Determining the effects of meteorological conditions on West Nile virus seasons in Washington State

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Abstract

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West Nile virus (WNV) is a global threat to public health. *Culex pipiens* and *Culex tarsalis,* the WNV vectors found in Washington state (WA), activity has been linked to environmental variables in previous research across the world but little has been done in WA. Understanding how environmental factors affect WNV vectors may help guide public health efforts to stem its spread. We examined mosquito and climate data over 10 years (2008-2017) to examine how temperature and precipitation, during multiple periods within each year, affect *C. pipiens* and *C. tarsalis* in four counties in South-Central WA and an aggregate of the counties. We used multivariate linear regression models to explore the relationships between temperature, precipitation and WNV seasons. We further examined the relationship between a WNV season's length and weekly mosquito infection rates. Results differed between counties, but warmer temperatures in the calendar month prior to the start of a WNV season appeared to be strongly linked to delayed season start-times and shorter season durations. Longer seasons were also associated with higher mean-weekly mosquito infection rates. Our research may provide insight into how local environmental conditions influence WNV seasons and how seasons affect mosquito infection rates.

INTRODUCTION

Arthropod-borne viruses (arboviruses) cause substantial morbidity and mortality to humans and other animals. Arboviruses are spread when arthropods blood-feed on infected vertebrate hosts and then pass the virus on to humans or other mammals[1]. Examples of arboviruses include dengue fever, yellow fever, Japanese encephalitis, and West Nile Virus (WNV)[2], and each one is responsible for multiple epidemics around the world. Among the arboviral diseases, WNV is the most globally widespread[1] as it exists on every continent except Antarctica.

Since its introduction to the United States (US) in 1999 in New York, over 48,000 people have been infected with WNV nationally (as of 2017), and the virus has been reported in almost every state in the continental US[3]. Between 1999 and 2017 almost 23,000 individuals developed the more serious neuroinvasive form of WNV, and around nine percent of those that developed the neuroinvasive form died[3]. Washington state (WA) experienced its first human WNV case in 2006, and since that time (through 2017), there have been 109 total human cases of WNV in WA, with over half of those progressing to the neuroinvasive form[4]. Unfortunately, there is neither a human vaccine nor any specific treatment for WNV[5] infections. The only methods currently available to combat WNV infection are vector control and public education to prevent exposure to WNV vectors[6].

Vector-borne diseases, like WNV, are particularly susceptible to variations in climate. The United Nations' Intergovernmental Panel on Climate Change (IPCC) lists vector-borne diseases as one of the top issues most likely to be influenced by climate change[7]. As such, temperature and precipitation have been studied in relation to WNV and other vector-borne diseases in many areas around the world[1,6-12]. Temperature is known to affect the rate of development, longevity, oviposition, and blood feeding habits of mosquito vectors. Studies show that as temperatures warm (between ~60ºF and 86ºF) *Culex* mosquitos (WNV vectors) experience higher growth rates, shorter intervals between feeding times, and increased viral replication[8,9]. Shorter viral development periods have been linked to higher probabilities of human WNV infection[10]. However, daily average temperatures above ~86ºF have also been shown to decrease *Culex* population survival proportions and longevity[6,8,9]. Precipitation has been shown to affect *Culex* mosquitoes, although the effects are not consistent between species. In Northeast Illinois, *Culex pipiens* populations have been found to decrease following heavy rainfall periods while *Culex tarsalis* populations tended to thrive[9]. Additionally, a study in Italy found that warmer temperatures earlier in the year were associated with earlier and longer WNV seasons for *C. pipiens*, while precipitation earlier in the year was associated with later and shorter WNV seasons for *C. pipiens*[6]. In California, the hot-bed of WNV activity in the US, warmer winters were associated with earlier WNV activity, higher mosquito infection rates, and subsequently higher numbers of human infections[11,12].

Though the total number of human WNV cases in WA is lower than that reported in other states (WA ranks 41 out of 49 among states reporting at least one WNV case[3]), it is nonetheless a concerning public health problem. Until recently, human in-state acquired cases have been limited to geographic areas that lie east of the Cascades (i.e., Eastern WA). However, the WNV vectors in WA (*C. pipiens* and *C. tarsalis*) both reside in Western WA, where most of the state's population also resides. What's more, in 2018 the first human locally-acquired WNV case was reported in King County and the first WNV-positive mosquitos were detected in Pierce County, both counties in Western WA[4]. Whether this is due to changes in the climate has yet to be determined. Still, if WNV became endemic to Western WA, over five million people could be at risk of infection every year. It is imperative that we expand our knowledge on how climate changes may have an effect on WNV, especially as it begins to appear in new areas within the state. While numerous studies have examined environmental factors in an attempt to predict the spread of WNV to humans, few have examined this specifically in WA. Further, there have been no studies, to our knowledge, that have examined the association between environmental factors and WNV seasons in WA.

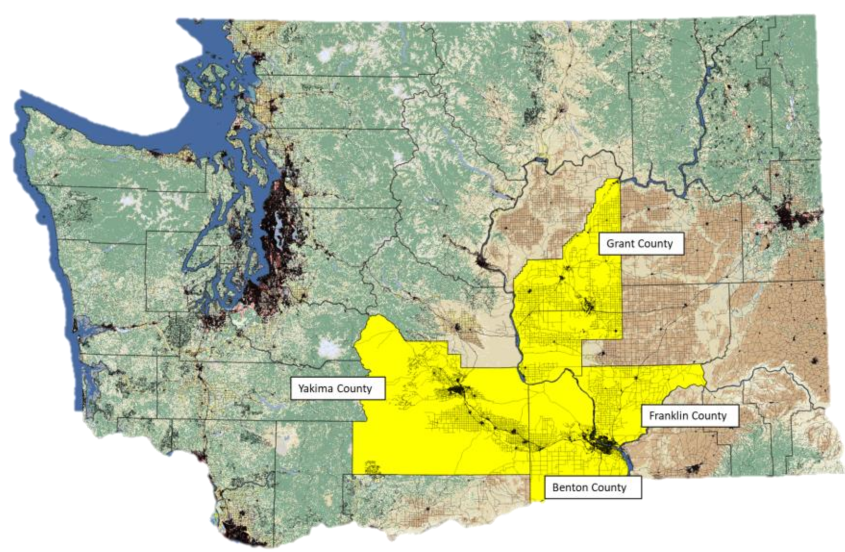
The objective of this analysis was to determine how different temperature and precipitation factors affected the two WNV mosquito vectors (*C. pipiens* and *C. tarsalis)* in eastern WA. Our work is intended to spur research on the impacts of local environmental conditions on WNV to inform the State's public health response to WNV, including preparations for potential spread of WNV into Western WA. Specifically, we aimed to: (1) determine if temperature or precipitation factors are associated with WNV season start; (2) determine if temperature or precipitation factors are associated with WNV season duration; and (3) determine if WNV season duration was associated with mosquito infection rates, using mean weekly maximum likelihood estimates (MLE), for each mosquito species.

METHODS

Study Design

We conducted an exploratory study to examine how temperature and precipitation affect the start and duration of WNV seasons for *C. pipiens* and *C. tarsalis* between 2008 and 2017 in four WA counties: Benton, Franklin, Grant, and Yakima (Figure 1). We also explored the association between a WNV season's duration and mosquito infection rates in each of the four counties. We chose these four counties because they have consistent and complete mosquito trapping reports within the period of this study, and have all reported a WNV-positive mosquito pool during the study period. Only data collected from weather stations or mosquito trapping locations within the geographic boundaries of each county were included in the study. Year was defined as January 1 to December 31 using the Gregorian Calendar system.

Figure 1. Washington State county map



Exposure and Outcome

The variables used in our study were derived from results of previous research examining relationships between temperature and precipitation measured during multiple periods within a year and WNV season start/length or WNV-vector activity[6,8-10]. We examined two exposures for Aims 1 and 2: temperature and precipitation (both continuous variables). Temperature was defined in three ways: 1) *degree days*, the sum of the difference between maximum temperature and 60ºF, if maximum temperature was equal-to or above 60ºF, for each day between March and April of each year and county; 2) *lag-month temperature*, the mean monthly temperature (ºF) based on mean daily temperature of the month prior to the first WNV-positive mosquito detected, per year; and 3) *start-month temperature*, the mean monthly temperature (ºF) based on mean daily temperature of the month of the first WNV-positive mosquito detected, per year. We also defined precipitation in three ways: 1) *mean total daily precipitation*, the mean of the total daily rainfall (inches) for each day between May and June of each year, 2) *lag-month precipitation*, the mean of the total daily rainfall (inches) for each day of the month prior to the first WNV-positive mosquito detected, per year, and 3) *start-month precipitation*, the mean of the total daily rainfall (inches) for each day of the month of the first WNV-positive mosquito detected, per year. The exposure variable for Aim 3 was WNV *season duration*, defined as the total number of days from the first detected WNV-positive mosquito to the last detected WNV-positive mosquito (aggregated species). Each row in the dataset represented a single year for a single county (and an aggregated county row), with the corresponding temperature, precipitation data, and WNV season duration.

The outcomes for Aim 1 and 2 were *start of the WNV season* and *WNV season duration*, respectively. The start of a WNV season was defined as the number of days in a calendar year between January 1 and the first WNV-positive mosquito detected, by county and species. Season duration is defined above in the exposure variable for Aim 3. The outcome for Aim 3 was mosquito infection rate (mean weekly MLE) of mosquitos infected with WNV. Mosquito infection rate was defined by aggregating the mean MLE per week over the duration of a WNV season for each year, county, and a county aggregate. We used an MLE calculation tool developed by Dr. Brad Biggerstaff at the CDC to determine mosquito infection rates that avoids the assumption of one positive mosquito per pool tested[13]. MLE was calculated per county and by an aggregate of the counties, per season, and incorporated all WNV-tested mosquito pools per season (*C. pipiens* and *C. tarsalis* were combined for MLE calculations).

Data Collection

Temperature and precipitation data were obtained from Oregon State University's PRISM Climate Group[14]. PRISM uses a combination of data from ground-based weather stations and a climate-elevation regression method to accurately model local climates in the US. Temperature and precipitation values were obtained from PRISM using inverse-distance squared weighting methods for each county. Mosquito infection data was obtained from WA State Department of Health's (DOH) Environmental Public Health department. The data included all mosquitos trapped and tested for WNV, using either the Rapid Analyte Measurement Platform (RAMP®, Response Biomedical Corp., Burnaby, Canada), or reverse transcription-polymerase chain reaction (RT-PCR) testing.

Data Analysis

Association of Temperature and Precipitation with WNV Season Start (Aim 1) and Duration (Aim 2)

Temperature and precipitation were assumed to be confounders of one another, so we used multiple linear regression to examine the association between our exposures and outcomes. We analyzed separate models for each county (and for the counties in aggregate) and mosquito species for Aims 1 and 2. Each model included the Aim-specific outcome (season start day for Aim 1 and season duration for Aim 2) along with one temperature and one precipitation variable. To identify which temperature and precipitation variable to include in our multivariate models, we used simple linear regression to examine the association between each of three temperature and precipitation variable (defined above) and our outcomes. The temperature and precipitation variables with the lowest P-value under the F-test (at $\alpha = 0.05$ level) in simple linear regression was included in the final multivariable linear regression model. All regression models were complete case analyses (i.e., years that were missing data for variables were dropped from the models). The model equations, for each county and aggregate, were as follows:

Aims 1 and 2: *C. pipiens*

$Y_{Start} = \beta_0 + \beta_1 X_{Temperature} + \beta_2 X_{Precipitation}$

$Y_{Duration} = \beta_0 + \beta_1 X_{Temperature} + \beta_2 X_{Precipitation}$

Aims 1 and 2: *C. tarsalis*

$Y_{Start} = \beta_0 + \beta_1 X_{Temperature} + \beta_2 X_{Precipitation}$

$Y_{Duration} = \beta_0 + \beta_1 X_{Temperature} + \beta_2 X_{Precipitation}$

Association of WNV Season Duration with Mean Weekly MLE (Aim 3)

We used simple linear regression to examine the association between WNV season duration and weekly MLE, stratified by county. Mosquito species were aggregated when creating the season duration variable due to several years of missing data for one or both species within the counties. The model equations, for each county and aggregate, were as follows:

Aim 3

$Y_{MLE} = \beta_0 + \beta_1 X_{Season\ Duration}$

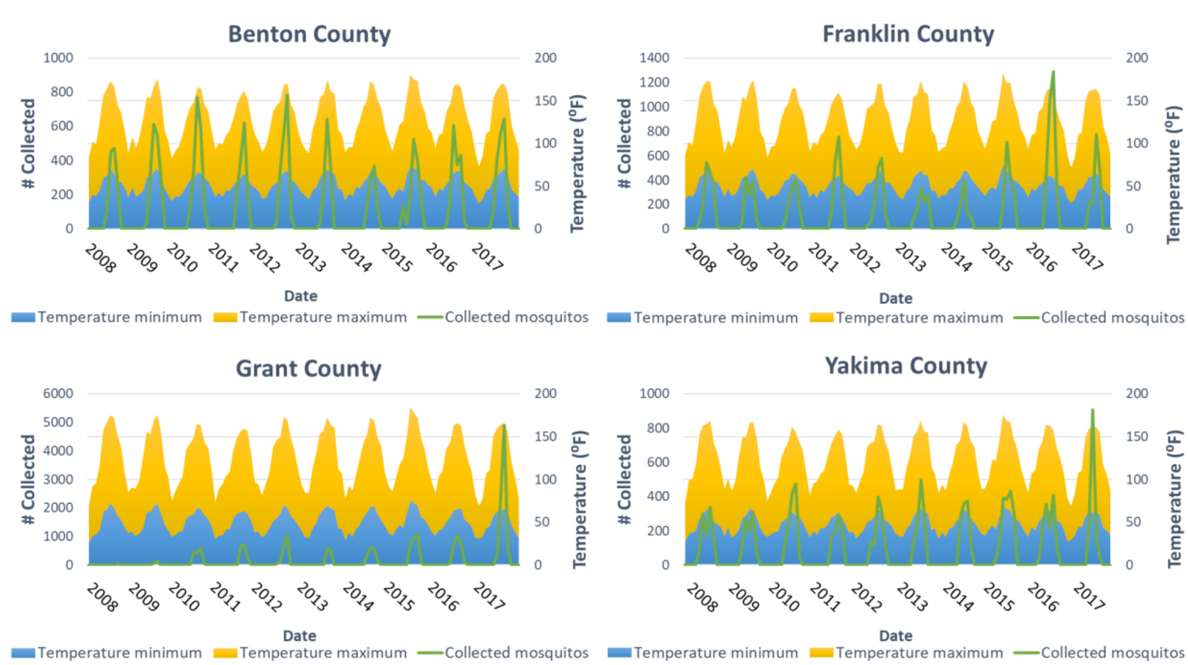
For each regression model, we report the slope (i.e., parameter estimate) of the association between each exposure variable and the outcome, with corresponding 95% confidence intervals and p-values. All analyses were conducted in R version 3.4.1.

RESULTS

Descriptive statistics of mosquito collection trends and meteorological conditions, stratified by county, are illustrated in Table 1 and Figures 2 and 3. Total mosquitos collected varied between each county, species and year. Yakima County collected more *C. pipiens* mosquitos, on average, than the other three counties (mean = 8,887), while Grant County collected the most *C. tarsalis* mosquitos (mean = 14,759) (Table 1). However, Grant County also collected the fewest *C. pipiens* on average (mean = 664). Grant County also had the highest mean percentage of WNV-positive *C. tarsalis* mosquito pools (32%) and the lowest mean number of WNV-positive *C. pipiens* mosquito pools (1%), while Yakima had the highest mean number of WNV-positive *C. pipiens* mosquito pools (14%). Each county reported at least one year with no WNV-positive mosquito pools detected for either species. Benton County had the highest number of human WNV infections between 2008 and 2017 (n=32) while Franklin County had the lowest (n=2). Temperature ranges between each county were similar (Figure 2). Each county had a mean temperature between 50ºF and 52ºF with mean minimum temperatures ranging from 5ºF to 9ºF and mean maximum temperatures ranging from 100ºF to 101ºF (Figure 2). Precipitation was also similar between counties (Figure 3). The mean total annual precipitation for all four counties was between 7 inches and 9 inches.

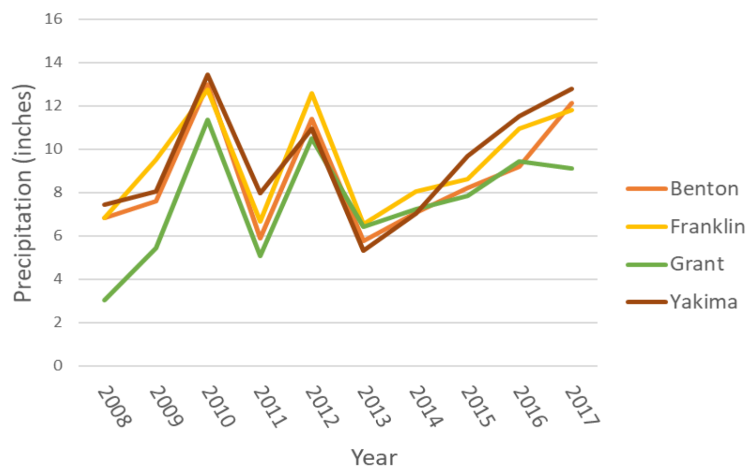
Association of Temperature and Precipitation with WNV Season Start and Duration

Figure 2. Meteorological and mosquito-collection trends by county, 2008-2017



We performed univariate analyses for each county, the county aggregate, and species for our outcomes of WNV season start and WNV season duration to determine which temperature and precipitation variables to include in the multivariate models (Tables 2 and 3).

Figure 3. Total annual precipitation by county, 2008-2017



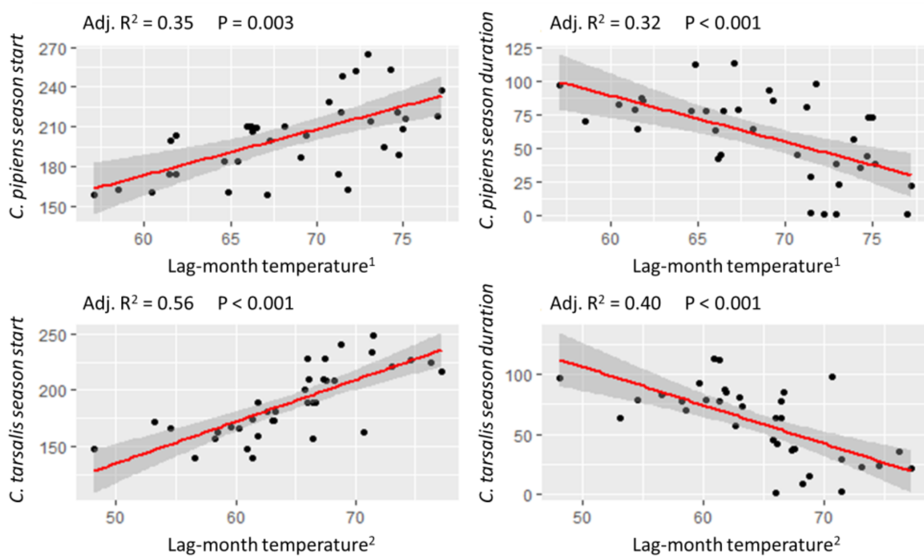
In the *C. pipiens* univariate models, degree days and lag-month temperature had the lowest P-values of the three temperature variables in four models each, while lag-month precipitation had the lowest P-value of the three precipitation variables in six models (Table 2). Only the county aggregate and Franklin County had significant associations between both temperature and precipitation and a season's start (Aggregate: lag-month temperature and precipitation; Franklin: start-month temperature and daily mean precipitation). Temperature was significantly associated with the aggregate of counties, Franklin, and Yakima County in regard to season duration (Aggregate/Yakima: lag-month; Franklin: start-month). Only Benton had a significant association between precipitation and season duration (daily mean precipitation).

In the *C. tarsalis* univariate models, lag-month temperature had the lowest P-value of the three temperature variables in eight models while start-month precipitation had the lowest P-value of the three precipitation variables in six models. The aggregate of counties, Franklin, and Grant County all demonstrated significant associations between temperature, precipitation and season start (Aggregate/Franklin: lag-month temperature and start-month precipitation; Grant: lag-month temperature and precipitation). The aggregate, Benton and Grant County showed significant associations between temperature and precipitation and season duration (Aggregate: lag-month temperature and start-month precipitation; Benton: lag-month temperature and daily mean precipitation; Grant: lag-month temperature and precipitation).

Using the results of the univariate models, we created multivariate models for each outcome, county and species using one temperature and one precipitation variable in each model (Tables 4 and 5). For *C. pipiens*, after adjusting for precipitation, each extra degree of mean lag-month temperature was associated with a 3.46-day-later WNV season start (95% CI: 1.44, 5.47) in the county aggregate and a 5.28-day-later season start in Yakima County (95% CI: 2.24, 8.31) (Table 4 and Figure 4). One degree higher of mean lag-month temperature was also associated with a shorter WNV season in the aggregate of counties (-4.19 days; 95% CI: -6.39, -2.00) and Yakima County (-5.21 days; 95% CI: -6.82, -3.60), adjusting for precipitation. Benton and Yakima counties both saw shorter WNV seasons in association with each extra 0.001 inches of precipitation (-1.81 days and -1.61 days, respectively), after adjusting for temperature, though in Benton we observed an association with daily mean precipitation between May and June while in Yakima there was an association with mean start-month precipitation.

When exploring *C. tarsalis* (Table 5), in both the aggregate of counties (Figure 4) and Yakima we observed an association with a later season start (3.54 days and 6.61 days, respectively) for each higher degree of mean lag-month temperature (Aggregate) or mean start-month temperature (Yakima), adjusting for start-month precipitation. In the county aggregate and Benton County we observed an association with shorter season (-3.27 days and -6.94 days, respectively) for each higher degree of mean lag-month

Figure 4. WNV season start and duration plots of aggregate of counties, by mosquito species



[1] Adjusting for lag-month precipitation
[2] Adjusting for start-month precipitation

temperature, adjusting for precipitation. Only in Franklin County did we see an association between both temperature and precipitation and season duration.

<u>WNV Season Duration and Mosquito Infection Rate</u>

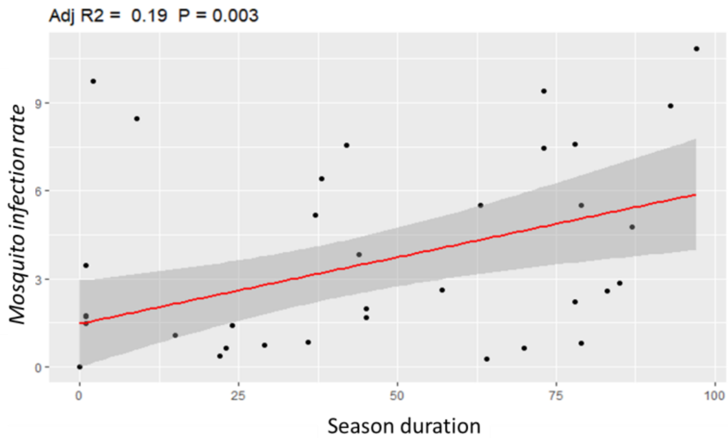
We found that for each one-day increase in WNV season duration for all counties in aggregate, the mosquito infection rate increased by 0.05 (95% CI: 0.02, 0.07) (Table 6 and Figure 5). In stratified analyses, there was a statistically significant association observed between WNV season duration and mosquito infection rate for Benton (MLE= 0.08; 95% CI: 0.03, 0.12) and Franklin (MLE = 0.06; 95% CI: 0.01, 0.11) but not for Grant or Yakima counties.

DISCUSSION

Our study demonstrates that temperature and precipitation may both play a role in determining when a WNV season will start and how long it will last within these four counties in WA, though temperature appears to have a greater impact. We found that, when examining all counties in aggregate, each mosquito species has a later WNV season start and shorter WNV season duration for every one degree higher of mean lag-month temperature. Our findings in the aggregate also suggest that a longer WNV season may contribute to higher mosquito infection rates. Taken together, our results may provide key insights into the complexity of WNV ecology and may help inform public health program planning for WNV control in Washington State.

Our primary finding – that higher temperatures may be associated with a later WNV season start and shorter season duration – runs counter to our prior hypothesis that that higher temperatures would lead to earlier and longer seasons. The most likely explanation for this is due to our methods for creating the season start and season duration outcome variables. We used the day of the first detected WNV-positive mosquito as our season start. If the first mosquito is detected later in the year when temperatures are already warmer then an association could naturally develop between higher temperatures, later season start, and shorter season duration (a late-starting season may see a shorter season duration). An alternative explanation could be that our hypothesis was based on evidence from studies done in areas with much different characteristics compared to the counties in our study (different temperature ranges, amount of rainfall, landcover, etc.). It may be that variations in environmental and terrain characteristics, even small ones, between different areas greatly influence changes in WNV vector activity. Studying WNV at different geographical scales may help clarify this.

Figure 5. Plot of Aggregate of Counties: WNV season duration vs. mosquito infection rate (MLE[1])



[1] Mosquito infection rate calculated using maximum likelihood estimate (MLE)

Results from our county-specific analyses are somewhat unclear, as we noted different associations between temperature and precipitation and WNV season start and duration by county. These differences by county may have resulted from variability in the completeness of the mosquito collection data. *C. pipiens* models for Franklin and Grant counties had four or more years with no WNV-positive *C. pipiens* detected, and Grant had no *C. pipiens* collections at all in three separate years. Similarly, Benton and Franklin counties each had five years with no WNV-positive *C. tarsalis* detected. To adequately explore the relationships between temperature, precipitation and WNV seasons more mosquito collection data may be required. However, similar to the county aggregate, mean lag-month temperature seemed to affect *C. pipiens* season start and duration in Yakima County, the county with the most robust collection data. Interestingly, the temperature and precipitation exposure variables for *C. pipiens* in Yakima County appear to be stronger in the multivariate model than in separate univariate models. This may suggest that, at least in Yakima County, temperature and precipitation act together to influence *C. pipiens*. In other county models (e.g., Franklin *C. pipiens* and Grant *C. tarsalis*), the associations from multivariable models are not statistically significant while those from univariate models are. It is difficult to determine the cause of the changes in significance due, in part, to our small sample sizes for individual counties (i.e., n<10). One possibility is that either temperature or precipitation, alone, was a stronger predictor of the outcomes. By adding a second variable we reduced statistical power to detect a significant association. The county-specific results could also misrepresent the true associations between temperature, precipitation and WNV seasons given the lack of collection data. Further analyses that include more years of collection data may further elucidate these associations.

Our findings of temperature and precipitation and WNV season start and duration are somewhat contrary to other studies that have examined the relationship between environmental conditions and WNV seasons. A 2014 study by Rosa et al. in Northwestern Italy found that increased “late” precipitation (number of days with precipitation between May and July) was associated with longer seasons for *C. pipiens*, whereas we found, in Benton, an association between mean daily precipitation between May and June and shorter seasons (Table 4). Differences between our study and that by Rosa and colleagues could be due to inherent differences in the researched geographical areas of each study (Northwestern Italy and Central Washington), different methods of assessing “early” and “late” temperatures or precipitation, or differences in mosquitos of each region. Rosa et al. (2014)’s study also examined *C. pipiens* mosquitos, but it is unclear if these are the same as the *C. pipiens* found in Washington. It is also possible that WNV ecology, as mentioned previously, is more nuanced than we realized.

Our analysis of WNV season duration and mosquito infection rates matched our prior hypothesis that a longer season may contribute to higher mosquito infection rates. We found a positive association between season duration and mosquito infection rates in the aggregate of counties (Figure 5), Benton, and Franklin counties. Though the predicted change in infection rate is less than 0.1 for each extra day of a WNV season, this change is significant. According to the California WNV Risk Assessment, an infection rate of

5 or greater (per 1,000 mosquitos) indicates an extremely high probability of human WNV transmission[15]. Our aggregate model estimates that a 75-80 day WNV season may see mean-weekly mosquito infection rates of 5/1,000.

A study in Central Canada by Chen and colleagues identified an association between higher mean lag-month temperatures, mean lag-month precipitation and *C. tarsalis* infection rates[10]. Higher mean lag-month temperatures were associated with higher infection rates, while higher mean lag-month precipitation was associated with lower infection rates. Our study found a negative association between mean lag-month temperature and *C. tarsalis* season duration (in aggregate), but a positive association between season duration and mosquito infection rates. Our results do not necessarily match those of the Chen study, which could be due to differences in environmental conditions between the study areas (Central Canada and Central Washington), or could be due to our methods used to create the season duration variable. Further studies have shown how temperatures above 86ºF can lead to lower mosquito survival[9,10,16]. Though the counties in our study did not often see mean daily temperatures above 86ºF, they did frequently see maximum daily temperatures above this level. This may partially explain why we found higher temperatures to be associated with later season start-times and shorter seasons. Examining the relationship between high temperatures (> 86ºF) and WNV seasons may shed light on this.

This study has several strengths. Our study is the first, to our knowledge, to explore the relationship between environmental variables and WNV seasons in Washington and in the US. It is also the first to explore how the duration of a WNV season is related to mosquito infection rates. Since the counties used in our study have similar temperature and precipitation trends, we were able limit any unmeasured confounding and use a county aggregate to supplement missing data to create an overall prediction for WNV seasons and mosquito infection rates within the study area. Our study also has several limitations. First, our methods used to create the season start and season duration outcome variables may have induced associations that otherwise would not have appeared. In future research, examining temperature as an outcome and the start-date as an exposure variable, or exploring how the degree days (≥ 60) of an entire season are related to season duration may be more prudent. Second, mosquito results were obtained from multiple testing sites and different mosquito testing methods were used across counties and years, which may have influenced our results. We assumed that the two mosquito testing methods (RAMP and RT-PCR) were equivalent in their accuracies of detecting WNV. However, RAMP tests with values < 50 'RAMP units' may produce more false-negative results than the RT-PCR method[17]. Since there was no analysis examining how many mosquito pools were tested using either of the two testing methods we are unable to determine the extent to which our study was impacted (i.e., if RAMP testing was used extensively we may have unknowingly counted a significant number of WNV-positive mosquito pools as negative instead of positive). Future research should not assume consistency between results of different testing methods and should include sensitivity analyses to compare the results of each method. Third, mosquito control is non-systematic across MCDs and jurisdictions. Effective mosquito control may alter the duration of a WNV season if infected mosquitos are killed before becoming trapped. Each county has its own MCD and may have more than one, and nonsystematic trapping by MCD and county may have influenced how early a WNV-positive mosquito was detected (sampling bias). We found that mosquito trapping was inconsistent between counties and between years which may have altered the results of our analyses. Fourth, there were several years of missing mosquito data that most likely weakened the results of county-specific models, though our aggregate models were able to act as an adjunct. Fifth, we were not able to include data on bird populations, which may be an important confounder in our analysis. Birds, specifically sparrows, robins and crows, are the preferred hosts of WNV and the preferred animals from which *C. pipiens* and *C. tarsalis* obtain blood meals[18]. Changes in avian habitats or migration due to shifts in weather conditions may have altered the ability for *C. pipiens* and *C. tarsalis* to blood-feed on the birds and obtain WNV from them. This in turn may have affected the timing and duration of WNV seasons depending on the presence of a suitable host population. Sixth, we did not examine how temperatures above 86ºF affect WNV seasons or mosquito infection rates. As mentioned above, previous studies have found *Culex* survival and longevity decrease with high daily average temperatures[6-10]. The counties included in this study frequently experienced temperatures above 86ºF in the summer and this may partially explain our unexpected results. Seventh, we also did not assess the effects of land cover in relation to our outcomes. Previous studies found associations between different types of land cover and WNV mosquito activity[1,6]; however, we were able to limit this confounding by adjusting for county in aggregate models. Finally, because we only obtained data from four Washington counties, our results cannot necessarily be extrapolated to other counties in the state or to other states within the country.

In conclusion, we found that temperature and precipitation may be important factors in WNV season start and duration, and that season duration may affect mosquito infection rates. Given the lack of research into the associations between climatic variables and WNV seasons, our study should function as a catalyst for generating hypotheses into this field. Since we did not examine climatic variables in relation to mosquito abundance, another potential indicator for WNV activity[15], future research building on this study should include analyses on this. Future research could focus on examining and comparing differing degree-day models (60ºF vs. 70ºF vs. 80ºF) against mosquito infection rates and abundance. Subsequent research could also explore mean monthly temperature and precipitation at more time-points within a year (i.e., divide a year into 8-week blocks) and determine how the variables at each time-point affect mosquito abundance and infection rates both during and after the corresponding time-point. As noted in the limitations, we never studied how daily mean temperatures above 86ºF alter mosquito activity. It could be important to study these high temperatures in relation to season duration and mosquito abundance, as well. Using logistic regression, or other non-linear models, to examine mean monthly temperatures against mosquito abundance could also provide further understanding of WNV dynamics as the relationships may not be linear in nature. These data could inform predictions of potential WNV mosquito activity, perhaps even human infections, given future projected climate scenarios, and could help us prepare for the future of WNV[19]. Understanding seasonal trends of WNV ahead of time allows for more timely public messaging and awareness campaigns by public health agencies, which could lead to reduced human WNV infections.

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Table 1. Mosquito-collection and WNV characteristics by county, 2008-2017

County	Year	C. pipiens collected	C. tarsalis collected	C. pipiens pools* collected (% positive)	C. tarsalis pools collected (% positive)	C. pipiens positive pools	C. tarsalis positive pools	Human WNV infections
Benton	2008	6252	899	131 (10)	23 (0)	13	0	0
	2009	6485	6195	185 (30)	162 (31)	56	51	9
	2010	8318	6920	191 (1)	179 (2)	2	4	0
	2011	5031	2470	128 (0)	64 (0)	0	0	0
	2012	5336	1497	120 (0)	38 (0)	0	0	1
	2013	3707	1548	83 (1)	41 (0)	1	0	0
	2014	3167	1894	68 (13)	43 (9)	9	4	7
	2015	3181	3084	83 (13)	81 (17)	11	14	12
	2016	3802	3226	108 (15)	82 (11)	16	9	3
	2017	6307	5128	151 (6)	123 (0)	9	0	0
Mean		5158.6	3286.1	124.8 (8.9)	83.6 (7)	11.7	8.2	3.2
Franklin	2008	2470	3500	64 (0)	86 (0)	0	0	0
	2009	3962	8815	93 (12)	193 (18)	11	34	0
	2010	4073	8387	95 (0)	202 (0)	0	0	0
	2011	5716	4907	139 (1)	125 (0)	1	0	0
	2012	4122	5983	102 (0)	150 (0)	0	0	0
	2013	8136	6805	187 (2)	159 (1)	3	2	0
	2014	1343	4611	33 (30)	117 (13)	10	15	0
	2015	2393	6049	61 (8)	146 (8)	5	12	2
	2016	2522	2215	58 (9)	57 (2)	5	1	0
	2017	1117	2277	29 (0)	56 (0)	0	0	0
Mean		3585.4	5354.9	86.1 (6.2)	129.1 (4.2)	3.5	6.4	0.2
Grant	2008	73	131	3 (33)	5 (20)	1	1	0
	2009	0	1245	0 (0)	26 (35)	0	9	1
	2010	122	23873	4 (0)	505 (22)	0	112	1
	2011	0	16757	0 (0)	344 (1)	0	3	0
	2012	0	22412	0 (0)	452 (0)	0	0	0
	2013	638	15804	13 (8)	318 (< 1)	1	1	0
	2014	415	19258	9 (0)	404 (8)	0	31	1
	2015	524	15034	11 (36)	309 (33)	4	103	4
	2016	3456	20641	77 (10)	446 (10)	8	43	0
	2017	1409	12435	34 (0)	273 (5)	0	14	0
Mean		663.7	14759	15.1 (8.7)	308.2 (13.44)	1.4	31.7	0.7
Yakima	2008	19691	7198	407 (8)	154 (4)	33	6	2
	2009	9191	5056	188 (41)	108 (45)	77	49	21
	2010	15991	7827	371 (1)	199 (1)	3	2	0
	2011	4423	1832	111 (0)	53 (2)	0	1	0
	2012	10290	4425	220 (2)	105 (1)	4	1	1
	2013	9711	5199	207 (2)	123 (1)	4	1	0
	2014	2878	2476	69 (2)	62 (0)	1	0	1
	2015	7644	1992	163 (3)	50 (0)	5	0	2
	2016	5799	1997	129 (6)	47 (6)	8	3	1
	2017	3247	4663	88 (5)	124 (5)	4	6	0
Mean		8886.5	4266.5	195.3 (7)	102.5 (6.5)	13.9	6.9	2.8

*Pools collected consist of approximately 50 mosquitos or less

Table 2. Univariate association between temperature and precipitation, among *C. pipiens*, with the start and length of a WNV Season, for all counties in aggregate and stratified by county[Δ]

Outcome: Start of WNV Season				Outcome: Length of WNV Season			
Variable (n)	**Coefficient and 95% CI**	**P-value**	**Adjusted R^2**	**Variable (n)**	**Coefficient and 95% CI**	**P-value**	**Adjusted R^2**
All Counties				**All Counties**			
Lag temperature[1] (27)	3.32 (1.96, 4.68)	< 0.001*	0.48	Lag temperature (27)	-3.31 (-4.83, -1.79)	< 0.001*	0.42
Lag precipitation[2] (27)	-900 (-1,553, -246)	0.01*	0.21	Lag precipitation (27)	706 (-33.81, 1,446)	0.06	0.10
Stratified Counties†				**Stratified Counties†**			
Benton				**Benton**			
Degree days[3] (8)	-0.13 (-0.25, -0.01)	0.04*	0.47	Degree days (10)	0.18 (-0.08, 0.43)	0.15	0.14
Lag precipitation (8)	-1,240 (-3,417, 936)	0.21	0.12	Daily mean precipitation (10)	-1,988 (-3,612, -365)	0.02*	0.44
Franklin				**Franklin**			
Month temperature[4] (6)	-5.39 (-7.47, -3.32)	0.002*	0.91	Month temperature (6)	3.83 (0.32, 7.33)	0.04*	0.62
Daily mean precipitation[5] (6)	2,497 (720, 4,274)	0.02*	0.74	Daily mean precipitation (10)	-1,463 (-3,032, 105)	0.06	0.29
Grant				**Grant**			
Degree days (4)	-0.16 (-0.39, 0.07)	0.10	0.72	Degree days (10)	0.20 (-0.01, 0.42)	0.06	0.30
Lag precipitation (4)	-978 (-7,692, 5,737)	0.59	-0.25	Lag precipitation (4)	2,077 (-9,948, 14,101)	0.54	-0.18
Yakima				**Yakima**			
Lag temperature (9)	5.29 (2.95, 7.63)	0.001*	0.77	Lag temperature (9)	-5.20 (-7.43, -2.96)	0.001*	0.79
Lag precipitation (9)	-711 (-1,952, 531)	0.21	0.09	Month precipitation[6] (9)	-1,589 (-5,698, 2,520)	0.39	-0.02

[Δ]Different outcomes represent results from separate univariate linear regression models with temperature and precipitation as primary exposures.

* Statistically significant at $\alpha = 0.05$ level

[1] Lag temperature = mean monthly temperature (°F) based on mean daily temperature of the month prior to the first WNV-positive mosquito detected, per year

[2] Lag precipitation = mean of the total daily rainfall (in.) for each day of the month prior to the first WNV-positive mosquito detected, per year

[3] Degree days = sum of the difference between maximum temperature and 60°F, given maximum temperature ≥ 60°F, for each day between March and April of each year

[4] Month temperature = mean monthly temperature (°F) based on mean daily temperature of the month of the first WNV-positive mosquito detected, per year

[5] Daily mean precipitation = mean of the total daily rainfall (in.) for each day between May and June of each year

[6] Month precipitation = mean of the total daily rainfall (in.) for each day of the month of the first WNV-positive mosquito detected, per year

†Each county is a separate model

Table 3. Univariate association between temperature and precipitation, among *C. tarsalis*, with the start and length of a WNV Season, for all counties in aggregate and stratified by county[Δ]

Outcome: Start of WNV Season				Outcome: Length of WNV Season			
Variable (n)	**Coefficient and 95% CI**	**P-value**	**Adjusted R^2**	**Variable (n)**	**Coefficient and 95% CI**	**P-value**	**Adjusted R^2**
All Counties				**All Counties**			
Lag temperature[1] (27)	3.66 (2.74, 4.58)	< 0.001*	0.72	Lag temperature (27)	-3.25 (-4.32, -2.18)	< 0.001*	0.60
Month precipitation[2] (27)	-1,254 (-2,136, -372)	0.01*	0.23	Month precipitation (27)	1,022 (125, 1,919)	0.03*	0.15
Stratified Counties†				**Stratified Counties†**			
Benton				**Benton**			
Lag temperature (5)	4.69 (0.69, 8.68)	0.03*	0.76	Lag temperature (5)	-5.54 (-8.47, -2.61)	0.01*	0.90
Month precipitation (5)	-3,680 (-10,160, 2,800)	0.17	0.36	Daily mean precipitation[5] (10)	-1,988 (-3,612, -365)	0.02*	0.44
Franklin				**Franklin**			
Lag temperature (5)	3.1 (0.75, 5.45)	0.02*	0.81	Degree days[6] (10)	0.18 (0.02, 0.35)	0.03*	
Month precipitation (5)	2,466 (639, 4,294)	0.02*	0.81	Daily mean precipitation (10)	-1,463 (-3,032, 105)	0.06	0.29
Grant				**Grant**			
Lag temperature (9)	3.44 (1.05, 5.83)	0.01*	0.57	Lag temperature (9)	-3.56 (-5.93, -1.19)	0.01*	0.59
Lag precipitation[3] (9)	-1,890 (-3,015, -765)	0.005*	0.65	Lag precipitation (9)	1,843 (597, 3,090)	0.01*	0.58
Yakima				**Yakima**			
Month temperature[4] (8)	5.82 (2.54, 9.09)	0.005*	0.72	Lag temperature (8)	-3.33 (-6.27, -0.40)	0.03	0.49
Month precipitation (8)	-1,414 (-3,359, 531)	0.13	0.24	Month precipitation (8)	964 (-997, 2,924)	0.27	0.06

[Δ] Different outcomes represent results from separate univariate linear regression models with temperature and precipitation as primary exposures.

* Statistically significant at $\alpha = 0.05$ level

[1] Lag temperature = mean monthly temperature (°F) based on mean daily temperature of the month prior to the first WNV-positive mosquito detected, per year

[2] Month precipitation = mean of the total daily rainfall (in.) for each day of the month of the first WNV-positive mosquito detected, per year

[3] Lag precipitation = mean of the total daily rainfall (in.) for each day of the month prior to the first WNV-positive mosquito detected, per year

[4] Month temperature = mean monthly temperature (°F) based on mean daily temperature of the month of the first WNV-positive mosquito detected, per year

[5] Daily mean precipitation = mean of the total daily rainfall (in.) for each day between May and June of each year

[6] Degree days = sum of the difference between maximum temperature and 60°F, given maximum temperature ≥ 60°F, for each day between March and April of each year

†Each county is a separate model

Table 4. Multivariate association between temperature and precipitation, among *C. pipiens*, with the start and length of a WNV Season, for all counties in aggregate and stratified by county[Δ]

Outcome: Start of WNV Season			
Variable (n)	**Coefficient and 95% CI**	**P-value**	**Adjusted R^2**
All Counties (27)		< 0.001*	0.46
Lag temperature[1]	3.46 (1.44, 5.47)	0.002*	
Lag precipitation[2]	74.08 (-710, 858)	0.85	
Stratified Counties†			
Benton (8)		0.06	0.54
Degree days[3]	-0.12 (-0.24, 0.001)	0.05*	
Lag precipitation	-916 (-2,598, 765)	0.22	
Franklin (6)		0.01*	0.91
Month temperature[4]	-7.95 (-16.59, 0.70))	0.06	
Daily mean precipitation[5]	-1,334 (-5,673, 3,004)	0.40	
Grant (4)		0.38	0.56
Degree days	-0.19 (-1.28, 0.91)	0.27	
Lag precipitation	583 (-14,394, 1,556)	0.71	
Yakima (9)		0.01*	0.74
Lag temperature	5.28 (2.24, 8.31)	0.01*	
Lag precipitation	-8.60 (-810, 793)	0.98	

Outcome: Length of WNV Season			
Variable (n)	**Coefficient and 95% CI**	**P-value**	**Adjusted R^2**
All Counties (27)		< 0.001*	0.43
Lag temperature	-4.19 (-6.39, -2.00)	< 0.001*	
Lag precipitation	-474 (-1,328, 379)	0.26	
Stratified Counties†			
Benton (10)		0.03*	0.54
Degree days	0.13 (-0.06, 0.33)	0.14	
Daily mean precipitation	-1,811 (-3,343, -280)	0.03*	
Franklin (6)		0.11	0.62
Month temperature	-0.52 (-15.11, 14.07)	0.92	
Daily mean precipitation	-2,267 (-9,587, 5,052)	0.40	
Grant (4)		0.34	0.65
Degree days	0.34 (-1.45, 2.13)	0.25	
Lag precipitation	-769 (-25,198, 23,659)	0.76	
Yakima (9)		<0.001*	0.90
Lag temperature	-5.21 (-6.82, -3.60)	<0.001*	
Month precipitation[6]	-1,613 (-2,971, -254)	0.03*	

[Δ]Different outcomes represent results from separate multivariate linear regression models with temperature and precipitation as primary exposures.

* Statistically significant at $\alpha = 0.05$ level

[1] Lag temperature = mean monthly temperature (°F) based on mean daily temperature of the month prior to the first WNV-positive mosquito detected, per year

[2] Lag precipitation = mean of the total daily rainfall (in.) for each day of the month prior to the first WNV-positive mosquito detected, per year

[3] Degree days = sum of the difference between maximum temperature and 60°F, given maximum temperature ≥ 60°F, for each day between March and April of each year

[4] Month temperature = mean monthly temperature (°F) based on mean daily temperature of the month of the first WNV-positive mosquito detected, per year

[5] Daily mean precipitation = mean of the total daily rainfall (in.) for each day between May and June of each year

[6] Month precipitation = mean of the total daily rainfall (in.) for each day of the month of the first WNV-positive mosquito detected, per year

†Each county is a separate model

Table 5. Multivariate association between temperature and precipitation, among *C. tarsalis*, with the start and length of a WNV Season, for all counties in aggregate and stratified by county[Δ]

Outcome: Start of WNV Season				**Outcome: Length of WNV Season**			
Variable (n)	**Coefficient and 95% CI**	**P-value**	**Adjusted R^2**	**Variable (n)**	**Coefficient and 95% CI**	**P-value**	**Adjusted R^2**
All Counties (27)		< 0.001*	0.71	**All Counties** (27)		< 0.001*	0.58
Lag temperature[1]	3.54 (2.42, 4.67)	< 0.001*		Lag temperature	-3.27 (-4.58, -1.97)	< 0.001*	
Month precipitation[2]	-121 (-773, 530)	0.70		Month precipitation	-25.89 (-783, 731)	0.94	
Stratified Counties†				**Stratified Counties†**			
Benton (8)		0.10	0.80	**Benton** (5)		0.03*	0.94
Lag temperature	3.77 (-2.19, 9.73)	0.11		Lag temperature	-6.94 (-11.69, -2.19)	0.02*	
Month precipitation	-1,665 (-7,545, 4,216)	0.35		Daily mean precipitation[5]	639 (-994, 2,273)	0.23	
Franklin (5)		0.11	0.78	**Franklin** (10)		0.01*	0.64
Lag temperature	1.55 (-7.12, 10.22)	0.52		Degree days[6]	0.16 (0.03, 0.29)	0.02*	
Month precipitation	1,332 (-5,548, 8,211)	0.49		Daily mean precipitation	-1,262 (-2,412, -111)	0.04*	
Grant (9)		0.03*	0.61	**Grant** (9)		0.03*	0.57
Lag temperature	1.02 (-4.16, 6.21)	0.65		Lag temperature	-1.98 (-7.49, 3.54)	0.41	
Lag precipitation[3]	-1,417 (-4,118, 1,284)	0.25		Lag precipitation	928 (-1,943, 3,799)	0.46	
Yakima (8)		0.03*	0.68	**Yakima** (8)		0.10	0.45
Month temperature[4]	6.61 (1.02, 12.23)	0.03*		Lag temperature	-4.41 (-9.38, 0.57)	0.07	
Month precipitation	381 (-1,635, 2,397)	0.65		Month precipitation	-691 (-3,139, 1,756)	0.50	

[Δ] Different outcomes represent results from separate multivariate linear regression models with temperature and precipitation as primary exposures.

* Statistically significant at $\alpha = 0.05$ level

[1] Lag temperature = mean monthly temperature (°F) based on mean daily temperature of the month prior to the first WNV-positive mosquito detected, per year

[2] Month precipitation = mean of the total daily rainfall (in.) for each day of the month of the first WNV-positive mosquito detected, per year

[3] Lag precipitation = mean of the total daily rainfall (in.) for each day of the month prior to the first WNV-positive mosquito detected, per year

[4] Month temperature = mean monthly temperature (°F) based on mean daily temperature of the month of the first WNV-positive mosquito detected, per year

[5] Daily mean precipitation = mean of the total daily rainfall (in.) for each day between May and June of each year

[6] Degree days = sum of the difference between maximum temperature and 60°F, given maximum temperature ≥ 60°F, for each day between March and April of each year

†Each county is a separate model

Table 6. Association between WNV season duration[1] and mean weekly MLE, for all counties in aggregate and stratified by county[Δ]

Outcome: Mean Weekly MLE			
(n)	**Coefficient and 95% CI**	**P-value**	**Adjusted R^2**
All Counties (40)	0.05 (0.02, 0.07)	0.003*	0.19
Stratified Counties†			
Benton (10)	0.08 (0.03, 0.12)	0.004*	0.63
Franklin (10)	0.06 (0.01, 0.11)	0.02*	0.47
Grant (10)	0.02 (-0.08, 0.12)	0.67	-0.10
Yakima (10)	0.04 (-0.04, 0.11)	0.28	0.04

[1] Season duration = number of days between first detected WNV-positive mosquito and the last detected WNV-positive mosquito

[Δ] Different outcomes represent results from separate linear regression models with WNV season duration as the primary exposure

* Statistically significant at α = 0.05 level

† Each county is a separate model