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Distribution of bird reservoir hosts for West Nile Virus in Montana

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Distribution of bird reservoir hosts

for West Nile Virus in Montana

Submitted in partial fulfillment of the requirements for graduation with honors from the
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This thesis for honors recognition has been approved for the Department of Natural Sciences by:
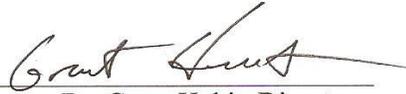
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Abstract

West Nile virus (WNv) is an arthropod-borne flavivirus that was first detected in the United States in 1999. By 2004 it had spread to 48 states and, to date, it has been the source of greater than 29,000 cases of human infection. WNv is maintained through an avian-mosquito transmission cycle, with birds serving as the primary vertebrate hosts for the amplification of the virus. Previous studies have shown Passeriform birds to be some of the most virulent competent hosts for the virus, producing adequate viremia counts for continuation of the transmission cycle. Associations were analyzed between mosquito populations and total bird abundance, Passeriformes abundance, and virulent competent bird species abundance using data collected from sites across Montana. No temporal correlation was found between bird and *Culex tarsalis* (the most important mosquito vector in Montana) mosquito numbers across the summer sampling period. However, a positive correlation was observced between passerine abundance and total mosquito numbers. These findings suggest that passerine abundance is associated with mosquito population numbers, which may result in an increased risk for WNv transmission.

Introduction

West Nile virus (WNv) is an arthropod-borne flavivirus. Arthropod-borne or arboviruses refer to viruses that use arthropods, such as insects, to transmit the virus upon biting, allowing the virus to enter the hosts' circulatory system. Flaviviruses refer to the viral family *Flaviviridae*, which often lead to encephalitis or acute inflammation of the brain (Lindenbach et al. 2007). WNv was first detected in North America in 1999 in New York City and by 2004 it covered 48 states (Ezanwa et al. 2007). Although WNv does not prominently infect humans, from the 1999 outbreak to 2010, there were 29,858 cases of WNv infection of which 1,166 of those cases were fatal (CDC).

In an arbovirus transmission the arbovirus, the arthropod vector, and the vertebrate host have a complex relationship involving viral competency and susceptibility of the vertebrate host. Transmission requires three essential factors to become established and maintain a presence in a population. 1.) Vertebrate hosts must produce high levels of viremia to ensure other vectors become infected (Pfeffer and Dobler 2010), 2.) WNv must be maintained in an avian-mosquito transmission cycle, primarily transmitted through mosquito vectors of *Culex* species (Hurlbut 1956, Hubalek 2008), and 3.) avian species must be able to serve as natural reservoir hosts (Seidowski et al. 2010). The likelihood of human infection appears to increase as environmental conditions promote viral amplification to surpass avian and mosquito populations and overflow into subsidiary host groups (Ezanwa et al. 2007).

Although some bird species, primarily corvids and birds of prey, can succumb to infection, most wild avian species are asymptomatic. These species, particularly passerines or perching song birds, are capable of becoming the primary viral competent

vertebrate hosts, producing viremias high enough to support the transmission cycle (Pfeffer and Dobler 2010). Because of the dependence on birds in the transmission cycle, WNv is often considered a dispersing arbovirus. Its ability to disperse over the total continental U.S.A, southern parts of Canada and parts of Central and South America suggests viral movement to be via the migration patterns of competent bird species (Pfeffer and Dobler 2010).

Due to the importance of avian reservoirs and their influence on overall viral amplification and movement of WNv across North America, numerous studies have been performed to better understand the role of birds in WNv amplification and transmission. While infection of West Nile virus has been detected in small mammals and rodents, these organisms do not produce high enough viremia to ensure amplification and passage of the virus compared to Passerine birds which are considered the main vertebrate host for WNv (Hubalek 2008).

Furthermore, community level models that analyze the interactions between viruses and their vectors can provide useful tools for gaining an understanding of disease outbreak (Orme-Zavaleta et al. 2006).   Understanding the modes of interaction between a vector and host, whether through habitat, migration, or diet, establishes a pivotal role in gaining a further grasp of risk assessment and analysis of viruses and diseases. According to Weaver et al., 2010, appreciation of the concept that the introduction and migration of large and "non-immune" populations of birds serve as efficient maintenance hosts for viruses is critical to the mapping and characterizing of a virus.

The purpose of my study was to analyze avian populations at study sites located across Montana that are known to have mosquito populations with *Culex* vectors. After

identifying bird species, total abundance of birds, and the number of Passeriformes at each field site, bird data was compared to against total mosquito populations and *Culex tarsalis* populations. Relationships between avian densities and mosquito densities were examined to test the hypothesis that mosquito population numbers would be proportional to avian population numbers. In addition, data was analyzed to determine whether or not relationship between abundance of passerine species and abundance of mosquitoes exists in order to better describe high-risk spots for WNv.

Materials and Methods

GIS Mapping and Establishment of Sampling Sites

Two distinct locations in Montana were selected as field sites: Canyon Ferry Lake and Ninepipe National Wildlife Refuge. Within each location 2 mosquito traps were placed. With the mosquito traps serving as the first bird sample sites, 5 additional sites were randomly placed around each trap within a 1000 meter buffer zone. Each sample site was circular with a diameter of roughly 100 meters, and sample points were not allowed to overlap. GIS mapping was used to insure accuracy of placement. With 2 mosquito traps at each locations and 6 birding sites around each trap, there were a total of 24 bird sample locations.

Avian Data Collection

Visual Avian Identification

Sampling started on July 7, 2010 and was completed weekly until August 24, 2010. Sampling time was typically between 7 a.m. to 10 a.m., when

birds are most active. Sampling took place for 10 minutes within each center of the 100 meter sampling sites. Birds were visually identified to species. The total number of each species was recorded into two distance categories; greater than 50 meters from the sampling point and less than 50 meters from the sampling point. The birds identified while perching or passing through the sample point were placed within the less than 50 meters category, while the birds seen passing outside the sample point were recorded in the greater than 50 meters category. The Sibley Field Guide to Birds, Nikon 8 x 40 binoculars, and the online Cornell Lab of Ornithology were used to confirm visual species identification.

Auditory Avian Identification

Birds that were vocalizing but not observed visually were identified using songs and calls. The Sibley Field Guide to Birds, The Cornell Lab of Ornithology, and auditory recording devices were used in supplementing the accuracy of auditory species identification.

Recording of Data

Data were compiled and placed into Excel documents. The total number of each species was recorded from July 7, 2010 to August 24, 2010. Data were further subdivided by family, and birds known to be virally competent. Data were compared to mosquito numbers from mosquito traps operating on the same dates as bird sampling.

Mosquito Sampling

Mosquitoes were collected at 6 sites in Montana, from July 7, 2010 to August 24, 2010 on the nights prior to bird sampling. Trapping was performed with CDC light traps using dry ice as a CO_2 source. The CDC traps were set up near dusk and then collected the following morning before performing bird sampling. The mosquitoes were frozen at -20ºC upon returning to the laboratory. After 2 days, the mosquitoes were sorted and quantified by species using dissection microscopy.

Statistical Analysis

To observe overall temporal trends, graphical analysis was used to plot mosquito and bird abundance over time. Pearson's correlation analysis was used to test for associations between mosquito and bird abundance. The total number of mosquito species and the total number of *C. tarsalis* were compared to the total number of bird species, the total number of individual birds, the number of passerine bird species and individuals and the number of virulent competent species and individuals. Finally, multivariate analysis of variance (MANOVA) was used to test for mosquito and bird differences between trapping locations.

3. Results

Graphical analysis revealed an overall slight decline in the total number of bird species, number of passerine species and number of virulent competent species over the course of the summer (figures 3-6). Pearson's correlation analysis found no significant correlation between bird numbers and the number of *C. tarsalis*. However, there was a significant positive association between the number of passerine species and the total number of mosquitoes. This association may be due to site effects. MANOVA revealed a

significant difference between trapping locations (Wilk's lambda = .29 p =.003). Canyon

Ferry had significantly higher number of passerine species (F = 4.94 P = .008) and total

number of mosquitoes (F = 6.05, p = .003) than Ninepipe, but also a significantly lower

number of *C. tarsalis* (F = 7.09, p = .001) (Table 1).

4. Discussion

The amplification and migration of WNv is thought to be promoted and

maintained via bird reservoir hosts in the wild. In addition, Passeriform birds are

considered to have a higher reservoir competency for the virus than other avian families

(Komar et al. 2003). The principal goal of this study was to examine this relationship and

numerically analyze any correlation that exists between mosquito populations and the

bird reservoir host populations found in Montana. What was observed over the 8 week

sampling period was that no temporal relationship existed between *Culex* mosquito

abundance and avian numbers at the chosen test sites. It was hypothesized that at the start

of the sampling period, total bird numbers would start high, and as *Culex* mosquito

numbers increased and peaked midway into the sampling period the bird numbers would

decline causing the mosquitos to move toward other vertebrates such as horses and

humans and away from birds for blood meals. As figures 3-6 show, this hypothesis was

observed to be wrong as bird numbers remained relatively constant while *Culex* mosquito

numbers increased, peaked, and then declined independently. These observations may, in

part, be due to the environmental conditions that were present during the sampling period

across Montana. A relatively wet and mild summer that extended into the fall months was

experienced and, consequently, bird numbers appeared to remain high into the fall

months.

A positive correlation was observed between total mosquito numbers and total Passeriformes abundance. These findings support the hypothesis that there would be a large presence of passerine species in the areas with larger populations of mosquitoes. However, it is likely that the overall association observed between total number of mosquitoes and passerine abundance is due to site differences between Ninepipe and Canyon Ferry. Although Canyon Ferry had relatively few *Culex tarsalis* throughout the summer, a relatively mild, wet summer allowed other mosquito species to become abundant. Canyon Ferry had significantly higher numbers of mosquitoes as well as higher numbers of passerine species, thus explaining the overall association observed in the data.

The viral cycle between competent bird hosts and mosquito vectors undoubtedly occurs for the virus to maintain its presence in Montana. Despite the lack of correlation that was observed between bird numbers and *Culex* mosquitos over the course of the summer, this study has helped demonstrate that passerine birds are prevalent during the season when total mosquito numbers are high. Further investigation during more typical summer seasons and at different locations may help explain how the virus maintains its presence in Montana.

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Table 1: Pearson Correlation Coefficients and Associated P-values
(MOSTOT = Mosquito Total; MOSCUL = Culex Total; BIRDSPP = Bird Species Total; BIRDTOT = Bird Total; PASSSPP = Passerine Species Total; PASSTOT = Passerine Total; VIRSPP = Virulent Competent Species Total; VIRTOT = Virulent Competent Total

	MOSTOT	MOSCUL	BIRDSPP	BIRDTOT	PASSSPP	PASSTOT	VIRSPP	VIRTOT
MOSTOT	1	-0.2918	0.1689	0.1463	0.456	0.3256	-0.1172	-0.2877
MOSCUL	p=.132	1	-0.2279	-0.1846	-0.2423	-0.1513	-0.0811	-0.0935
BIRDSPP	p=.390	p=.243	1	0.4579	0.8021	0.3267	0.7162	0.2915
BIRDTOT	p=.457	p=.347	p=.014	1	0.3173	0.8327	0.47	0.6575
PASSSPP	p=.015	p=.214	p=.000	p=.100	1	0.3762	0.44	-0.0089
PASSTOT	p=.091	p=.442	p=.090	p=.000	p=.048	1	0.2643	0.1661
VIRSPP	p=.552	p=.682	p=.000	p=.012	p=.019	p=.174	1	0.4682
VIRTOT	p=.138	p=.636	p=.132	p=.000	p=.964	p=.398	p=.012	1
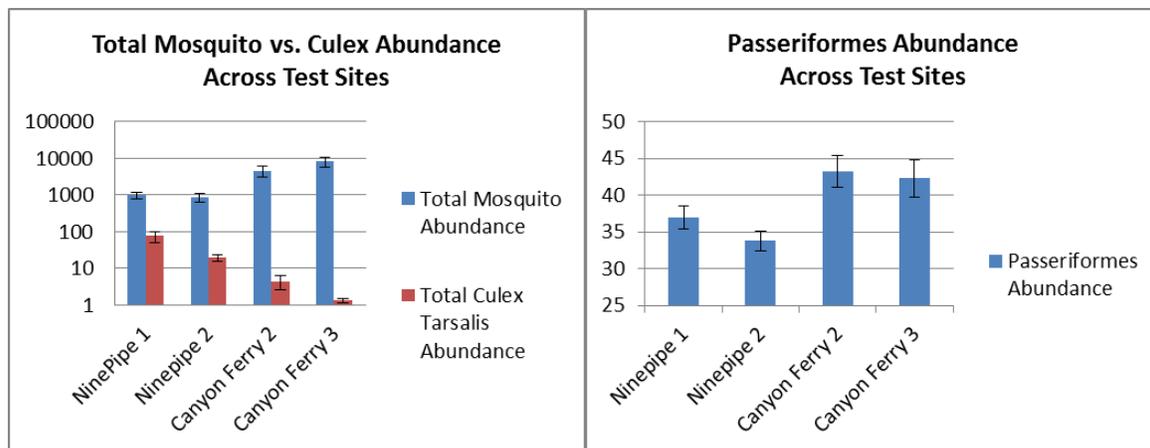
Figures 1-2: Absolute abundance of both mosquitos and Passeriformes across test sites in Montana
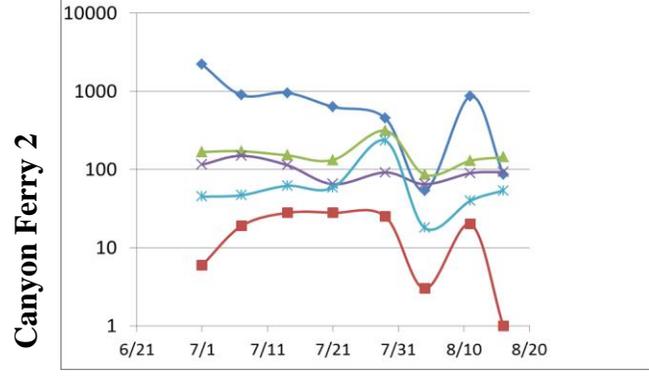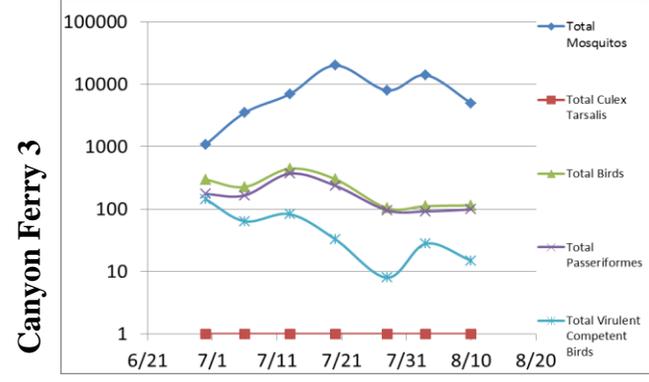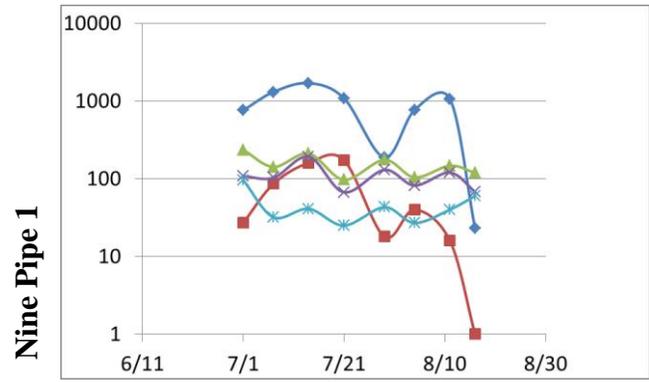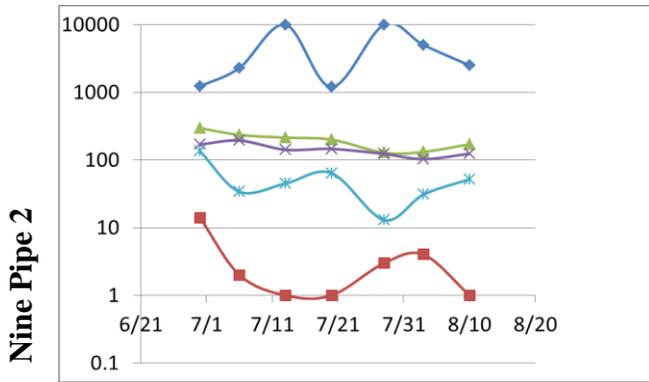
Figures 3-6: Graphical analysis of avian species against mosquito population numbers over time at 4 study sites in Montana.