5-2020

Contrasting Seasonal Presence of West Nile Virus in Mosquitos Between Coastal and Inland Communities of New Jersey

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Abstract

In 1999, West Nile Virus (WNV) was introduced to New York City, most likely by an infected bird or mosquito. Since then, West Nile has been detected in every one of the forty-eight continental states of the US, and outbreaks have occurred yearly. Mosquitos play an important role as the main vector for WNV transmission to mammals, birds and some reptiles. As New Jersey is the most densely populated state in the United States, WNV is an ever-present public health concern. High human population density may increase the probability of coming in contact with a WNV positive mosquito. The prevalence of WNV in most states, including New Jersey, fluctuates from year to year, both in humans and mosquitos. As a coastal state, New Jersey has many unique habitats including dune communities and salt marshes, which are home to distinct species of potential WNV vector such as *Ochlerotatus taeniorhynchus*. Coastal locations also typically have divergent climate patterns compared to more inland locations. The ocean has a large heat capacity that will slow the rate at which the temperature changes on nearby land providing relatively more stable weather conditions than that of an inland community. It is presently unclear how environmental variance between coastal and inland locations may influence WNV presence within potential vectors. In order to determine the impact of climate variance on WNV presence and duration in mosquitos, weekly mosquito surveillance data from two distinct New Jersey counties between 2002 and 2019 was used to assess potential relationships between climate variance and the seasonal presence of WNV in mosquitos. The climate variables analyzed include precipitation and temperature from 2002-2019 in each county. As differences between species richness of the counties may also influence the presence of WNV, mosquito community composition was also

assessed between the counties. I found that Monmouth County has thirteen additional species than Hunterdon. Three of these thirteen additional species are saltmarsh or shoreline specialists and all of them are known to be vectors for WNV. Additionally, I have identified trends of WNV presence in response to yearly climatic variation and discuss how these findings may be used to predict and prevent future WNV outbreaks in New Jersey.

MONTCLAIR STATE UNIVERSITY

Contrasting seasonal presence of West Nile Virus in mosquitos between coastal and inland communities of New Jersey

By Domenica Mousa

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science

May 2020

College of Science and Mathematics

Department of Biology

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Montclair, NJ

2020

Acknowledgements

I would like to thank my thesis advisor Matthew L. Aardema for his time, patience, guidance, and support throughout my master's and thesis work. Through his mentorship I have had the opportunity to explore the complex ecological relationship of vector disease presence in New Jersey. I would also like to thank my committee members, Jennifer Krumins and Matthew Schuler, for their thoughts, comments and assistance.

A special thanks to all the members of the New Jersey Mosquito Control Association, Monmouth County Mosquito Control and Hunterdon County Mosquito Control for the continual guidance and have provided the surveillance data.

I would like to acknowledge the financial support provided for this project by the Montclair State University Wehner Student Research Program and The New Jersey Mosquito Control Association Daniel M. Jobbins Scholarship.

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Introduction

In 1999, West Nile Virus (WNV) was introduced to New York City, most likely by an infected bird or mosquito, however there is no clear evidence to name an exact culprit. Since then, West Nile has been detected in every one of the 48 continental states of the U.S. and as a result, outbreaks have occurred every summer since 1999. *Culex* mosquitos are the main WNV vectors in the Northeastern region of the United States. Together, *Cx. pipiens* and Cx. *restuans* mosquitos are responsible for 80% transmission of WNV in North America (Ciota et al., 2013). Female *Cx. pipens* prefer to obtain their blood meals from birds, small mammals and occasionally humans (Campbell et al. 2002). The cycle of WNV between mosquitos and their food source is the perfect cocktail for sustainability of the virus against mosquito control efforts. As a reservoir host, birds serve as a "hideaway" for WNV until an uninfected mosquito bites them. They then may go on to transmit the pathogen to other disease reservoirs, beginning the cycle again. Humans, however, are dead end hosts and do not aid the cycle of transmission between vector and host.

According to the CDC, there have been reported cases of WNV in New Jersey each year since 2000. Although its wide-spread presence, WNV still impacts bird populations and could be a public health concern for humans (Ladeau, 2007) (Paz, 2019). Therefore, monitoring and surveillance are very important. Monitoring allows state and county mosquito control agencies evaluate the patterns and trends of WNV in mosquitos. Surveillance may also provide insight into which habitats may be more productive in WNV transmission and the species that reside or reproduce in those habitats. Surveillance allows officials to implement control efforts in order to reduce mosquito populations and subsequently the prevalence and transmission of WNV. Due to the nature of the WNV transmission cycle, it is very unlikely that officials will be able to break that cycle, but it is possible to limit the viremia, presence of virus in the blood, in the population. However, if officials are able to understand the dynamics of WNV presence in the mosquito population in a particular site, more efficient mosquito control efforts can be made. Mosquito populations and WNV presence may be influenced by many factors such as climate.

It is important to understand the climatic parameters that increase the prevalence of WNV. Mosquito density is influenced by climatic parameters and food availability at larval and adult stages. As ectotherms, a mosquito's development from egg to reproductive adult relies on environmental conditions such as temperature and precipitation to provide pools of water to lay eggs (Mosquito Control, 2019). The prevalence of WNV at a particular site may be related with mosquito density, or environmental factors such as periods of drought (Gu et. al, 2006). Periods of drought can cause areas of creeks and rivers to slow creating stagnant pools and decrease the numbers of larvae-eating fish (Barik, 2015). High temperatures aid in faster replication of WNV resulting in a higher viral load in birds and mosquitos (Johnson et. al, 2013). In drought situations, birds tend to congregate at common water sources which may be the same location in which adult mosquitos are emerging. Habitual high temperatures worldwide may influence sea level rise and increase the incidence of coastal flooding for breeding as well (GH, 2019). However, the summer of 2018 had one of the highest numbers of WNV cases in Monmouth County and was also one of the wettest summers.

These observations defy popular belief, but there may be more parameters than just rainfall that influence WNV transmission. Climate change is impacting mosquito populations worldwide (HG, 2019). Subarctic regions are experiencing higher incidence of mosquito-borne illness that some scientist believe may be attributed to climate change (Anderson et. al, 2016). Climate change increases the temperature during the summer months; this increase in temperature may shorten the incubation periods of a mosquito's life cycle so they are reproducing at faster rates. It is imperative to note that WNV has a complex transmission cycle with numerous factors in addition to the environment making it susceptible to ecological and environmental factors (GH, 2019). There are many factors, abiotic and biotic, that influence the transmission and amplification of WNV in a system including daylength, temperature, rainfall, wind speeds, vegetation, vector-viral competence, population of host species, population of predators, parasites, immune susceptibility and human interference (GH, 2019). Mosquitos have a very short life cycle, for example, in temperate regions, most *Culex* species development take about 7-10 days from egg to reproductive adults (Mosquito, 2019). Furthermore, viruses replicate expeditiously; when both are interconnected in their life history traits, adaptability and evolution can occur very quickly. Epstein et. al (2001) suggests that as the climate changes, there will be shifts in the geographic range of vectors as well as increasing biting rates and shortened incubation periods of the pathogen itself. According to these claims, it is probable that New Jersey and the rest of the United States will experience an increase in WNV, and other mosquito borne diseases in mosquitos and consequently humans (Epstein, 2001).

An entomologist, J.B. Smith, of Rutgers University published the first report on mosquito detection and identification. Smith identified 35 species of mosquitos in 1904. Soon after, he became the founder of "a nationwide mosquito control movement" (Farajollah et. al, 2012). In 1912, New Jersey started the first mosquito control programs in the country that was implemented by The State Mosquito Control Commission (SMCC), The New Jersey Department of Environmental Protection (NJDEP), New Jersey Department of Agriculture (NJDA), and the New Jersey Department of Health and Senior Services (NJDHSS). Currently, New Jersey has one of the most comprehensive mosquito control programs in the United States. Each county in New Jersey has a mosquito control program that conducts mosquito density and disease surveillance as well as practices habitat modification, larvicide and adulticide. Methods of larvicide could include biological control such as the use *Bacillus thuringiensis* subspecies *israelensis* (BTI) or pesticide applications (CDC, 2016). Adulticide methods include the use of a pesticide that specifically targets adult mosquitos usually applied in a liquid form by a fogger or pressurized sprayer (CDC, 2016).

The number of species found in New Jersey is higher than ever before at 63 species (Ciota et. al, 2013). New Jersey being the most densely populated state in the United States, is a public health concern because residents are living relatively close to each other which increases the possibility of increased infection by contact with an infected mosquito. The increase in the number of mosquito species found indicates that mosquitos are adapting to urbanized areas and utilizing artificial containers, stagnant swimming pools, clogged drains and even potholes for breeding grounds in densely populated urban areas (Final, n.d.).For example, a species recently introduced into New Jersey in 2009, *Aedes albopictus,* is expanding its geographic range and population numbers since its introduction (Farajollahi et. al, 2012). The combination of a highly adaptive viral vector and a dense human population is a concern for public health of the community and wildlife that reside there.

Two counties in NJ that differ in demographics and water coverage are Monmouth County and Hunterdon County. Monmouth County is located along the shoreline of central New Jersey and covers 472 square miles of land with a population of over 630,000 people as of 2010 (Monmouth, 2018; see Figure 1). Monmouth County is a densely populated county that sits along the Jersey Shore making it a very active region in the summer months when WNV is most prevalent. The towns within Monmouth County have major outdoor tourist attractions such as the Jersey Shore and Six Flags Great Adventure Theme Park. As a coastal community, it is expected that Monmouth County provides a unique coastal environment that is not present non-coastal counties in NJ. Coastal regions provide a specific brackish habitat for specialized mosquito species (e.g. *Ochlerotatus taeniorhynchus*) to inhabit. Additionally, if it is found that the specialized coastal species may also be vectors for WNV, this may suggest a difference in the transmission cycle between a coastal and inland community.

Hunterdon County is located west of Monmouth County in central New Jersey. Hunterdon County covers 428 square miles of land with a population of over 125,000 people as of 2017 (Hunterdon, n.d.; see Figure 1). Hunterdon County is located on the western border of New Jersey adjacent to Pennsylvania. Hunterdon County also provides many outdoor activities that may increase human exposure to WNV in the summer months. However, as an inland community, Hunterdon might harbor fewer species of mosquito due to lack of saline habitat in the county.

The Monmouth and Hunterdon County's Mosquito Control Divisions have hundreds of sites combined across the counties that are surveyed for adult mosquitos and routinely tested for evidence of WNV and other mosquito-borne pathogens. Monmouth and Hunterdon County use a variety of trap types with different methods to attract a variety of mosquito species, such as CDC, BSG (BG-Sentinel), Gravid, UV light, CO2, baited EVS (Encephalitis Virus Surveillance) traps (mosquito.org, n.d.). Once live mosquitos are acquired, they are then sorted by location, gender and species. Pools of up to 50 females are tested together for WNV by immunofluorescent antibody (IFA) and polymerase chain reaction (PCR). If presence of disease is detected, adulticide efforts are used in the affected area with a NJDEP registered and regulated pesticide.

The objective of this study is to identify, analyze and determine any trends of WNV prevalence in two (coastal and inland) New Jersey counties in response to climatic changes from 2002-2019. The use of environmental data to examine trends in WNV numbers will provide a better understanding of what to expect in future years as global climate change transforms Earth's climate and ultimately weather patterns in NJ. I anticipate that high temperatures will have a positive relationship with mosquito density and WNV presence regardless of location. I expected that substantial rainfall along with high temperatures will also have a positive relationship with mosquito density and WNV presence regardless of location. It is also expected that Monmouth County will have higher species richness than Hunterdon County due to proximity to saltmarsh habitats.

Materials and Methods

Mosquito Surveillance Data

I obtained mosquito surveillance data for 2002-2019 from Hunterdon County and Monmouth County. Officials from both Hunterdon and Monmouth County sent me excel files via email that contained the mosquito trap surveillance data. Both counties have comparable mosquito control policies and procedures. Specifically, mosquito traps are placed throughout the county for general population analysis and pathogen screening. The mosquito traps that were utilized used a number of different methods to attract different species of mosquitos. Traps such as CDC, BSG (BG-Sentinel), Gravid, UV light, CO2, baited EVS (Encephalitis Virus Surveillance) were used. Trap placement was determined on a number of factors, including general surveillance, known areas to be densely populated, previous incidence of disease, or residents request. The mosquitos in the traps are collected weekly and taken back to the Hunterdon County Mosquito Control work building or Monmouth County Mosquito Control work building for identification. Mosquitos are then sexed by licensed mosquito specialists. Female mosquitos are combined into pools of up to fifty individuals by species. *Culex* species are generally pooled together as a genera due to the difficulty of identification to the species level.

The pools are then placed into containers and labeled with the collection date and species or genera of mosquito contained. The containers of mosquitos are then sent to the New Jersey Department of Environmental Protection: New Jersey Mosquito Commission and tested using immunofluorescent antibody (IFA) and polymerase chain reaction (PCR) to amplify and detect the absence or presence of WNV in the tested pools. The results are then sent back to the counties which the mosquitos were from for recordkeeping and adulticiding efforts. If positive test results are found, adulticiding efforts are made in order to control the pathogen's presence.

Climatological Data

Climatological data were obtained from the National Weather Service (National, n.d.). In order to download the data, I used the Climate Data Online (CDO) Department and used the Search Tool. The Search Tool required information entry regarding the dataset, date range and location of climate data one is trying to obtain. My entries were entered as followed: Select Weather Observation Type/Dataset: Daily Summaries, Select Date Range: 2002-01-01 to 2019-12-31, Search for: Counties, Enter a Search Term: Hunterdon County, NJ or Monmouth County, NJ. After all the climate data requirements were entered, I selected search and selected the appropriate location according to the map. I then clicked "Add to Cart" and entered Select the Output Format: Custom GHCN-Daily CSV and clicked continue. On the following screen, my selections were as follows, Station Detail & Data Flat Options: Station Name, Select data types for custom output: Precipitation and Air Temperature and clicked continue. On the following page, I entered the email address in which I wanted the data file sent to and proceeded to Submit Order. Moments later, I received an email with the option to download the CSV file with the selected data. I completed this process for each county, Monmouth County, NJ and Hunterdon County, NJ.

Data Organization

The mosquito surveillance data and climatological data were organized into weeks in order to normalize the data among years. I classified a week as a group of seven consecutive days starting on January 1 of each year. The data were organized in this way so that we were able to compare years without any confusion. There were no mosquitos collected in any February in either county and therefore, for the purpose of this study 2004, 2008, 2012, 2016 were not treated as leap years.

The mosquito surveillance data files that were sent from the counties were organized to only include the date of collection, species of pool tested and WNV test results (positive or negative). The climatological data were transferred into an excel file. Once in the excel file, the data was organized into sheets which contained one year per sheet, each sheet in the file was named with the year of data that was contained in it (i.e 2002, 2003, 2004 etc...to 2019). The daily summaries for maximum temperature and minimum temperature were standardized by using the function =AVERAGE to select the beginning and ending date of a given week. This was done for each week in every year from 2002-2019 for both Hunterdon and Monmouth County. I chose a representative site for total rainfall for each county that was surveyed weekly from 2002-2019. Monmouth County's representative rainfall site was site Freehold Marlboro (40.31358°N, 74.25106°W). Hunterdon County's representative rainfall site was site Flemington 5NNW (40.57401°N, 74.88156°W). I then found the total rainfall for each week for each year 2002-2019 for each site.

WNV Duration

In order to determine potential differences in the WNV presence in mosquitos between these two New Jersey counties, I determined the average start week, average duration and average end week that WNV was observed in sampled mosquitos for each county. The start week was defined as the first week of a given year when WNV presence was detected in mosquitos. The start weeks for each year were entered into an excel spreadsheet. I used the function =AVERAGE to select years 2002-2019 to find the average start week. The duration (weeks) for each year were entered into an excel spreadsheet. I used the function =AVERAGE to select years 2002-2019 to find the average duration of WNV. The end week was defined as the last week of a given year when WNV presence was detected in mosquitos. The end weeks for each year were entered into an excel spreadsheet. I used the function =AVERAGE to select the years 2002-2019 to find the average end week. These steps were done for each county and entered into a chart (see Figure 2).

In order to create a visual representation for the start, end and duration of WNV presence in mosquitos I created Gantt charts for each county. In order to create a Gantt chart, I entered the years 2002-2019 ascending in column A, the corresponding start week in column B and the corresponding end week in column D. In the first cell of column C I entered the function =D1-B1; then selected that cell and dragged to the last cell (2019). This calculated the duration for each year. To create the chart, I selected the cells in column B, C and D and clicked Insert, Insert Bar Chart then Stacked Bar Chart. Next, I selected the data in the graph that represented the start week or Series 1 (blue) and in the Format Tab selected Shape Fill and then No Fill. I repeated this step with the end week or Series 3 (grey). By using the Format Tab, I adjusted the axes to reflect the data with the weeks on the x-axis and the years along the y-axis, the ranges of the axes and color of the bar. I completed this process with Monmouth County negative WNV, Monmouth County positive WNV, Hunterdon County negative WNV, and Hunterdon County positive WNV. I then superimposed each county's chart so that there was one chart for each county. Both negative and positive results were used in this case to show when counties began and ended surveillance in comparison to the actual start and end of the WNV presence in mosquitos. Lastly, the average start week and average end week for each county was displayed on the Gantt chart by colored lines corresponding to the colors in Table 1 (Monmouth County = purple, Hunterdon County = orange; see Figure 3).

Regression Analysis

I examined the number of rainfall days in each year from 2002-2019 for each county. I counted the number of days with recorded precipitation from May 1st to September 30th of each year in both counties. I entered the values in an excel spreadsheet aside the corresponding year. Next, I counted the weeks of positive WNV for each year and entered those values aside the corresponding year and rainfall days. I did this for each Hunterdon County and Monmouth County. I then created a scatter plot in excel for both counties. I added chart titles, axis titles, appropriate axes ranges, R-squared value, and linear trendline; see Figures 3 and 4.

Statistical Analysis

I examined the differences between the weather variance during the start and end of WNV presence in mosquitos with the previous and subsequent weather variance. To do this, I first chose the duration that WNV was present in mosquitos for each year in each county. I examined the two weeks prior to WNV presence (1A) and compared it to the two weeks prior to that (2A) for each county. Then, I examined the last two weeks of WNV presence (1B) and the two weeks prior to that (2B) for each county; see Figure 4. Two-week intervals prior and following positive WNV to illustrate the average lifecycle of a give mosquito from egg to adult. The idea of two weeks allows a given mosquito to develop into an adult and the ability and time to feed to potential pick up WNV. I created an excel spreadsheet, with a total of four sheets: Monmouth County 1A and 2A, Monmouth County 1B and 2B, Hunterdon County 1A and 2A, Hunterdon County 1B and 2B so that comparisons were straightforward.

Each sheet contained the weekly average minimum temperature, weekly average maximum temperature, and weekly total rainfall for the corresponding weeks for each year 2002 to 2019. Representative sites for rainfall were used for the statistical analysis as stated previously. I calculated the standard deviation of the weekly average maximum temperature and weekly total rainfall, and weekly temperature range for Monmouth County 1A, 2A, 1B, 2B and Hunterdon County 1A, 2A, 1B, 2B. Total rainfall was found for the corresponding weeks in Monmouth County 1A, 2A, 1B, 2B and Hunterdon County 1A, 2A, 1B, 2B. Then, using a Paired T-Test, I found the P-values for Monmouth County 1A, 2A, 1B, 2B and Hunterdon County 1A, 2A, 1B, 2B; see Tables 3 and 4.

There is a level of variance in respect to the minimum and maximum temperature in a given period of time. Narrow ranges in temperature fluctuations may be related to presence of WNV in mosquitos. I determined the weekly temperature range (WTR), difference between the minimum and maximum temperature. I determined the WTR for weeks 2A, 1A, 2B, and 1B for each Hunterdon County and Monmouth County for each year 2002-2019. I entered these values in an excel spreadsheet and used the =AVERAGE function to determine the average of the values for 2A, 1A, 2B and 1B. I then determined the standard deviation of these values by using the =STDEV function; see Tables 2 and 3. The P-values that are less than 0.05 are highlighted in yellow to show that those variables, according to this data, are statistically significant, with the exception of the P-Value for average weekly temperature range in weeks 2A and 2B. This paired t-test statistical analysis allowed me to determine the difference in weather variance and determine if temperature or rainfall were more variable and therefore may have had a more significant impact on the start of WNV.

Species Richness Analysis

Species richness may have an impact on the presence of WNV in a given county. In order to determine the differences in species richness between the two counties. I selected the species found in Monmouth County and Hunterdon County from 2002 to 2019. I then entered them into an excel spreadsheet with a column for each county. I then organized them by alphabetical order to determine the differences in species found between the two counties. I used these data to research the species that were absent in Hunterdon County to determine if the difference in habitat may be the reason for this or if there may be another confounding factor; see Table 4.

Results

WNV Duration

It is evident that WNV presence in mosquitos differed between Monmouth County and Hunterdon County. According to this study, WNV presence in mosquitos on average occurred earlier in Monmouth County at week 28 (July 9 - July 15) and persisted in the mosquito population for about 10 weeks, ending on average in week 38 (September 17 - September 23). In contrast, Hunterdon County experienced the start of WNV presence in mosquitos on average a week later than Monmouth County at week 29 (July 16 - July 22). The average duration in Hunterdon County was 11 weeks and had an average end week of week 40 (Oct 1 - Oct 7). Hunterdon County on average had a slightly longer WNV season in mosquitos than Monmouth County; see Table 1. There was a difference in the start, duration and end week of WNV presence in mosquitos between Monmouth County and Hunterdon County.

Regression Analysis

The regression analysis for total days of rainfall allowed to determine if multiple rainfall days are related with WNV presence in mosquitos rather than total rainfall overall. Total rainfall days between May 1st and Sept 30th were seen to be more strongly consistent with weeks of WNV presence in Hunterdon County than Monmouth County. The r-squared value in the Figure 3: Hunterdon County was 0.1513 which is greater than the r-squared value in Figure 4: Monmouth County of 0.0073. This analysis suggests that total number of rainfall days may be more necessary in Hunterdon County than Monmouth County for WNV presence in mosquitos.

Paired T-test Climate Variation

Paired t-test statistical analysis allowed for the comparison between the weather conditions of Monmouth County and Hunterdon County. Average minimum temperature (P-value: 0.1345), average maximum temperature (P-value: 0.822), total rainfall (P-value: 0.7199) and average weekly temperature range (0.0548) for weeks 2A and 1A in Hunterdon County had P-Values greater than 0.05. Whereas in Monmouth County weeks 2A and 1A had an average minimum temperature (P-value: 0.0044) and average maximum temperature (P-value: 0.0077) has P-Values less than 0.05 but the total rainfall (P-value: 0.4899) and average weekly temperature range (0.6377) were greater than 0.05; see Table 2. Monmouth County for weeks 2A and 1A had higher standard deviations than Hunterdon County for average minimum temperature, average maximum temperature and average weekly temperature range; see Table 2. Standard deviation for total rainfall in Hunterdon County for weeks 2A and 1A were higher than Monmouth County. Average minimum temperature (P-value: 0.0032) and average maximum temperature (P-value: 0.008) for weeks 2B and 1B in Hunterdon County had P-Values less than 0.05. Total rainfall (P-value: 0.1516) and average weekly temperature range (P-Value: 0.2350) for weeks 2B and 1B in Hunterdon County had a P-Value greater than 0.05. For weeks 2B and 1B in Monmouth County average minimum temperature (P-value: 0.0001), average maximum temperature (P-value: 0.0001), average rainfall (P-value: 0.0031) and average weekly temperature range (P-Value: 0.0057) all had P-Values of less than 0.05; see Table 3. Contrasting with weeks 1A and 2A, Hunterdon County for weeks 2B and 1B had higher standard deviations than Hunterdon County for average minimum temperature, average maximum temperature, and total rainfall.

Species Richness

There is an evident difference in the species richness between Monmouth County and Hunterdon County. All the species that were found in Hunterdon were also present in Monmouth County. However, there were thirteen additional species found in Monmouth County that were not present in Hunterdon County; see Table 4. Three of the additional thirteen species found in Monmouth County were identified as salt marsh specialist species

(*Ochlerotatus cantator*, Ochlerotatus sollicitans*, Ochlerotatus taeniorhynchus**; see Table 4). Ocherlotatus is sometimes categorized as a subgenus of Aedes, however, for the purpose of this paper they will be referred to as genus *Ochlerotatus* (Reinert, 2000).

Discussion

WNV Duration & Paired T-test Climate Variation

Hunterdon County seemed to have a later start and additionally a longer WNV season in mosquitos than Monmouth County. It is difficult to determine the cause; however, the relationship is interesting and may be attributed to the multiple factors including the difference in species richness and climate variables. Overall, the data suggest that Hunterdon County seems to have more variable weather conditions than Monmouth County. Although Monmouth County and Hunterdon County are relatively close in proximity to each other, the Atlantic Ocean may be acting as a stabilizer of environmental variation in Monmouth County. The ocean has a large heat capacity that will slow the rate at which the temperature changes on nearby land providing relatively more stable weather conditions than that of an inland community (Stammer et al, 2019). Thus, the proximity of the Atlantic Ocean may be indirectly influencing the dynamics of mosquito density and therefore WNV in Monmouth County.

Hunterdon County displayed P-values in weeks 1A and 2A that were all greater than 0.05, which suggests that this dataset is not statistically significant and that we cannot reject the null hypothesis, that there is no difference. Therefore, the data suggests that environmental conditions weeks 1A and 2A in Hunterdon County were not significantly different. Nonetheless, the P-Value for the average weekly temperature range was 0.0548 and may not be statistically significant but it is biologically significant. The impact of temperature ranges on mosquito development may not be a definite cut off. Due to the closeness of 0.0548 to 0.05, this figure is considered significant for the purpose of this study.

Monmouth County displayed two P-values in weeks 1A and 2A for average minimum temperature and average maximum temperature that were less than 0.05. These low P-values suggest that we reject the null hypothesis. For weeks 1A and 2A, it is reasonable to suggest that average minimum temperature and average maximum temperature may have had a greater influence in the start of WNV presence in mosquitos in Monmouth County. According to the data presented, it is reasonable to suggest that temperature may have a greater influence than rainfall in WNV presence in the mosquito community in Monmouth County and Hunterdon County, New Jersey. Hunterdon County displayed two P-values in weeks 2B and 1B for average minimum temperature and average maximum temp that were less than 0.05. These low P-values suggests that we reject the null hypothesis. However, the P-values for total rainfall and average weekly temperature range in weeks Hunterdon County 2B and 1B were greater than 0.05. Additionally, all four P-values for Monmouth County were less than 0.05. These findings suggest that average minimum temperature, average maximum temperature, total rainfall and average weekly temperature range in Monmouth County may have influenced the start of WNV in weeks 2A and 1A due to the low P-values. However, in weeks 2B and 1B, the low P-values suggests that average minimum temperature and average maximum temperature may have an influence on WNV in both counties. Total rainfall may have also been related the presence of WNV in Monmouth County for weeks 2B and 1B. Total rainfall was analyzed using a single representative site in each county. Therefore, the rainfall is most likely not consistent throughout the county in a given week in either county. Rainfall could influence the results to be more skewed toward the region of the representative site rather than the county as a whole.

There is a noticeable difference in the mechanisms by which WTR may impact WNV presence in mosquitos. The temperature fluctuations may influence the presence of WNV by influencing the mosquito population directly. Large fluctuations in temperature in a short period of time may be too drastic for a mosquito to withstand. As expected, the weeks 2A and 1B had larger WTR than weeks 1A and 2B. This may suggest that large temperature fluctuations are not ideal for mosquitos and therefore WNV presence in mosquitos. The According to this study, a WTR greater than 22.38°C may have a negative influence on mosquitos and their ability to carry WNV. However, the WTRs are very close in number, so it is difficult to determine an exact WTR.

WNV and Rainfall Regression

There is a difference between the regression of total rainfall days and duration of positive WNV in Hunterdon County and Monmouth County. The data presented suggests that total number of rainfall days in Hunterdon County have a large impact on WNV presence in mosquitos than Monmouth County. Monmouth County is at a relatively low altitude of 107 meters compared to Hunterdon County of 264 meters (Monmouth, 2018) (Hunterdon, n.d.). Altitude may also influence more flowing water from high to low altitudes in Hunterdon County more than Monmouth County. Monmouth County also has more standing freshwater than Hunterdon County. For these reasons, total number of rainfall days may not be as essential for mosquitos in Monmouth County due to increase standing water.

Species Richness

There is an obvious difference in the richness diversity in Monmouth County and Hunterdon County. As expected, Monmouth County provides a unique habitat that Hunterdon County does not seem to have. Thus, Monmouth County is able to support a more diverse set of mosquito species. Thirteen additional species were found in Monmouth County, and three of those were identified as saltmarsh specialist species which are known vectors in New Jersey for WNV. The other ten species reside inhabitants such as freshwater marsh, acid water bogs, tree holes, grassland depressions and floodwater ponds (bugguide.net, n.d.) (NEW JERSEY’S, n.d.). However, it is important to note that Monmouth County contains more urbanized and developed regions than Hunterdon County. The increased development of Monmouth County allows for additional habitat and egg-laying areas such as containers which are commonly found in urbanized areas. Mosquito abundance and species richness in rural areas will differ from those or urban areas. Some environmental factors that may influence mosquito abundance and species richness could be “land use, vegetation and hydrological factors” (Ferraguti et. al, 2016). It is also possible that the variation in species between the counties may influence difference in the start, duration and end of WNV presence in mosquitos.

There are many factors that influence WNV in a given population. For the purpose of this study, it is difficult to draw any direct conclusions to the presence and dynamics of the relationship between climate and WNV presence in mosquitos. Confounding factors may include additional abiotic factors such as atmospheric pressure, wind speed and availability of larval habitat or biotic factors such as overwintering vectors and predators such as larvae-eating fish or insectivorous birds and bats. The previous year's control efforts may have an influence on the number of overwintering individuals. The data shown in this study indirectly shows relationships between climate variance and presence of WNV in mosquitos. Ultimately, for the presence of WNV to exist in a mosquito, there needs to be a mosquito present. The age of a mosquito is crucial to the ability of that mosquito to be a vector for WNV. A female mosquito's chances of contracting WNV increases which each host she feeds. This study may infer the mosquitos tested for WNV are also older in age therefore becoming more efficient vectors than those who are younger and did not feed. The longevity of the mosquito herself will directly impact the ability for her to become a WNV vector. This study aimed to determine factors that may influence mosquito density to consider WNV presence consistent with these findings.

Additionally, the results presented in this study only reflect conclusions drawn from the data analyzed and climate variation (temperature and precipitation) being the influencing factor. According to the data presented, temperature seems to have a greater influence on WNV presence in mosquitos than total rainfall. In the future, I would like to explore the rainfall ranges that which WNV presence was consistent with. Rainfall is integral for mosquito development; determining how much could vary between species, but there may be a specific weekly range needed. I would also like to explore bird densities in comparison to the start, duration and end of WNV. Birds are known as reservoir hosts of WNV where the pathogen can amplify before being transmitted to an uninfected mosquito. Although significant mortality rates are seen in American Crows and other corvid species, it is thought that migrating waterfowl species may have the ability to carry WNV to and from their migration site (Peterson et al, 2004) (McLean, 2002). By using eBird.org, I could record the overall bird densities and species richness seen during the start, duration and end of WNV. It will be interesting to note any avian species presence consistent with the start, duration or end of WNV in Monmouth and Hunterdon County.

Mosquitos are known as the deadliest animal in the world. As vectors for lethal pathogens worldwide, it is important to understand their biology and mechanisms of disease transmission. In this study, I aimed to understand the relationships that may be present between WNV presence in mosquitos and climate variation. It may be reasonable to suggest that as climate change continues to impact the Earth to experience warmer and wetter summers, mosquito populations may increase and therefore, WNV presence in mosquitos may increase by sheer number of individuals infected or duration present. Identification and understanding of the relationships that may or may not be present is beneficial for mosquito control and public health officials. The knowledge of these relationships may also be used to predict future WNV outbreaks and could be used to implement increased control efforts to prevent these possible outbreaks.

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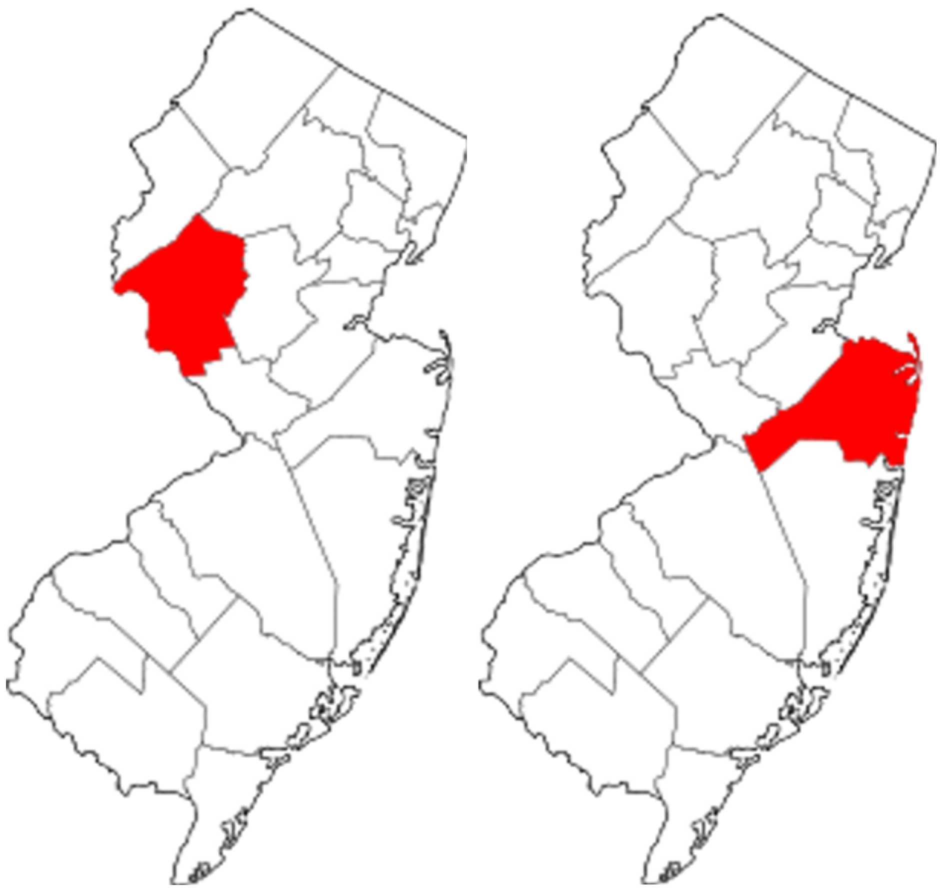
Figures

Figure 1: Hunterdon County (left) and Monmouth County (right)



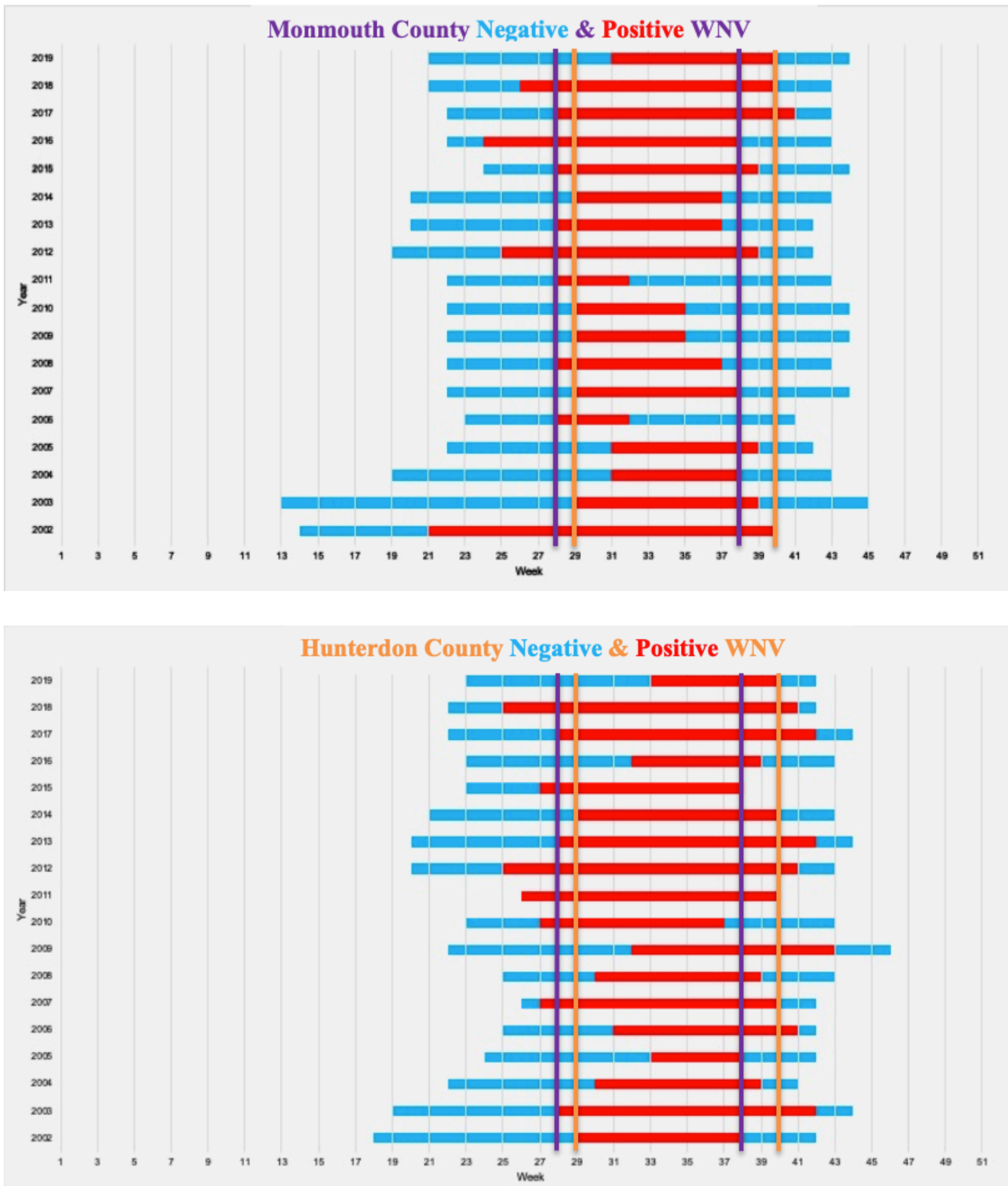


Figure 2: Positive and Negative WNV testing in Hunterdon County and Monmouth County

This figure shows the weekly testing results from 2002-2019 in Hunterdon County and Monmouth County. The blue bars represent the negative results for WNV testing, red bars represent the positive results for WNV testing. Purple lines represent the start and end of WNV in Monmouth County, Orange lines represent the start and end of WNV in Hunterdon County – color coordinated with Table 1.

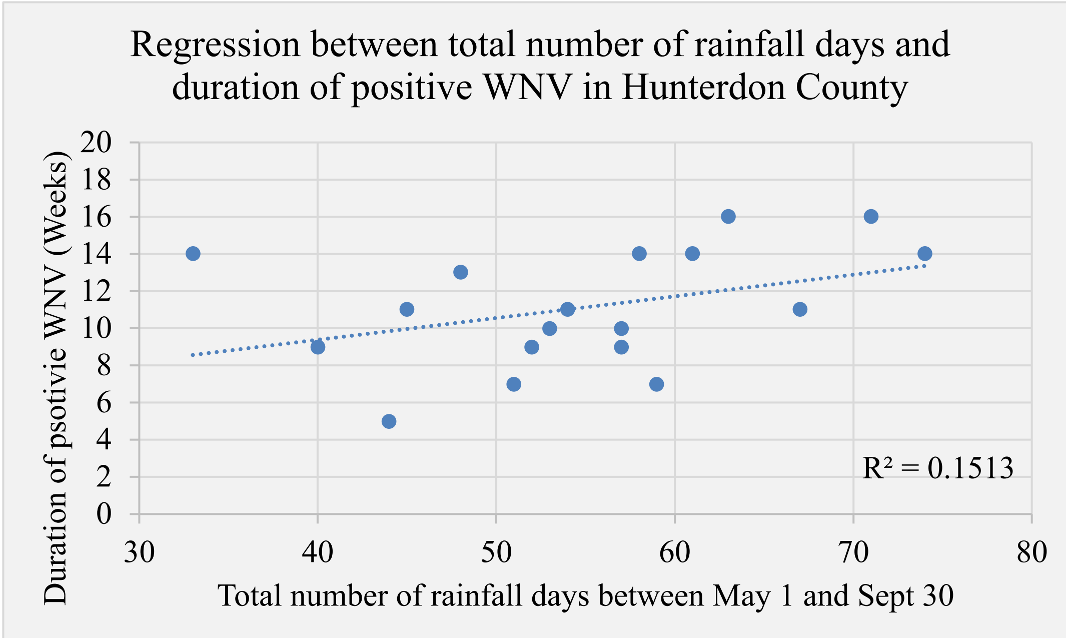


Figure 3: Rainfall and WNV Regression Hunterdon County

This figure shows the regression between Duration of positive WNV and Total number of rainfall days in Hunterdon County

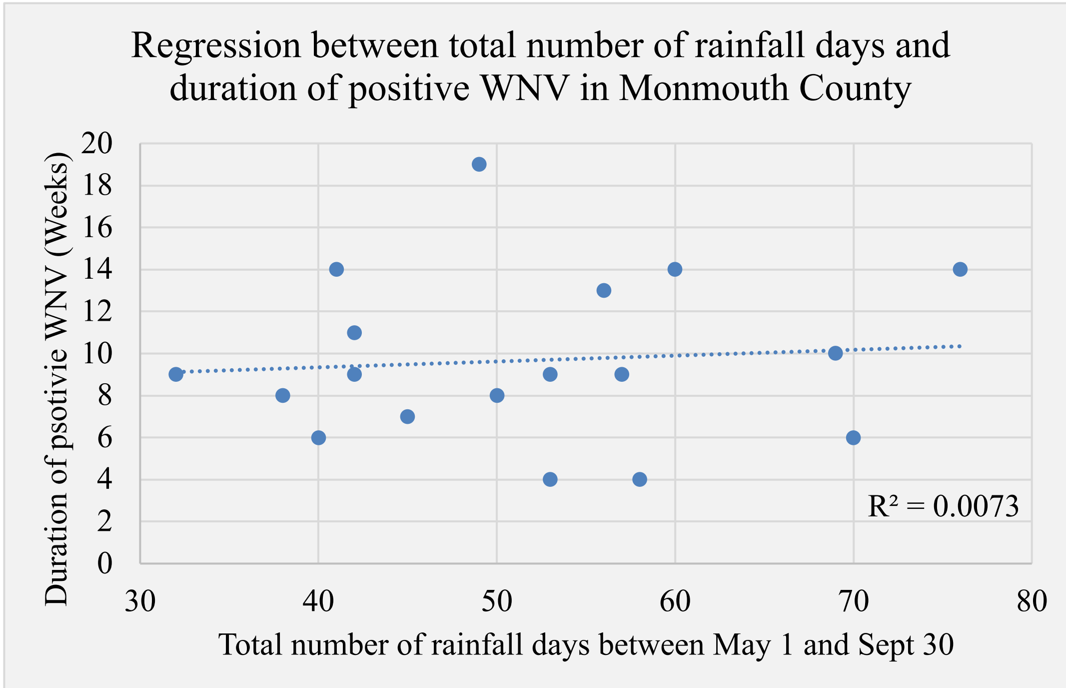


Figure 4: Rainfall and WNV Regression Monmouth County

This figure shows the regression between Duration of positive WNV and Total number of rainfall days in Monmouth County

Figure 5: Visual representation of positive WNV statistical analysis.

2A: 2 weeks prior to 1A, 1A: 2 weeks prior to the start of positive WNV, 2B: 2 weeks prior to 1B, 1B: last 2 weeks of positive WNV

Tables

Table 1: Start, duration and end of WNV in Monmouth and Hunterdon County

	Monmouth County	**Hunterdon County**
Average start of WNV presence in mosquitoes	July 9 – July 15 (Week 28)	July 16 – July 11 (Week 29)
Average duration of WNV presence in mosquitos	10 weeks	11 weeks
Average end week of WNV presence in mosquitos	Sept 17 – Sept 23 (Week 38)	Oct 1- Oct 7 (Week 40)

Table 2: 2A and 1A: 4 Weeks Prior to Start of Positive WNV

Table shows the value, standard deviation and p-value for the average min temp, average max temp and total rainfall for weeks 2A and1A in Monmouth and Hunterdon County. The colors correspond with the color blocks in Figure 3. The P-values that are less than 0.05 (statistically significant) are highlighted in yellow.

	Hunterdon			**Monmouth**		
	2A	**1A**	**P-Value**	**2A**	**1A**	**P-Value**
Avg Min Temp	16.31°C (SD: 1.82)	17.13°C (SD: 2.12)	0.1345	15.56°C (SD: 3.75)	17.64°C (SD: 2.67)	0.0044
Avg Max Temp	28.14°C (SD: 2.01)	28.96°C (SD: 2.31)	0.822	26.36°C (SD: 3.59)	28.01°C (SD: 2.54)	0.0077
Total Rainfall	59.28 mm (SD: 30.4)	65.3 mm (SD: 54.1)	0.7199	50.81 mm (SD: 32.8)	60.02 mm (SD:29.9)	0.4899
Avg Weekly Temp Range	22.38°C (SD: 2.64)	20.75°C (SD: 3.01)	0.0548	22.32°C (SD: 3.70)	21.72°C (SD: 3.30)	0.6277

Table 3: 2B and 1B: Last 4 Weeks of Positive WNV

Table shows the value, standard deviation and p-value for the avg min temp, avg max temp, total rainfall and avg weekly temp range for weeks 2B and 1B in Monmouth and Hunterdon County. The colors correspond with the color blocks in Figure 3. The P-values that are less than 0.05 (statistically significant) are highlighted in yellow.

	Hunterdon			**Monmouth**		
	2B	**1B**	**P-Value**	**2B**	**1B**	**P-Value**
Avg Min Temp	12.08℃ (SD: 3.23)	10.46℃ (SD: 2.88)	0.0032	17.36℃ (SD: 2.04)	15.25℃ (SD 2.37)	0.0001
Avg Max Temp	25.17℃ (SD: 3.49)	22.91 ℃ (SD: 3.89)	0.008	28.06℃ (SD: 2.23)	24.43℃ (SD: 2.45)	0.0001
Total Rainfall	31.97 mm (SD: 35.6)	54.11 mm (SD: 54.4)	0.1516	22.09 mm (SD: 19.6)	46.47 mm (SD: 29.4)	0.0031
Avg Weekly Temp Range	25.10℃ (SD: 3.35)	26.10℃ (SD: 2.73)	0.2360	22.14℃ (SD: 2.31)	24.70℃ (SD: 3.32)	0.0057

Table 4: Mosquito species of Monmouth County and Hunterdon County

Saltmarsh specialist species only found in Monmouth County are indicated by *.

Monmouth County	**Hunterdon County**
Aedes albopictus	*Aedes albopictus*
Aedes vexans	*Aedes vexans*
Anopheles barberi	*Anopheles barberi*
Anopheles bradleyi	*Anopheles bradleyi*
Anopheles crucians	*Anopheles crucians*
Anopheles earlei	*Anopheles punctipennis*
Anopheles punctipennis	*Anopheles quadrimaculatus*
Anopheles quadrimaculatus	*Coquillettidia perturbans*
Coquillettidia perturbans	*Culex species*
Culex species	*Culiseta inornata*
Culiseta inornata	*Ochlerotatus canadensis*
Culiseta melanura	*Ochlerotatus japonicus*
Ochlerotatus abserratus	*Ochlerotatus sticticus*
Ochlerotatus atlanticus	*Ochlerotatus triseriatus*
Ochlerotatus canadensis	*Ochlerotatus trivittatus*
*Ochlerotatus cantator**	*Psorphora ciliata*
Ochlerotatus excrucians	*Psorphora columbiae*
Ochlerotatus grossbecki	*Psorphora ferox*
Ochlerotatus hendersoni	*Psorphora howardii*
Ochlerotatus japonicus	*Uranotaenia sapphirina*
*Ochlerotatus sollicitans**	
Ochlerotatus sticticus	
*Ochlerotatus taeniorhynchus**	
Ochlerotatus thibaulti	
Ochlerotatus triseriatus	
Ochlerotatus trivittatus	
Orthopodomyia signifera	
Psorphora ciliata	
Psorphora columbiae	
Psorphora cyanescens	
Psorophora ferox	
Psorophora howardii	
Uranotaenia sapphirina	