January 2013

The Ecology Of Host-Seeking Mosquitoes Within The Red River Valley Of Central North Dakota

Joseph Orlo Mehus

THE ECOLOGY OF HOST-SEEKING MOSQUITOES WITHIN THE RED RIVER VALLEY OF CENTRAL NORTH DAKOTA

by

Joseph Orlo Mehus
Bachelor of Science, Mayville State University, 2004

A Dissertation

Submitted to the Graduate Faculty

of the

University of North Dakota

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Grand Forks, North Dakota
August
2013

This dissertation, submitted by Joseph Orlo Mehus in partial fulfillment of the requirements for the degree of Doctor of Philosophy from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

Dr. Jefferson Vaughan, Chairperson

Dr. Vasyl Tkach

Dr. Diane Darland

Dr. Robert Newman

Dr. David Bradley

This dissertation meets the standards for the appearance, conforms to the style and format requirements of the Graduate School of the University of North Dakota, and is hereby approved.

Dr. Wayne Swisher,
Dean of the Graduate School

July 23, 2013
Date

PERMISSION

Title The Ecology of Host-seeking Mosquitoes within the Red River Valley of Central North Dakota

Department Biology

Degree Doctor of Philosophy

In presenting this dissertation in partial fulfillment of the requirements for a graduate degree from the University of North Dakota, I agree that the library of this University shall make it freely available for inspection. I further agree that permission for extensive copying for scholarly purposes may be granted by the professor who supervised my dissertation work, or in his absence, by the Chairperson of the department or the dean of the Graduate School. It is understood that any copying or publication or other use of this dissertation or part thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of North Dakota in any scholarly use which may be made of any material in my dissertation.

Joseph Orlo Mehus
06 June 2013

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would to thank my advisor, Jefferson Vaughan, for his support and guidance throughout both my undergraduate and graduate research projects here at UND. Thank you to my committee: V. Tkach, R. Newman, D. Darland, D. Bradley for their time and effort assisting me with this research program. Thank you to the UND Biology Department, the faculty, and the staff; especially J. Lewis, A. Dumas, M. Pung, and K. Drees. I thank the numerous undergraduates and graduates students of the Vaughan lab for their laboratory contributions. Thanks to the graduate students, past and present, of the UND Biology Department. Thank you to North Dakota Experimental Program to Stimulate Competitive Research (ND EPSCoR) and University of North Dakota Graduate School for funding this research. Thank you to my parents Jeroy and Debbie Mehus, and other family and friends who have supported me throughout this work. Finally, thank you to Anthony Schroeder for bringing me into his family, providing enormous amounts of support, and allowing me to spend time with his beautiful daughters Peyton and Marley, who always brightened my days and provided me with years of entertainment and much love.

ABSTRACT

Host-seeking mosquitoes are taxing for people and wildlife alike in the Red River Valley (RRV). During the summer massive numbers of mosquitoes swarm the RRV, yet little is known about the ecology and biology of the mosquito species that inhabit this area. This research will help to fill some of those knowledge gaps by studying the ecology of host seeking mosquitoes in the RRV.

Host-seeking mosquitoes were collected using CO_2-baited MMX™ traps. Trapping was conducted in two very different rural settings within the RRV. One site, a 40-acre hardwood forest with closed canopy, the other a farmstead consisting of open agricultural fields interspersed with forested wind-rows. Trapping was conducted 2-3 times weekly throughout the mosquito season (May through August). Each night's catch was sorted, counted and identified to species. During sorting, all engorged and partially engorged mosquitoes were removed, identified to species and stored at -80ºC.

DNA was extracted from individual mosquito blood meals and analyzed via polymerase chain reaction (PCR) assays multiple times to determine the host feeding preferences and parasitic infection status of the host. The first round of PCR assays determined the host species from which the blood originated (e.g., deer, dog, human, etc.). Analyzing the host composition of many mosquito blood meals produced information on the preference of host species that were most commonly fed upon by the various mosquito species within their natural environment. The following rounds of

PCR assays examined mosquito blood meals for the presence of blood-borne pathogens (*e.g.*, filarial nematodes, avian malaria, etc.). This process, known as xenomonitoring, uses mosquitoes as a sampling tool to acquire blood samples from wildlife without having direct contact with the vertebrate host. Thus, xenomonitoring is an indirect way of estimating the prevalence of infection among vertebrate populations.

Mosquito counts from the forest and farm sites along with Grand Forks "Skeeter Meter" counts from the years of 2002-2010 were used to construct predictive models to understand the effects of climate on mosquito population dynamics and abundance throughout the summer. Generalized linear models are used to determine how climate variables play roles on everyday mosquito activity, while cross-correlation maps were used to determine correlation values of preceding weather variables to trap counts. This allowed for the determination of which climate variables can be used to predict how mosquito populations will fluctuate in the future.

This research provides a critical foundation by describing the species composition of mosquitoes that inhabit two unique rural study sites within the RRV. Species composition is crucial to the initial component of mosquito-borne vector transmission of diseases, presence of mosquito vectors. Building from the composition, this study provides information describing the population trends of multiple mosquito populations throughout the summers of 2009-2011 at these two rural sites. Because mosquito population trends differed between sites, several meteorological variables were identified as affectors of mosquito abundance and activity. By understanding how these meteorological factors affect mosquito populations, vital data is provided for the

future design of predictive models that will allow for focused mosquito control, but also lend information in potential disease risk-assessment map production.

To further build on the potential for zoonotic and enzootic pathogen transmission, it is important to understand the feeding habits of local mosquito species. These feeding preferences determine which hosts are more commonly fed upon by given mosquito species and offer a background to determine which vector transmitted diseases are currently present in the RRV as well as potential diseases, that upon introduction to the region, which could be transmitted within the valley.

CHAPTER I

SPECIES COMPOSITION AND PHENOLOGY OF HOST-SEEKING MOSQUITOES IN TWO UNIQUE RURAL HABITATS OF THE RED RIVER VALLEY OF NORTH DAKOTA

Abstract

North Dakota, specifically the Red River Valley, is a highly understudied region in terms of mosquito based research. This study lays the foundations of mosquito ecology and biology by investigating the species composition between two contrasting habitat types, a typical Farming/agricultural setting and a heavily forested, riparian zone located near the Goose River in Steele County, North Dakota. Over 125,000 mosquitoes were collected belonging to 20 species. Of the total collected, 57% (n=70,730) were identified as *Aedes vexans*, 19% (n=23,155) *Ae. excrucians*, and 11% (n=14,042) *Culex tarsalis*. Within the Forest site, *Ae. excrucians* was the most abundant species (n=23,008; 55%) followed by *Ae. vexans* (n=8,301; 20%), *Ae. triseriatus* (n=3,914; 9%) and *Coquillettidia perturbans* (n=3,606; 9%). Species composition at the Farm site was dissimilar to the Forest site with the major population being *Ae. vexans* (n=62,429; 75%), *Cx. tarsalis* (n=13,804; 17%), *Culiseta inornata* (n=1,932; 2%) and *Ae. dorsalis* (n=1,470; 2%). In addition to having unique species composition between sites, each of these mosquito species displayed differing population trends throughout the summer with *Ae. excrucians* being the first to emerge

in early June from the Forest and *Ae. vexans* being the earliest of the Farm mosquito species.

Introduction

Since 2003, the northern Great Plains states of North Dakota, South Dakota and Nebraska have consistently experienced some of the highest incidence of West Nile virus infections in humans within the United States. Despite this, the mosquito fauna of North Dakota has been little studied. This chapter provides the first detailed description of the adult mosquito fauna within rural areas of the central Red River Valley of eastern North Dakota.

The Red River Valley (RRV) is a large prehistoric lakebed (Lake Aggassiz) that extends from southern North Dakota northward into southern Manitoba, Canada. The topography is extremely flat and the thick soil is fertile but does not drain well. Consequently, there are vast areas of potential mosquito breeding habitat within the RRV. The RRV is primarily rural and the main landscape feature consists of large-scale crop production (sugar beets, potatoes). Agricultural sections are delineated by a grid-like network of raised gravel roadbeds, often flanked by low-lying ditches that can serve as mosquito larval habitats. Other low-lying areas within agricultural fields can also contribute to mosquito breeding. The farmsteads managing the crop production typically consist of several buildings surrounded by windrows of trees. Presumably, farmsteads are the site of most of the mosquito transmission of West Nile virus to humans (i.e., epizootic transmission) within the RRV.

Interweaved within the grid of agricultural fields, is a secondary habitat consisting of small slow-moving streams that meander and eventually drain into the

Red River flowing north into Lake Winnipeg, Canada. These streams often support areas of forest along their banks. These small tributaries typically flood during the spring snow melt and leave behind woodland pools and water-filled tree-holes that can serve as breeding habitat for mosquito species different from those breeding in the sun-lit agricultural habitat. Although not generally visited by humans during peak mosquito season, many wildlife species take up residence within these riparian habitats, as food and water resources are plentiful. With ample breeding sites and many species of wildlife to feed upon, these forested, riparian zones present potential areas of enzootic transmission of mosquito-borne pathogens.

Within the state of North Dakota, up to 40 species of mosquitoes have been reported (Darsie and Anderson 1985, Darsie and Ward 1989) and during the summer months the Red River Valley (RRV) supports vast numbers of mosquitoes. One of the most important roles of mosquitoes is as vectors of disease in wildlife, livestock and humans. In terms of disease transmission, it is essential to understand the composition and distribution of mosquito species as not every species of mosquito transmits every pathogen. Some regions may be more or less likely to support transmission of a given pathogen based on the presence/absence of a mosquito species. In addition, the degree of viral competency has been shown to differ between mosquito species (Meyer et al. 1988, Turell et al. 1996, Turell et al. 2000, Turell et al. 2001), indicating that differing mosquito species may have varying impacts on viral transmission.

The importance to determine which species inhabit the understudied, rural RRV of North Dakota, and identify potential candidate vector mosquito species is crucial.

This study analyzes the mosquito species composition and population dynamics between two unique rural sites in the RRV of North Dakota.

Material and Methods

Study Sites. Mosquitoes were collected from two unique, rural sites within the RRV in Steele County, North Dakota. Steele County is an agricultural region that comprises 712 square miles and has a total population of 1,975 people living within 4 communities and multiple farmsteads (U.S. Census Bureau, 2010). Crops commonly grown in the county include: wheat, barley, corn and soybeans. Insecticides are commonly applied once during the summer using ground and aerial spraying.

Both sites are located west of Hatton, ND (47.64°N, 97.46°W), a small rural community (pop. ~ 700) located within Traill County (Fig. 1.1). The first site, located 8.45 km southwest of Hatton, ND, is a hardwood forest with a semi-closed canopy and thick underbrush (hereafter referred to as the Forest site). Green Ash (*Fraxinus pennsylvanica)*, Boxelder (*Acer negundo)*, Oak (*Quercus mongolica)* and American Elm (*Ulmus americana)* are the predominant tree species found within the Forest. The lone building on this site is a hunting cabin that is only occupied during the North Dakota deer hunting season (November). The Forest is bordered along the southern edge by the northern branch of the Goose River, which commonly overflows its banks each spring, leaving low lying forest flooded throughout summer.

The second site, an agricultural ecosystem (from here on out referred to as the Farm), is located 1.61 km west of Hatton, ND, and is surrounded by cropland and shelterbelts. A small grove of trees partitions the buildings from the surrounding fields. This type of landscape is more typical of North Dakota. Buildings include a residential

home, an unoccupied pole barn and chicken coop, large equipment storage shed, and three granaries. Boxelder, American Elm, Chinese Elm (*Ulmus parvifolia*), Russian Olive (*Elaeagnus angustifolia*), Blue Spruce (*Picea pungens*) trees and Lilac (*Syringa vulgaris*) bushes are common on the Farm. A small coulee collects overland floodwater from surrounding fields and borders the Farm along the north and eastern limits. This coulee retains water throughout the summer months. No livestock are raised on the Farm. Mosquito control is absent at both sites.

Mosquito Collection: Mosquitoes were collected using battery operated CO_2-baited Mosquito Magnet X traps (MMX) (Woodstream Lititz, PA) from late May through mid-August of 2009, 2010, and 2011. Traps were placed throughout both sites 2-3 times per week and operated from 1800 to 0800 hr. In addition to MMX traps, a hand-operated, battery powered aspirator (33 cm diameter, 91.44cm length) (Metropolitan Mosquito Control District, St. Paul, MN) was used weekly (15 min duration) to collect resting mosquitoes around buildings, trees and underbrush at both sites during the summer of 2011.

Mosquitoes were transported to the laboratory and placed in -20°C freezers to immobilize mosquitoes. Mosquitoes were transferred into enamel pans and subsequently to plastic baggies for long term storage in -80°C freezers. Using dissecting scopes and dichotomous keys, mosquitoes were identified to species (Darsie Jr. and Ward 2005).

Images and Statistics: Images were obtained through Google Earth ™ (Google, Mountain View, CA, United States of America). Statistical analyses were performed using the R software package (R Development Core Team 2007). Mosquito

species diversity at each site was quantified by computing Shannon indices (alyoung.com), using the following formula:

$$-\sum_{i=1}^{s} = p_i \ln p_i$$

where, ***p*** is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), ***ln*** is the natural log, **Σ** is the sum of the calculations, and ***s*** is the number of species. Higher indices indicate greater species diversity than do lower indices. To quantify the degree of similarity between the mosquito communities found within the two habitats, Sorenson's coefficient was computed using the following formula:

2 x (no# of species in common) / (no# Forest species collected) +

(no# Farm species collected)

Complete community overlap = 1, whereas complete community dissimilarity = 0.

Results

Species Diversity. In this study, 125,695 mosquitoes (20 species) were identified from the two rural sites. Three species of mosquitoes, *Aedes vexans* (n=70,730, 57%), *Ae. excrucians* (n=23,155, 19%) and *Culex tarsalis* (n=14,042, 11%), composed the majority of the total collection (Table 1.1). Darsie and Ward 2005 reported 26 species of mosquitoes within the state of North Dakota. Using this as a comparison tool, seven additional species were found that were not named in Darsie and Ward 2005, including *Ae. aurifer, Ae. hendersoni, Ae. triseriatus, Ae. trivatattus, Anopheles quadrimaculatus, Culiseta minnesotae and Cx. territans*. All of these species, except *Ae. aurifer*, have previously been reported (Post and Munro 1949,

Darsie and Anderson 1985, Darsie and Ward 1989). Of the 20 mosquito species collected at both sites, there were 13 species in common. Overall, the mosquito communities were fairly similar between Farm and Forest (Sorenson's coefficient = 0.79), however the Forest site had a greater diversity of mosquito species (18 species, Shannon index = 1.35) than did the Farm site (15 species, Shannon's index = 0.86).

During the years of 2009 and 2011, mosquitoes were collected using MMX traps for the population trends within the Forest. From mid-June through July, mosquito numbers peaked and consistently collected between 350-550 mosquitoes per trap per week. Numbers of mosquitoes declined to nearly zero approximately two weeks into August.

Comparison of Mosquito Collection Techniques. In 2011, two types of mosquito collection were employed. The MMX trap is a CO_2 based trap operated during the night. Thus, MMX traps collected primarily female mosquitoes seeking blood (=host-seeking segment of the population). Aspiration of vegetation during the day collected resting mosquitoes that were not actively hunting but were either newly emerged, blood engorged or gravid (Fig. 1.2). Relative densities of host-seeking mosquitoes were similar between sites but relative densities of resting mosquitoes were over five times greater in the Forest than at the Farm site (Table 1.2). Blood-fed mosquitoes were collected using both techniques however significantly more were collected using the aspirator as compared to the MMX traps combined at each site (Kruskal-Wallis, χ^2=7.0886, df=1, p=0.0078).

Forest Site. Using the combination of MMX traps and aspirator, 18 species of mosquitoes were collected within the Forest site (Table 1.1) (n=41,755). Predominant

species were *Ae. excrucians* (55%), *Ae. vexans* (20%), *Ae. triseriatus* (9%) and *Coquillettidia perturbans* (9%). MMX traps collected 18 species while aspiration of vegetation collected only 12 species. There was not a significant difference between the number of individual mosquitoes caught using MMX traps and the aspirator (Kruskal-Wallis, χ^2=0.1304, df=1, p=0.7180).

Within the Forest, each of the major mosquito species shows unique population fluctuations throughout the summer months. The first of the major mosquito species to appear was *Ae. excrucians* (mid-June) (Fig. 1.3A). By the first week of July the number of questing females depleted to half of the peak number. Two smaller peaks of host-seeking *Ae. excrucians* occurred during the second and fourth weeks of July. By mid-August the population of *Ae. excrucians* dropped to almost zero. Host-seeking *Ae. vexans* first appeared at the end of June and peaked the first week of July, approximately one month later than *Ae. excrucians*. The initial numbers of *Ae. vexans* dropped sharply and within a week there was a subsequent peak (Fig. 1.3A). By the fourth week of July, numbers of *Ae. vexans* decreased, and like *Ae. excrucians* are no longer present by mid-August.

The secondary species, *Ae. triseriatus* and *Co. perturbans*, also showed unique emergence and peak patterns (Fig. 1.3B). *Aedes triseriatus* numbers were low until the first week of July when the population started to increase. By the third week of July, *Ae. triseriatus* numbers peaked but, unlike *Ae. excrucians* and *Ae. vexans*, remain high for approximately one week before dropping gradually to zero in mid-August. Initial emergence of *Co. perturbans* began in late June and gradually peaked the second week of July. *Coquillettidia perturbans,* like *Ae. triseriatus,* showed a similar tendency to

sustain high numbers for approximately one week. After the extended peak, just as the other species, numbers of *Co. perturbans* dropped to nil by mid-August.

Farm Site. Using the combination of MMX traps and aspirator, 15 species of mosquitoes were identified at the Farm site (Table 1.1) (n=82,940). Predominant species were *Ae. vexans* (75%), *Cx. tarsalis* (17%), *Cs. inornata* (2%) and *Ae. dorsalis* (2%). The MMX traps collected 15 species while the aspirator collected 9 species. During 2011, significantly more mosquitoes were collected using MMX traps versus the aspirator (Kruskal-Wallis, χ^2=4.4179, df=1, p=0.03556).

Host-seeking *Ae. vexans* were abundant at the Farm in early June and peaked by the third week of June (Fig. 1.4A). A smaller, less intense peak occurred during the first week of July. Two unique characteristics to the Farm population of *Ae. vexans* are the emergence weeks before the Forest population and abundance during peak times are 5-8 times higher than the peaks of *Ae. vexans* within the Forest. After the second peak, numbers drop and remain at these lower levels through August. Appearance of host-seeking *Cx. tarsalis* appeared later in the season as their populations started to increase during the end of June and peaked within the first week of July (Fig. 1.4A). Abundance declined after this peak but approximately one month later (first week in August) there occurred a larger, additional peak. It appears there may have occurred an elevation in numbers after this second peak, but is unknown as sampling finished before the end of August. The abundance of host-seeking *Cx. tarsalis*, although smaller than *Ae. vexans*, was almost 60 times higher than the numbers of *Cx. tarsalis* collected from the Forest site.

The secondary species of the Farm have less defined trends. The *Ae. dorsalis* population fluctuated throughout the summer (Fig. 1.4B), but at much lower intensities than that of *Ae. vexans* or *Cx. tarsalis*. It would appear that throughout the summer in the RRV, *Ae. dorsalis* questing female numbers may have up to three peaks. It also appears that each of these peaks is approximately one month apart. *Culiseta inornata* showed an initial peak prior to the beginning of the sampling season (Fig 1.4B). The population remained low throughout June and July but showed a defined peak the first week of August. A subsequent peak may have occurred after the third week of August, but it was not determined due to end of field season.

Engorged mosquitoes were also collected via both collection methods. While the MMX traps collected a larger number mosquitoes than the aspirator overall, the numbers of engorged mosquitoes collected using the aspirator was nearing a significantly larger number than that of MMX traps (MMX n=125, Aspirator n= 1716; χ^2=3.7844, df=1, p=0.0517).

Discussion

Mosquito-borne pathogens are common throughout the world. The success of these pathogens depends on both biotic factors (host susceptibility/competency/ intensity of viremia/parasitemia, competency of vector) and abiotic factors (rainfall, temperature). All of these factors mean little in disease transmission if vector arthropods are not present within a region. Not only does presence of the vector play an important role, but also the abundance of the vector species.

This study investigated the composition and population dynamics of mosquito species within the two rural habitat types found within the RRV. Seven species of

mosquitoes were identified which were not in the newest distribution maps of Darsie and Ward 2005. These include: *Ae. aurifer, Ae. hendersoni, Ae. triseriatus, Ae. triviatattus, An quadrimaculatus, Cs. minnesotae* and *Cx. territans*. All of these species, except *Ae. triseriatus*, were relatively rare and represented less than 1% of the total catch for all years within the sampling areas. The low population numbers in combination with species habitat preference may be why these mosquitoes are not always collected and reported. North Dakota claims a mere 4.4% of total land as Forest, which is the preferred habitat of *Ae. triseriatus* as well as *Ae. trivittatus* (Siverly 1972). Thus collection sites may influence prior reports of their presence.

Another caveat to mosquito population studies is the style of trap used for collection. To reduce variation due to trap preference we used a combination of two trap types to collect mosquitoes, CO_2-baited MMX traps and vacuum aspiration of resting mosquitoes. Using this combination of trapping styles, almost 42,000 mosquitoes were collected from the Forest site. Most of these mosquitoes were *Ae. excrucians* (55%). *Aedes excrucians* is one of the most widely distributed *Aedes* mosquito and prefers a flooded, wooded habitat (Means 1979), which describes the Forest site. Since *Ae. excrucians* is univoltine, we can surmise that the first population spike (4^{th} week in June, Fig 1.3A) is the new cohort of the summer. There are two subsequent peaks in female, host-seeking *Ae. excrucians* throughout the summer. Because *Ae. excrucians* is univoltine, these subsequent peaks were probably parous females seeking another blood meal. Even though this species is univoltine, multiple blood meals are likely taken and egg numbers build through the summer in depressions and low-lying ground as they are the overwintering stage that await spring flooding.

Aedes excrucians has also been implicated in the transmission of various parasites. In fact, *Ae. excrucians* were classified as having high vector potential for dog heartworm (*Dirofilaria immitis*) (Arnott and Edman 1978). Dog heartworm has been found within local pet populations (personal communications with veterinarians) and it is likely that *Ae. excrucians* is one of the local vectors.

In addition to the transmission of dog heartworm, *Ae. excrucians* also shows the potential of becoming a vector to various viruses. In experimental studies, Sindbis virus (Ockelbo subtype) produced disseminated infections in 100% of the *Ae. excrucians* that fed on viremic chicks (Turell et al. 1990). The Ockelbo virus was first isolated in Sweden in 1982. This virus is endemic to Scandinavia and causes inflammatory responses such as rash and fever upon human infection. This northern Great Plains of the United States is highly populated with individuals with Scandinavian background, and many people plan trips to Norway/Sweden to visit relatives and homelands. If active virus was transported either through infectious mosquito or active viremia in humans, the RRV could be a prospective entry point for a widespread Ockelbo epidemic.

Aedes vexans were also recovered from the Forest site. *Aedes vexans* is among the most prevalent mosquito species in urban areas of the RRV previously studied (Deckert 1995, Bell et al. 2005). *Aedes vexans* is known as a flood water mosquito and lays eggs in a variety of substrates such as shallow depressions and within cracks in soils that are commonly flooded such as in fields and ditches (Horsfall et al. 1973). Eggs are less common oviposited in areas of high vegetation and permanent bodies of water. While some *Ae. vexans* may be actively breeding within the Forest, it seems

more likely that these populations move into or are blown into the Forest (Fig 1.3A). This is supported by the appearance of *Ae. vexans* at the Farm site early in June (Fig 1.4A), preceding the appearance of *Ae. vexans* in the Forest (Fig. 1.3A). It may also be hypothesized that if *Ae. vexans* breed within the Forest, the development of larvae may have been delayed by cool temperatures of the water flooding the Forest floor.

Aedes vexans has shown, at least in the RRV, they will inhabit any of our local landscapes (urban, forested, agricultural). Moreover, they will likely be the dominant species of mosquito in the region. *Aedes vexans* has been shown to be an unlikely vector to such viruses as West Nile virus as it seems the mosquito is refractory to initial infection, but if virus is somehow permitted across the midgut epithelial cells of the mosquito (loss of midgut integrity, Vaughan et al. 2012), the infection of the mosquito can occur and virus may be transmitted. Even if the percentage of mosquitoes that may have compromised midguts is low, the sheer number of *Ae. vexans* provides more than an abundance of new potential, initially refractory vectors.

Both *Ae. triseriatus* and *Coq. pertubans* displayed a single generation per year with host-seeking females appearing rather late in the season (Fig. 1.3B). *Aedes triseriatus* is a tree-hole breeder and the Forest habitat provides many tree-holes in which gravid females can oviposit. The eggs, are the overwintering stage and are typically deposited along the bottom and sides of these tree-holes. Eggs hatch only after they become submerged. While the ground within the Forest is often damp or flooded, tree-holes often remain dry until summer rains fill them. The delayed emergence of *Ae. triseriatus* within the Forest is likely due to the time it takes for the tree-holes to fill above the level at which the eggs are deposited. As spring continues,

rainfall rates tend to increase (USGS NPWRC), filling these tree-holes, and allowing for development of larvae.

The reasons underlying the late-season emergence of a single generation of *Coquillettidia perturbans* are quite different (Fig 1.3B). *Coquillettidia perturbans* is unique in that the larvae have specialized siphons which are used to pierce aquatic plants such as Cattails (*Typha angustifolia*), and larval respiration occurs through the plant and larvae do not have to surface for oxygen (Bosak and Crans 2002). This species overwinters as larvae in association with the roots and mud below the frost level of the vegetation inhabited bodies of water (Rademacher 1979). Because of the extremely low temperatures in the RRV during the winter months (often below -17°C), frost can penetrate 1.37 meters (USGS NPWRC). Even with snow pools of water originating early (March/April), it may take many weeks for substrate below these pools to thaw. Once the substrate has thawed, overwintering *Co. perturbans* larvae resume development. The slow thaw of the substrate below the flooded surface of the Forest can explain the emergence of *Co. perturbans* at the beginning of July.

General mosquito population trends at the Farm site show multiple peaks throughout the summer. The Farm population of host-seeking *Ae. vexans* peaks a full 3 weeks earlier (3rd week in June) than the Forest population. Approximately 75% of the mosquitoes collected from the Farm were *Ae. vexans* (Table 1.1). Ditches and cropland that are routinely flooded during the spring provide regions that produce massive numbers of *Ae. vexans*. The second peak of *Ae. vexans* during the first week of July is likely a combination of parous females and a new cohort of host-seeking females as *Ae. vexans* is a multivoltine species. Even though *Ae. vexans* is common at both Forest and

Farm sites, the seasonal dynamics at the Farm is uniquely different from that within the Forest.

The second most abundant species at the Farm was *Culex tarsalis* which is the local West Nile virus within the RRV (Bell et al. 2005). Mated, adult females are the overwintering stage of *Cx. tarsalis*. These females break diapause (time of reduced physiological activity to retain viability through winter) as photoperiod and temperature increase, likely through the associated increase of juvenile hormone and reduction of fat bodies (Bennington et al. 1958, Mitchell 1981), and begin searching for a blood meal. The first peak of *Cx. tarsalis* occurs during the first week of July. This cohort of mosquitoes has potential to be of mixed ages, young from overwintering females who find blood meals early after breaking diapause, and females who are late to break diapause (Reisen et al. 2003). It is probable that that second peak of *Cx. tarsalis*, 1st week of August, represent brood from mosquitoes that successfully engorged during the first peak. It is possible that some of the *Cx. tarsalis* in the second peak were from the initial peak, but unlikely that these mosquitoes would survive for an entire year. Because *Cx. tarsalis* is multivoltine (Buth et al. 1990), it is plausible that the host-seeking females in the second peak will feed and give rise to females that have increased fat body production, decreased tendency for blood feeding, and reduced gonoactivity due to decrease in temperature and photoperiod, in preparation for diapause (Harwood and Takata 1965, Mitchell 1981).

Blood feeding patterns of female *Culex tarsalis* have shown that this species feed primarily on birds (Mehus and Vaughan 2013) and to lesser extent mammals. This species also tends to reside in non-forested, agricultural or urban regions of the RRV.

Because of these reasons, *Cx. tarsalis* has the opportunity to transmit many zoonotic diseases. Within the region, *Cx. tarsalis* is the most competent vector for zoonotic viruses and *Plasmodium*.

It would seem that the *Culex* populations, both *Cx. tarsalis* and *Cx. pipiens*, in North Dakota would all be nulliparous at spring emergence, yet WNV is common within the state. There are many theories as to what can spur the viral transmission cycle in northern regions such as vertical viral transmission (Blackmore and Winn 1956, Bailey 1978, Goddard 2003, Reisen 2006), immigration of birds containing active virus (Rappole et al. 2000, Peterson et al. 2003, Dusek et al 2009) and overwintering of virus in vertebrate hosts (Owen et al. 2012, Nemeth et al. 2009, Miller et al. 2003, Steinman et al. 2006). Other viruses that can potentially be transmitted via infectious bite of Cx. tarsalis include: Rift Valley Fever virus, Western Equine Encephalitis virus, and St. Louis Encephalitis virus (Turell et al. 2010, Hammon et al. 1941, Hammon and Reeves 1943, Hammon and Reeves 1943).

Culex tarsalis has also been implicated in the transmission of haemosporidian parasites such as avian *Plasmodium* (Christensen et al. 1983, Work et al. 1990). Avian *Plasmodium* has been confirmed within the RRV and it is believed that there is active transmission of this parasite in the RRV (Mehus and Vaughan Unpub). House sparrows (*Passer domesticus*) have been found to be infected with avian *Plasmodium* in the RRV. These birds are year round residents, often found at bird feeders throughout the winter months. For these birds to become infected, they need to be bitten by a mosquito with sporozoites within the salivary glands. Christenson 1983 has shown that both *Cx. pipiens* and *Cx. tarsalis* are capable vectors for avian Plasmodium. Given our

large numbers of *Cx. tarsalis* it seems probable that the likely vector species of avian *Plasmodium* is *Cx. tarsalis*.

Culiseta inornata, representing a mere 2% of the total mosquito population, was the third most abundant population at the Farm. This species showed a bimodal peak during the summers of 2010 and 2011 (Fig 1.4B). It appears that *Cs. inornata* develops a peak in abundance in May before collections began. Although the population numbers are low, they are consistent throughout the summer until the first week in August when the second peak occurs.

Culiseta inornata overwinters as mated females (Siverly 1972) as does *Cx. tarsalis*, yet female *Cs. inornata* appear to break diapause over a month earlier than female *Cx. tarsalis*. This phenomenon has also been noted within the neighboring state of Minnesota (Barr 1958). There may be many hypotheses why *Cs. inornata* break diapause before *Cx. tarsalis* such as quicker metabolism of plant fluid reserves (food supply for winter) throughout overwintering or an increase in sensitivity to photoperiod.

Culiseta inornata within the RRV feed primarily on WTD as well as cows and to a lesser extent smaller mammals such as house cats (Mehus and Vaughan 2013). Avian and human blood meals were observed in Manitoba, but were in very small percentages. Even though WNV is common in North Dakota, it is unlikely that even though *Cs. inornata* may transmit WNV, its feeding patterns (mainly mammalian) would only constitute it as a minor or low risk mosquito (Goddard et al. 2002).

Aedes dorsalis is the fourth most populous species from the Farm site. This mosquito species showed an early peak in population before sampling began in late

May (Fig 1.4B). By the time sampling had begun, the population gradually decreased until the end of June. During the first week of July, there occurred a population spike. There was one smaller peak of questing females during the beginning of August but is more than likely females looking for a second blood meal as the eggs from the previous group are more than likely set up for diapause as the egg is the overwintering stage. Eggs of *Ae*. *dorsalis* are usually deposited in most soils in depressions in ditches, fields and roads which frequently collect water in the spring and summer.

It was believed that as these pools fill with water they are rich in oxygen and as the oxygen levels are depleted via increased bacterial growth, they stimulate the hatching of the larvae from the egg (Gjullin et al. 1941). Further studies have shown that it may not necessarily due to low levels of oxygen associated with the hatching of eggs, but the bacteria themselves, compounds released from the bacteria, or from the plant material associated with the pool of water (Punnusamy et al. 2011). Whichever the case, it is easy to believe egg hatch is early for our *Aedes* mosquitoes due to the high levels of organic matter within our rich soils which is naturally degraded via aerobic bacteria.

Females *Ae*. *dorsalis* have been shown to feed on WTD in the region, and will bite during the day or the night. If mammals are not available for feeding upon, these mosquitoes will also feed on birds. Because these mosquitoes will also feed upon man, there is the potential for disease transmission to humans from animals. These mosquitoes have been known to transmit zoonotic viruses such as LaCrosse (California Encephalitis) (Turell et al. 1982) as well as Western Equine Encephalitis (Fulhorst et al. 1994, Kramer et al. 1998).

These two study sites offered distinctive species composition. The Forest site provided species that are typically found in heavily wooded areas such as *Ae. excrucians*, *Ae. triseriatus*, *Ae. hendersoni*, and *Co. perturbans*. These species are common vectors of various enzootic diseases such as heartworm (*Ae. excrucians*), Eastern Equine Encephalitis (*Co. perturbans*) (Turell 2005), and La Crosse virus (*Ae. triseriatus*) (Grimstad et al. 1977). Given the introduction to the area, these riparian areas offer great habitats for the transmission of multiple enzootic diseases.

The Farm site provided many species of mosquitoes, with composition and abundance differing from that at the Forest site. The most abundant species at the Farm was *Ae. vexans*, which is one of the most widely distributed mosquitoes in the world. The second most abundant mosquito species is *Cx. tarsalis*, a mosquito with great vector competency for multiple pathogens ranging from WNV to avian *Plasmodium*. Records from the CDC have shown that human cases of WNV occur within the region, and avian *Plasmodium* has been detected in blood meals of *Cx. tarsalis*, showing probable transmission in the RRV.

Acknowledgements

Thank you to Sarina Bauer, Stephen Greimen, Jeffrey Bell, and Alex Droske for assistance in identifying mosquitoes in addition to Ting-Wu Chuang for providing R-code structure for CCMs.

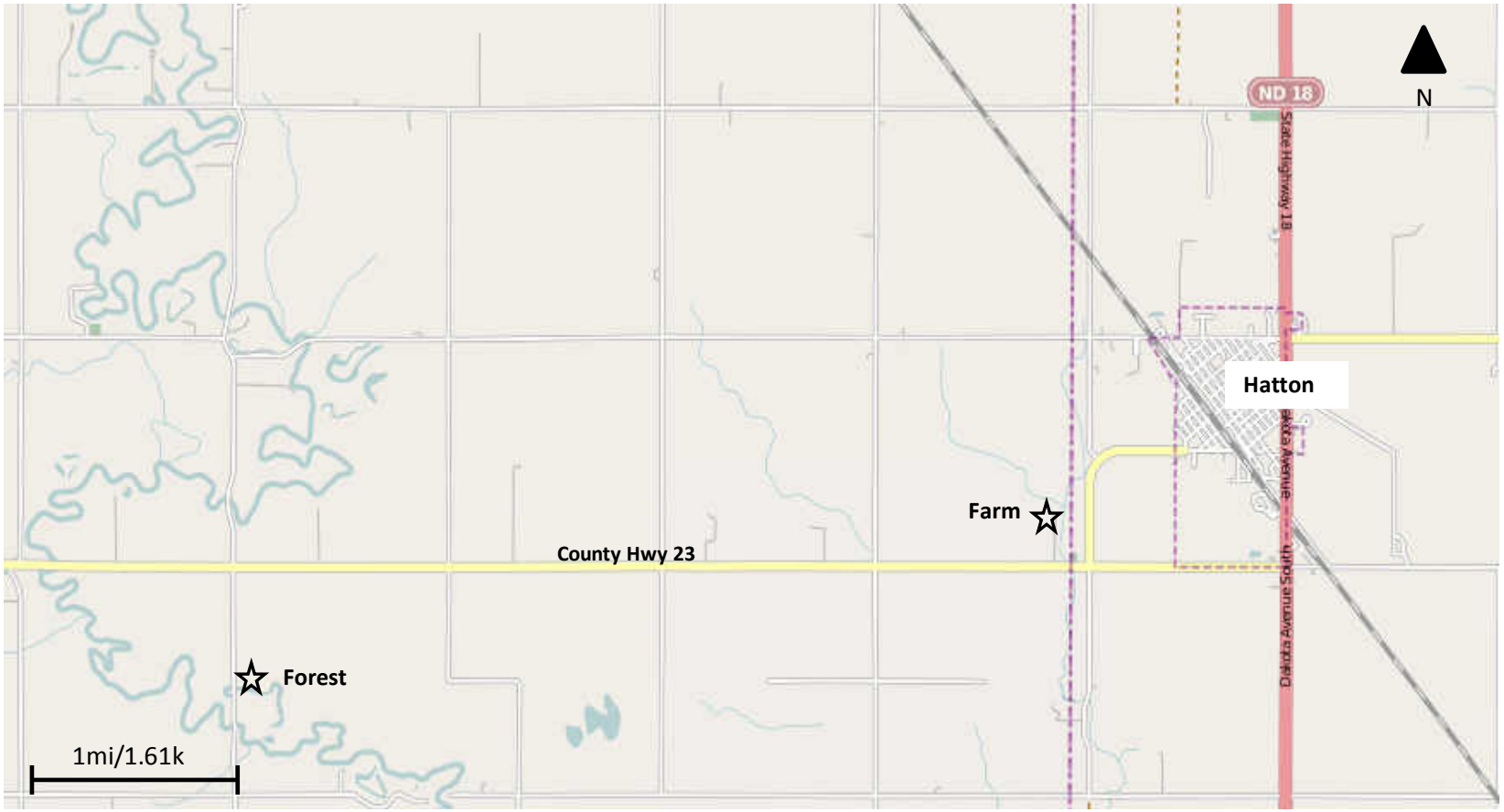


Figure 1.1. Location of Farm and Forest sites. Farm location is 1.60km west of Hatton, ND, USA. The Forest site is located 8.05km west and 0.80km south of Hatton, ND, USA.

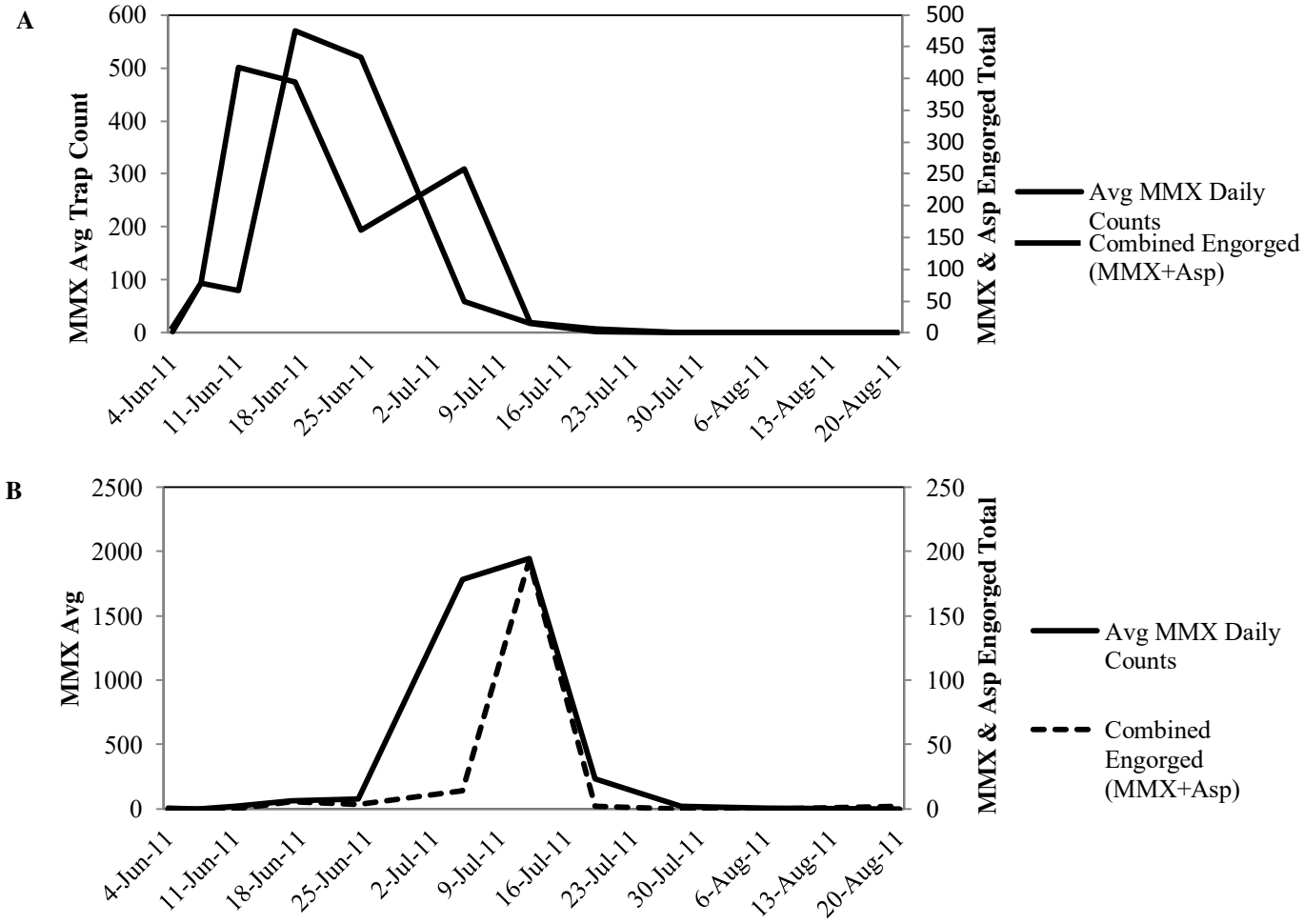


Figure 1.2. Host-seeking activity (MMX counts) and collection of engorged mosquitoes from June-August 2001. Host-seeking activity of the primary species (*Ae. excrucians*, Forest and *Ae. vexans*, Farm) precedes the collection of engorged mosquitoes by approximately one week for both species. A) *Ae. excrucians* from Forest site. B) *Ae. vexans* from the Farm site.

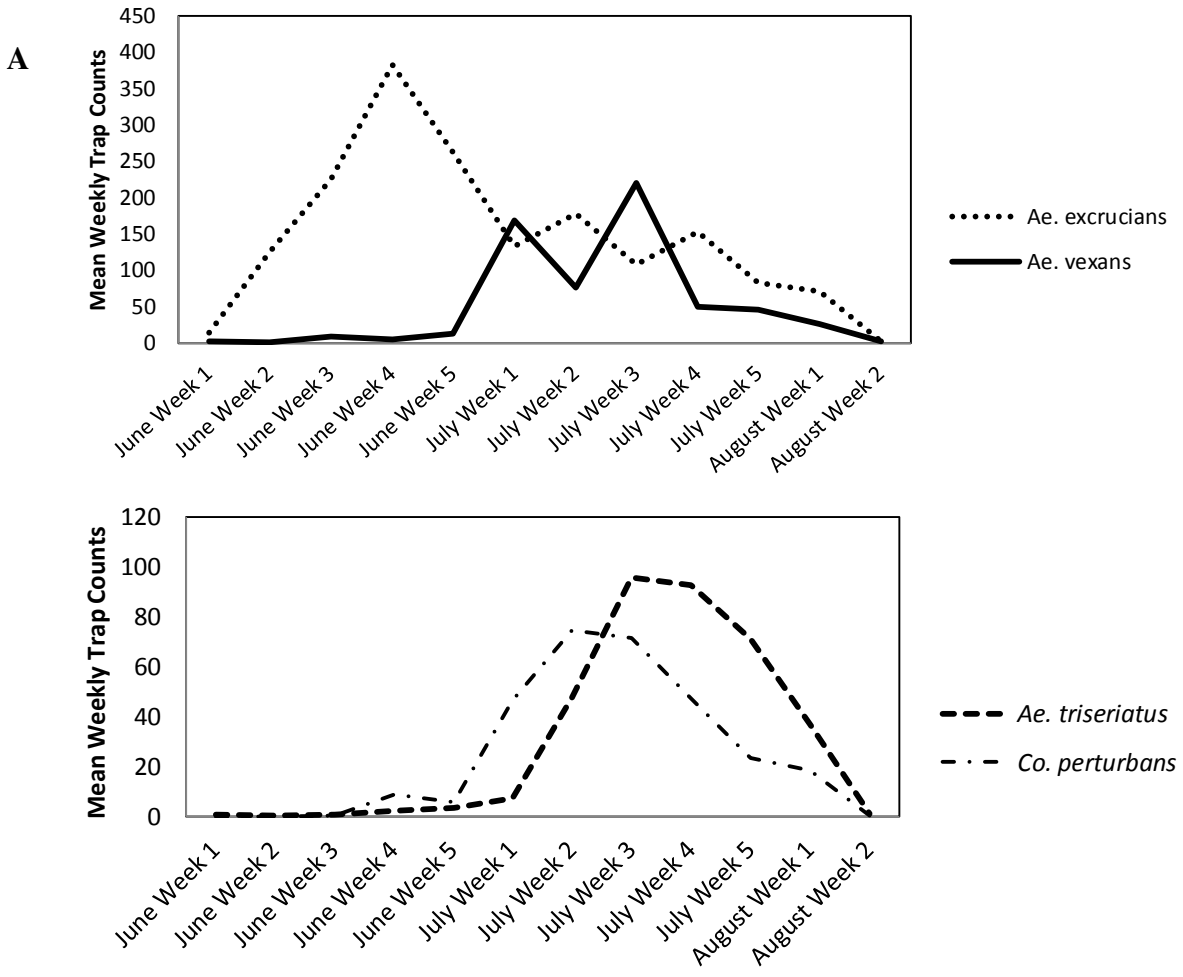


Figure 1.3. Population trends in host-seeking mosquitoes within the Forest site during 2009 and 2011. A) Dominant two mosquito species *Ae. vexans* and *Ae. excrucians*. B) Secondary species *Ae. triseriatus* and *Co. perturbans*.

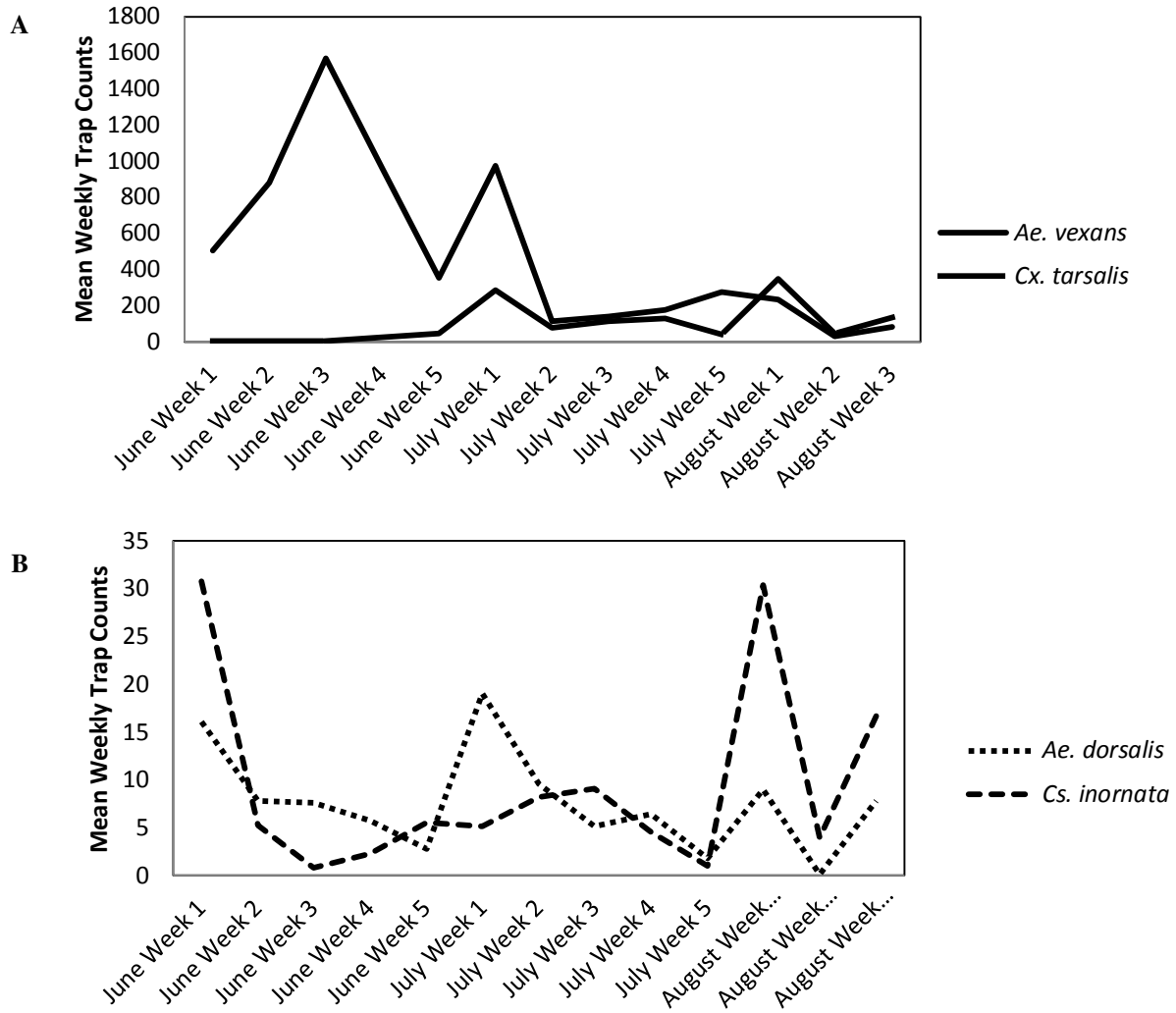


Figure 1.4. Population trends in host-seeking mosquitoes at the Farm during 2010 and 2011. A) Dominant two mosquito species *Ae. vexans* and *Cx. tarsalis*. B) Secondary mosquito species *Ae. dorsalis* and *Cs. inornata*.

References

Arnott, J.J., J.D. Edman. 1978. Mosquito vectors of dog heartworm (*Dirofilaria immitis*) in western Massachusetts. J Am Mosq Cont Assoc. 2:222-230

Bailey, C. L., B. F. Eldridge, D. E. Hayes, D. M. Watts, R. F. Tammariello, J. M. Dalrymple. 1978. Isolation of St. Louis encephalitis virus from overwintering *Culex pipiens* mosquitoes. Science. 199(4335):1346-1349

Bar, A. R. 1958. The mosquitoes of Minnesota (Diptera: Culicidae:Culicinae). Technical Bulletin 228. University of Minnesota Agricultural Experiment Station.

Blackmore, J. S., J. F. Winn. 1956. A winter isolation of Western Equine Encephalitis virus from hibernating *Culex tarsalis* Coquillett. Exp. Biol. Med. 91(1):146-148

Bell, J. A., N. J. Mickelson, J. A. Vaughan. 2005. West Nile virus in host-seeking mosquitoes within a residential neighborhood in Grand Forks, North Dakota. Vector Borne Zoonotic Dis. 5(4):373-82

Bennington, E. E., C. A. Sooter, H. Baer. 1958. The diapause in adult female *Culex tarsalis* Coquillett (Diptera: Culicidae). Mosquito News. 18(4):299-304

Bosak, P. J., W. J. Crans. 2002. The structure and function of the larval siphon and spiracular apparatus of *Coquillettidia perturbans*. J. Am. Mosq. Control Assoc. 18(4): 280-83

Buth, J. L., R. A. Brust, R. A. Ellis. 1990. Development time, oviposition activity and onset of diapause in *Culex tarsalis, Culex restuans* and *Culiseta inornata* in southern Manitoba. J. Am. Mosq. Control Assoc. 6(1): 55-63

Christensen, B. M., H. J. Barnes, W. A. Rowley. 1983. Vertebrate host specificity and experimental vectors of *Plasmodium* (*Novyella*) *kempi* sp. n. from the eastern wild turkey in Iowa. J. Wildl. Dis. 19(3):204-13

Darsie Jr., R. F., A. W. Anderson. 1985. A revised list of the mosquitoes of North Dakota, including new additions to the fauna. J. Am. Mosq. Control Assoc. 1(1):76-9

Darsie Jr., R. F., R. A. Ward. 1989. Review of new Nearctic mosquito distributional records north of Mexico, with notes on additions and taxonomic changes of the fauna, 1982-89. J. Am. Mosq. Control Assoc. 5(4):552-557

Darsie Jr., R. F., R. A. Ward. 2000. Summary of new distribution records for mosquito species in the United States and Canada for the period 1981-99. J. Am. Mosq. Control Assoc. 16(1):1-4

Darsie Jr., R. F., R. A. Ward. 2005. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. University Press of Florida, Gainesville, Florida

Deckert, W.A. 1995. The occurrence of mosquitoes in the central Red River Valley in 1992-1993. Dissertation. University of North Dakota. Grand Forks.

Dusek, R. J., R. G. McLean, L. D. Kramer, S. R. Ubico, A. P. Dupuis II, G. D. Ebel, S. C. Guptill. 2009. Prevalence of West Nile virus in migratory birds during spring and fall migration. Am. J. Trop. Med. Hyg. 81(6):1151-1158

Fulhorst, C. F., J. L. Hardy, B. F. Eldridge, S. B. Presser, W. C. Reeves. 1994. Natural vertical transmission of Western Equine Encephalomyelitis virus in mosquitoes. Science. 263(5147):676-678

Gjullin, C. M., C. P. Hegarty, W. B. Bollen. 1941. The necessity of a low oxygen concentration for the hatching of *Aedes* mosquito eggs. J. Cell. & Comp. Physiol. 17(2):193-202

Goddard, L. B., A. E. Roth, W. K. Reisen, T. W. Scott. 2002. Vector competence of California mosquitoes for West Nile virus. Emerg. Infect. Dis. 8(12):1385-1391

Goddard, L. B., A. E. Roth, W. K. Reisen, T. W. Scott. 2003. Vertical transmission of West Nile virus by three California *Culex* (Diptera: Culicidae) species. J. Med. Entomol. 40(6):743-746

Grimstad, P. R., G. B. Craig, Jr., Q. E. Ross, T. M. Yuill. 1977. Aedes triseriatus and La Crosse virus: geographic variation in vector susceptibility and ability to transmit. Am. J. Trop. Med. Hyg. 26(5): 990-6

Harwood, R. F., N. Takata. 1965. Effect of photoperiod and temperature on fatty acid composition of the mosquito *Culex tarsalis*. J. of Insect Physiol. 11(6):711-716

Hammon, W. M., W. C. Reeves, B. Brookman, E. M. Izumi, C. M. Gjullin. 1941. Isolation of the viruses of Western Equine and St. Louis Encephalitis from *Culex tarsalis* mosquitoes. Science. 94(2440):328-330

Hammon, W. M., W. C. Reeves. 1943. Laboratory transmission of St. Louis Encephalitis virus by three genera of mosquitoes. J. Exp. Med. 78(4):241-253

Hammon, W. M., W. C. Reeves. 1943. Laboratory transmission of Western Equine Encephalomyelitis virus by mosquitoes of the genera *Culex* and *Culiseta*. J. Exp. Med. 78(6):425-434

Horsfall, W. R., H. W. Fowler, Jr., L. J. Moretti, J. R. Larsen. 1973. Bionomics and embryology of the inland floodwater mosquito *Aedes vexans*. Board of Trustees of the University of Illinois. University of Illinois Press. p.7

Kotchman, L. A. 2010. North Dakota statewide assessment of Forest resources and Forest resource strategy. NDSU-North Dakota Forest Service. Bottineau, ND.

Kramer, L. D., W. K. Reisen, R. E. Chiles. 1998. Vector competence of *Aedes dorsalis* (Diptera: Culicidae) from Morro Bay, California, for Western Equine encephalomyelitis virus. J. Med. Entomol. 35(6):1020-1024

Means, R. G. 1979. Mosquitoes of New York Part I. The genus *Aedes* (Meigen) with identification keys to genera of Culicidae. The University of the State of New York. The State Education Department. State Science Service, New York State Museum. Albany, New York

Mehus, J. O., J. A. Vaughan. 2013. Molecular identification of vertebrate and hemoparasite DNA within mosquito blood meals from eastern North Dakota. Vector Borne Zoonotic Dis. *In press*

Meyer, R. P., J. L. Hardy, S. B. Presser, and W. K. Reisen. 1988. Preliminary evaluation of the vector competence of some southern California mosquitoes to western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses. Proc. Calif. Mosq. Vector Control Assoc. 56: 42–48.

Miller, D. L., M. J. Mauel, C. Baldwin, G. Burtle, D. Ingram, M. E. Hines II, K. S. Frazier. 2003. West Nile virus in Farmed alligators. Emerging Infectious Diseases 9:794-799

Mitchell, C. J. 1981. Diapause termination, gonoactivity and differentiation of host-seeking behavior from blood-feeding behavior in hibernating *Culex tarsalis* (Diptera: Culicidae). J. Med. Entomol. 18:386-394

Nemeth, N., G. Young, C. Ndaluka, H. Bielefeldt-Ohmann, N. Komar, R. Bowen. 2009. Persistent West Nile virus infection in the house sparrow (Passer domesticus). Arch. Virol. 154(5):783-789

Peterson, A. T., D. A. Vieglais, J. K. Andreasen. 2003. Migratory birds modeled as critical transport agents for West Nile Virus in North America. Vector Borne Zoonotic Dis. 3(1):27-37

Ponnusamy, L., K. Boroczky, D. M. Wesson, C. Schal, C. S. Apperson. 2011. Bacteria stimulate hatching of Yellow Fever mosquito eggs. PloS ONE. 6(9):e24409. Doi:10.137/journal.pone.0024409

Post, R. L., J. A. Munro. 1949. Mosquitoes of North Dakota. North Dakota Agricultural Experiment Station Bimonthly Bulletin. 11(5):173-183

Rademacher, R. E. 1979. Studies of overwintering larvae of *Coquillettidia perturbans* mosquitoes in Minnesota. Mosquito News. 39(1):135-136

Rappole, J. H., S. R. Derrickson, Z. Hubalek. 2000. Migratory birds and spread of West Nile Virus in the western hemisphere. Emerging Infectious Diseases. 6(4):319-328

Reisen, W. K., H. D. Lothrop, B. Lothrop. 2003. Factors influencing the outcome of mark-release-recapture studies with *Culex tarsalis* (Diptera: Culicidae). J. Med. Entomol. 40(6):820-829

Reisen, W. K., Y. Fang, H. D. Lothrop, V. M. Martinez, J. Wilson, P. O'Connor, R. Carney, B. Cahoon-Young, M. Shafii, A. C. Brault. 2006. Overwintering of West Nile virus in southern California. J. Med. Entomol. 43(2):344-355

Siverly, R. E. 1972. Mosquitoes of Indiana. Indiana State Board of Health. Indianapolis, Indiana.

Steinman, A., C. Banet-Noach, L. Simanov, N. Grinfeld, Z. Aizenberg, O. Levi, D. Lahav, M. Malkinson, S. Perk, N. Y. Shpigel. 2006. Experimental infection of common garter snakes (*Thamnophis sirtalis*) with West Nile virus. Vector Borne Zoonotic Dis. 6(4)361-368

Turell, M. J., J. L. Hardy, W. C. Reeves. 1982. Stabilized infection of California Encephalitis virus in *Aedes dorsalis*, and its implications for viral maintenance in nature. Am. J. Trop. Med. Hyg. 31(6):1252-1259

Turell, M. J., J. O. Lundstrom, B. Niklasson. 1990. Transmission of Ockelbo virus by *Aedes cinerous, Ae. communis* and *Ae. excrucians* (Diptera: Culicidae) collected in an enzootic area in central Sweden. J. Med. Entomol. 3:266-8

Turell, M. J., S. M. Presley, A. M. Gad, S. E. Cope, D. J. Dohm, J. C. Morrill, and A. R. Arthur. 1996. Vector competence of Egyptian mosquitoes for Rift Valley Fever virus. Am. J. Trop. Med. Hyg. 54: 136-39

Turell, M. J., M. O'Guinn, J. Oliver. 2000. Potential for New York mosquitoes to transmit West Nile virus. Am. J. Trop. Med. Hyg. 62: 413-414

Turell, M. J., M.L. O'Guinn, D. J. Dohm, J. W. Jones. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. J. Med. Entomol. 38:130-134

United States Census Bureau. 2010. Steele County, North Dakota. http://factfinder2.census.gov/faces/nav/jsf/pages/searchresults.xhtml

United States Geological Survey. 2006. Northern Prairie Wildlife Research Center. Climate of North Dakota. http://www.npwrc.usgs.gov/resource/habitat/climate/precip.htm

United States Geological Survey. 2013. West Nile Virus Historical. http://diseasemaps.usgs.gov/wnv_historical.html

Vaughan, J. A., J. O. Mehus, C. M. Brewer, D. K. Kvasagar, S. Bauer, J. L. Vaughan, H. K. Hassan. T. R. Unnasch, J. A. Bell. 2012. Theoretical potential of passerine filariasis to enhance the enzootic transmission of West Nile virus. J. Med. Entomol. 49(6):1430-41

Work, T. M., R. K. Washino, C. Van Riper 3rd. 1990. Comparative susceptibility of Culex *tarsalis, Anopheles franciscanus*, and *Culiseta inornata* (Diptera: Culicidae) to *Plasmodium relictum* (Haemosporidia: Plasmodiiae). J. Med. Entomol. 27(1):68-71

Table 1.1. List of mosquito species found within two rural sites in Steele Co., ND from 2009-2011.

	Forest Site				Agriculture Site				
	MMX Traps		Aspirator		MMX Traps		Aspirator		
Mosquito Species	2009	2011	2011	Totals	2010	2011	2011	Totals	Grand Total
Ae. aurifer	68	4	1	73 (<1)	0	3	0	3 (<1)	76 (<1)
Ae. canadensis	36	18	1	55 (<1)	668	296	11	975 (1)	1030 (1)
Ae. dorsalis	23	28	4	55 (<1)	1140	323	7	1470 (2)	1525 (1)
Ae. excrucians	16100	3199	3709	23008 (55)	76	29	42	147 (<1)	23155 (19)
Ae. flavescens	89	39	10	138 (<1)	706	61	17	784 (1)	922 (1)
Ae. hendersoni	9	21	0	30 (<1)	0	0	0	0 (0)	30 (<1)
Ae. nigromaculis	0	0	0	0 (0)	1	0	0	1 (<1)	1 (<1)
Ae. triseriatus	3424	490	0	3914 (9)	721	46	49	816 (1)	4730 (4)
Ae. trivatattus	79	0	22	101 (<1)	0	0	0	0 (0)	101 (<1)
Ae. vexans	3419	3787	1095	8301 (20)	53481	8304	644	62429 (75)	70730 (57)
An. earlei	0	0	0	0 (0)	0	2	0	2 (<1)	2 (<1)
An. punctipennis	6	8	0	14 (<1)	14	1	0	15 (<1)	29 (<1)
An. quadrimaculatus	34	7	0	41 (<1)	13	3	0	16 (<1)	57 (<1)
An. walkeri	3	0	0	3 (<1)	0	0	0	0 (0)	3 (<1)
Co. perturbans	2537	1065	4	3606 (9)	172	328	6	506 (1)	4112 (3)
Cs. inornata	1985	119	15	2119 (5)	1901	29	2	1932 (2)	4051 (3)
Cs. minnesotae	3	0	0	3 (<1)	0	0	0	0 (0)	3 (<1)
Cx. pipiens	0	17	34	51 (<1)	0	8	32	40 (<1)	91 (<1)
Cx. tarsalis	161	74	3	238 (1)	13548	256	0	13804 (17)	14042 (11)
Cx. territans	1	0	4	5 (<1)	0	0	0	0 (0)	5 (<1)
Tot. Mos.	27977	8876	4902	41755	72441	9689	810	82940	124695
Tot. Trap Events	105	22	11		117	21	11		
Avg Mos./Trap Night	266	403	446		619	461	74		

Numbers in parentheses following totals is the percentage of the total mosquitoes from each site. Grand total includes counts from both sites.

Table 1.2. Relative abundance of adult mosquitoes collected concurrently at two different habitats throughout the summer 2011, Steele County, ND. Values indicate the number of mosquitoes collected per sampling interval (number of trap-nights [MMX traps] or trapping sessions [aspiration]).

	Farm Site	Forest Site
Host-seeking population (collected *via* MMX traps at night)	461 (21)	403 (22)
Resting population (collected *via* vacuum aspiration of vegetation during the day)	74 (11)	446 (11)

CHAPTER II

DETERMININATION OF METEOROLOGICAL VARIABLES THAT PLAY PREDICTIVE ROLES ON ADULT MOSQUITO ACITIVITY IN THE RED RIVER VALLEY OF NORTH DAKOTA

Abstract

The development of pathogen risk-assesment trends includes multiple factors including vector competence, presence of the vector arthropod, feeding preference of the arthropod as well as the behavior of the vector. This study provides information about the host-seeking activity of multiple mosquito species from two rural sites located in Steele Co., North Dakota based on meteorological data. One of these study sites is a densely forested habitat, the other a large agricultural farm habitat surrounded by cropland. *Aedes excrucians, Ae. vexans, Ae. triseriatus* and *Coquillettidia perturbans* were identified as the most abundant species, while *Ae. vexans, Culex tarsalis, Ae. dorsalis* and *Culiseta inornata* were the most abundant at the Farm site. Mosquito trap counts from the city of Grand Forks, ND, USA were also collected and analyzed for the years 2002-2010.

Regression modeling was used to identify concurrent weather variables and daily trap counts for each of the predominant species for both sites. Photoperiod was significantly associated with trap counts of *Ae. excrucians* ($p=0.0013$). *Ae. vexans* from the Forest was positively associated with insect degree days ($p<0.0001$), minimum temperature ($p=0.0002$), and photoperiod ($p=0.0005$) but negatively associated with

bare soil temperature (p=0.0132), maximum humidity (p=0.0321) and wind speed (p=0.0036). The population of *Ae. vexans* from the Farm site showed associations to similar meteorological variables as did the Forest population. The local West Nile virus vector, *Cx. tarsalis*, displayed positive associations with turf soil temperature (p<0.0001), average humidity (p<0.0001) and maximum temperature (p=0.0009), while negatively associated with rainfall (p=0.0003) and dew point (p=0.0017). Because mosquito counts for Grand Forks were not identified to species, total counts were used in regression modeling and both average temperature (p=0.0324) and minimum temperature (p=0.0033) were positively associated with trap counts.

Meteorological variables were used demonstrated time-lagged affects ranging from 1-30 days prior to mosquito trap counts by using cross-correlation map analyses. *Ae. excrucians* was negatively correlated with precipitation and minimum temperature at day 29 prior to trap date while negatively associated with relative and average humidity 2-4 weeks prior to trap date. *Ae. vexans* from the Forest site were highly correlated with multiple meteorological variables including precipitation, minimum temperature, relative humidity, maximum humidity and dew point thoughtout the 30 days prior to trap date. *Ae. vexans* from the Farm was correlated to the same variables, but at reduced significance of correlation. *Cx. tarsalis* was negatively correlated to rainfall 2-3 days prior to trap date as well as negatively correlated to wind speed throughout the 30 day period. *Cx. tarsalis* also showed strong correlations between trap counts and turf soil temperature (day 6), maximum humidity (days 4-30) and relative humidity (days 1-30).

Because Grand Forks mosquito counts were inclusive of all species, CCMs were analyzed by month. The meteorological variables influencing trap counts varied by month. In addition, total mosquito counts were analyzed for both Farm and Forest sites. These total count CCMs were compared to individual CCMs for mosquito species. These total CCMs displayed blended results of the predominant species from each site. Grand Forks mosquito count CCMs were compared then to correlations to mosquito species CCMs resulting in the confirmation of hypothesized mosquito population trends within the city.

Introduction

In 2002, West Nile virus was first detected in North Dakota (www.cdc.gov). From 2002-2011, North Dakota has been within the top five states in the USA for human cases of WNV (per capita) eight times within the ten year span (Table 2.1). Within the USA, the major vectors of this virus belong to the genus *Culex* (Turell et al. 2005). It has been reported that there are 5 species within the *Culex* genus inhabiting North Dakota: *Cx. pipiens, Cx. restuans, Cx. salinarius, Cx. tarsalis,* and *Cx. territans* (Darsie and Anderson 1985). In addition, West Nile virus has also been detected within non-vector mosquito species in the generas *Aedes, Anopheles, Coquillettidia*, and *Culiseta*, all genera of which are found within North Dakota.

While numbers of mosquitoes are generally "high" during the summer, there are fluctuations in the populations of mosquitoes throughout the mosquito season in North Dakota (late May through August) (Mehus unpubl. data) (Fig 2.1). Climatic variables (degree-day cooling, air temperature, precipitation) have previously been analyzed in time series studies in attempts to unravel the complex patterns of *Cx. pipiens* and *Cx.*

restuans abundance (Trawinski and Mackay 2008 [New York, NY], Wang et al. 2011 [Ontario, Canada]), and Chuang et al. (2012) has recently shown positive associations between precipitation events several weeks prior to increased numbers of *Cx. pipiens/restuans* in Michigan.

Recently, cross-correlation maps have been utilized to visually display how time lagged meteorological variables are associated with mosquito abundance (Curriero et al. 2005, Shone et al. 2006, Chuang et al. 2012, Lebl et al. 2013), and using variations of their methods, we investigated the mosquito population dynamics of multiple local mosquito species including *Aedes dorsalis, Ae. excrucians, Ae. triseriatus, Ae. vexans, Co. perturbans, Cs. inornata,* and *Cx. tarsalis*. We also investigated impacts of meteorological variables on day-to-day mosquito abundance/activity using regression based models, as well as long term weather variables influence on mosquito abundance using cross-correlation techniques which analyzes larval development and to a lesser extent adult mostquito responses. By understanding the effects weather has on mosquito populations, we can better understand strategies for viral and pathogen risk assessment throughout the summer as well as inform local mosquito control groups.

Materials and Methods

***Study Sites*.** Three different study sites were utilized for this study. Mosquitoes were collected from two unique, rural sites within the RRV in Steele County, North Dakota. Two sites were located west of Hatton, ND in Steele County (47°38'20"N, 97°27'28"W), a small rural community (pop. ~ 700) located within Traill County. The first site, located 8.45 km southwest of Hatton, ND, was a hardwood forest with a semi-

closed canopy and thick underbrush. Green Ash (*Fraxinus pennsylvanica)*, Boxelder (*Acer negundo)*, Oak (*Quercus mongolica)* and American Elm (*Ulmus Americana)* were the predominant tree species found within the forest. Mosquitoes were collected from this site during the summers of 2008 and 2010

The second site, an agricultural ecosystem (from here on out referred to as the farm), was located 1.61 km west of Hatton, ND, and is surrounded by cropland and shelterbelts. A small grove of trees partitioned the farm from the surrounding fields. This type of landscape is more typical of North Dakota. A small coulee collects overland floodwater from surrounding fields and borders the farm along the north and eastern limits. This coulee retains water throughout the summer months. No livestock is raised on the farm. Mosquito control is absent at both the forest and farm sites. Mosquitoes were collected from the farm site during the summers of 2009 and 2010.

The third site from which mosquito data were collected was the city of Grand Forks, North Dakota (47°55'31" N, 97°1'57"W). Grand Forks (pop. ~ 52,838, area=52.03km^2) (U.S. Census Bureau, 2010) is located in Grand Forks county along the eastern edge of North Dakota and lies adjacent to the Red River. Mosquito control is used in the city and includes larviciding and ground/aerial spraying at the discretion of the Grand Forks Public Health Department (GFPHD).

Mosquito Collection. Mosquitoes from Steele County were collected using battery operated CO_2-baited Mosquito Magnet X traps (MMX) (Woodstream Lititz, PA) from late May through mid-August of 2009, 2010, and 2011. Traps were placed throughout both sites 2-3 times per week and operated from 1800 to 0800 hr. Mosquitoes were transported to the laboratory and placed in -20°C freezers to

immobilize mosquitoes. Mosquitoes were transferred into enamel pans and subsequently to plastic baggies for long term storage in -80°C freezers. Using dissecting scopes and dichotomous keys, mosquitoes were identified to species (Darsie Jr. and Ward 2005).

Mosquitoes from Grand Forks city were collected using CDC New Jersey light traps (NJLTs) throughout the city limits. Average daily mosquito counts for 2002-2010 produced 24,785 mosquitoes and a total of ~7,360 trap nights. Trap count averages were used to account for addition of new traps throughout the 9 year study period. Trap counts were obtained through the Grand Forks Public Health Department (GFPHD), Grand Forks Mosquito Control website (www.gfmosquito.com). Individual mosquito species were not identified by GFPHD, thus counts include female mosquitoes of multiple species.

***Weather Variables*.** Values for meteorological variables were taken from the North Dakota Agricultural Weather Network Center (NDAWN Center)(http://ndawn.ndsu.nodak.edu/). NDAWN Center has many weather stations that record both hourly and daily meteorological measurements throughout North Dakota and western Minnesota. These records were complete with no missing dates or measurements. Fifteen meteorological variables were initially investigated, including: precipitation (rain), maximum daily temperature (mxtemp), minimum daily temperature (mntemp), average daily temperature (avgtemp), bare soil temperature (bstemp), turf soil temperature (tstemp), wind speed (wind), dewpoint (dew), wind chill (windc), relative humidity (relhum), average daily humidity (ahum), maximum daily humidity

(mxhum), minimum daily humidity (mnhum), insect degree-days (IDD40) and photoperiod (hours of daylight) (photo).

The weather station nearest the Steele county sites is located near Mayville, North Dakota (47º29'59"N, 97 º19'32"W) at 290m elevation. The Mayville weather station is located 24.14km and 32.19km from farm and forest sites, respectively. The Grand Forks weather station located in Grand Forks, ND (47 º50'28"N, 97 º4'16"W) at an elevation of 257m. Individual trap locations were not disclosed to us, but the weather station location is approximately 6.44km south of the city limits.

***Statistical Analyses*.** General linear models were produced to determine the influence of daily weather variables on concurrent mosquito counts. Weather variables were analyzed for correlation and variables that displayed over 90% correlation to each other were then compared to mosquito counts. Of the correlated variables, the variable with the highest correlation value to mosquito count were used in regression models. Regressions were estimated for specific mosquito species from the farm and forest sites only. Martin et al. (2005) described the use of zero-inflated models, and their criteria were used to determine if zero-inflated models should be used based on the presence of either high numbers of true zero counts (low frequency of organism occurrence/no organisms present) or false zero counts (organism at site but not during trapping/organism failed to identify). Full models were reduced stepwise until the best AIC score was reached. Regression-based models were produced using the R software ("pscl" package) and models confirmed with SAS software (SAS Institute Inc., Cary, NC, USA).

Farm, forest and Grand Forks mosquito data sets were analyzed to determine associations between adult mosquito abundance and any preceding meteorological conditions that might have contributed to that abundance (e.g., meteorological factors affecting larval development and survival, etc.). Using the methods described recently by Chuang et al. (2012), individual cross correlation models (CCMs) were developed for each site and for each meteorological variable throughout the summer months. The CCMs produced in this study display the degree of correlation that existed between a particular trap count on a particular day (abundance) and the daily weather conditions (e.g., humid or dry, windy versus calm, etc.) over a time interval varying from 1-30 days. Correlations were computed for each of 15 meteorological variables that occurred each day, up to 30 days, prior to the trap day. Using CCMs allows for graphical representation of association values. Cross-correlation maps were produced using the R software (R Development Core Team 2007) in association with "mass" package.

Results

From the forest site (years 2009 and 2011), 18 species of mosquitoes were identified (n=9,321; 131 trap nights). The predominant species were *Ae. excrucians* (n=4,241; 46%), *Ae. vexans* (n=2,527; 27%), *Ae. triseriatus* (n=887; 10%) and *Co. perturbans* (n=984; 11%). Average numbers of mosquitoes per trap night were as follows: 32.37 *Ae. excrucians* , 19.29 *Ae. vexans* , 6.77 *Ae. triseriatus* and 7.51 *Co. perturbans* .

From the farm site (years 2010-2011), 15 species of mosquitoes were identified (n=24,799, 134 trap nights). The predominant species were *Ae. vexans* (n=19,389;

78%), *Cx. tarsalis* (n=3,451; 14%), *Cs. inornata* (n=505; 2%) and *Ae. dorsalis* (n=494; 2%). Average numbers of mosquitoes per trap night were as follows: 144.69 *Ae. vexans* , 25.75 *Cx. tarsalis* , 3.77 *Cs. inornata* and 3.67 *Ae. dorsalis* .

From the NJLT in Grand Forks, (years 2002-2010), 24,785 mosquitoes were trapped (7,360 traps nights). The average trap count for the entire period was 3.37 mosquitoes.

General mosquito abundance trends are unique to forest, farm, and Grand Forks sites. Within the forest (Fig 2.1A), we noted an initial increase in mosquito abundance starting early June. Around mid-June mosquito numbers peak and remain at elevated levels until the end of July. During August, mosquito numbers gradually decline to zero.

Farm mosquito abundance rises more quickly and peak approximately one week earlier than the forest population (Fig 2.1B). This population shows an initial peak (3rd week of June), then the population drops until second and third peaks are achieved the first weeks of July and August, respectively. Even with the second and third peaks, there is a general decline in mosquito numbers after the initial peak in early summer.

The Grand Forks population of mosquitoes is unique compared to the other sites. While the initial peak is similar in timing (end of June) (Fig 2.1C) to forest and farm sites, numbers tend to decline through August. If the temperatures continue to remain warm into September, a second peak is noted in the first half of the month.

To determine how various species within Steele County fluctuated throughout the summer, mosquitoes were identified, counted and the four species with the most abundant numbers were plotted. Figure 2.2 depicts the primary and secondary species

identified from the forest site and Figure 2.3 shows fluctuations of primary and secondary species at the farm site. Mosquito count data were used in generalized linear models (GLMs) to determine which meteorological variables were statistically associated with day-to-day trap counts. Individual species counts that contained many zeros were considered zero-inflated, and were run as either zero-inflated Poisson (ZIP) or zero-inflated negative binomial (ZINB) mixture models dependent upon calculation of overdispersion.

Concurrent Weather Conditions and Daily Trap Count Variability

Forest Mosquitoes

Generalized linear regressions were used to indicate which weather variables prevailing at the time of mosquito trapping significantly influenced the outcome of trapping (i.e., daily trap counts). Model summaries and statistical values for Forest mosquito species are presented in Appendix A. The only meteorological variable significantly correlated to *Ae. excrucians* trap counts was photoperiod (Photo; $p=0.0013$) (Table 2.2). Daily trap counts of *Aedes vexans*, the second most populous species, were significantly correlated to bare soil temperature (bstemp;$p=0.0133$), insect degree-days (IDD40; $p<0.0001$), minimum daily temperature (mntemp, $p=0.0002$); maximum daily humidity(mxhum; $p=0.0321$), photoperiod (photo, $p=0.0005$), and wind ($p=0.0036$).

Similarly, *Aedes triseriatus* daily counts were related to bare soil temperature (bstemp $p=0.0002$), insect degree-days (IDD40; $p<0.0001$), photoperiod (photo; $p<0.0001$), and (relhum $p=0.0114$).

In contrast, trap counts for *Co. perturbans* were negatively associated with bare soil temperature (bstemp; $p<0.0001$),), insect degree-days (IDD40; $p<0.0001$), rain ($p=0.0047$), relative humidity (relhum; $p=0.0055$); wind ($p<0.0001$), and positively associated with turf soil temperature (tstemp; $p<0.0001$); and average daily humidity (avghum; $p=0.0144$)

Farm Mosquitoes

Complete model summaries and statistical values for Farm mosquitoes can be found in Appendix B . Daily trap counts for *Ae. vexans* at the farm site were negatively influenced by bare soil temperature (bstemp $p<0.001$) (Table 2.3.), minimum daily humidity (mnhum; $p<0.0001$), rain ($p=0.0186$), relative humidity (relhum $p=0.0027$). Conversely, daily trap counts for *Ae. vexans* was positively influenced by insect degree-days (IDD40; $p<0.0001$), minimum daily temperature (mntemp; $p<0.0001$), maximum daily humidity (mxhum; $p<0.0001$), and photoperiod (photo; $p<0.0001$).

Daily trap counts for *Cx. tarsalis* at the farm were negatively influenced by both rain ($p=0.0003$) and dewpoint ($p=0.0017$). Conversely, daily trap counts for *Cx. tarsalis* were positively influenced by turf soil temperature (tstemp; $p<0.0001$), average daily humidity (ahum; $p<0.0001$), and maximum daily temperature (mxtemp; $p=0.0010$).

Daily trap counts for *Ae. dorsalis* were negatively influenced by bare-soil temperature (bstemp; $p<0.0001$) and average daily humidity (ahum; $p=0.0007$). Although not highly significant, rain ($p=0.0786$) also seems to be playing a negative role and removal of this variable increased the AIC score. Daily trap counts for *Ae. dorsalis* were positively influenced by minimum daily temperature (mntemp;

p=0.0022) and insect degree-days40 (IDD40; p=0.0035). Maximum temperature (mxtemp) (NB; s.e.=0.0462; n.d.=72.16; z=1.87; AIC=270.58; p=0.0611) positively influenced trap counts, but not significantly.

Daily trap counts of *Cs. inornata* trap counts were not positively influence by any meteorological variable, but were negatively influenced by insect degree-day 40 (IDD40 p=0.0055), photoperiod (p=0.0005) and rain (p=0.0243).

Grand Forks Mosquitoes

Using regression analysis, it was found that daily trap counts for the Grand Forks mosquitoes were positively affected by two different temperature variables: average temperature (Avgtemp;p=0.0324) (Table 2.4) and minimum temperature (mntemp; p=0.0033) (Appendix C)

Time-lagged Mosquito Count Variability

Forest Mosquitoes

To determine which meteorological variables could be used to forecast the abundance of mosquitoes, CCMs were developed for the four most abundant mosquito species. *Aedes excrucians*, by far the most abundant mosquito species, was positively correlated with photoperiod for days 10-12 prior to trap date (Table 2.5). Interestingly, factors usually associated with increased mosquito numbers such as rain, mntemp, avghum, relhum, and IDD40 all influenced counts in a negative manner. Wind speed showed a slightly positive influence on *Ae. excrucians* counts, but only for a short period approximately 2 weeks prior to trapping. Cross-corellation maps for *Ae. excrucians* can be found in Appendix D.

Aedes vexans showed almost the opposite response to meteorological variables than *Ae. excrucians*. In fact, rain, mntemp, relhum, avghum, IDD40 and photo all showed highly positive influences on *Ae. vexans* trap count numbers (>0.6350). CCMs for *Ae. vexans* are reported in Appendix E. (Table 2.5)

The tree-hole breeder, *Ae. triseriatus*, showed similar responses to meteorological variables as *Ae. vexans*. Rain, mntemp, relhum, IDD40 and photo all influenced trap counts in a positive manner. Relhum, also showed a period of negative influence 8 days prior to trap day. The variable wind also had a negative influence around 27-30 days before trap day. (Table 2.5) (Appendix F)

Following suit, *Co. perturbans* was positively correlated with rain, mntemp, relhum, avghum, IDD40 and photo. Similarly to *Ae. triseriatus*, relhum showed a period of negative influence on *Co. perturbans* around but around 28 days in advance of trap date. Wind speed showed a negative influence on trap counts for the majority of the 30 day period, but with strongest correlation at day 29. Table 2.5, Appendix G.

Farm Mosquitoes

The common species between the farm and forest, also the most abundant at the farm, was *Ae. vexans*. Not surprising, between the two sites, cof this species were associated with similar sets of variables. At the farm site, rain, mntemp, relhum, mxhum, and photo all showed positive association with trap counts. These variables seemed to influence daily trap count to a lesser degree at the farm versus the forest site (Appendix H and E). Wind speed had a negative influence on trap count approximately 1 and 3 weeks prior to trap day (data not shown). Table 2.6, Appendix H.

The local WNV vector, Cx. tarsalis, was negatively impacted by wind during days 16-18 prior to trap date. Rain was also a negative factor a few days before trapping, but showed a positive correlation slightly over 3 weeks before catch day. Turf-soil temperature, relhum, mxhum, IDD40 and photoperiod all had strong positive effects on trap counts. Wind had a strong negative effect on trap counts of *Cx. tarsalis*. Table 2.6, Appendix I.

Aedes. dorsalis, had differing outcomes from similar meteorological variables. Average temp, relhum, mnhum, IDD40, and photo had negative impacts on mosquito trap counts. Wind was the only factor that played a positive role in mosquito counts for the first couple days prior to catches while other meteorological factors showed marginal negative correlations or no correlation. (Table 2.6, Appendix J)

Culiseta inornata showed a contrasting affect of weather variables than did *Ae. vexans* and Cx. tarsalis from the farm site, but similar to *Ae. dorsalis*. Mntemp, avghum, rain, wind, and photo all showed differing degrees of negative influence. The only variable that showed a strictly positive effect was IDD40. Few of the variables (rain, relhum, avghum, and wind) displayed mixed roles in trap counts having both positive and negative influences throughout the preceding 30 day period. (Table 2.6, Appendix K)

Grand Forks Mosquitoes

Because the mosquito counts for the Grand Forks data set were not identified to species, daily trap counts were analyzed as one set. To try to obtain as much detail about the mosquitoes of Grand Forks, the mosquito counts were divided by month to determine if there was variability throughout the mosquito season (Table 2.7,

Appendices L-O). During the month of June, rain (15-30 days), mntemp (1-30 days), avghum (10-17days), IDD40 (1 day) and photo (17 days) all had positive influences on daily trap counts. The only variable that showed a negative relationship with count data in June was wind (5-13 days).

In July, there was a slight shift of meteorological variables that significantly influenced trap counts. Not surprising, the most influential variable was rain (1-30 days). Minimum temperature again was an important factor, but instead of the entire 30 day lag, it is most influenced by the first 12 days prior. Wind speed, previously a negative factor, now showed a positive influence from 11-30 days prior. Also reversing roles was the once positive role of IDD40, which is now negative in July. While avghum seemed to be the humidity variable most influential on trap counts during June, there appears to be a switch to mnhum in July for the preceding 3 week period.

In August, many of the variables that had earlier been highly correlated became insignificant. For instance, the variable rain, that had correlation coefficients of 0.5742 and 0.5200 for the months of June and July respectively, had a non-significant negative correlation coefficient of -0.1248. Rain also showed a negative impact on August trap counts at 3-4 weeks lag time. All three measurements of humidity (avghum, mxhum, and mnhum), were negatively correlated to mosquito counts during August. Both IDD40 and photo had previously been significant factors for trap counts, but now display absolute correlation values < 0.1.

Although August showed general declines in meteorological influence on trap counts, September showed a general return to previous climate based influence. Rain again showed a highly positive influence on mosquito counts. Minimum temperature,

mxhum and photo also showed positive associations with September trap counts. Insect degree-day 40 (IDD40) on the preceding days have negative correlation values.

Discussion

In this study, multiple meteorological variables were analyzed to determine if they influenced mosquito trap counts and thus abundance of host-seeking mosquitoes. To determine the role on adult female mosquitoes, regression models were used to evaluate how meteorological variables affected daily activity of questing females. While this information is unique and overall interesting, it does not help much in the long term forecasting of mosquito abundance, or allow for a timely focus for mosquito control or risk assessment. To aid in identification of informative forecasting variables, CCMs were utilized to analyze a 30-day pre-trap date time lag.

Rain has often been associated with mosquito abundance because of the aquatic nature of the larvae. Previous studies have implied that rainfall may have either positive effects on mosquito counts by providing breeding places, but may also cause flooding, thereby washing away mosquito larvae and reducing trap counts (Gubler et al. 2001, Woodruff et al. 2002, Kelly-Hope et al. 2004, Gardner et al. 2012). Reduced mosquito activity may also be affected by rain as a hindrance to flight; although usually not lethal to mosquitos given the mosquito is not in close proximity to the ground or a hard structure in which the mosquito may collide (Dickerson et al. 2012).

Humidity is important to mosquito survival in many aspects. For instance, high humidity may be an indication that breeding sites are losing water due to evaporation (Wu et al. 2007). It does not appear that relative humidity (rh) has an impact on actual flight (Rowley and Graham 1968) of mosquitoes, but it has been shown that mosquitoes

avoid flight during times of humidity extremes (Muirhead-Thomson 1938, Murty et al. 2010), possibly to maintain water equilibrium within the mosquito body.

While dew point has been reviewed in association with WNV transmission rates (DeGroote et al. 2008, Soverow et al. 2009) dew point has not been actively reviewed in association with mosquito counts in terms of direct relationship to trap counts. It can be assumed though, that if there have been associations between WNV transmission and dew point, there is an association between dew point and mosquitoes, especially those involved with WNV transmission.

Wind and wind speed have been implicated in the spread of CO_2 and heat, two of the major attractants that mosquitoes use for host location (Carde et al. 2008), and mosquitoes can maneuver both horizontally and vertically to locate upwind hosts on which to feed upon (Spitzen et al. 2012). Decreased wind speed may prevent proper CO_2 dispersal and limit the mosquito's ability to locate available hosts. Previous studies have also indicated that different mosquito species have unique abilities to fly within differing speeds of wind (Gjullin et al. 1961, Haufe 1966, Grimstad and DeFoliart 1974).

Degree-days and temperature have widely been established as factors that can initiate and play positive or negative roles in insect development and activity. For example, *Ae. vexans* larvae do not develop at temperatures lower than approximately 5ºC (41ºF), and highest developmental rates occur at 25ºC (77ºF) (Trpis and Shemanchuk 1970) and flight activity increases two-fold when temperatures are above 19ºC (Bidlingmayer 1974).

Photoperiod is often associated with insect degree-days. Photoperiod tends to increase and decrease at regular intervals, but this variable plays a crucial role in physiological changes in mosquito metabolism that can either induce or break mosquito diapause (Barr 1958, Bennington et al. 1958, Harwood and Takata 1965, Mitchell 1981, Reisen et al. 2003). Photoperiod is a seasonal factor particularily prior to adult emergence.

Daily Mosquito Counts. Plotting mosquito trap counts as a measure of adult mosquito abundance (*i.e*., the dependent variable) against the meteorological conditions (*i.e*., independent variables) present at the time on which trapping was conducted may not provide very useful or predictive information on which to forecast future adult mosquito abundance. That is because adult mosquito abundance is largely driven by the size, survival and developmental rate of the immature population. However, such an analyses can provide useful information on specific meteorological conditions that may favor (or inhibit) the flight and/or host-seeking behavior of adult mosquitoes on a given night. My analyses suggest that different mosquito species may respond differently to various meteorological conditions.

Forest Mosquitoes

Aedes excrucians is one of the earliest mosquitoes to appear within the forest site (Mehus unpub. data), appearing in early June. The only meteorological variable to impact daily trap counts is photoperiod (Appendix A). This may be due to the life history of the larval development of this species. *Aedes excrucians* is widely known as a snow pool mosquito with larvae starting development as snow starts melting. As larvae hatch while temperatures are low, it would seem that the activity of these

mosquitoes is not directly associated with temperature as long as larval habitat is present.

Aedes vexans daily trap counts show closer relationships with meteorological variables. Both IDD40 and photo period are positively associated with daily trap counts, and it is plausible that these factors initiate early development and are associated due to an autocorrelation of increased population as these variables generally increase during the mosquito season. Minimum daily temperature is a measurement of air temperature, and as minimum daily temperatures increased the capture of host-seeking *Ae. vexans* also increased. Conversely, increasing bare soil temperatures actually reduced trap counts. At both the Forest and Farm sites, mosquitoes were trapped with CO_2 emitting MMX traps. Because MMX traps do not utilize heat to attract mosquitoes, increased bare soil temperatures may actually increase mosquito activity and lead them to the vicinity of the trap, but since traps are located 3-4ft above the ground surface, mosquitoes may remain close to the ground and not be drawn into the trap.

Daily trap counts of *Ae. triseriatus* were highly influenced by both photoperiod and IDD40. Within the forest, *Ae. triseriatus*, like *Ae. vexans*, showed a decrease in trap counts while bstemp increases. This may be related to the means of sampling as it is hypothesized to be for *Ae. vexans*. Relative humidity also plays a significant role in reduced trap counts of *Ae. triseriatus*. As previously mentioned, this may be an effect of reduced flight activity to maintain mosquito hydration.

The fourth and final species discussed from the forest site is *Cs. inornata*. This species is unique in that two humidity measurements seem to be influencing daily trap

counts, but in contrasting ways. Maximum daily humidity increases trap counts while minimum daily humidity reduces daily trap counts for *Cs. inornata*. It would appear that while having higher upper limits of humidity are beneficial to *Cs. inornata*, prolonged or higher levels of mnhum for the day may halt activity, causing avoidance of flight. It seems, from this study, that *Cs. inornata* prefers the cycling of high mxhum and lower mnhum values. It is plausible that increased mnhum values provide excess build up of condensation on vegetation reducing resting locations for adult *Cs. inornata* causing them to travel elsewhere or become trapped or damaged in the excess condensation. Wind was a highly positive significant variable to *Cs. inornata*. Because of the nature of the forest site, *Cs. inornata* may not be able to detect CO_2 plumes given off by the traps without the aid of wind movement.

Farm Mosquitoes

Ae. vexans in both sites demonstrated significantly reduced daily trap counts in association with bstemp (Appendix B). It would appear that although *Ae. vexans* responded positively to high levels of humidity (mxhum) the population does not prefer high levels of mnhum, avoiding flight and capture. Without the protection of the canopy and dense underbrush of the forest, it appears that rain negatively influences trap counts, likely due to increased exposure to raindrops. It is likely that mntemp increases activity of *Ae. vexans*, thus increasing daily trap counts.

Culex tarsalis, as previously explained, shows a negative relationship between daily counts and rain. Also, an increase in dew point (likely due to condensation on resting surfaces (Harwood and Halfhill 1960)) decreases *Cx. tarsalis* activity. Unlike other species previously discussed, this species shows positive correlation to surface

and air temperature variables, tstemp and mxtemp. While surface temperatures increase activity close to the ground, it may be due to the aggressive nature of this mosquito that draws it vertically into the suction of the trap.

As with the previously described *Aedes* mosquitoes, it appears that increased bstemps reduces daily trap counts of *Ae. dorsalis*, while air temperature (mntemp) increases trap counts. Insect-degree days also positively influence trap counts and likely plays a role in mosquito larval development and adult emergence at approximately the same time. Unlike other mosquitoes, *Ae. dorsalis* does not react positively to increased humidity.

Finally, the *Cs. inornata* population at the farm is likely to be the first mosquitoes active during the spring. While IDD40 and photo may initially spark development and break diapause, as abundance decreases in early June, both photo and IDD40 continue to increase, leading to a negative association. Continuing with the trend, rain also relates to a negative trap count of *Cs. inornata*.

Grand Forks Mosquitoes

The nine year mosquito daily trap counts were analyzed using a negative binomial, generalized linear model based on overdispersion (Appendix C). For this data set, it appears that there are only two meteorological variables that influenced trap count. These variables are avgtemp and mntemp, with mntemp showing stronger significance (0.0324 and 0.0033 respectively). This model does not show either IDD40 or photo as influences on trap count, nor any of the humidity variables or rain. It may be that by grouping all mosquito species together into one large count, the specificity of

variable impact is reduced, creating a model that is less intense due to a blending of meteorological variable effects on differing species of mosquitoes.

Cross-Correlation Map Analysis. These analyses were undertaken to determine if specific meteorological conditions, existing up to 30 days in advance of mosquito trapping, could be correlated in a predictive manner to forecast adult mosquito abundance. Although some meteorological conditions preceding mosquito trapping could conceivably influence the emigration and immigration of adult mosquitoes into a particular trapping zone (*e.g.*, strong windstorms), it is more probable that precedent weather exerts its greatest effect on the immature mosquito population or breeding period. Furthermore, it might be anticipated that some meteorological variables may more influential than others and that there may be key meteorological variables that ultimately determine the survival or developmental rate of immature mosquitoes. This in turn, will determine the abundance of adult mosquitoes.

Forest Mosquitoes

All forest mosquito CCMs can be found in Appendices D-G. The snowpool mosquito, *Aedes excrucians*, actually shows a negative response to rain approximately 28-29 days prior to trapping. While it seems beneficial to have added moisture in the larval habitat, additional rain causes the local river system to flood, washing larvae away. Around day 18, rain does have a beneficial effect, and is probably good for maintaining breeding pools. *Aedes excrucians*, whose larvae are frequently found in 4ºC pools of water, are negatively affected by an increase in mntemp (day 29), probably due to stress from water temperatures reaching critical levels. Minimum temperature does also play a role 3 days prior to trapping, around the time these

mosquitoes will be hatching and mating before looking for a bloodmeal. Both relhum and avghum show negative influences on time-lagged trap counts 2-4 weeks before trap day. It is likely that this increase in humidity is due to moisture evaporating from breeding pools, thus reducing larval survival. Because *Ae. excrucians* is an early season, univoltine mosquito and has only one hatch during the summer, it is expected that as those mosquitoes age and die through the summer and as IDD40 continues to increase we would see a negative response to IDD40 based on the life history of the species.

Aedes vexans, the most abundant mosquito species in the region, showed variation between day-to-day and time-lagged trap counts. While rain on a daily count basis proved a negative effect on adult mosquito behavior, over the 6-29 day lag period, rain is highly associated with trap counts and larval mosquito survival and development. This is likely the result of the life history pattern of breeding site selection of the species. *Aedes vexans* is a floodwater mosquito and eggs are laid in soils just higher than water levels and intermittent added moisture aids in keeping the pools from drying. Minimum daily temperature is strongly associated with trap count 2-3 weeks after the temperature increase. During this time, it is likely that the increase in temperature does not affect imagos, but more likely causes increased larval development rates. In contrast to day-to-day counts, relhum and mxhum have a positive influence on trap counts for the majority of the 30 day period. The combination of increased humidity and dew point likely helps to retain water holes used for breeding and larval development.

Aedes triseriatus are tree-hole breeding mosquitoes whose overwintering stage is the egg, which are placed in dry tree-holes. It is no surprise then, that 24-27 days prior to trapping, rain is an important variable. Overall, relhum acts positively on trap counts, as does dew point, probably aiding the retention of water in the tree-holes. Mntemp also acts positively 23-27 days prior to trap day, likely stimulating egg hatch and larval development. IDD40 is important for the entire 30 day period, while photoperoid plays a larger impact 2-4 weeks prior to emergence, again, likely stimulating egg hatch and development of larval *Ae. triseriatus*.

Coquillettidia perturbans is a unique mosquito in that the overwintering stage is the larvae, which attach themselves to flooded vegetation via specialized siphons used for breathing (Rademacher 1979). Because the breeding pools stay flooded through the winter, the positive role of rain 24-27 days prior may actually aid by increasing temperature of breeding pools and substrate where immature stages of *Co. perturbans* are found. Mntemp, IDD40, and photo all are likely to play roles in breaking diapause in larval mosquitoes. In fact, all variables except wind showed positive influences. Because these breeding pools may be only damp or under a few centimeters deep within the forest, increased wind may dry out grounds where larvae are present, increasing larval mortality.

Total mosquito counts were used in the development of CCMs for the Forest site (Appendix P). These maps inially seemed to have unique correlations to meteorological variables until CCMs for the four most abundant mosquito species were simultaneously compared. Total count CCMs for the Forest show correlation values approximately halfway between those of *Ae. excrucians* and *Ae. vexans*. This effect is

likely due to two components, abundance of given mosquito species and the correlation of those mosquito populations to meteorlogical variables. As an example, *Ae. excrucians* showed almost no correlation to precipitation except the negative correlation from days 28-29 (-0.4574) while *Ae. vexans* showed an extremely high correlation value (0.7638) for days 6-29. Because of the high prevalence and lack of correlation of *Ae. excrucians* to rain along with the lower prevalence and the high correlation of *Ae. vexans* to rain we wind up with a blended CCM for total counts. This was a similar trend with other meteorological variables within the site.

Farm Mosquitoes

All CCMs for the farm mosquitoes can be found in Appendices H-K. The population of *Ae. vexans* at the farm site showed the same responses to meteorological variables as the forest population. The difference between the two groups is the degree of significance of each of these variables. There are a few possibilities why these results may have occurred. One of the main reasons is that *Ae. vexans*, in our region, is an open space, open environment mosquito, preferring habitats such as this agricultural site over the forest site (Mehus unpub data). In the open environment, they are more exposed to all meteorological variables, increases the importance of variables within the Forest. Rain was important in both farm and forest populations, 9-27 days and 6-24 days, respectively. This is likely the timing of initial flooding of soils containing overwintering eggs or eggs laid throughout the summer as *Ae. vexans* is multivoltine.

Local WNV vector *Cx. tarsalis* shows an interesting response to the rain variable. Although, there are times rain events appear significant (23 days, rs=0.3096), overall rain assumes a negative role or is not strongly correlated in either direction.

This is likely due to the fact that *Cx. tarsalis* overwinter as mated female mosquitoes. These mated females need to first acquire a blood meal and produce eggs before a new cohort of *Cx. tarsalis* appears. Since *Cx. tarsalis* oviposite egg rafts on permanent bodies of water, it is less likely that rain events will be significant except in extreme cases such as drought.

Ae. dorsalis appears early in the season, but is also multivoltine, having multiple broods throughout the summer. For the first 24 days lagged from trap date, rain aids in increasing mosquito trap counts. Like *Ae. vexans*, *Ae. dorsalis* is a floodwater mosquito, and relies heavily on multiple rain events to replenish breeding pools. None of the temperature variables, humidity variables, IDD40 or photoperiod plays strong roles in the abundance of this mosquito species, in fact, most have slightly negative roles. As an example, avgtemp which is usually positively associated with mosquito counts, negatively affects *Ae. dorsalis*, likely due to the fact these mosquitoes are more abundant during early spring while temperatures are still mild. While multiple broods are likely during the summer, hatch events demonstrated in our regions have shown decreased emergence abundances later in the summer. It is possible that increased temperatures increase physiological stress to larvae, and mortality increases, decreasing the percentage of imagos attained. It may also be that during the summer months, there is an increase of other mosquito species' larvae that may out-compete *Ae. dorsalis* larvae for resources.

The earliest mosquito species found within the majority of the RRV is *Cs. inornata*. The mated adult females break diapause early in the spring, often before all the snow has melted. These females find a blood meal, produce eggs and lay their eggs

on permanent to semi-permanent bodies of water. This may explain why for the majority of the 30 day lag period, rain is shown as a negative variable, with rain only acting as an obstacle to overwintered females. As with *Ae. dorsalis*, most of the climate based variables displayed either no correlation or slightly negative correlations to *Cs. inornata* trap counts. Besides rain, the most influential of the meteorological variables was photoperiod, having a strong negative relationship with *Cs. inornata* trap counts, again likely due to the early season nature of this mosquito species.

One of the most interesting discoveries during this study was the relationship between climate-based variables and early-season versus later-season mosquito species. If we look at species such as *Ae. triseriatus, Ae. vexans, Co. perturbans* and *Cx. taralis* we note the importance of temperature, humidity as well as IDD40 and photoperiod. These late-season mosquitoes also show general negative tendencies towards wind. Except for *Cx. tarsalis*, these late-season mosquitoes rely heavily on rain events to replenish breeding pools. *Culex tarsalis* does not rely on rain because of its tendency to lay egg rafts on permanent bodies of water.

Early season mosquitoes, Ae. *dorsalis, Ae. excrucians* and *Cs. inornata* are not as influenced by climatic variables, but more highly upon more stable variables such as IDD40 or photoperiod. Temperature and humidity were either not correlated with trap counts or were negatively associated with trap counts, showing that as temperature and humidity increase through the summer months, the general numbers of *Ae. dorsalis, Ae. excrucians* and *Cs. inornata* decline.

When CCMs were run on total mosquito counts for the Farm site (Appendix Q) the resemblance to the *Ae. vexans* CCMs (Appendix H) were quickly noted. The

meteorological variables rain and minimum temperature showed the same days of highest correlation (9-27 and 6-6) and only slightly reduced correlation coefficients ($0.4653<0.5172$ and $0.4665<0.4983$ respectively). With almost 80% of the total mosquito population being *Ae. vexans*, it is plausible that in CCMs for total counts the correlations would favor those of *Ae. vexans*.

Grand Forks Mosquitoes

Cross-correlation analyses were used to determine how time-lagged weather variables were influencing mosquito counts in the city of Grand Forks. Because individual species were not identified, counts were analyzed by month to determine if there were differing weather impacts on trap counts throughout the summer. During the month of June, rain was highly correlated with mosquito trap counts two to four weeks prior to trap date. Most of the mosquitoes collected in the city of Grand Forks are floodwater mosquitoes, more specifically *Ae. vexans,* and precipitation is likely involved in the flooding of breeding grounds containing the overwintering egg stages. For the entire 30-day lag period, minimum temperature was highly correlated with trap counts, influencing both larval development by warming soils and increase adult activity. To maintain larval development, it is essential that these breeding grounds retain moisture. It is possible that higher average humidity amounts help to maintain these breeding grounds by releasing moisture into the environment during the cool summer evenings. In June, the role of wind on trap counts is negative. The high winds in the RRV have a drying effect on the environment, specifically on soils and shallow depressions, regions often associated with larval *Ae. vexans*.

The weather variables that are highly correlated to trap counts in July are similar to those in the month of June. Rain is again the most correlated value to July trap counts, but for the entire 30-day period prior to trapping. The most probable reason for the increase in days for which rain is important from June to July is the maintenance of dwindling breeding grounds due to increased temperatures in the month of July. Minimum temperature again is positively correlated to trap counts, yet at a reduced lag period. This reduction may be a product of soils and breeding pools warming gradually through the month of June. Unlike wind speed in June, wind in July is positively correlated to trap counts. Since many of the breeding grounds for these floodwaters are outside city limits, it is possible that mosquitoes reaching adulthood from these outlying regions are carried by the wind into city limits.

During the month of August, highly correlated weather variables such as rain, temperature and humidity drop in association values, yet one variable, wind, still plays a positive role on trap counts, suggesting that the mosquitoes entering traps are likely being blown in from outside city limits. The decreased correlation values also are characteristic of a previously discussed mosquito species, *Cx. tarsalis*. It is probably not coincidental then that by the end of July and beginning of August, *Ae. vexans* numbers start to decrease and there is an increase in the percentage of *Cx. tarsalis* recovered in traps (Deckert 1995; Bell et al. 2005) and we may be seeing this population switch within the cross-correlation maps.

By the beginning of September *Cx. tarsalis* populations in the region have declined (Mehus unpub. data), and a new and likely last cohort of newly emerged floodwater mosquitoes appears. This dynamic of mosquito populations is visualized in

the cross-correlation maps for September as rain, minimum temperature and maximum humidity regain positive correlation values to trap counts. By dividing the mosquito trap counts of Grand Forks by month, it was possible to determine which mosquito species was likely active.

Acknowledgements

Thank you to Sarina Bauer, Stephen Greimen, Jeffrey Bell and Alex Droske for assistance in identifying mosquitoes in addition to Ting-Wu Chuang for providing R-code structure for CCMs.

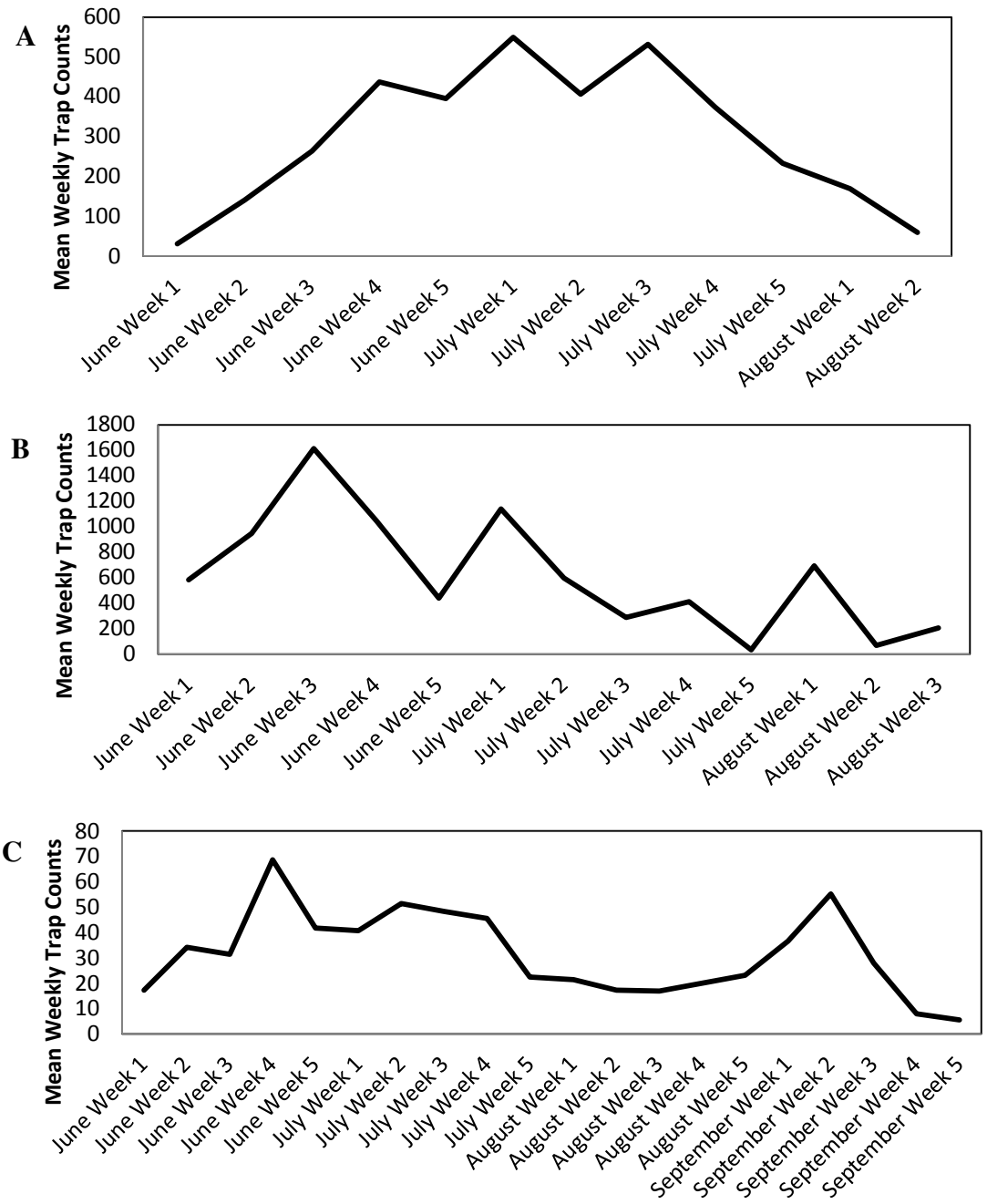


Figure 2.1. Weekly trends in total mosquito population at forest, farm and city of Grand Forks sites. A) Mosquito species mean weekly counts within the forest site. B) Mean weekly mosquito counts within the farm site. C) Mean weekly mosquito counts within the city of Grand Forks ND (2002-2010).

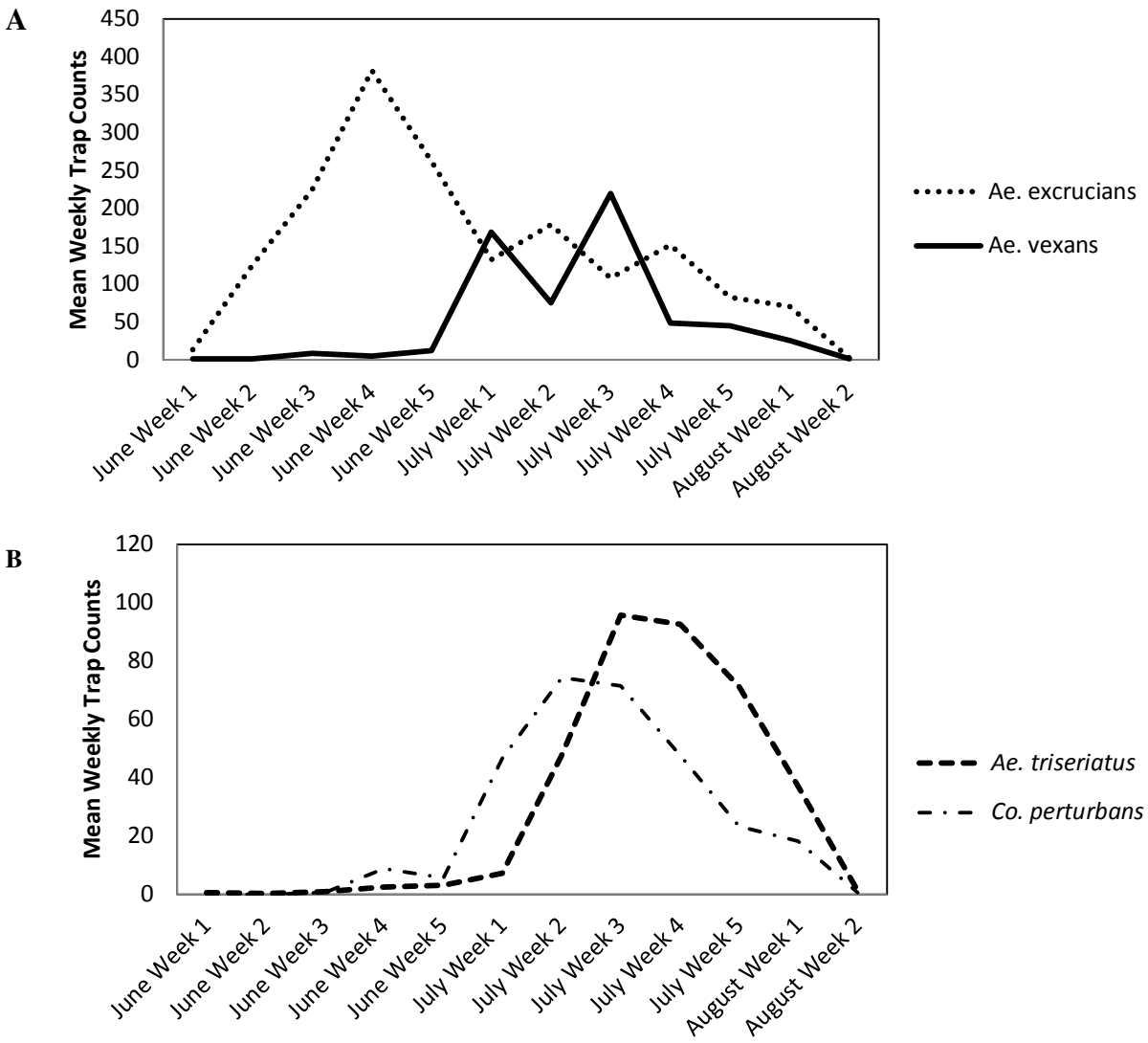


Figure 2.2. Trends in mosquito species dynamics with the forest. A) Top two mosquito species showing unique peak times for *Ae. vexans* and *Ae. excrucians*. B) Secondary species within the forest site showing overlapping peaks for *Ae. triseriatus* and *Co. perturbans*.

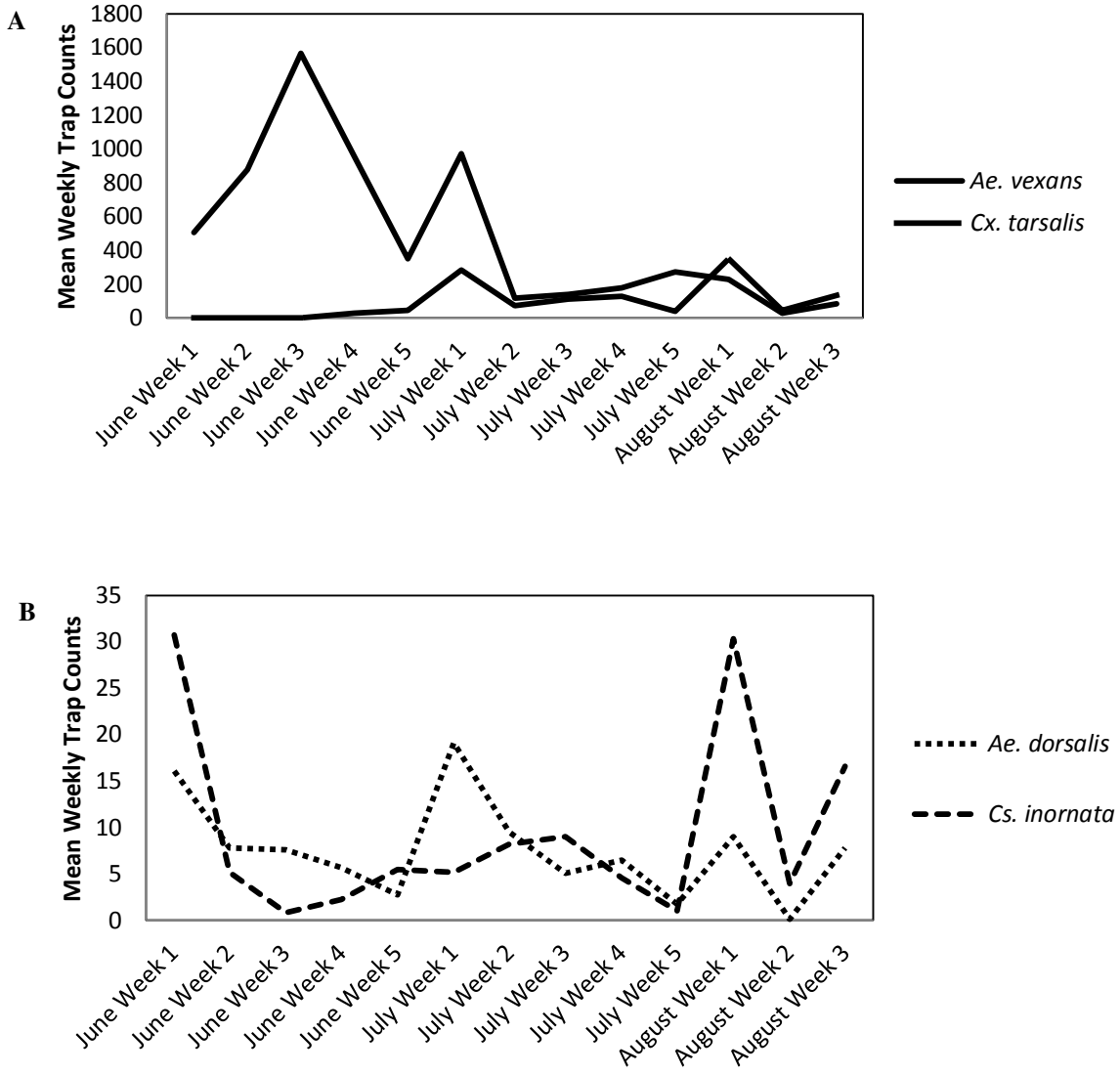


Figure 2.3 Trends in mosquito populations at the farm during the summers of 2010 and 2011. A) Predominant mosquito species dynamics showing multiple peaks in host-seeking *Ae. vexans* and *Cx. tarsalis*. B) Secondary mosquito populations showing early peaks with unique delayed peaks for *Ae. dorsalis* and *Cs. inornata.*

Table 2.1. States with the highest per capita (incidence/100,000 people) human cases of WNV from 2002-2011. Information obtained via CDC website.

Rank	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
1	NE (8.80)	SD (136.04)	AZ (6.81)	SD (29.51)	ID (77.48)	**ND (56.52)**	**ND (5.63)**	NE (2.87)	AZ (2.60)	MS (1.75)
2	LA (7.32)	NE (111.70)	SD (6.62)	**ND (13.51)**	**ND (21.38)**	WY (33.84)	SD (4.88)	SD (2.60)	SD (2.45)	NE (1.57)
3	IL (7.06)	**ND (96.59)**	CO (6.32)	ND (10.70)	NE (19.98)	SD (26.28)	NE (2.62)	ID (2.45)	NE (2.13)	AZ (1.06)
4	MS (6.72)	WY (74.49)	NM (4.62)	LA (3.78)	WY (12.62)	MT (20.94)	ID (2.54)	WY (2.14)	CO (1.60)	NE (0.59)
5	MI (6.13)	CO (65.07)	**ND (3.14)**	MT (2.67)	AR (11.37)	CO (11.99)	MS (2.21)	CO (2.07)	**ND (1.33)**	**ND (0.58)**
-	**(10) ND (2.66)**							**(20) ND (0.15)**		

Population data acquired via the United States Census Bureau

Table 2.2. Summary of mosquito species generalized linear models analyzing meteorological variables affecting daily mosquito trap counts from the forest site.

Model Components	AIC Score	Estimates	Standard Error	Z-value	p-value
Ae. excrucians	339.12				
Photo		0.0102	0.0032	0.2200	0.0013
Year		0.0350	0.0014	24.8690	<0.0001
Ae. vexans	259.76				
Bstemp		-0.1555	0.0628	-2.476	0.0132
IDD40		0.0045	0.0011	4.018	<0.0001
Mntemp		0.0835	0.0230	3.632	0.0002
Mxhum		-0.0913	0.0426	-2.143	0.0321
Photo		0.0343	0.0099	3.466	0.0005
Wind		-0.2043	0.0702	-2.911	0.0036
Year		0.3072	0.0055	56.344	<0.0001
Ae. triseriatus	215.94				
Bstemp		-0.2378	0.0643	-3.698	0.0002
IDD40		0.0076	0.0011	7.195	<0.0001
Photo		0.0377	0.0083	4.518	<0.0001
Relhum		-0.0773	0.0306	-2.530	0.0114
Co. perturbans	220.43				
Bstemp		-0.4093	0.0778	-5.260	<0.0001
IDD40		-0.0021	0.0005	-4.161	<0.0001
Rain		-5.7930	2.0490	-2.827	0.0047
Relhum		-0.0826	0.0298	-2.775	0.0055
Tstemp		5.6960	0.0738	7.715	<0.0001
Wind		-0.4010	0.0860	-4.667	<0.0001
Avghum		0.0663	0.0271	2.446	0.0145
Year		-0.6852	0.0223	-3074.540	<0.0001

Table 2.3. Summary of mosquito species generalized linear models analyzing meteorological variables affecting daily mosquito trap counts from the farm site.

Model Components	AIC Score	Estimates	Standard Error	Z-value	p-value
Ae. vexans	517.04				
Bstemp		-0.3092	0.0690	-4.480	<0.0001
IDD40		0.0028	0.0007	3.965	<0.0001
Mnhum		-0.1307	0.0243	-5.370	<0.0001
Mntemp		0.2686	0.0532	5.052	<0.0001
Mxhum		0.5120	0.1069	4.790	<0.0001
Photo		0.0282	0.0066	4.298	<0.0001
Rain		-3.7021	1.5732	-2.353	0.0186
Relhum		0.1873	0.0625	-2.996	0.0027
Cx. tarsalis	369.92				
Rain		-6.3070	1.7439	-3.617	0.0003
Tstemp		0.4231	0.0840	5.040	<0.0001
Avghum		0.1874	0.0445	4.211	<0.0001
Dew		-0.2644	0.0843	-3.137	0.0017
Mxtemp		0.2242	0.0680	3.297	0.0009
Ae. dorsalis	270.58				
Avghum		-0.1085	0.0317	-3.423	0.0006
Bstemp		-0.2055	0.0510	-4.031	<0.0001
IDD40		0.0007	0.0003	2.923	0.0035
Mntemp		0.1331	0.0435	3.062	0.0022
Mxhum		0.0866	0.0462	1.873	0.0611
Rain		-2.2712	1.2912	-1.759	0.0786
Cs. inornata	259.17				
IDD40		-0.0010	0.0004	-2.776	0.0055
Photo		-0.0140	0.0040	-3.491	0.0005
Rain		-2.2010	0.9777	-2.251	0.0244
Year		-2.3340	0.4426	-5.480	<0.0001

Table 2.4. Summary of generalized linear model analyzing meteorological variables affecting daily mosquito trap counts from Grand Forks, North Dakota.

Model Components	AIC Score	Estimates	Standard Error	Z-value	p-value
Grand Forks	6590.8				
Avgtemp		0.0286	0.0134	2.139	0.0324
Mntemp		0.0386	0.0131	2.939	0.0033

Table 2.5. Correlation coefficients of mosquito abundance and weather variables for the forest mosquito species (2009 and 2011). Numbers in parentheses represent lag days.

Weather Variable	*Aedes excrucians*	*Aedes vexans*	*Aedes triseriatus*	*Coquillittidea perturbans*
Rain	-0.4574 (28-29)	0.7638 (6-29)	0.5479 (24-27)	0.6010 (24-27)
Mntemp	-0.3961 (29-29)	0.8610 (14-20)	0.7243 (23-27)	0.6682 (24-27)
Relhum	-0.6233 (18-30)	0.6554 (5-27)	0.3679 (21-23)	0.4529 (21-23)
Wind	0.3231 10-12	0.4991 (26-29)	-0.5196 (27-30)	-0.4462 (29-29)
Dew	-0.4451 (23-24)	0.8628 (14-28)	0.6178 (23-27)	0.6132 (23-27)
Avghum	-0.5078 (20-30)	0.7261[α] (9-29)	0.4931 (15-27)	0.4405 (15-27)
IDD40	-0.4377 (30-30)	0.6350 (1-1)	0.4657 (6-6)	0.3862 (1-1)
Photo	0.6418 (10-12)	0.6391 (29-29)	0.5996 (29-29)	0.6460 (29-30)

[α]-Mxhum substituted due to stronger correlation coefficient

Table 2.6. Correlation coefficients of mosquito abundance and weather variables for the farm mosquito species (2010-2011). Numbers in parentheses represent lag days.

Weather Variable	*Aedes vexans*	*Culex tarsalis*	*Aedes dorsalis*	*Culiseta inornata*
Rain	0.5172 (9-27)	-0.4124 (2-3)	0.4139 (1-24)	-0.4969 (9-30)
Mntemp	0.4983 (6-6)	0.7584[€] (6-6)	-0.3797 (4-5)	-0.4466 (1-2)
Relhum	0.3826 (1-7)	0.6122 (1-30)	-0.1947 (12-28)	-0.2621 (2-3)
Wind	0.3231 (21-21)	-0.6376 (16-18)	0.4663 (1-2)	-0.4843 (28-28)
Dew	0.4140 (6-7)	0.7408 (6-7)	-0.2448 (4-4)	-0.4134 (2-2)
Avghum	0.3521[α] (1-7)	0.5484 (4-30)	-0.2352[β] (28-28)	-0.4148 (2-2)
IDD40	0.1602 (1-1)	0.5680 (1-1)	-0.1049 (1-1)	0.1864 (30-30)
Photo	0.4862 (24-24)	0.7090 (30-30)	-0.1560 (29-29)	-0.4843 (23-23)

[α]-Mxhum substituted due to stronger correlation coefficient

[β]-Mnhum substituted due to stronger correlation coefficient

[€]-Tstemp substituted due to stronger correlation coefficient

Table 2.7. Correlation coefficients of mosquito abundance and weather variables for Grand Forks mosquito (2002-2010). Numbers in parentheses represent lag days.

Weather Variable	June	July	August	September
Rain	0.5742 (15-30)	0.5200 (1-30)	-0.1248 (25-25)	0.6251 (7-26)
Mntemp	0.5780 (1-30)	0.4336 (1-12)	-0.2369[¥] (5-6)	0.5815 (1-10)
Wind	-0.3787 (5-13)	0.4224 (11-30)	0.4442 (6-16)	-0.1778 (4-4)
Dew	0.6001 (1-27)	0.2664 (1-4)	-0.2387 (5-12)	0.6127 (1-17)
Avghum	0.5040 (10-17)	0.3385[β] (1-23)	-0.3040[α] (6-15)	0.5485[α] (8-15)
IDD40	0.4661 (1-1)	-0.2651 (30-30)	0.0755 (15-15)	-0.4609 (19-19)
Photo	0.3126 (17-17)	0.1844 (2-2)	-0.0663 (28-29)	0.3709 (29-29)

[α]-Mxhum substituted due to stronger correlation coefficient

[β]-Mnhum substituted due to stronger correlation coefficient

[¥]-Mxtemp substituted due to stronger correlation coefficient

References

Barr, A. R. 1958. The mosquitoes of Minnesota (Diptera: Culicidae:Culicinae). Technical. Bulletin 228. University of Minnesota Agricultural Experiment Station.

Bennington, E. E., C. A. Sooter, H. Baer. 1958. The diapause in adult female *Culex tarsalis* Coquillett (Diptera: Culicidae). Mosquito News. 18(4):299-304

Bidlingmayer, W. L. 1974. The influence of environmental factors and physiological stage on flight patterns of mosquitoes taken in the vehicle aspirator and truck, suction, bait and New Jersey light traps. J. Med. Entomol. 11(2):119-46

Carde, R. T., M. A. Willis. 2008. Navigational strategies used by insects to find distant, wind-borne sources of odor. J. Chem. Ecol. 34:854-866

Center for Disease Control. 2012. West Nile Virus. http://www.cdc.gov/westnile/index.html

Chuang, T. W., E. L. Ionides, R. G. Knepper, W. W. Stanuszek, E. D. Walker, M. L. Wilson. 2012. Cross-correlation map analyses show weather variation influences on mosquito abundance patterns in Saginaw County, Michigan, 1989-2005. J. Med. Entomol. 49(4):851-858

Curriero, F. C., S. M. Shone, G. E. Glass. 2005. Cross correlation maps: a tool for visualizing and modeling time lagged associations. Vector Borne Zoonotic Dis. 5:267-275

Darsie Jr., R. F., A. W. Anderson. 1985. A revised list of the mosquitoes of North Dakota, including new additions to the fauna. J. Am. Mosq. Control Assoc. 1(1):76-9

Darsie Jr., R. F., R. A. Ward. 2005. Identification and geographical distribution of the mosquitoes of North America, north of Mexico. University Press of Florida, Gainesville, Florida

Degroote, J. P., R. Sugumaran, S. M. Brend, B. J. Tucker, L. C. Bartholomay. 2008. Research landscape, demographic, entomological and climatic associations with human disease incidence of West Nile virus in the state of Iowa, USA. International J. of Health Geograph. 7:19 doi:10.1186/1476-072X-7-19

Dickerson, A. K., P. G. Shankles, N. M. Madhavan, D. L. Hu. 2012. Mosquitoes survive raindrop collisions by virtue of their low mass. Proc. Natl. Acad. Sci. USA. 109(25): 9822-9827

Gardner, A. M., G. L. Hamer, A. M. Hines, C. M. Newman, E. D. Walker. M. O. Ruiz. 2012. Weather variability affects abundance of larval *Culex* (Diptera: Culicidae) in storm water catch basins in suburban Chicago. J. Med. Entomol. 49(2):270-76

Gjullin, C. M., R. I. Sailer, A. Stone, B. V. Travis. 1961. The mosquitoes of Alaska. Agricultural Research Service. United States Department of Agriculture. No. 182: 98

Grand Forks Public Health Department. 2012. Grand Forks Mosquito Control. www.gfmosquito.com

Grimstad, P. R., G. R. Defoliart. 1974. Mosquito nectar feeding in Wisconsin in relation to twilight and micro-climate. J. Med. Ent. 11:691-698

Gubler, D. J., P. Reiter, K. Ebi, W. Yap, R. Nasci, J. Patz. 2001. Climate variability and change in the United States: potential impacts on vector- and rodent-borne diseases. Environ. Health Perspecti. 109:223-33

Harwood, E. F., J. E. Halfhill. 1960. Mammalian burrows and vegetation as summer resting sites of the mosquitoes *Culex tarsalis* and *Anopheles freeborni*. Mosquito News. 20(2): 174-178

Harwood, R. F., N. Takata. 1965. Effect of photoperiod and temperature on fatty acid composition of the mosquito *Culex tarsalis*. J. of Insect Physiol. 11(6):711-716

Haufe, W. O. 1966. Synoptic correlation of weather with mosquito activity. Biometeorology, Part II. S. W. Tromp and W. H. Weihe (ed.) Pergamon Press, Oxford, 523-540

Kelly-Hope, L. A., D. M. Purdie, B. H. Kay. 2004. Ross River virus disease in Australia 1886-1998, with analysis of risk factors associated with outbreaks. J. Med. Entomol. 41: 133-50

Lebl, K. K. Brugger, F. Rubel. 2013. Predicting *Culex pipiens/restuans* population dynamics by interval lagged weather data. Parasit. Vectors. 6(1):129 Epub

Martin, T. G., B. A. Wintle, J. R. Rhodes, P. M. Kuhnert, S. A. Field, S. J. Low-Choy, A. J. Tyre, H. P. Possingham. 2005. Zero-tolerance ecology: improving ecological inference by modeling the source of zero observations. Ecology Letters. 8: 1235-1246

Mitchell, C. J. 1981. Diapause termination, gonoactivity and differentiation of host-seeking behavior from blood-feeding behavior in hibernating *Culex tarsalis* (Diptera: Culicidae). J. Med. Entomol. 18:386-394

Murty, U. S., M. S. Rao, N. Arunachalam. 2010. The effects of climatic factors on the distribution and abundance of Japanese encephalitis vectors in Kurnool district of Andhra Pradesh, India. J. Vector Borne Dis. 47: 26-32

North Dakota Agricultural Weather Network Center. 2012. NDAWN Center. Ndawn.ndsu.nodak.edu

Rademacher, R. E. 1979. Studies of overwintering larvae of *Coquillettidia perturbans* mosquitoes in Minnesota. Mosquito News. 39(1):135-136

Reisen, W. K., H. D. Lothrop, B. Lothrop. 2003. Factors influencing the outcome of mark-release-recapture studies with *Culex tarsalis* (Diptera: Culicidae). J. Med. Entomol. 40(6):820-829

Rowley, W. A., C. L. Graham. 1968. The effect of temperature and relative humidity on the flight performance of female *Aedes aegypti*. J. Insect Physiol. 14: 1251-57

Shone, S. M., F. C. Curriero, C. R. Lesser, G. E. Glass. 2006. Characterizing population dynamics of *Aedes sollicitans* (Diptera: Culicidae) using meteorological data. J. Med. Entomol. 43: 393-402

Soverow, J. E., G. A. Wellenius, D. N. Fisman, M. A. Mittleman. 2009. Infectious disease in a warming world: how weather influenced West Nile virus in the United States (2001-2005). Environ. Health Perspect. 117(7): 1049-52

Spitzen, J. C. W. Spoor, G. Grieco, C. ter Braak, J. Beeuwkes. 2013. A 3D analysis of flight behavior of *Anopheles gambiae* sensu strict malaria mosquitoes in response to human odor and heat. PLoS ONE 8(5):e62995. Doi:10.1371/journal.pone.006295

Muirhead Thomson, R. C. 1938. The reactions of mosquitoes to temperature and humidity. Bulletin of Entomol. Research. 29(2): 125-140

Trawinski, P. R., D. S. Mackay. 2008. Meteorologically conditioned time-series predictions of West Nile virus vector mosquitoes. Vector Borne Zoonotic Dis. 8: 505-521

Turell, M. J., D. J. Dohm, M. R. Sardelis, M. L. Oguinn, T. G. Andreadis, J. A. Blow. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. J. Med. Entomol. 42:57-62

Trpis, M., J. A. Shemanchuk. 1970. Effect of constant temperature on larval development of *Aedes vexans* (Diptera: Culicidae). The Canadian Entomologist. 102(8): 1048-51

United States Census Bureau. 2010. Steele County, North Dakota. http://factfinder2.census.gov/faces/nav/jsf/pages/searchresults.xhtml

Wang, J., N. H. Ogden, H. Zhu. 2011. The impact of weather conditions of *Culex pipiens* and *Culex restuans* (Diptera: Culicidae) abundance: a case study in Peel Region. J. Med. Entomol. 48:468-475

Woodruff, R. E., C. S. Guest, M. G. Garner, N. Becker, J. Lindesay, T. Carvan, K. Ebi. 2002. Predicting Ross River virus epidemics from regional weather data. Epidemiology. 13: 384-93

Wu, P. C., H. R. Guo, S. C. Lung, C. Y. Lin, H. J. Su. 2007. Weather as an effective predictor for occurrence of dengue fever in Taiwan. Acta Tropica. 103: 50-7

APPENDIX A

Forest Mosquito Daily Trap Count Regressions

Zero-inflated negative binomial regression model for *Ae. excrucians* from the forest site (AIC=339.12).

Model Components	Estimate	Std. Error	z value	Pr(>|z|)
photo	0.0102	0.0032	3.220	0.0013 **
Year	0.0350	0.0014	24.869	< 0.0001 ***
Log(theta)	-0.1944	0.1910	-1.018	0.30873

Photo= photoperiod (hours of daylight)
Year = year to year variability

Reduced zero-inflated negative binomial regression model for *Ae. vexans* from the Forest site. (AIC=259.76)

Model Components	Estimate	Std. Error	z value	Pr(>|z|)
bstemp	-0.1555	0.0628	-2.476	0.0132 *
IDD40	0.0045	0.0011	4.018	<0.0001 ***
mntemp	0.0835	0.0230	3.632	0.0002 ***
mxhum	-0.0913	0.0426	-2.143	0.0321 *
photo	0.0343	0.0099	3.466	0.0005 ***
wind	-0.2043	0.0702	-2.911	0.0036 **
Year	0.3072	0.0055	56.344	<0.0001 ***
Log(theta)	1.1490	0.1880	6.112	9.86e-10 ***

Bstemp= bare soil temperature
IDD40= insect degree days at 40ºF
Mntemp=minimum temperature
Mxhum= maximum humidity
Photo= photoperiod (hours of daylight)
Wind= wind speed
Year= year to year variability

Reduced negative binomial model model for Forest species *Ae. triseriatus* (AIC=215.94)

Model Components	Estimate	Std. Error	z value	Pr(>|z|)
bstemp	-0.2378	0.0643	-3.698	0.0002 ***
IDD40	0.0076	0.0011	7.195	<0.0001 ***
photo	0.0377	0.0083	4.518	<0.0001 ***
relhum	-0.0773	0.0306	-2.530	0.0114 *

Bstemp= bare soil temperature
IDD40= insect degree days at 40ºF
Photo= photoperiod (hours of daylight)
Relhum= relative humidity

Zero-inflated negative binomial regression model for *Co. perturbans* from the Forest site. (AIC=220.43)

Model Components	Estimate	Std. Error	z value	Pr(>|z|)
bstemp	-0.4093	0.0778	-5.260	<0.0001 ***
IDD40	-0.0021	0.0005	-4.161	<0.0001 ***
rain	-5.7930	2.0490	-2.827	0.0047 **
relhum	-0.0826	0.0298	-2.775	0.0055 **
tstemp	5.6960	0.0738	7.715	<0.0001 ***
wind	-0.4010	0.0860	-4.667	<0.0001 ***
Year	-0.6852	0.0223	-3074.540	<0.0001 ***
avghum	0.0663	0.0271	2.446	0.0145 *
Log(theta)	0.9809	0.1580	6.208	5.38e-10 ***

Avghum= average humidity
Bstemp= bare soil temperature
IDD40= insect degree days at 40ºF
Rain = precipitation
Relhum= relative humidity
Tstemp= turf soil temperature
Wind= wind speed
Year= year to year variability

APPENDIX B

Farm Mosquito Daily Trap Count Regressions

Negative binomial regression model for Farm population of *Ae. vexans*. (AIC=517.04)

Model Components	Estimate	Std. Error	z value	Pr(>|z|)
bstemp	-0.3092	0.0690	-4.480	<0.0001 ***
IDD40	0.0028	0.0007	3.965	<0.0001 ***
mnhum	-0.1307	0.0243	-5.370	<0.0001 ***
mntemp	0.2686	0.0532	5.052	<0.0001 ***
mxhum	0.5120	0.1069	4.790	<0.0001 ***
photo	0.0282	0.0066	4.298	<0.0001 ***
rain	-3.7021	1.5732	-2.353	0.0186 *
relhum	-0.1873	0.0625	-2.996	0.0027 **

Bstemp= bare soil temperature
IDD40= insect degree days at 40ºF
Mnhum= minimum humidity
Mntemp=minimum temperature
Mxhum= maximum humidity
Photo= photoperiod (hours of daylight)
Rain = precipitation
Relhum= relative humidity

Zero-inflated negative binomial model for *Cx. tarsalis* population from the Farm site. (AIC=359.92)

Model Components	Estimate	Std. Error	z value	Pr(>|z|)
rain	-6.3070	1.7439	-3.617	0.0003 ***
tstemp	0.4231	0.0840	5.040	<0.0001 ***
avghum	0.1874	0.0445	4.211	<0.0001 ***
dew	-0.2644	0.0843	-3.137	0.0017 **
mxtemp	0.2242	0.0680	3.297	0.0009 ***
Log(theta)	-0.3719	0.2269	-1.639	0.1012

Avghum= average humidity
Dew= dew point
Mxtemp= maximum temperature
Rain = precipitation
Tstemp= turf soil temperature

Reduced negative binomial regression model for *Ae. dorsalis* from the Farm site. (AIC=270.58)

Model Components	Estimate	Std. Error	z value	Pr(>|z|)
avghum	-0.1085	0.0317	-3.423	0.0006 ***
bstemp	-0.2055	0.0510	-4.031	<0.0001 ***
IDD40	0.0007	0.0003	2.923	0.0035 **
mntemp	0.1331	0.0435	3.062	0.0022 **
mxhum	0.0866	0.0462	1.873	0.0611 .
rain	-2.2712	1.2912	-1.759	0.0786 .

Avghum= average humidity
Bstemp= bare soil temperature
IDD40= insect degree days at 40ºF
Mntemp=minimum temperature
Mxhum= maximum humidity
Rain = precipitation

Reduced negative binomial model for *Cs. inornata* from the Farm site. (AIC=259.17)

Model Components	Estimate	Std. Error	z value	Pr(>|z|)
IDD40	-0.0010	0.0004	-2.776	0.0055 **
photo	-0.0140	0.0040	-3.491	0.0005 ***
rain	-2.2010	0.9777	-2.251	0.0244 *
Year	-2.3340	0.4426	-5.480	<0.0001 ***

IDD40= insect degree days at 40ºF
Photo= photoperiod (hours of daylight)
Rain = precipitation
Year= year to year variability

APPENDIX C

Grand Forks Mosquito Daily Trap Count Regression

Reduced negative binomial regression model for Grand Forks "Skeeter Meter"data for the years 2002-2010. (AIC=6590.80)

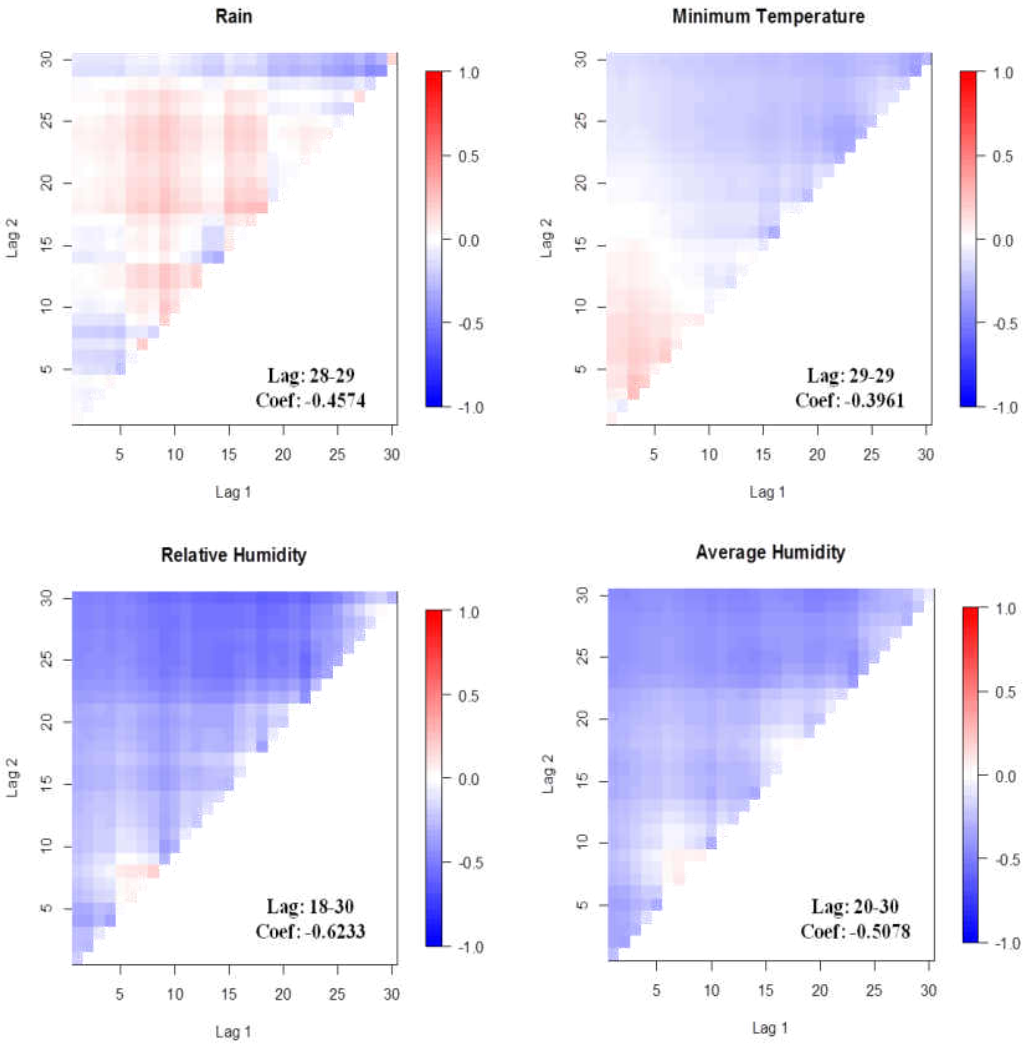
Model Components	Estimate	Std. Error	z value	Pr(>|z|)
avgtemp	0.0286	0.0134	2.139	0.0324 *
mntemp	0.0386	0.0131	2.939	0.0033 **

Avgtemp= average temperature
Mntemp=minimum temperature

APPENDIX D

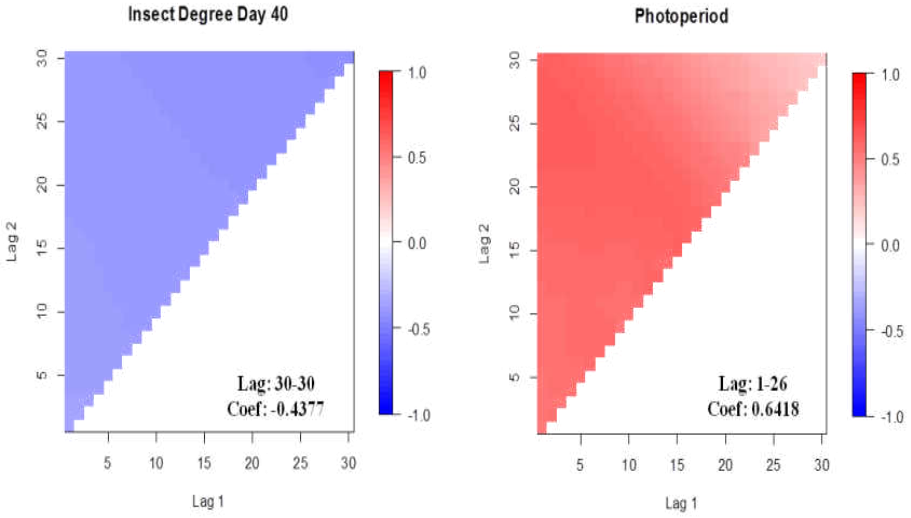
Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

Forest *Ae. excrucians*



Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

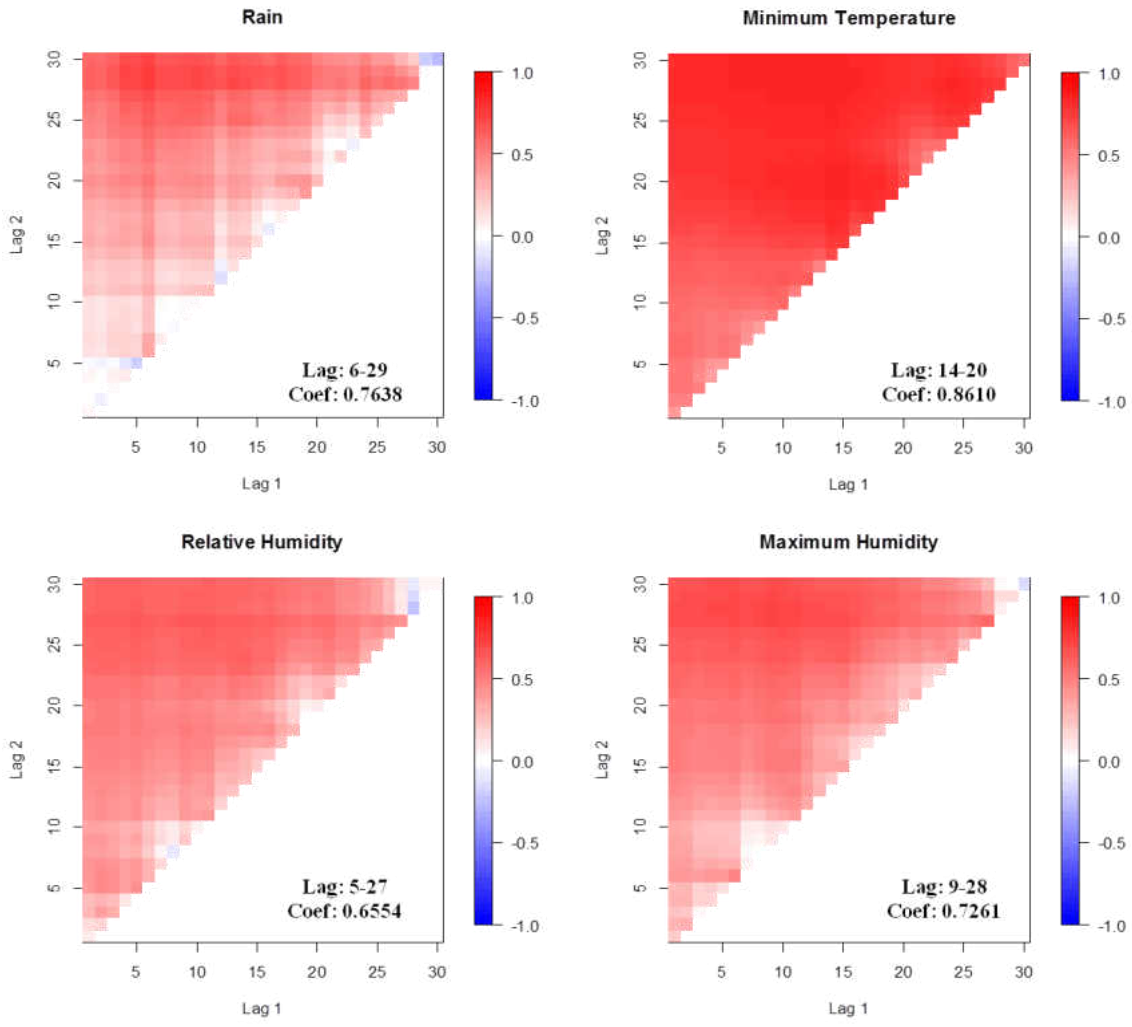
Forest *Ae. excrucians*



APPENDIX E

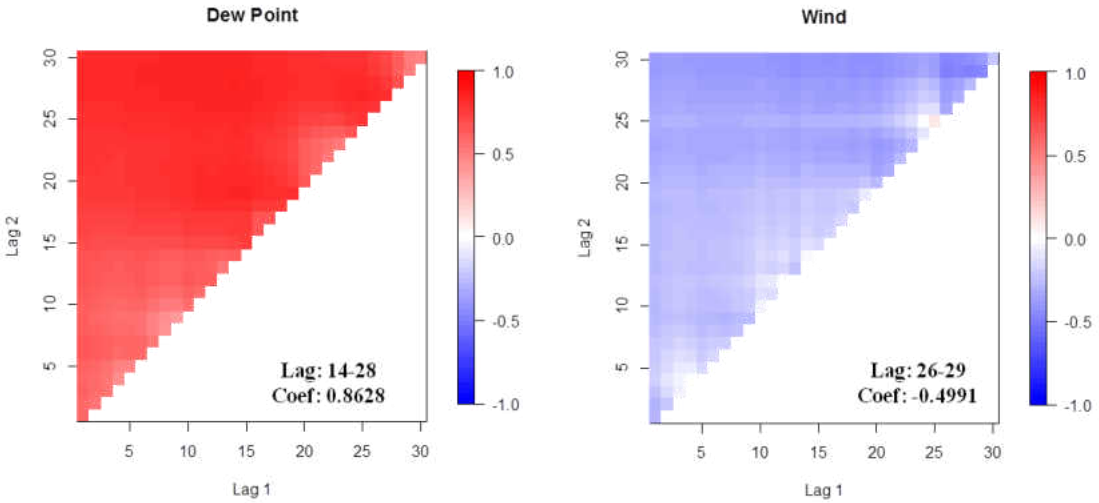
Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

Forest *Ae. vexans*



Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

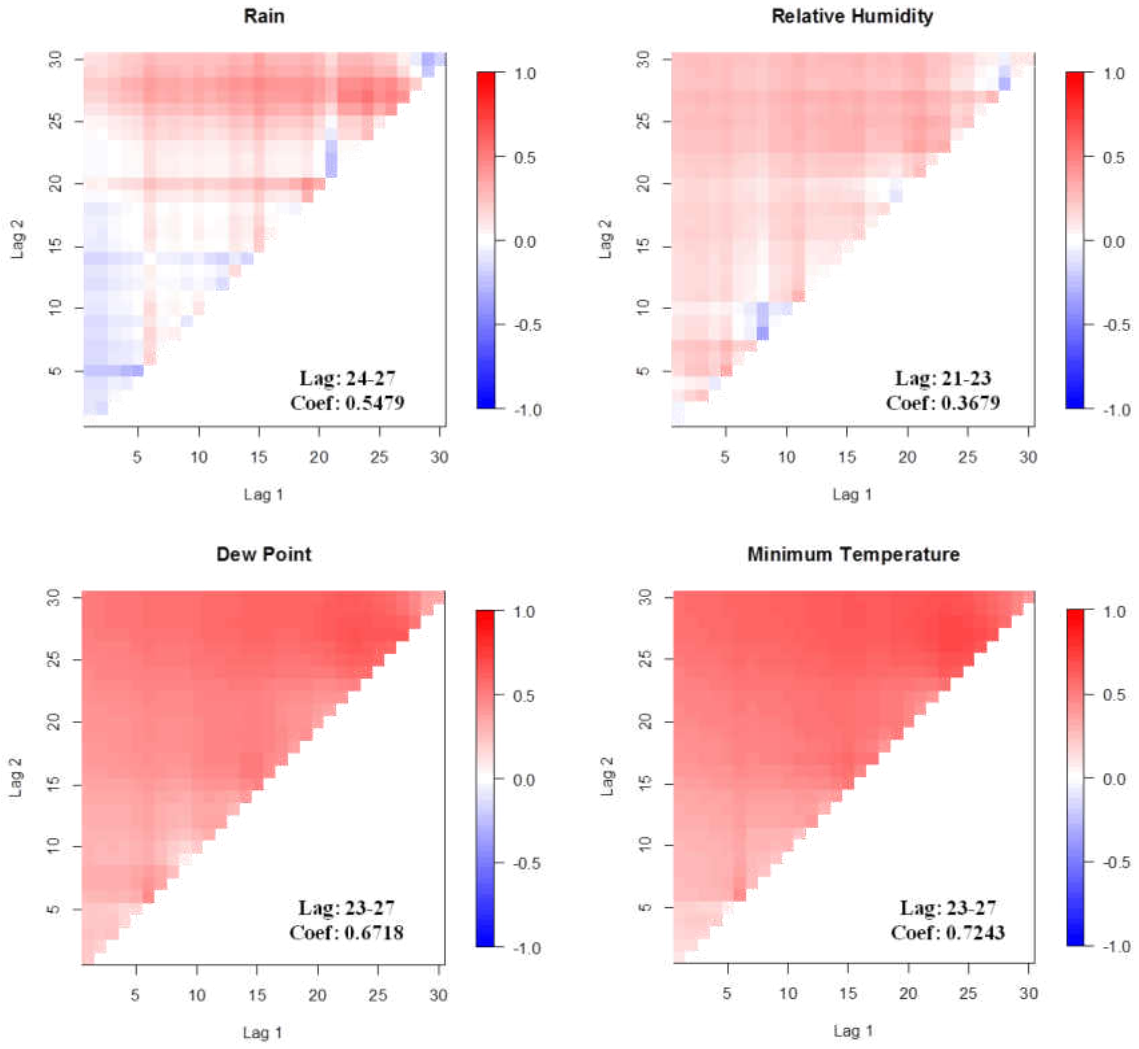
Forest *Ae. vexans*



APPENDIX F

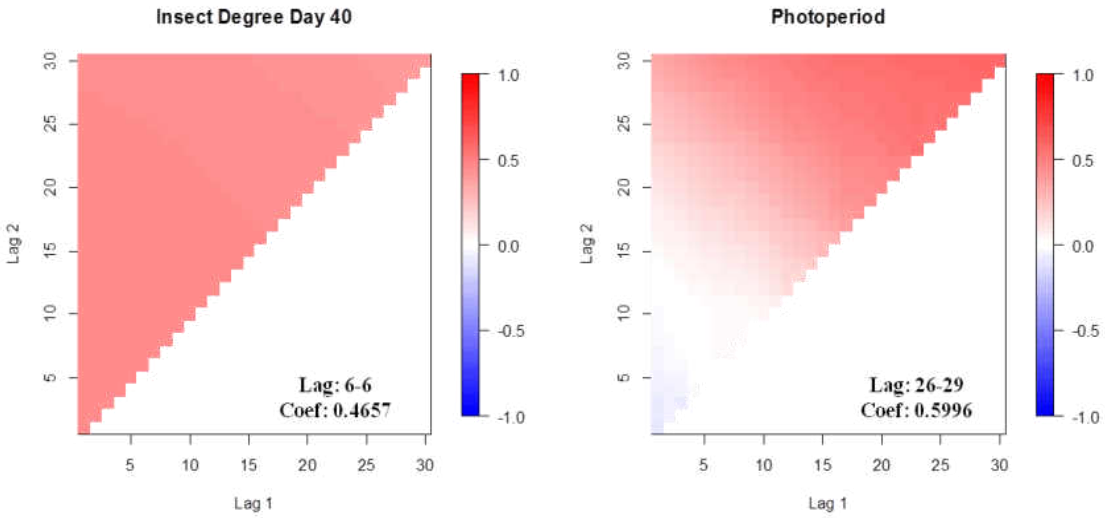
Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

Forest *Ae. triseriatus*



Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

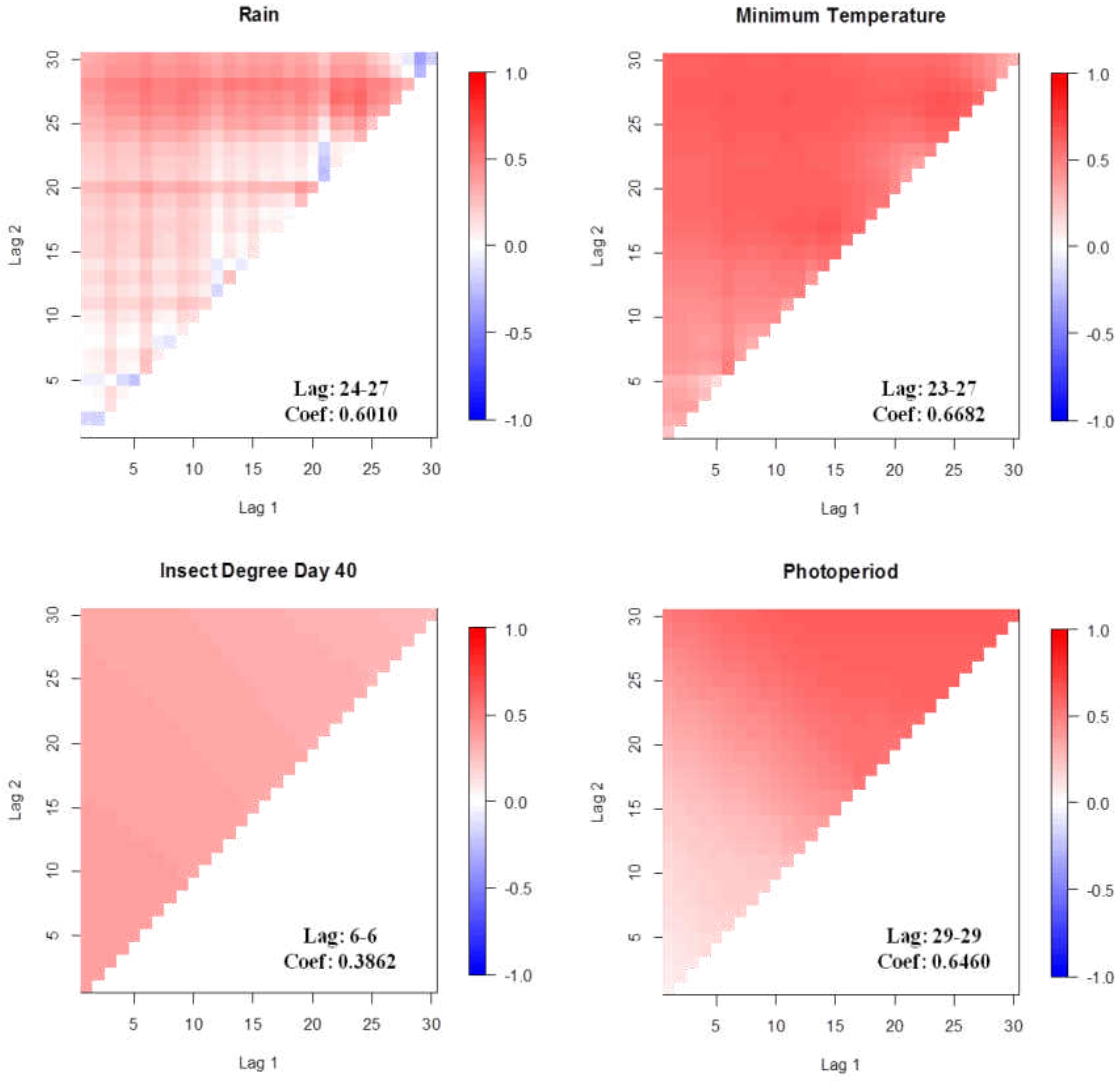
Forest *Ae. triseriatus*



APPENDIX G

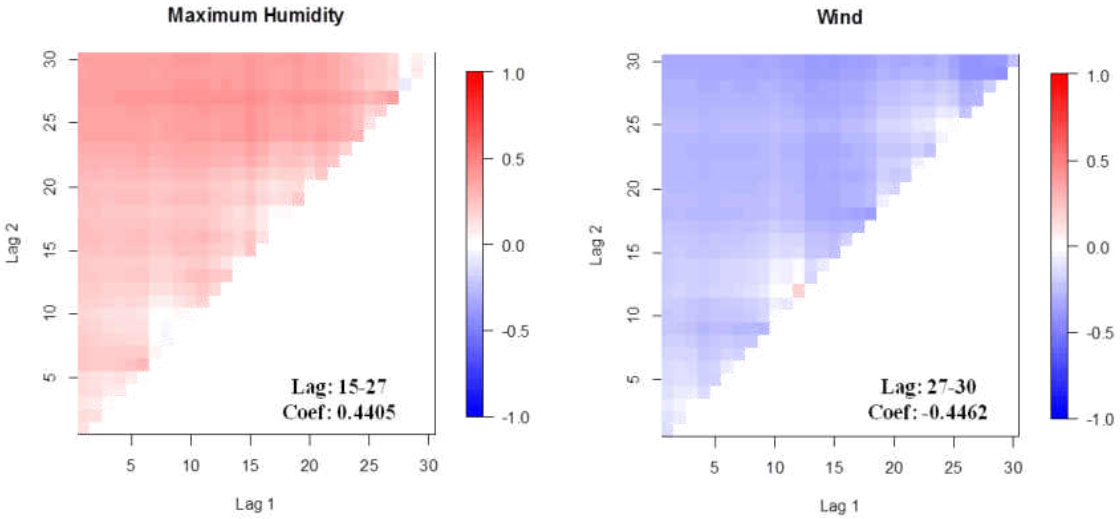
Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

Forest *Co. perturbans*



Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

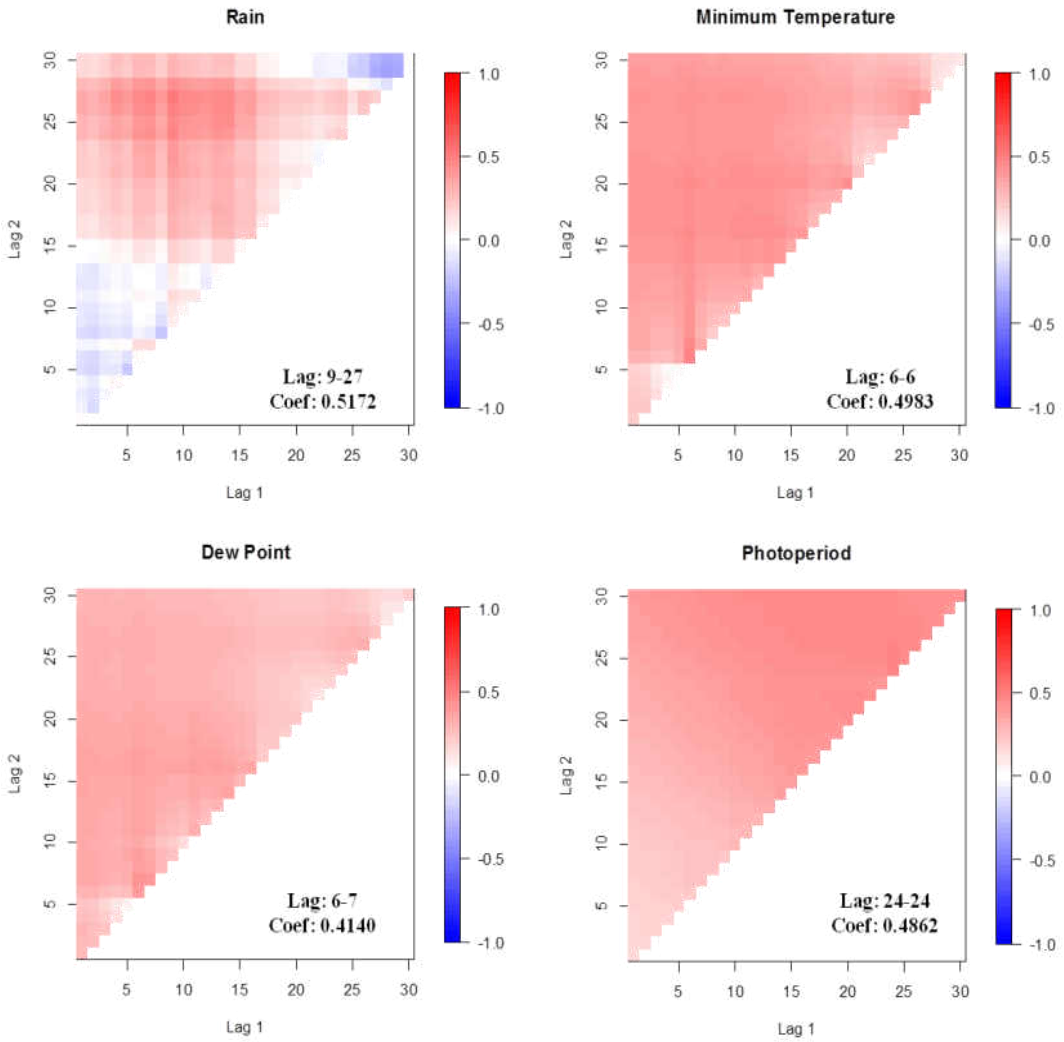
Forest *Co. perturbans*



APPENDIX H

Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

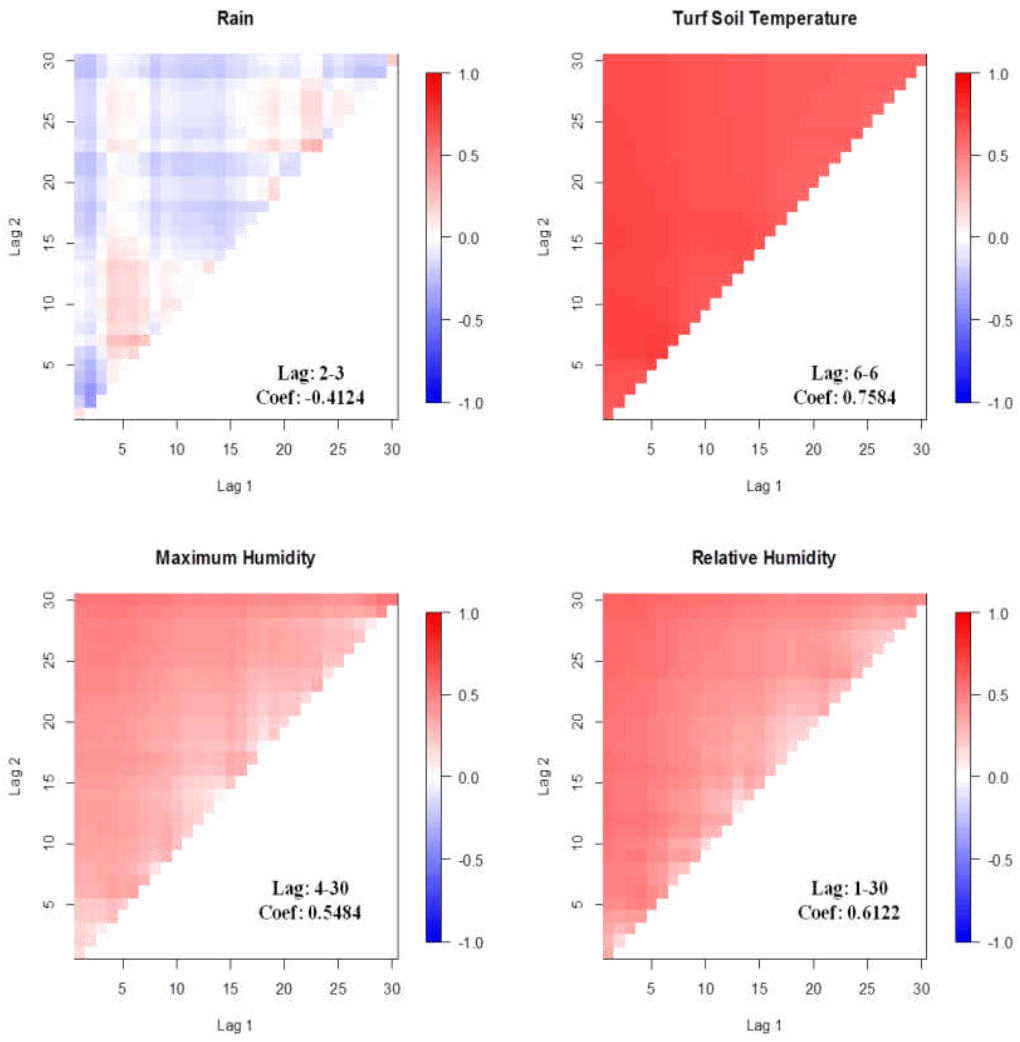
Farm *Ae. vexans*



APPENDIX I

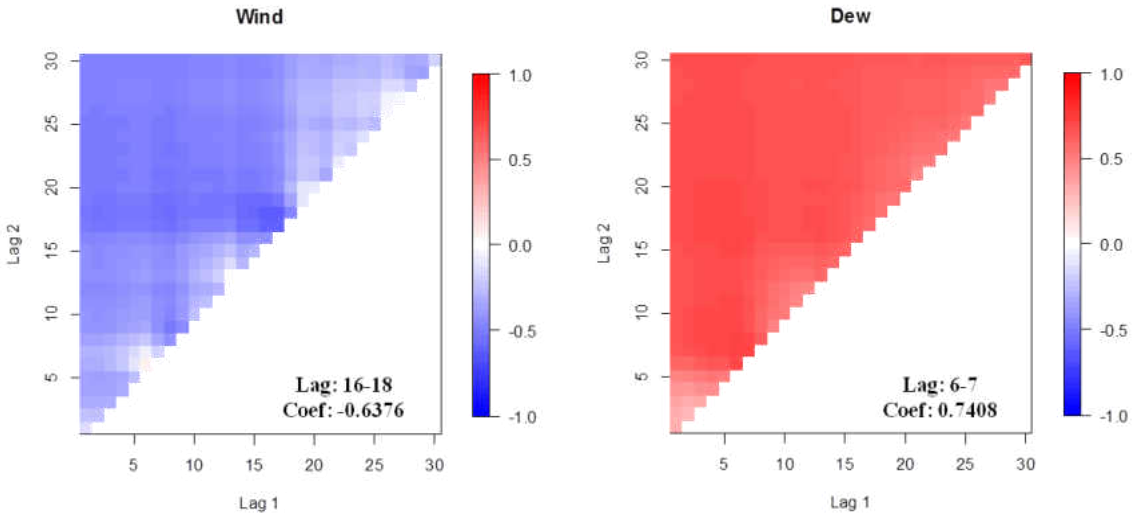
Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

Farm *Cx. tarsalis*



Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

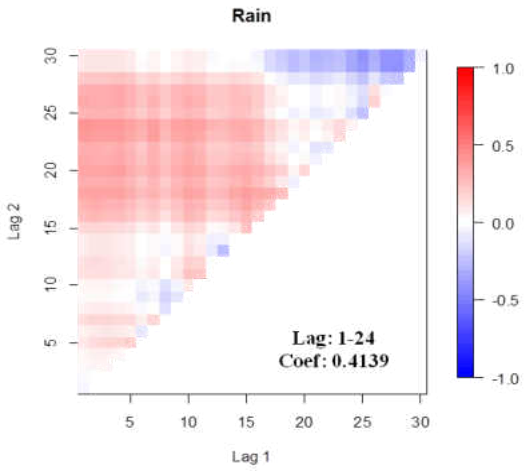
Farm *Cx. tarsalis*

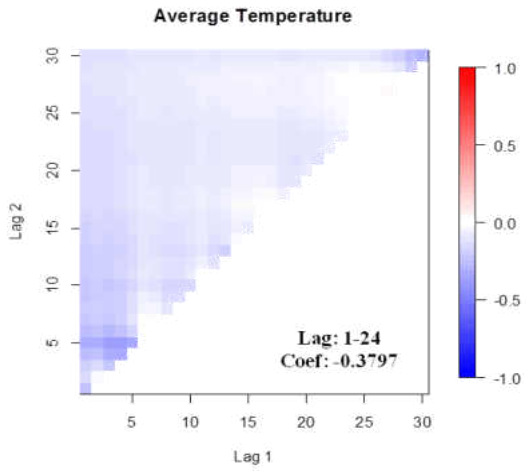


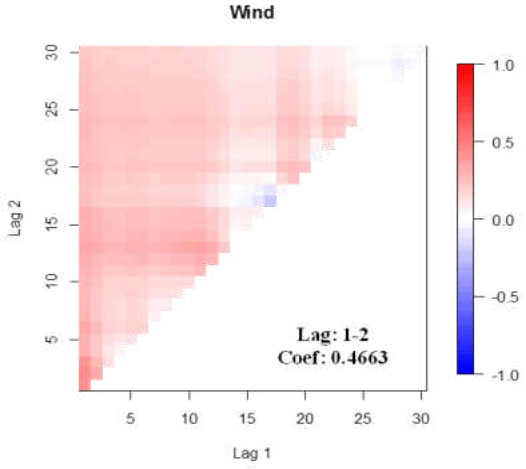
APPENDIX J

Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

Farm *Ae. dorsalis*



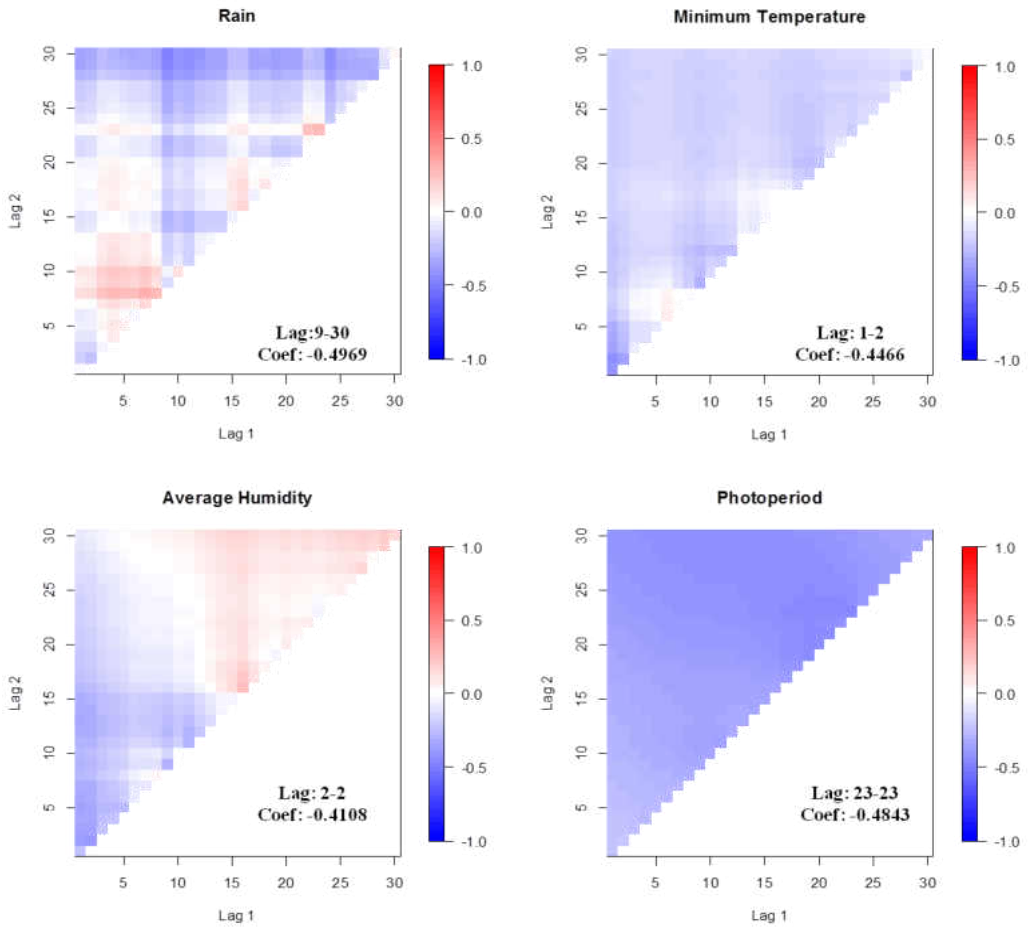




APPENDIX K

Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

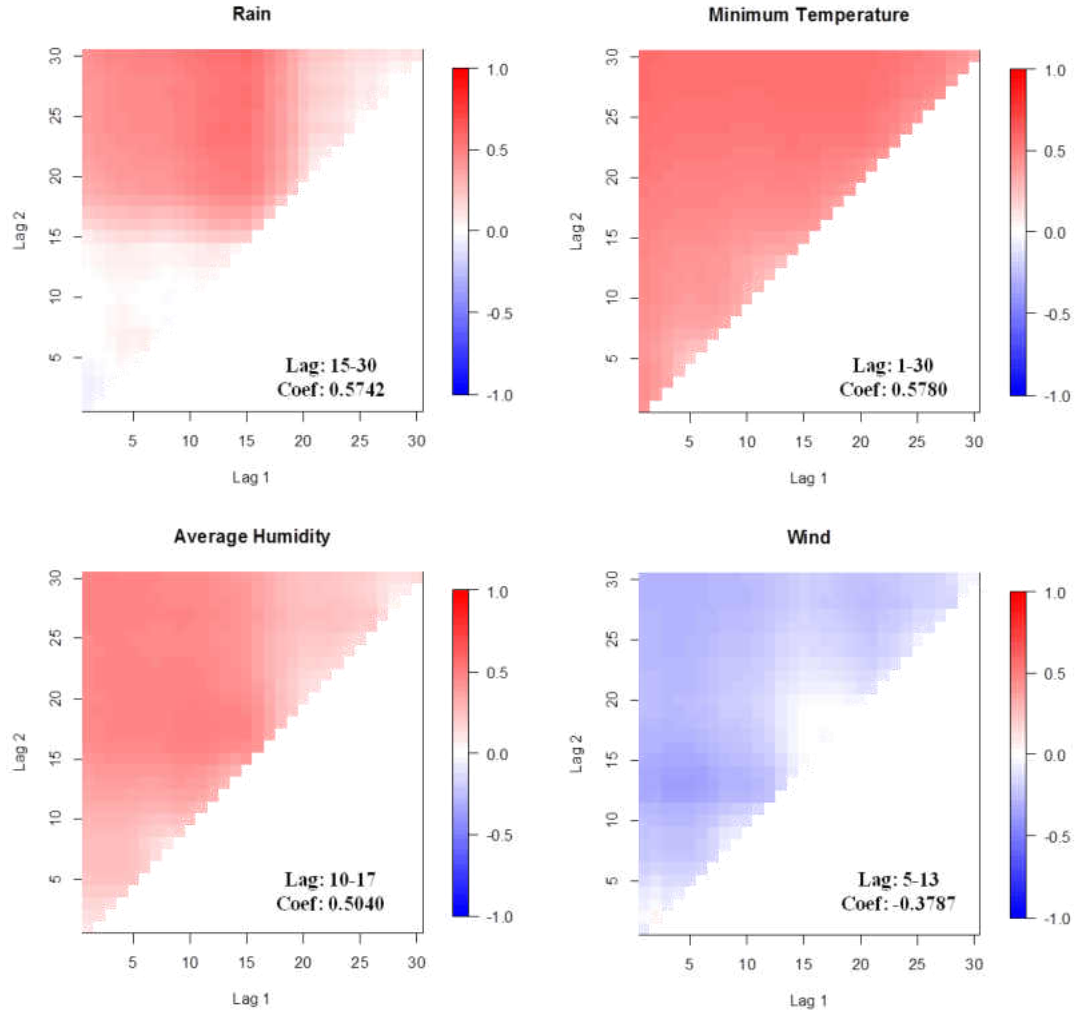
Farm *Cs. inornata*



APPENDIX L

Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

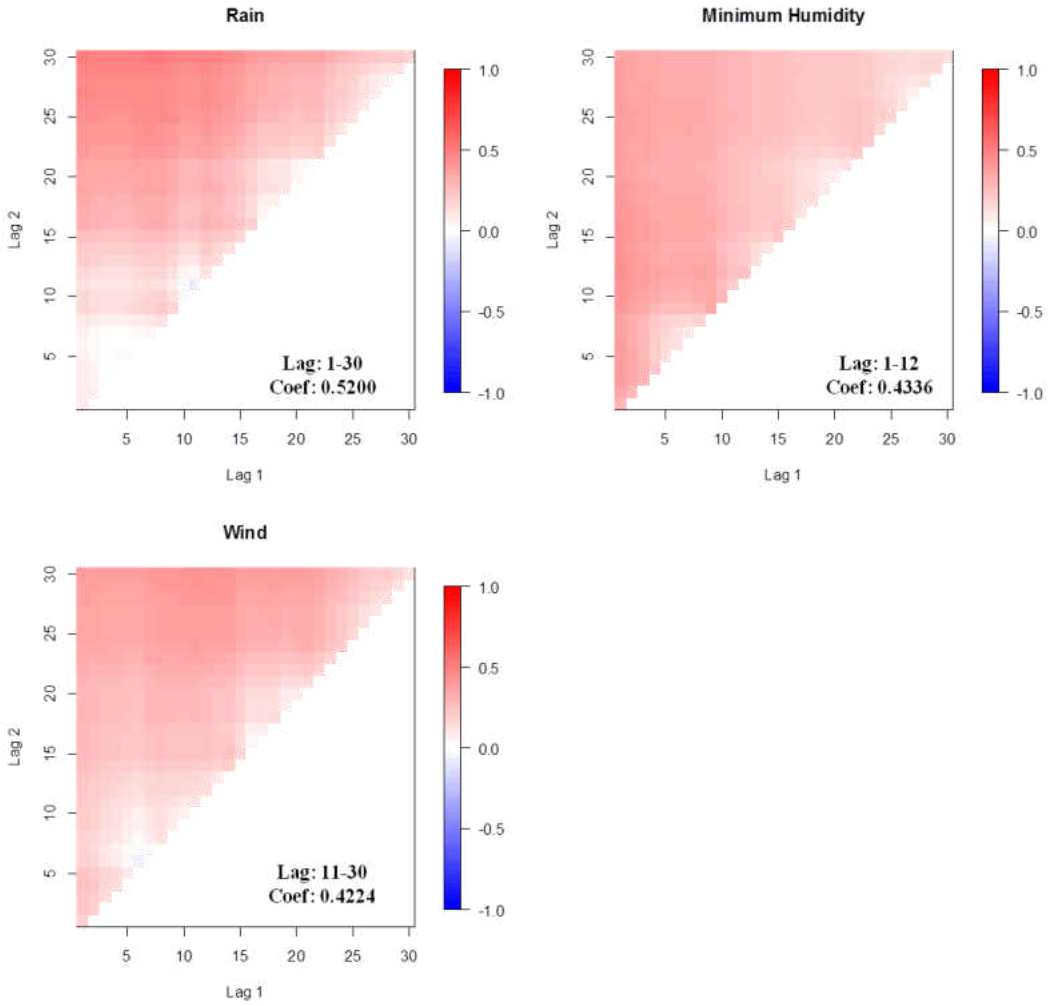
Grand Forks June



APPENDIX M

Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

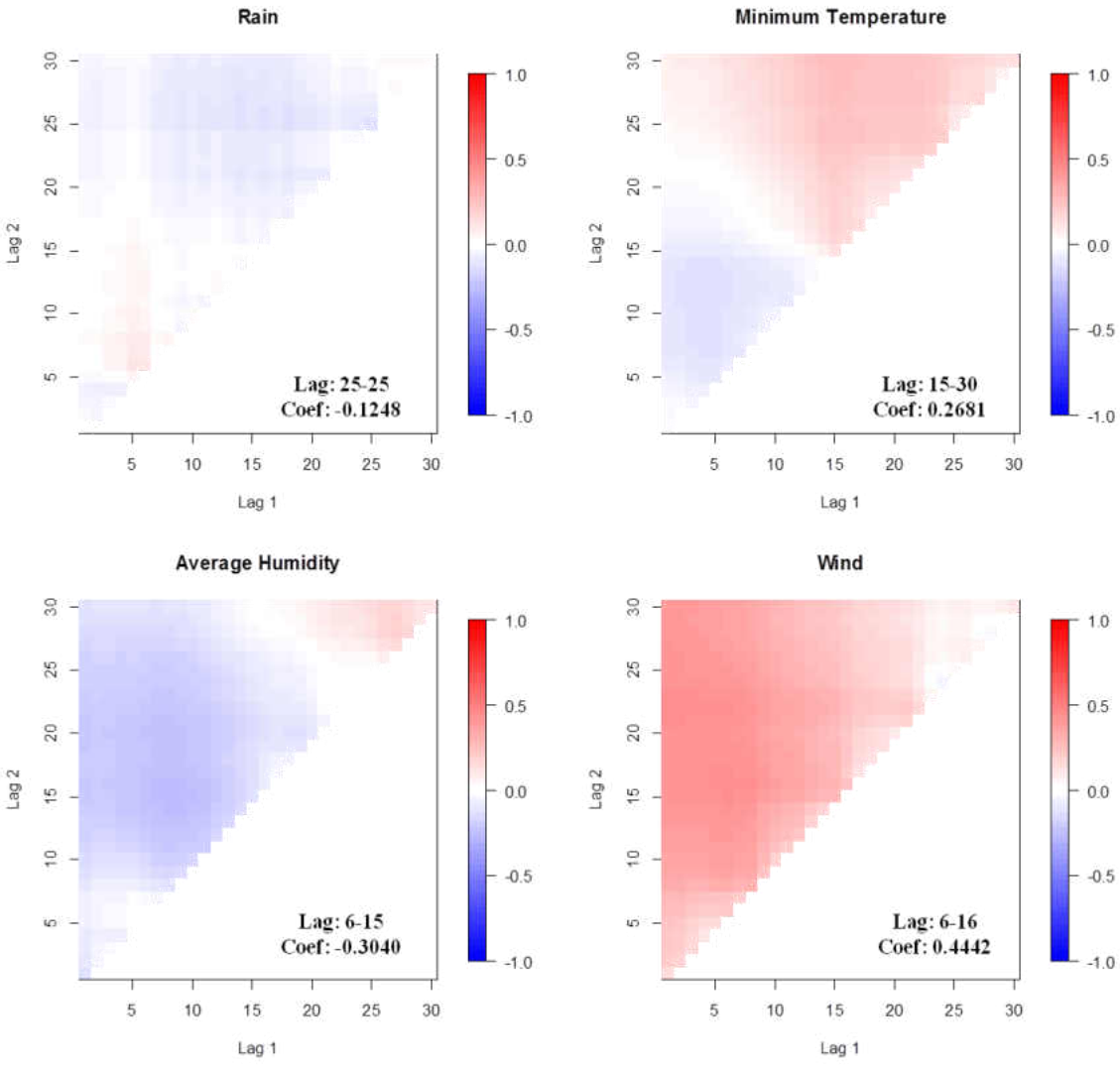
Grand Forks July



APPENDIX N

Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

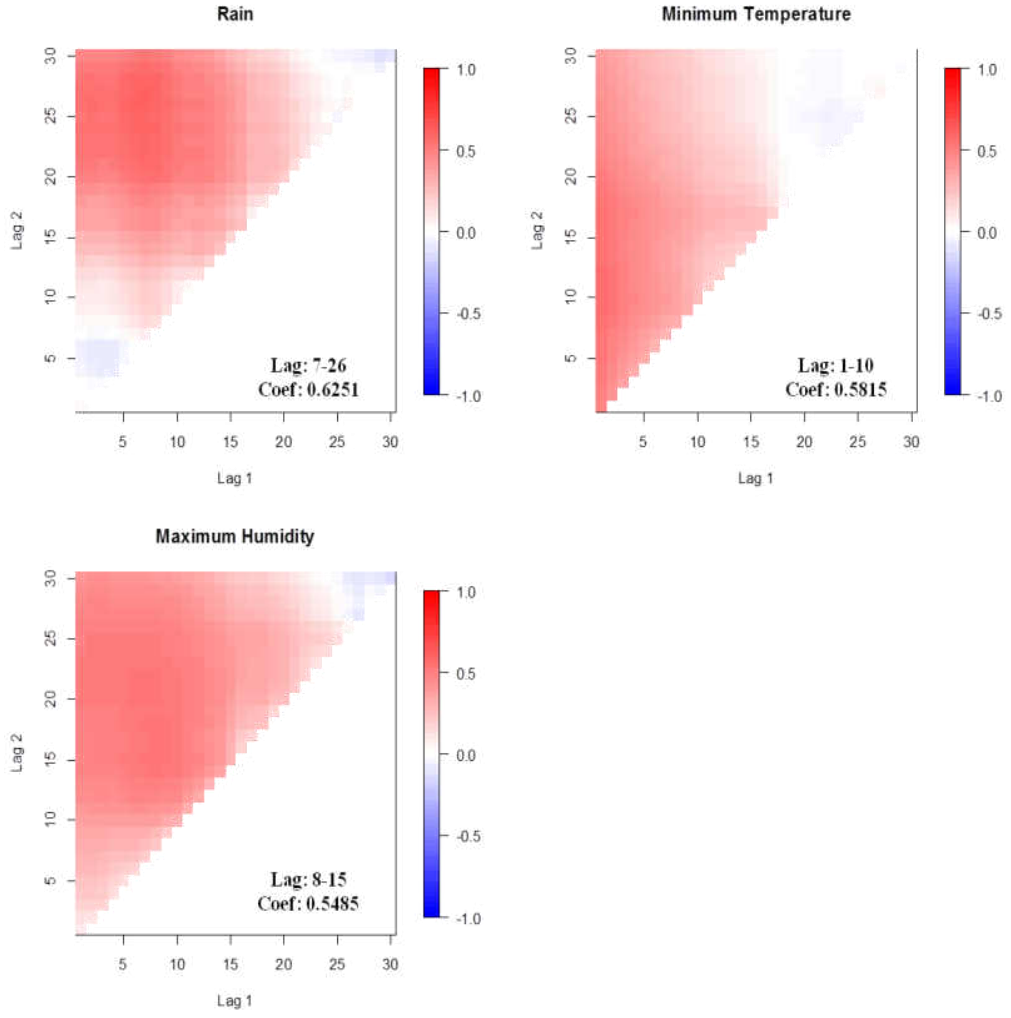
Grand Forks August



APPENDIX O

Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

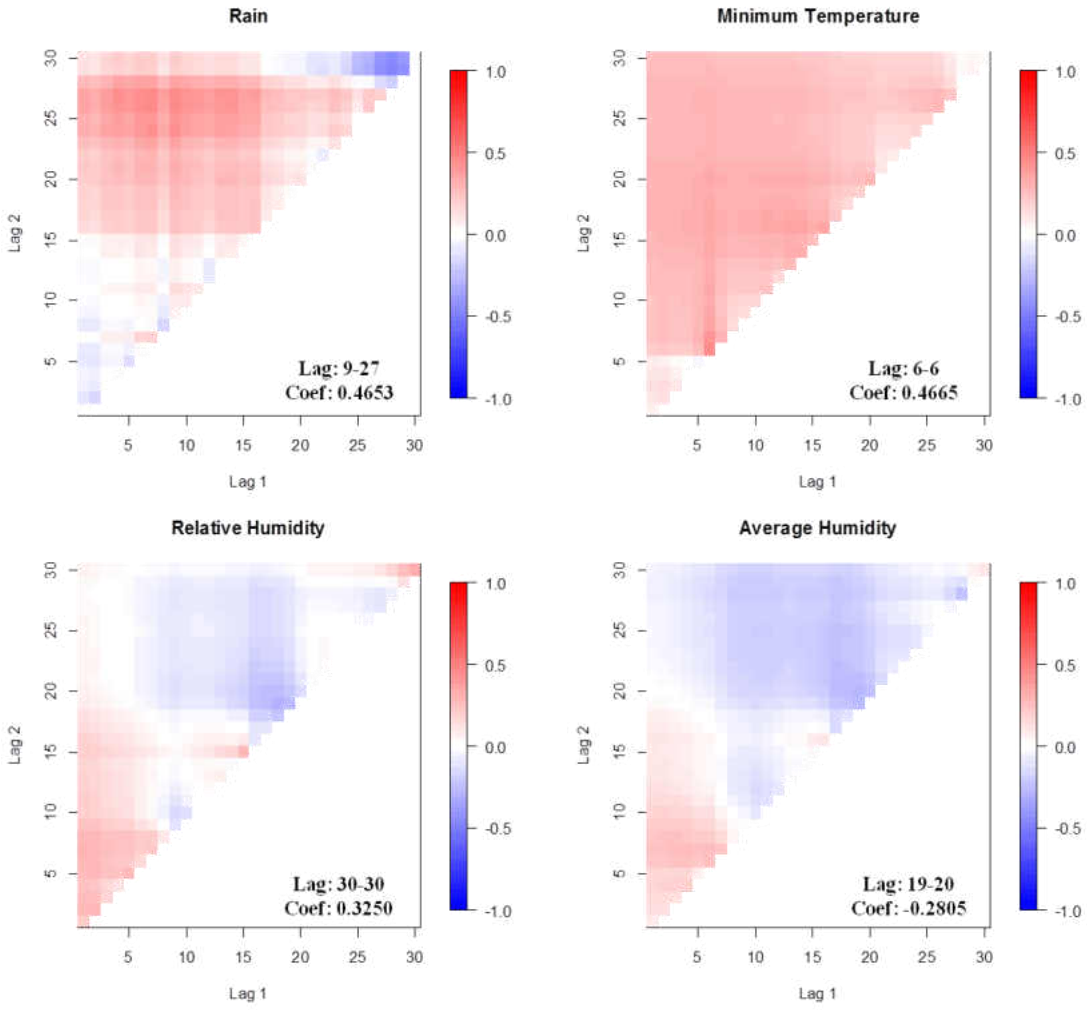
Grand Forks September



APPENDIX P

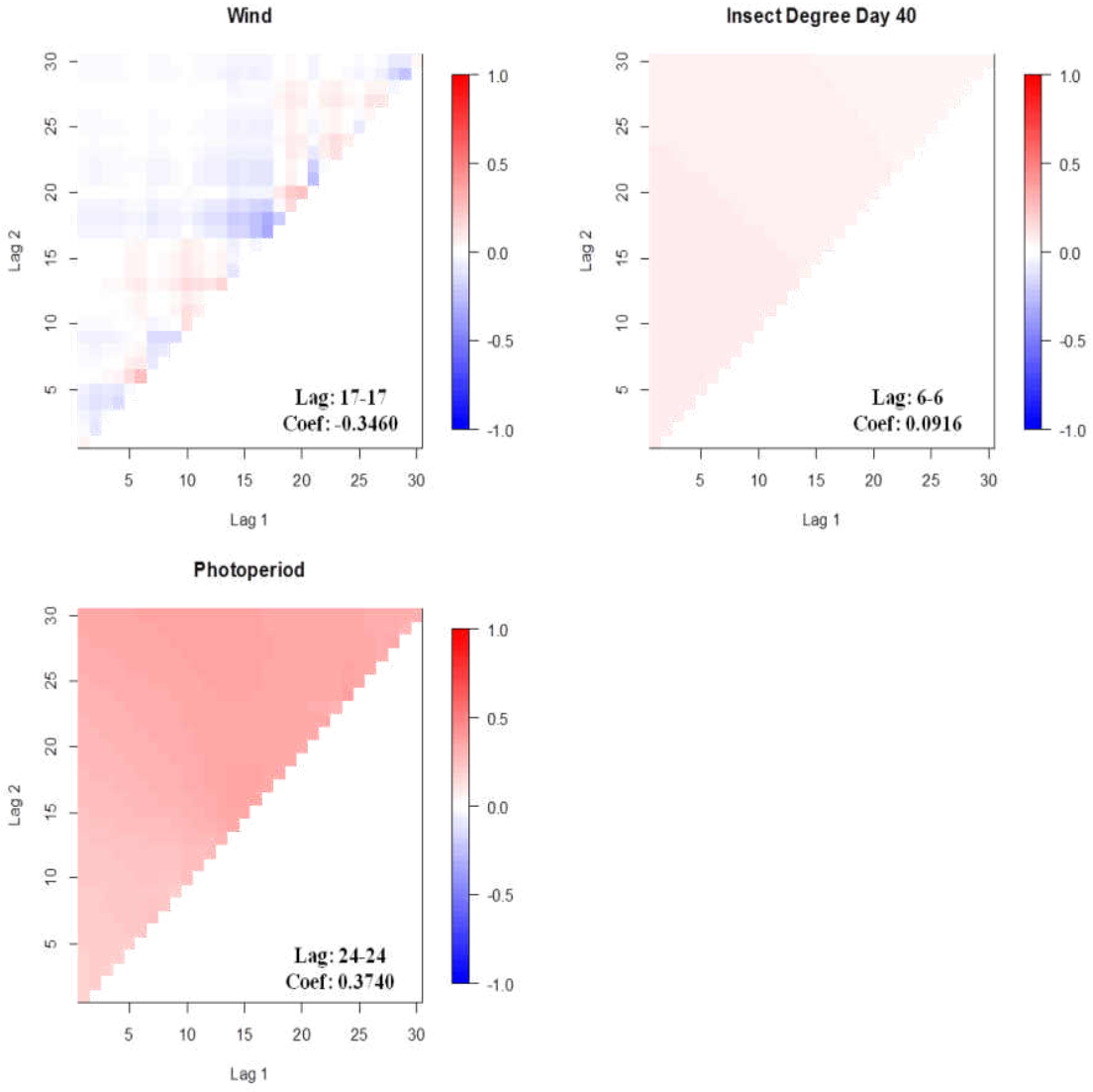
Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

Forest Total Mosquito Counts



Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

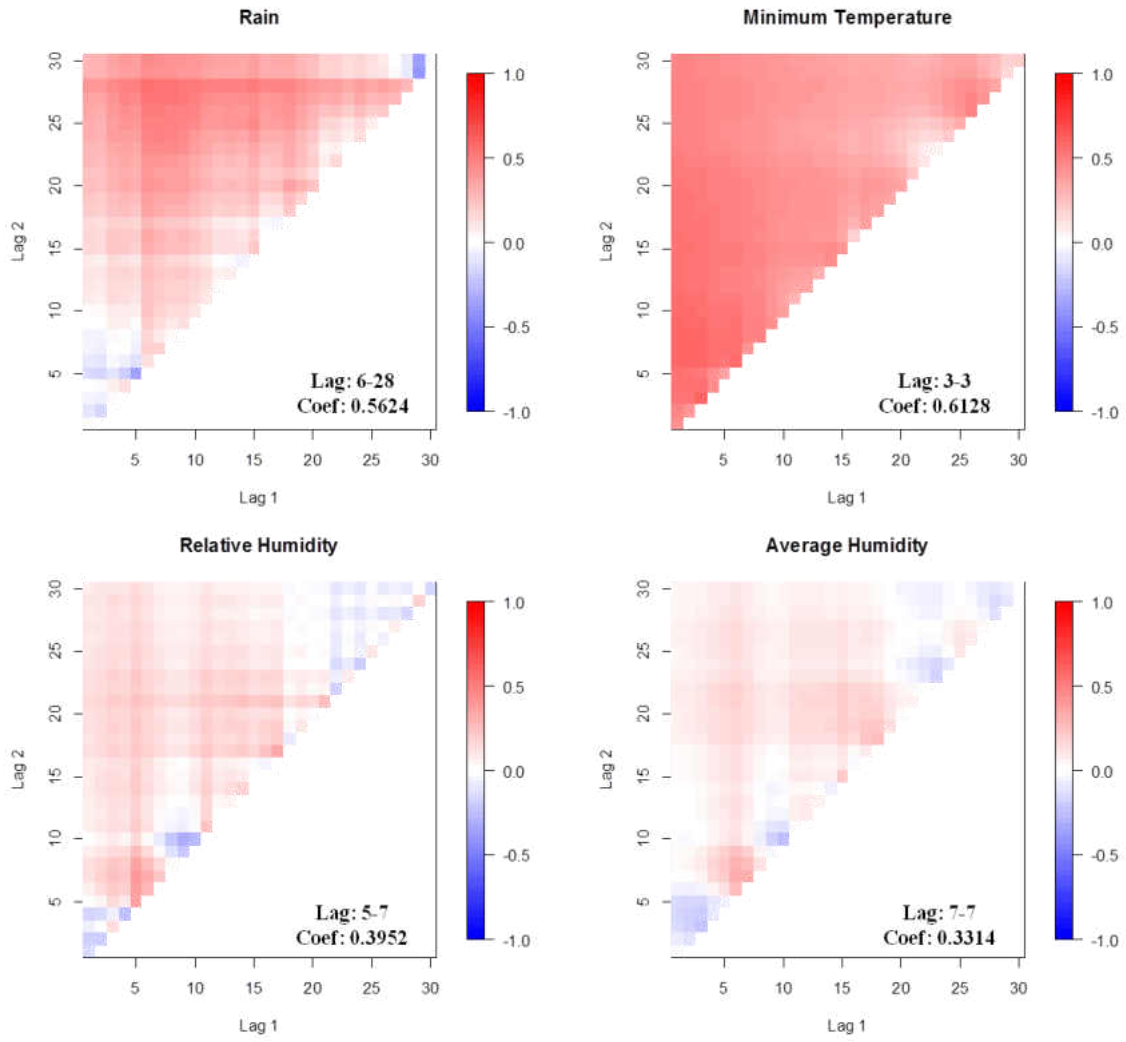
Forest Total Mosquito Counts



APPENDIX Q

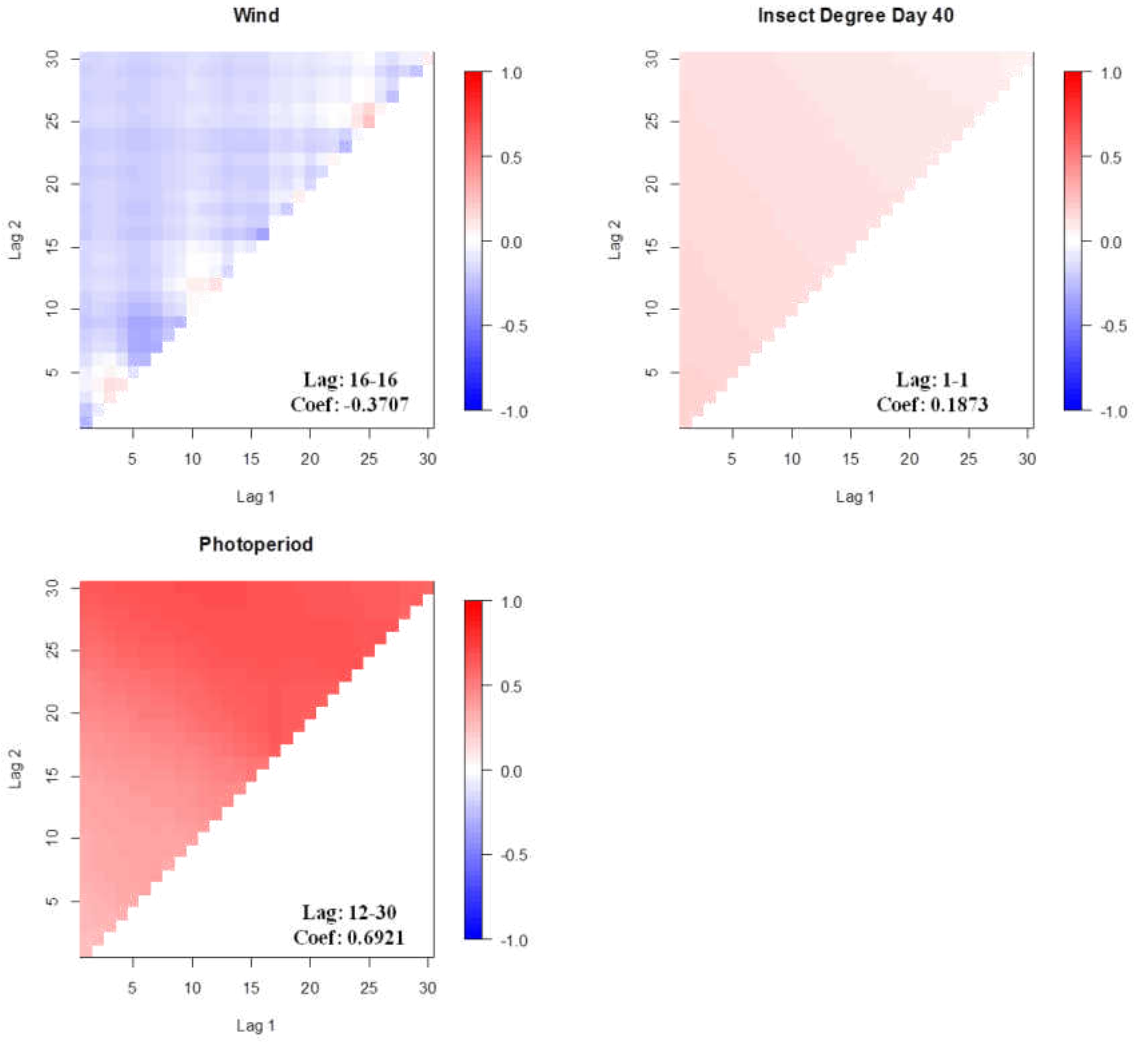
Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

Farm Total Mosquito Counts



Cross Correlation Maps Displaying Meteorological Variable Impact on Trap Counts

Farm Total Mosquito Counts



CHAPTER III

MOLECULAR IDENTIFICATION OF VERTEBRATE AND HEMOPARASITE DNA WITHIN MOSQUITO BLOOD MEALS FROM EASTERN NORTH DAKOTA

Abstract

To understand local transmission of vector-borne diseases, it is important to identify potential vectors, characterize their host feeding patterns, and determine if vector-borne pathogens are circulating within the region. This study simultaneously investigated these aspects of disease transmission by collecting engorged mosquitoes within two rural study sites in the central Red River Valley of North Dakota. Mosquitoes were identified, midguts were excised and the blood was expelled from the midguts. DNA was extracted from blood meals and subjected to polymerase chain reaction (PCR) and direct sequencing to identify the vertebrate origin of the blood. Using different primer sets, PCR was used to screen for two types of vector-borne pathogens, filarioid nematodes and haemosporidian parasites. White-tailed deer were the primary source of blood meals for the eight aedine mosquito species collected. None of the 288 deer-derived blood meals contained filarioid or haemosporidian DNA. In contrast, 18 of 32 *Culex tarsalis* and 3 of 3 *Cx. pipiens* blood meals contained avian blood, representing 8 different species of birds. Of 24 avian-derived blood meals

examined, 12 contained *Plasmodium* DNA, three of which also contained *Leucocytozoon* DNA (i.e., dual infection). Potential confounding effects resulting from parasite acquisition and development from previous blood meals (e.g., oocysts) were eliminated because host blood had been removed from the midguts prior to DNA extraction. Thus specific parasite lineages/species could be unequivocally linked to specific vertebrate species. By combining mosquito identification with molecular techniques for identifying blood meal source and pathogens, a relatively small sample of engorged mosquitoes yielded important new information about mosquito feeding patterns and hemosporidia infections in birds. Thorough analyses of wild-caught engorged mosquitoes and other arthropods represent a powerful tool in understanding the local transmission of vector-borne and zoonotic diseases.

Introduction

The Red River Valley (RRV) is plagued by mosquitoes every summer, yet knowledge of mosquito fauna and biology within the region is marginal. Within the city of Grand Forks, the mosquito fauna is dominated by three species; two floodwater species, *Aedes vexans* and *Ae. dorsalis* which are present throughout the summer, and *Culex tarsalis* which is abundant during mid to late summer and is the primary vector for West Nile virus (Deckert 1995, Bell et al. 2005, Bell et al. 2006). Preliminary studies suggest that species richness of mosquitoes is greater in the surrounding rural areas than within the city (Vaughan, unpubl. data). Almost nothing is known about the blood feeding patterns of mosquitoes within the rural RRV. Blood feeding patterns can incriminate mosquito species involved in various mosquito-borne diseases. In addition to the limited knowledge of mosquito biology, the diversity of zoonotic pathogens in

the RRV remains understudied. Surveying vertebrate populations for blood pathogens can be difficult and time-consuming. Instead, we examined the blood meals of wild-caught mosquitoes. The DNA within each blood meal was analyzed by polymerase chain reaction (PCR) and sequencing to identify its vertebrate host origin. Polymerase chain reaction and sequencing have also been used to identify various types of hemoparasites within mosquito blood meals (Chanteau et al. 1994, Farid et al. 2007, Massey et al. 2007, Bartlett-Healy et al 2009, Chambers et al. 2009, Kim et al. 2009, Latrofa et al. 2012). This is known as 'molecular xenomonitoring'. Using these techniques, we determined the blood feeding patterns of local mosquito species as well as the prevalence and species identity of hemoparasites within birds fed on by mosquitoes.

Materials and Methods

Mosquito collection

Mosquitoes were collected from two sites within Steele County, North Dakota. The first site was a 40-acre hardwood forest with a closed canopy and thick underbrush located 8.4 km southwest of Hatton, ND. The site is typically flooded in early spring by the Goose River, leaving behind breeding pools within the forest throughout the summer. The second site was a farmstead located 1.6 km west of Hatton, ND. The farm was surrounded by open cropland and is more typical of the RRV landscape. Mosquito control was absent at both sites.

Host-seeking mosquitoes were collected using three battery operated, CO_2-baited Mosquito Magnet X traps (MMX) (Woodstream Lititz, PA) spaced ca. 200 m apart. Traps were deployed 2-3 times per week and operated from 1800 to 0800 h,

from late May through August. The MMX traps were operated at the forest site during 2009 and 2010, and at the farm site during 2011. In 2011, resting mosquitoes were collected at both sites from underbrush, tree holes and around the bases of trees and buildings with a battery-operated vacuum aspirator. The aspirator was constructed of lightweight aluminum with fan, and a 33cm diameter circular collection area by personnel at the Metropolitan Mosquito Control District, St. Paul, MN. Insects were transported to the laboratory and placed in -20°C freezers for immobilization. Mosquitoes were identified to species (Darsie Jr. and Ward 2005) and stored at -80°C. Besides mosquitoes, the MMX traps also collected large numbers of host-seeking black flies (Simuliidae). Blackflies were identified to species (Adler et al. 2004) but were not processed or analyzed for vertebrate or hemoparasite DNA.

DNA Extraction

Mosquitoes were dissected in phosphate buffered saline using jeweler's forceps. Midguts were excised. split open and gently pressed against the inside of 1.5mL microtubes to expel the midgut contents. Nucleic acids were extracted using a guanidine/ethanol protocol (Tkach and Pawlowski 1999).

Identification of vertebrate DNA

To identify the vertebrate origin of each blood meal, mitochondrial cytochrome b and cytochrome oxidase subunit I genes were amplified using previously described protocols (Townzen, Brower and Judd 2008). Successful amplifications were visualized by gel electrophoresis. PCR products were cleaned with ExoSAP-IT (Affymetrix, Santa Clara, CA) according to the manufacturer's protocol and sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems Inc. -

ABI, Foster, CA) and a 3100 Genetic Analyzer (Applied Biosystems Inc. - ABI, Foster, CA). Sequences were analyzed and trimmed to no fewer than 300 base pairs using the BioEdit program (Ibis Biosciences, Carlsbad, CA). Sequences were aligned to published sequences available via the National Center for Biotechnology Information (NCBI) using Basic Local Alignment Search Tool (BLAST). Host sequences were considered a match at or above 98% base pair matches.

Identification of Parasite DNA

The DNA extracts were screened for filarioid nematode and hemosporidian DNA using different primer sets (Table 3.1). For filarioid nematodes, PCR primers were designed based on conserved sequences of the 18s ribosomal RNA genes of *Loa loa*, *Brugia pahangi*, *Onchocerca cervicalis*, *Chandlerella quiscali* (GenBank Accession numbers: DQ094173.1, EU496884.1, DQ094174.1). When tested, the primers successfully amplified 18s rRNA gene fragments of five different genera from four different subfamilies of filarioid nematodes, including Dirofilariinae (*Dirofilaria immitis, Waltonella* sp.), Onchocercinae (*Brugia pahangi*), Splendidofilariinae (*Chandlerella quiscali*) and Lemdaninae (*Eufilaria* sp.), indicating their utility for detecting a broad taxonomic range of filarioid species (Fig. 3.1A). The primers exhibited high sensitivity when tested against two-fold dilutions of DNA extracted from *Chandlerella quiscali* microfilariae recovered from the lungs of a Common Grackle (*Quiscalus quiscula*) (Fig. 3.1B). To screen for *Babesia* parasites, a set of semi-nested primers was developed using sequences of the 18s rRNA genes of *Babesia* spp. (GenBank Accession numbers: AY046577.1, AY237638.1, AB190459.1, HQ184411.1) and tested using DNA from *B. microti* (kind gift of S. Telford III). The

thermocycling protocols for the filarioid and the *Babesia* PCR were identical; 95°C for 2 min for initial activation followed by 40 cycles of 95°C for 45 sec, 55°C for 30 sec, 72°C for 45 sec with a one-time final extension at 72°C for 7 min. Detection of the cytochrome b gene from *Plasmodium, Leucocytozoon*, and *Haemoproteus* utilized the previously described protocol and primers of Hellgren, Waldenstrom and Bensch (2004).

Results

MMX traps collected 8,855 and 9,687 mosquitoes from the forest and farm sites respectively, but few were blood-fed (1.3 and 0.1%, respectively). Vacuum aspiration collected less mosquitoes (4,898 and 810 from the forest and farm sites respectively) but the proportion blood-fed was greater (30 and 31%, respectively). Of 1,841 engorged mosquitoes collected, DNA was extracted and PCRs were run on 770 individual mosquitoes to determine blood meal origin. Of these, usable DNA was recovered from 523 (68%) of the extracts. The low recovery rate may be due to the digestion of blood as well as low levels of DNA since partial blood meals were also categorized as engorged. Of 523 extracts with usable DNA, 416 were selected for sequencing. Of the 416 DNA extracts that were sequenced, 391 (94%) provided sufficiently high-quality sequences to be aligned with vertebrate host sequences in the NCBI database.

Mosquito Host Feeding Patterns

The majority of engorged mosquitoes (75%) collected at the forest site were *Ae. excrucians*, most of which (94%) fed on white-tailed deer (WTD) (Table 3.2). Similarly, the host feeding patterns observed for *Ae. canadensis*, *Ae. flavescens*, *Ae.*

triseriatus, Cs. inornata and *Co. perturbans* suggest that the main blood source for mosquitoes at the forest site was WTD.

Most (74%) of the engorged mosquitoes collected at the farm site were *Ae. vexans* the majority of which (70%) fed on WTD, although other blood sources were also utilized including cow, dog, rabbit and human (Table 3.3). With the notable exception of the two *Culex* species, most of the mosquitoes collected at the farm site were decidedly mammophilic. *Culex tarsalis* was the second most numerous mosquito species collected at the farm site and utilized a wide range of host animals (Table 3.3). Only 16% of *Cx. tarsalis* blood meals were from WTD. The majority of *Cx. tarsalis* blood meals (56%) were avian-derived. Robins, house sparrows, and grackles constituted >60% of all avian-derived blood meals taken by *Cx. tarsalis*. Even so, the bird species composition (N=9) within *Cx. tarsalis* blood meals was diverse. *Culex pipiens* also seem to feed preferentially on birds although the numbers collected were low (Table 3.3).

Parasite Detection

From the 255 blood meals derived from WTD, we suspected we might find DNA evidence for two parasite species; namely, *Setaria yehi*, a mosquito-borne filarioid nematode of North American cervids, and *Babesia odocoilei*, a tick-borne hemosporidian of WTD. We found no DNA evidence of either parasite in mosquito blood meals.

From the 24 blood meals derived from birds, we suspected we might find DNA evidence for two parasite groups; namely, filarioid nematodes (*i.e.*, blood microfilariae) and hemosporidians (*i.e.*, *Plasmodium*, *Leucocytozoon* or *Haemoproteus*). None

contained filarioid or *Haemoproteus* DNA. However, three (12%) of the 24 bird-derived blood meals contained *Leucocytozoon* DNA and 12 (50%) contained *Plasmodium* DNA (Table 3.4). *Leucocytozoon* DNA was present in two of the four blood meals taken from Common Grackles and one of the five blood meals taken from American Robins (*Turdus migratorius*). *Plasmodium* DNA was present in three of the six blood meals taken from American Robins, all four blood meals taken from Common Grackles, one of the three blood meals from Cedar Waxwings (*Bombycilla cedrorum*), three of the five blood meals from House Sparrows, and the single blood meal taken from Green Heron (*Butorides virescens*). When the 12 *Plasmodium* DNA sequences were compared with DNA sequences available from the NCBI database, two separate *Plasmodium* lineages emerged, both without formal species status. One lineage was present in a *Cx. tarsalis* blood meal taken from an American Robin. This lineage was 99% identical to the sequence of a *Plasmodium* isolate recovered from a White-throated Thrush (*Turdus assimilis*) in Costa Rica (Accession JN819347). The other 11 *Plasmodium* sequences were identical to each other and to the sequence of a *Plasmodium* isolate recovered from a Barred Owl (*Strix varia*) in Wisconsin (Accession EU627827). The fact that the second *Plasmodium* lineage was recovered from blood meals derived from 4 different families of passerine birds, a heron and an owl indicates that this particular *Plasmodium* lineage has a broad host range. Three of the 12 blood meals (25%) contained both *Plasmodium* and *Leucocytozoon* DNA, including one blood meal from an American Robin and two blood meals from Common Grackles. This suggests that haemosporidian polyparasitism among some passerine species within the RRV may be common.

Discussion

Molecular analyses of blood meals from wild-caught mosquitoes yielded new information on local zoonotic disease ecology. First, PCR and sequencing of vertebrate mitochondrial genes yielded information on the blood feeding patterns of local mosquito species. This is important in defining the transmission ecology of mosquito-borne diseases within a region and helps predict the likelihood of success for exotic mosquito-borne pathogens that may move into a region. For example, we found that deer were the main blood source for many aedine mosquito species in the RRV. This presents a favorable ecological setting for certain arboviruses such as Jamestown Canyon virus (see Andreadis et al. 2008) or perhaps even Rift Valley fever virus (see Iranpour et al. 2011) should these arboviruses become introduced in the RRV.

Second, once the host origin of a blood meal was identified, it was then possible to target the types of hemoparasites that might be present within a particular blood meal. By using different primer sets on the same DNA extracts, we were able to link the presence of certain hemoparasites to specific vertebrate species (i.e., molecular xenomonitoring). Although mosquitoes are the raw material for analyses, it is important to remember that molecular xenomonitoring does not provide information on a mosquito's ability to transmit the hemoparasites it has ingested (i.e., molecular xenomonitoring is not vector competence). Molecular xenomonitoring merely substitutes for the direct capture and bleeding of vertebrates and thus it should be interpreted as an indirect way of estimating the prevalence and diversity of hemoparasites circulating within specific vertebrate populations. Because it is an indirect measurement, the accuracy and reliability of molecular xenomonitoring can be

influenced by several factors including obvious things such as the age of the blood meal and the sample size, as well as less-obvious factors such as sample processing and the unique biology of the hemoparasite and host under investigation.

For example based on the assays of 288 deer-derived blood meals, we found no evidence of *Babesia* or filaroid nematode infections in the local WTD population. This may indeed be the case for *B. odocoilei* because the vector tick, *Ixodes scapularis*, has only recently become established in the RRV (Russart and Vaughan, unpubl. data). However, the reliability of our xenomonitoring to assess microfilarial infections in WTD is less certain. Reported prevalences of the cervid filarioid, *S. yehi,* in WTD are 16% (n=84) and 27% (n=1,045) from Illinois and the southeastern USA, respectively (Prestwood and Pursglove 1977, Cook, et al. 1979). The prevalence of *S. yehi* in California black-tailed deer is 40% (n=488) (Weinmann, et al. 1973). Thus, one would expect that xenomonitoring of 288 deer-fed mosquitoes would result in detecting at least some *S. yehi* parasites. However, there are two confounding factors related to the biology of the parasite and the size of the host. First, *S. yehi* microfilariae quickly penetrate the midgut after ingestion by *Aedes* spp. mosquitoes (Lee 1971). Engorged mosquitoes in our studies, many of which likely fed at night, were collected in the morning and transported to the laboratory prior to dissections. The delay between mosquito ingestion of deer blood and mosquito dissection may have allowed microfilariae sufficient time to exit the midgut and escape detection. Second, deer are large animals and a single animal can serve as the blood source for many mosquitoes. Thus, the prevalence of pathogens within the blood meals of mosquitoes feeding on large herding animals (particularly if mosquitoes were collected at the same time and

location) may not directly correlate with the prevalence of pathogens within the vertebrate population in general. This is probably of lesser concern when considering pathogens within blood meals derived from small or solitary host species.

We also failed to detect filarioid nematode DNA in avian-derived blood meals. In this case, concerns over microfilariae exiting the midgut and escaping detection are unwarranted because only a small fraction of ingested passerine microfilariae successfully exit the midguts of local *Culex* mosquitoes (Vaughan et al. 2012). Failure to detect filarioid DNA in avian-derived blood meals was most likely due to the low number of samples represented per bird species. The greatest numbers of con-specific blood meals were from American Robins (n=6), House Sparrows (n=5) and Common Grackles (n=4) (Table 3.4). But the estimated prevalence of filarioid infections for these bird species within the RRV is less than 20% (Vaughan et al. 2012). Such small samples sizes fall below the theoretical limit of detection. Obviously, the use of molecular xenomonitoring to detect low prevalence infections among a diverse community of host species requires larger sample sizes than those used here.

In contrast, molecular xenomonitoring detected haemosporidian infections in 12 of the 24 avian-derived blood meals examined (Table 3.4), indicating a high prevalence of haemosporidian infections in local bird populations. The dominant haemosporidian consisted of a single lineage of *Plasmodium* capable of infecting a broad taxonomic range of birds. Active transmission of this *Plasmodium* lineage almost certainly occurred at this site as indicated by the high abundance of an ornithophilic and competent vector (*e.g., Culex tarsalis*; see Work, Washino and Van Riper 1990) and the fact that three of the five blood meals containing House Sparrow blood also

contained *Plasmodium* DNA. Unlike other bird species identified in this study, House Sparrows are non-migratory residents and thus are unlikely to have acquired their infections elsewhere.

In addition, a substantial proportion (25%) of haemosporidian infections within mosquito blood meals consisted of *Plasmodium* and *Leucocytozoon*, indicating that polyparasitism of local birds was common. The MMX traps used to collect mosquitoes also collected enormous numbers of black flies. Black flies are the vectors of *Leucocytozoon* parasites (Valkiunas 2005). A large sample (>800) of black flies was examined. All belonged to a single ornithophilic species, *Simulium johannseni*. Their appearance in late May and early June corresponded temporally with the detection of *Leucocytozoon* in avian-derived blood meals (Table 3.4). Thus in addition to *Plasmodium*, active transmission of *Leucocytozoon* may also have occurred at the site.

One of our objectives was to use molecular xenomonitoring to link specific parasites to specific host species. In the case of haemosporidia in avian-derived blood meals, the link was definitive (Table 3.4) because of the way in which mosquitoes were processed– i.e., blood meal contents were removed from mosquito midgut tissue prior to nucleic acid extraction. Alternative ways in which engorged mosquitoes can be processed for DNA extraction include using whole mosquitoes or whole abdomens separated from the head/thorax region. The optimal method depends on the object of the study. If the object is merely to identify the vertebrate host origin of the blood, then the quickest method (*i.e.,* whole mosquito) may be preferred. However if the object is to link blood meal pathogens to the vertebrate species from which the blood originated,

then the method of sample preparation should be dictated by the type of pathogen being monitored.

There are five types of pathogens that can be taken up in a mosquito blood meal; arboviruses, bacteria, trypanosomes, hemosporidians and microfilariae. With bacteria and trypanosomes, it does not matter if the mosquito is dissected. That is because blood-borne bacteria (*e.g.,* rickettsiae, spirochetes, *etc*.) and most trypanosomes either never leave the mosquito digestive tract or, if they do (*e.g*, Salivaria trypanosome *Trypanosoma brucei*), they do not exit the gut until the blood meal has been digested beyond the point of accurately determining its vertebrate origin.

With hemosporidia and arboviruses, it is crucial to rid the sample of mosquito midgut tissue prior to extraction. As part of their normal development, hemosporidian oocysts stay attached to the outside of the gut for many days. Similarly, arborviruses infect midgut epithelium long after the viremic blood meal has been digested and the mosquito has oviposited. If an oocyst or arbovirus-infected mosquito takes a second blood meal from a different host species, then confusion may arise as to the host origin of the pathogen. For example, Ejira et al. (2011a, b) identified avian *Plasmodium* DNA in the severed abdomens of engorged mosquitoes containing blood identified molecularly as being from cow and sitka deer. Avian *Plasmodium* DNA could not have originated from the blood of a ruminant. Instead it probably originated from oocysts attached to the outside of the midguts (*i.e.,* previous blood meal from a bird), not from the blood inside the midguts. Similarily, Santiago-Alarcon et al. (2012) analyzed severed abdomens of 105 engorged *Culicoides* gnats. Four were concurrently positive for the DNA of humans and the DNA of avian *Plasmodium* and *Haemoproteus*

parasites. Since avian hemosporidians do not infect humans, the only logical sequence of events that could account for such a result would be if the gnats had first fed on gametocytemic birds, the parasites underwent sporogonic development to oocysts and then the infected gnats fed on a human. Without first separating the ingested blood from midgut tissue, it is impossible to unequivocally link a hemosporidian species (or an arbovirus) identified in an engorged abdomen to the vertebrate host species represented within the blood meal.

The filarioid nematodes are the most problematic because the nematodes' location within the mosquito can vary. In some filarioid/mosquito combinations, most if not all ingested microfilariae exit the bloodmeal into the hemocoel within hours after being ingested (Wharton 1957, Laurence and Pester 1961). In other combinations, few if any succeed in leaving the midgut (Ewert 1966, Zielke 1992). To account for such variability, severing abdomens from thorax might be the preferred method.

In summary, we successfully used DNA recovered from the midguts of wild-caught mosquitoes to simultaneously determine the host feeding patterns of local mosquitoes and conduct a survey of blood-borne parasites within local wildlife. Careful examination of relatively few specimens yielded important new information about the local transmission of avian hemosporidian parasites. Application of this technique can contribute to an increasing understanding of the transmission ecology of zoonotic diseases. However, certain considerations should be taken into account regarding how samples from the field are prepared prior to the extraction of nucleic acids.

Acknowledgments

We thank Dr. Sam Telford III, Tufts University, for the generous gift of *Babesia microti* samples used as a positive control in the development of our PCR tests. Sarina Bauer, Stephen Greiman, Alex Droske, Bo Chung, Jeffrey Bell, and Max Tkach assisted in setting traps, sorting and identifying mosquitoes. We thank Dr. Vasyl Tkach for assisting in primer development. We thank the Metropolitan Mosquito Control District, St. Paul, MN for the loan of their mosquito aspirator. Financial support was provided by UND Graduate School Fellowship (JOM), ND EPSCoR Graduate Student Research Assistantship (JOM), and NIH grant R03 AI092306 (JAV).

References

Adler, P, DC Currie, M Wood, L Zettler, DH Janzen. The Black Flies (Simuliidae) of North America. Cornell University Press, Ithaca. 2004

Andreadis, TG, JF Anderson, PM Armstrong, and AJ Main. Isolations of Jamestown Canyon virus (Bunyaviridae: Orthobunyavirus) from field-collected mosquitoes (Diptera: Culicidae) in Connecticut, USA: A ten-year analysis, 1997-2006. Vector Borne Zoonotic Dis 2008; 8:175-188.

Bartlett-Healy K, W Crans, R Gaugler. Vertebrate hosts and phylogenetic relationships of amphibian trypanosomes from a potential invertebrate vector, *Culex territans* Walker (Diptera: Culicidae). J Parasitol 2009; 95:381-387.

Bell, JA, NJ Mickelson, and JA Vaughan. West Nile virus in host-seeking mosquitoes within a residential neighborhood in Grand Forks, North Dakota. Vector Borne Zoonotic Dis 2005; 5:373-82

Bell, JA, CM Brewer, NJ Mickelson, GW Garmen, and JA Vaughan. West Nile virus epizootiology, Central Red River Valley, North Dakota and Minnesota, 2002-2005. Emerg Infect Dis 2006; 8:1245-47

Chambers, EW, SK McClintock, MF Avery, JD King, MH Bradley, MA Schmaedick, PJ Lammie, and TR Burkot. Xenomonitoring of *Wuchereria bancrofti* and *Dirofilaria immitis* infections in mosquitoes from American Samoa: trapping considerations and comparison of polymerase chain reaction assays with dissection. Am J Trop Hyg 2009; 5:774-81

Chanteau, S, P Luguiaud, AB Failloux, SA Williams. Detection of *Wuchereria bancrofti* larvae in pools of mosquitoes by the polymerase chain reaction. Trans R Soc Trop Med Hyg 1994; 6:665-66

Cook, TW, BT Ridgeway, R Andrews and J Hodge. Gastro-intestinal helminths in white-tailed deer (Odocoileus virginianus) of Illinois. J Wildl Dis 1979; 15:405-08

Darsie Jr., RF., and RA. Ward. Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico. Gainesville, FL: University Press of Florida, 2005.

Deckert, WA. The occurrence of mosquitoes in the central Red River Valley area in 1992-1993. Dissertation. University of North Dakota, Grand Forks 1995

Ewert, A. Comparative migration of microfilariae and development of *Brugia pahangi* in various mosquitoes. Am J Trop Med Hyg 1966, 14:254-259.

Farid, HA, ZS Morsy, H Helmy, RM Ramzy, M El Setouhy, and GJ Weil. A critical appraisal of molecular xenomonitoring as a tool for assessing progress toward elimination of lymphatic filariasis. Am J Trop Med Hyg 2007; 4:593-600

Hellgren, O, J Waldenstrom, and S Bensch. A new PCR assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. J Parasitol 2004; 90:797-802.

Iranpour M, MJ Turell, and LR Lindsay. Potential for Canadian mosquitoes to transmit Rift Valley fever virus. J Am Mosq Control Assoc. 2011, 27: 363-369.

Kim, KS, Y Tsuda, T Sasaki, M Kobayashi, and Y Hirota. Mosquito blood-meal analysis for avian malaria study in wild bird communities: laboratory verification and application to *Culex sasai* (Diptera:Culicidae) collected in Tokyo, Japan. Parasitol Res 2009; 5:1351-57

Lee, D. The role of the mosquito, *Aedes sierrensis*, in the epizootiology of the deer body worm, *Setaria yehi*. Thesis. University of California, Berkely 1971

Latrofa, MS, S Ciocchetta, G Annoscia, F Dantas-Torres, S Ravagnan, G Capelli and D Otranto. Molecular xenomonitoring of *Dirofilaria immitis* and *Dirofilaria repens* in mosquitoes from north-eastern Italy by real-time PCR coupled with melting curve analysis. Parasit Vectors 2012; 5:76

Laurence BR, and FRN Pester. The behavior and development of *Brugia patei* (Buckley, Nelson and Heisch, 1958) in a mosquito host, Mansonia uniformis (Theobold). J Helmintol 1961; 35:285-300

Massey, B, DM Gleeson, D Slaney, and DM Tompkins. PCR detection of *Plasmodium* and blood meal identification in a native New Zealand mosquito. J Vector Ecol 2007; 1:154-56

Prestwood, AK and SR Pursglove. Prevalence and distribution of *Setaria yehi* in southeastern white-tailed deer. J Am Vet Med Assoc 1977; 171:933-35

Santiago-Alarcon D, P Havelka, HM Schaefer, and G Segelbacher. Bloodmeal analysis reveals avian *Plasmodium* infections and broad host preferences of *Culicoides* (Diptera: Ceratopogonidae) vectors. PLoS One 2012; 7:e31098.

Tkach, V, and J Pawlowski. A new method of DNA extraction from the ethanol-fixed parasitic worms. Acta Parasitol 1999; 44:147-148.

Townzen, J. S., A. V. Brower, and D. D. Judd. Identification of mosquito bloodmeals using mitochondrial cytochrome oxidase subunit I and cytochrome b gene sequences. Med Vet Entomol 2008: 386-393.

Valkiunas, G. *Avian Malaria Parasites and Other Haemosporidia*. Washington DC: CRC Press; 2005.

Vaughan, J. A., J.O. Mehus, C. M. Brewer, D. K. Kvasager, S. Bauer, J. L. Vaughan, H. K. Hassan, T. R. Unnasch, J. A. Bell. Theoretical potential of passerine filariasis to enhance the enzoonotic transmission of West Nile virus. J Med Entomol 2012; 6:1430-41

Weinmann, CJ, JR Anderson, WM Longhurst and G Connolly. Filarial worms of Columbian black-tailed deer in California. 1. Observations in the vertebrate host. J Wildl Dis 1973; 9:213-20

Wharton, RH. Studies on filariasis in Malaya: the efficiency of *Mansonia longipalpis* as an experimental vector of *Wuchereria malayi*. Ann Trop Med Parasitol 1957; 51:422-439.

Work, TM, RK Washino, and C Van Riper. Comparative susceptibility of *Culex tarsalis*, Anopheles franciscanus and Culiseta inornata (Diptera: Culicidae) to *Plasmodium relictum* (Haemosporidia: Plasmodiiae). J Med Entomol 1990; 27:68-71.

Zielke E. Observations on the midgut penetration by *Wuchereria bancrofti* microfilariae in mosquitoes of varying susceptibilities to infection. Mosq Borne Dis Bull 1992; 9:60-3.

Table 3.1. Primers used in molecular analyses of mosquito blood meals.

Target Organism	*Target Gene*	*Primer Name*	*5'-3' Primer Sequence*	*Amplicon Size (bp)*
Vertebrate*	mtDNA cytochrome b	Forward- Cyt b	GAGGMCAAATATCATTCTGAGG	457
Vertebrate*	mtDNA cytochrome b	Reverse- Cyt b	TAGGGCVAGGACTCCTCCTAGT	457
Vertebrate*	mtDNA cytochrome oxidase 1	Forward- COI_long	AACCACAAAGACATTGGCAC	663
Vertebrate*	mtDNA cytochrome oxidase 1	Reverse- COI_long	AAGAATCAGAATARGTGTT	663
Vertebrate*	mtDNA cytochrome oxidase 1	Forward- COI_short	GCAGGAACAGGWTGAACCG	324
Vertebrate*	mtDNA cytochrome oxidase 1	Reverse- COI_short	AATCAGAAYAGGTGTTGGTATAG	324
Filaria	18s rRNA	Forward- Chand FO	GAGACCGTTCTCTTTGAGGCC	580
Filaria	18s rRNA	Reverse- Chand RO	GTCAAGGCGTANNTTTACCGCCGA	580
Babesia	18s rRNA	Forward- BabF 1102-1122	GACTAGGGATTGGAGGTCGTC	739 & 240
Babesia	18s rRNA	Reverse- BabR 1841-1818	GACCACCACCCAAAGAATCAA	739
Babesia	18s rRNA	Reverse- BabR 1342-1318	GGTCCGAATAATTCACCGGATCAC	240
Haemosporidia**	mtDNA cytochrome b	Forward- HaemNFl	CATATATTAAGAGAAITATGGAG	570
Haemosporidia**	mtDNA cytochrome b	Reverse- HaemNR3	ATAGAAAGATAAGAAATACCATTC	570
*Leucocytozoon***	mtDNA cytochrome b	Forward- HaemFL	ATGGTGTTTTAGATACTTACATT	478
*Leucocytozoon***	mtDNA cytochrome b	Reverse- HaemRL2	CATTATCTGGATGAGATAATGGIGC	478
Plasmodium / *Haemoproteus***	mtDNA cytochrome b	Forward- HaemF	ATGGTGCTTTCGATATATGCATG	480
Plasmodium / *Haemoproteus***	mtDNA cytochrome b	Reverse- HaemR2	GCATTATCTGGATGTGATAATGGT	480

* Townzen et al. 2008 ** Hellgren et al. 2004

Table 3.2. Mosquito feeding habits at the Forest site in 2009 and 2011, Steel Co., ND, USA.

Host	*Ae. excrucians*	*Ae. triseriatus*	*Ae. vexans*	*Ae. canadensis*	*Ae. flavescens*	*Cs. inornata*	*Co. perturbans*	*Total*
White-tailed deer	104	17	3	2	1	6	3	136
Cow	2					2	1	5
Human	1						1	2
Raccoon	2							2
American Mink							1	1
Common Yellow-throat	1							1
Total	110	17	3	2	1	8	6	147

Table 3.3. Mosquito feeding habits at the farm site in 2010 and 2011, Steele Co., ND, USA.

Host	*Cx. tarsalis*	*Cx. pipiens*	*Ae. canadensis*	*Ae. dorsalis*	*Ae. excrucians*	*Ae. flavescens*	*Ae. triseriatus*	*Ae. vexans*	*Cs. inornata*	*Total*
Deer	5		11		1		3	125	4	149
Cow	1						1	21		23
Dog	5			2		1	1	14		23
Cat			2			1	2	1	1	7
Rabbit	2							11		13
Human	1						1	4		6
AmericanRobin	5		1							6
House Sparrow	5									5
Cedar Waxwing	3									3
Mourning Dove								1		1
AmericanGoldfinch	1									1
Chipping Sparrow	1									1
Cliff Swallow	1									1
CommonGrackle	1	3								4
Green Heron	1									1
Total	32	3	14	2	1	2	8	177	5	244

Table 3.4. Detection of haemosporidian DNA within mosquito blood meals of avian origin. Steele Co., ND, USA.

Month	*Day*	*Year*	*Mosquito Species*	*Avian Host Species*	*Plasmodium*	*Leucocytozoon*
June	9	2010	Ae. vexans	Mourning Dove		
	11	2011	Cx. pipiens	Common Grackle	+	+
	11	2011	Cx. pipiens	Common Grackle	+	
	17	2011	Cx. pipiens	Common Grackle	+	
	17	2011	Ae. canadensis	American Robin	+	+
July	1	2010	Cx. tarsalis	Common Grackle	+	+
	1	2010	Cx. tarsalis	Green Heron	+	
	7	2010	Cx. tarsalis	Cliff Swallow		
	7	2010	Cx. tarsalis	American Robin	+	
	8	2010	Cx. tarsalis	American Robin		
	8	2010	Cx. tarsalis	House Sparrow		
	15	2010	Cx. tarsalis	American Robin	+	
	15	2010	Cx. tarsalis	American Robin		
	26	2010	Cx. tarsalis	American Robin		
August	2	2010	Cx. tarsalis	Chipping Sparrow		
	6	2010	Cx. tarsalis	American Goldfinch		
	6	2009	Ae. excrucians	Common Yellowthroat		
	6	2010	Cx. tarsalis	Cedar Waxwing	+	
	6	2010	Cx. tarsalis	Cedar Waxwing		
	6	2010	Cx. tarsalis	Cedar Waxwing		
	6	2010	Cx. tarsalis	House Sparrow	+	
	6	2010	Cx. tarsalis	House Sparrow	+	
	6	2010	Cx. tarsalis	House Sparrow		
	8	2010	Cx. tarsalis	House Sparrow	+	

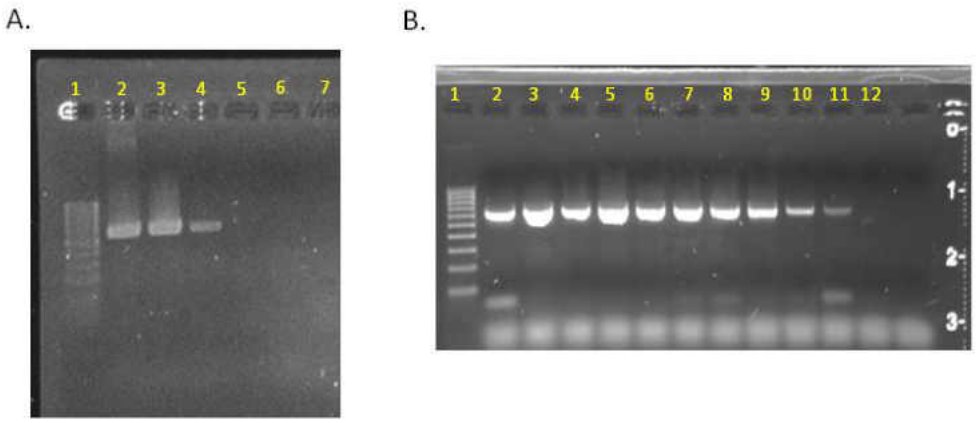


Figure 3.1. Xenomonitoring PCR using 18s rRNA gene. A.) Lane 1- ladder, lane 2- avian filarid (*Eufilaria* sp.), lane 3- frog filarid (*Waltonella* sp.), lane 4- avian filarid (*Chandlerella* sp.), lane 5- engorged *Cx. pipiens* (host=mouse), lane 6-mouse blood, lane 7- unengorged *Ae. vexans.* B) Sensitivity of PCR to *Ch. quiscali* microfilaria in sample. Lane 1- ladder, lanes 2-12 represeting 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.125 microfilaria respectively. PCR is sensitive to pick up less than 1 microfilaria in each 5µl sample.

EPILOGUE

Summary of Research Findings

Mosquitoes in the Great Plains are highly understudied, especially in the state of North Dakota. Although there have been a handful of papers discussing the mosquitoes in the Red River Valley (RRV), they have been limited to only the city limits of Grand Forks, North Dakota. Also, incomplete and inaccurate mosquito identifications have made the records and information provided unreliable. These inaccuracies provide an unstable foundation on which to build solid statements about the mosquitoes of the region and the potential role in disease transmission in wildlife, livestock and humans. The research described here had three main objectives 1) Identify the species composition between two rural habitats, 2) Determine which meteorological variables have impacts on day-to-day and time-lagged mosquito counts and 3) Analyze mosquito blood meals for host feeding habits and blood-borne parasite DNA.

The goal of the first chapter was to collect and identify mosquitoes collected from Farm and Forest habitats. I identified over 125,000 mosquitoes from the two sites belonging to 20 different species. The two sites shared 13 species between the two, yet the Forest site had a greater diversity of mosquito species. This suggests that even though forested regions within North Dakota are few and far between, the mosquito populations within these areas are more numerous than agricultural sites. This study also provided a comparison between mosquito collection techniques for both host-

seeking and engorged, resting mosquito populations. Mosquitoes actively looking for vertebrate hosts were collected in the CO_2-baited MMX traps while mosquitoes that had previously taken blood and were digesting and producing eggs were collected at a higher frequency via aspiration.

In addition to revealing the species composition between these two study sites, I also used multiple meteorological variables in regression modeling to determine their influence on adult, host-seeking mosquito activity. While these day-to-day models are interesting, they do not provide long range predictive efficacy. To increase predictive range, cross-correlation map (CCM) analyses were utilized. CCMs provided the ability to perceive mosquito count responses to meteorological variables ranging from 1-30 days prior to trap date. Not only do different mosquito species respond uniquely to varying meteorological factors, but mosquitoes of the same species respond differently given the habitat they reside within. *Ae. vexans* populations from the Forest and Farm sites were highly correlated to similar meteorological variables (precipitation, minimum temperature, relative humidity), yet the correlation between counts and variables was less significant at the Farm site. This may be the result of a higher exposure to the elements of climate at the Farm site that the Forest site may not be as exposed to, such as wind affects. It also appears that while viewing mosquito species grouped together (using non-species specific trap counts), the CCMs tend to favor the correlations of the most prevalent mosquito species in the habitat. By identifying individual species responses to meteorological variables, it allows some insight to the composition of mosquito counts that, at the time of collection, were not identified to species.

The third chapter of this study provides critical information about the feeding habits of local mosquito species. Not only is this information important to determine which mosquito species commonly feed upon humans, but also provides details on the feeding habits of mosquitoes that do not often encounter human blood sources. Aedine mosquitoes inhabiting these two rural sites commonly fed upon one of the most numerous mammals present, white-tailed deer. The local *Culex* population displayed a much wider variety of hosts, 60% avian and 40% mammalian. While avian samples were more common that mammalian blood meals, there was no one bird species that was preferred. Xenomonitoring of blood meals for hemoparasites was also used to determine if parasite DNA could be recovered from mosquitoes. While no mammalian-dervied blood meals contained filarial nematode DNA nor haemosporidian DNA, avian samples provided some positive results. Avian *Plasmodium* DNA was recovered from 12 of 24 bird-derived blood meals. In addition, three of the avian blood meals that contained *Plasmodium* also showed successful amplification of *Leucocytozoon* DNA, proving there is blood-borne polyparacitism in local bird species.

Future Studies

While this research provides the foundation to many aspects to mosquito ecology, disease biology and pathogen transmission, there are many potential studies or areas of this research that may be expounded. The two major areas of future direction I am most interested include 1) A more intense collection and study of engorged mosquitoes and 2) Incorporation of CCM data into regression modeling to allow for mosquito abundance forecasting.

Engorged mosquitoes provide a source of data that can be used in multiple studies such as species composition. By not specifically targeting host-seeking mosquitoes, many mosquito species can be recovered by collecting engorged mosquitoes in habitats where they are likely to be resting and digesting blood meals. This study did offer some insight to the feeding habits of *Culex* mosquito populations in the RRV, the sample size of this study is low. By focusing efforts on the aspiration collection method, we can more intensely study the *Culex* populations that act as the local vectors of West Nile virus and likely avian *Plasmodium*. Viral pathogen detection within mosquito blood meals could also be added to the regime of xenomonitoring.

Not only are engorged blood meals important sources of parasite DNA, but they can also offer potential research opportunities in blood meal digestion/DNA recovery studies. From this study, we know that not every mosquito that contains blood provides ample DNA for amplification. By developing a grading scale of the degraded blood meal, we can potentially identify day of the feeding or age of the blood meal as well as determine a cut off point to which blood meals should no longer be used as DNA sources for PCRs to identify vertebrate hosts or xenomonitoring.

Another potential branch of this research is to combine results of CCMs and regression based modeling. CCMs provide correlation between trap counts and meteorological variables ranging from 1-30 days before trap date. Upon identification of significant time-lagged correlations between trap counts and weather variables, the values of the meteorological variables over the range of significantly correlated dates can be incorporated into the full regression models. By determining these correlated

dates and incorporating the values into regression models we can forecast at a higher level of specificity how mosquito populations will be affected by weather.