Impact of Environmental Factors on Mosquito Population Abundance and Distribution in King County, Washington

Julia J Weicheld

A thesis
submitted in partial fulfillment of the
requirements for the degree of

Master of Science

University of Washington
2015

Committee:
Charles D. Treser
John Scott Meschke
Elizabeth Dykstra

Program Authorized to Offer Degree:
School of Public Health- Environmental Health

University of Washington

Abstract

Impact of Environmental Factors on Mosquito Population Abundance and Distribution in King County, Washington

Julia J Weicheld

Chair of the Supervisory Committee:
Senior Lecturer Charles D. Treser, MPH
Department of Environmental and Occupational Health Sciences

Background: Climate, land cover, and other environmental factors have been shown to have a direct impact on the epidemiology of vector-borne diseases. Warming temperatures combined with other effects of climate change and changes in land use have the potential to amplify vector mosquito populations and transmission of arboviruses in King County, Washington. This research aims to provide insight into vector populations that may govern vector-borne disease transmission in King County.

Methods: Mosquitoes were trapped at selected areas in King County in summer 2014. Additional mosquito data for King County were gathered and assessed for quality and completeness. Identical sites sampled in 2003 and 2014 were directly compared to determine any changes in mosquito abundance and diversity over an 11-year period. Temperature, precipitation, and land cover data were obtained and investigated for their influence on mosquito abundance using correlative and regression analyses.

Results: The correlative analysis found mosquito abundance was significantly positively associated with percent med-high developed land cover, maximum temperature, and minimum temperature variables. Mosquito abundance metrics were found to be negatively correlated with percent forested land cover and average weekly precipitation. Mosquito abundance was significantly higher in 2003 than in 2014, but was unexplained by changes in land cover or climate.

Conclusions: Mosquito populations appear to be impacted by the climate and land cover variables studied, but other factors not examined in this study may have greater impacts.

Table of Contents

Introduction 7
Landscape Epidemiology 8
Climate Change in Washington State 8
Mosquito-Transmitted Diseases of Concern for Washington State 9
West Nile Virus 9
St. Louis Encephalitis Virus Western Equine Encephalitis Virus 9
Travel-Associated Diseases and Potential for Endemic Emergence 10
Vector-borne Disease and Mosquito Control in Washington State 11
Mosquito Species Behavior 11
Effect of Temperature 13
Effect of Precipitation 13
Effect of Land Cover and Use 14
Need for Location-Specific Models 15
Preliminary Studies in King County, WA 15
Methods 17
Mosquito Surveillance 2014 17
Sampling Sites 17
Mosquito Sampling, Identification, and WNV Testing 19
WA DOH Mosquito Surveillance Dataset 20
Selection Criteria for Incorporation into Multiple Regression Model 21
Environmental Factors Datasets and Analysis 22
Land Cover 22
Climate Data 23
Statistical Analysis 26
2003 versus 2014 Comparison 26
Correlatvie Analysis and Generalized Additive Model 27
Results 28
Mosquito Surveillance 2014 28
Adults 28
Larvae 30
2003 versus 2014 Comparison 31

Mosquito Distribution 2003, 2008, 2014 .... **36**
Environmental Factors and Mosquito Abundance **37**
Land Cover 37
Temperature 39
Precipitation 39
Regression Model **40**
Discussion **41**
Aim III: Limitations and Future Directions **42**
Limitations 42
WA DOH Mosquito Dataset Discussion and Future Directions 43
Aim I: Mosquito Surveillance 2014 .... **44**
Aim I: 2003 versus 2014 Comparison **45**
Aim II: Environmental Factors and Mosquito Abundance **46**
Conclusions **48**
References **49**
Appendix **53**
Attribute Table of Selected Sampling Sites in King County **53**
Sites and Mosquito Abundance Metrics 53
Sites with Percent Land Cover Classification Values 54
Map of All King County Data Points from the WA DOH Dataset **55**
ArcGIS Workflow **56**
Mosquito Larvae **57**
Land Cover Classification, All Sites **58**
Climate Variables Correlation Coefficients by Year **58**
All Correlations- 2003, 2008, 2014 Combined **60**

Acknowledgements

I would like to thank the following organizations and people for making this study possible:

Washington State Department of Health, Zoonotic Disease Program

- Elizabeth Dykstra
- Susan Brush
- David Kangiser

University of WA Department of Environmental and Occupational Health Sciences

Advisors

- Chuck Treser
- Scott Meschke

Undergraduate Field Sampling Interns

- Kevin Chung
- Logan Kegley

Meschke Lab Staff

- Nicola Beck
- Alexandra Kossik
- Christa Fagnat

Fellow DEOHS Graduate Students

University of WA Statistical Consulting Service

- JooYoon Han
- Paul Sampson
- Bowen Wang

GIS Help

- Edmund Seto
- UW GIS Lab

Introduction

Climate, land cover, and other environmental factors have a direct impact on the epidemiology of vector-borne diseases (WHO, 2000). Climate affects the geographic and temporal distribution of arthropod vectors, as well as the lifecycle characteristics, dispersal patterns, and transmission of vector-borne diseases (Gould and Higgs, 2009). Land cover and built environment may affect the transmission dynamics of vector-borne disease by acting as environmental reservoirs where hosts, vectors, and pathogens come into contact. Environmental changes such as climate change and urbanization may affect the transmission of vector-borne diseases. These changes can result in altered interactions among vectors, animal reservoirs, pathogens, and human hosts (Institute of Medicine, 2003). Because of these altered interactions, certain vector-borne diseases may increase in some regions, while decreasing in others.

Findings from the Washington Climate Change Impacts Assessment suggest that the Pacific Northwest is likely to experience a 1.1 °C to 4.7 °C increase in temperature by 2070 (Dalton et al., 2013). Warming temperatures combined with other effects of climate change and changes in land use have the potential to amplify vector mosquito populations and transmission of arboviruses such as West Nile Virus in Washington. The relationship of vector-borne disease transmission with the environment is complex and highly localized. To adequately prepare for the effects of climate and other environmental change, vector populations and disease transmission dynamics need to be studied on a local scale.

This thesis aims to provide insight into vector populations that may transmit vector-borne disease in King County, Washington State. The study was conducted in conjunction with the Washington State Department of Health (WADOH) Zoonotic Disease Program. The project consisted of three aims:

Aim I: *Survey the current mosquito population at selected sites in King County and compare results with data collected from 2003;*

Aim II: *Associate mosquito populations with current and historical climate and land cover data; and,*

Aim III: *Assess the quality and completeness of the existing data.*

For the first aim, mosquito surveillance was conducted during the summer of 2014 at ten sites over fifteen weeks. Land cover and climate variables were analyzed for associations with mosquito abundance data. Additionally, some of the sites selected were identical to sites sampled previously by DEOHS students, so an analysis directly comparing these sites was performed. The second aim uses a dataset compiled by WADOH containing mosquito population data in conjunction with climate and land cover datasets collected over roughly the past ten years. These data were analyzed using correlative analysis and a generalized additive regression model to elucidate trends between mosquitoes and the local environment of King County. The third aim assessed the quality and completeness of mosquito data for King County and made recommendations for future research. The information generated by this study can be used to help guide state and county mosquito abatement efforts. The study also provides the groundwork for the future development of a model that could predict the risk of vector-borne disease using climate change scenarios and other environmental factors for regions of the state.

Landscape Epidemiology

This project uses a landscape epidemiology approach to better understand vector-borne disease transmission in Washington. Landscape epidemiology aims to locate centers of disease transmission where vector, host, and pathogen interact within a distinct landscape. The spatially defined focus, sometimes called the 'nidus' of transmission, can be characterized by vegetation, climate, elevation, built environment, and other ecological factors (Reisen, 2010). This project specifically focuses on how mosquito populations are affected by the environment in which they live. Arthropod vector populations are particularly important to study from an environmental health perspective because they can be targeted by vector control strategies, which are generally the most effective way to control an outbreak of vector-borne disease. To better understand the epidemiology of mosquitoes and environment, climate and land cover datasets are analyzed in relation to mosquito abundance, species, and location data. Geographical information systems, remote sensing, and modern computing allow for enhanced analysis of environmental factors and vector population dynamics (Reisen, 2010). In this project, geographic information systems were used to spatially relate land cover datasets, weather station datasets, and mosquito population data. Associations between these environmental factors and mosquito population data were calculated using R.

Climate Change in Washington State

The Pacific Northwest is likely to experience an annual warming of 1.1 °C to 4.7 °C (2 °F to 8.5 °F) by 2070, with the lower end possible only if greenhouse gas emissions are significantly reduced. There is less consensus regarding average precipitation effects, but models are unanimous in predicting that the state will experience drier summers. The greatest changes in temperature and precipitation are expected to occur in the summer season, specifically increased temperatures and decreased precipitation. Overall there is predicted to be an increase in extreme heat events, and a decrease in extreme cold events.

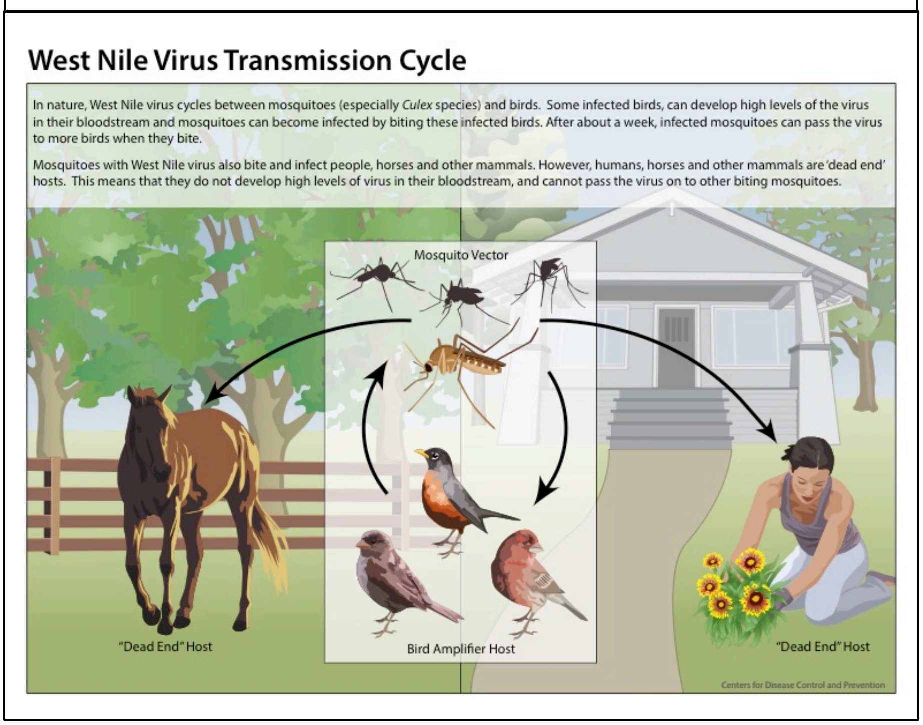
Other consequences of climate change that will not directly effect vector populations but will affect Washington include increased snowmelt and sea-level rise (Dalton et al., 2013).

Mosquito-Transmitted Diseases of Concern for Washington State

West Nile Virus

West Nile Virus (WNV) is an arboviral disease affecting birds, horses, and humans. The transmission cycle consists of an avian reservoir and mosquito vector. Historically, WNV epidemics were constrained to areas of Africa, Europe, the Middle East, and parts of Asia (Rossi et al., 2010). In 1999, an unprecedented outbreak of WNV occurred in New York, which subsequently spread across the nation the following few years. WNV first appeared in Washington in 2002, after it was identified in two birds and two horses. The first human cases in the state occurred in 2006 (WADOH). Infection with WNV may cause a febrile illness, or it may result in a more severe neuroinvasive disease such as encephalitis or meningitis which can be fatal. It is estimated 80% of people infected with WNV do not show symptoms. Since its introduction into the U.S. there have been an estimated 3 million cases of WNV in humans. Although there is a vaccine for horses, no human vaccine is yet available. Vector control strategies are the primary method used to reduce the spread of this pathogen (WHO, 2011; CDC, 2013).

Figure 1. West Nile Virus Transmission Cycle. Source: CDC



St. Louis Encephalitis Virus and Western Equine Encephalitis Virus

St. Louis Encephalitis (SLE) and Western Equine Encephalitis Virus (WEE) are arboviral diseases closely related to WNV that are also maintained in a mosquito-bird cycle. Clinically, SLEV has been shown to affect humans, and WEE affects both humans and horses.

St. Louis Encephalitis has primarily occurred in eastern and central states, where episodic urban-centered outbreaks have occurred cyclically since the 1930s. In the rural west, including the central valleys of Eastern WA, transmission patterns have followed more of an endemic pattern. From 2004-2013, 92 SLE cases nationwide were reported to the CDC. It is estimated that less than 1% of SLE infections are clinically apparent and the vast majority of infections remain undiagnosed. However when the disease is clinically apparent, it most often results in encephalitis and the overall case-fatality ratio is 5-15%. (CDC, 2009). SLE was last detected in sentinel chickens in Benton County, WA in 2005 (WADOH, 2015). No in-state acquired infections have occurred since the 1970s (Bost, 2004).

Western Equine Encephalitis is usually reported in states and Canadian provinces west of the Mississippi River. The disease is generally considered to be less serious than SLEV in humans, but more severe in horses. The mortality rate in humans ranges from 1-5%, but is considerably higher in horses (Pratt & Moore, 1993). A WEE vaccine is available for horses, but not humans (MDH, 2005). Before the introduction of WNV, the last human case of mosquito-borne encephalitis in Washington was a WEE infection reported in 1988 (WADOH, 2015). Typically, less than 5 WEE human cases per year are reported nationwide. No human cases have been reported since 1999 (ODH, 2015).

Travel-Associated Diseases and Potential for Endemic Emergence

It is worth noting the recent emergence of endemic cases of Dengue and Chikungunya elsewhere in the US. These two diseases typically only occurred as travel-associated illnesses in the states, but were recently found to occur locally in areas of Florida. Chikungunya was first detected locally in Florida in Summer 2014, and Dengue in the Summer of 2009. The most common vectors for these diseases are *Aedes aegypti* and *Aedes albopictus* (FloridaHealth, 2015). Currently neither of these species have been reported anywhere in Washington state. Typically, there are between 0-20 cases of travel-associated Dengue fever reported per year in Washington. Travel-associated cases of Chikungunya were very rare in Washington until 2014, when 11 cases were identified in travelers returning from the Caribbean (WADOH, 2015). Both Dengue and Chikungunya are human-mediated diseases, so in addition to the mosquito vectors being present, a significant proportion of the human population has to be infected for the diseases to be established in a new area. At this point, it is unlikely that Dengue or Chikungunya will occur locally in Washington. However, if the mosquito vectors are introduced to the state by travel, trade, or other means, it is possible that they could become established and potentially carry the diseases.

Vector-borne Disease and Mosquito Control in Washington State

In Washington, vector-borne disease has historically remained at low levels. West Nile Virus is currently the mosquito-transmitted disease of most concern across the nation and in the Pacific Northwest. The highest number of WNV cases in the Pacific Northwest occurred in 2006, at 1,068 cases. Of these cases, 996 occurred in Idaho, which led the nation that year. The most significant year for WNV in WA was 2009, during which 38 human cases were reported (CDC, 2013). As of 2012, 27 potential WNV vector species have been identified in the state (WADOH). *Culex pipiens* and *Culex tarsalis* are considered to be Washington's most efficient bridge vectors for WNV (WADOH, 2008). From 2008 to 2014, 575 mosquito pools tested positive for WNV in Washington State. Of the positive pools, 41% were *Culex pipiens*, and 59% were *Culex tarsalis*. There was also one pool that tested positive for SLE, which consisted of *Culex tarsalis* mosquitoes (WADOH).

Currently 13 counties in WA have mosquito control districts, 9 of which are in eastern Washington. Eastern Washington is primarily an arid environment, but irrigation practices used for agriculture in the region increase mosquito densities and the potential for vector-borne disease transmission. The region has experienced WEE and SLE outbreaks in the past. Three eastern Washington counties, Grant, Chelan, and Walla Walla, have multiple mosquito control districts. Western Washington historically has not experienced much mosquito-borne disease. Just four of the western Washington counties have mosquito control districts: Clark, Cowlitz, Skamania, and portions of the Island Counties. King and Pacific counties control mosquitoes on an as-needed basis (Sames, 2007). In 2001, WADOH began a WNV Surveillance Program (Bost, 2004). In 2008, WADOH released the document, "Guidance for Surveillance, Prevention, and Control of Mosquitoborne Disease" (WA DOH, 2008). The document outlines surveillance, mosquito control activities, alert levels, and phased response protocols for mosquito-borne disease outbreaks in the state.

Mosquito Species Behavior

One reason mosquito population fluctuations are so difficult to predict is because each species has its own distinct habitat and behavioral preferences. When examining climate and other environmental effects, it is important to consider the variability among mosquito species. The following list outlines the habitat and behavioral preferences of some of the most frequently caught mosquitoes in King County.

***Culex pipiens (*subspecies *Culex pipiens pipiens*):** An urban dwelling mosquito that prefers to breed in city drains, artificial containers, and catch basins rich with organic matter. Commonly known as the 'northern house mosquito', it is present in roughly the northern half of the United States and in southern British Columbia. The flight range is generally ¼-1 mile. In warm weather, eggs hatch in 1-2 days, and development from larva to adult takes 8-10 days (EPA, 2004; Pratt & Moore, 1993). It is a bridge vector that feeds on birds and humans, and is important in the transmission of WNV and SLE to humans (Hamer et al., 2008).

***Culex tarsalis*:** Commonly known as the 'encephalitis mosquito', *Cx. tarsalis* is considered the most important vector of WEE and WNV to man and horses in the western US. It is a rural vector that mostly breeds in temporary pools of standing water in upland vegetative ecotones, including irrigated agricultural areas (EPA, 2004; Reisen, 2002). It is widely distributed west of the Mississippi River, and is probably the most widespread mosquito in Washington state (WADOH, 2008). *Cx. tarsalis* commonly feeds on birds, domesticated animals, and humans, and (EPA, 2004; Pratt & Moore, 1993). Dispersion studies have shown *Cx. tarsalis* can fly as far as 11 miles, but most remain within a mile of their breeding place. Eggs usually hatch within 48 hours, and the larval and pupal stages develop rapidly (Pratt & Moore, 1993).

***Coquillettidia perturbans*:** Commonly known as the 'irritating mosquito', *Cq. perturbans* is a fierce biter but not a common vector of disease. This species breeds in marshes, ponds and lakes that have a thick growth of cattails or other aquatic vegetation. Larvae attach to the stalks of vegetation and do not need to rise to the surface to breathe because they have a breathing tube adapted for penetrating plant tissue and obtaining oxygen through the plant. Larval development is much slower than in other mosquito species, and typically there is only one generation of adults per year. Adults emerge in the late spring/early summer in large numbers, and the flight range is 1-5 miles (Pratt & Moore, 1993; WADOH, 2008). *Cq. perturbans* was once considered a potential vector for WNV, but it exhibits a substantial salivary gland barrier, thereby preventing virus transmission(Sardelis et al., 2001).

Culiseta incidens: Commonly known as the 'cool weather mosquito', *Cs. incidens* is often trapped in King County, especially later in the season. The species is peridomestic, and is distributed in the western United States. Larvae have been found in a variety of environments including brackish water on the coasts, snow pools in the mountains, and bird baths, ornamental ponds, and rain barrels in more urban areas. Under optimum

conditions development from egg to adult takes about two to three weeks. Flight range is limited to 5 miles or less. *Cs. incidens* is reported to feed more frequently on fowl and domestic animals than humans (Pratt & Moore, 1993; Solano County, 2005).
Although repeatedly tested for arboviruses, it has not been found naturally infected with WEE, SLEV, or WNV except in very rare instances (Reisen et al., 2006).

Effect of Temperature

Environmental temperature can affect the infection, dissemination, and transmission of arboviruses, as well as the lifecycle and behavior of the vectors. In laboratory experiments, higher temperatures yielded faster and increased dissemination of WNV in *Cx. pipiens* mosquitoes, and increased transmission of WNV by *Cx. tarsalis* mosquitoes (Dohm et al., 2002; Reisen et al., 2006). In warmer climates, adult female mosquitoes digest blood faster and feed more frequently, increasing transmission further. If water temperatures also rise, larval development happens more quickly and the number of female adult mosquitoes seeking a blood meal at a given time increases (WHO, 2000).

Several studies have found increased WNV transmission and/or mosquito populations in years with high temperatures in the northern latitudes (Chen et al., 2013; Ruiz et al., 2010). In contrast, southern latitudes show increased vector mortality in high temperature years due to the already high temperatures present in these regions (Morin & Comrie, 2013). However, a decline in vector mortality does not necessarily indicate a decrease in WNV transmission (Epstein, 2001). Increased annual temperatures have also been shown to lengthen the seasonality of vector species. A model using a projected climate change scenario predicted a seasonality for the WNV vector *Culex p. quinquefasciatus* that extended well into spring and fall (Morin & Comrie, 2013). Warm winters followed by hot, dry summers are believed to favor WNV transmission cycles. The initial 1999 outbreak in New York that introduced WNV to the US occurred in a year marked by these conditions, and subsequent spreading proliferated most in years with similar climate conditions.(Epstein, 2001).

Effect of Precipitation

The effect precipitation has on mosquito populations and vector-borne disease is less well defined than the effects of temperature. Precipitation is generally considered to benefit mosquito populations because it creates sites for mosquitoes to lay their eggs and larva to develop. For example, *Cx. tarsalis* benefits from increased rain because it creates breeding sites in rural areas (Reisen, 2002). Arid environments experience the greatest surge in mosquito populations following rainfall because areas of standing water are created that weren't previously available. On the other hand, environments that experience high precipitation regularly may experience a decrease in mosquito populations after a heavy rainfall event because the rain flushes mosquito larvae out of their standing water environments. *Cx. pipiens* prefers to breed in urban containers rich in organic matter, so higher than average rainfall may dilute or flood these containers making them less desirable to breed in.

It is drought, not rainfall that appears to facilitate the spread of WNV. *Cx. pipiens* may directly benefit from drought conditions because of the concentration of organic matter in urban basins. Drought can also decrease the number of mosquito predators such as frogs and dragonflies that are a threat to vectors breeding in wetland areas (Epstein, 2001). Drought is also thought to bring mosquitoes and avian reservoirs into closer proximity, so that the virus circulates more easily. The combination of increased vector populations and increased contact with birds following drought was associated with a higher risk of SLE in humans (Shaman et al., 2005). The same effect has been suggested for WNV (Wang et al., 2010). There is evidence for increased WNV cases following drought in Washington. A study looking at environmental factors and WNV prevalence on a county-by-county basis in the Pacific Northwest found decreased precipitation was associated with a higher prevalence of WNV infection. However, the study did not find a direct association between precipitation and vector abundance, nor for precipitation and abundance of several avian reservoirs (Crowder et al., 2013).

Effect of Land Cover and Use

Land use patterns have been associated with vector populations and vector-borne disease dynamics. Changes to the physical environment can result in altered interactions among vectors, reservoirs, pathogens, and human hosts (Institute of Medicine, 2003). Climate effects can be exacerbated or minimized by the current land cover. Urbanization and agricultural intensification have been linked with WNV prevalence in areas of the United States (Bowden et al., 2011; Brown et al, 2009). In Washington, orchard habitats have been associated with a greater prevalence of WNV in birds and horses. The abundance of *Cx. pipiens* and *Cx. tarsalis* was also significantly higher in orchard environments. The study also found an increased number of WNV infected vector mosquitoes in areas with vegetable/forage crop and natural land cover, but did not find an increase in WNV infections in bird, horse, or human populations in these areas. This suggests that these areas do not promote WNV transmission as well as orchards (Crowder et al., 2013). To better understand vector population dynamics and vector-borne disease transmission potential, land use patterns need to continue to be studied in relation to these factors.

Need for Location-Specific Models

Although many studies have been published looking at the associations between environmental factors and WNV transmission, broad patterns remain elusive because of variable results (Ruiz et al., 2010). Local climate data and land cover play important roles in mosquito population dynamics, making each region distinct in how it may experience vector-borne disease. Climate change also has a regional effect, further emphasizing the need for location-specific models addressing these two coinciding public health threats. Currently it is difficult to develop a public health response because of the lack of location-specific models and the lack of quantitative data needed in these models (Morin & Comrie, 2013).

Climate models require data sets with high spatial resolution and continuous sampling over time so that future climate scenarios can reasonably be predicted. Choice of geographic scale and units can profoundly impact the outcome and quality of an analysis. Issues of scale remain a challenge in integrating mosquito and climate models because vector surveillance often occurs at the GPS coordinate level, whereas climate and other environmental data is often collected at the zip code level, county level, or higher. Problems may also exist if the weather data is collected sporadically or on a weekly scale rather than daily. If a geographic scale is too coarse it can obscure fine spatial and temporal patterns (Ruiz et al., 2010). It is likely that the environmental factors occurring directly at or around the mosquito sampling sites are the most responsible for affecting the population dynamics measured at those sites. Unfortunately, it is extremely labor intensive to produce environmental data sets at such a fine scale. Advancements need to be made in the integration of vector population data with projected climate scenarios, and localized models will provide the most useful results.

Preliminary Studies in King County, Washington

DEOHS collected mosquito surveillance in King County, WA from 2002-2006. Surveillance was performed weekly at as many as 19 locations throughout the county. Surveillance data served as the basis of the Masters theses of Amanda Zych in 2002 and Heather Bost in 2004, and was the focus of the paper "Climatic and landscape correlates for potential West Nile virus mosquito vectors in the Seattle region" (Peccoraro et al., 2007). Some smaller studies were also conducted at the time, including one that looked at mosquito species diversity in two ecologically distinct sites, the Union Bay Wetland Area and Lake City Pond (Stevens, 2004). The data collected from King County during this time has good identification of site factors, spatial coordinates, and species-level mosquito data. Additionally, sampling involved collection of both adults and larvae in contrast to most

WADOH locations where only adult sampling was performed. Due to budget constraints, surveillance ceased after 2006, until the summer of 2013 when sampling was re-initiated at a few sites.

Bost's thesis focused on comparisons of mosquito abundance at detention pond and non-detention pond sites. She found that mosquito abundance was significantly higher at the pond sites. Additionally, she briefly examined vector abundance and climate trends. Bost found a general trend of increased vector abundance with increased temperature, but no statistically significant correlations of vector abundance with temperature or precipitation were found.

In this study, four of the sites sampled by Bost in 2003 were resampled during the Summer of 2014 and results are directly compared. Environmental datasets and analyses were recollected and performed to match the parameters used in the current study.

Methods

Mosquito Surveillance 2014

Sampling Sites

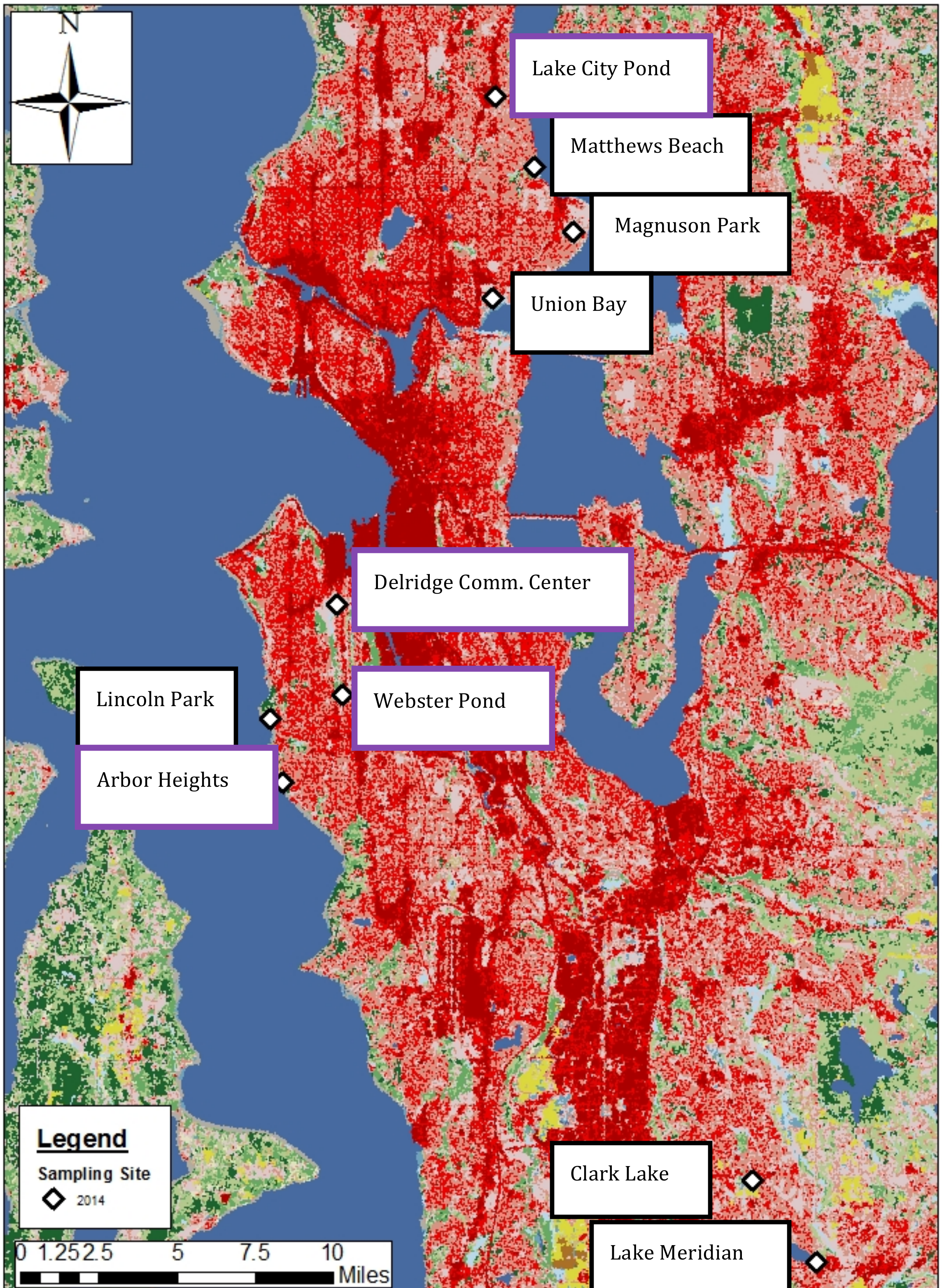
Mosquitoes were sampled at 10 sites over 15 weeks during the summer of 2014. Adults were sampled at all of the sites consistently over the entire period. Mosquito larvae were sampled at a few of the sites, and not always as consistently. Sampling strategy is discussed further in the next section. Sampling sites were located in King County in the greater Seattle area. Sampling was conducted at the sites listed in Table 1.

Table 1. Mosquito Sampling Sites 2014

Site Name	**Site Code**	**Larva sampling?**
Lake City Pond	WEHS0082	Yes
Magnuson Park	WEHS0083	Yes
Matthews Beach	WEHS0084	No
Union Bay Wetland Area	WEHS0085	Attempted
Arbor Heights	WEHS0086	No
Delridge Community Center	WEHS0087	No
Lincoln Park	WEHS0088	No
Webster Street Pond	WEHS0089	Attempted
Clark Lake Park	WEHS0090	Attempted
Lake Meridian	WEHS0091	Attempted

A map of the sampling sites is shown in Figure 2. Sites were chosen based on previous sampling, public accessibility, and visual inspection of the landscape. A more detailed analysis of the land cover was performed later on in the project, and is described in the 'Environmental Factors Datasets and Analysis' section. WEHS0082, WEHS0086, WEHS0087, and WEHS0089 were sampled by Bost in 2003. WEHS0082 and WEHS0089 contain detention ponds, whereas WEHS0086 and WEHS0087 do not. All four of these sites are in residential areas. Of the remaining sites, WEHS0083, WEHS0085, and WEHS0090 have some wetland characteristics based on visual inspection of the sites. WEHS0084 and WEHS088 are located in public parks in woodland areas near the coast. WEHS0091 is located at a public park near a large lake. All of the sites are located in either urban or suburban areas. More detailed descriptions of the study sites can be found in the supplementary files.

Figure 2. Map of 2014 Mosquito Sampling Sites with Land Cover. Sites highlighted in purple were sampled in 2003 and 2014.



Mosquito Sampling, Identification, and WNV Testing

Adults

Adult mosquitoes were sampled using Encephalitis Virus Surveillance CO_2 light traps according to methods outlined by CDC (2013). These traps are baited with dry ice as a CO_2 source and light, and are designed to attract host-seeking female mosquitoes. These traps are advantageous because they collect a wide range of mosquito species, which provides information about both primary and secondary vectors and a better understanding of the species composition in an area (CDC, 2013). Traps were set out once a week for approximately 13 hours over night at each site, and collected in the morning. Two traps were used at each site to get a better estimation of mosquito numbers. For the analysis, the data from the two traps are averaged and presented as 'Average Catch per trap night'. On a few occasions one of the traps did not operate properly (ran out of batteries, catch bag fell off etc.); in those cases only the data for the functional trap is included. A detailed log of mosquito sampling including dates with trapping errors is available in the supplementary files.

Adults were brought back to the lab and identified to the species level using a dichotomous key adapted from Darsie, 2004. A cold chain procedure was used to keep the mosquitoes alive and preserve any virus, if present. Live mosquitoes were knocked out using dry ice and stored in a cooler until identification. Identification was performed using a dissecting microscope on a modified chill table. Male mosquitoes and arthropods other than mosquitoes were discarded. Mosquito species that were less common or more difficult to identify were sent to WA DOH for confirmation. Pools of female *Cx. pipiens* or *Cx. tarsalis* with $\geq$12, but $\leq$ 50 mosquitoes were tested for WNV. When pools exceeded 50, a random subsample of 50 were tested. Other mosquito species are not considered important vectors of WNV in Washington, so were not tested.

WNV testing was performed using the Rapid Analyte Measurement Platform (RAMP®) WNV test (Response Biomedical Corp., Burnaby, Canada) according to the manufacturer's instructions. The RAMP test uses WNV-specific antibodies conjugated to fluorescent latex particles to determine the status of a sample (Kesavaraju et al., 2012). The WADOH criteria was used to determine positives, which considers a RAMP value $\geq$300 positive for WNV. During the summer 2014 season, 21 pools of *Cx. pipiens* were tested for WNV. All pools tested negative for WNV. All mosquito surveillance data was reported to WA DOH as part of their WNV surveillance program, and from there reported to CDC and incorporated into national ArboNET surveillance data and mapped through USGS.

Larvae

Mosquito larvae were sampled at the sites containing potential larval habitat denoted in Table 1. Sampling was performed using the dipping method (O'Malley, 1995). We had trouble sufficiently surveying the sites for larval habitat. WEHS0082 was the best surveyed, with likely all potential areas of standing water accounted for. This site was sampled consistently from Week 4 through Week 15. Larval habitat surrounding the traps at WEHS0083 were also well accounted for, and were sampled consistently over the same period. Sites listed in Table 1 as 'Attempted' were sampled at least once over the season, but were not sampled consistently because either no larvae were found or the original sampling site dried up later in the season. However, it is likely that other larval habitat existed at these sites that were not accounted for.

Mosquito larvae that were successfully caught in the field were brought back to the lab and reared to adults using larval growth chambers. Chambers were placed on a windowsill with natural light, and larvae were fed fish food. Larvae that emerged to adults were identified using the methods outlined above. WNV testing was not performed because vertical transmission is rare (CDC, 2013). We had a large number of larvae in the growth chambers that did not mature to adults. Reasons for this remain unclear, but may have been due to water conditions or the fish food being contaminated. Results from the larvae sampling study are not considered generalizable due to errors in sampling and rearing, but are presented descriptively in the Results section.

Washington DOH Mosquito Surveillance Dataset

WADOH compiled a dataset of all mosquito surveillance data collected in the state from 2008-2014. This study focused attention on King County because it is the most populous county in Washington and is where the field research was performed in summer 2014. WADOH was later able to provide a dataset for King County for 2006-2007. All data collected in 2003 for King County as recorded in Heather Bost's thesis was also added to the dataset. The data was assessed for accuracy and completeness using Google Maps, ArcGIS, and Microsoft Excel. Flags for location accuracy and sampling frequency were incorporated into the dataset. Flags are outlined in Table 2. The dataset is available in the supplementary files.

The third scientific aim of this thesis was to address the quality and completeness of the WADOH dataset for King County and other select areas of WA State. Commentary addressing the third aim is presented in the Discussion.

Table 2. Location and Sampling Frequency Flags	
Flag Type	**Description**
Location	Flags based on the scale of location accuracy. Labeled categorically.
E	*Exact.* The exact GPS coordinates of the site are known.
SA	*Street Address.* The street address of a site is known, and a GPS point is generated using the address. Site may be located anywhere within that address property.
V	*Vicinity.* The site is located somewhere in the vicinity of the GPS point provided. Can be a large area. Ex) Cougar Mountain
ZC	*Zip Code.* The zip code where sampling occurred is known. A random GPS point (often in the middle of the zip code) is provided.
Sampling Frequency	Refers to the number of times a site was sampled throughout the season. Labeled discrete numerically.

Selection Criteria for Incorporation into Multiple Regression Model

A regression model was developed to assess the impacts that climate and land cover have on mosquito abundance in King County. This will be discussed in more detail under *Statistical Methods*. Because the WADOH dataset is not consistent in space or time, i.e. there are no sites that were consistently sampled at the same location over the same time period over all the years, selection criteria were established for data points deemed suitable for inclusion in a regression model. Firstly, mosquito data was excluded if it contained any of the following:

- Data was a larval sample
- Data was for male mosquitoes
- If mosquito control methods were used, such as fogging
- If comments reported that a trap broke or malfunctioned
- If there was contradictory info in the data point, i.e. comments say 9 mosquitoes in trap but number lists 7

After the initial exclusion criteria, the data was examined according to location and sampling frequency flags. For final inclusion in the model, it was determined that the data must meet both of the following requirements:

- Location data is at the exact GPS coordinate level
- The site was sampled at least 12 times over a three month period

This resulted in 44 sites being included in the analysis from the years 2003, 2008, and 2014. All of these sites were sampled using the same method (CO_2 light traps) and field protocol. With the exception of WEHS0082, WEHS0086, WEHS0087, and WEHS0089, each site was sampled over just one sampling season. The location of these sites is displayed on the map in Figure 3. A map showing the excluded points and a detailed attribute table of the selected sites are available in the Appendix.

Environmental Factors Datasets and Analysis

Land Cover

The National Land Cover Database 2011(NLCD 2011) was used to conduct an analysis of the land cover surrounding the mosquito sampling sites. The NLCD 2011 consists of spatial raster datasets and their corresponding metadata which classify the land cover of the United States according to pixels. A modified version of the Anderson Land Cover Classification System was used to classify pixels of land at a spatial resolution of 30 m according to 16 different land cover classes (Jin et al., 2013). The NLCD 2011 Land Cover and the NLCD 2001 to 2011 Land Cover Change datasets were used because these datasets most closely resembled the years the selected mosquito sampling data was conducted in King County. For the purpose of this analysis, only land cover classes that were present in the sampling area were used. Land cover classes were aggregated to produce the seven land cover classes described in Table 3.

Table 3. Land Cover Classification. Modified from the Anderson Land Cover Classification System

Developed Med-High	Impervious surfaces account for 50-100% of the total cover. Includes highly developed areas where people reside or work in high numbers, and areas with a mixture of constructed materials and vegetation.
Developed Open-Low	Impervious surfaces account for <20-49% of the total cover. Includes areas with a mixture of constructed materials and vegetation, and areas with a mixture of some constructed materials, but mostly vegetation in the form of lawn grasses.
Total Developed	The sum of the medium-high and open-low developed land covers classes.
Forested	Areas dominated by trees generally greater than 5 meters tall, and greater than 20% of total vegetation cover. Includes deciduous, evergreen, and mixed forest.
Wetlands	Areas where the soil or substrate is periodically saturated with or covered with water. Includes woody wetlands and emergent herbaceous wetlands.
Open Space	Includes area of shrub/scrub, cultivated crops, pasture/hay, and grassland and herbaceous areas.
Open Water/Barren Land	Areas of open water with <25% cover by vegetation on soil, and areas of barren rock/sand/clay where vegetation accounts for <15% of the total cover.

The land cover analysis was performed using ArcGIS Version 10.2. It was assumed that the land cover in 2011 was the same in 2008 and 2014, and that the land cover in 2001 was the same in 2003. The analysis was only performed at sites where location data was classified as 'Exact'. Buffers were used to determine the percent of each land cover class at each of the sites. Buffers with a radius of 50 m, 300 m, and 500 m were used, so that the distance(s) that had the best fit in the regression model could be evaluated. The maximum radii of 500m was selected based on Crowder et al. (2013), who chose this distance because mosquito dispersal typically occurs over distances <0.5 km. The percent land covers in the total area of each of these buffers were determined using zonal statistics tools in the Spatial Analyst extension in ArcGIS. A detailed description of the methodology for this analysis using ArcGIS is available in the Appendix.

Climate Data

Climate data was obtained from the National Oceanic and Atmospheric Administration's (NOAA) National Centers for Environmental Information website (NCEI, formerly known as NCDC) (NOAA, 2015). After consideration of other possible finer scale climate datasets, data from the NOAA's weather stations were selected because they provide high quality data and use consistent sampling methods and locations for all of the years studied. Four NOAA weather stations were selected to include in the analysis based on the following criteria:

- The weather station contains daily precipitation, maximum temperature, and minimum temperature data
- The weather station sampled consistently from at least 2003- 2015
- The weather station was within approximately a 10-mile radius of a mosquito sampling site
- The weather station was identified as the closest station to a mosquito sampling site within a 10-mile radius

The stations selected are displayed in Table 4 and in Figure 3. Daily summaries were averaged at each weather station to get weekly values of maximum temperature (Tmax), minimum temperature (Tmin), and precipitation (Precip). Tmax, Tmin, and Precip were then averaged between all four stations and used in the analyses. One and two week lags for all three variables were calculated and used in the analyses. In addition to these variables, a Degree Weeks base 63 (DW_63) was calculated. The degree weeks method was selected based on Ruiz et al. (2010), which is an adaptation of the more common degree-day. The metric was calculated as:

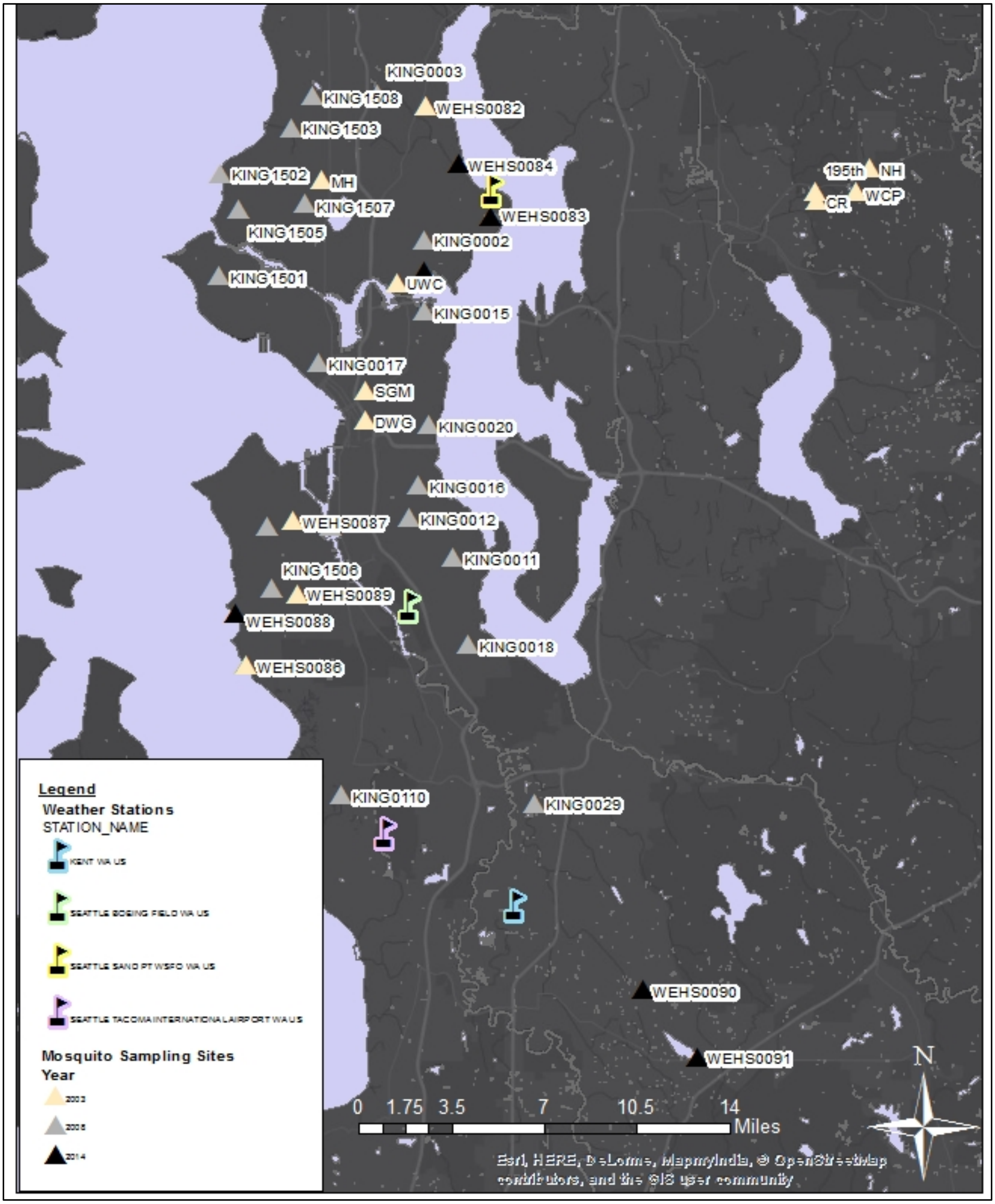
$$DW = \begin{cases} [0.5\ (T_{mean} - T_{min})] - T_{base} & \text{if } T_{mean} > T_{base} \\ 0 & \text{if } T_{mean} < T_{base} \end{cases}$$

Where $T_{base} = 63$ deg F. T_{base} is the temperature that represents the lower temperature threshold for development. When temperatures drop below the lower threshold, development of the insect stops (UC IPM, 2014). The temperature 63 deg F was chosen based on studies by Trawinski & MacKay (2008; 2009). The authors determined a cooling degree-days base 63 at a lag of zero weeks was one of the most significant meteorological variables for predicting the abundance of the combined group of *Cx. pipiens* and *Cx. restuans*.

Table 4. NOAA Weather Stations used in Analysis

Station Name	**Latitude**	**Longitude**	**Station ID**
KENT WA US	47.4172	-122.2433	GHCND:USC00454169
SEATTLE BOEING FIELD WA US	47.53028	-122.30083	GHCND:USW00024234
SEATTLE SAND PT WSFO WA US	47.6872	-122.2553	GHCND:USW00094290
SEATTLE TACOMA INTERNATIONAL AIRPORT WA US	47.44444	-122.31389	GHCND:USW00024233

Figure 3. Map of Selected Mosquito Sampling Sites included in the multiple regression model from years 2003, 2008, 2014. There were 44 points used in total, with 39 being unique sites.



An interactive version of this map is available at: https://www.google.com/maps/d/edit?mid=zMqC-VirD_9c.kbGipTvGAY7M&usp=sharing

Statistical Analysis

2003 versus 2014 Comparison

Adult mosquito sampling results were compared in 2003 and 2014 at the WEHS0082, WEHS0086, WEHS0087, and WEHS0089 sites. These sites are denoted in Figure 2. Results were compared over the same weeks of the year where the sites were sampled in both 2003 and 2014. For WEHS0082, WEHS0087, and WEHS0089 these were weeks 27-39, and for WEHS0086 it was weeks 31-39. This allowed for a direct comparison of the sites because mosquitoes were sampled at the same locations, using the same protocol, over the same time period. We were obviously not able to control for climate and other environmental factors that may have differed between the sites over the years, but we did compare the seasonal means of some of these factors between the two years using a Mann Whitney U-test.

Total mosquito abundance was determined using the 'Average Catch per Trap Night' mentioned previously. Vector abundance was determined by adding the total number of vector species caught per night at a site, and dividing it by the number of traps. Vector species were defined as *Culex pipiens* and *Culex tarsalis*. An initial assessment of the mosquito metrics data showed the data was highly skewed to the right, so the data were transformed using the log transformation:

$$\log data = \log(data + 1)$$

This transformation helped make the data normally distributed in some but not all cases. When data was normal, a Student's t-Test was used, and when it was nonparametric a Mann-Whitney U-Test was performed. These tests were used to compare the mosquito metrics between the two years at the seasonal level over all the sites, and at each site individually. Student's t-Tests and Mann Whitney U-Tests were also used to compare Tmax, Tmin, Precip, and %Med-high developed land cover at the three buffer levels in 2003 and 2014 over all the sites. Med-high developed land cover was the only land cover variable assessed because it was the only one that differed in 2003 and 2014 at the four sites. All tests were performed using R version 3.1.

The population composition of mosquito species was also assessed at these sites in 2003 and 2014. The percent of the population represented by each species was determined using the method described by Bost in her thesis (2004). Although not a statistical test, this allowed us to examine the species composition at the sites, and determine if there were any changes in species diversity over the intervening years.

Correlative Analysis and Generalized Additive Model

A generalized additive model (GAM) model was developed to assess the impact selected environmental factors have on *Culex pipiens* abundance. The model used environmental variables and other necessary predictors to predict the *Cx. pipiens* abundance on a weekly scale. GAM was selected because it allows covariates depending on non-linear smooth functions to be incorporated in the model. *Cx. pipiens* was selected rather than total mosquito abundance because other mosquito species could have conflicting preferences to certain environmental variables that would affect the model's performance.

Selection of environmental factors to include in the model was based on a Principal Components Analysis (PCA), which looked at the correlation of the climate and land cover variables with the mosquito abundance metrics and each other. When conducting the PCA for climate variables, the mean of the log-transformed abundance metric for each site was subtracted from each average catch observed. This allowed identification of possible associations without variation from spatial effects.

The mosquito data points included in this model are displayed in Figure 3. All land cover variables at the 500 m buffer were included in the model except total developed land cover because it was highly correlated with many of the other land cover variables. Because Tmax, Tmin, and DW_63 were highly correlated, these variables were excluded and instead the average of Tmax and Tmin by week was used as a predictor at 0, 1, and 2-week lags. A figure depicting these correlations is available in the Appendix. The Precip variable at 0, 1 and 2-week lags was included as mentioned previously. Temporal effects were also considered in respect to week and year. It was determined that mosquito abundance generally follows a non-linear quadratic curve over the season when assessed by week. A smooth was added to this 'week' predictor when it was incorporated in the model. A 'year' predictor was also added to account for unexplained variation between years. Interaction terms were also considered in the model, and were selected based on a forward stepwise regression method.

The regression model can be described as:

$$Y_{i,j} = year_i + smooth(week_j) + LC_{i,x} \dots + +Temp_{i,j,x} \dots + Precip_{i,j,x} \dots + Temp_{i,j,x} \times Precip_{i,j,x} \dots$$

Where Y = log(*Cx. pipiens* abundance+1) in respect to i= year, and j= week; LC = land cover in respect to year; *Temp*= temperature in respect to year and week; *Precip*= precipitation in respect to year and week; and *Temp* x *Precip* represents an interaction term in respect to year and week. The analysis was performed using R version 3.1. Final model selection and the output of the models are presented in the results.

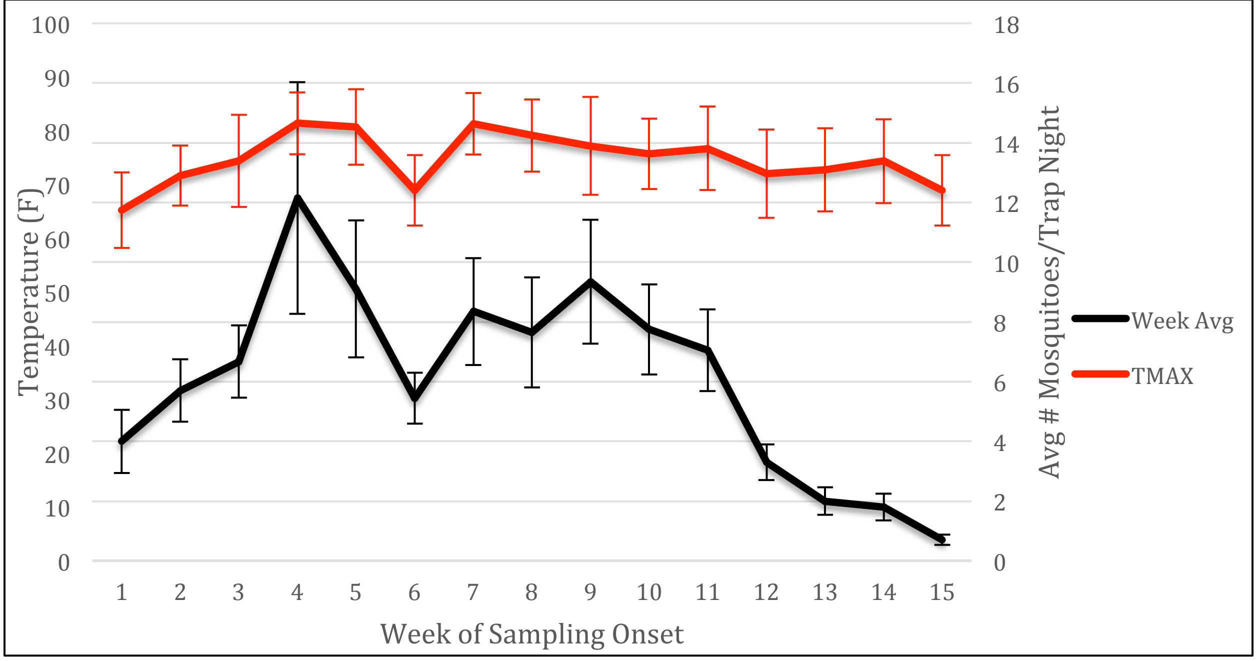
Results

Mosquito Surveillance 2014

Adults

Generally speaking, the mosquito trend over the summer followed a quadratic curve where counts started low, eventually increased and peaked, and then dropped off again. As evidenced by Figure 4, the mosquito counts fluctuated from this trend at some weeks, particularly week 4 where there was a sharp peak, and then week 6 where there was a sharp decrease. The maximum temperature also displayed a sharp decrease at week 6, suggesting it may have played a role in the decreased mosquito abundance. The impact Tmax and other environmental variables have on adult mosquito abundance is explored further in the 'Environmental Factors and Mosquito Abundance' section.

Figure 4. 2014 Avg Mosquito Catch/Trap Night and Avg Tmax by Week. Standard deviation bars shown for Tmax and standard error bars shown for Week Avg.



Mosquito abundance varied greatly between sites, with WEHS0082 having the highest number of mosquitoes caught, and WEHS0084 having the lowest number of mosquitoes caught. The total number of mosquitoes caught over the season by site is displayed in Figure 5.

Culex pipiens was the dominant mosquito species caught over the 2014 season. Total mosquito abundance was correlated with *Cx. pipiens* abundance (r=0.76, p=<0.001), as *Cx. pipiens* was the dominant mosquito at most of the sampling sites. The sites where *Cx. pipiens* was less dominant were the sites with the lowest catch, WEHS0084 and WEHS0086, and the sites WEHS0085 and WEHS0090. The breakdown of mosquito diversity for all the sites over the season is displayed in Figure 6.

Figure 5. 2014 Seasonal Mosquito Distribution by Site.

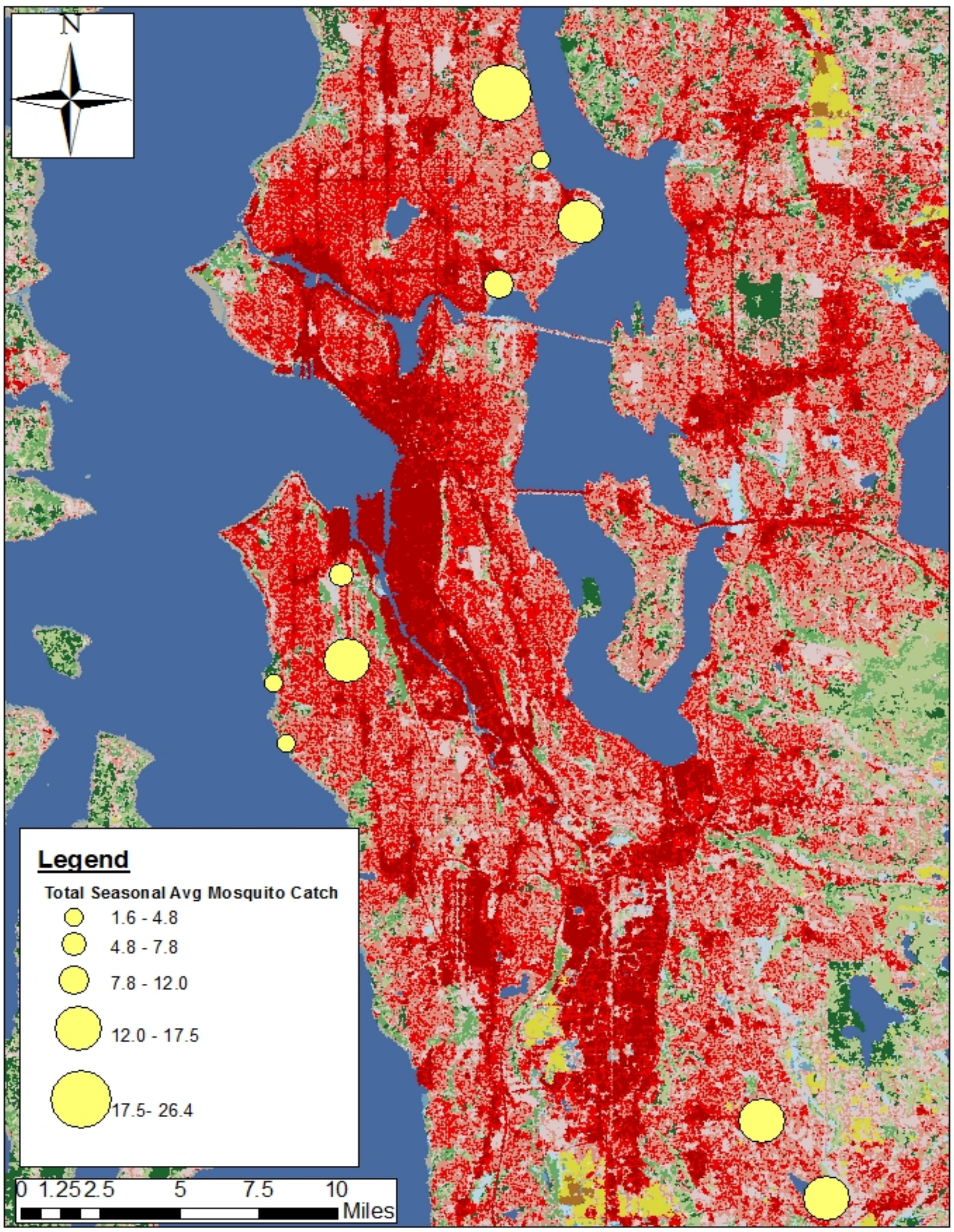
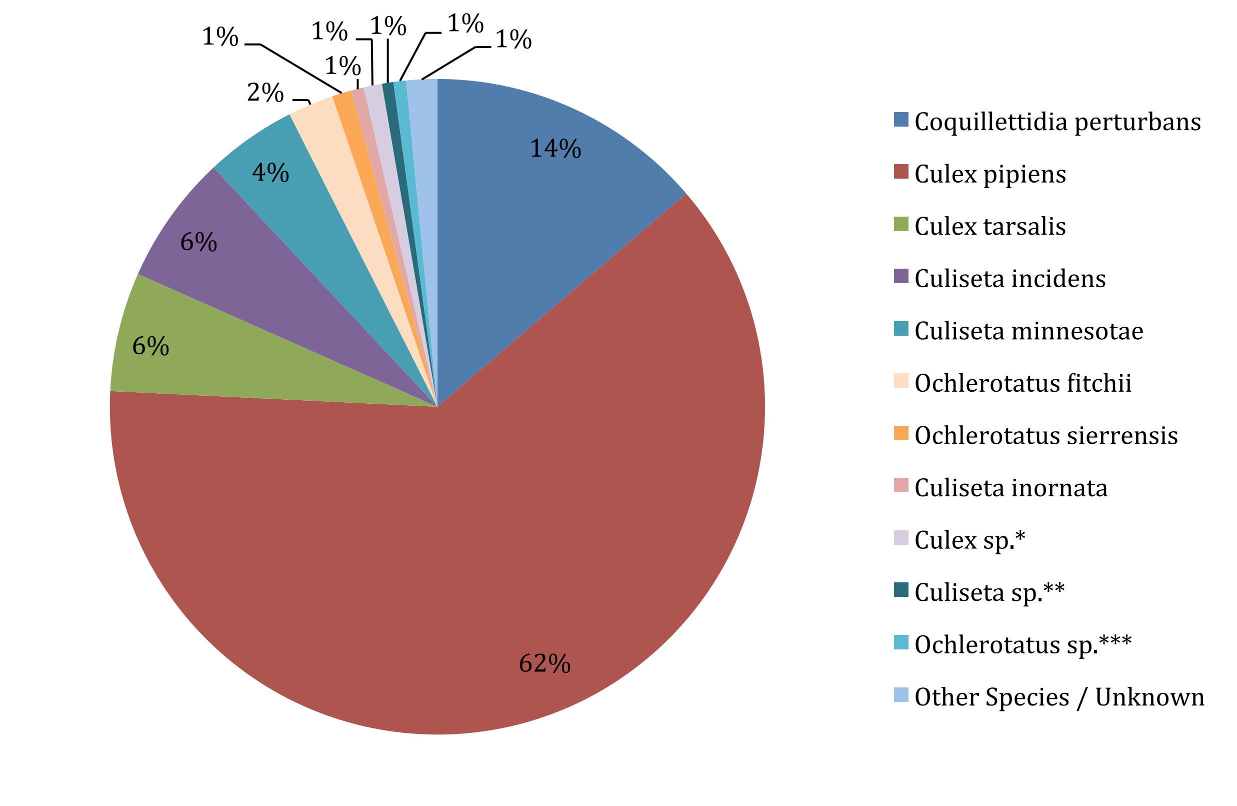


Figure 6. 2014 Total Adult Mosquito Diversity. Species composition of the total number of adult mosquitoes caught by species over the 2014 season. n= 1770.



* Includes Culex sp. identified to genus level, *Culex salinarius,* and *Culex territans*

** Includes Culiseta sp. identified to genus level and *Culiseta particeps*

*** Includes Ochlerotatus sp. identified to genus level, *Oc. increpitus*, and *Oc. japonicus*

Larvae

The diversity of mosquito larvae species caught in 2014 is displayed in Figure 7. The majority of the larvae were caught at the WEHS0083 site (n=94). Although the majority of the larvae were unable to be identified to species, the species that were identified at WEHS0083 were roughly split between *Cx. territans* and *Cx. tarsalis,* with some *Cx. pipiens* identified (n=18, n=17, n=5, respectively). All the larvae successfully identified to the species level at WEHS0082 were *Cx. pipiens* (n=30). The larvae abundance trend over the season is shown in Figure 8. When the larva abundance is graphed over the season with precipitation, some of the peaks in larvae appear to be associated with the average precipitation at the current week, others are more associated with average precipitation at a one-week lag, and others still are associated with precipitation at a two-week lag. Figures depicting this are available in the Appendix.

Figure 7. 2014 Total Larvae Mosquito Diversity. Total number of larval mosquitoes caught by species over the 2014 season. n=153.

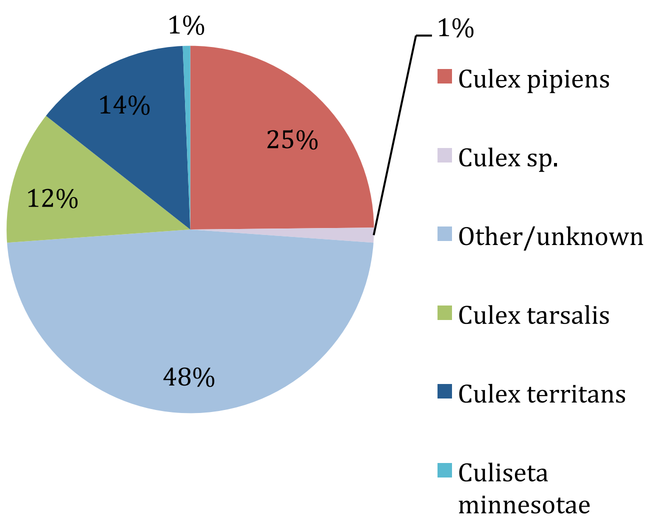
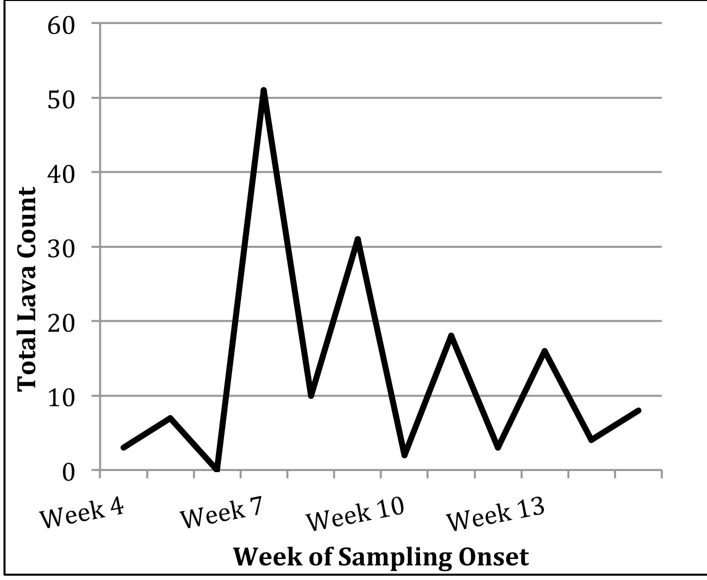


Figure 8. 2014 Total Larva Count all Sites and Species, by Week.



2003 versus 2014 Comparison

The mosquito abundance trends for the 2003 and 2014 sites are shown in Figure 9. The results of the Student t-Test found that mosquito abundance was significantly higher in 2003 than in 2014 when all four sites were considered (mean 2003 =15.5, mean 2014=7.1, p=<0.001). Descriptive statistics for 2003 and 2014 along with the results of all the Student t-Tests and Mann Whitney U-tests comparing mosquito abundance are presented in Table 5. A boxplot showing the difference in mosquito abundance is presented in Figure 9. The trends in mosquito abundance by week are presented in Figure 10.

Temperature, precipitation, degree weeks, and land cover were examined for 2003 and 2014. Percent med-high land cover was the only land cover variable assessed because there were no other changes in land cover between 2003 and 2014. Seasonal means and the results of the Mann Whitney U-Test are presented in Table 6. The Tmax and Precip trends over the 2003 and 2014 season are presented in Figures 11 and 12. There was a significant difference in the Tmin for 2003 and 2014, but otherwise no significant differences in the environmental factors were found. There was a significant drop in temperature during Week 30 in 2014, which was not observed in 2003.

The mosquito species composition in 2003 and 2014 are presented in Figure 13 and Figure 14, respectively. *Cx. pipiens* was the dominant mosquito species both years, although in 2003 it comprised 86% of the adult mosquito population whereas in 2014 it comprised

79% of the adult population. Both years *Cs. incidens* comprised a significant portion of the remaining population. In 2014 *Cs. minnesotae* comprised 10% of the population, whereas in 2003 it was not present at all.

Figure 9. Boxplot of Total Mosquito Abundance 2003 and 2014.

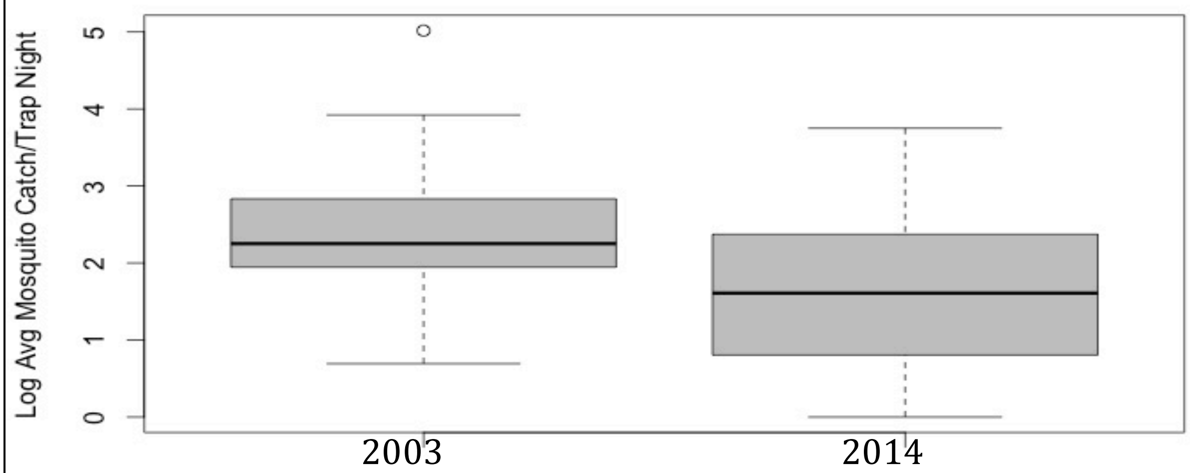


Figure 10. Avg Mosquito Catch/Trap Night by Week 2003 and 2014. Standard error bars displayed.

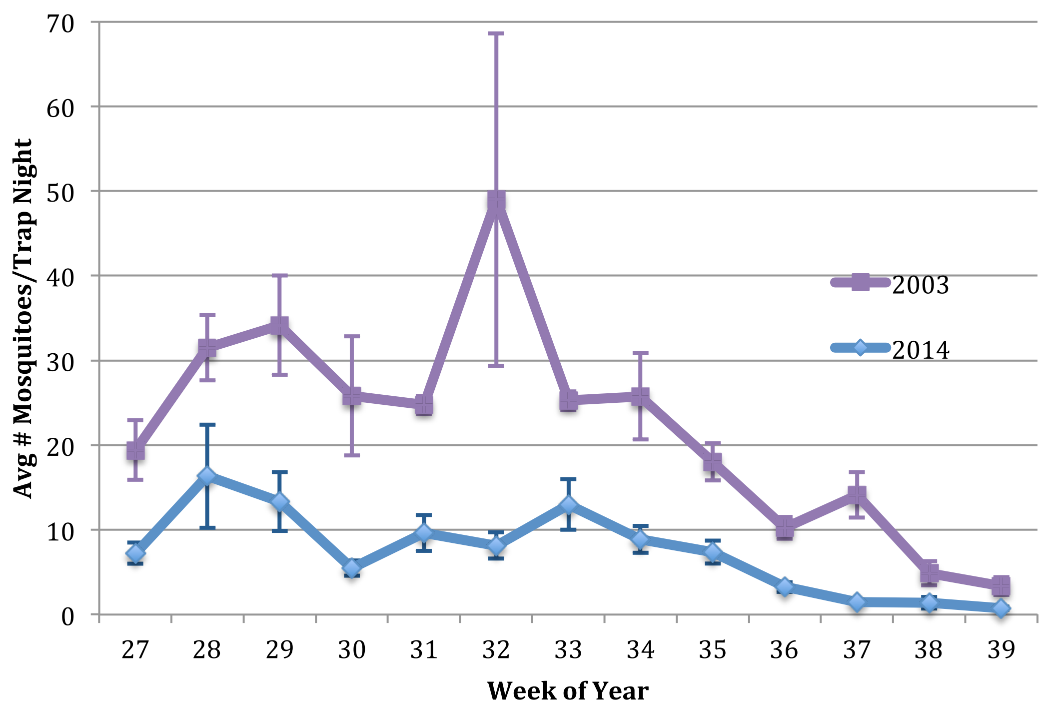


Table 5. Descriptive Statistics and Student t-Test/Mann Whitney U-Test Results Comparing Mosquito Abundance in 2003 and 2014. Items denoted with (*) indicate a Mann Whitney U-Test was performed.

		Mean	Median	SE	P-Value
ALL SITES **Avg Catch/trap night all mosquito species**	2003	15.5	8.5	3.3	**<0.001**
	2014	7.1	4.0	1.2	
ALL SITES **Avg Catch/trap night vector species**	2003	12.9	5.5	3.4	**0.029**
	2014	5.7	3.0	1.1	
WEHS0082 **Avg Catch/trap night all mosquito species**	2003	33.9	23.0	10.6	0.084
	2014	13.7	11.5	3.1	
WEHS0082 **Avg Catch/trap night vector species**	2003	33.7	22.0	10.6	0.076
	2014	12.7	11.5	3.0	
WEHS0086 **Avg Catch/trap night all mosquito species**	2003	12.2	12	1.7	**<0.001**
	2014	2.2	1	0.7	
WEHS0086 **Avg Catch/trap night vector species***	2003	4.0	3	1.4	**0.010**
	2014	1.2	0	0.6	
WEHS0087 **Avg Catch/trap night all mosquito species***	2003	8.4	8.5	1.1	**0.012**
	2014	4.1	3.3	1.1	
WEHS0087 **Avg Catch/trap night vector species**	2003	1.6	1.6	0.22	0.260
	2014	1.2	1.2	0.22	
WEHS0089 **Avg Catch/trap night all mosquito species**	2003	5.9	6.5	1.0	0.797
	2014	6.8	5.0	1.9	
WEHS0089 **Avg Catch/trap night vector species**	2003	5.4	5.0	1.1	0.381
	2014	3.9	3.0	1.0	

Figure 11. Tmax and Tmin trends for 2003, 2008 and 2014 over weeks 25-39. Standard deviation bars displayed.

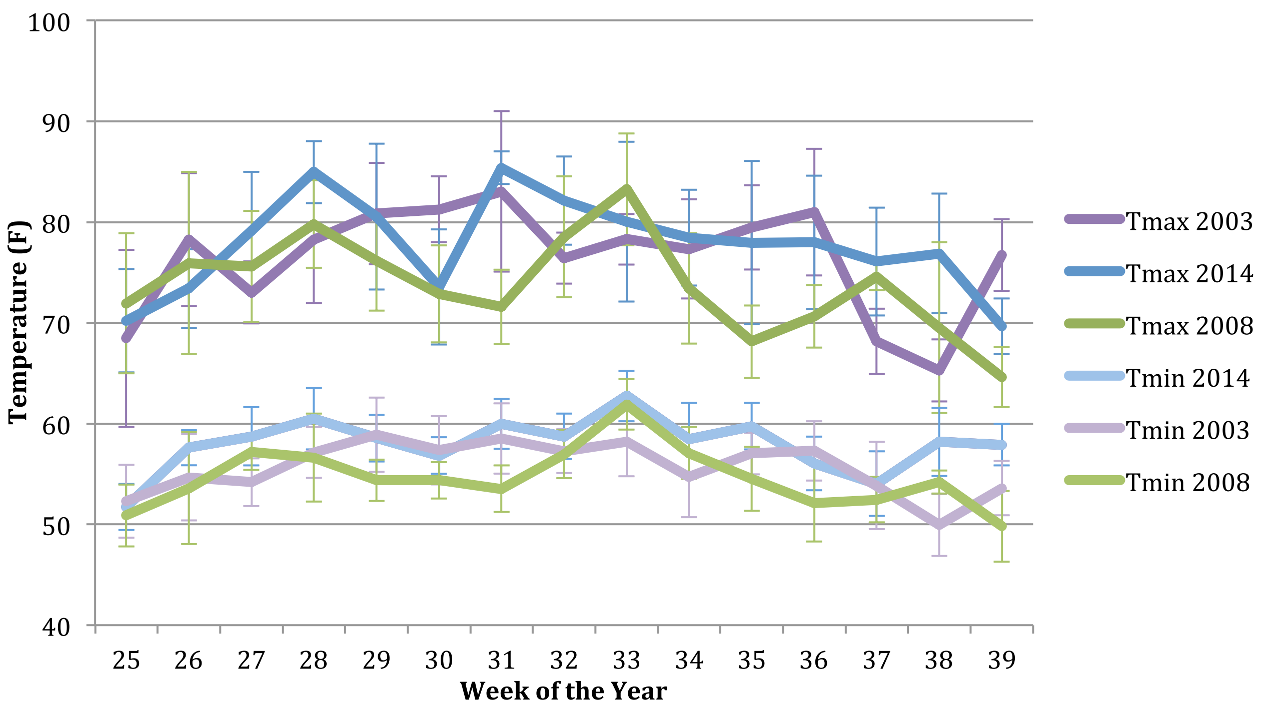


Figure 12. Precip trends for 2003, 2008, and 2014 over weeks 25-39. Standard deviation bars displayed.

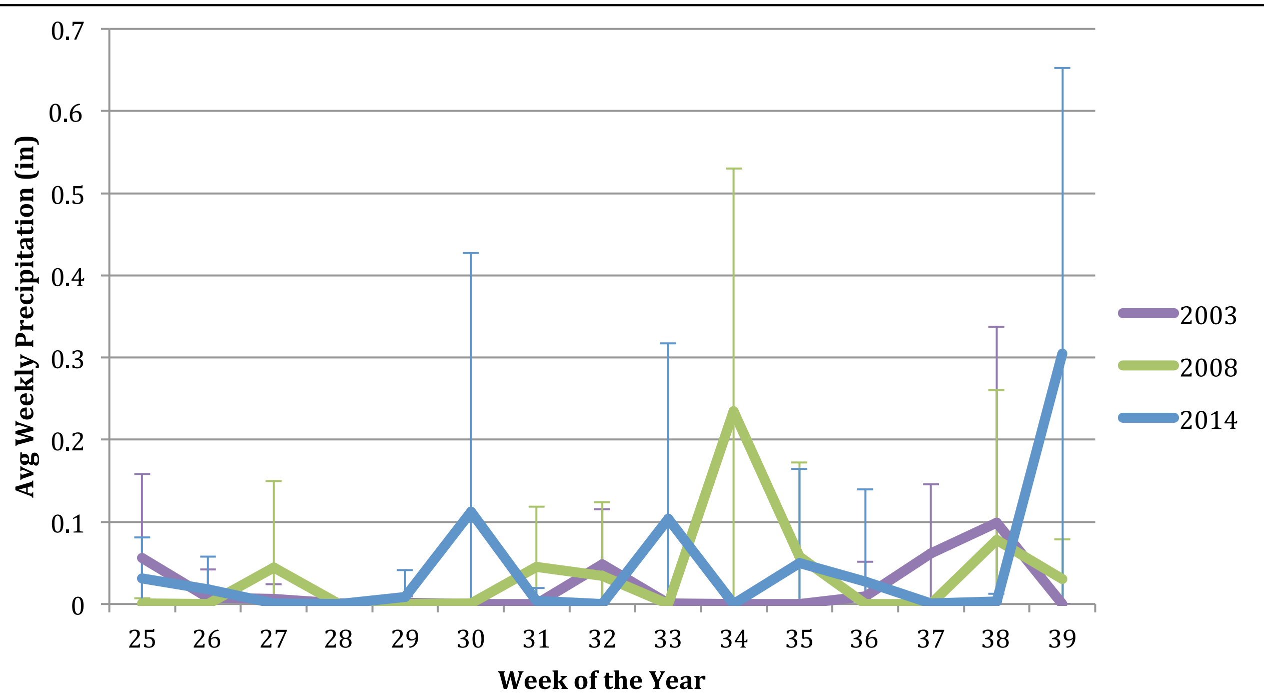


Table 6. Comparison of selected environmental factors in 2003 and 2014. Descriptive statistics and Mann-Whitney U-test results presented.

	2003		2014		**Mann-Whitney U-Test**
	Mean	SD	**Mean**	SD	**p-value**
Tmax (°F)	76.8	5.1	78.7	4.0	0.16
Precip (in) (Excluding last 2 weeks)	0.015	0.02	0.027	0.03	0.47
Tmin (°F)	56.0	2.8	58.5	2.4	<0.001
%Med-high developed LC (500m buffer)	35.4%	18.4%	37.1%	16.8%	0.12

Figure 13. Mosquito Population Composition of the WEHS0082, WEHS0086,WEHS0087, and WEHS0089 Sites in 2003. n= 1292

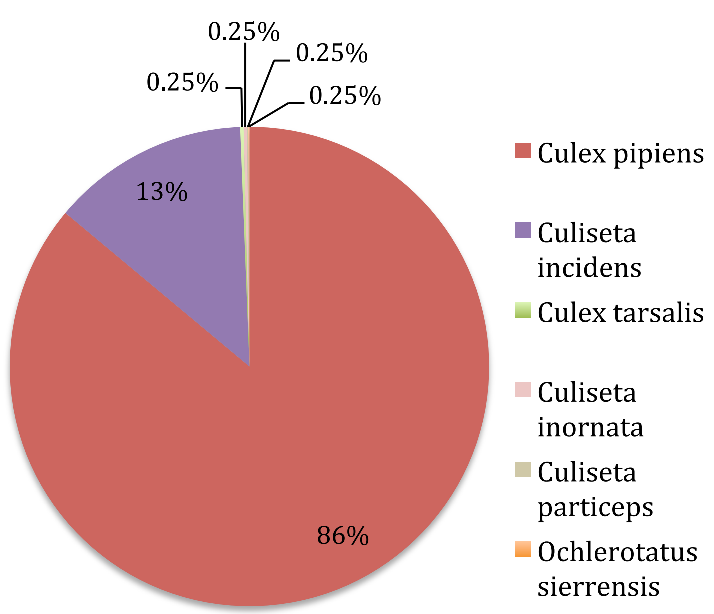
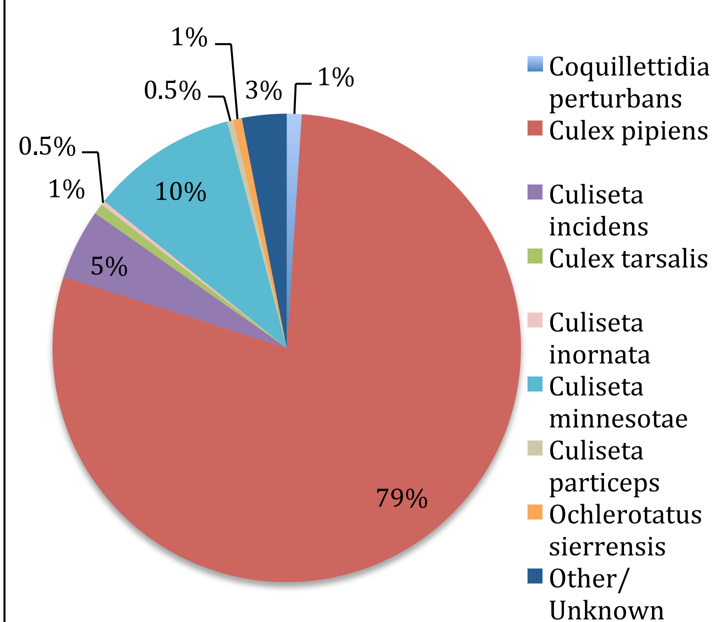


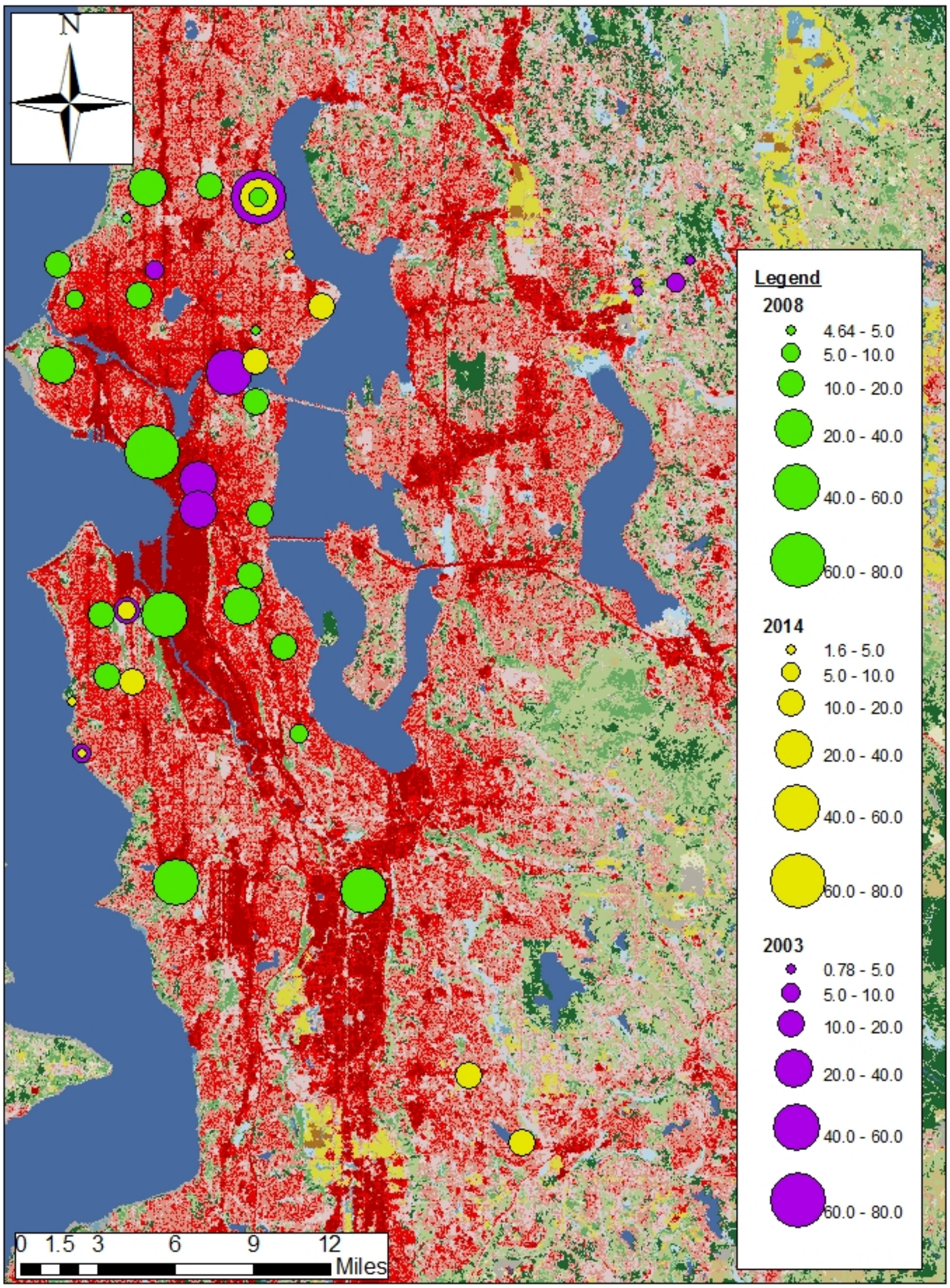
Figure 14. Mosquito Population Composition of the WEHS0082, WEHS0086,WEHS0087, and WEHS0089 Sites in 2014. n= 682



Mosquito Distribution 2003, 2008, 2014

The distribution of mosquito abundance spatially and temporally was examined for the selected sites in 2003, 2008, and 2014. The seasonal averages are displayed in Figure 15. A PowerPoint showing the temporal distribution of mosquito abundance over weeks 27-39 is available in the supplementary files.

Figure 15. Distribution of Total Adult Mosquito Abundance for Years 2003, 2008, and 2014. Seasonal Means presented.



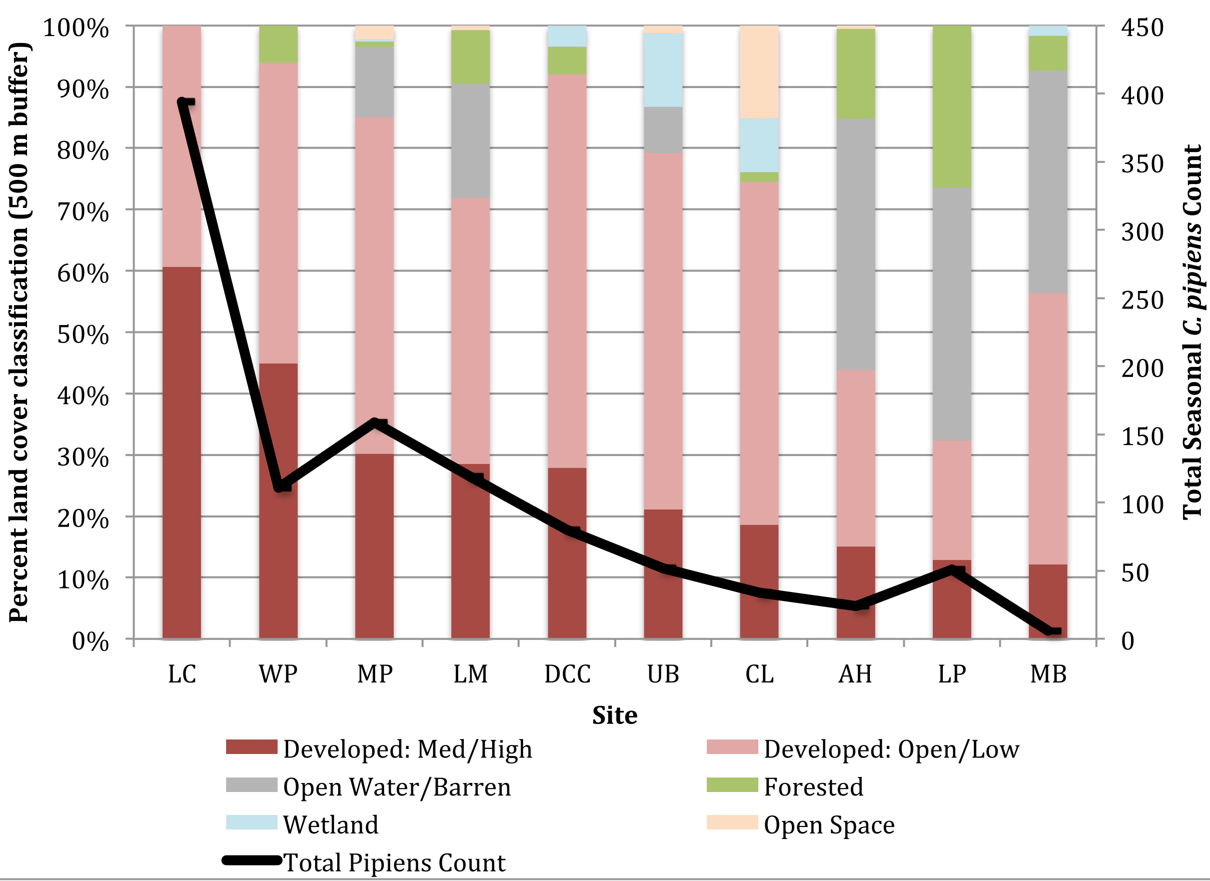
Environmental Factors and Mosquito Abundance

Associations of climate and land cover variables with total mosquito abundance and *Culex pipiens* abundance were examined using correlative analysis and visual inspection of scatterplots of subsets of data. This section of the results also serves as the principal correlative analysis for the regression model.

Land Cover

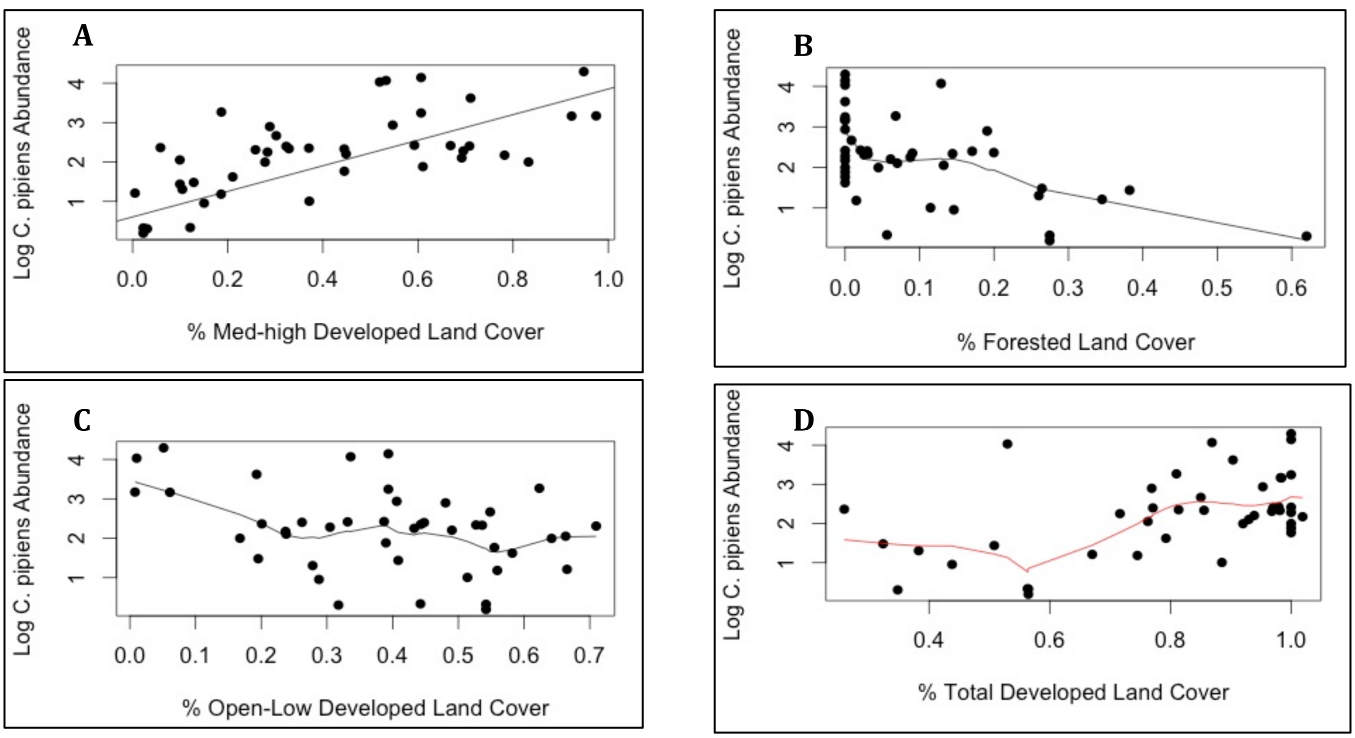
The land cover composition of the sites in 2003, 2008, and 2014 were comprised mainly of developed land cover. The percent breakdown of the various land cover classes in the 2014 sites is displayed in Figure 16. The land cover composition of the remaining sites can be found in the Appendix. Of the buffer levels examined, the 500 m buffer showed the clearest associations with the mosquito abundance metrics, so land cover compositions at this level were used in the analyses.

Figure 16. 2014 Land Cover Classification by Site with Total Seasonal Cx. pipiens Count



The land cover variable most highly correlated with total mosquito abundance was med-high developed land cover(r= 0.52 , p= <0.001, all years). The association of med-high developed land cover with *Cx. pipiens* abundance was even stronger (r=0.68, p=<0.001, all years). The total *Cx. pipiens* count at each site sampled during the 2014 season is displayed with the land cover for these sites in Figure 16. The *Cx. pipiens* trend very closely follows the percent med-high developed land cover. Percent forested land cover had a negative association with total mosquito abundance (r= -0.47, p= 0.001, all years) and *Cx. pipiens* abundance (r= -0.56, p=<0.001). Developed open-low land cover was weakly negatively associated with *Cx. pipiens* abundance (r=-0.38, p= 0.01), but not with total mosquito abundance (p>0.05). Both total mosquito abundance and *Cx. pipiens* abundance showed a dichotomous correlative relationship with total developed land cover, where sites with less than 60% total developed cover were negatively associated with increased total development, and sites that had greater than 60% total developed land were positively associated with increased total development. Scatterplots of *Cx. pipiens* abundance with land cover are displayed in Figure 17. The remaining land cover variables were not present in enough sites to make confident decisions about the relationships of these variables with mosquito abundance.

Figure 17. Scatterplots of *Cx. pipiens* abundance with: A) Med-high developed land cover, B) Forested land cover C) Open-low developed land cover and D) Total developed land cover



Temperature

The temperature trends over the 2003, 2008, and 2014 seasons were relatively similar, with 2014 generally having the highest average weekly temperatures and 2008 having the lowest. As mentioned previously, 2014 had a steep drop in Tmax during Week 30, which neither 2003 nor 2008 experienced. Tmin and Tmax trends for the three seasons are shown in Figure 11. The correlation coefficients for total mosquito abundance and *Cx. pipiens* abundance with the temperature variables were similar, with *Cx. pipiens* abundance having slightly stronger associations (total mosquito abundance range: r= 0.25-0.33; *Cx. pipiens* range: r= 0.32-0.38, all years). All temperature variables were at least slightly positively associated with mosquito abundance metrics, except in 2014 when total mosquito abundance was not significantly associated with Tmax at the 2-week lag (r= 0.07, p=0.4). The association of temperature variables with mosquito abundance metrics varied somewhat by year, with 2003 yielding the strongest associations with temperature variables. The temperature variable with the strongest association varied by year and mosquito abundance metric, but when looking at the data over all the years, both metrics were most strongly associated with Tmin at the 1-week lag and least associated with Tmin at the 2-week lag. The association of total mosquito abundance and *Cx. pipiens* abundance with the temperature variables over all the years is shown in Table 7. The correlation coefficients by year are available in the Appendix.

Precipitation

The precipitation trends from 2003, 2008, and 2014 were less consistent than those seen with temperature. Figure 12 displays the seasonal precipitation trends over weeks 25-39. There were many zeroes in the data because rainfall is commonly sparse during the summer season in Seattle. The precipitation variables and mosquito metrics were weakly associated. No significant associations between mosquito abundance metrics and precipitation variables were found in 2008 (p>0.05). In 2003 and 2014, the precipitation variables most associated with total mosquito abundance and *Cx. pipiens* abundance differed, but for the most part were slightly negatively correlated with precipitation. Looking at the data over all three years, both mosquito abundance metrics were most strongly associated negatively with precipitation at the 0-week lag (total mosquito abundance: r= -0.16, p<0.001, *Cx. pipiens* abundance: r= -0.14, p<0.001). The association of total mosquito abundance and *Cx. pipiens* abundance with the precipitation variables over all the years is shown in Table 7.

Table 7. Spearman's Correlation Coefficients (*r*) of Association for Total Mosquito Abundance and *Culex pipiens* Abundace with Temperature Variables. Strongest associations highlighted in blue, weakest highlighted in red.					
		Total Mosquito Abundance		***Culex pipiens* Abundance**	
Tmax		*r*	*p*-value	*r*	*p*-value
	0 week lag	0.32	<0.001	0.34	<0.001
	1 week lag	0.32	<0.001	0.37	<0.001
	2 week lag	0.27	<0.001	0.33	<0.001
Tmin					
	0 week lag	0.32	<0.001	0.38	<0.001
	1 week lag	**0.33**	**<0.001**	**0.38**	**<0.001**
	2 week lag	0.25	<0.001	0.32	<0.001
DW_63					
	Current week	0.32	<0.001	0.33	<0.001
Precip					
	0 week lag	**-0.16**	**<0.001**	**-0.14**	**<0.001**
	1 week lag	-0.15	<0.001	-0.13	0.001
	2 week lag	-0.08	0.04	-0.11	0.006

Regression Model

The following regression model was selected:

$$Y \log(Cx.pip\ abundance\ + 1) =$$
$$year + smooth(week) + LC(DMH) + LC(DML) + LC(F) + LC(OS) + LC(OW) + AvgTemp +$$
$$AvgTemp_{1weeklag} + Precip + Precip_{1weeklag} + Precip_{1weeklag} \times AvgTemp + Precip_{1weeklag} \times LC(DMH)$$

The model R^2 = 0.49. The output of the regression model is displayed in Table 8. The regression model found significant associations with the land cover variables($p<0.0005$), but not with any of the climate variables or with the year variable ($p>0.05$). All of the land cover variables were found to be negatively associated with *Cx. pipiens* abundance. Table 8 presents the geometric means of the estimates of association, with percent med-high developed land cover (B500_DMH) having the strongest association. This association estimates the geometric mean of *Cx. pipiens* abundance +1 is $e^{-3.02}$ = 0.05 times the geometric mean of *Cx.* Pipiens +1 of every 1 unit decrease in med-high developed land cover when all other covariates are the same. Although this effect is statistically significant, the impact of the association is not strong. The interaction term $Precip_{1weeklag} \times LC(DMH)$ was also found to be significant in the model, but again was a weak association equaling an $e^{-5.91} = 0.0027$ change in geometric mean. The results of the regression model contradict many of the results of the correlative analysis, and the model fit accounts for less than half of the variation observed. Reasons for this are discussed further in the Discussion.

Table 8. **Output of Regression Model**. Estimates and 95% CIs are presented as the geometric mean of *Cx. pipiens* abundance +1

	Estimate	95% CI	P-value
Year 2008	0.16	(-0.06, 0.39)	0.15
Year 2014	-0.09	(-0.38, 0.20)	
B500_DMH	-3.02	(-4.49, -1.55)	<0.0005
B500_DML	-5.87	(-7.48, -4.26)	<0.0005
B500_F	-5.44	(-7.01, -3.87)	<0.0005
B500_OS	-10.11	(-13.33, -6.89)	<0.0005
B500_OW	-5.66	(-7.40, -3.92)	<0.0005
AVGTEMP	0.002	(-0.04, 0.04)	0.93
AVG1LAGTEMP	-0.02	(-0.06, 0.01)	0.24
PRCP_WEEK_AVG	-0.28	(-1.58, 1.02)	0.67
PRCP_1WEEK_LAG	-11.63	(-41.30, 18.04)	0.44
p1_AVGTEMP	0.19	(-0.27, 0.65)	0.41
p1_DMH	-5.91	(-11.33, -0.49)	0.03

Discussion

For Aim I of this thesis, the current mosquito population in King County was surveyed and selected sites in 2003 and 2014 were compared. Mosquito abundance was found to be significantly higher in 2003 than in 2014 at these sites. In Aim II, analyses were performed to determine the associations mosquito abundance metrics have with land cover, temperature, and precipitation. Mosquito abundance was positively associated with developed land cover and temperature. Less developed land covers and precipitation exhibited negative trends with mosquito abundance. This discussion section opens with Aim III, where the limitations of the current study and an assessment of the WADOH dataset are presented.

Aim III: Limitations and Future Directions

For Aim III, an overview and assessment of the WADOH mosquito dataset is provided and suggestions on how it can best be utilized in future projects are made. Initially we hoped to be able to incorporate the mosquito data along with selected environmental factors into an existing predictive risk model. Whether or not the potential for the mosquito data to be used in that way will be discussed. The limitations of the current study will also be discussed.

Limitations

There are several limitations to the research presented in this study. First, it should be acknowledged that the data was examined on a weekly scale. Mosquito abundance was determined using the average catch per trap night, which was considered to be the average catch for that week. Daily climate data values were averaged to get a weekly value, when there may have been considerable variation within one week. Standard deviation and standard error are presented in our results to help account for this. However, we were not able to account for daily changes in temperature or precipitation that may have affected the mosquitoes. Additionally, our regression model does not account for unexplained effect by site or spatial autocorrelation. The vast majority of the sites' locations differed by year, so it is possible spatial effects not explained by land cover could potentially be confounding 'year' and other variables in the model.

Another limitation is the scalability of the climate and land cover data. We were only able to incorporate data from four weather stations, which were averaged. Because of the spatial location and number of weather stations, inverse distance weighting methods were considered unlikely to increase the quality of the data. Ideally, more weather stations with data consistent from at least 2003-present will be able to be used in future studies, and inverse distance weighting or kriging methods can be utilized. The best solution would be to take weather measurements at the sampling sites, however high quality measurements require expensive equipment. Because most of the mosquito sampling sites are public, this is not attainable. The land cover data we utilized was fine scaled at a 30 m resolution, but because mosquitoes are so environmentally-niche driven, even this scale may not have been fine enough. Generation of a land-cover dataset fine enough for this analysis is likely impractical, but merging different existing land cover and shapefile datasets could produce finer-scale data.

Our analysis looked at a few environmental factors that could be affecting mosquito populations. In reality, there are many more contributing factors. Some other factors commonly used in mosquito studies include humidity, elevation, soil wetness, and anthropogenic factors (Reisen, 2010). Our analysis looked at climate data on the temporal scale affecting a single mosquito lifecycle. Many studies have found associations with mosquito numbers at greater lags, or attributed a year's mosquito count to a specific seasonal condition, i.e. a warm winter. The body of literature examining mosquito population and climate effects has yet to, and will likely never, reach a consensus on what period of climate is most effective in predicting mosquito patterns. Therefore a 2-week lag period was considered reasonable for this study. The precipitation data for the Seattle Region contains many zeroes during the summer months, which complicates analyses. In the future a mixture model that determines the probability of zero and the distribution of the data when it is positive would help resolve this issue (Hyndman, 2010).

WA DOH Mosquito Dataset Discussion and Future Directions

There are many benefits that could be gained by making use of the WA DOH mosquito dataset. Almost all of the mosquitoes are identified to the species level, the only exception being when the specimens were too damaged to identify to that level of detail. Many of the specimens include WNV testing results, so in areas where there are positive pools of mosquitoes, metrics accounting for infection potential such as Minimum Infection Rate (MIR) can be incorporated in statistical models. Another major benefit of this dataset is that all the mosquito-sampling points are specified by the exact date they were captured. This allows for models to be developed on a daily scale, which is the most accurate temporal scale when developing explanatory and predictive models (Reisen, 2010).

The dataset from WA DOH is high quality and contains minimal errors, however the usability of the data in all locations comes into question in regards to spatial resolution and temporal coverage. In King County, most of the problems experienced were due to these issues. Originally there were 177 sites included in the King County dataset. However, our previously mentioned exclusion criteria reduced the number of sites we were able to use in our analyses to 44. Of the excluded sites, 110 were sampled less than ten times throughout the season, including 68 that were only sampled once or twice. Sites that are sampled infrequently over a season are difficult to draw conclusions from regarding the seasonality of the mosquitoes at the site, let alone the potential impacts environmental factors could be having on the population. Of the sites with twelve or more sampling dates, seven had to be excluded because they were identified spatially at the zip code level. It may be worthwhile in the future to identify these sites, if possible, to a more specific location so that they can be included in the analysis.

Another issue with the King County dataset that makes it difficult to use in predictive risk modeling is the variability of sampling sites over the years. While the inconsistency in sampling sites did not hinder our ability to look at the impacts of environmental factors on mosquito abundance, it would affect the capability of a model to predict risk accurately (Diuk-Wasser et al., 2006). At this point in time, I do not think a model predicting mosquito populations in King County is possible to develop. More data from sites conserved over the years are needed. I recommend mosquito sampling continue in King County, and that site selection should incorporate some of the sites used in this analysis, as well as new sites with more diverse land cover classifications. Additional environmental datasets including finer scale climate datasets should be sought out.

I recommend turning attention away from King County, and instead looking at other counties in WA for future projects. On a state level, the current level of mosquito data in WA is not enough to create a predictive model for the state. Currently, ten of WA's counties contain 1,000 or more mosquito sampling points. The counties with the most mosquito data are Benton and Grant counties, which both have over 10,000 data points, followed by Franklin and Yakima counties which have between 5,000 and 8,000 points. These counties have mosquito control districts, which aid in the collection of this data. These counties also make up the current endemic region for WNV in the state. WNV-positive mosquito pools could be used to calculate a MIR for mosquito populations in the region, which could potentially be used to predict WNV risk. Benton, Grant, Franklin, and Yakima counties also have a large number of sites that are conserved from 2008-2014. A future student would have to take a more detailed look at the data from these counties to determine how usable they are, but I find it highly likely they could be incorporated in models that quantify the impact climate and other environmental factors have on mosquito populations, that could then be used to develop predictive risk models under future climate change scenarios.

Aim I: Mosquito Surveillance 2014

In 2014 mosquito abundance was generally low. *Cx. pipiens* was the dominant mosquito species in the adult samples. Sites with the highest catch were dominated by *Cx. pipiens*, whereas sites with lower catches showed a greater diversity of species. Other adult species commonly caught at the sites include *Cx. tarsalis, Cs. incidens, Cq. perturbans* and *Cs. minnesotae.* Of the larvae identified to species level, the majority were *Cx. pipiens*, *Cx.*

tarsalis, and *Cx. territans*. *Cx. territans* is an amphibian-biter and is not usually caught in CO_2 traps, which is why we did not collect many of this species as an adult (Darsie, 2004). *Cq. perturbans* larvae live in reeds in marshy areas; we did not gather reeds and sample them for larvae in this study, which is why they are excluded from our findings. The mosquito species collected reflect what we would expect for an urban environment.

Adult mosquito abundance showed some relationships with temperature, precipitation, and land cover. In week 6 of sampling (week of the year 30), there was a sharp drop in Tmax and in mosquito numbers (Figure 4). There was also an increase in precipitation that week (Figure 12). Mosquito abundance, especially *Cx. pipiens* counts were generally higher in areas with more med-high developed land cover (Figure 16). Mosquito larvae numbers appeared to be related to precipitation, but spiked at different points related to 0, 1 and 2-week lags. These results suggest the interaction of temperature and precipitation has a greater effect on populations than temperature or precipitation alone.

In 2014, no WNV-positive mosquito pools were found. Previous years data have also found a lack of WNV prevalence in mosquito, bird, and human populations in the county (WADOH, 2013).

Aim I: 2003 versus 2014 Comparison

The results of the four-site comparison found that mosquito abundance was significantly higher in 2003 than in 2014. Looking at the environmental factors, there was not much difference between the years (Table 6). These findings suggest that mosquito populations may be affected by factors other than the ones we examined. Because we examined identical sites and the land cover did not significantly differ over the years, it is unlikely that site is the primary contributor to the difference in counts. It is more likely climate factors that are playing a role. Seasonal effects we did not examine, such as a warm winter or spring, could account for the differences. Another possibility is that in 2014 there was a significant drop in temperature early in the season, which was not experienced in 2003. It could be that this drop had lasting effects on the seasons' mosquito numbers. It is also possible there were changes in vegetation at the sites the NLCD did not pick up. Photo evidence and personal accounts suggest there were vast changes in vegetation at the WEHS0082 and WEHS0089 sites from 2003 to 2014. A detailed vegetation survey would have to be performed to truly understand the impacts of the microhabitats on mosquito populations at these sites.

Cx. pipiens was the dominant mosquito at the sites both years, although there was a greater diversity of species in 2014 than in 2003 (Figures 13 and 14). This follows the pattern mentioned previously where higher catches are more dominated by *Cx. pipiens*, and lower catches are more diverse. This suggests the diversity is due more to a lack of *Cx.*

pipiens than a true greater diversity of species. However, in 2014 additional species were identified that were not present in 2003. The most notable is *Cs. minnesotae*, which was found in high numbers at the WEHS0089 site in 2014. *Cs. minnesotae* is not a known WNV vector, and is generally found in wooded or semi-permanent marshy areas in places with cooler climates (Trimble, 1972). It is possible that this is an emerging species in King County.

Aim II: Environmental Factors and Mosquito Abundance

The correlative analysis showed positive associations of mosquito abundance with percent med-high developed land cover, Tmax, Tmin, and DW_63. Percent forested land cover and Precip exhibited negative relationships with mosquito abundance. The regression model did not find any significant associations of temperature or precipitation with *Cx. pipiens* abundance when controlling for the other variables. Additionally, the model found that all land cover variables were negatively associated with *Cx. pipiens* abundance and that weekly precipitation at the 1-week lag strengthened the negative relationship of med-high developed land cover with *Cx. pipiens* abundance.

The results of the regression model conflict with the correlative analysis, and do not make much sense considering what we know about mosquito ecology. Some possible reasons for why the model did not perform well are because site effect and spatial autocorrelation were not considered. Because most of the sites are unique, it is possible that some of the variation in mosquito abundance is explained by site for reasons not due to land cover. However, it is doubtful this is the primary contributor considering Aim I found significant differences in mosquito abundance in sites with the same location. Because of this, it would be expected that the year variable in our model would explain most of the variation in mosquito numbers. However in our model it was not determined to be significant, probably because it was confounded by site which we did not account for. Developing a covariate that could effectively account for site would likely vastly improve the R^2 of this model and allow us to see additional associations that were deemed insignificant in the current model. Unfortunately considering our time constraints this was not possible. The explanation of interactions between land cover, temperature, and precipitation was also limited in our approach because interaction terms were selected by minimizing the Akaike information criterion (AIC) rather than by scientific reason.

The degree-weeks metric did not provide as much insight into our research aims as we hoped it would. It did not show associations with mosquito abundance any more significantly than Tmax or Tmin, and because it was highly correlated with these variables it was excluded from the regression analysis. Using a degree-day metric may have been more beneficial, but considering the weekly scale of the mosquito sampling data and other climate data, the usability of a degree-days metric is questionable.

Considering the results of just the correlative analysis, the mosquito abundance metrics showed the strongest association with the percent med-high developed land cover variable (Figure 16). This agrees with the notion that *Cx. pipiens* is the dominant mosquito in the Seattle region, and prefers to live in highly developed areas where man-made containers with organic matter are abundant. The correlative analysis with land cover also showed that percent forested and open-low developed land cover (in the case of *Cx. pipiens* abundance) exhibited negative relationships with mosquito abundance. These results suggest the more forested or 'low developed' an area is, the less advantageous it is for *Cx. pipiens*. According to the NLCD, open-low developed land cover includes areas like parks and grassy fields that may in fact be 'too natural' for *Cx. pipiens*, even though it is classified as developed.

The correlative analysis also showed an interesting dichotomous trend with total developed land cover, where mosquito traps in areas with <60% developed land had a negative association with developed land cover, but traps in areas with >60% developed land had a positive association. This was explored further to determine if species diversity played a role in this trend. Ten data points were identified as having <60% developed land cover. Upon further inspection one of these sites (KING0019) was considered to be an outlier because it was actually highly developed being located on the Duwamish Waterway, and was excluded from the subsequent analysis. A ratio of *Cx. pipiens*: all other species was calculated by dividing the seasonal average *Cx. pipiens* abundance by the seasonal average abundance of all other species at each site. The average *Cx. pipiens*: other species ratio at the sites with >60% developed land cover was 12.7, and was 1.4 at the sites with < 60% developed land cover. This suggests *Cx. pipiens* is present about nine times as much in sites where developed land makes up over half the land cover than in sites where developed land is less than 60% of the total. This contrast in species diversity could account for the different associations of the mosquitoes with developed land cover noted in the correlative analysis.

Again considering just the results of the correlative analysis, the temperature variables were found to have slightly positive associations with mosquito abundance and the precipitation variables were found to have slightly negative associations with mosquito abundance. The associations were relatively similar among all lags. The results of our temperature analysis agree with other scientific findings that warmer temperatures contribute to increased mosquito numbers. Precipitation was found to have a slightly negative impact, which is somewhat contradictory to mosquito ecology but altogether not surprising considering the varied results characterizing this relationship in the literature. Heavy rainfall events have been shown to have negative impacts on adult populations (Jones et al., 2012), and it is also possible that because rainfall is King County is often associated with cooler temperatures, the drop in temperature could be responsible for this trend rather than rainfall alone. The impacts of temperature and precipitation we found in our analysis were relatively low. Crowder et al. (2013) did not find any significant associations of mosquito populations with climate in WA, but did find that climate altered WNV prevalence. They offered the suggestion that climate may alter mosquito or bird activity which affects the transmission of WNV, rather than quantitatively affecting vector or host abundances. Our results suggest that immediate weather patterns may have some effect on vector populations, but that land cover and longer-term climate patterns we did not assess may play a bigger role in predicting vector abundance.

Conclusions

This study provided insight into mosquito population diversity, distribution, and abundance in King County, Washington. We were able to discern trends for mosquito abundance with land cover and climate factors, and determined that the mosquitoes in the area are most strongly affected by land cover variables. Areas with high development were associated with greater numbers of mosquitoes. We determined that mosquito populations increase with increased temperature, and possibly decrease with increased precipitation. It was also suggested that one specific dramatic climate event, like the temperature drop during week 30 of 2014, could have lasting effects on a season's mosquito numbers. This study also suggests that mosquito populations are likely affected by variables we did not examine. Long-term climate patterns are likely a primary contributor to the differences observed in mosquito numbers by year.

At this point there is no evidence of WNV in King County, but considering our results and the modeled impacts of climate change for the Pacific Northwest, it is likely that mosquito numbers will rise. If WNV were to occur in the Seattle region, it would likely take on an urban transmission cycle where developed and residential areas pose the greatest risk for WNV amplification and transmission. Because our results show higher vector numbers in areas with higher development, public health measures should be taken to reduce mosquitoes in these areas if an outbreak were to occur. To aptly prepare King County for possible changes in mosquito populations, future research should focus on polishing the regression model developed in this study, and incorporating additional environmental variables to gain a better understanding of how mosquito populations are affected by their environment.

References

1. Bost H (2004). A comparison of West Nile Virus vector mosquito populations in sites with and without storm water drainage ponds. *Masters Thesis.* University of Washington
2. Bowden SE, Magori K, Drake JM (2011). Regional differences in the association between land cover and West Nile virus disease prevalence in humans in the United States. *Am J Trop Med Hyg., 84*: 234-238
3. Brown HE, Childs JE, Diuk-Wasser M, Fish D (2008). Ecological factors associated with West Nile virus transmission, Northeastern United States. *Emerg Infect Dis, 14*:1539-1545.
4. CDC, Centers for Disease Control and Prevention (2009). St Louis Encephalitis. Accessed from: http://www.cdc.gov/sle/index.html
5. CDC (2013). West Nile virus in the United States: Guidelines for surveillance, prevention, and control. Accessed from: http://www.cdc.gov/westnile/resources/pdfs/wnvguidelines.pdf
6. Chen CC, Jenkin E, Epp T, Waldner C, Curry PS, Soos C (2013). Climate Change and West Nile virus in a highly endemic region of North America. *Int J. Environ. Res. Public Health, 10*: 3025-3071.
7. Crowder DW, Dykstra EA, Brauner JM, Duffy A, Reed C, Martin E, Peterson W, Carriere Y, Dutilleul P, Pwen JP (2013). West Nile virus prevalence across landscapes is mediated by local effects of agriculture on vector and host communities. *PLOSone, 8*(1).
8. Dalton MM, Mote PW, Snover AK (2013). Climate Change in the Northwest. *Island Press*: Oregon Climate Change Research Institute, 271 pgs.
9. Darsie RF, Ward RA (2004) Identification and geographical distribution of the mosquitoes of North America, north of Mexico. Gainesville: University of Florida Press. 400 pp.
10. Diuk-Wasser MA, Brown HE, Andreadis TG, Fish D (2006). Modeling the spatial distribution of mosquito vectors for West Nile Virus in Connecticut, USA. *Vecotr-borne and Zoonotic Diseases, 6*(3): 283-295.
11. Dohm DJ, O'Guinn ML, Turell MJ (2002). Effect of environmental temperature on the ability of *Culex pipiens* (Diptera: Culicidae) to transmit West Nile virus. *J. Med. Entomol., 39*(1):221-225.
12. EPA, US Environmental Protection Agency (2004). Wetlands and West Nile Virus. Office of Water. Accessed from: http://www.epa.gov/owow/wetlands/pdf/WestNile.pdf
13. Epstein PR (2001). West Nile virus and the climate. *Journal of Urban Health: Bulletin of the NY Academy of Medicine, 78*(2):367-371.

14. FloridaHealth (2015). Dengue Fever. Accessed from: http://www.floridahealth.gov/diseases-and-conditions/dengue/
15. Gould EA, Higgs S (2009). Impact of climate change and other factors on emerging arbovirus diseases. *Trans R Soc Trop Med Hyg, 103*(2): 109-121.
16. Institute of Medicine (2003). *Microbial threats to health: Emergence, detection, and response.* National Academy of Sciences, Washington DC.
17. Hyndman, RJ (2010, Aug 13). Transforming data with zeroes (Web log post). Retrieved from: http://robjhyndman.com/hyndsight/transformations/
18. Jin, S., Yang, L., Danielson, P., Homer, C., Fry, J., and Xian, G. 2013. A comprehensive change detection method for updating the National Land Cover Database to circa 2011. *Remote Sensing of Environment*, 132: 159 – 175.
19. Jones CJ, Lounibos LP, Marra PP, Kilpatrick AM (2012) Rainfall influences survival of *Culex pipiens* mosquitoes in a residential neighborhood in the mid-Atlantic USA. *J Med Entomol, 49*: 467–473. doi: 10.1603/me11191
20. Kesavaraju B, Farajollahi A, Lampman RL, Hutchinson MH, Krasavin NM, Graves SE, Dickson SL (2012). Evaluation of a Rapid Analyte Measurement Platform for West Nile Virus Detection Based on United States Mosquito Control Programs. *Am J Trop Med Hyg., 87*(2):359-363.
21. MDH, Minnesota Department of Health (2005). Western Equine Encephalitis Fact Sheet. Infectious Disease Epidemiology, Prevention and Control (IDEPC) Division. Accessed from: http://www.health.state.mn.us/divs/idepc/diseases/weencephalitis/wee.html
22. Morin CW & Comrie AC (2013). Regional and seasonal response of a West Nile virus vector to climate change. *PNAS Early Edition.*
23. NOAA, National Oceanic and Atmospheric Administration, National Centers for Environmental Information (2015). Climate Data Online. Retrieved from: http://www.ncdc.noaa.gov/cdo-web/
24. ODH, Ohio Department of Health (2015). Western equine encephalitis virus disease. Accessed from: http://www.odh.ohio.gov/pdf/IDCM/wee.pdf
25. O'Malley C (1995). Seven ways to a successful dipping career. *Wing Beats, 6(4)*:23-24.
26. Pratt HD & Moore CG (Revised 1993). Mosquitoes of Public Health Importance and Their Control. Self-Study Course 3013-G Vector-Borne Disease Control. CDC. US Department of Health and Human Services.
27. Reisen WK (2002). *Culex tarsalis:* History, biology, ecology, public health and control. *Excerpt from:* National West Nile Virus conference call July 10, 2002.
28. Reisen WK, Fang Y, Martinez VM (2006). Effects of temperature on the transmission of West Nile virus by *Culex tarsalis*(Diptera: Culicidae). *J. Med. Entomol., 43*(2): 309-317.

29. Reisen WK, Fang Y, Martinez VM (2006). Vector competence of Culiseta incidens and Culex thriambus for West Nile virus. *J Am Mosq Control Assoc., 22*(4): 662–665.
30. Reisen WK (2010). Landscape epidemiology of vector-borne diseases. *Annual Review of Entomology*, *55*: 461 -483.
31. Rossi SL, Ross TM, Evans JD. (2010).West Nile Virus. *Clin Lab Med, 30* :47-65.
32. Ruiz MO, Chaves LF, Hamer GL, Sun T, Brown WM, Walker ED, Haramis L, Goldberg TL, Kitron UD (2010). Local impact of temperature and precipitation on West Nile virus infection *Culex* species mosquitoes in northeast Illinois, USA. *Parasit Vectors, 3(19)*. doi:10.1186/1756-3305-3-19.
33. Sames WJ, Duffy A, Maloney FA, Townzen JA, Brauner JM, McHugh CP, Lilja, J (2007). Distribution of mosquitoes in Washington State. *Journal of the American Mosquito Control Association, 23*(4): 442-448.
34. Sardelis MR, Turell MJ, Dohm DJ, O'Guinn ML (2001). Vector Competence of Selected North American Culex and Coquillettidia Mosquitoes for West Nile Virus. *Emerg Infect Dis.* Available from http://wwwnc.cdc.gov/eid/article/7/6/01-0617
35. Shaman J, Day JF, Stieglitz M (2005). Drought-induced amplification and epidemic transmission of West Nile virus in southern Florida. *J. Med. Entomol.*, 42(2): 134-141.
36. Solano County Mosquito Abatement District (2005). Mosquito Notes. Type: Cool-Weather Mosquito. Accessed from: http://www.solanomosquito.com/mosquitoes_coolweather.html
37. Trimble RM (1972). Occurrence of *Culiseta minnesotae* and *Aedes trivittatus (Diptera:Culicidae)* in Manitoba including a list of mosquitoes from Manitoba. *Can. Ent., 104*: 1535-1537.
38. UC IPM, University of California Agriculture and Natural Resources (2014). Degree-Days. Retreived from: http://www.ipm.ucdavis.edu/WEATHER/ddconcepts.html
39. Wang G, Minnis RB, Belant JL, Wax CL (2010). Dry weather induces outbreaks of human West Nile virus infections. *BMC Infectious Diseases, 10*(38).
40. WADOH, Washington State Department of Health (2008). Guidance for Surveillance, Prevention, and Control of Mosquitoborne Disease. Zoonotic Disease Program. Accessed from: http://www.doh.wa.gov/portals/1/Documents/Pubs/333-149.pdf
41. WADOH (2013). West Nile virus surveillance maps and statistics. Zoonotic Disease Program. Accessed from: http://www.doh.wa.gov/DataandStatisticalReports/DiseasesandChronicConditions/WestNileVirus.aspx
42. WADOH (2015). Arboviral disease (except West Nile Virus and Yellow Fever) reporting and surveillance guidelines. Zoonotic Disease Program. Accessed from: http://www.doh.wa.gov/Portals/1/Documents/5100/420-046-Guideline-Arbo.pdf

43. White DJ (2006). Vector Surveillance for West Nile Virus*. Annals of the New York Academy of Sciences, 951*: 74–83.
44. WHO, World Health Organization. Githeko AK, Lindsay SW, Confalonieri UE, Patz JA (2000). Climate change and vector-borne diseases: A regional analysis. *Bulletin of the World Health Organization, 78*(9): 1136-1147.
45. WHO (2011). West Nile Virus. World Health Organization Media Center. Accessed from:http://www.who.int/mediacentre/factsheets/fs354/en/

Appendix

Attribute Table of Selected Sampling Sites in King County

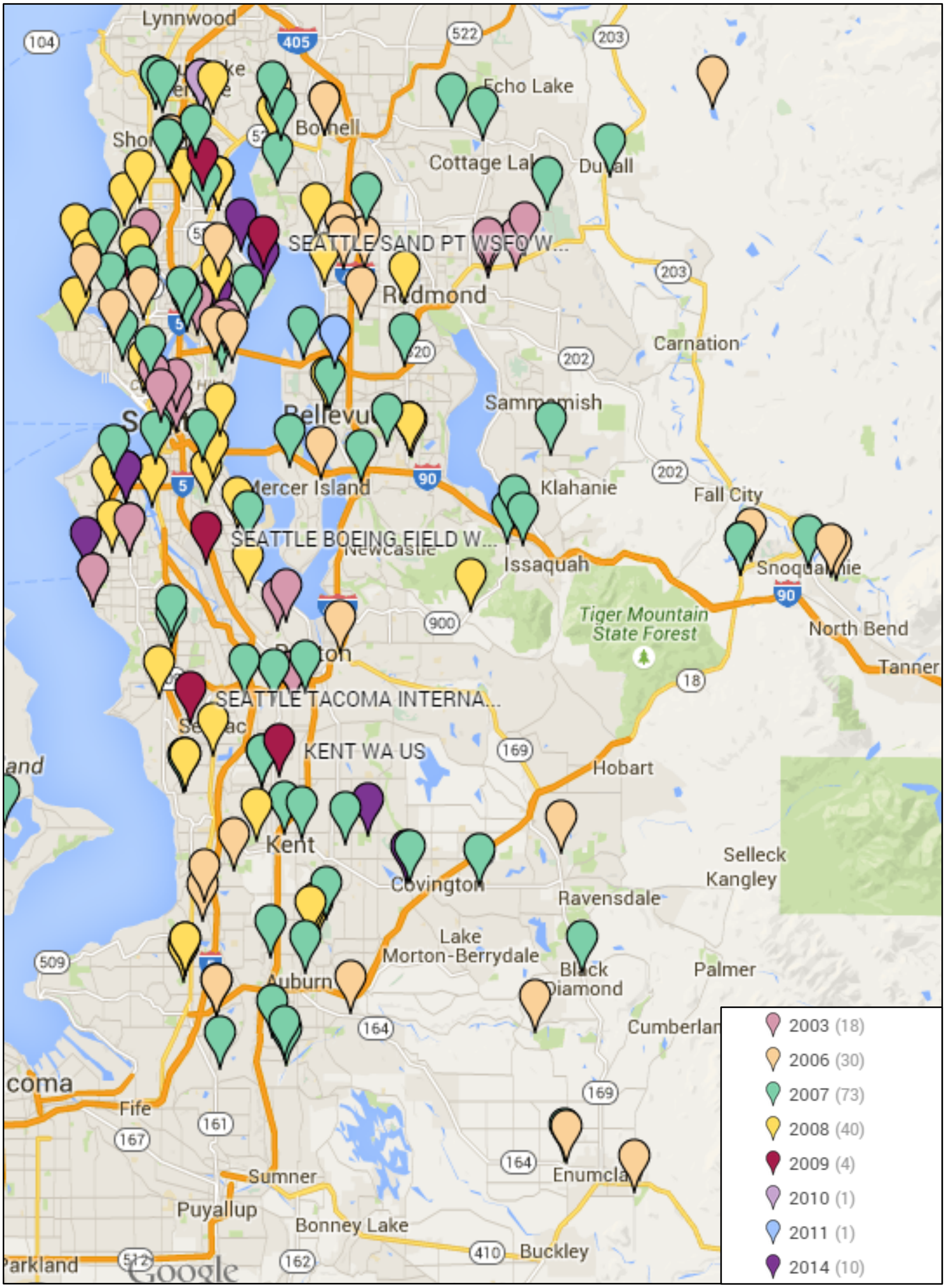
Sites and Mosquito Abundance Metrics

Site	Year	Latitude	Longitude	Location_Flag	Freq_sampling	Pipiens_Sum	Pipiens_Avg	Total_Mosq_Sum	Total_Mosq_Avg	Other Species	Ratio Pip:Other
KING1502	2008	47.6936	-122.4039	E	14	135	9.642857143	158	11.28571429	1.642857143	5.869565217
WEHS0088	2014	47.5275	-122.3958	E	15	51	3.4	72	4.8	1.4	2.428571429
NH	2003	47.6954	-122.0492	V	14	5	0.357142857	45	3.214285714	2.857142857	0.125
WEHS0086	2003	47.5079	-122.3900	E	13	35	2.692307692	125	9.615384615	6.923076923	0.388888889
WEHS0086	2014	47.5079	-122.3900	E	15	24	1.6	53	3.533333333	1.933333333	0.827586207
KING1503	2008	47.7109	-122.3652	E	14	45	3.214285714	65	4.642857143	1.428571429	2.25
KING0019	2008	47.5607	-122.3438	E	15	829	55.26666667	868	57.86666667	2.6	21.25641026
WEHS0084	2014	47.6971	-122.2739	E	15	6	0.4	24	1.6	1.2	0.333333333
195th	2003	47.6868	-122.0793	SA	13	5	0.384615385	18	1.384615385	1	0.384615385
CR	2003	47.6833	-122.0785	SA	14	3	0.214285714	11	0.785714286	0.571428571	0.375
WCP	2003	47.6865	-122.0571	SA	14	33	2.357142857	129	9.214285714	6.857142857	0.34375
WEHS0091	2014	47.3599	-122.1436	E	14	119	8.5	204	14.57142857	6.071428571	1.4
WEHS0090	2014	47.3852	-122.1734	E	15	34	2.266666667	227	15.13333333	12.86666667	0.176165803
KING0015	2008	47.6416	-122.2931	E	14	95	6.785714286	213	15.21428571	8.428571429	0.805084746
KING0003	2008	47.7235	-122.3186	E	14	240	17.14285714	257	18.35714286	1.214285714	14.11764706
KING1501	2008	47.6551	-122.4048	E	13	130	10	272	20.92307692	10.92307692	0.915492958
WEHS0085	2014	47.6569	-122.2928	E	15	61	4.066666667	180	12	7.933333333	0.512605042
KING0029	2008	47.4558	-122.2325	E	12	303	25.25	517	43.08333333	17.83333333	1.41588785
KING0020	2008	47.5989	-122.2906	E	14	133	9.5	171	12.21428571	2.714285714	3.5
WEHS0083	2014	47.6774	-122.2562	E	15	201	13.4	263	17.53333333	4.133333333	3.241935484
KING0012	2008	47.5639	-122.3009	E	14	131	9.357142857	499	35.64285714	26.28571429	0.355978261
KING0110	2008	47.4590	-122.3379	E	12	689	57.41666667	710	59.16666667	1.75	32.80952381
KING0018	2008	47.5158	-122.2688	E	15	26	1.733333333	79	5.266666667	3.533333333	0.490566038
UWC	2003	47.6525	-122.3077	V	13	473	36.38461538	531	40.84615385	4.461538462	8.155172414
WEHS0087	2014	47.5625	-122.3649	E	14	89	6.357142857	109	7.785714286	1.428571429	4.45
KING1504	2008	47.5607	-122.3790	E	15	108	7.2	177	11.8	4.6	1.565217391
WEHS0089	2014	47.5351	-122.3622	E	15	121	8.066666667	213	14.2	6.133333333	1.315217391
KING1508	2008	47.7228	-122.3536	E	14	250	17.85714286	374	26.71428571	8.857142857	2.016129032
WEHS0087	2003	47.5625	-122.3649	E	14	127	9.071428571	207	14.78571429	5.714285714	1.5875
KING0016	2008	47.5758	-122.2957	E	14	141	10.07142857	144	10.28571429	0.214285714	47
KING1506	2008	47.5371	-122.3760	E	14	144	10.28571429	168	12	1.714285714	6
WEHS0089	2003	47.5351	-122.3622	E	14	130	9.285714286	154	11	1.714285714	5.416666667
SGM	2003	47.6118	-122.3252	SA	12	274	22.83333333	287	23.91666667	1.083333333	21.07692308
DWG	2003	47.6004	-122.3251	SA	13	295	22.69230769	303	23.30769231	0.615384615	36.875
WEHS0082	2014	47.7189	-122.2916	E	16	394	24.625	423	26.4375	1.8125	13.5862069
KING0002	2008	47.6686	-122.2932	E	13	63	4.846153846	65	5	0.153846154	31.5
KING0011	2008	47.5486	-122.2768	E	15	153	10.2	178	11.86666667	1.666666667	6.12
KING0017	2008	47.6222	-122.3506	E	19	1371	72.15789474	1436	75.57894737	3.421052632	21.09230769
KING1505	2008	47.6802	-122.3945	E	14	123	8.785714286	136	9.714285714	0.928571429	9.461538462
KING1507	2008	47.6821	-122.3578	E	13	83	6.384615385	166	12.76923077	6.384615385	1
WEHS00821	2008	47.7189	-122.2916	E	14	78	5.571428571	84	6	0.428571429	13
MH	2003	47.6912	-122.3491	SA	14	109	7.785714286	136	9.714285714	1.928571429	4.037037037
WEHS0082	2003	47.7189	-122.2916	E	14	867	61.92857143	874	62.42857143	0.5	123.8571429

Sites with Percent Land Cover Classification Values

Site	B500_DMH	B500_DML	B500_TD	B500_F	B500_W	B500_OS	B500_OW
KING1502	0.05855339	0.20091849	0.25947187	0.19977038	0.0815155	0	0.32376579
WEHS0088	0.128527	0.195402	0.323929	0.264368	0	0	0.411703
NH	0.03061224	0.31746032	0.34807256	0.61989796	0.06122449	0.07142857	0
WEHS0086	0.10459184	0.27822581	0.38281764	0.26020408	0	0.00510204	0.34438776
WEHS0086	0.15032	0.287846	0.438166	0.146055	0	0.00533	0.410448
KING1503	0.09954233	0.40846682	0.50800915	0.38215103	0.10983982	0	0
KING0019	0.51958525	0.01036866	0.52995392	0	0.05184332	0.01843318	0.39976959
WEHS0084	0.121068	0.442326	0.563394	0.056244	0.017159	0	0.363203
195th	0.02252252	0.54204204	0.56456456	0.27477477	0	0.11111111	0
CR	0.02252252	0.54204204	0.56456456	0.27477477	0	0.11111111	0
WCP	0.00507614	0.66535433	0.67043047	0.34517766	0.02791878	0	0.02030457
WEHS0091	0.283629	0.432742	0.716371	0.087591	0	0.007299	0.184567
WEHS0090	0.18609	0.559211	0.745301	0.015038	0.088346	0.151316	0
KING0015	0.09931507	0.66324201	0.76255708	0.13242009	0.09703196	0	0.00799087
KING0003	0.28850575	0.48045977	0.76896552	0.1908046	0.04022989	0	0
KING1501	0.32302406	0.44788087	0.77090493	0.17067583	0	0.05841924	0
WEHS0085	0.210526	0.582043	0.79257	0	0.120743	0.012384	0.074303
KING0029	0.18663595	0.62327189	0.80990783	0.06797235	0.11059908	0.00691244	0
KING0020	0.37070938	0.44279176	0.81350114	0.09038902	0.0194508	0	0.08810069
WEHS0083	0.301987	0.548344	0.850331	0.008609	0.003311	0.023179	0.11457
KING0012	0.32875143	0.52691867	0.8556701	0.1443299	0	0	0
KING0110	0.53279632	0.33601841	0.86881473	0.12888377	0	0.0023015	0
KING0018	0.37155963	0.51376147	0.8853211	0.1146789	0	0	0.02293578
UWC	0.7106599	0.19291339	0.90357328	0	0	0	0
WEHS0087	0.278459	0.641856	0.920315	0.044658	0.035026	0	0
KING1504	0.69195402	0.23793103	0.92988506	0.07011494	0	0	0.13563218
WEHS0089	0.448705	0.490155	0.93886	0.06114	0	0	0
KING1508	0.54691076	0.40617849	0.95308925	0	0	0	0.04691076
WEHS0087	0.25831202	0.70967742	0.96798944	0.02557545	0.04347826	0	0
KING0016	0.70823799	0.26201373	0.97025172	0.02974828	0	0	0
KING1506	0.5924225	0.3869116	0.9793341	0.0206659	0	0	0
WEHS0089	0.44501279	0.53629032	0.98130311	0.03069054	0	0	0
SGM	0.97461929	0.00787402	0.98249331	0	0	0	0
DWG	0.92307692	0.06097561	0.98405253	0	0	0	0
WEHS0082	0.606557	0.393443	1	0	0	0	0
KING0002	0.44520548	0.55479452	1	0	0	0	0
KING0011	0.66857143	0.33142857	1	0	0	0	0
KING0017	0.94863014	0.05136986	1	0	0	0	0
KING1505	0.6954023	0.3045977	1	0	0	0	0
KING1507	0.83238312	0.16761688	1	0	0	0	0
WEHS00821	0.61028571	0.38971429	1	0	0	0	0
MH	0.78205128	0.23694779	1.01899907	0	0	0	0
WEHS0082	0.6870229	0.35177866	1.03880156	0	0	0	0

Map of All King County Data Points from the WA DOH Dataset



ArcGIS Workflow

Workflow for determining percent land cover classification in ArcMap Version 10.2

NLCD can be downloaded here: http://www.mrlc.gov/nlcd11_data.php

1. **Clip Raster to shapefile**
   - Need shapefile of WA State/County.
     - Can be found here: http://guides.lib.washington.edu/content.php?pid=78069&sid=577938
   - Use Clip tool under Data management tools
2. **Multiple buffer analysis:**
   - Use multiple ring buffer tool
   - Created different layers for each year
   - Use explode tool in advanced editing to get separate object IDs for all rings
   - Buffers created: 50,100,200,300,400,500m
     - Use Dissolve ALL

*For sites that are close enough that they overlap in the same year, but were individually sampled, use Dissolve NONE. This will create buffers that each have their own rings, but rings are not hollow so need to subtract out

3. **Landcover attribute extraction**
   - Use Extract by Attributes tool to extract land cover for the classifications you are defining
   - The value or ObjectID represent the Anderson Land cover classification
     - The following were used:

     a) developed: open space(21), low(22), medium(23), high(24) intensity
     b) forest: mixed(43),deciduous(41),evergreen(42)
     c) wetlands: woody wetlands(90), emergent herbaceous wetlands(95)
     d) grassland/open: grassland/herbaceous(71), pasture/hay(81), shrub/scrub (52), cultivated crops (82)
     e) other: open water(11), barren land (31)
   - Example of extract by attributes query:

     Wetlands: "VALUE" = 90 OR "VALUE" = 95

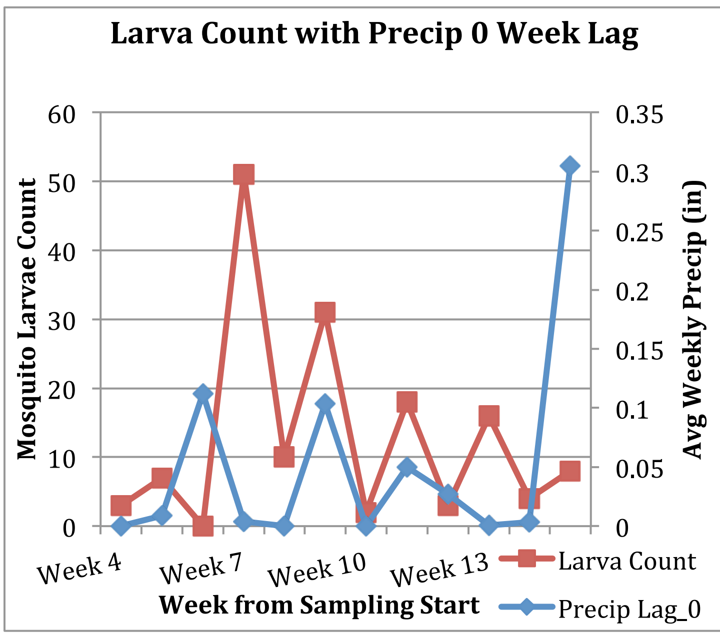
4. **Zonal Statistics as Table**
   - Do for all sites at all buffer distances
   - Need to do each land cover class independently
   - In 'Environments' always set processing extent and raster analysis to same raster layer

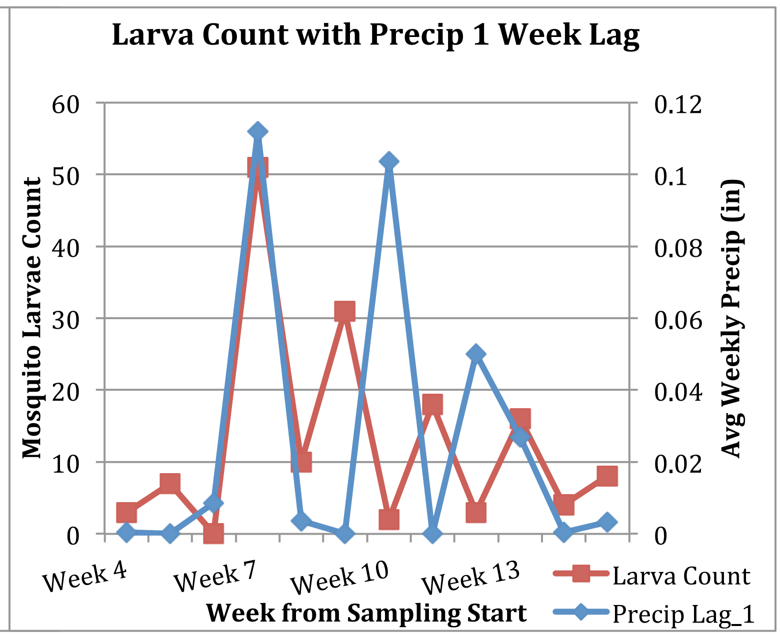
- Need to know total pixel count for each buffer distance (and or combined), can calculate by just using NLCD as input raster
- In Zonal Statistics as Table Tool: Input raster(the buffers you create), give it a zone field (id in buffer table), input value raster is land use

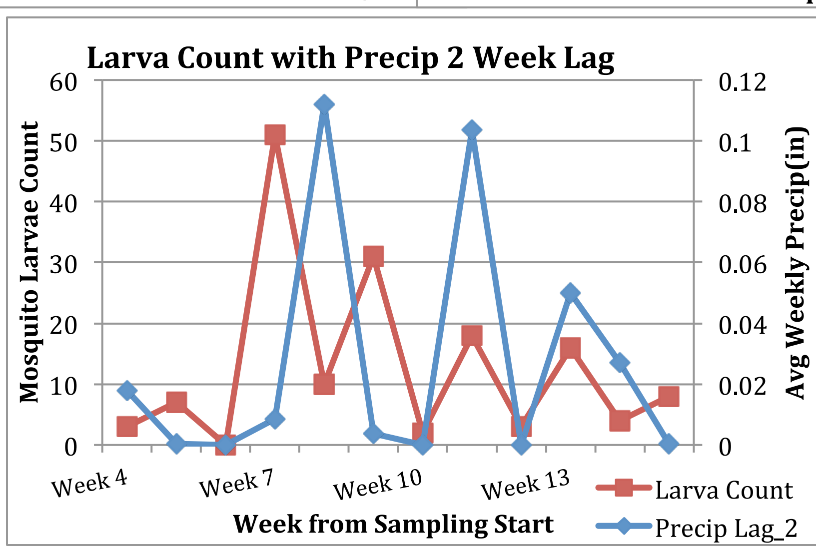
5. **Final Processing**

- To determine the percent of each land cover class in each buffer at each site, you need to divide the pixel count you received from zonal statistics by the total pixel count for that buffer distance at that site
    - The total number of pixels will vary by site, so cannot just calc # of pixels in a 500m buffer and assume it will be the same throughout. Need to calc for each site individually

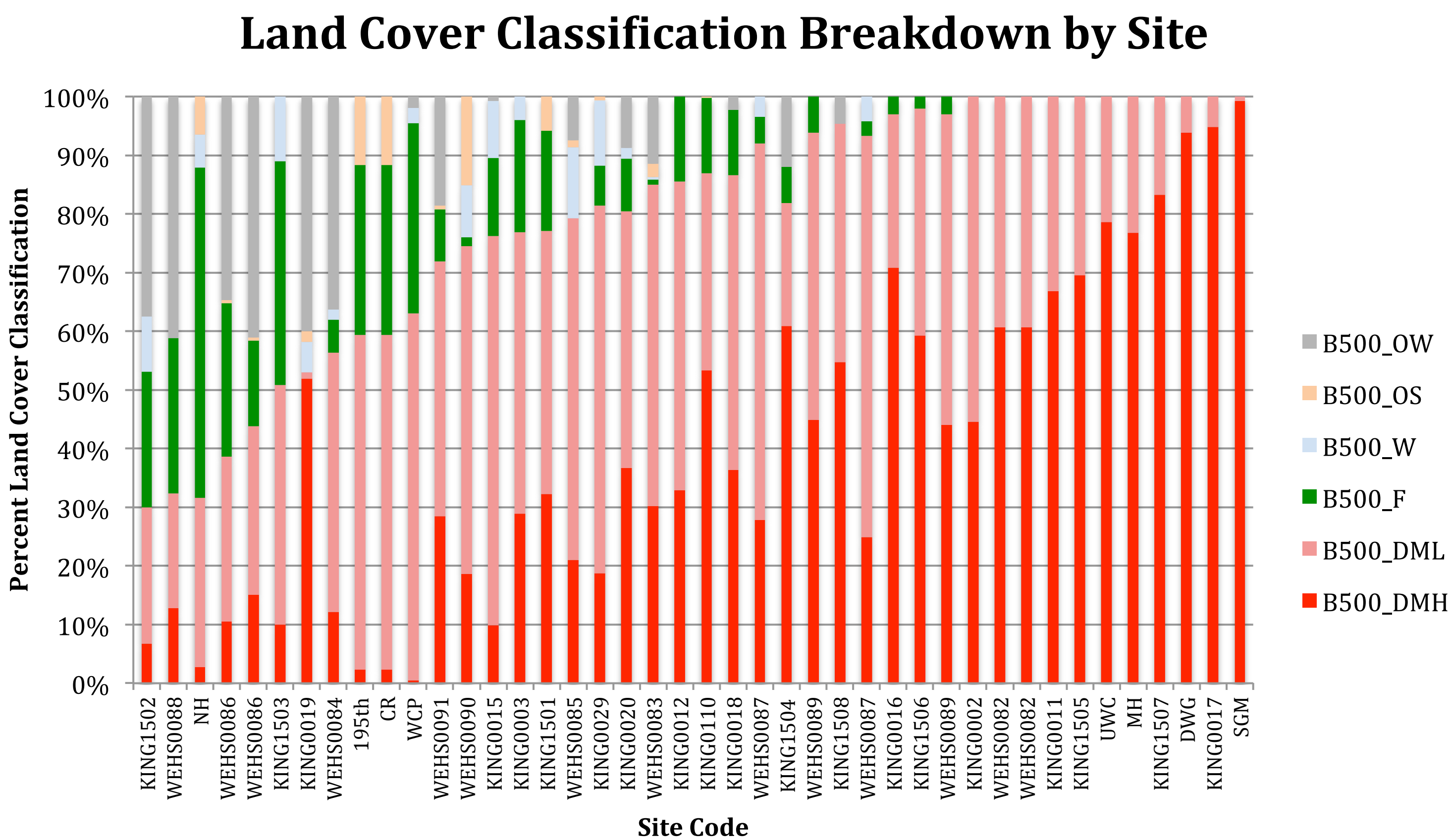
Mosquito Larvae







Land Cover Classification, All Sites



Climate Variables Correlation Coefficients by Year

Temperature- Total Mosquito

			r	p
2014	Tmax			
		0 week lag	0.43	<0.001
		1 week lag	0.17	0.04
		2 week lag	0.07	0.4
	Tmin			
		0 week lag	0.33	<0.001
		1 week lag	0.24	0.004
		2 week lag	0.19	0.02
	DW_63			
		0 week lag	**0.45**	**<0.001**
2008	Tmax			
		0 week lag	0.26	<0.001
		1 week lag	0.35	<0.001

Temperature- Pipiens

			r	p
2014	Tmax			
		0 week lag	0.35	<0.001
		1 week lag	0.18	0.03
		2 week lag	0.26	0.001
	Tmin			
		0 week lag	0.3	<0.001
		1 week lag	0.27	<0.001
		2 week lag	0.32	<0.001
	DW_63			
		0 week lag	**0.38**	**<0.001**
2008	Tmax			
		0 week lag	0.32	<0.001
		1 week lag	0.41	<0.001

		2 week lag	0.28	<0.001			2 week lag	0.32	<0.001
	Tmin					**Tmin**			
		0 week lag	0.35	<0.001			**0 week lag**	**0.54**	**<0.001**
		1 week lag	**0.36**	**<0.001**			1 week lag	0.41	<0.001
		2 week lag	0.27	<0.001			2 week lag	0.32	<0.001
	DW_63					DW_63			
		0 week lag	0.31	<0.001			0 week lag	0.35	<0.001
2003	Tmax				2003	Tmax			
		0 week lag	0.51	<0.001			0 week lag	0.53	<0.001
		1 week lag	0.49	<0.001			1 week lag	0.5	<0.001
		2 week lag	0.47	<0.001			2 week lag	0.47	<0.001
	Tmin					**Tmin**			
		0 week lag	0.54	<0.001			0 week lag	0.43	<0.001
		1 week lag	**0.55**	**<0.001**			**1 week lag**	**0.57**	**<0.001**
		2 week lag	0.46	<0.001			2 week lag	0.48	<0.001
	DW_63					DW_63			
		0 week lag	0.46	<0.001			0 week lag	0.48	<0.001
All Years	Tmax				All Years	Tmax			
		0 week lag	0.32	<0.001			0 week lag	0.34	<0.001
		1 week lag	0.32	<0.001			1 week lag	0.37	<0.001
		2 week lag	0.27	<0.001			2 week lag	0.33	<0.001
	Tmin					**Tmin**			
		0 week lag	0.32	<0.001			**0 week lag**	**0.38**	**<0.001**
		1 week lag	**0.33**	**<0.001**			**1 week lag**	**0.38**	**<0.001**
		2 week lag	0.25	<0.001			2 week lag	0.32	<0.001
	DW_63					DW_63			
		0 week lag	0.32	<0.001			0 week lag	0.33	<0.001

Precipitation

2014	**Pipiens**	r	p	**Total Mosquito**	r	p
	0 week lag	**-0.25**	**0.003**	**0 week lag**	**-0.34**	**<0.001**
	1 week lag	0.22	0.006	1 week lag	0.12	0.15
	2 week lag	0.08	0.342	2 week lag	0.1	0.23

2008

	0 week lag	0.1	0.1	0 week lag	0.03	0.27
	1 week lag	-0.08	0.14	1 week lag	-0.11	0.07
	2 week lag	-0.08	0.16	2 week lag	-0.05	0.38
2003						
	0 week lag	-0.41	<0.001	0 week lag	-0.36	<0.001
	1 week lag	**-0.45**	**<0.001**	**1 week lag**	**-0.43**	**<0.001**
	2 week lag	-0.38	<0.001	2 week lag	-0.38	<0.001
All years						
	0 week lag	**-0.14**	**<0.001**	**0 week lag**	**-0.16**	**<0.001**
	1 week lag	-0.13	0.001	1 week lag	-0.15	<0.001
	2 week lag	-0.11	0.006	2 week lag	-0.08	0.04

All Correlations- 2003, 2008, 2014 Combined