting for differences among observers to estimate the mean abundance per BBS route for each year (LaDeau et al. 2007). The observer adjusted mean abundance was used to evaluate the abundance changes among years. We then fit the BBS data before the emergence of WNV in the Canadian prairies (1986 to 2001 for Saskatchewan and Manitoba and 1986 to 2002 for Alberta). The bird abundance models using data before WNV incursion were applied to predict the probability distributions of expected abundance after the incursion of WNV of each selected birds (LaDeau et al. 2007, Wheeler et al. 2009). Abundance of the species was considered to have been significantly impacted by WNV when observed abundance fell outside the 95% Bayesian credible intervals from the posterior abundance distributions in years after the first emergence of WNV (LaDeau et al. 2007). The Poisson distribution with overdispersion was used for model construction of BBS counts data. The model equation is as below:

$$\log(\lambda_{j,t}) = \alpha_j + \beta_j(t - t^*) + \gamma_t + \kappa + \eta I(j,t) + \varepsilon_{j,t}$$

This model describes the expected bird counts $\lambda_{j,t}$ in a specific route/observer and year, denoted as *j* and *t* respectively. Here, α_j is the route specific intercept, β_j is the slope of linear population trend centered at year t* in route j. t* was set as 1994 (middle year of BBS dataset for model construction), which is a baseline year from which change is measured. The year effects, γ_t, are random effects for variation among years with mean zero normal distribution. I(*j*, *t*) is an indicator (dichotomous variable) for the event that the count was in the first year of service of an observer and $\varepsilon_{j,t}$ are error terms with zero mean normal distribution. The standard vague priors are used for all hyperparameters with normal distribution that mean is set as zero and 1,000 for standard deviation. Variances of the year effects (γ_t) and error terms ($\varepsilon_{j,t}$) are with flat inverse gamma distributions with mean of 1 and variance of 1,000. Models were fitted by Markov Chain Monte Carlo (MCMC) with Gibbs sampler method and five independent Markov chains using the WinBUGS program (Lunn et al. 2000). Based on the preliminary analyses, the convergence of all BBS data were achieved before 8000 iterations. Each chain was run for 50,000 iterations following a 15,000 iteration 'burn-in'. The performance of five MCMC chains were assessed by Gelman-Rubin diagnostics for convergence of MCMC chains and autocorrelation plots through visual inspection (Link and Sauer 2002, Toft et al. 2007). For each selected species, a model with and without the climate variable (κ) were constructed. Model predictive ability were evaluated by deviance information criterion (DIC) (Spiegelhalter et al. 2002). A difference of DIC value between two models (with and without the climate variable) larger than 5 was considered to be substantial.

6.2.3 The relationship between bird species diversity and WNV infection rate in *Cx. tarsalis*

6.2.3.1 Changes of bird community composition among years

Birds counted on each BBS route in the Canadian prairies from 2005 to 2008 were used to estimate indices of avian community composition for each route. Community composition indices included absolute abundance of passerines, absolute abundance of non-passerines, passerine to non-passerine population ratio, species richness, and Shannon-Weiner species diversity. Bird community composition indices were logarithm transformed, except the Shannon-Weiner species diversity, which was exponential transformed, to normalize the data distribution. Repeated measures analysis of variance (ANOVA) was applied to assess the changes in avian community composition among years. Years between 2005 and 2008 were treated as a within-subjects variable (Swaddle and Calos 2008). The Huynh-Feldt correction was used to adjust the p value of repeated measures ANOVA, if the sphericity was violated (Girden 1992).

We then interpolated the avian community composition indices of each route in ArcGIS 9.2 (Environmental System Research Institute, California) using the inverse distance weighted method (Sauer et al. 1995) for the following determination of the effects of avian community composition on WNV infection rate in *Cx. tarsalis*.

6.2.3.2 WNV infection rate in *Cx. tarsalis*

We selected records of *Cx. tarsalis* collected in August from 2005 to 2008, from collection sites located less than 50 km from any selected BBS survey routes with BBS survey conducted in the matched year (Figure 6.1). For estimating WNV infection rate, *Cx. tarsalis* in each mosquito collection were grouped into pools for testing WNV infection by

reverse transcription polymerase chain reaction (RT-PCR) (Lanciotti et al. 2000, Drebot et al. 2003, Gu et al. 2004). Pooled WNV infection rate (per 1000 individuals) in *Cx. tarsalis* was computed using PooledInfRate (version 3.0), a Microsoft® Excel plug-in (Biggerstaff 2006), by Maximum Likelihood (ML-IR) and minimum infection rate (MIR) methods (Chiang and Reeves 1962, Biggerstaff 2006). The records with samples less than 100 female *Cx. tarsalis* per site per month were excluded from the analysis to prevent potential outliers and incorrect estimation of WNV infection rate in *Cx. tarsalis* resulting from small sample size (Gu and Novak 2004, Chen et al. 2012).

Values of avian community composition indices were extracted from the interpolated layers by matched location and year of mosquito collection sites. We applied a generalized linear model (GLM) with negative binomial distribution to assess the effects of bird community composition on WNV infection rate in *Cx. tarsalis* (Chen et al. 2012). In this model, WNV infection rate in *Cx. tarsalis* was set as the dependent variable and bird community composition indices were treated as explanatory variables. Initially, each explanatory variable was evaluated individually in the GLM. Variables with P-values larger than 0.2 were excluded for further model construction and analysis (Dohoo et al. 2009b). Explanatory variables selected in the WNV infection rate models were based on the Wald test (Dohoo et al. 2009a) and threshold for significance of the P-value was set at 0.05. The AICc (Akaike information criterion corrected for finite sample sizes) values were used to assess model fit (Burnham and Anderson 2002). Additionally, we controlled for monthly mean temperature, precipitation, and land cover types as possible confounding variables during model construction (Chen et al. 2012). Statistical analyses were performed with the SAS statistical software package, version 9.2 (Statistical Analysis System, Cary, NC).

6.2.3.3 Correlation between host dominance and competence

To assess the applicability of the last criterion of the dilution effect for WNV transmission in the Canadian prairies, we determined whether the competence indices of bird species (based on experimental infections by Komar et al (2003, 2005)) were correlated with the relative abundance of birds in the Canadian prairies using the Spearman rank correlation test.

6.3 Results

6.3.1 Impact of WNV on selected bird population abundance

From 1986 to 2010, there were 1621 surveys, produced by 161 observers on 95 BBS routes. Most of the missing data were distributed between 1986 and 1990. Average annual counts for each species are shown in Figure 6.2. All MCMC chains converged adequately according to the Gelman-Rubin diagnostics. The DIC values for models of each species of bird with or without the climate variable were not significantly different (Table 6.1). The climate variable did not improve model predictive ability for any species, and thus was not included in final models. Before the incursion of WNV into the Canadian prairies, abundance of corvid species (black-billed magpie and blue jay), was increasing or, for the American crow, relatively stable (Table 6.1); however, following introduction of WNV and contrary to the projected trend, abundance of theis highly susceptible corvid species decreased (Figure 6.2). For the other susceptible species, the observed abundance of the American robin and house wren was somewhat decreased compared to projected abundance following 2002, while the common grackle and house sparrow were declining but increased in abundance after 2002. Observed abundance of all 4 of the resistant icterid bird species was greater than projected abundance following incursion of WNV (Figure 6.2); brown-

headed cowbird and red-winged blackbird were decreasing in abundance prior to introduction of WNV and, contrary to the projected trend, increased following introduction of WNV (Table 6.1; Figure 6.2). For the 4 species of conservation concern, the observed abundance of ferruginous hawk and loggerhead shrike was less than that of projected abundance following 2002, while Swainson's hawk remained stable and Baird's sparrow showed an increase following 2002.

None of the selected species exhibited significant declines in abundance following the emergence of WNV in the prairies (Figure 6.2). However, the lowest estimates of abundance for the majority of species occurred after the incursion of WNV, whether examining a 15 year period (13 of 15 species from 1996 to 2010) or a 25 year period (9 of 15 species from 1986 to 2010) (Table 6.1, Figure 6.2). The lowest population count for black-billed magpies occurred in 1996 with 11.6 individuals per BBS route; however, this low abundance was approached again in 2006, 2008 and 2009 at 11.7, 11.9 and 11.7, respectively, following the emergence of WNV.

6.3.2 Bird community composition and WNV infection rate

In 2007, the number of human cases and infection rates in *Cx. tarsalis* were very high (Table 6.2). The sphericity was satisfied in all tests of the repeated measure ANOVA (Table 6.2). There was no significant difference in annual variation in bird community composition indices between 2005 and 2008 (Table 6.2).

In total, 482 mosquito collections from 2005 to 2008 in August were included in the data analysis. None of the bird community composition indices were associated with WNV infection rate in *Cx. tarsalis*, even when mean temperature, precipitation and land cover types were controlled as possible confounding factors (Table 6.3).

Competency of bird species for WNV was not correlated with abundance (Spearman

rank correlation coefficient was 0.12), suggesting that species with high competency were not the dominant species.

6.4 Discussion

In the first part of this study, we assessed the effect of WNV on susceptible and resistant wild bird populations in a region of conservation importance. We found no significant effect of WNV on abundance of selected bird species; however, following WNV incursion, noticeable population declines were observed in the highly susceptible corvid species, in contrast to the increasing trends in abundance predicted for these species prior to the introduction of WNV. In contrast, all of the resistant species (icterid blackbirds) increased in abundance following introduction of WNV, even though these species were declining prior to introduction of WNV. In addition, ferruginous hawk and loggerhead shrike, both species of conservation concern, declined below projected values following introduction of WNV. Since the susceptibility of these threatened species cannot be determined through experimental infections, this evidence, though circumstantial, may be the best available demonstration of the impact of WNV on conservation of threatened avian species.

Other studies in the USA using BBS data and Bayesian models have detected dramatic population declines in some bird species following the emergence of WNV, particularly in corvids. Impact of WNV on bird populations can vary regionally within the same bird species (LaDeau et al. 2007, Wheeler et al. 2009, Foppa et al. 2011). Regional heterogeneity of WNV impact on bird abundance may result from underlying differences in WNV prevalence (LaDeau et al. 2007), avian population abundance (Wheeler et al. 2009), and the distribution, abundance and feeding preference of competent vectors (LaDeau et al. 2007, Wheeler et al. 2009). In the Canadian prairies, the effects of WNV on the abundance of wild birds may have been buffered by the relatively low prevalence of the pathogen, shorter

transmission seasons, relatively low abundance of corvids, and dispersal from the nearby boreal forest, where WNV is not established. For example, significant population declines observed in several corvid species in Kern County, California, may have been due to higher infection rates in *Cx. tarsalis* and the longer WNV season in California compared to the Canadian prairies (Wheeler et al. 2009). Spatial heterogeneity of bird population abundance could also influence the WNV impact on bird populations. Wheeler et al.(2009) found that more widely distributed species experienced less population impacts by WNV. Population abundance of corvids is relatively low in the prairie ecozone compared to surrounding habitats (Sauer et al. 2011). Although corvid species were the most commonly found dead birds due to WNV infection in the study region, dispersal of individuals from nearby habitats (Withey et al. 2005), particularly individuals from northern boreal forest habitat, may have replenished the WNV depleted population and mitigated the impacts of WNV.

Changes in host abundance due to a pathogen could lead to substantial changes in the composition of the host community. Pathogen induced changes in community composition have been observed in many pathogen-host systems (LaDeau et al. 2007, Collinge et al. 2008). However, in the study, there were no significant annual differences in bird community composition indices even though significant annual variation was observed for the number of human cases and WNV infection rate in *Cx. tarsalis*, the primary vector for WNV in the Canadian prairies. This may reflect the choice of bird species selected; i.e., we may have missed the most susceptible and/or most competent species, or the factors discussed above that may have buffered bird populations from the effects of WNV.

Since we observed no significant changes in bird community composition associated with WNV in the Canadian prairies, it is not surprising that changes in bird community composition were not linked to WNV infection rate in *Cx.tarsalis*. Most studies which found an inverse relationship between bird diversity and WNV prevalence in mosquitoes were

conducted in the eastern United States with *Cx. pipiens*, an urban dwelling mosquito species (Kilpatrick et al. 2006, Reisen and Brault 2007), as the primary vector (Swaddle and Calos 2008, Allan et al. 2009). Urban environments generally have lower bird diversity and are dominated by a few human-adapted species (Miller et al. 2003, Crooks et al. 2004); therefore, these results may not be extrapolated to the Canadian prairies, a very different habitat and climate with different bird community composition and vector density. This study found no evidence to support the association between bird community composition and WNV infection rate in *Cx. tarsalis* in the Canadian prairies, even when the environmental or climatic variables were controlled in the model.

The dilution effect, or the protective effects of biodiversity in transmission of vector-borne diseases, has been postulated to affect pathogen transmission in many vector-borne pathogens, including WNV (Ostfeld and Keesing 2000b, Keesing et al. 2006, Ostfeld 2009). Several studies have been conducted at different scales and in many regions in the United States to evaluate the possible role of the dilution effect on WNV; however, the influence of dilution effect still remains controversial (Ezenwa et al. 2006, Swaddle and Calos 2008, Allan et al. 2009, Loss et al. 2009). This study suggests that one of the most important criteria for the dilution effect does NOT apply to WNV transmission in the Canadian prairies; namely, there was only a weak correlation between avian competence and abundance. This finding indicated that a species with high competence index was not necessary to be a dominant species in the Canadian prairies (Loss et al. 2009). When the optimal host is not a dominant species and can only occur in a highly diverse community, increasing the host diversity may also increase the infection prevalence (Ostfeld and Keesing 2000b).

This study finds no evidence to support the influence of dilution effect on WNV infection rate in *Cx. tarsalis* in the Canadian prairies. These findings were similar to other

recent studies which concluded that WNV prevalence was not directly related to the host diversity or community composition, but to the specific bird species which were highly competent for WNV, and also the preferred host of vectors (Hamer et al. 2011, Simpson et al. 2012). Future work to determine the avian species driving transmission in this unique ecosystem should focus on experimental demonstration of host competence and feeding preference of *Cx tarsalis*, and field sampling to determine prevalence of exposure in species that might not previously have been considered important for WNV transmission.

The approach that we have used to determine the effects of a newly introduced vector borne disease on wildlife populations, and conversely the effects of changes in wildlife community composition on disease transmission, is robust and can be applied to other vector-borne diseases transmitted between birds and *Cx. tarsalis* in North America, such as St. Louis encephalitis and western equine encephalitis (Hongoh et al. 2009). Such tools are increasingly important as climate and landscape change drive the complex ecology of vector-borne diseases, which may lead to unexpected declines in wildlife populations and increased risk of exposure in naïve human populations.

6.5 References

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Table 6.1 Fitted population trends based on breeding bird survey data from 1986, and deviance information criterion (DIC) values of population abundance models, for selected bird species in the Canadian prairies before WNV incursion in 2002 (2003 in Alberta)

Species	DIC		Population trend (95% credible interval)	Year of minimum abundance	
	With climate	Without climate		15 years	25 years
WNV Susceptible					
American crow	7108.02	7107.84	-0.13 (-1.65,1.26)	2008	2008
Black-billed magpie	5145.13	5145	2.85 (1.45, 4.32)	1996	1986
Blue Jay	1319.94	1319.48	1.78 (-1.89, 5.61)	2006	2006
American robin	5946.81	5947.24	1.72 (0.48, 2.96)	2008	1986
Common grackle	2767.61	2768.43	-2.26 (-5.24, 0.84)	2002	2002
House sparrow	6644.29	6644.36	-5.52 (-7.38, -3.61)	2009	2009
House wren	5957.37	5957.55	-0.94 (-2.49, 0.73)	2004	2004
WNV Resistant					
Brown-headed cowbird	6399.73	6399.92	-4.16 (-5.82, -2.51)	2001	2001
Brewer’s blackbird	6686.74	6687.09	0.03 (-1.53, 1.52)	2005	1989

Red-winged blackbird	7909.52	7908.43	-1.26 (-2.4, -0.08)	2003	2003
Yellow-headed blackbird	5056.27	5056.04	-7.47(-10.04, -4.54)	2003	2003
Conservation concern					
Baird’s sparrow	1810.33	1811.27	-8.63 (-12.72, -4.27)	2002	2002
Ferruginous hawk	702.82	704.78	4.559 (-1.08, 10.48)	2007	1990
Swainson’s hawk	3068	3067.28	-1.7 (-3.75, 0.42)	2008	2008
Loggerhead shrike	1213.07	1213.75	1.96 (-2.11, 6.19)	2007	1991

Table 6.2 WNV infection rate in *Cx. tarsalis* and bird community composition indices from 2005 to 2008 in the Canadian prairies

WNV intensity and bird community composition indices	Average (SD[1]); n = 56 BBS routes for each year				Sphericity p value[2]	ANOVA p value[3]
	2005	2006	2007	2008		
WNV infection rate in *Cx. tarsalis* (1/1000)	2.56 (4.0)	9.26 (7.07)	19.67 (14.76)	0.62 (1.49)		
Human cases of WNV	128	111	2,315	29		
Passerines absolute abundance	670.9 (397.8)	717.4(522.8)	685.7 (473.9)	659.6 (316.9)	0.18	0.47
Non-passerines absolute abundance	274.8 (267.4)	278.6 (266.6)	326.8 (361.7)	257.9 (206.1)	0.22	0.13
Passerines to non-passerines abundance ratio	3.7 (2.4)	4.1 (3.3)	3.2 (2.0)	3.8 (2.3)	0.70	0.10
Species richness	57.4 (12.1)	57.4 (12.7)	58.1 (11.9)	56.0 (11.4)	0.08	0.15
Shannon-Weiner species diversity	3.2 (0.4)	3.2 (0.4)	3.2 (0.5)	3.2 (0.4)	0.70	0.52

[1] SD = Standard deviation.

[2] Chi-Square probability of Mauchly's sphericity test.

[3] Results of testing the differences in community composition indices among years by repeated measure ANOVA

Table 6.3 Effects of bird community composition on WNV infection rate in *Cx. tarsalis*, evaluated using generalized linear models.

Model	Wald test p value (n = 482)		AICc	
	Without envir. vars.[1]	Envir. vars.	Without envir. vars.	Envir. vars.
Null model (no variable included)			2347.9	2342.5
Passerine abundance	0.2593	0.7437	2348.6	2344.5
Non-passerine abundance	0.3575	0.6855	2348.9	2344.4
Passerine to non-passerine abundance ratio	0.2687	0.5982	2348.7	2344.3
Shannon-Weiner diversity	0.5386	0.145	2349.6	2342.5
Species richness	0.5443	0.5631	2349.5	2344.3

[1]Envir. vars. indicates the environmental variables including mean monthly temperature, monthly mean precipitation and land cover types that were controlled in the model.

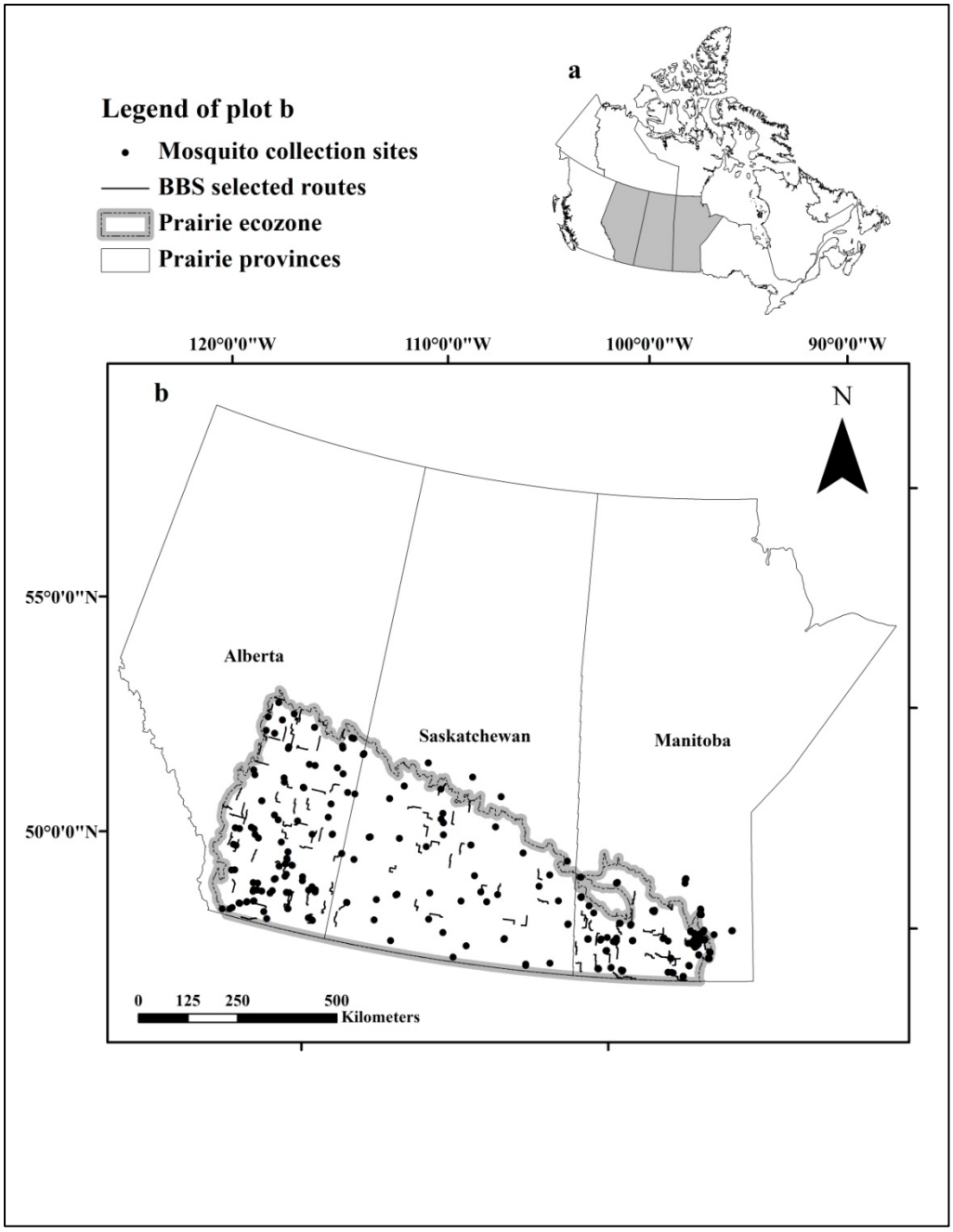

Figure 6.1 Study area. Distribution of selected breeding bird survey routes with at least 10 surveys that were conducted between 1986 and 2010, and mosquito collection sites from 2005 to 2008 in the Canadian prairies (provinces of Alberta, Saskatchewan, and Manitoba). a. Location of prairie provinces (grey) in Canada; b. Enlargement of prairie provinces

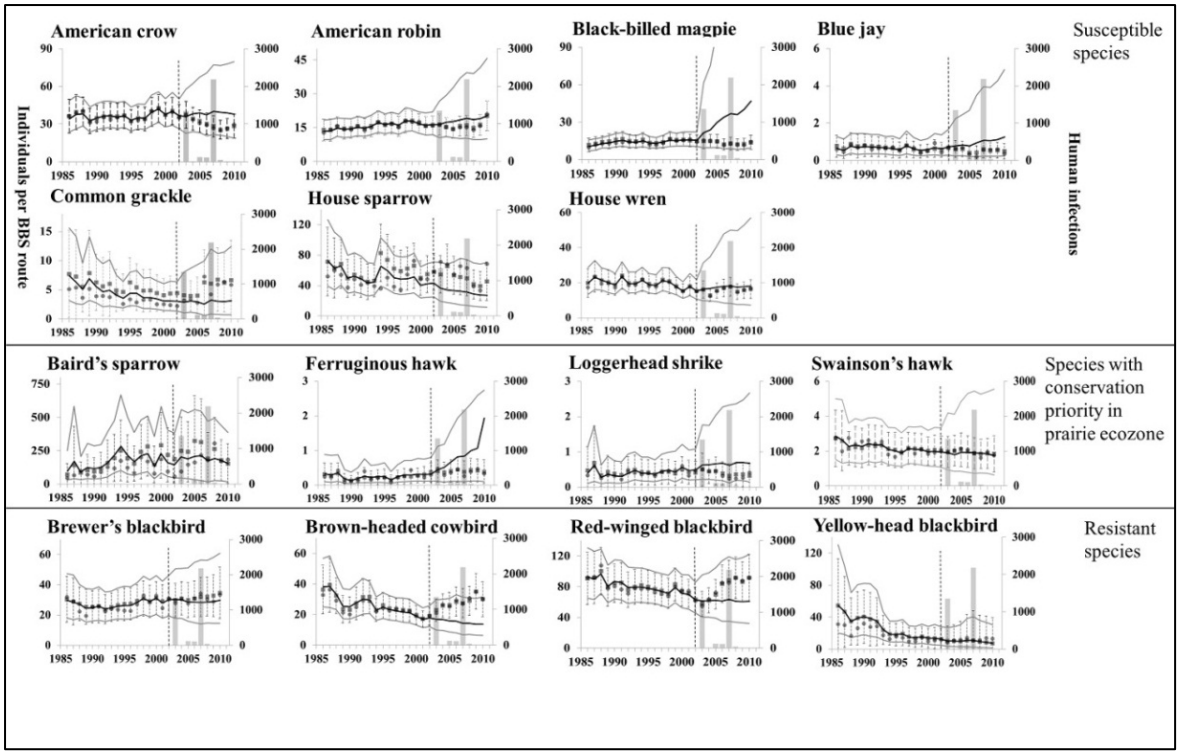

Figure 6.2 The effect of WNV on the abundance of selected bird species. Projected and observed mean population abundance of selected bird species in the Canadian prairies from 1980 to 2010. The projected mean abundance fitted with data from 1986 to 2002 by Bayesian hierarchical regression is represented by a solid black line (upper and lower grey lines represent 95% confidence intervals). Squares represent the time series mean abundance fitted with data from 1986 to 2010 and adjusted for observer differences. Error bars show 2 standard errors. Circles represent the observed mean abundance in each year. Vertical grey columns show the number of human infections in each year and the vertical dashed line indicates the first detection of WNV (year 2002) in this area.

Chapter 7 Summary and Conclusions

7.1 Background

West Nile virus (WNV) has induced unexpected impact on humans, domestic animals and wildlife since its introduction into North America. The impact of WNV on its hosts is even more severe within the North America continent than in the Old World regions (Petersen and Roehrig 2001, Hayes and Gubler 2006). The Canadian prairies have been identified as the "hotspot" of WNV with the highest human incidence of WNV infection in Canada. Nevertheless, information about factors affecting WNV transmission and the impacts of WNV on birds in this region remains limited.

For WNV, environmental factors can dramatically affect the abundance of competent vectors as well as the transmission intensity of pathogens (Reisen 1995, Reisen et al. 2006, Reisen et al. 2008, Chuang et al. 2011). In addition, bird species are known to be the primary reservoir hosts for amplification and transmission of WNV and susceptible to WNV. Predictive models are commonly used to evaluate the effects of abiotic and biotic factors on the transmission intensity of WNV, and such models can be applied for risk prediction by parameterizing them with selected climate and landscape variables. As such, a predictive model comprising habitat and climatic factors as explanatory variables can be applied to evaluate the potential effects on the distribution of WNV due to climate change. Furthermore, predictive model can be used to assess the effects of WNV on the abundance and community composition of bird hosts.

The first objective of this thesis was to determine the effects of abiotic and biotic factors on the abundance of *Culex tarsalis* (Diptera: Culicidae) and WNV transmission

intensity in the Canadian prairies. Secondly, models of two different time scales, weekly and monthly, were constructed for predicting WNV infection rate in *Cx. tarsalis* by utilizing geographic information system (GIS), remote sensing, and spatial analysis. The monthly model specifically was then applied to evaluate the effects of future climates on the distribution of WNV. In addition, this thesis assessed the effects of WNV on the abundance of bird species, and conversely, the effects of changes in bird species composition on transmission of WNV.

7.2 Summary of highlights from each chapter

7.2.1 Predicting weekly variation of West Nile virus infection in *Culex tarsalis*

It was expected that a more detailed epidemic process could be forecasted with the weekly model, a finer temporal scale compared to monthly model. Using mosquito data collected between 2005 and 2008, this study constructed models integrating abiotic and biotic factors to predict the WNV infection rate in *Culex tarsalis* Coquillett on a weekly time scale.

During the study period, the highest observed mean weekly WNV infection rate in *Cx. tarsalis* was at week 34 (late August) in 2005 - 2007. However, in 2008, there was a lower WNV infection rate as compared to other years. The *Cx. tarsalis* infection rate intensified with rising *Cx. tarsalis* abundance and mean temperature lagged from 1 to 8 weeks, but declined with elevating mean precipitation, which lagged from 2 to 6 weeks. Furthermore, this study found that *Cx. tarsalis* abundance had a significant positive association with WNV infection rate only when time lagged precipitation was controlled. This finding indicated that time lagged precipitation was a 'distorter variable' which alters the association between *Cx. tarsalis* abundance and the WNV infection rate. The aggregation of bird hosts and vectors due to decreased precipitation or oviposition sites might be the reason for this finding.

This study showed the variation of WNV infection rate and clarified how weather influenced WNV infection rate based on a weekly time scale in the Canadian prairies, a highly endemic region of North America. An understanding of the role of lagged weather variables was essential for providing sufficient lead time to predict WNV occurrence, and implementing intervention measures to protect public health and animal.

7.2.2 Modeling monthly variation of *Culex tarsalis* abundance and West Nile virus infection rate in the Canadian prairies

The monthly model was constructed in order to compare the resulting variables of importance with the weekly model. In addition, due to the limitation of predicted future climate datasets, the monthly model was the finest temporal scale to be applied to predict the effects of future climate change on WNV. In this study, abiotic and biotic factors were used as explanatory variables to construct a generalized linear mixed model for predicting WNV risk. This study applied the data of WNV infection rate in *Cx. tarsalis*, collected from 2005 to 2008 in the Canadian prairies, as an indicator of WNV transmission intensity and a dependent variable.

For comparing the differences of effects of explanatory factors between *Cx. tarsalis* abundance and WNV infection rate, this study also constructed models to clarify the effects of these factors on *Cx. tarsalis* abundance. In the constructed models, *Cx. tarsalis* abundance and WNV infection rate increased directly with increasing mean temperature and time lagged mean temperature. Increasing precipitation was associated with a greater *Cx. tarsalis* abundance and, paradoxically, a lowered WNV infection rate. In addition, increased temperature fluctuation and wetland land cover were associated with decreased infection rate in the *Cx. tarsalis*.

The constructed monthly models can be applied to predict *Cx. tarsalis* abundance and WNV transmission in the Canadian prairies and provide valuable information of WNV risk for public health interventions. Furthermore, these models are critical tools for assessing the possible effects of climate change on the vector abundance and the distribution of WNV.

7.3.4 Climate change and West Nile virus

The studies in this thesis have identified the significant effects of climate factors on the distribution of *Cx. tarsalis* and WNV in the Canadian prairies. Therefore, it is expected that the observed and projected changes in future climate could dramatically change the current distribution of WNV. Furthermore, changes in climate may alter the distribution of habitat for vectors. To evaluate the possible effects of climate change on the risk of WNV, this study first used models to predict the distribution of grassland, the primary habitat for *Cx. tarsalis*, in the Canadian prairie provinces. The monthly models and biological thresholds were then applied to predict the spatial and temporal distribution of *Cx. tarsalis* and WNV infection rate in *Cx. tarsalis* in the prairie provinces. This study selected one median and two extreme (wet, cool; dry, warm) outcome scenarios to represent future climate conditions in the 2020s (2010 -2039), 2050s (2040-2069) and 2080s (2070-2099) time slices.

In currently endemic regions of the Canadian prairies, the projected WNV infection rate under the median outcome scenario in 2050 was 18 times higher than under current climate conditions; even under the cool, wet scenario, projected infection rates never dropped below current infection rates. This finding suggesting enhanced transmission intensity of the virus in currently endemic regions under climate change. Seasonal availability of *Cx. tarsalis* infected with WNV extended from June to August to include May and September. Under the criterion of presence of predicted grassland distribution, models, with the exception of the

cool, wet scenario in the 2020s time slice, predicted the northward range expansion from 1.06-2.56 times for *Cx. tarsalis* and 1.08-2.34 times for WNV compared to the current distribution area. These findings predict future public and animal health risk associated with WNV and other viral encephalitis for which *Cx tarsalis* serves as a vector in the Canadian prairie provinces, as well as identify newly vulnerable regions in a future of rapid climate and landscape change.

7.3.5 The relationship between West Nile virus, bird abundance and communities

Impacts of WNV on avian fauna have been observed in different regions in North America. Declines of abundance of susceptible bird species caused by WNV may further influence the bird community composition; in turn, loss of biodiversity might affect the transmission of WNV through a dilution effect. This study used the North American Breeding Bird Survey (BBS) data to evaluate the effect of WNV on abundance of selected bird species in the Canadian prairies, as well as annual changes of bird community composition. Conversely, this study assessed the effects of bird community composition on WNV infection rate in the *Cx. tarsalis*.

Although many selected species in this study were at their lowest abundance in the past 15 or 25 years after the incursion of WNV into the Canadian prairies, there were no statistically significant declines in abundance of selected WNV susceptible avian species associated with the emergence of WNV. However, the abundance of WNV resistant birds, such as the brown-headed cowbird, increased significantly after the introduction of WNV. While an epidemic outbreak of WNV occurred in 2007 in the study area, analyses utilizing repeated measure analysis of variance (ANOVA) indicated that bird community composition indices had not changed significantly from 2005 to 2008. Furthermore, results indicated that

no bird community composition indices were associated with WNV infection rate in *Cx. tarsalis*, even when the mean temperature, precipitation and land cover types were controlled for as potential confounding factors.

Findings in this study suggest trends in the effect of WNV on the abundance of selected birds and bird community composition, none of which were significant. In addition, there is no evidence to support any association between bird community composition and WNV infection rate in *Cx.tarsalis* in the Canadian prairies. Other extrinsic and intrinsic factors, such as habitat, temperature, precipitation, host distribution and host preference of vectors, may have played a more important role in WNV transmission and its impact on avifauna in this highly endemic region.

7.4 Conclusion

This thesis applied long term mosquito surveillance data, North American Breeding Bird Survey data, as well as various environmental factors to evaluate many aspects of WNV transmission in its enzootic cycle. The studies in this thesis revealed the factors, including lagged temperature, precipitation, vector abundance, and habitat, affecting WNV transmission in different time scales and showed little impact of WNV on the abundance of selected bird species including endangered species. Integrated mapping techniques and predictive models identified the southern regions of the Canadian prairies are with high WNV transmission intensity. In addition, this thesis showed the increased transmission intensity of WNV in the current endemic area and expansion of spatial and temporal distribution of WNV into the current WNV-free area and time periods under climate change conditions.

7.4.1 Appling current knowledge to WNV interventions in the Canadian prairies

The studies in this thesis have identified the factors affecting WNV transmission and constructed models to predict the risk of WNV. By integrating mapping techniques and predictive models, public health professionals can identify the areas and time periods of potentially high WNV transmission intensity and implement intervention measures for preventing human infection.

The epidemic season of WNV in the Canadian prairies is generally between June and August. It is important to recognize the timing and locations to conduct the WNV prevention measures in order to optimize the capacity and increase the efficiency of WNV intervention. In this section, a flow chart of decision making for WNV intervention in the Canadian prairies is created based on the constructed predictive model and up-to-date scientific knowledge about WNV with *Cx. tarsalis* as the primary vector (Figure 7.1).

In Figure 7.1, the timeline was set prior to the occurrence of *Cx. tarsalis* and ended at the identification of risk area of WNV and interventions. Each step in the flow chart has a criterion to determine the direction that follows. The first step of the flow chart is to determine environment temperature thresholds that are higher than 14ºC and lower than 35 ºC, required for the emergence of adult *Cx. tarsalis* from hibernation (Reisen 1995). The next step is to estimate the accumulative degree days (ADD) for the development of the life cycle of *Cx. tarsalis* from egg hatch to pupation, in order to determine the presence of first generation *Cx. tarsalis*. Although the ADD for the development of *Cx. tarsalis* might differ between populations and regions, the estimated ADD is generally over 200 (Reisen 1995). Following the presence of the first generation *Cx. tarsalis*, the ADD for WNV in *Cx. tarsalis* should be estimated to verify the suitability of environmental temperature for WNV transmission. WNV transmission is considered possible when the temperature is higher than

14.3 ºC, which is the minimum temperature for WNV amplification to occur in *Cx. tarsalis* (Reisen et al. 2006), and at least 82 degree days are accumulated to complete the extrinsic incubation period of the virus in a 12 day feeding period of life cycle for a female *Cx. tarsalis* (Reisen et al. 1993, Schrag et al. 2011). The surveillance of dead birds or mosquitoes infected with WNV is then recommended to detect early WNV transmission (Eidson et al. 2001, Julian et al. 2002, Brownstein et al. 2004). In this stage, the application of the monthly model of WNV infection rate could facilitate the identification of target areas and time periods for conducting WNV surveillance. In the final step, the application of monthly model is still recommended to predict the intensity of WNV transmission and identify the risk areas and times in the Canadian prairies prior to the epidemics.

This flow chart of decision making is created specifically for WNV transmitted primarily by *Cx. tarsalis*. This flow chart would need to be modified for other WNV-vector systems, or other diseases vectored by *Cx. tarsalis*, as many parameters used in the flow are specific to this host-pathogen system. Nonetheless it is a template that could be adapted as needed for any vector-borne pathogen of public health significance, particularly as climate change continues to rewrite the distribution and patterns of transmission of vector borne diseases globally.

7.5 Future directions

The studies in this thesis have led to a better understanding of factors affecting WNV risk in the Canadian prairies. By applying the constructed models, WNV risk can be predicted prior to disease outbreaks, which provides lead time for conducting intervention strategies. However, predictive models constructed in this thesis are focused on the WNV with *Cx. tarsalis* as the primary vector in the Canadian prairies. Caution should be taken

when applying the models for predicting other WNV-vector system or pathogens transmitted by *Cx. tarsalis*. Although the predictive models are constructed and validated with observed mosquito and WNV infection rate data collected in the Canadian prairies, continuous surveillance of *Cx. tarsalis* abundance and WNV prevalence, particularly in the areas of the southern Canadian prairies with high transmission intensity of WNV and northern boundary of current WNV distribution, should be maintained. Surveillance in these regions could provide independent data for external validation and improve the predictability, as well as verify the predicted enhanced endemic amplification and expansion of its distribution under future climate changes (Süss et al. 2008, Luz et al. 2010). In addition, small spatial scale surveillance could facilitate evaluation of localized factors which effect the distribution of vectors and WNV and identify the specific sites for conducting any intervention measures.

Although this study demonstrated that changing climate and habitat can drastically alter the current distribution of *Cx. tarsalis* and WNV, a number of other factors could also affect the ecology of WNV, and may in turn be affected by climate conditions. These factors include ability of hosts to migrate, disperse and adapt to changing local environments, host resistance to disease, biotic interactions, evolutionary change, other anthropogenic alternations of environment, and efforts of disease control (Pearson and Dawson 2003, Lafferty 2009). Lacking information on how these factors relate to climate factors and their effect on WNV could limit the accuracy of predictions of the distribution of WNV under climate change scenarios. In addition, northward expansion of the grassland, *Cx. tarsalis* and WNV are predicted in this study; however, the precise lag time of habitat change (i.e. from boreal forest to grassland) and subsequent dispersal of vectors and hosts to newly suitable habitat remain unclear.

Host competence for WNV is different among bird species (Komar et al. 2003, Komar et al. 2005). The difference in host competence is one criterion of dilution effect essential to vector-borne pathogens (Ostfeld and Keesing 2000, Ostfeld 2009). However, studies have demonstrated that the majority of transmission of WNV can be attributed to a few "super spreader" host species, even uncommon species (Kilpatrick et al. 2006, McKenzie and Goulet 2010, Hamer et al. 2011). The feeding preference of the primary vector species determines what competent birds are significant in maintenance and amplification of WNV transmission as well as the species at risk of infection (Thiemann et al. 2012). Studies have shown that the blood-feeding pattern of *Cx. tarsalis* is highly specific and varies between different areas (Lee et al. 2002, Kent et al. 2009, Thiemann et al. 2012). Information about the blood-feeding pattern of *Cx. tarsalis* remains scarce in the Canadian prairies. Studies on the blood-feeding pattern of *Cx. tarsalis* in this ecoregion can provide valuable insights into WNV transmission in its enzootic cycle, and its possible impact on the abundance of susceptible bird species, as well as the timing of spillover into humans and other incidental hosts. This is all essential information for wildlife conservation and human disease intervention.

7.6 Reference

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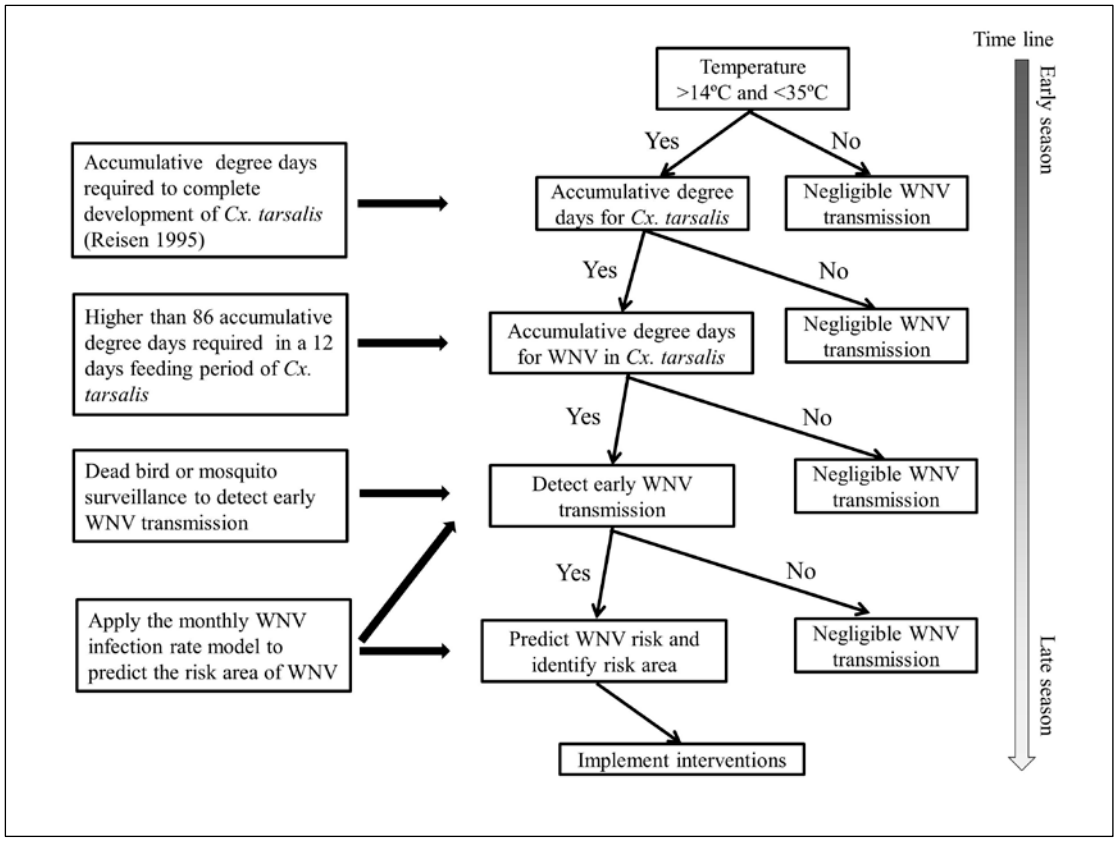

Figure 7.1 Flow chart of decision making for WNV control and interventions in the Canadian prairies.

Appendix

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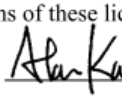
Signature of copyright holder or representative: 

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