EXPLORING MOSQUITO DIVERSITY AND DYNAMICS ACROSS WASHINGTON STATE AS THEY RELATE TO WEST NILE VIRUS TRANSMISSION

By

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ABSTRACT

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Preventing mosquito-borne disease is dependent on knowledge of mosquito fauna, activity patterns, and the efficacy of management techniques. This project investigated these aspects of mosquito biology in Washington State (WA), in light of the recent spread of West Nile virus (WNV). In WA there are 44 recorded species of mosquitoes, but the distribution of species across the state is not well documented. To examine mosquito diversity in southeastern WA, mosquito-trapping surveys were conducted in five counties. The surveys resulted in the collection of seventeen species (8 new species records for four counties). Mosquito species diversity and activity patterns were examined in three areas of the state representing different levels of WNV activity. The diversity of "bridge" vector mosquitoes (bird and mammal feeders) was roughly equal in areas of differing WNV activity, however, abundance and activity of selected species was greater in areas with greater WNV transmission. In addition, activity levels (trap night catch and mosquito abundance) were positively associated with temperature. This temperature relationship helps explain the observation of higher mosquito activity levels in areas with greater WNV transmission. Mosquito control in WA is accomplished primarily with larvicide *Bacillus thuringiensis israelensis* (*Bti*). Populations of *Culex pipiens* mosquitoes collected from Grant County (frequent *Bti* use), Spokane County (no *Bti* use) and Whitman

County (no *Bti* use) were analyzed for development of *Bti* resistance. Based on comparisons of resistance among populations, no development of resistance was evident. The results of these studies have improved the knowledge of mosquito biology in WA and have provided future direction for studies concerned with pathogen transmission by mosquitoes in this state.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

LIST OF FIGURES

PAGE

CHAPTER ONE

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are a large and well-known group of insects that are found on every continent except Antarctica (Mullen and Durden 2002). The family is divided into two subfamilies based on larval morphology. The subfamily Anophelinae contains three genera and the subfamily Culicinae contains 41 genera, making up about 3,490 recognized species worldwide (Harbach 2007). One hundred seventy four species and subspecies in 14 genera are recorded in North America (Darsie and Ward 2005) and 44 species from six genera (*Aedes*, *Anopheles*, *Coquillettidia*, *Culex*, *Culiseta*, and *Ochlerotatus*) are recorded in Washington State (WA) (Sames et al. 2007). Some of the more important species in WA include *Aedes vexans* (Meigen), *Coquillettidia perturbans* (Walker), *Culex pipiens* Linnaeus, and *Cx. tarsalis* Coquillett, all of which are potential vectors of West Nile virus (WNV). *Aedes vexans* is one of the "floodwater" mosquitoes and can be found in large numbers in irrigated and inland floodwater areas. *Coquillettidia perturbans* breeds in marshes, ponds, and lakes. This species requires permanent bodies of water that have cattails or other aquatic vegetation that the larvae penetrate with specialized siphons to obtain oxygen. *Culex pipiens* is commonly called the "Northern House Mosquito" and is most abundant in urban and suburban areas. *Culex tarsalis* is probably WA's most widespread species and can be found in a wide variety of habitat, including artificial containers and permanent or semi-permanent waters that are either clean or polluted.

Adults can be distinguished from other Diptera by their characteristic wing venation, the scales along the wing veins, and the long proboscis (Triplehorn and Johnson 2005). The aquatic habitats of the larvae and pupae range from permanent surface water (e.g. pools, streams, swamps, and lakes) to temporary surface water (e.g. flood water, vernal pools, and

tidal pools in salt marshes) to various natural and artificial containers (e.g. tree holes, leaf axils, mollusk shells, pitcher plants, and discarded tires) (Mullen and Durden 2002). The chemical characteristics of the water inhabited by mosquitoes also vary widely. Larvae can be found in clear water with low organic concentrations to water with high organic concentrations, fresh water to brackish water, acidic water, and polluted water. The only absolute requirement of any developmental site is that it must maintain at least a film of water for the duration of the immature stages to allow for development (Mullen and Durden 2002).

Mosquito larvae develop through four instars and most species are free swimming. They require access to the water surface to breath, which is done through a tube called the siphon in culicines, or through spiracular lobes in anophelines (Becker et al. 2003). Species of *Coquillettidia* and *Mansonia* remain submerged and have a siphon that can pierce plant tissue from which the larvae obtain oxygen. Harbach (2007) reported that *Aedeomyia* larvae use enlarged antennae for respiration and that some species have enlarged anal papillae that enable them to obtain oxygen from the water (Harbach 2007). Larvae feed on microorganisms, algae, protozoa, and detritus (Becker et al. 2003), which they filter from the water surface or water column, or collect from submerged substrates. Predacious larvae, such as *Toxorhynchites* and some *Psorophora* species are predators of invertebrates, including other mosquito larvae. The time required to complete the life cycle depends on temperature and food resources. Typically, larvae hatch from eggs within 2-3 days after the eggs are laid. Eggs of floodwater species that are laid on the soil must delay hatching until they are inundated by water. The larval stage generally lasts 7-14 days and the pupal stage 1-4 days. The average life span of the adult is 1-2 months (WA DOH 2008a).

Pupae also require access to the water surface to breath. The siphon of the larvae is replaced with a pair of respiratory trumpets through which pupae obtain oxygen. *Coquillettidia* and *Mansonia* pupae have specialized respiratory trumpets that penetrate plant tissue to obtain

oxygen. Pupae of these genera remain attached to the host plant and do not surface until the adults are ready to emerge. Pupae do not feed (Becker et al. 2003).

Male mosquitoes are typically smaller than females and emerge one to two days before the females. Mating usually occurs a few days after adult emergence. Both genders feed on sugar obtained from flower nectar, honeydew, rotting fruit, or other natural sugar sources for the energy needed for mating, dispersal, and seeking hosts (Harbach 2007). Only females are blood-feeders. Females of some species can produce their first batch of eggs without taking a blood meal (autogenous), whereas others require a blood meal for egg development (anautogenous). Autogeny occurs at low frequencies among many mosquito species, and is enhanced by good larval nutrition, a carbohydrate source upon adult eclosion, and the opportunity to mate (Mori et al. 2008).

Host preferences vary widely, depending on the innate preference of the mosquito and the availability of hosts when and where the mosquito is active (Mullen and Durden 2002). Mosquitoes locate hosts by orienting to host-associated volatile cues such as CO_2, lactic acid, and octenol, as well as by using visual and thermal cues. Depending on the species of mosquito, hosts include many species of vertebrates such as mammals, birds, lizards, toads, and snakes. Non-vertebrates such as nymphal cicadas, lepidopterous larvae, and mantids can be hosts as well (Harbach 2007). Mosquitoes also vary in the time of day they actively seek hosts. There are diurnal, crepuscular, and nocturnal host seeking species.

Eggs are laid two to four days after a blood meal and, depending on the species, will be deposited on the surface of the water or on moist soil in areas that will eventually be flooded by snow melt, rising rivers, or irrigation. Eggs that are laid on the water surface are glued together to form rafts (*Culex, Culiseta, Coquillettidia*) or are laid singly (*Anopheles*). Eggs that are laid on the soil (*Aedes, Ochlerotatus*) are laid singly and will not hatch until inundated by water. As a result floodwater species undergo diapause as eggs before hatching. Species that lay their eggs in this manner are known as “floodwater” mosquitoes because the mosquitoes don’t

appear until after dry ground has been flooded. These eggs can remain viable for several years. Many species of *Aedes* will not hatch until the water oxygen content is low, ensuring that the larvae will not hatch in running water or deep water in which the larvae cannot survive (Becker et al. 2003).

Mosquitoes are the most important arthropods affecting human health (Mullen and Durden 2002). The blood feeding habits of the females make many species of mosquitoes important as nuisance pests. Their greatest impact, however, comes from their ability to vector the pathogens that cause human diseases such as malaria, yellow fever, dengue fever, filariasis, and encephalitis, which cause the deaths and debilitation of millions of individuals every year. Well over 100 species of mosquitoes are pathogen vectors (Harbach 2007).

Mosquito-borne diseases have not been of great concern in WA for several decades. From 1939 to 1942 annual epidemics of mosquito-borne encephalitis resulted in 151 human cases (Hammon et al. 1945). St. Louis encephalitis and western equine encephalitis were last reported in 1972 and 1988, respectively (WA DOH 2008a), and the last endemic case of malaria was reported in 1944 (Gjullin and Yates 1945). However, with the introduction of West Nile virus (WNV) into New York in 1999 and its rapid spread across the United States – WNV was first detected in WA in 2002 – mosquito surveillance and management efforts have become more important. Sixty-four species of mosquitoes have been found infected with WNV in the United States (CDC 2009); 22 of these species are found in WA. Because of their broad host range and their wide distribution throughout the state, *Cx. pipiens* and *Cx. tarsalis* are WA's most efficient WNV vector species (WA DOH 2008a).

Washington State is divided by the Cascade Mountain Range into two regions that differ in climate. West of the Cascades a marine west coast climate predominates with mild temperatures, frequent cloud cover, and long lasting drizzles; summer is the driest season. East of the Cascades the climate is drier and has wider temperature extremes. Geography differs between these regions as well. Western WA has an additional mountain range that

parallels the Cascades, the Olympic Mountains, and the Puget Sound Lowlands. Eastern WA is dominated by the Columbia Plateau that includes the Palouse country in the southeast corner of the state. A portion of the Rocky Mountains cuts across the northeast corner of the state, and the northern end of the Blue Mountains stretches into the southeast corner. These differing climates and geographies provide for a diverse mosquito fauna within the state. Most of the 44 mosquito species recorded from the state are found on both sides of the Cascades, however, there are two species (*Culiseta particeps* (Adams) and *Ochlerotatus togoi* (Theobald)) that have been recorded from the west side only, and two species (*Oc. canadensis canadensis* (Theobald) and *Oc. cataphylla* (Dyar)) that have been recorded only from the east side.

In 1957, the first mosquito control districts (MCD) were formed in seven counties of south-central WA. There are now 16 MCDs throughout the state. The majority of mosquito management efforts focus on controlling mosquitoes while they are in the immature stages as they are concentrated, accessible, and unable to disperse. Controlling the adult stage is done only when populations become so large that they cause extreme annoyance or when the threat of disease transmission is high. These efforts are often complicated by the declining susceptibility of mosquito populations to the insecticides used in chemical control efforts. This genetically based phenomenon is known as insecticide resistance, and it eliminates one of the most effective tools for managing mosquito populations and the pathogens they vector. Insecticide resistance has been a problem in all insect groups that serve as vectors (Brogdon and McAllister 1999). Preventing, or at least delaying, resistance must be a consideration when developing effective integrated mosquito management plans.

Where MCDs exist and surveillance takes place, the mosquito fauna is well known. However, little surveillance occurs in the absence MCDs, and in some areas of the state the mosquito fauna is not well documented. Such is the case for southeast WA. The following chapters document efforts to increase the knowledge of the mosquito species in this part of the state. I also explore some of the possible components driving the transmission of WNV within

the state and test mosquitoes for the development of resistance to a commonly used insecticide.

CHAPTER TWO

DISTRIBUTION OF MOSQUITOES IN SOUTHEAST WASHINGTON STATE

Introduction

Central to the management of West Nile virus (WNV) and other mosquito-borne diseases is an accurate knowledge of the mosquito fauna. Mosquitoes vary widely in spatial, temporal, and trophic requirements, and in their capacity to vector disease causing pathogens. Without knowledge of the endemic species, it is impossible to know when, where, or if mosquito management efforts are required.

Approximately 44 species of mosquitoes have been recorded from Washington State (WA) (Sames et al. 2007). Mosquito surveillance within WA has been patchy and inconsistent, depending on budgets. Surveillance was conducted in 13 counties in 2008 - when WNV surveillance began - rose to 31 counties in 2003, then fell to 16 counties by 2008. Areas with greatest surveillance are those covered by mosquito control districts and large urban areas. No surveillance activities have been conducted in Asotin, Garfield, and Columbia counties in the southeast corner of the state since at least 2001 (Brauner 2008). Prior to this survey only two mosquito species were known from Asotin County, three from Garfield County, five from Columbia County, and fourteen from Whitman County (Sames et al. 2007). These numbers are certainly low and this lack of knowledge of the mosquito species found in southeast WA hinders effective integrated mosquito management. Inadequate knowledge of county mosquito distributions impacts the ability to determine species occurrence and distribution within the state and the ability to identify geographic areas where mosquito species occur. This makes pinpointing where potential vector populations occur difficult.

Methods and Materials

Adult Mosquito Surveillance

In order to improve our understanding of the diversity and distribution of mosquito species found in the southeast corner of WA, weekly sampling was conducted at three sites from March through September 2008 using two types of traps. Encephalitis Virus Surveillance traps were placed at each site and baited with dry ice and light. Also placed at each site were Reiter/Cummings gravid traps baited with a mixture of rabbit pellet food and water that was allowed to ferment for at least two days. One liter of the mixture was then placed into the trap tub and water was added until the water level was within two inches of the bottom edge of the tube entering the collection chamber. Both traps were set for approximately 17 hours one night per week.

Weekly survey sites included Camp William T. Wooten State Park, Columbia County; Spring Lake, Columbia County; and the city of Pullman, Whitman County (Table 2a and Fig. 2a). Satellite images and photographs of each site are shown in Figures 2b and 2c, 2d and 2e, and 2f and 2g for Camp Wooten, Spring Lake, and Pullman, respectively. Sites were selected for importance of mosquito-borne disease risk, security from vandalism of surveillance equipment, and ease of access. In addition, adult mosquitoes were sampled for one night each at Turnbull National Wildlife Refuge, Spokane County; Moses Lake, Grant County; and a site approximately 2 km south of the Elmer C. Huntley-Central Ferry Bridge on the Snake River, Garfield County (Table 2a and Fig. 2h).

Larval Mosquito Surveillance

Larval mosquitoes were collected from habitat near the weekly surveillance sites as well as from habitat along routes to and from the sites.

Mosquito Activity

In order to present adult mosquito activity over the trapping season, standardized catch trap means were plotted with mean temperature and mean precipitation by week for those mosquito species that were collected more than once during the season (Fig. 2i).

Results and Discussion

Adult Mosquito Surveillance

Table 2b presents the mosquito species collected from the three weekly surveillance sites, Turnbull National Wildlife Refuge, and the Moses Lake area. A total of six genera and 11 species were collected from the three weekly surveillance sites. An additional five species were collected from the single night trapping sites. Only one mosquito (*Culiseta inornata* (Williston)) was collected from the Garfield County site. The survey resulted in nine new county records from four counties. Table 2c presents Eastern WA mosquito species by county, with new county records (†) included. Also included in Table 2c is a new Asotin County record of *Culex pipiens* Linnaeus collected by the WA Department of Health in 2007 that was never formally reported.

Two species, *Cx. pipiens* and *Aedes vexans* (Meigen), were collected in Pullman but not at either of the other two sites. Spring Lake and Camp Wooten are located in the Tucannon River drainage at the northern end of the Blue Mountains, where few individuals live and no large industrial, civil, or agricultural activities exist, resulting in relatively pristine riparian areas with clear water that is low in organic content and pollution. Vegetation in this area is of the Ponderosa Pine Type (Highsmith and Bard 1973) and consists of yellow pine trees, cottonwood trees, and various species of shrubs, including chokecherry, wild rose, and willows. Pullman, on the other hand, has a relatively large population and more industrial, civil, and agricultural activities that result in water with high organic content and pollution. The plant community in

this area is dominated by agricultural crops with patchy forested areas. Since *Cx. pipiens* larvae prefer water containing organic pollutants (Harmston and Lawson 1967), Pullman provides suitable habitats for this species while Spring Lake and Camp Wooten do not. *Aedes vexans* larvae occur in transient water such as pools created by flood water, rain-filled depressions, and ponded irrigation water (Harmston and Lawson 1967). The Tucannon River drainage is relatively steep and well drained so the preferred habitat of this species is relatively rare compared to the less well drained, rolling hills of the Palouse that surround Pullman.

Species that were collected at Spring Lake and Camp Wooten but not at Pullman included *Anopheles punctipennis* (Say) and *Cx. territans* Walker. *Anopheles punctipennis* larvae are found in cool, fresh water habitats with permanent or semi-permanent water such as margins of slow moving streams, permanent pools, clear shaded pools, and roadside ditches (Harmston and Lawson 1967, Gjullin and Eddy 1972). Larvae of *Cx. territans* are found in permanent to semi-permanent pools containing considerable vegetation (Gjullin and Eddy 1972). As described above, the Tucannon River drainage provides more of the type of habitat that these species prefer than does Pullman and the surrounding area.

Larval mosquito surveillance

Included in Table 2c is a new Columbia County record (Curl Lake, Table 2a) of *Ae. cinereus* Meigen. Larvae of this species were collected on 13 May 2008 from a partially shaded, temporary pool with heavy emergent vegetation.

Mosquito activity

Mosquitoes were collected between week 19 (6 May 2008) and week 40 (30 Sep 2008). Mean temperatures on those dates were 10.8°C and 10.2°C, respectively. Species that develop in permanent or semi-permanent water (*An. freeborni* Aitken*, An. punctipennis*, and *Coquillettidia perturbans* (Walker)) were active for a longer period and more consistently than

species that develop in more transient water (*Cx*. *pipiens*, *Cx. tarsalis* Coquillett, and *Ochlerotatus sierrensis* (Ludlow)). None of the species collected, with the possible exception of *Cs. inornata,* seemed to be strictly dependent on precipitation. *Culiseta inornata* was collected four weeks after rainfall that occurred on week 21 and then again three weeks after rainfall that occurred on week 30. The shorter delay from precipitation to mosquito activity on the later collection could be the result of higher temperatures that shortened development time. This temporal distribution could also be driven by temperature or day length, as adults of this species have been reported to aestivate (Gjullin and Gains 1972, Harmston and Lawson 1967). *Ochlerotatus increpitus* (Dyar) has a similar bimodal distribution. This species inhabits temporary pools filled by snow melt, rainwater, and stream overflow so tends to appear early in the season (Harmston and Lawson 1967). Bohart and Washino (1978) found *Oc. increpitus* in pools under moderate to dense shade. The later peak may be due to these types of situations providing habitat later in the season.

The collection of nine mosquito species from sites where they were not previously known to occur has expanded the knowledge of the mosquito fauna in southeast WA, which improves the ability for mosquito management personnel to implement effective integrated mosquito management practices. Future surveillance efforts are needed to further increase the knowledge of mosquito species composition and distribution in this part of the state.

Location	Latitude (°)	Longitude (°)	Elevation (meters)	Max Temp	Min Temp	Mean Temp
Camp Wooten	N 46.239142	W 117.694508	892	33.5	-2.0	13.0
Central Ferry Bridge	N 46.655278	W 117.785000	198	-	-	-
Curl Lake	N 46.254429	W 117.673492	787	-	-	-
Moses Lake	N 47.048856	W 119.556039	345	20.5	4	11.3
Spring Lake	N 46.332994	W 117.676376	635	33.5	-4.0	13.7
Pullman	N 46.726447	W 117.139727	760	33	-3.0	13.0
Turnbull NWR	N 47.416111	W 117.53000	686	28.5	11.7	17.8

Table 2a. Mosquito collection sites: latitude, longitude, elevation, and temperatures. Suveillance took place between 21 Mar 2008 and 30 Sep 2008. All sites are in Washington State. Coordinate readings taken with DeLorme Earthmate GPS PN-20 Handheld GPS, 15 m accuracy, WGS84.

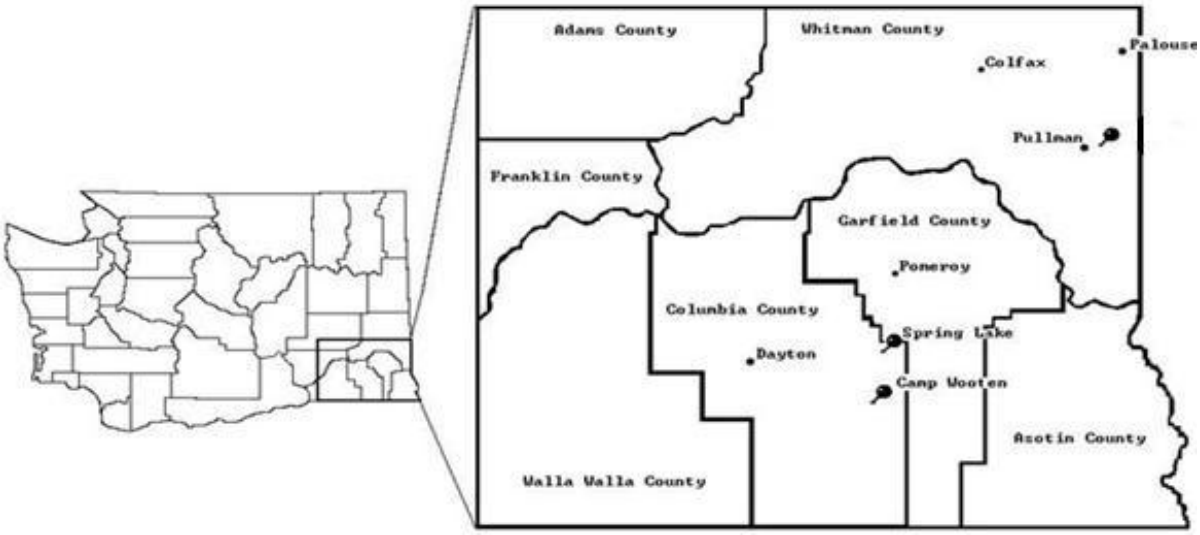


Figure 2a. Locations of weekly mosquito surveillance sites. Surveillance occurred between 21 Mar 2008 and 30 Sep 2008. All sites are in Washington State. Coordinate readings taken with DeLorme Earthmate GPS PN-20 Handheld GPS, 15 m accuracy, WGS84.

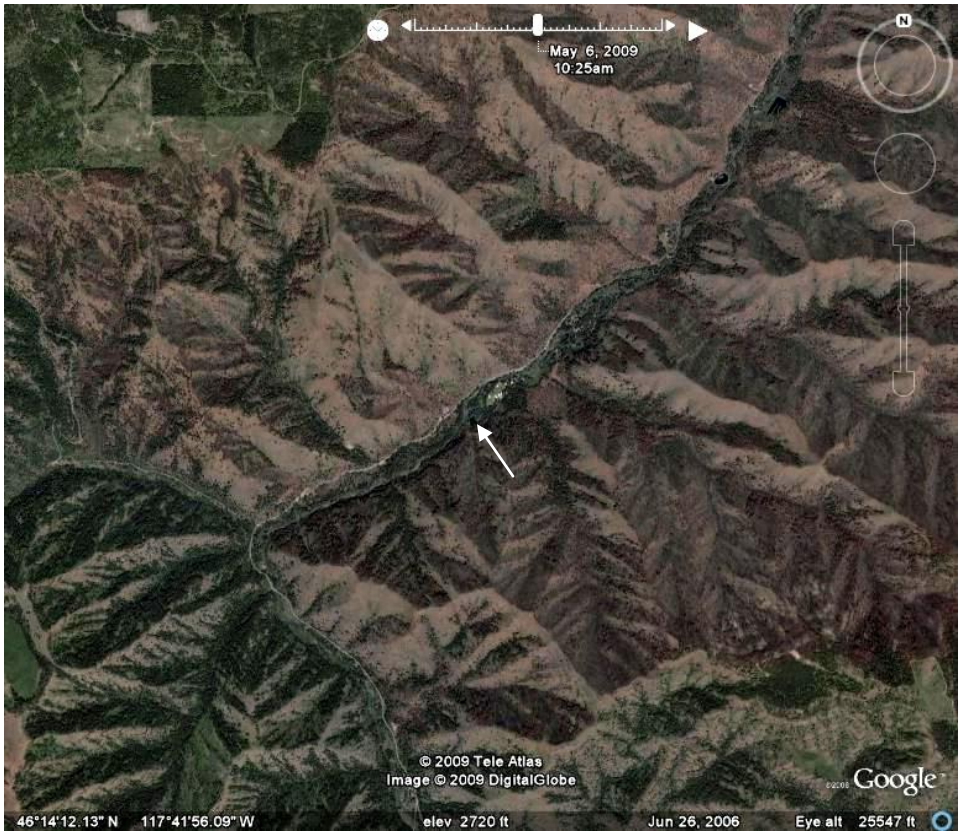


Figure 2b. Satellite image of ca. 3 km radius around Camp W.T. Wooten State Park. Arrow indicates location of pond.

Figure 2c. Photograph of pond at Camp W.T. Wooten State Park.

Figure 2d. Satellite image of ca. 3 km radius around Spring Lake.
Arrow indicates location of lake.

Figure 2e. Photograph of Spring Lake.

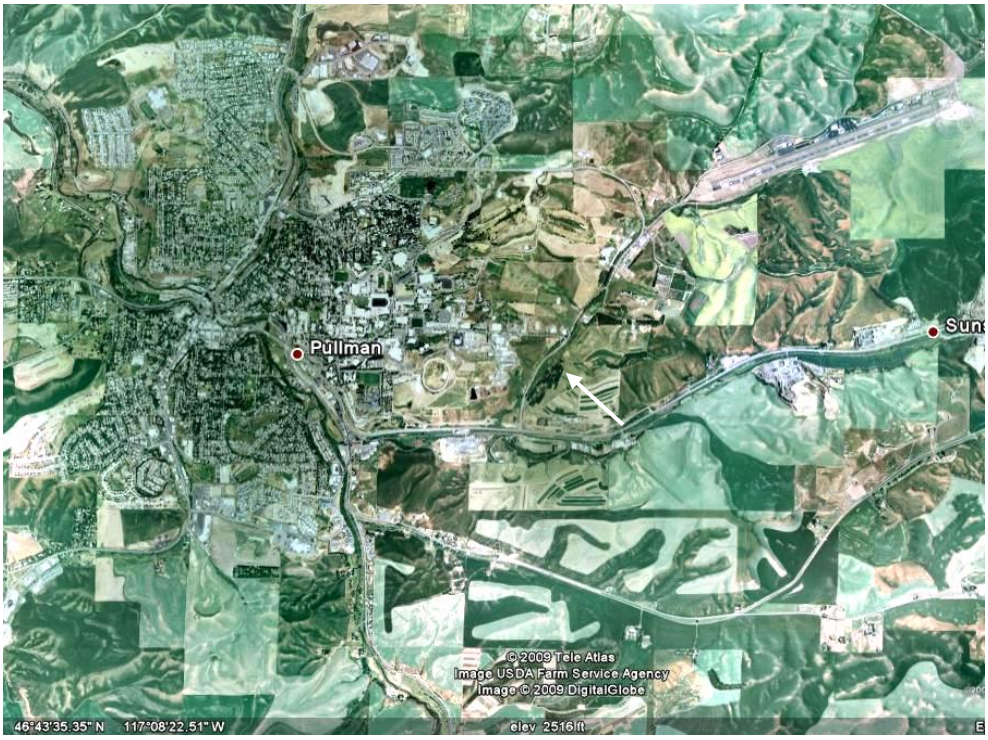


Figure 2f. Satellite image of ca. 3 km radius around the Pullman survey site. Arrow indicates location of pond.

Figure 2g. Photograph of the pond at the Pullman survey site.

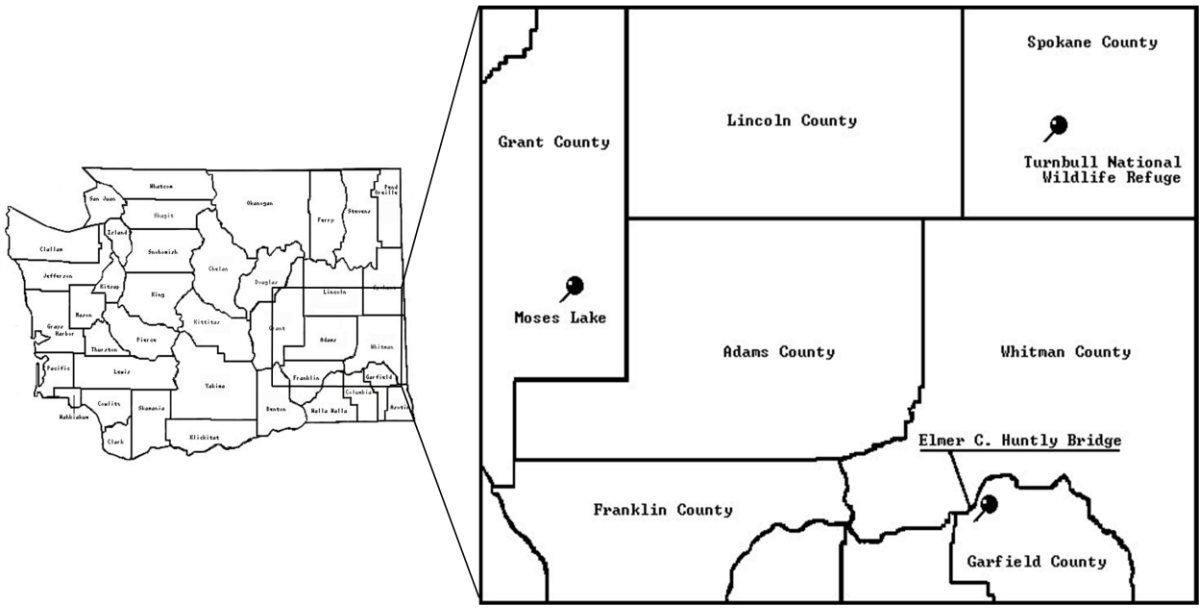


Figure 2h. Locations of additional, single night mosquito survey sites. Collection at Turnbull NWR, Moses Lake, and Elmer C. Huntley Bridge occurred on 14 Aug 2008, 21 Aug 2008, and 1 Sep 2008, respectively. All sites are in Washington State. Coordinate readings taken with DeLorme Earthmate GPS PN-20 Handheld GPS, 15 m accuracy, WGS84.

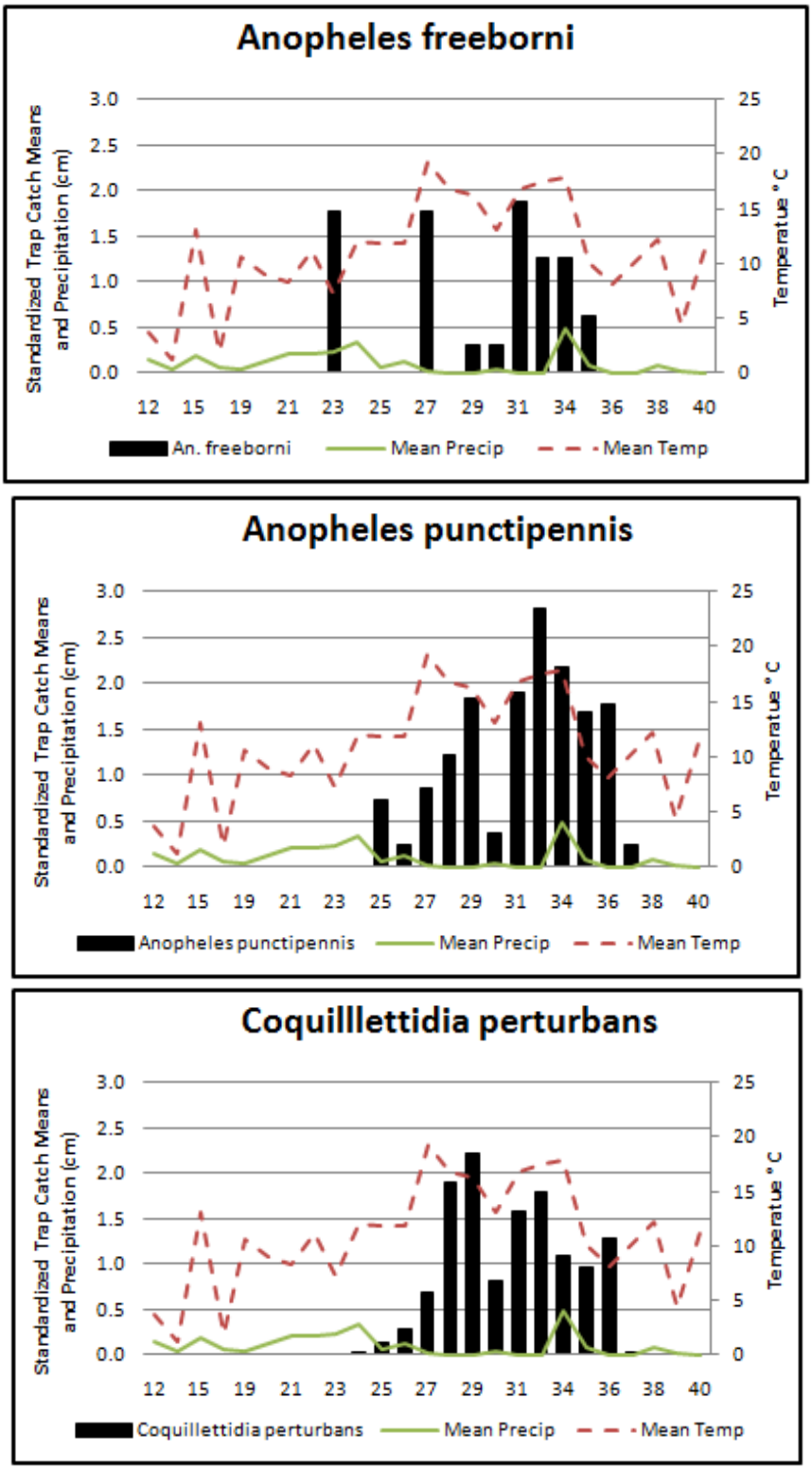


Figure 2i. Mosquito activity, mean precipitation, and mean temperature. Surveillance took place between 21 Mar 2008 and 30 Sep 2008 at three weekly surveillance sites in Washington State.

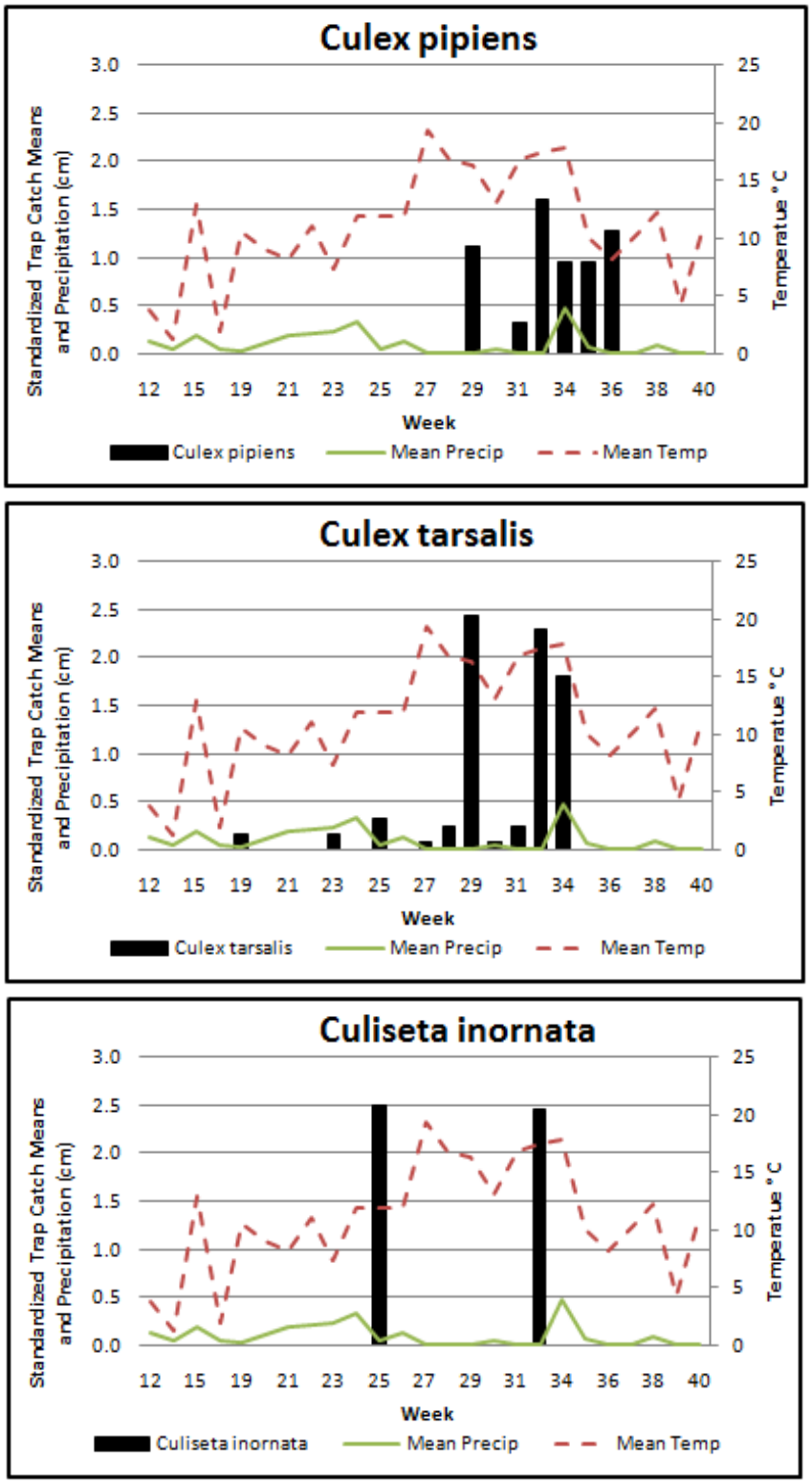


Figure 2i, continued. Mosquito activity, mean precipitation, and mean temperature. Surveillance took place between 21 Mar 08 and 30 Sep 08 at three weekly surveillance sites in Washington State.

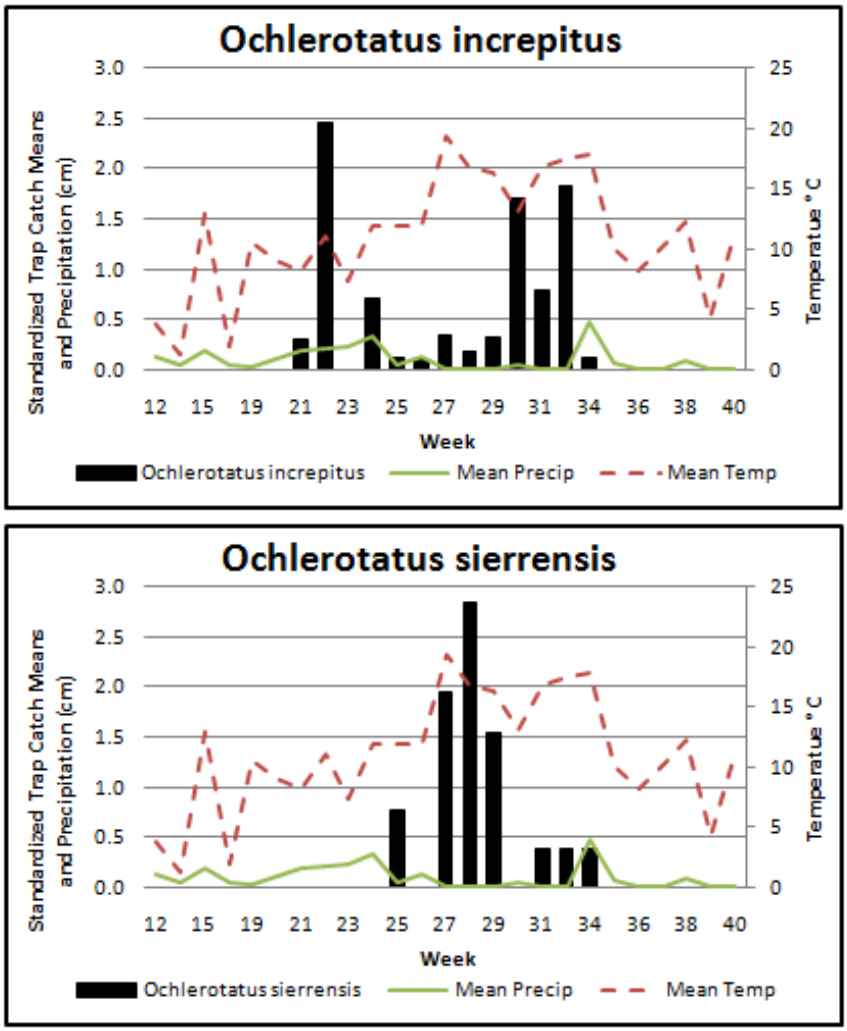


Figure 2i, continued. Mosquito activity, mean precipitation, and mean temperature. Surveillance took place between 21 Mar 2008 and 30 Sep 2008 at three weekly surveillance sites in Washington State

Survey Location	*Aedes vexans*	*Anopheles freeborni*	*Anopheles punctipennis*	*Coquillettidia perturbans*	*Culex pipiens*	*Culex tarsalis*	*Culex territans*	*Culiseta incidens*	*Culiseta inornata*	*Culiseta minnesotae*	*Ochlerotatus dorsalis*	*Ochlerotatus excrucians*	*Ochlerotatus fitchii*	*Ochlerotatus increpitus*	*Ochlerotatus melanimon*	*Ochlerotatus sierrensis*
Camp W.T. Wooten State Park			•			•	•	•	•					•		•
Spring Lake		•	•	•		•		•	•					•		•
Pullman	•	•		•	•	•		•	•					•		•
Turnbull National Wildlife Refuge		•		•		•	•					•	•			
Moses Lake	•	•		•	•	•			•	•	•				•	

Table 2b. Mosquito species collected from all survey sites.
Surveillance took place between 21 Mar 2008 and 30 Sep 2008.
All sites are in Washington State.

Eastern Washington Counties	Adams	Asotin	Benton	Chelan	Columbia	Douglas	Ferry	Franklin	Garfield	Grant	Kittitas	Klickitat	Lincoln	Okanogan	Pend Oreille	Spokane	Stevens	Walla Walla	Whitman	Yakima
Aedes cinereus				●	†		●			●	●			●	●	●	●	●		●
Aedes vexans	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Anopheles earlei														●	●	●	●			
Anopheles freeborni	●	●	●	●	●		●	●		●	●		●	●	●	●	●	●	●	●
Anopheles occidentalis			●					●						●	●		●			
Anopheles punctipennis			●	●	†			●	●		●			●		●		●	●	●
Coquillettidia perturbans	●		●		†		●	●		●	●			●		●		●	●	●
Culex apicalis																				●
Culex boharti													●							
Culex pipiens	●	†	●	●			●	●		●	●	●	●	●	●	●	●	●	●	●
Culex stigmatasoma			●									●						●		●
Culex tarsalis	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Culex territans			●	●	†		●			●	●			●	●			●		●
Culiseta impatiens				●							●						●	●		●
Culiseta incidens			●	●		●	●	●	●	●	●	●	●	●		●	●	●	●	●
Culiseta inornata	●		●	●	†	●		●	†	●	●	●	●	●		●	●	●	●	●
Culiseta minnesotae			●	●						†	●				●	●				●
Culiseta morsitans				●							●					●				●
Culiseta particeps																				
Ochlerotatus aboriginis				●			●				●	●				●		●	●	●
Ochlerotatus aloponotum					●															●
Ochlerotatus campestris			●					●		●				●		●			●	
Ochlerotatus canadensis							●			●				●	●	●	●			
Ochlerotatus cataphylla			●	●							●						●			
Ochlerotatus communis			●	●			●			●	●		●	●	●		●			●
Ochlerotatus dorsalis	●		●	●		●		●		●	●			●		●	●	●		●
Ochlerotatus excrucians				●							●		●	●	●	●				●
Ochlerotatus fitchii			●	●			●			●	●		●	●	●	●	●		●	●
Ochlerotatus flavescens				●						●			●	●		●				●
Ochlerotatus hexodontus				●			●				●	●		●	●					●
Ochlerotatus impiger				●			●							●	●					●
Ochlerotatus implicates				●			●							●	●					●
Ochlerotatus increpitus			●	●	●		●	●		●	●	●	●	●	●	●	●	●	●	●
Ochlerotatus intrudens				●			●								●					●
Ochlerotatus j. japonicus																				
Ochlerotatus melanimon	●		●			●		●		●				●		●		●		●
Ochlerotatus nevadensis				●													●			●
Ochlerotatus nigromaculis	●		●					●		●	●						●	●		●
Ochlerotatus pullatus				●										●	●	●				
Ochlerotatus sierrensis			●	●	†			●		●		●	●	●		●	●	●	●	●
Ochlerotatus spencerii idahoensis				●						●	●			●	●					●
Ochlerotatus sticticus			●	●			●	●			●			●	●	●		●	●	●
Ochlerotatus togoi																				
Ochlerotatus ventrovittis				●							●				●					

Table 2c. Eastern Washington State mosquito species by county.
Surveillance took place between 21 Mar 2008 and 30 Sep 2008.
† indicates new county records. ● indicates previous
records based on Sames et al. (2007)

CHAPTER 3

COMPONENTS OF WEST NILE VIRUS TRANSMISSION IN WASHINGTON STATE

Introduction

Emerging infectious diseases (EID) remain a leading cause of death worldwide with vector-borne diseases being responsible for almost 29% of the EID events in the last decade (Jones et al. 2008). The development of spatial and temporal models that provide the capability to describe, explain, predict, and communicate vector-borne disease risk and the outcome of interventions (Kitron 2000) is hindered by a lack of reliable data for probable pathogen exposure locations (Eisen and Eisen 2007). These models are important for disease prevention because vector-borne disease risk is spatially and temporally heterogeneous, depending on geographical and environmental conditions that affect arthropod vectors.

Pathogen transmission by an arthropod vector is dependent on a number of factors, including: ability of the arthropod to carry the pathogen, host preference, vector density, and distribution. Effective transmission is dependent on the competency of an arthropod to vector the pathogen. Vector competence is a physiological property of the arthropod that reflects its ability to become infected with (or carry) a pathogen and then transmit it to another host. Being a competent vector does not mean that a mosquito will play a significant role in pathogen transmission. Mosquito species vary in habitat preference, host preference, population density, and activity patterns (e.g. day versus night), all of which impact the likelihood that pathogen transmission will occur. These factors all play a role in how important any one species is in transmitting a pathogen and will determine what species are critical vectors for a given area.

West Nile virus (WNV) is currently the most widely distributed arbovirus in the world, occurring on all continents except Antarctica (Kramer 2008). The virus was initially isolated in Uganda in 1937 and up until the mid-1990s it was associated with mild to moderate febrile

disease in humans in Africa, the Middle East, and Eastern Europe. Since then it has been increasingly associated with severe human illness across the Mediterranean Basin, including outbreaks in Romania (1996), Russia (1999), France (2000), and Israel (2000) (Zeller and Schuffenecker 2004).

In the United States (U.S.), WNV was first detected in New York State in 1999 and spread quickly across the country, reaching the west coast by 2002 (Kramer 2008). By 2004 all 48 contiguous states had detected the virus. West Nile virus activity in Washington State (WA) has been lower than in its bordering states. The first detection in WA was in 2002 when two birds and two horses tested positive. The first human cases were reported in 2006, when infections were confirmed in three individuals. An additional three human cases occurred in 2008, which was the most active WNV season for WA since monitoring began in 2001. Whereas WA has had a total of six reported human cases over the 7-year period following initial detection, the bordering states of Oregon (OR) and Idaho (ID) have had 124 (OR DHS 2009) and 1,181 reported cases (USGS 2009), respectively, in the same period of time. Although WNV first appeared in WA the same year as it appeared in ID, and two years before it appeared in OR, annual total reported cases in WA have been fewer than in the latter two states until 2008, which was the first year that positive cases rose above the low historical numbers. However, human cases were still lower in WA. California has had far more WNV activity than WA, ID, and OR since 2003 (Table 3a). Current distribution and frequency of WNV detection in mosquitoes, horses, and birds indicate the virus is now established in south-central WA and is making inroads into western WA (WA DOH 2008b). The south-central area of WA has had most of the WNV activity and historical outbreaks of western equine encephalitis and St. Louis encephalitis have also occurred in this area of the state during the late 1930s and early 1940s (Reeves 1987).

Investigations into the low activity of WNV in WA are absent in the published literature. One possible explanation is that the mosquito vectors in WA are different than in the

surrounding states. The primary vectors of WNV in WA are *Culex pipiens* Linnaeus and *Cx. tarsalis* Coquillett (WA DOH 2008a). Recent studies have shown that activity of species other than those identified as the primary vectors may be more important in WNV transmission. For example, Rochlin et al. (2009) showed that locations with elevated human risk of WNV within Suffolk County, New York had the least abundant number of *Cx. pipiens* and *Cx. salinarius* Coquillett, which were the suspected vectors for that area.

Another potential explanation is that heterogeneous mosquito habitat across the state results in disjunct mosquito population dynamics that have impaired the establishment of robust WNV transmission cycles. Washington State is divided by the Cascade Mountain Range into two regions that differ in climate. West of the Cascades a coastal climate predominates with mild temperatures, frequent cloud cover, and long lasting drizzles; summer is the driest season. East of the Cascades the climate is consistently drier and has wider temperature extremes. Western WA has an additional mountain range (Olympic Mountains) that parallels the Cascades, and the Puget Sound Lowlands, which together provide wide variation in elevation and vegetation. Eastern WA is dominated by the Columbia Plateau that includes the Palouse region (rolling hills, un-irrigated agriculture) in the southeast corner of the state. A portion of the Rocky Mountains cuts across the northeast corner of the state, and the northern end of the Blue Mountains stretches into the southeast corner. These differing climates and geographies provide for a diverse mosquito fauna and produce differing mosquito activities within the state that impact WNV transmission.

To determine the factors affecting emerging WNV transmission in WA, mosquito surveillance and environmental data from three geographically and environmentally distinct areas of the state were analyzed. Two hypotheses regarding vector activity and WNV transmission were tested: (i) Areas with a higher diversity of bridge vector species have a higher incidence of WNV cases; (ii) Variation in vector abundance and activity correlates with temperature and humidity.

Methods

Interstate comparisons of WNV activity and vector diversity

WNV activity

The dates and locations (counties) of WNV activity were obtained from the U.S. Geologic Service (USGS) Disease Maps web site (USGS 2009) for WA, ID, and OR. Public attention to WNV tends to increase after veterinary and human cases increase and people are more likely to notice and report dead birds than they were prior to these cases. Therefore, WNV bird positive cases were left out of the 2008 data as they are not a reliable indicator of pathogen dynamics. Also excluded were data from sentinel chickens, as not all states have a sentinel chicken program.

Mosquito diversity

Comparisons of mosquito diversity between WA, ID, and OR were based on the reports of Sames et al. (2007), Brothers and Darsie (2003), and Darsie and Ward (2005), respectively.

Comparisons of WNV activity and vector diversity within WA

Total WNV activity

The dates and locations of WNV activity were obtained from the USGS Disease Maps web site (USGS 2009).

2004 and 2005 mosquito and WNV data

The relative abundance of mosquito species between counties with WNV activity and those without WNV activity was compared using mosquito data obtained from the 2004-2005 Surveillance Program Report: West Nile virus Environmental Surveillance in WA (WA DOH

2006). A county was considered to have WNV activity if WNV was detected any year from 2002-2008. Only counties and mosquito species that were included in the report were used in the analysis. Mosquito species were grouped into putative vectors (empirical evidence for role in transmission), suspected vectors (demonstration of WNV in vector; limited evidence of transmission), and those not suspected to be vectors (CDC 2009).

Mosquito diversity

Comparison of mosquito diversity in counties with WNV activity was conducted using mosquito species information taken from Sames et al. (2007) and WNV activity information from the USGS Disease Maps web site (USGS 2009). Mosquito diversity and seasonal activity within WA for the year 2008 was analyzed using data gleaned from mosquito surveillance records of mosquito control districts, public health departments, military installations, and surveillance conducted by the author (see Chapter 2).

Data were grouped into one of three zones within the state, based on geography (Fig. 3c). The West zone was in the southwest portion of the state, west of the Cascade mountain range. Mosquito data for the West zone were available from Cowlitz, King, and Thurston counties as well as from various military installations around the Puget Sound. The South-central zone was on the Columbia Plateau, which is dominated by irrigated agriculture and native shrub-steppe. Mosquito data for the South-central zone were available from Benton, Franklin, and Yakima counties. The Southeast zone was in the southeast corner of the state and includes the areas of the Palouse and the northern end of the Blue Mountains. Mosquito data for the Southeast zone were available from Columbia and Whitman counties.

Species included in the zone comparisons were limited to those collected from all zones and missing from not more than one county within a zone. Mosquito species that fit these criteria were *Aedes vexans* (Meigen), *Coquillettidia perturbans* (Walker), *Cx. pipiens*, *Cx.*

tarsalis, and *Culiseta inornata* (Williston). *Aedes vexans* was not collected in the Southeast zone during the time frame that was analyzed.

Due to considerable variation in trapping effort (dates trapping conducted and number of traps deployed) among sites across the state, comparisons were limited to a thirteen week period between 15 June 2008 and 13 September 2008 and only for collection sites (zip codes) that did not miss more than four weeks of surveillance during that period. In addition, collection sites were selected that had similar numbers of traps (n = 20-33), so that trapping effort was not significantly different among sites.

Temperature and precipitation data were obtained from weather stations that were within the collection site (zip code), or the weather station that was closest if there was not one available in that zip code (IPPC 2009). Temperature data for the Southeast zone were obtained from digital temperature data loggers placed with the mosquito traps. To compare 2008 precipitation with average precipitation, data were obtained from the National Weather Service Advanced Hydrologic Prediction Service web site (NWS 2009).

Vector Type diversity

Mosquitoes were grouped into one of three vector types, reflecting the potential to transmit WNV: enzootic vectors (bird feeders), bridge vectors (bird and mammal feeders), or non-vectors (exclusive mammal feeding species). Groupings were based on the work of Turell et al. (2005), Gjullin and Eddy (1972), Harmston and Lawson (1967), Belton (1983), and Bohart and Washino (1978). Opportunistic feeders were grouped with bridge vectors. Species that prefer bird or mammal hosts were placed into either the enzootic or bridge group, based on vector competence and relative host preference. Diversity within the vector type was characterized using Simpson's Index (Simpson 1949), Shannon-Weaver Index, and evenness (Weaver and Shannon 1949).

Analysis

Minitab 15® (Minitab Inc., State College, PA) software was used to perform all statistical analyses.

2004 and 2005 mosquito and WNV data

Statistical analysis to determine significance between mosquito abundance in counties with WNV activity and counties without WNV activity was done using the Kruskal-Wallis test.

Mosquito diversity

Trap Night Catch (the proportion of deployed traps that caught a mosquito species) was used to determine variation in spatio-temporal activity of species between zones. Trap Night Catch was positively correlated with abundance. Binary logistic regression was used for statistical analysis.

General linear model analysis was used to determine if statistically different variations in temperature and precipitation existed between zones.

Binary logistic regression was initially run with the following predictors: week, temperature, precipitation, and mean catch. For each species, any significant predictors from the initial regressions were included in subsequent regressions with interaction terms. Only samples with >1 trap per zip code were used.

Results

Interstate comparisons of WNV activity and vector diversity

WNV activity

From 2002 through 2008 there were a total of 6 reported human cases of WNV in WA, 121 in OR, and 1,184 in ID. Annual WNV cases for WA, ID, OR, and California (CA) are shown

in Table 3a. On the west coast of the U.S., WNV was first detected in 2002 in northern WA and southern CA. Activity stalled in WA, but became well established in CA and seems to have moved northward into OR and WA from there. Maximum WNV activity for ID and OR occurred in 2006, whereas maximum activity to date for WA was in 2008. The 2008 weekly WNV activity for all WA, ID, and OR is shown in Figure 3a. Washington State had more WNV activity than ID and OR. The bulk of activity in ID was from human cases with only one veterinary case. Oregon activity was split evenly between positive mosquito pools and human cases. There were no veterinary cases in OR. Washington State activity, on the other hand, came mostly from positive mosquito pools and horse cases. There were three human cases in WA. Both human cases and peak activity appeared later in WA than in ID and OR.

Mosquito diversity

Forty-four species of mosquitoes are recorded from WA, 51from ID, and 40 from OR. Many species have not been recorded from all three states (Table 3b). There are four species that are common to ID and OR that have not been recorded in WA. Of these, only *Cx. restuans* Theobald has been implicated as a WNV vector. Idaho has ten species that have not been recorded from WA or OR. Of these, *Cx. erythrothorax* Dyar, *Cx. salinarius* Coquillett, *Oc. provocans* (Walker), and *Oc. trivittatus* (Coquillett) have been found to be infected with WNV. Oregon has two species that have not been recorded from WA or ID. *Anopheles franciscanus* McCracken and *Orthopodomyia signifera* (Coquillett) have both been found to be infected with WNV.

Comparisons of WNV activity and vector diversity within WA

Total WNV activity

From 2002-2008 WNV was detected in 14 counties of WA (Fig. 3b). Half of these counties had only a single incident of WNV activity. Washington State annual WNV activity by county is shown in Table 3c. The occurrence of WNV throughout the state has been inconsistent through time, with the exception of Yakima County, which has had WNV activity every year since 2005. The majority (84%) of WNV activity has occurred in the south-central portion of the state.

2004 and 2005 mosquito and WNV data

Eleven of fourteen WNV-positive counties, and fourteen of twenty-five WNV-negative counties in WA were included in the comparisons of mosquito activity data for years 2004 and 2005 (Fig. 3b). Table 3d shows the relative abundance of mosquitoes from counties included in the 2004-2005 report. For putative WNV vectors (*Cx. pipiens* and *Cx. tarsalis*), mean mosquito catch per county was roughly 1.5x higher in WNV-positive counties compared to WNV-negative counties (Wilcoxan H = 5.16; DF = 1; P = 0.023). For suspected WNV vectors (20 species), mean mosquito catch per county was roughly 5.7x higher in WNV-positive counties compared to WNV-negative counties (Wilcoxan H = 3.65; DF = 1; P = 0.056). These differences are driven by only a few species that are found in much greater numbers in counties with WNV. For example, *Oc. sticticus* (Meigen), *Cq. perturbans*, and *Ae. vexans* (Meigen) had 222-fold, 14-fold, and 10-fold greater abundance, respectively, in counties with WNV activity. A few species were more abundant in counties with no WNV activity, but in much lower numbers, e.g. *Oc. dorsalis* (Meigen) and *Oc. nigromaculis* (Ludlow). Mean mosquito catch per county for species never documented to carry WNV (non-suspect) did not differ between counties with and without WNV activity (Wilcoxan H = 0.74; DF = 1; P = 0.388).

Mosquito diversity

Mosquito species were not evenly distributed among counties with WNV activity (Table 3e). Mosquito fauna for species known to be infected with WNV did not differ substantially between zones except for *Oc. nigromaculis* (Ludlow) which was found in all of the South-central zone counties, but in only one county outside this zone. *Ochlerotatus melanimon* (Dyar) was also found in three out of four South-central zone counties, but in only one county outside this zone. This was also the case with *Oc. spencerii idahoensis* (Theobald). Species that were not found in the South-central zone but have been reported from at least one county in other areas include *Oc. japonicus japonicus* (Theobald), *Oc. nevadensis* (Chapman & Barr), *Cs. particeps* (Adams), *Oc. aloponotum* (Dyar), *Cx. boharti* Brookman & Reeves, *Oc. intrudens* (Dyar), *Oc. pullatus* (Coquillett), *An. earlei* Vargas, *Oc.implicatus* (Vockeroth), and *Oc. togoi* (Theobald). Since these species did not occur where WNV activity was the highest, they were ignored as WNV vectors in WA.

Washington State precipitation in 2008 was normal to less than normal in all three zones (Fig. 3d) (NWS 2009). The maximum precipitation difference for the time frame analyzed was 0.45 cm. Variation in precipitation (Fig. 3e) was not statistically significant between zones ($P = .077$). Temperature was statistically different ($P < 0.001$) between zones with the South-central zone having higher temperatures (maximum difference = 10.7°C, minimum difference = 2.0°C) than the other two zones (Fig. 3f).

Trap night catch variation of species between zones was statistically different for four of the five species with *Ae. vexans, Cs. inornata*, and *Cx. tarsalis* being more abundant in the South-central zone (Table 3f and Fig. 3g, 3h, and 3i). *Culex pipiens* was less abundant in the Southeast zone, but not statistically different between the South-central and West Zones (Fig. 3j) and *Cq. perturbans* showed no statistical difference in any zone (Fig. 3k).

Table 3g shows the statistical values for effect of week, temperature, mean catch, interactions of week and mean catch, and interactions of temperature and mean catch on trap

night catch. Blank cells indicate that there was no significant correlation in the initial predictors and data were not subsequently analyzed. *Coquillettidia perturbans* was not significantly affected by any of the initial predictors so was not included. *Aedes vexans* and *Cx. pipiens* trap night catches were significantly correlated with all predictors, with the exception of the interaction of week and mean catch on *Cx. pipiens*. *Culiseta inornata* trap night catch was significantly correlated with temperature, mean catch, and the temperature and mean catch interaction. *Culex tarsalis* trap night catch was significantly correlated with temperature and temperature and mean catch interaction, but not with week, mean catch, or week and mean catch interaction.

Vector Type Diversity

A total of twenty-four mosquito species were collected during the surveillance efforts in the three zones (Table 3h). *Culiseta minnesotae* Barr and *Oc. aboriginis* (Dyar) were not included in the analysis because no definitive information could be found regarding host preference. *Culex territans* Walker prefers cold-blooded hosts, so was also excluded from analysis. Of the remaining 21 species, nearly half prefer mammal hosts. Bridge vector species outnumbered enzootic vector species by almost three to one.

The diversity of vector type from each zone is shown in Figures 3l, 3m, and 3n for enzootic vectors, bridge vectors, and mammal feeding species respectively. These figures show the trap night catch (proportion of trap nights that a species was collected). For enzootic vectors, all three zones were dominated by *Cx. pipiens.* Bridge vector species were fewest in the Southeast zone and no species were unique to that zone. The West zone had seven species whereas the South-central zone had six species. Five species (*Ae. vexans*, *Cq. perturbans*, *Cs. inornata, Cx. tarsalis*, and *Oc. dorsalis*) were common to the latter two zones. The South-central zone had one unique species (*Oc. melanimon*) and the West zone had two unique species (*Oc. j. japonicus* and *Oc. sierrensis*).

The abundance of each species within the bridge vector type from each zone is shown in Figure 3o. Each zone was dominated by a single species: *Cq. perturbans* was the dominant species in the West and Southeast zones and *Ae. vexans* was the dominant species in the South-central zone. Mean catch per trap for *Ae. vexans, Cx. tarsalis,* and *Cx. pipiens* was significantly greater in the South-central zone while *Cq. perturbans* mean catch per trap was roughly equal (Fig.3p).

The Simpson's Index, Shannon-Weaver Index, and evenness for bridge vectors, enzootic vectors, and mammal feeders by zone are shown in Table 3i, 3j, and 3k, respectively. Bridge vector diversity was highest in the South-central zone followed by the West and Southeast zones, respectively. There was essentially no enzootic vector diversity in any of the zones. Mosquitoes that prefer mammal hosts were most diverse in the West zone and least diverse in the Southeast zone.

Discussion

Risk of exposure to arthropod disease vectors and the pathogens they carry is spatially heterogeneous in the U.S. (Eisen and Eisen 2007). This heterogeneity is due to geographical and environmental conditions that affect arthropod vectors and makes developing risk models and risk maps difficult. This pattern of heterogeneity holds true for WNV transmission in WA. West Nile virus activity in WA is lower than in the bordering states and the majority of activity has been focused in the south-central portion of the state.

The most active WNV season in WA to date was 2008, which was the first year that WNV activity in WA exceeded the activity of its bordering states. The downtrend in peak activity in ID and OR may be the result of bird hosts gaining immuno-competence against the virus, which would lead to a reduction in the numbers of all of the positive WNV categories, as there would be fewer infective birds on which mosquitoes would feed and therefore fewer infective mosquitoes to transmit the pathogen (Reisen and Brault 2007). Most of the WNV activity in ID

has occurred in the southern part of the state, and in OR the bulk of activity has been in the eastern half of the state. In WA most of the activity has been in the south-central portion. All of these areas have some irrigated agricultural land.

One possible explanation for the difference in WNV transmission in WA is that the mosquito fauna is different relative to ID and OR. West Nile virus is normally maintained in an enzootic cycle between birds and ornithophilic mosquitoes. If a mosquito becomes infected from feeding on an infective bird and then feeds on a horse or a person, it can transmit the virus to either of the latter, accidental hosts. However, not all mosquitoes feed on a wide variety of hosts. Some prefer to feed on birds, whereas others prefer mammals, amphibians, or reptiles. Some species are more opportunistic and will readily feed on a wider range of hosts. Therefore some species are important for maintaining and amplifying the virus in its normal avian hosts (enzootic vectors) while other species are important for transmitting the virus to accidental hosts such as horses and humans (bridge vectors). Both types are necessary for WNV activity to persist and be a health risk to humans.

Of the species that are unique to OR, *An. franciscanus* and *Or. signifera* occur only in the southwest part of OR and do not play a role in WNV transmission where it is the heaviest. Similarly, the four species that are unique to ID do not have distributions (Darsie and Ward 2005) that would account for that state's WNV activity. *Culex restuans*, a putative WNV vector in the northeastern U.S. (Brown et al. 2008), occurs in ID and OR but not in WA. This species has a distribution that is continuous from ID's eastern border to OR's Pacific coast (Darsie and Ward 2005), but only in the southern portion of these states. The distribution of *Cx. restuans* stops south of where much of the WNV activity occurs in both states. Therefore, it seems unlikely that this species is one of the primary WNV vectors in WA's bordering states and it does not explain the differences in WNV activity between states. *Culex pipiens* and *Cx. tarsalis* occur in all three states and can serve as enzootic and bridge vectors (Kramer et al. 2008, Turell et al. 2005).

Difference in mosquito fauna is one possible explanation for the heterogeneity of WNV transmission within WA as well. Of the three species that have a limited range outside of the South-central zone, only *Oc. nigromaculis* is significantly more abundant in that zone, based on the 2004-2005 mosquito data. However, this species has not been implicated as an important WNV vector. As with the differences between WA and its bordering states, it is unlikely that the differences in WNV activity between these zones are due to differences in the mosquito fauna alone. However, bridge vector diversity may play a role. The diversity indices support our hypothesis that areas with a higher diversity of bridge vector species have a higher incidence of WNV cases.

Another possible explanation is that the abundances of WNV vectors are different between zones. Since three of the four species that were significantly more abundant in the South-central zone are putative WNV vectors, this would seem to be a reasonable explanation. This explanation is also supported by the 2004-2005 data that showed higher abundances of putative and suspect WNV vectors in counties with WNV activity. Bridge vector species abundance is different among common species between zones; however, I feel that trap night catch is a more reliable indication of vector presence since it represents temporal activity. Interestingly, *Ae. vexans* makes up 26% of the bridge vector species trap night catch in the South-central zone but 65% of the total mosquitoes collected. Tiawsirisup et al. (2008) reported that the WNV vector competence of *Ae.vexans*, which primarily feeds on mammals but will also feed on birds, approaches that of *Cx. pipiens*. Currently it is not being tested for WNV infection in WA.

The differing abundances of bridge vector species between zones leads to the question as to why these mosquitoes are more abundant in the South-central zone. Trap night catch of four of the five species analyzed was positively correlated with temperature and temperature was significantly warmer in the South-central zone. The exception was *Cq. perturbans,* which was not significantly different in abundance between zones. This species develops in

permanent bodies of water that have emergent, or floating, vegetation that is used by the larvae to obtain oxygen through a specialized siphon that penetrates plant tissue (Gjullin and Eddy 1972). Temperature in these larger, permanent bodies of water is slower to fluctuate with ambient temperature and these bodies of water do not dry out. This provides these larvae a more stable, long lasting environment in which to develop.

These findings support our hypothesis that variation in vector abundance and activity correlate with temperature. However, it does not support correlation with humidity. Precipitation was used as a proxy for humidity, as data for the latter were not readily available. Precipitation was not significantly different between zones; however, it may not be a reliable indicator of humidity, particularly in the irrigated agricultural environment of the South-central zone. Not only could irrigation and the crops it produces provide seasonally consistent habitat for mosquito larvae to develop, it could also provide conditions (e.g. shade, relative humidity, etc.) that enable the adult females to live longer, increasing their reproductive potential and possibly their vectorial capacity.

Although there appears to be a large difference in precipitation on week 34, it is only a function of the scale of the graph, as the difference is less than 0.5 cm. There was very little precipitation during the time frame that was analyzed. Analysis of relationships between precipitation - and by proxy, humidity - and mosquito activity were not possible because precipitation was not significantly different between zones. Such relationships may exist.

These findings indicate that there is an increased risk of WNV activity in areas of WA with higher temperatures and that some bridge vector species, as well as *Cx. pipiens*, are more active in these areas.

Studies beyond these findings are needed to improve our ability to predict WNV risk and control WNV activity in WA. First, given the abundance and seasonal consistency of *Ae. vexans,* this species should be looked at more closely to determine if it is a potentially important

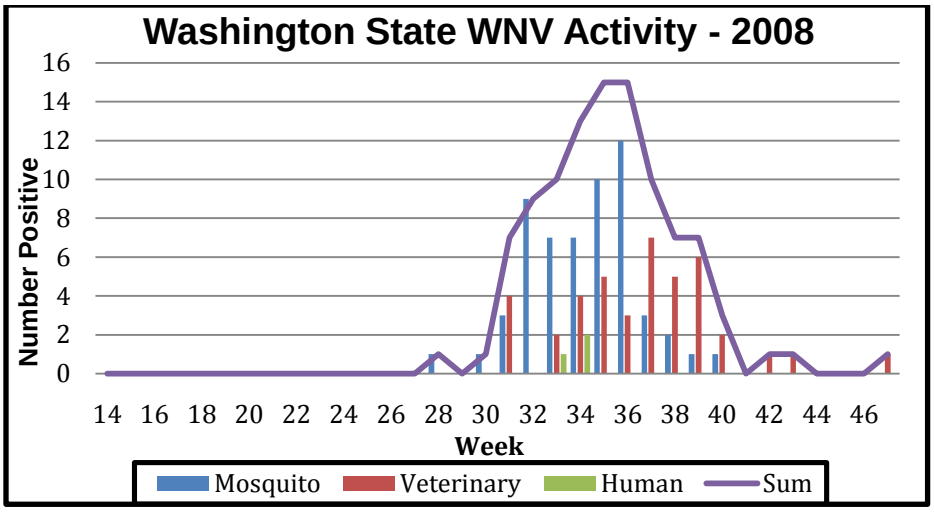
vector of WNV in the South-central zone. Currently it is not considered to be an important vector and is not tested for WNV.

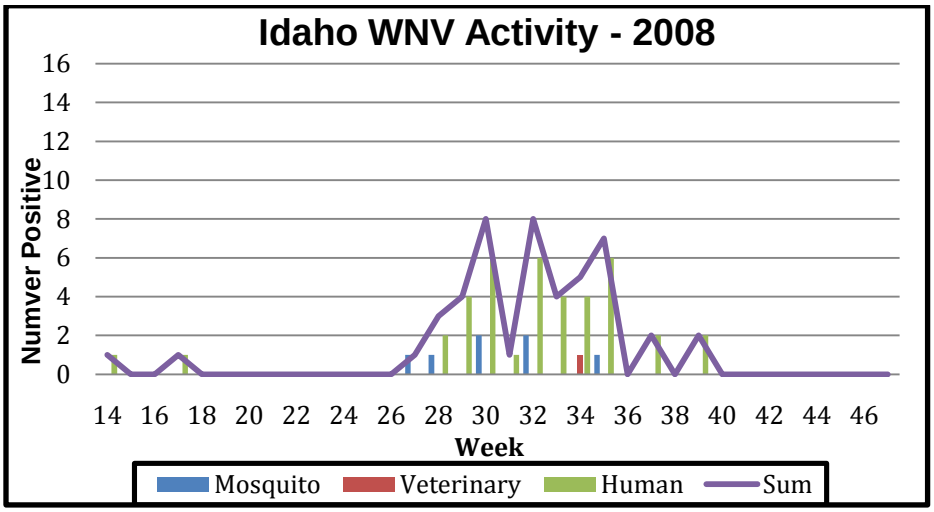
The possibility of a relationship with irrigated agriculture deserves more study and may show that variation in vector abundance is correlated with humidity.

Even within areas such as the zones analyzed, the environment is highly heterogeneous. Vector control efforts and WNV risk assessment would benefit from more finely scaled study of environmental factors.

Year	Case Type	**Washington**	Total	**Idaho**	Total	**Oregon**	Total	**California**	Total
2002	Mosquito		4		1		0		1
	Bird	2							
	Veterinary	2		1					
	Human							1	
2003	Mosquito		0		1		0	32	132
	Bird							96	
	Veterinary							1	
	Human			1				3	
2004	Mosquito		0		33		53	1136	5687
	Bird			7		19		3232	
	Veterinary			23		31		540	
	Human			3		3		779	
2005	Mosquito	2	4	19	161	10	78	1215	5649
	Bird	1		15		15		3046	
	Veterinary	1		114		46		508	
	Human	0		13		7		880	
2006	Mosquito	0	22	236	1697	22	151	820	2634
	Bird	13		127		25		1446	
	Veterinary	6		338		35		90	
	Human	3		996		69		278	
2007	Mosquito	0	10	26	189	28	128	1003	2832
	Bird	1		15		55		1395	
	Veterinary	9		16		19		54	
	Human	0		132		26		380	
2008	Mosquito	57	125	7	51	16	34	2003	5043
	Bird	24		4		2		2531	
	Veterinary	41		1				64	
	Human	3		39		16		445	

Table 3a. Annual West Nile virus activity.
Activity is separated by case type and annual totals for
Washington State, Idaho, Oregon, and California.
Veterinary cases include equine, canine,
feline, bat, squirrel, rabbit, raccoon,
and “other” (Lindsey 2009).





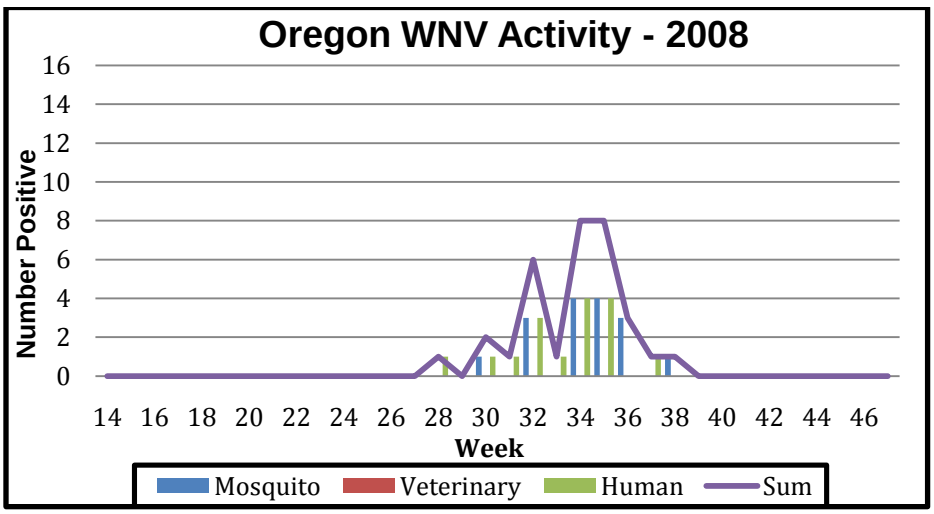


Figure 3a. Weekly 2008 WNV activity for Washington State, Idaho, and Oregon. Dead bird and sentinel chicken data is excluded.

	Washington	Idaho	Oregon
Species known to be infected with WNV			
Anopheles franciscanus			•
Culex apicalis	•		
Culex erythrothorax		•	
Culex restuans		•	•
Culex salinarius		•	
Culex stigmatosoma	•		•
Culiseta morsitans	•	•	
Culiseta particeps	•		•
Ochlerotatus canadensis	•	•	
Ochlerotatus j. japonicus	•		
Ochlerotatus provocans		•	
Ochlerotatus sticticus	•	•	
Ochlerotatus trivittatus		•	
Orthopodomyia signifera			•
Species not known to be infected with WNV			
Anopheles earlei	•	•	
Anopheles occidentalis	•		•
Culiseta alaskaensis		•	
Ochlerotatus aloponotum	•		•
Ochlerotatus decticus		•	
Ochlerotatus euedes		•	
Ochlerotatus hendersoni		•	•
Ochlerotatus mercurator		•	
Ochlerotatus niphadopsis		•	•
Ochlerotatus pionips		•	•
Ochlerotatus punctor		•	
Ochlerotatus schizopinax		•	
Ochlerotatus sierrensis	•	•	
Ochlerotatus spencerii idahoensis	•	•	
Ochlerotatus togoi	•		
Ochlerotatus ventrovittis	•	•	

Table 3b. Mosquito species differences between Washington State, Idaho, and Oregon. Species are separated by those known to be infected with West Nile virus (WNV) and those that have not been found to be infected with WNV. Species common to all three states are not included (Sames et al 2007, Brothers and Darsie 2003, Darsie and Ward 2005).

		South-Central Zone				Western Washington and Pend Oreille County											
		Benton	Grant	Kittitas	Yakima	Clark	Cowlitz	Island	King	Lewis	Pend Oreille	Pierce	Snohomish	Thurston	Whatcom	Case Totals	Year Totals
2002	Mosquito															0	4
	Bird										1		1			2	
	Veterinary							1							1	2	
	Human															0	
2005	Mosquito				2											2	4
	Bird				1											1	
	Veterinary				1											1	
	Human															0	
2006	Mosquito															0	22
	Bird				3		1	1	6				2			13	
	Veterinary				5				1							6	
	Human					1						2				3	
2007	Mosquito															0	10
	Bird				1											1	
	Veterinary				9											9	
	Human															0	
2008	Mosquito	14	2		41											57	125
	Bird	10	1		5				3	1		3		1		24	
	Veterinary	4	10	1	26											41	
	Human				2				1							3	
County Totals		28	13	1	96	1	1	2	11	1	1	5	3	1	1	165	165
Area Totals		138				27											

Table 3c. WNV activity for all Washington State counties with West Nile virus (WNV) activity, 2002 – 2008 (USGS 2009). Counties are separated into the South-central zone and other counties with WNV activity.

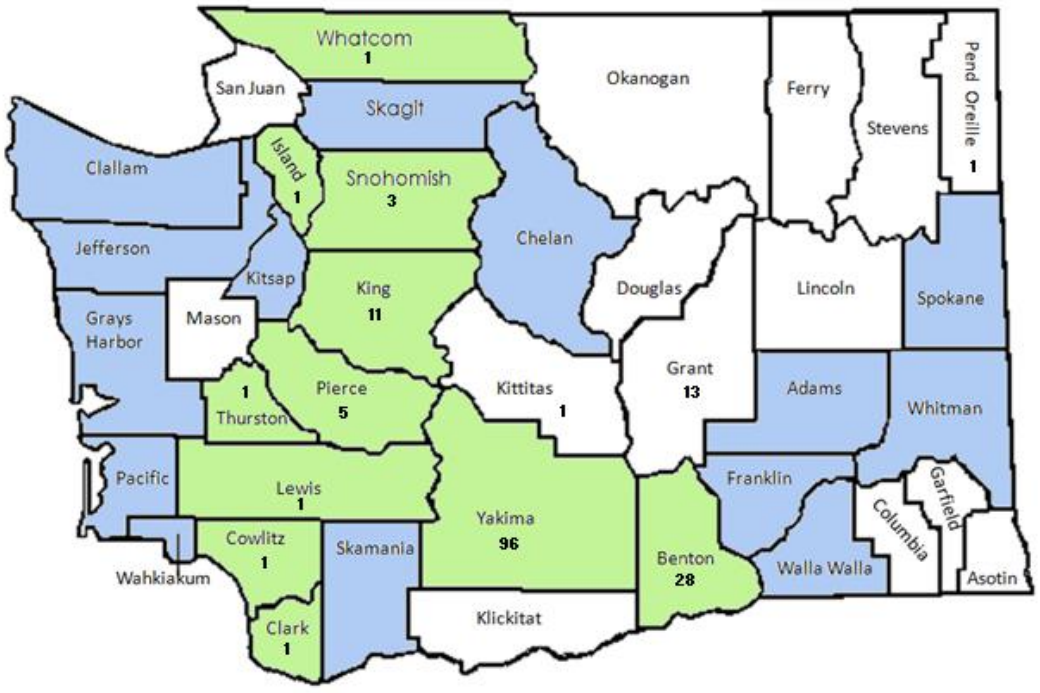


Figure 3b. Washington State counties included in the 2004-2005 Surveillance Program Report: West Nile virus Environmental Surveillance in WA (WA DOH 2006). Green indicates counties included in the report that have had WNV activity. Blue indicates counties included in the report with no WNV activity. Numbers within counties indicate the total WNV activity in that county from 2002-2008.

	Putative		Suspect																				
	Cx. pipiens	*Cx. tarsalis*	*Ae. cinereus*	*Ae. vexans*	*An. freeborni*	*An. punctipennis*	*Cq. perturbans*	*Cx. salinarius*	*Cx. stigmatosoma*	*Cs. impatiens*	*Cs. incidens*	*Cs. inornata*	*Cs. morsitans*	*Cs. particeps*	*Oc. dorsalis*	*Oc. fitchii*	*Oc. j. japonicus*	*Oc. melanimon*	*Oc. nigromaculis*	*Oc. sticticus*	*Oc. territans*	*Oc trivittatus*
	Counties with no WNV Activity																						
	19,045	13,037	56	6,278	3,403	120	2,425	18	0	55	338	1,025	38	156	4,828	700	0	20	578	35	7	0
Total	32,082		20,080																				
Mean/County	2,917		1,434																				
	Counties with WNV Activity																						
	45,750	15,472	79	60,416	1,290	863	35,505	0	56	84	4,128	2,096	16	389	1,643	893	8	16	1	7,764	6	2
Total	61,222		115,255																				
Mean/County	4,373		8,233																				

Table 3d. Relative abundance of mosquitoes from counties included in the 2004-2005 Surveillance Program Report: West Nile virus environmental surveillance in Washington State (WA DOH 2006). Mosquito abundance is separated into counties with WNV activity and those without WNV activity. Mosquito species are separated into putative vectors, suspect vectors, and non-suspect vectors.

	Non-suspect														
	An. earlei	*Cx. boharti*	*Cx. minnesotae*	*Oc. aboriginis*	*Oc. aloponotum*	*Oc. campestris*	*Oc. cataphylla*	*Oc. communis*	*Oc. excricians*	*Oc. flavescens*	*Oc. hexodontus*	*Oc. impiger*	*Oc. increpitus*	*Oc. pullatus*	*Oc. sierrensis*
	Counties with no WNV Activity														
No WNV	0	0	128	611	0	302	0	0	203	9	0	0	701	0	108
Total	2,062														
Mean/County	187														
	Counties with WNV Activity														
	11	11	118	311	4	0	77	374	0	0	4	209	1,292	47	405
Total	2,863														
Mean/County	260														

Table 3d cont'd. Relative abundance of mosquitoes from counties included in the 2004-2005 Surveillance Program Report: West Nile virus environmental surveillance in Washington State (WA DOH 2006). Mosquito abundance is separated into counties with WNV activity and those without WNV activity. Mosquito species are separated into putative vectors, suspect vectors, and non-suspect vectors.

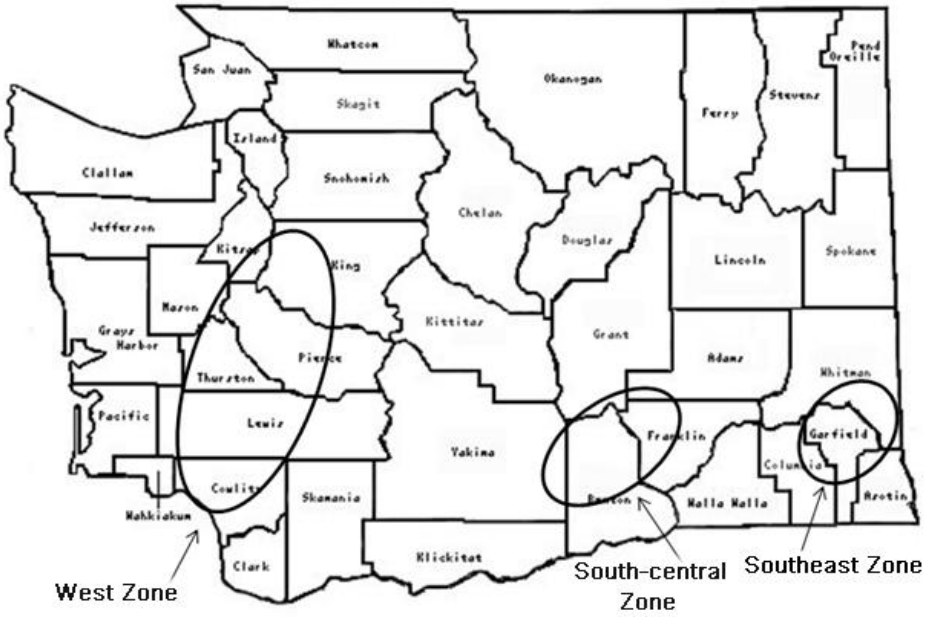


Figure 3c. Zones of West Nile virus, mosquito composition, and mosquito activity comparison. Zones were based on locations of available mosquito data.

	West Zone						South-central Zone				Northern counties			
	Clark	**Cowlitz**	**King**	**Lewis**	**Pierce**	**Thurston**	Benton	Grant	Kittitas	Yakima	Island	Pend Oreille	Snohomish	Whatcom
Species known to be infected with WNV														
Culex pipiens	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Culex tarsalis	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Aedes cinereus	●	●	●	●	●	●		●	●	●	●	●	●	●
Culex territans	●	●	●	●	●	●	●		●	●	●	●	●	●
Culiseta inornata	●	●	●	●	●	●	●	●	●	●	●		●	●
Ochlerotatus fitchii		●	●	●	●	●	●	●	●	●	●	●	●	●
Aedes vexans	●	●	●		●		●	●	●	●	●	●	●	●
Anopheles freeborni	●	●	●		●		●	●	●	●	●	●	●	●
Anopheles punctipennis	●	●	●	●	●	●	●		●	●	●		●	●
Ochlerotatus sticticus	●	●	●		●	●	●		●	●	●	●	●	●
Ochlerotatus dorsalis		●	●		●	●	●	●	●	●	●		●	●
Coquillettidia perturbans	●	●	●		●	●		●		●	●		●	●
Culiseta impatiens	●	●	●	●	●				●	●			●	●
Culiseta morsitans			●		●	●			●	●	●	●	●	●
Culex stigmatosoma		●	●	●	●		●			●				
Ochlerotatus nigromaculis					●		●	●	●	●				
Ochlerotatus melanimon							●	●		●	●			
Ochlerotatus j. japonicus			●		●								●	
Ochlerotatus canadensis								●				●		
Ochlerotatus nevadensis					●									
Species not known to be infected with WNV														
Culiseta incidens	●	●	●	●	●	●	●	●	●	●	●		●	●
Ochlerotatus increpitus	●	●	●		●	●	●	●	●	●	●	●	●	●
Ochlerotatus sierrensis	●	●	●	●	●	●	●	●		●	●		●	●
Ochlerotatus excrucians	●		●		●	●		●	●	●		●	●	●
Culiseta minnesotae		●	●		●		●		●	●	●		●	●
Ochlerotatus aboriginis		●	●	●	●	●			●		●		●	●
Ochlerotatus communis		●	●	●	●				●	●		●		●
Culiseta particeps	●	●	●		●	●					●		●	
Ochlerotatus aloponotum		●	●		●	●					●			●
Ochlerotatus hexodontus			●	●	●				●			●		●
Ochlerotatus spencerii idahoensis	●	●						●	●	●		●		
Anopheles occidentalis					●		●					●	●	●
Ochlerotatus impiger				●	●					●		●		●
Culex apicalis		●	●							●			●	
Culex boharti		●	●		●									●
Ochlerotatus campestris		●					●	●		●				
Ochlerotatus intrudens		●			●							●		●
Ochlerotatus ventrovittis					●				●	●		●		
Ochlerotatus flavescens					●			●		●				
Ochlerotatus pullatus			●		●							●		
Anopheles earlei												●	●	
Ochlerotatus cataphylla							●		●					
Ochlerotatus implicatus					●							●		
Ochlerotatus togoi											●			●

Table3e. Mosquito species that occur in Washington State counties with West Nile virus (WNV) activity. (Sames et al. 2007, USGS 2009). Counties are separated into West zone, South-central zone, and northern counties. Mosquito species are separated by those with WNV isolation and those without WNV isolation.

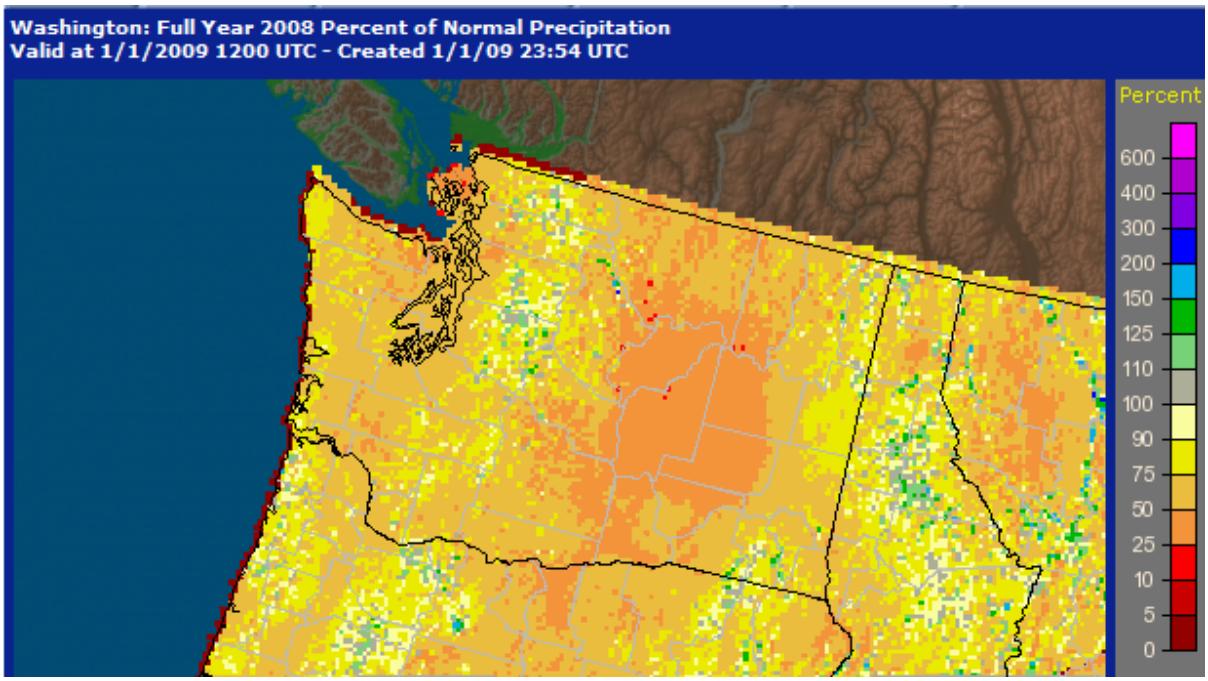


Figure 3d. Map of Washington State depicting the percent of normal precipitation in 2008. National Weather Service, Advanced Hydrologic Prediction Service (http://water.weather.gov/index.php)

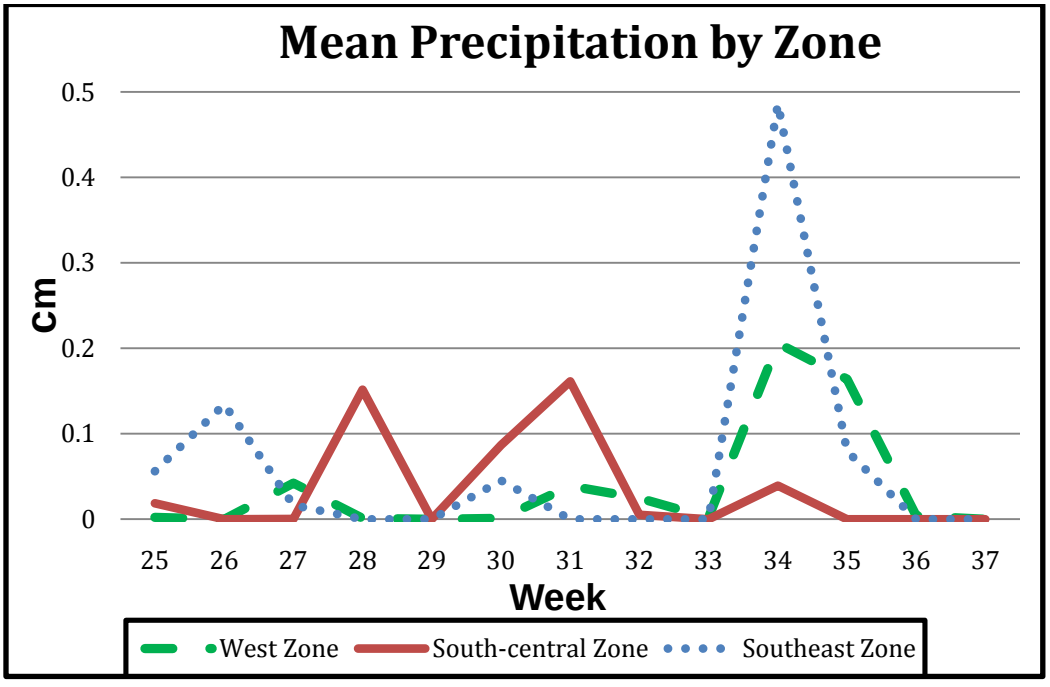


Figure 3e. Weekly mean precipitation by zone.

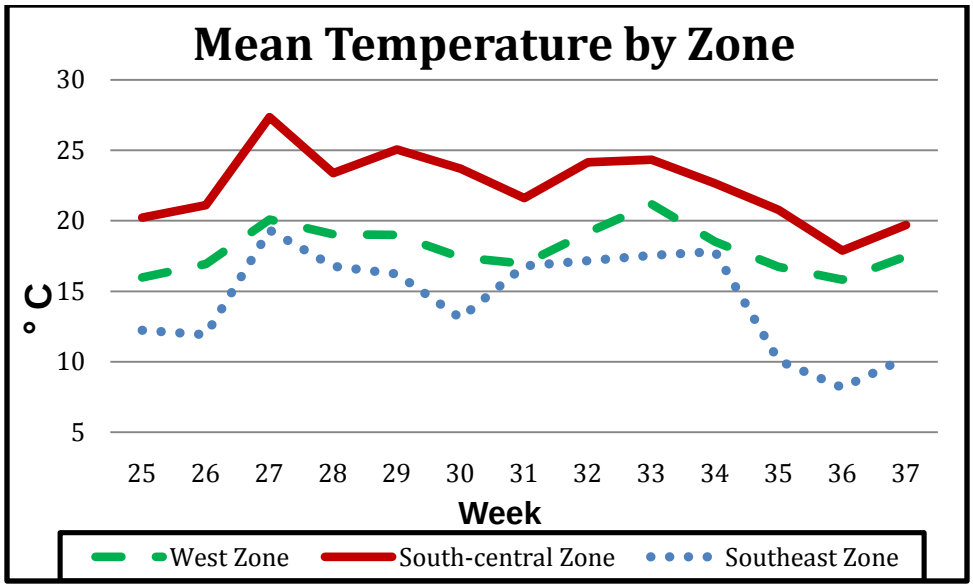


Figure 3f. Weekly temperature by zone.

	Zone				
	p-value	z-score	Coefficient	Concordance	DF
Ae. vexan	<0.001	9.01	1.008	54.5	1
Cq. perturbans	0.289	-1.06	-1.130	27.6	1
Cs. inornata	<0.001	7.27	0.926	52.6	1
Cx. pipiens	<0.001	-4.80	-0.524	31.6	1
Cx. tarsalis	<0.001	9.90	1.129	52.4	1

Table 3f. Statistical values of Binary Logistic Regression analysis of species variation by zone.

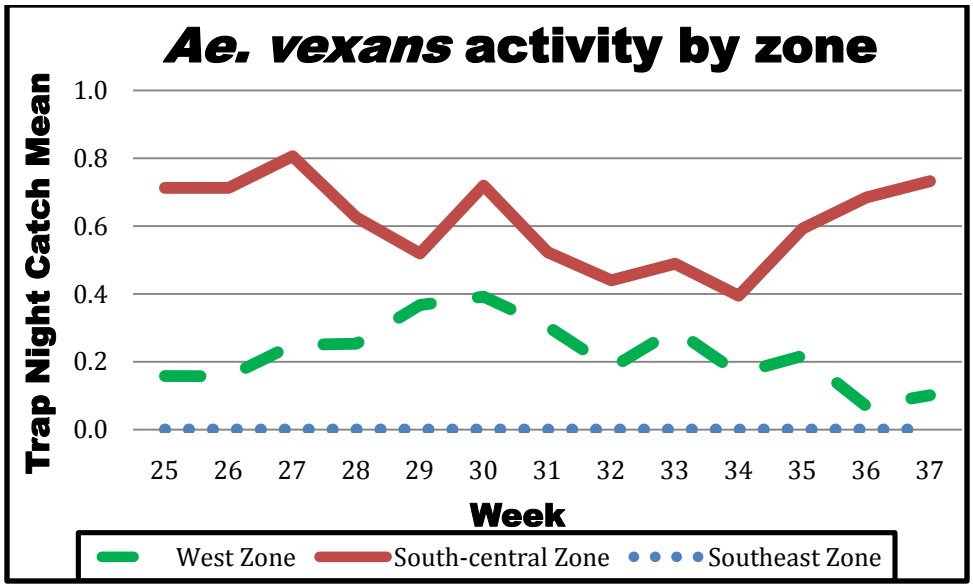


Figure 3g. *Aedes vexans* activity by zone.

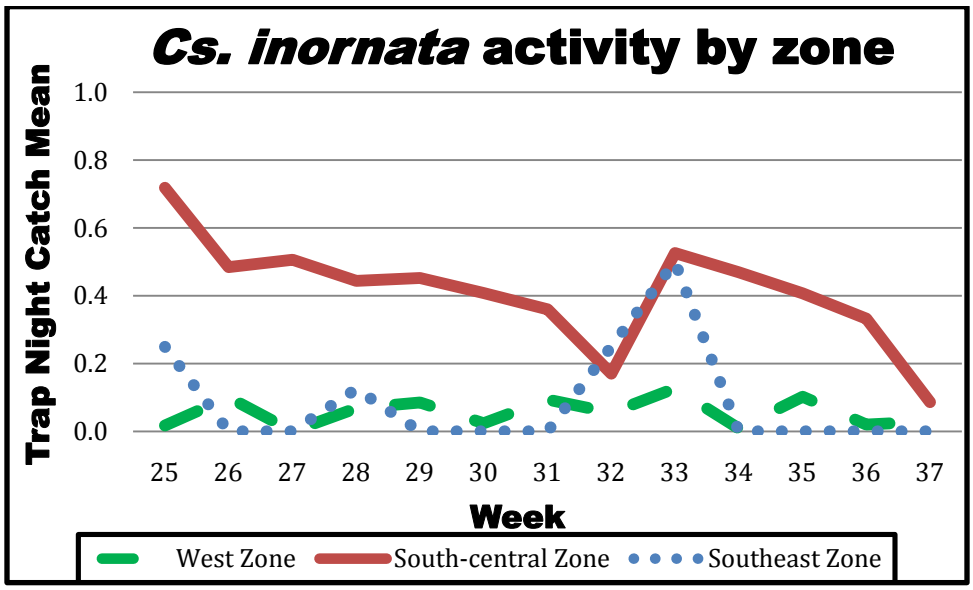


Figure 3h. *Culiseta inornata* activity by zone.

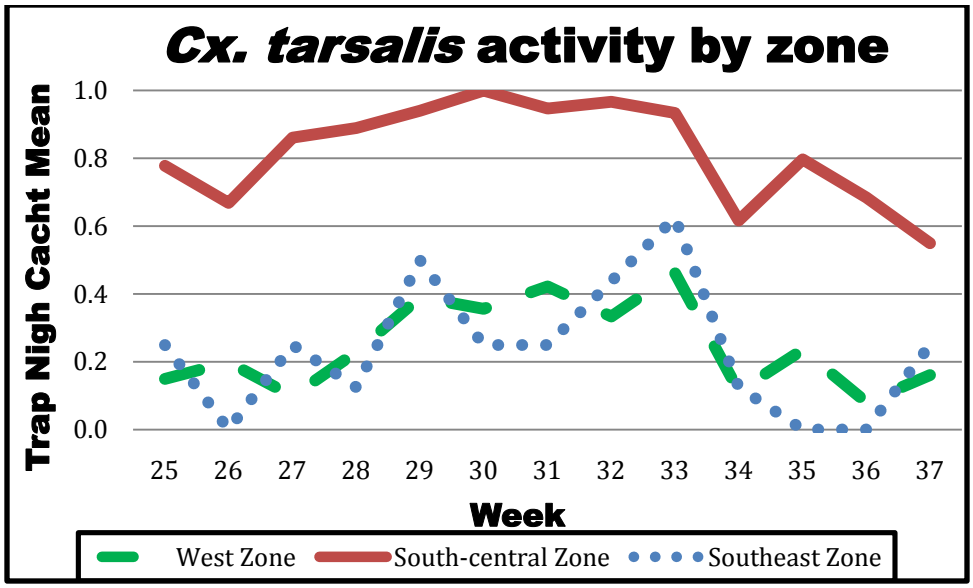


Figure 3i. *Culex tarsalis* activity by zone.

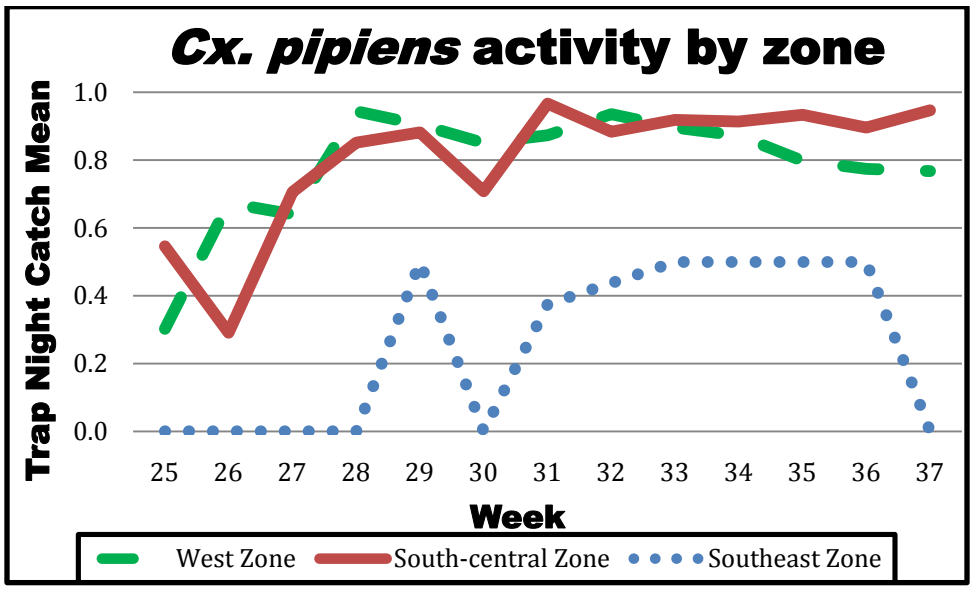


Figure 3j. *Culex pipiens* activity by zone.

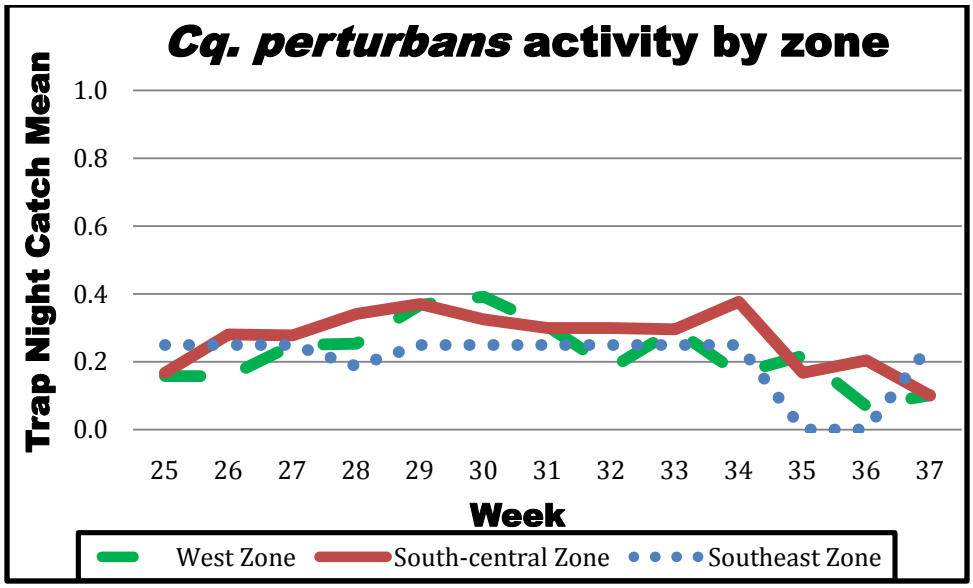


Figure 3k. *Coquillettidia perturbans* activity by zone.

		p-value	z-score	Coefficient	Concordance	DF
Ae. vexans	Week	<0.001	4.08	0.133	84.3	5
	Temperature	<0.001	8.57	0.285		
	Mean Catch	<0.001	6.01	0.207		
	Week*Mean Catch	<0.001	-5.18	-0.004		
	Temp*Mean Catch	<0.001	-3.99	-0.003		
Cs. inornata	Week				84.0	3
	Temperature	<0.001	5.90	0.246		
	Mean Catch	0.002	3.08	1.116		
	Week*Mean Catch					
	Temp*Mean Catch	0.022	-2.30	-0.037		
Cx. pipiens	Week	<0.001	5.02	0.137	79.7	5
	Temperature	<0.001	7.99	0.260		
	Mean Catch	0.005	0.02	0.058		
	Week*Mean Catch	0.088	-1.71	-0.001		
	Temp*Mean Catch	0.001	-3.19	-0.001		
Cx. tarsalis	Week	0.929	0.09	0.002	84.1	5
	Temperature	<0.001	10.03	0.350		
	Mean Catch	0.122	1.55	0.067		
	Week*Mean Catch	0.136	1.49	0.001		
	Temp*Mean Catch	<0.001	-3.76	-0.004		

Table 3g. Statistical values for effect of predictors on trap night catch.

Enzootic	Bridge	Mammal Feeder	Amphibian Feeder	Unknown
Cx. pipiens	*Ae. vexans*	*An. freeborni*	*Cx. territans*	*Cs. minnesotae*
Cx. stigmatosoma	*Cq. perturbans*	*An. punctipennis*		*Oc. aboriginis*
Cs. morsitans	*Cx. tarsalis*	*Cs. impatiens*		
	Cs. inornata	*Cs. incidens*		
	Oc. dorsalis	*Cs. particeps*		
	Oc. j. japonicus	*Oc. campestris*		
	Oc. melanimon	*Oc. communis*		
	Oc. sierrensis	*Oc. fitchii*		
		Oc. increpitus		
		Oc. sticticus		

Table 3h. Mosquito species in each vector type.
Based on Turell et al. (2005), Gjullin and Eddy (1972), Harmston and Lawson (1967), Belton (1983), and Bohart and Washino (1978). Table also includes one amphibian feeder (Crans 1970) and two species of unknown feeding preferences.

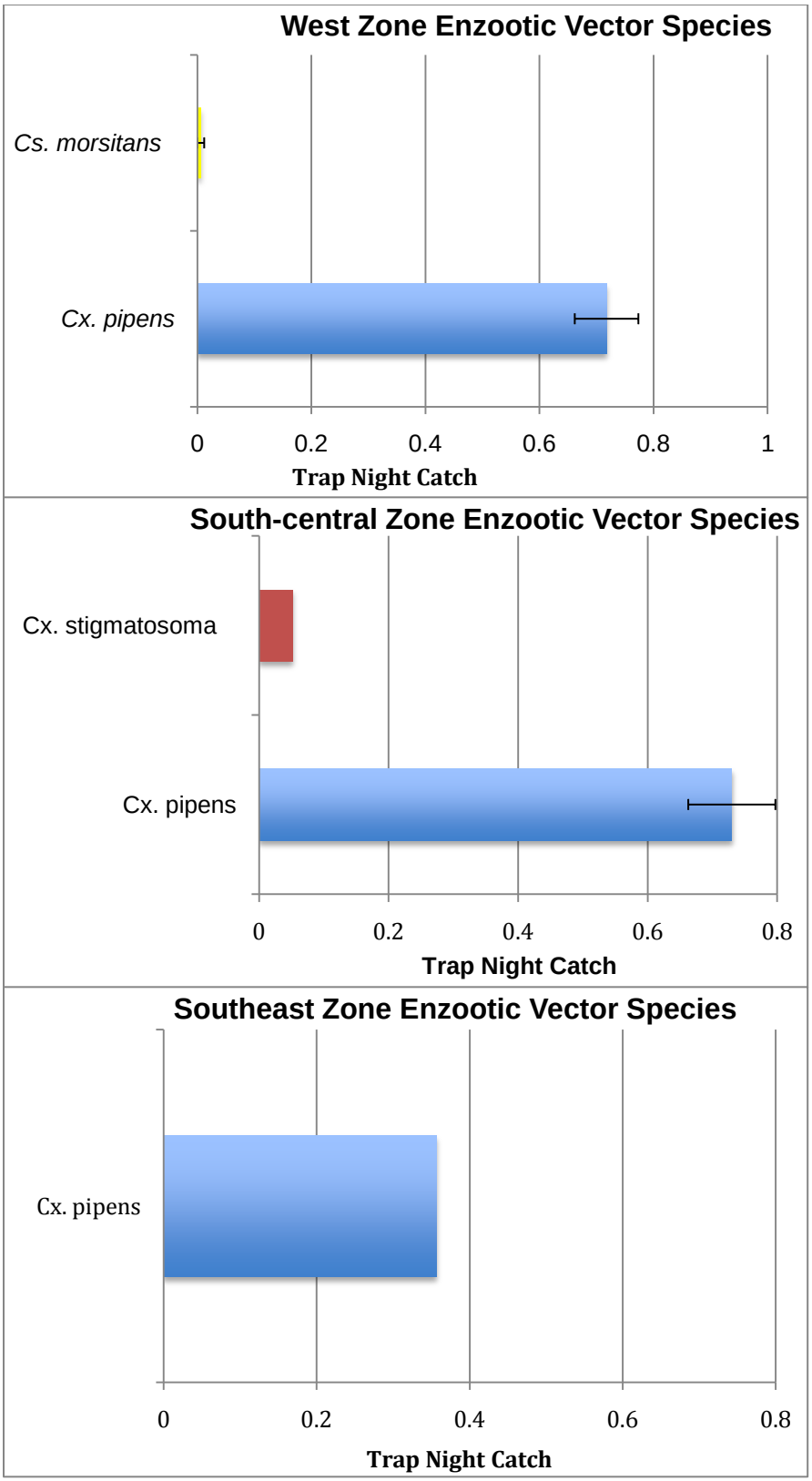
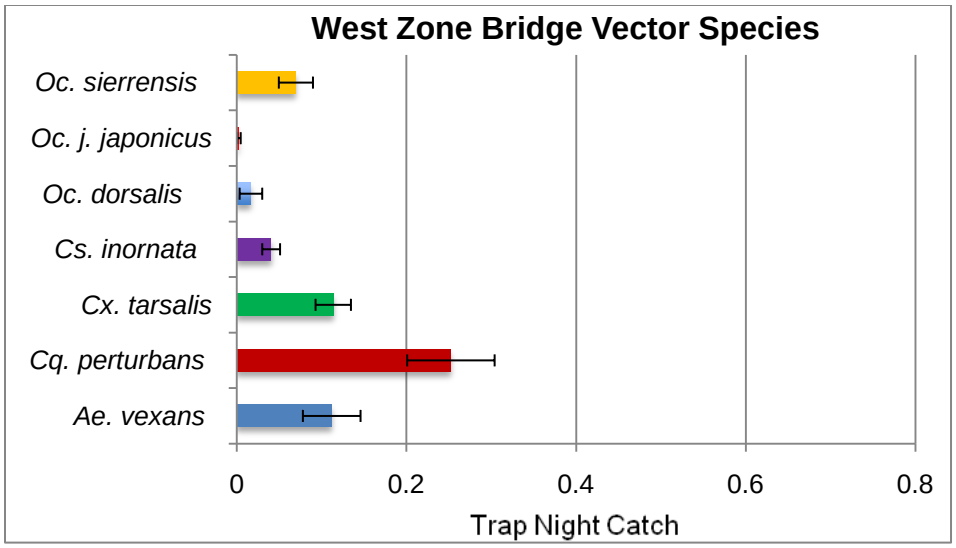
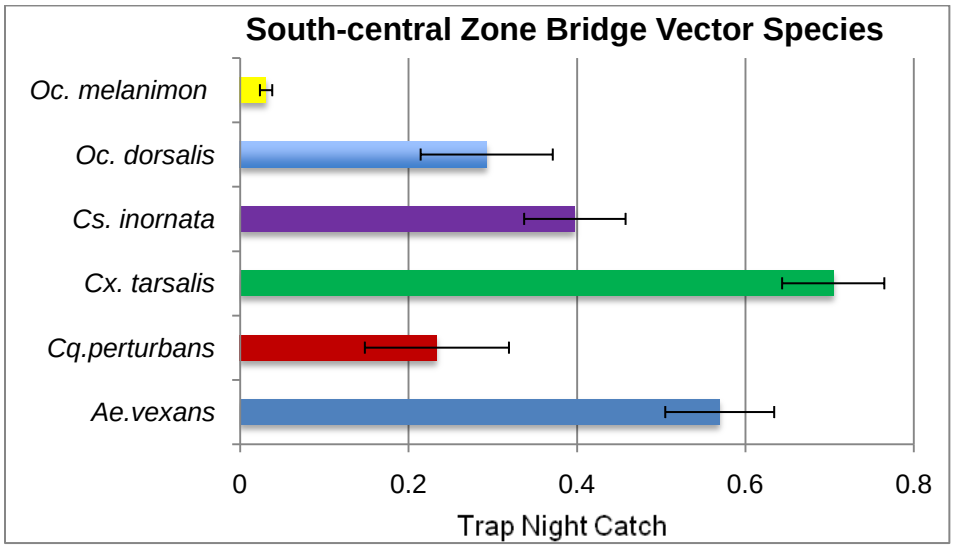


Figure 3l. Washington State enzootic vector diversity by zone.
Depicts the trap night catch (proportion of trap nights a species was collected).





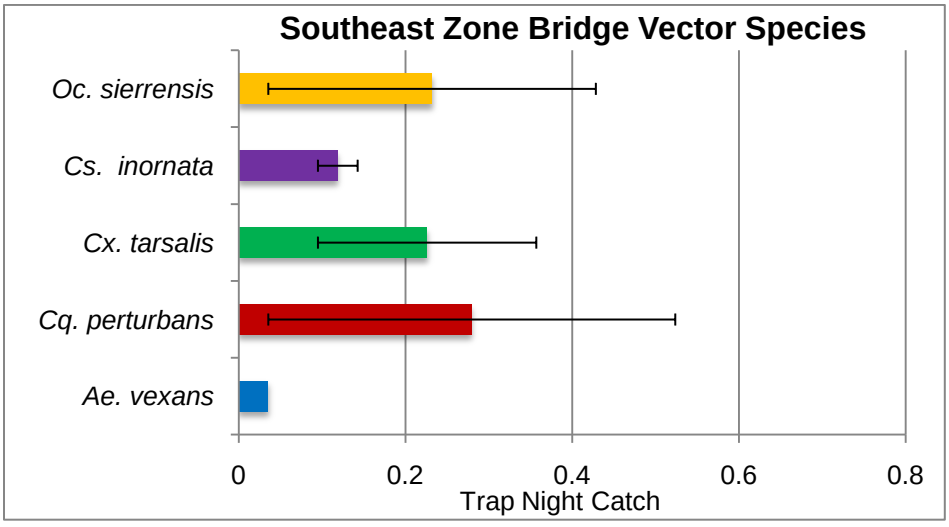
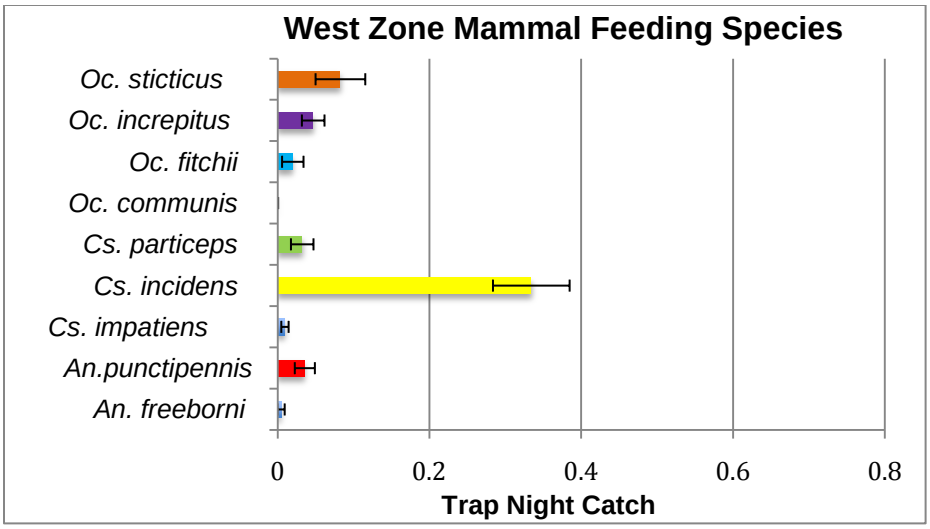
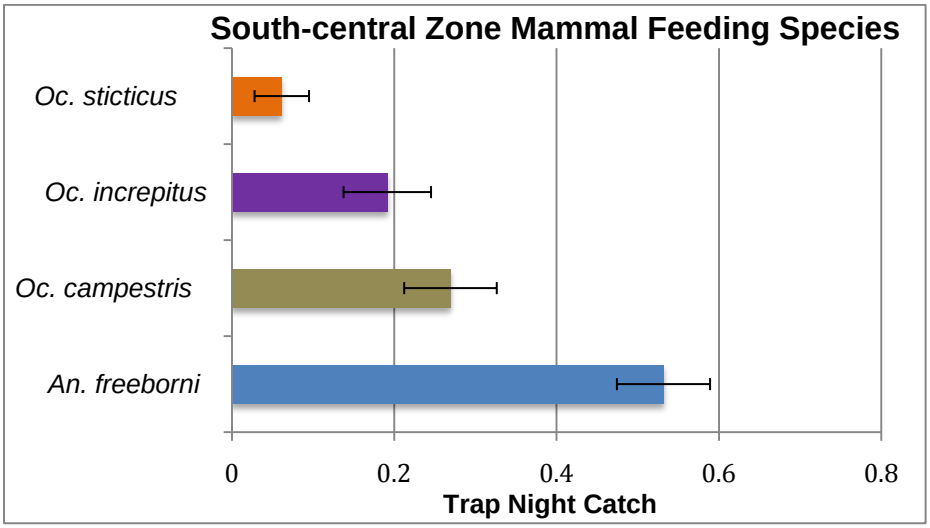


Figure 3m. Washington State bridge vector diversity by zone.
Depicts the trap night catch (proportion of trap nights a species was collected).





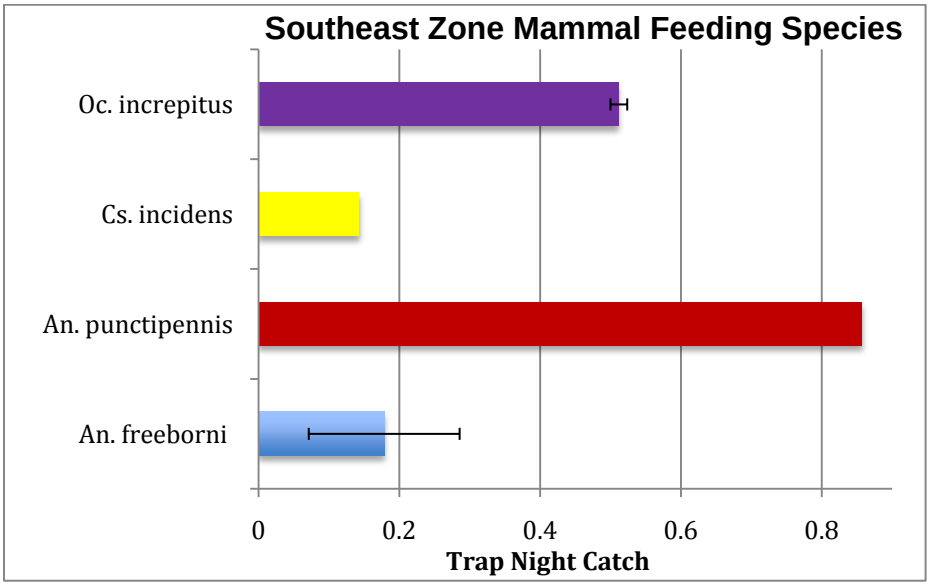
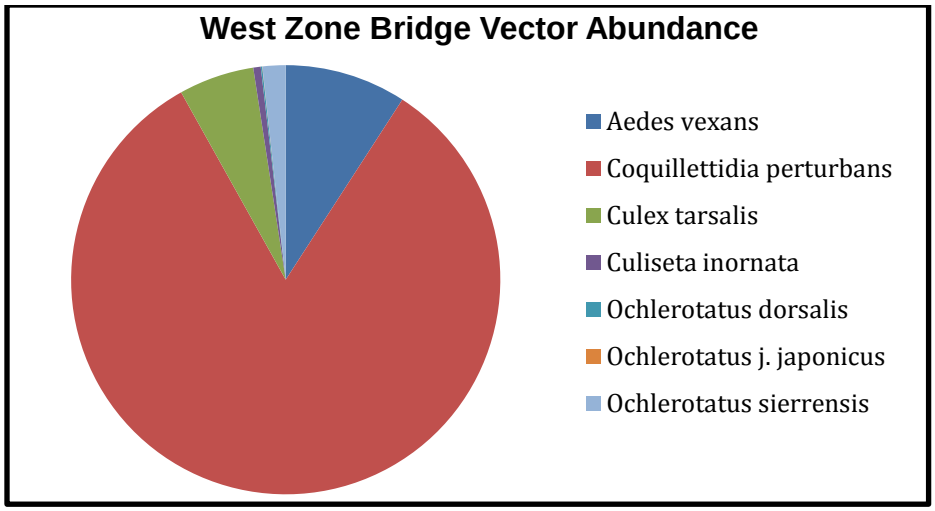
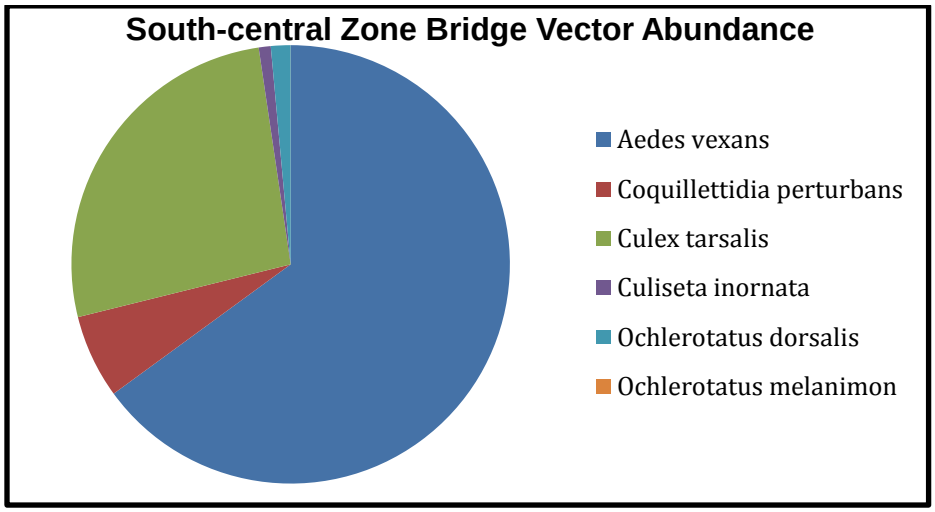


Figure 3n. Washington State mammal feeding species diversity by zone. Depicts the trap night catch (proportion of trap nights a species was collected).





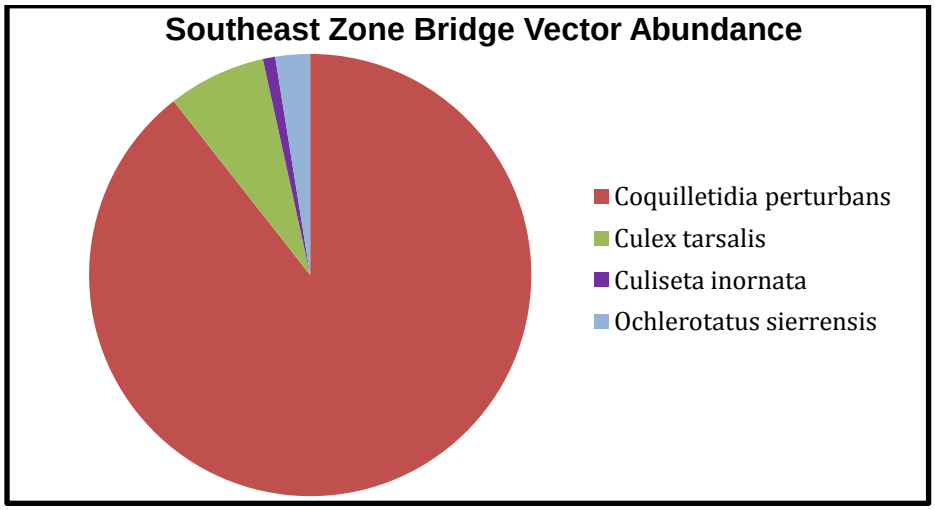


Figure 3o. Washington State bridge vector species abundance by zone.
Depicts total number of each mosquito species collected from each zone.

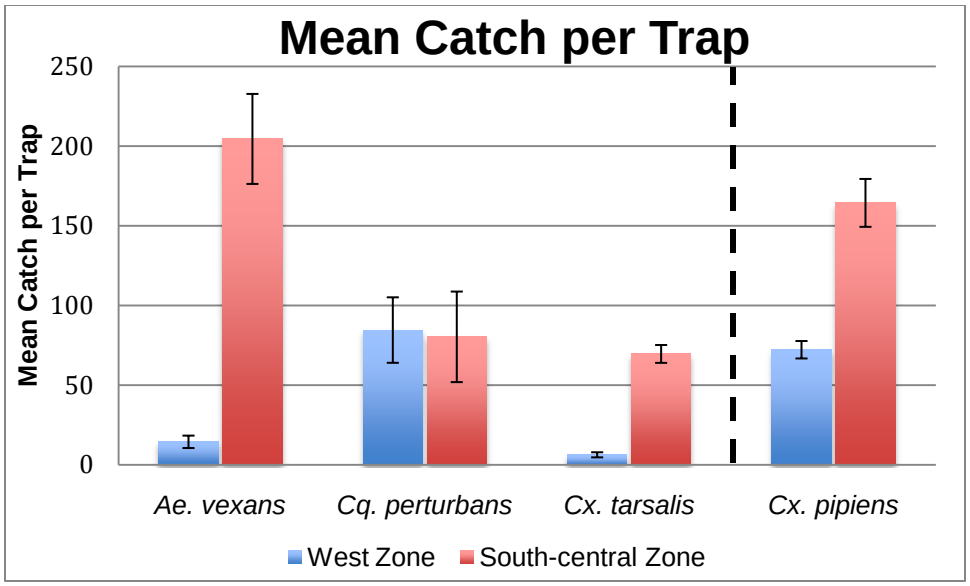


Figure 3p. Mean catch per trap of three prominent bridge vectors and an enzootic vector (*Culex pipiens*) from the West and South-central zones.

Zone	Simpson's	Shannon-Weaver	Evenness
Southeast	0.8051	0.4245	0.3823
South-central	0.4943	0.9094	0.3525
West	0.6967	0.6466	0.2728

Table 3i. Diversity Indices for bridge vectors by zone.

Zone	Simpson's	Shannon-Weaver	Evenness
Southeast	1.0000	0.0000	1.0000
South-central	0.9999	0.0003	0.5002
West	0.9998	0.0008	0.4966

Table 3j. Diversity Indices for enzootic vectors by zone.

Zone	Simpson's	Shannon-Weaver	Evenness
Southeast	0.8200	0.3786	0.4869
South-central	0.7330	0.4941	0.2300
West	0.5989	0.8478	0.2919

Table 3k. Diversity Indices for mammal feeding species by zone.

CHAPTER FOUR

TESTING FOR DEVELOPMENT OF *CULEX PIPIENS* RESISTANCE TO BACILLUS THURENGIENSIS ISRAELENSIS

Introduction

Since the discovery that the pathogens that cause infectious diseases can be transmitted by mosquitoes, there have been efforts to eradicate these vectors. Total eradication is no longer viewed as a viable objective. Management efforts now focus on preventing mosquito bites, reducing mosquito population density, minimizing mosquito-vertebrate contact, and reducing the longevity of female mosquito life spans (Mullen & Durden 2002). Historically, management efforts in the United States have included land reclamation projects to eliminate larval habitat and reliance on insecticide application for control of adult mosquitoes (Rose 2001). These efforts have evolved into a more environmentally sensitive integrated pest management scheme that includes habitat management, personal protection, biological control, chemical control, surveillance to determine when and where chemical control efforts are necessary, and monitoring the efficacy of any chemical control efforts taken. When insecticides are necessary, the products used are applied judiciously and most have a low environmental impact. An effective biological control product is the bacterium *Bacillus thuringiensis israelensis* (*Bti*). *Bti* toxicity is specific to certain Diptera larvae, including mosquitoes, and kills larvae by damaging the midgut epithelial lining, which leads to ionic imbalances and death. *Bti* has been used around the world since 1978 (Stark 2005) and has been proven to be effective with few non-target insect impacts (Tilquin et al. 2008).

A potential limitation of any pesticide is the development of target organism resistance, which makes the pesticide ineffective for controlling that pest. Chemical insecticides have been used against vector mosquitoes for the management of malaria and other mosquito-borne

diseases since the 1940's (Mittal 2003) and resistance has developed to every available class of insecticide (Hemingway et al. 2004). Resistance develops through two primary mechanisms. DNA mutations can decrease the sensitivity of the pesticide target site, and changes in detoxification enzymes can lead to an increase in pesticide detoxification activities by the insect (Boyer et al. 2007).

Despite extensive use of *Bti*, only one study (Paul et al. 2005) has reported mosquitoes resistant to this pesticide in the field. The lack of widespread resistance to *Bti* could be due to its complex mode of action, which involves synergistic interaction between up to four proteins (Becker and Margalit 1993). However, some Lepidoptera have developed resistance to *Bti* (Glare and O'Callaghan 1998), so it cannot be assumed that widespread resistance in mosquitoes targeted by this pesticide will not develop. The detection of resistance at early stages may allow for the extension of the effective life of pesticides by alerting vector control agencies that alternative management methods need to be employed (NRC 1986). The purpose of this study was to test for resistance in a population of *Culex pipiens* Linnaeus mosquitoes that are currently being exposed to *Bti*.

Methods and Materials

Mosquitoes

Mosquito eggs or larvae used for this study were collected from three different sites in eastern Washington State (WA): Moses Lake, Grant County; Deer Park, Spokane County; and Pullman, Whitman County (Table 4a and Figure 4a). The Grant County Mosquito Control District #1 conducts management efforts using *Bti* at the site where mosquito larvae were collected from Moses Lake, but no management efforts are employed at the latter two locations.

Rearing

Larvae collected from each site were maintained in the laboratory and reared through five generations. Larvae were reared in 23 cm by 33 cm enameled metal pans and were fed a three to one mixture of Kaytee Forti-Diet® mouse, rat, and hamster food and Coopers brewing yeast, respectively. Three grams of the powdered mixture was added to 100 ml de-ionized water. Each larval pan received 6 ml daily. Environmental conditions were kept at 29° C and a 14:10 light/dark schedule for the majority of the study. Dawn and dusk light was simulated one hour before lights on and one hour after lights out with a 15 watt incandescent light bulb and some shielding. Additional ultraviolet radiation was provided during light hours with a 60 watt incandescent black light bulb. Pupae were removed from rearing pans and placed in adult cages on the same day they appeared. Adults were maintained in the same environmental conditions and were fed an eight percent sucrose solution. Females were blood fed on white leghorn chickens for egg production. Chickens were placed inside the adult mosquito cages for less than 12 hours. Dawn and dusk light was provided in the same manner as described above for the larvae.

Bioassays

Groups of ten, fourth-instar larvae were placed in 100 ml of distilled water and the appropriate amount of insecticide (VectoBac® 12AS Aqueous Suspension, Valent Biosciences Corporation., Libertyville, IL) in 266 ml plastic cups. Larvae were subjected to the insecticide field application rate concentrations and a series of four, ten-fold dilutions. Control cups filled with 100 ml distilled water were provided for each replication. Mortality was recorded after 24 hours. The number of replications for each population and each generation depended on the number of larvae available. Larvae that survived the assays were discarded and not used to develop subsequent generations.

Statistical Analysis

Bioassay data for each population and F_1, F_3, and F_5 generations were pooled and probit analysis was conducted using Minitab 15® (Minitab Inc., State College, PA) software. Statistical significance of LC_{50} and LC_{95} values was based on non-overlap of 95% confidence intervals.

Results

Figures 4b and 4c show the LC_{50} and LC_{95} 95% confidence intervals, respectively. Too few larvae from the Pullman population were produced to include that population in the F_1 generation assays. There was a significant difference between the Moses Lake population and the Deer Park population in the F_1 generation. This difference disappeared in the F_3 and F_5 generations. Similarly, there was a significant difference between the Pullman population and the Deer Park population in the F_3 generation that disappeared in the subsequent generations. There was no significant difference between any of the populations in the F_5 generation.

Discussion

The standard protocols for *Bti* assays use groups of twenty larvae per concentration (Wirth et al. 2001). Given the limited number of larvae that were produced for this study, I was unable to use this many larvae. A protocol for methoprene bioassays used by Braga et al. (2005) used ten larvae per concentration and another protocol for methoprene bioassays used by Cornel et al. (2002) used fifteen larvae per concentration and probit analysis for the statistical method. Restrepo et al. (1997) used five first instar larvae and probit analysis to determine the toxicity of *Bacillus thurengiensis medellin* and Stevens et al. (2005) used ten larvae per concentration and probit analysis for *Bti* assays of *Chironomus tepperi* Skuse. Furthermore, using a known susceptible line of *Cx. quinquefasciatus* Say mosquitoes obtained from the University of California, Riverside, CA, bioassays were performed with ten larvae per cup for comparison to our bioassays of field populations. Probit analysis showed no significant

difference between the field populations and the susceptible line of mosquitoes, based on non-overlap of 95% confidence intervals (Figure 4d). Given the use of fewer than twenty larvae per test group for various pesticide bioassays in published studies and the fact that bioassays conducted on a known susceptible mosquito line were not significantly different, I am confident that using ten larvae per concentration was an appropriate method for this study. Additionally, using only ten larvae per concentration allowed more replicates to be performed.

The significant differences between the Moses Lake and Deer Park populations in the F_1 generation and between the Deer Park and Pullman populations in the F_3 generations could have occurred for various reasons. First, I was not able to rear a large number of larvae. This led to a small representation of the genetic variation in these populations and also limited the number of assays that could be conducted on any one population/generation combination. These limitations potentially increased sampling error and these assays may not have represented the population as a whole. Second, pesticide resistance often comes with a fitness cost. If a field population is under insecticide pressure, those individuals that have some resistance will be able to out-compete those that do not. Even though they are generally less fit, they will still be able to survive pesticide application and reproduce. When this population is brought into the lab and not subjected to insecticide pressure, those individuals that do not have resistance can out-compete those that do have resistance, since they are not under insecticide pressure and are fitter than those with resistance. This can cause any significant differences to disappear. Third, when populations are brought into the lab and reared under ideal environmental conditions, their fitness increases and may enable them to withstand insecticide application to a greater degree. Fourth, even though every effort was taken to conduct assay under identical bioassay conditions, differences in bioassay conditions are always possible (Wirth et al. 2001).

Resistance is defined as the developmental ability of a strain to tolerate doses of toxins that usually are lethal for most individuals within a normal population (Mullin and Scott 1992).

However, normal populations can vary considerably in the amount of toxins that can be tolerated. Natural variation in susceptible *Cx. pipiens* mosquito populations to *Bti* has been reported to vary over 10-fold (Wirth et al. 2001) and tolerance in levels under this amount have not been considered as resistant. For example, Paul et al. (2005) reported *Cx. pipiens* resistance ratios of 6.6, 3.1, and 7.7 to phenothrin, *Bacillus sphaericus*, and methoprene, respectively, but considered this same population resistant only to *Bti* with a resistance ratio of 33. Tables 4b and 4c show the LC_{50} resistance ratios for each population and each generation of the mosquitoes from the current study compared to the susceptible strain of *Cx. quinquifasciatus* mosquitoes obtained from the University of California. All resistance ratios are well within the natural variation reported in the literature and below the resistance ratios of the published studies that do not consider populations to be resistant.

Based on data from this field population bioassay, I conclude that there is no significant difference in the susceptibility to *Bti* of these three mosquito populations and that there is no development of resistance in the Moses Lake population of mosquitoes.

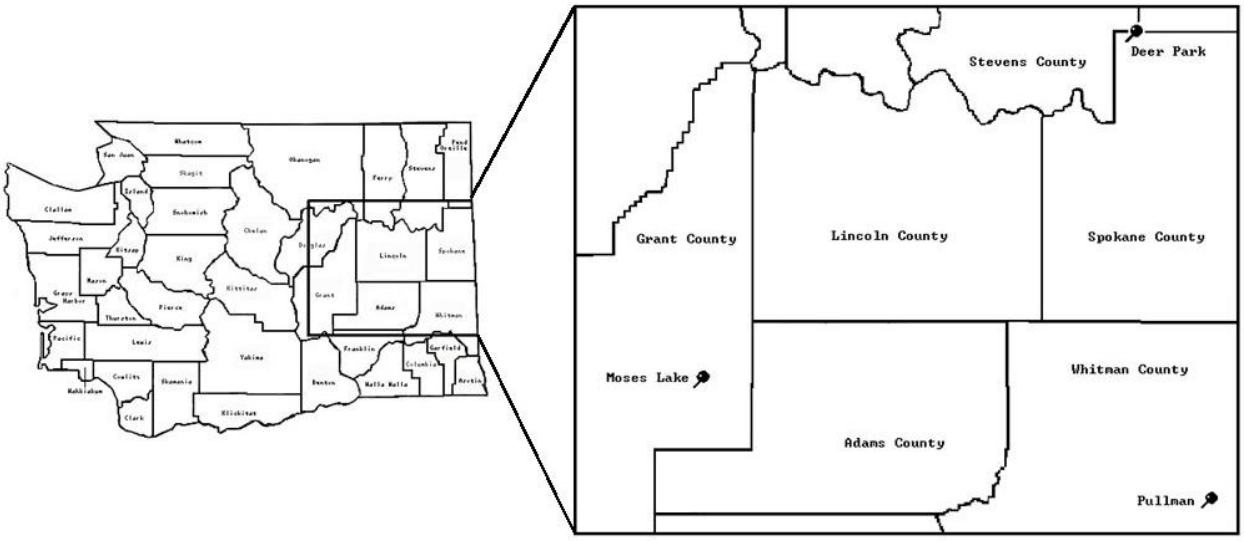


Figure 4a. Mosquito collection sites.
Mosquitoes were collected on 30 Aug 2008, 1 Sep 2008, and
30 Sep 2008 from Moses Lake, Deer Park, and Pullman, respectively.

Location	Latitude (°)	Longitude (°)	Elevation (meters)
Moses Lake	N 47.07773	W 119.21564	357
Deer Park	N 48.01935	W 117.4879	684
Pullman	N 46.75094	W 117.19113	762

Table 4a. Mosquito collection locations.
Mosquitoes were collected on 30 Aug 2008, 1 Sep 2008, and
30 Sep 2008 from Moses Lake, Deer Park, and Pullman, respectively.

Population Resistance Ratios	
Moses Lake	3.22
Deer Park	4.80
Pullman	4.39

Table 4b. Moses Lake, Deer Park, and Pullman
mosquito population *Bti* resistance ratios

Generation Resistance Ratios	
F_1	0.63
F_3	6.52
F_5	4.01

Table 4c. F_1, F_3, and F_5 mosquito generation *Bti* resistance ratios

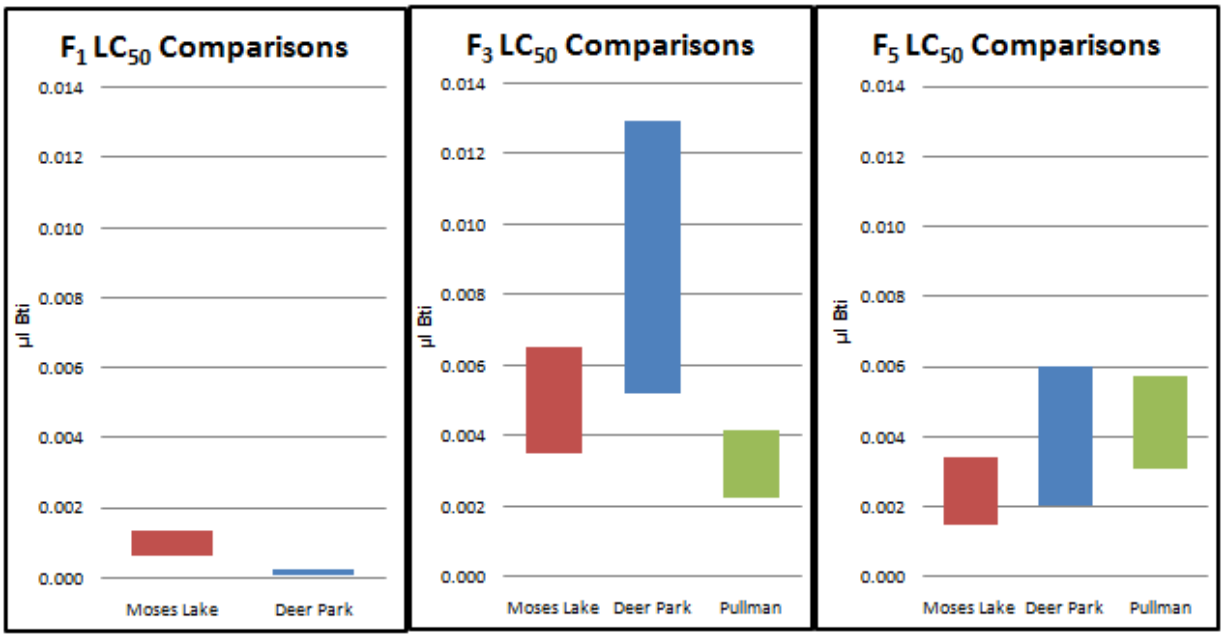


Figure 4b. *Bti* bioassay LC_{50} 95% confidence intervals for F_1, F_3, and F_5 mosquito generations. Too few F_1 larvae from the Pullman population were reared to include in that bioassay.

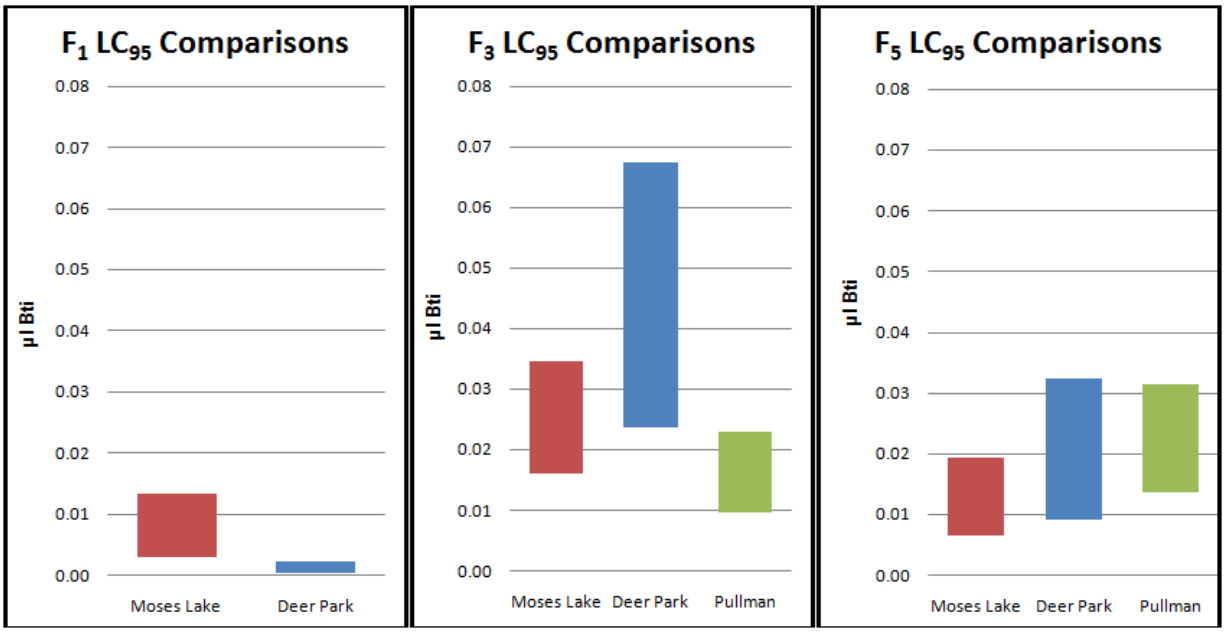


Figure 4c. *Bti* bioassay LC_{95} 95% confidence intervals for F_1, F_3, and F_5 mosquito generations. Too few F_1 larvae from the Pullman population were reared to include in that bioassay.

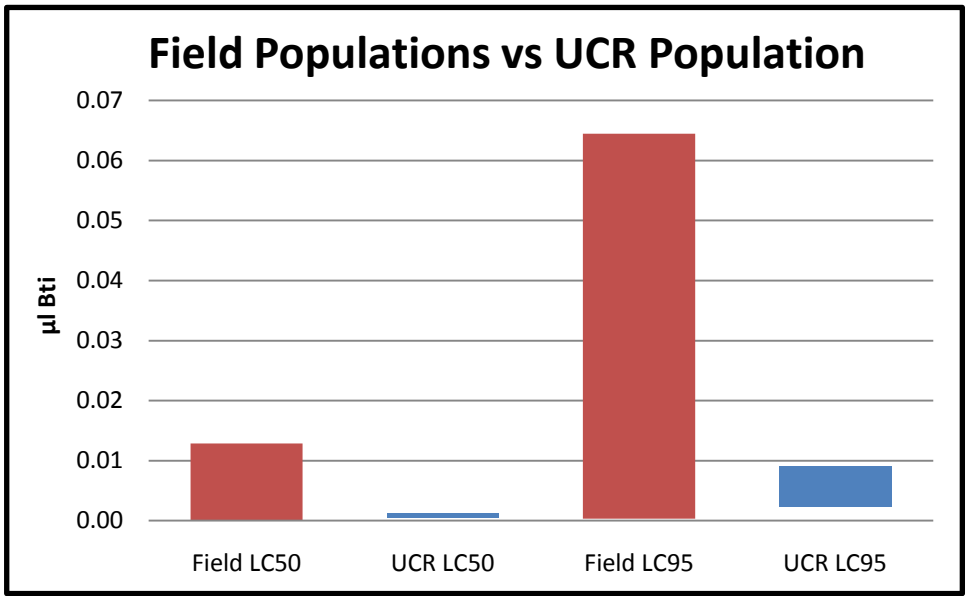


Figure 4d. 95% confidence interval range of field populations and UCR population.

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