Predictability of West Nile Virus Based on Climate Variables in Washington State

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Abstract

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This study assesses the association between regional, monthly climate variables, including mean, minimum, and maximum temperature and total precipitation, with the following outcome variables: regional, monthly mosquito population size; regional, monthly West Nile Virus (WNV) mosquito maximum likelihood estimates (MLE); and regional, monthly human WNV incidence between 2007-2017. This study used data provided by NASA's Goddard Earth Sciences Data and Information Services Center for climate data and the WA Department of Health for mosquito and human case data. The study plotted linear regressions of all of these associations using univariate analyses for every climate region of WA. Thus, regional, monthly climate was observed against regional, monthly mosquito population; regional, monthly mosquito MLE; and regional, monthly human WNV incidence, where county-level data were used as separate data points. This study found a negative relationship between regional, monthly precipitation and mosquito population, and a positive relationship between regional, monthly temperature and mosquito population. This study did not find any clear associations between regional, monthly climate and mosquito MLE or human WNV incidence. The study found a lack of association between regional, monthly mosquito population size and mosquito MLE, and a positive association between regional, monthly mosquito MLE and human WNV incidence.

Introduction

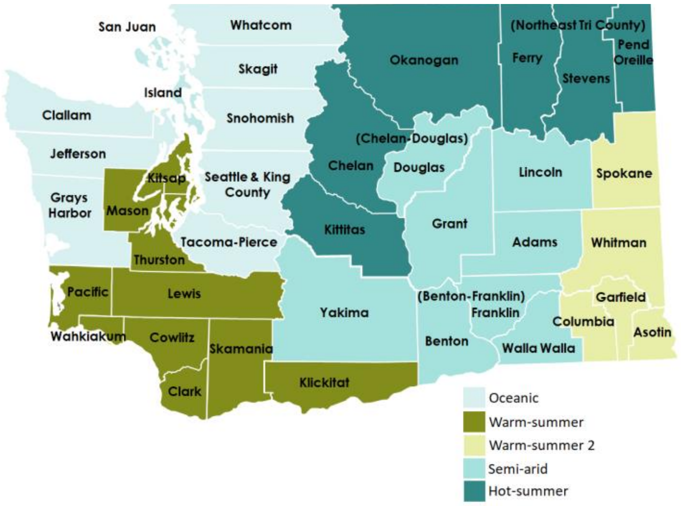
West Nile virus (WNV) first appeared in the United States in 1999 and in Washington (WA) in approximately 2002. The first human case in WA was reported in 2006.[1] Since 2014, the mean human WNV incidence in WA has increased.[4] The virus is transmitted from infected *Culex* mosquitoes *(Cx. pipiens* and *Cx. tarsalis* in in the Pacific Northwest) to birds, horses, and humans. In other areas of the United States, additional species of mosquitoes have been shown to transmit WNV.[2,3] Horses and humans are considered dead-end hosts, meaning that mosquitoes cannot become infected from biting either.[2] While most human cases tend to be asymptomatic, 20-30% develop acute systemic febrile illness, and a small portion of these cases go on to experience neuroinvasive disease like meningitis, encephalitis, or myelitis.[3]

Many studies have attempted to better understand how the virus and/or mosquito are affected by temperature and precipitation changes to help predict particularly dangerous years for transmission. These studies have shown that, in some regions, elevated annual temperatures, above mean winter temperatures, elevated annual precipitation, rural populations, and certain forms of agricultural land use are associated with WNV prevalence.[3,6,7,8] Other studies show that these factors are not significantly associated or are inversely associated with WNV prevalence.[5,7,8] Additionally, these studies show a clear difference in patterns of these associations from one region to another, implying that either some models needs to be validated or diverse regions must establish their own models for WNV.[7] In this regard, few studies have considered WNV in WA, due to the relatively low risk of WNV transmission. With continuous changes in global climate, however, it becomes increasingly important to understand how changes in WA’s climate may influence WNV incidence. This study has attempted to assess the associations between temperature and precipitation in different climate regions of the state and the incidence of WNV in humans and mosquitos.

Methods

Data: This descriptive study used available data from the WA State Department of Health (DOH) Public Health Issue Management System (PHIMS) for human WNV incidence, the Environmental Public Health Department at DOH for mosquito abundance and positive pools, and NASA's Goddard Earth Sciences Data and Information Services Center (*GES DISC*) for 2006-2017 weather station collected climate data in every county of WA (pulled May 2018). Human WNV incidence was defined as a WNV test positive in a human using PCR or IgM testing, both of which indicate acute infection as opposed to pre-existing infection. Human cases were counted in the total incidence if they were classified as probable or confirmed according to the Council for State and Territorial Epidemiologists (CSTE) case definition, or if they tested positive at a blood donation center. Mosquito WNV infection rate was measured using maximum likelihood estimates (MLE), which is based on minimum infection rate (MIR). MIR is calculated as the number of infected mosquitoes per every 1,000 tested ([number of positive pools/total specimens tested] x 1,000). The MIR model assumes that only one mosquito was tested positive for WNV per positive pool, however, and this is often not the case. We avoided this assumption by using a computer program developed by Dr. Brad Biggerstaff, Mathematical Statistician at CDC/DVBD, which calculates MLE.[10] MLE considers the possibility that more than one mosquito per mosquito pool tested positive and compensates for different pool sizes. MLE was calculated for every county, month and WNV-year using county-level data on the total numbers of mosquitos tested and total number of pools tested positive. Temperature metrics for every county, month, and year include the mean, minimum, and maximum monthly temperatures. Precipitation was defined as the total monthly precipitation for every county, month, and year. Thus, all data

Figure 1: Washington State Climate Region Map



Based on a Köppen climate types map created by Adam Peterson, 2016.[11]

for all variables (precipitation, temperature, mosquito population, mosquito MLE, and human WNV incidence) was aggregated on county, month and year.

Table 1: Summary of Regional Climate

Region	Precipitation Mean (inch)	Temperature Mean (F)	Temperature Maximum (F)	Temperature Minimum (F)
Hot Summer	2.498875	44.25468	54.31296	34.22937
Oceanic	4.658669	47.68861	55.19012	40.23178
Semi-arid	1.064985	50.06326	61.33817	38.78104
Warm-summer	6.088623	49.15256	57.94545	40.37978
Warm-summer2	2.074586	47.03613	56.57241	37.62049

Comment: The numbers presented in this table represent the average precipitation and temperature across all counties, months, and years in every region.

Due to the scarcity of data in some counties, this study analyzed relationships by region. To do so, this study plotted each linear regression using data from every county in each region to create regional results. Regions were defined by their Köppen Climate Type which was mapped out in Washington state by Adam Peterson.[11] Regions were divided by county groups as shown in *Figure 1*, and aggregated climate data for every region is summarized in *Table 1*. The study deviated from the Köppen Climate Type map by splitting the Warm-summer regions into two due to some observed differences in precipitation. Standardized mosquito collection and testing methods generated data for 25 of the 35 counties in WA from 2006-2017. Counties with no mosquito data were excluded from the study. Mosquito collection and testing capacity and month of collection differed by county. No mosquitos were collected between November-February of any year. While some were collected in March, April, May, and October, these were most often not tested for WNV. Mosquito records with missing data on collection county, month, or year were excluded.

Human WNV data was available for 117 cases between 2006 and 2017. Eleven human cases without a known state or county of exposure were excluded. These cases resided in primarily the Warm-summer and Semi-arid regions. Seven more cases for whom multiple counties were recorded as county of exposure were re-assigned a county of exposure that was most near their county of residence. Human and mosquito data availability is illustrated in *Figure A1* in the Appendix. Okanogan was the only county for which there was human data but no mosquito data. Fourteen counties had no recorded human cases but reported mosquito data. These counties remained in the study. Mosquito data was not collected in all counties of WA and therefore gaps in mosquito WNV surveillance data exist. Human WNV cases are reportable in every county and surveillance data is considered therefore complete. Climate data was available for almost all counties and months between 2006 and 2017.

Since WNV incidence occurs most frequently from June-October, this study has assumed November of the previous year to October of the year under investigation as a WNV-year using data from November 2006-October 2017. Human WNV incidence was aggregated by county, month, and WNV-year of symptom onset. Mosquito population size was calculated by summing all of the collected mosquitos in every county, month, and year. These calculation were not standardized, however, to correct for the differences in collection capacity between counties. Mosquito MLE was calculated using total number of mosquitos tested and number of pools tested positive for WNV in a given county, month, and WNV-year. Mosquito species (*Cx. pipiens* vs *Cx. tarsalis*) was also considered as a potential covariate in analysis while *a priori* confounders such as county, month and year were used to stratify data.

Analysis: The study examined each association for every region individually, using only the county-level data that pertained to each region. This was done to create enough data points for each linear regression of every association. Likewise, combining counties into regional groups enables the study to assess these associations among counties of similar climate. For every region in which data was available, this study has first attempted to examine the association of regional, monthly climate with mosquito population size. The study then examined the association of regional, monthly climate, along with mosquito population size, with regional, monthly mosquito WNV MLE. Lastly, the study attempted to examine the association of regional, monthly climate, along with mosquito population size and mosquito WNV MLE, with regional, monthly human WNV incidence.

Ultimately, this study created univariate linear regressions to compare each of the aforementioned associations using data on every available month and year in every county as individual data points for every region. *Figure A2* is a directed acyclic graph (DAG) of the causal pathways under investigation. Analysis was conducted using univariate linear regression with log transformed outcome variables (regional, monthly mosquito population, regional, monthly mosquito MLE, and regional, monthly human WNV incidence).

Therefore, the estimates produced in this study indicate a percentage change in the outcome variable as the exposure variables (regional, monthly total precipitation, regional, monthly mean, minimum, and maximum temperatures, regional, monthly mosquito population size, and regional, monthly mosquito WNV MLE) increase by one unit (degree temperature, inch precipitation, thousand mosquitos, or one mosquito per thousand, respectively). The final data was analyzed using R 1.1.456. Washington State IRB reviewed this study as exempt using a limited dataset prior to the start of the project.

<u>Results</u>: Results presented here are those for which there was enough data to generate a linear regression between the variables noted. Estimates generated from a combination of all available data*, meaning unstratified data for all counties, months, and years, are presented first. Estimates generated from data for mosquito species are presented second. Estimates generated from data for each of the five regions is presented last.

<u>Monthly Mosquito Population:</u> In general, *Cx. tarsalis* was collected in higher numbers than *Cx. pipiens*, and tested positive more often. This may have been due to biased collecting, testing, or due to a higher abundance of this mosquito. In every region, monthly mosquito population size was negatively associated with monthly precipitation (*Table 2*). When using all available data*, there were roughly 33 percent less mosquitos collected every month for every inch increase in total monthly precipitation. *Cx. tarsalis* generated a more negative association between regional, monthly precipitation and regional, monthly mosquito population. The Semi-arid and Warm-summer2 regions generated more negative associations between monthly precipitation and monthly mosquito population in comparison to the other regions, but Warm-summer2 did not generate a statistically significant association.

In every region, monthly mosquito population size was positively associated with monthly mean, minimum, maximum temperature (*Table 2*). When using all available data*, there were roughly 12, 14, and 10 percent more mosquitos collected for every degree increase in total monthly mean, minimum, and maximum temperature, respectively. *Cx. tarsalis* generated a more positive association between regional, monthly temperature and regional, monthly mosquito population. Comparing the regions, the Warm-summer region monthly mosquito population was least associated with monthly mean, minimum, and maximum temperature. The Oceanic region monthly mosquito population was most associated with monthly mean and maximum temperature, while the Hot-summer region monthly mosquito population was most associated with monthly minimum temperature. The Warm-summer2 region did not generate statistically significant results in any association.

Table 2: Regional Association between Total Mosquitos Collected and:

Mean Precipitation (inch)				**Mean Temperature (F)**			
	All Counties, Months, and Years				**All Counties, Months, and Years**		
	Slope Estimate	**R-squared**	**P-value**		**Slope Estimate**	**R-squared**	**P-value**
	0.6685227	0.09267	2.20E-16		1.122364	0.1953	2.20E-16
By Species		**By Species**					
Tarsalis	0.5603465	0.1489	2.20E-16	Tarsalis	1.14637	0.2283	2.20E-16
Pipiens	0.7492744	0.04506	2.32E-07	Pipiens	1.098516	0.1166	2.20E-16
	By Climate Region and Month				**By Climate Region and Month**		
Climate Region	**Slope Estimate**	**R-squared**	**P-value**	**Climate Region**	**Slope Estimate**	**R-squared**	**P-value**
Hot-summer	*0.988899105*	*6.7042E-05*	*0.93425483*	Hot-summer	1.173701792	0.149794022	4.93641E-05
Oceanic	0.818914208	0.021907294	0.043217805	Oceanic	1.21641283	0.161981443	1.12904E-08
Semi-arid	0.316234975	0.115350633	1.5646E-14	Semi-arid	1.133602202	0.275783007	1.16397E-35
Warm-summer	0.837032737	0.056944993	7.19831E-06	Warm-summer	1.088095456	0.068726187	1.0208E-06
Warm-summer2	*0.509047681*	*0.05223838*	*0.22443269*	*Warm-summer2*	*1.078381688*	*0.06144252*	*0.18659959*

Minimum Temperature (F)				**Maximum Temperature (F)**			
	All Counties, Months, and Years				**All Counties, Months, and Years**		
	Slope Estimate	**R-squared**	**P-value**		**Slope Estimate**	**R-squared**	**P-value**
	1.141487	0.1658	2.20E-16		1.098106	0.1992	2.20E-16
By Species				**By Species**			
Tarsalis	1.156132	0.1676	2.20E-16	Tarsalis	1.122455	0.2523	2.20E-16
Pipiens	1.118737	0.1063	1.39E-15	Pipiens	1.077312	0.1141	2.20E-16
	By Climate Region and Month				**By Climate Region and Month**		
Climate Region	**Slope Estimate**	**R-squared**	**P-value**	**Climate Region**	**Slope Estimate**	**R-squared**	**P-value**
Hot-summer	1.250224652	0.195045342	2.69176E-06	Hot-summer	1.088191925	0.081071766	0.00339512
Oceanic	1.239213745	0.132910567	2.90317E-07	Oceanic	1.175993622	0.169160137	4.99142E-09
Semi-arid	1.159545247	0.287700678	2.09024E-37	Semi-arid	1.109062145	0.254652634	1.23903E-32
Warm-summer	1.123276533	0.083779257	6.02323E-08	Warm-summer	1.06066908	0.053286414	1.81739E-05
Warm-summer2	*1.073883582*	*0.04045459*	*0.28652273*	*Warm-summer2*	*1.062394969*	*0.06453974*	*0.17551515*

Results shown in green indicate a positive associateion between the variables. Results shown in red indicate a negative association between the variables. *Reulsts italizised indicate a p-value greater then 0.05, implying statistical insignificanse.*

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*Meaning unstratified data for all counties, months, and years.

<u>Monthly Mosquito MLE</u>: When using all available data*, regional, monthly mosquito MLE was negatively associated with regional, monthly precipitation (*Table 3*). For every inch increase in total monthly precipitation, there were roughly 28 percent less mosquitos found to be infected with WNV (per every thousand tested). Both *Cx. tarsalis* and *Cx. pipiens* generated statistically insignificant associations between regional, monthly precipitation and regional, monthly mosquito MLE. The Hot-summer region had too little data to generate a significant association, as is indicated by the R-squared statistic of 1. The Semi-arid region did not generate a statistically significant association.

When using all available data*, regional, monthly mosquito MLE was positively associated with regional, monthly mean, minimum, and maximum temperature (*Table 3*). For every degree increase in total monthly mean, minimum, and maximum temperature, there were roughly 1, 2, and 1 percent more mosquitos found infected with WNV (per every thousand tested), respectively. Both *Cx. tarsalis* and *Cx. pipiens* generated statistically insignificant associations between regional, monthly mean, minimum, and maximum temperature and regional, monthly mosquito MLE. The Hot-summer region had too little data to generate any significant associations, as is indicated by the R-squared statistic of 1. The Semi-arid region did not generate any statistically significant associations between regional, monthly mean, minimum, and maximum temperature and regional, monthly MLE.

When using all available data*, regional monthly mosquito MLE was not associated with regional, monthly mosquito population. Assessing the *Cx. tarsalis* and *Cx. pipiens* mosquitos separately did not generate statistically significant results. While both the Hot-summer and Semi-arid regions generated a similar relationship as did all available data*, the Hot-summer region had too little data to generate a significant association, as is indicated by the R-squared statistic of 1.

Table 3: Regional Association between Maximum Likelihood Estimate (MLE) in Mosquitos and:							
Mean Precipitation (inch)				**Mean Temperature (F)**			
	All Counties, Months, and Years				**All Counties, Months, and Years**		
	Slope Estimate	**R-squared**	**P-value**		**Slope Estimate**	**R-squared**	**P-value**
	0.7239015	*0.005403*	*0.1619*		*1.015306*	*0.0009378*	*0.2813*
By Species				**By Species**			
Tarsalis	*0.6030833*	*0.005258*	*0.2458*	*Tarsalis*	*1.036905*	*0.02116*	*0.1175*
Pipiens	*0.399237*	*0.03768*	*0.05991*	*Pipiens*	*1.036355*	*0.02055*	*0.124*
	By Climate Region and Month				**By Climate Region and Month**		
Climate Region	**Slope Estimate**	**R-squared**	**P-value**	**Climate Region**	**Slope Estimate**	**R-squared**	**P-value**
Hot-summer	1.545662532	1	2.53943E-16	Hot-summer	1.206814955	1	6.51787E-15
Semi-arid	*0.681444303*	*0.01550006*	*0.10267607*	*Semi-arid*	*1.017482587*	*0.00893749*	*0.21600716*
Minimum Temperature (F)				**Maximum Temperature (F)**			
	All Counties, Months, and Years				**All Counties, Months, and Years**		
	Slope Estimate	**R-squared**	**P-value**		**Slope Estimate**	**R-squared**	**P-value**
	1.018082	*0.001537*	*0.2603*		*1.012356*	*0.005621*	*0.3172*
By Species				**By Species**			
Tarsalis	*1.033768*	*0.01041*	*0.1919*	*Tarsalis*	*1.035299*	*0.02739*	*0.08924*
Pipiens	*1.034088*	*0.01113*	*0.1885*	*Pipiens*	*1.034243*	*0.02547*	*0.1003*
	By Climate Region and Month				**By Climate Region and Month**		
Climate Region	**Slope Estimate**	**R-squared**	**P-value**	**Climate Region**	**Slope Estimate**	**R-squared**	**P-value**
Hot-summer	0.487902575	1	2.44632E-14	Hot-summer	1.091247883	1	3.3859E-15
Semi-arid	*1.020631057*	*0.00977724*	*0.195559*	*Semi-arid*	*1.01410987*	*0.00766312*	*0.25210494*
Mosquito Population							
	All Counties, Months, and Years						
	Slope Estimate	**R-squared**	**P-value**				
	0.9999511	0.04477	0.002521				
By Species							
Tarsalis	*0.9999564*	*0.009848*	*0.1971*				
Pipiens	*0.9999223*	*0.03388*	*0.07025*				
	By Climate Region and Month						
Climate Region	**Slope Estimate**	**R-squared**	**P-value**				
Hot-summer	0.9992337	1	5.92533E-16				
Semi-arid	0.999959236	0.035546982	0.012984758				
Results shown in green indicate a positive associateion between the variables. Results shown in red indicate a negative association between the variables. *Reulsts italisized indicate a p-value greater then 0.05, implying statistical insignificanse.*							

<u>Monthly Human WNV Incidence:</u> When using all available data*, regional, monthly human WNV incidence was negatively associated with regional, monthly precipitation, although this association was not statistically significant (*Table 4*). Both the Semi-arid and Warm-summer2 regions generated statistically

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*Meaning unstratified data for all counties, months, and years.

insignificant associations between regional, monthly precipitation and regional, monthly human WNV incidence.

When using all available data*, regional, monthly human WNV incidence was positively associated with regional, monthly mean, minimum, and maximum temperature, although these associations were not statistically significant. The Semi-arid region generated a positive association between regional, monthly mean, minimum, and maximum temperature and regional, monthly human WNV incidence, while the Warm-summer2 region generated no association between regional, monthly mean, minimum, and maximum temperature and regional, monthly human WNV incidence. However, both of these regions generated statistically insignificant associations.

When using all available data*, regional, monthly human WNV incidence was not observed to be associated with regional, monthly mosquito population, although all of the associations (all data, by mosquito species, and by region) were not statistically significant. When using all available data*, regional, monthly human WNV incidence was positively associated with regional, monthly mosquito MLE. For every additional 1 in 1,000 mosquitos that tested positive for WNV, there was 1 percent more human cases reported. While the association between regional, monthly human WNV incidence and regional, monthly mosquito MLE was not significant among *Cx. tarsalis* mosquitos, this association was significantly positive among *Cx. pipiens* mosquitos (5 percent more human WNV cases were reported with every additional 1 in 1,000 *Cx. pipiens* that was tested positive for WNV). The Semi-arid region shared this positive association between regional, monthly mosquito MLE and regional, monthly human WNV incidence. The Warm-summer2 regional generated a negative association between regional, monthly mosquito MLE and regional, monthly human WNV incidence.

Table 4: Regional Association between Human WNV Incidence and:

Mean Precipitation (inch)				**Mean Temperature (F)**			
	All Counties, Months, and Years				**All Counties, Months, and Years**		
	Slope Estimate	**R-squared**	**P-value**		**Slope Estimate**	**R-squared**	**P-value**
	0.8896119	*0.00119*	*0.2985*		*1.013896*	*0.01025*	*0.1802*
	By Climate Region and Month				**By Climate Region and Month**		
Climate Region	**Slope Estimate**	**R-squared**	**P-value**	**Climate Region**	**Slope Estimate**	**R-squared**	**P-value**
Semi-arid	*1.398988101*	*0.01246999*	*0.40397226*	*Semi-arid*	*1.008732604*	*0.00845645*	*0.49236631*
Warm-summer2	*1.356104445*	*0.09534524*	*0.38534422*	*Warm-summer2*	*0.985294805*	*0.02667448*	*0.65210887*
Minimum Temperature (F)				**Maximum Temperature (F)**			
	All Counties, Months, and Years				**All Counties, Months, and Years**		
	Slope Estimate	**R-squared**	**P-value**		**Slope Estimate**	**R-squared**	**P-value**
	1.015976	*0.009933*	*0.1832*		*1.011415*	*0.008886*	*0.1938*
	By Climate Region and Month				**By Climate Region and Month**		
Climate Region	**Slope Estimate**	**R-squared**	**P-value**	**Climate Region**	**Slope Estimate**	**R-squared**	**P-value**
Semi-arid	*1.013570095*	*0.01563577*	*0.34966105*	*Semi-arid*	*1.005430263*	*0.00427843*	*0.625674*
Warm-summer2	*0.990805644*	*0.00627596*	*0.82778763*	*Warm-summer2*	*0.984784692*	*0.04366553*	*0.56233576*
Mosquito Population				**MLE**			
	All Counties, Months, and Years				**All Counties, Months, and Years**		
	Slope Estimate	**R-squared**	**P-value**		**Slope Estimate**	**R-squared**	**P-value**
	1.000009	*0.003641*	*0.625*		1.06717	0.1554	0.0006211
By Species				**By Species**			
Tarsalis	*0.9999825*	*0.007149*	*0.6292*	*Tarsalis*	*1.048961*	*0.08259*	*0.05489*
Pipiens	*1.000051*	*0.0126*	*0.2444*	Pipiens	1.058561	0.1245	0.02701
	By Climate Region and Month				**By Climate Region and Month**		
Climate Region	**Slope Estimate**	**R-squared**	**P-value**	**Climate Region**	**Slope Estimate**	**R-squared**	**P-value**
Semi-arid	*0.999996561*	*0.00057199*	*0.8650213*	Semi-arid	1.070775055	0.180567639	0.001516148
Warm-summer2	0.995196964	0.948500493	0.005038956	Warm-summer2	0.751798591	0.934585773	0.00724508

Results shown in green indicate a positive associateion between the variables. Results shown in red indicate a negative association between the variables. *Reulsts italisized indicate a p-value greater then 0.05, implying statistical insignificanse.*

Limitations

This study has attempted to assess the relationship between many variables for which data were often limited. As such, spurious associations may have been discovered in this study that are the product of too few observations rather than true associations. First, there were very few human WNV cases in WA between the 2007-2017 WNV-years. This reduces the power of the study to find statistically significant associations with human WNV incidence. Mosquito data were also limited to 25 of WA's 39 counties because not all counties collected mosquitoes for testing. Additionally, regional results were obtained using county-level data. As some county divisions do not directly correspond with climate region divisions, the regions used in this study may not represent the actual climate regions that reflect the ecology of WNV mosquito species.

*Meaning unstratified data for all counties, months, and years.

Second, counties that collected and tested mosquitoes often did so inconsistently, implying that testing may have been biased to certain months or regions. This limitation impacts measures of mosquito population size, as reported population size is directly related to number of trap-nights in every county. The mosquito population size modeled here is likely greatly biased by the variation of trap-nights across counties. Mosquito data was sometimes recorded inexactly, as well. In some cases, mosquito pools were collected with a tested pool size recorded but no testing complete, while in other cases a pool size recorded as zero included a test result. Such information was removed before MLE calculations were made, but such inconsistency in data alludes to other potential data inaccuracies gone unnoticed. Some MLE calculations were also unattainable because the only one pool was tested for a given county, month, and year, and as a rate, MLE needs multiple pools collected in the same county, month, and year to generate an estimate. Thus, some county, month, years may have had confirmed WNV but no MLE scores to use in regression analyses. Climate data was robust except for Wahkiakum County, which had sparse climate information, and Kitsap County, which had two missing temperature variables for August 2008. As with mosquitoes, climate data collection sites, collection methods, and collection months were not entirely consistent between the 2007-2017 WNV-years.

Lastly, this study also had limitations in its design in that WNV incidence could be impacted by data not collected for this study, such as data on bird populations or human behavior. For example, bird populations play an integral role in the WNV transmission cycle. It is possible that migration patterns and mating seasons with some bird populations may affect the mosquito WNV MLE and human WNV incidence. Likewise, human behavior patterns may affect the likelihood that humans expose themselves to WNV environments such as lakes, orchards, farms, and forests. Therefore, associations between precipitation or temperature variables and mosquito WNV MLE or human WNV incidence may be more attributable to other variables, and accounting for such confounders may contribute to generating more significant results. This study also did not consider how climatic conditions in previous months may have influenced the outcomes observed. Thus, monthly data aggregation may have impacted the ability to observe genuine associations present between the variables analyzed.

Discussion

While some significant associations were observed between regional, monthly climate and regional, monthly mosquito population size, regional, monthly mosquito MLE, and regional, monthly human WNV incidence, in most cases insufficient data were available to generate significant associations. Across all data except that of the Warm-summer2 region, regional monthly mosquito population size was negatively associated with regional, monthly precipitation and positively associated with regional, monthly mean, minimum, and maximum temperature. This means that more mosquitoes are observed across most of WA when conditions are drier and warmer. This may be because either the hot, dry summers in the majority of WA counties supports the growth of mosquito populations, or that lower monthly precipitation is associated with higher monthly mosquito populations because higher amounts of precipitation may wash away mosquito eggs. Regional differences in these associations may occur because of differences in mosquito habitat preference or collection.

Across all data, no statistically significant associations were found between regional monthly mosquito MLE and regional, monthly precipitation and mean, minimum, and maximum temperature. This was due to the limited amount of MLE data available across WA. The Semi-arid region collected and tested mosquitos the most thoroughly, and thus most MLE estimates were generated for this region. While other regions may have collected and tested mosquitoes, they did so infrequently and sparsely. Thus, no other region had enough MLE estimates to generate significant linear regressions. That said, the MLEs generated for the Semi-arid region were not enough to create statistically significant associations between regional, monthly climate and regional, monthly mosquito MLE. Thus, more mosquito collection may be necessary to observe the association between WA climate and WNV incidence.

Across all regions, no statistically significant associations were found between monthly precipitation and mean, minimum, and maximum temperature and monthly human WNV incidence. While data was available for all regions and months, the limited number of human WNV cases was insufficient to create statistically significant associations between climate and human WNV incidence. The lack of any clear associations could also be because the data aggregation by region and month may have affected the power of the analyses. Nevertheless, it is important to note that climate variables may influence human behavior, such as likelihood to go outdoors, which subsequently influences WNV risk. As such, future studies may consider incorporating variables on human behavior, such as outdoor activity, to better examine the association between climate and human WNV incidence.

Regional monthly mosquito population size was found to not be associated with regional, monthly mosquito MLE nor regional, monthly human WNV incidence. This observation was based on data from the Semi-arid and Warm-summer2 regions. It is possible that regional, monthly mosquito MLE and human WNV incidence are in fact unaffected by mosquito population size. This may be because WNV in mosquitos and humans is attributable to variables affecting the actual virus rather than the mosquito, or because of other aspects of WNV ecology and/or transmission dynamics that this study did not measure. Thus, mosquito population growth alone may not stimulate WNV replication or transmission. If this correlation is genuine, the first set of analyses testing associations between regional, monthly climate variables and total monthly mosquito population may insufficient to predict WNV infection, as they would have little to do with WNV activity in and of themselves. However, it is also possible that these associations are inaccurate due to the limited data on WNV incidence in WA. It is also possible that the division of data by region, month, or year has blurred or distorted any associations that may be otherwise present using the data from this study.

Unsurprisingly, the association between regional, monthly mosquito MLE and regional, monthly human WNV incidence was observed to be positive when using all available data*, although most of the data came from the Semi-arid region. *Cx. pipiens* had a more significant association between mosquito MLE and human WNV incidence than did *Cx. tarsalis.* Since *Cx. tarsalis* was tested positive for WNV more often, it is unlikely that *Cx. pipiens* is the preferred host for WNV in WA. Therefore, the significantly positive association between regional, monthly MLE and human WNV incidence in *Cx. pipiens* may be because this species comes into contact with humans more often than *Cx, tarsalis.* There may also be other regional differences that result in more human WNV cases in the Semi-arid region, where more *Cx. pipiens* may reside in higher numbers. The Warm-summer2 region generated a negative association between regional, monthly MLE and regional, monthly human WNV incidence. As mentioned previously, this study did not include data on human behavior. Therefore, some regions may observe difference in human behavior that may make their populations more or less susceptible to WNV when mosquito WNV MLE increases. In this case, we may find that when more mosquitos are tested positive for WNV, in some regions human are discouraged from participating in activities that may expose themselves to mosquitos, which would generate a negative association. On the other hand, it is also possible that when humans are found infected with WNV, more mosquitos are collected and tested for WNV, which would generate a positive association. Like with all data, results may have also been affected by any misclassification of human WNV county of exposure.

Overall, while associations observed within regions for climate variables may hold true with more robust data, because of the methodological limitations of this study definitive conclusions are not possible.

Conclusion

Improved data collection and completeness and further research are needed to understand the relationship between climate and WNV incidence in WA. It is possible that the division of data by region, month, or year in this study has blurred or distorted any associations that may be otherwise present using the data from this study. Likewise, variables not incorporated in this study may be play into the associations between climate patterns and WNV in mosquitos and humans in WA. This study suggested that there may be a lack of association between regional, monthly mosquito population and WNV incidence in mosquitos; however, this hypothesis should be further assessed. The study has also suggested that WNV incidence in mosquitoes correlates with WNV incidence in humans. This is biologically plausible, although it may possibly be due to biased testing of mosquitos (e.g., testing mosquitos after a human cases are reported). This study may encourage public health agencies to enhance mosquito collection and WNV testing in order to better model WNV occurrence in mosquitos and humans in WA state. Ultimately, this study may be improved upon if mosquito collection becomes more routine across all counties and months, and if variables about bird population and human behavior are included in future analyses.

*Meaning unstratified data for all counties, months, and years.

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Appendix

Figure A1: Availability of Data across Washington State

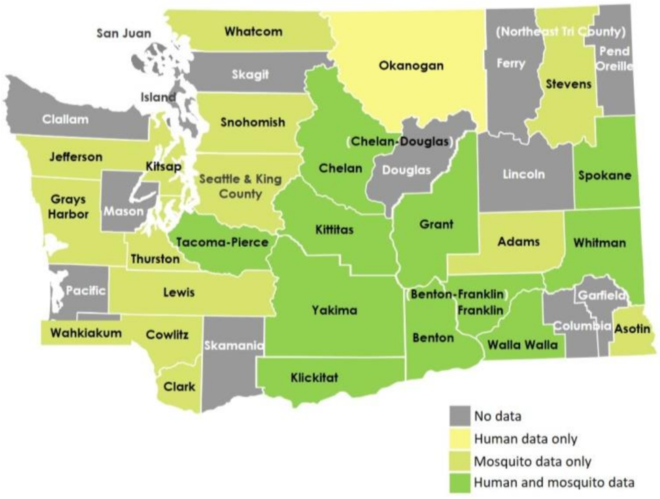

Figure A2: Causal Pathways under Investigation