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Ecological Effects On The Distribution of Culex Tarsalis Mosquitos

Paige Plancich
Carroll College, Helena, MT

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Paige Plancich

Department of Life and Environmental Sciences, Carroll College, Helena, MT

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ABSTRACT

West Nile Virus (WNV) is an increasing problem worldwide due to vector competence and enzootic transmissibility between vector and host. The most prevalent vector of WNV in the state of Montana is the *Culex tarsalis* mosquito, which is found in varying ecological conditions such as shallow, standing water and at high temperatures (14°C – 34°C). These conditions are not well understood in Montana, thus limiting predictions concerning potential outbreaks. This study tested the ecological effects on *C. tarsalis* and found that land cover and May precipitation were significant factors that affect the ability of *C. tarsalis* to thrive in Montana, with May precipitation being negatively associated with *C. tarsalis* abundance. Land cover classified as sagebrush steppe had a greater abundance of *C. tarsalis* than did lowland prairie grassland and wetland riparian, with modified agricultural lands having the lowest abundance of *C. tarsalis*. A better understanding of the predictive ecological factors of *C. tarsalis* will allow state officials to predict when outbreaks may become prevalent.

INTRODUCTION

West Nile virus (WNV) was first isolated in Uganda in 1937 and eventually spread to other parts of Africa, Asia, Europe, the Middle East and North America. Initially, the virus did not cause mortality rates to increase in any mammalian hosts (Molaei et al., 2010). However, in the mid-1990s a sporadic outbreak in human hosts led to encephalitic WNV and has since spread and ultimately caused an increased interest in understanding this infectious disease (Friesen and Johnson, 2014).

WNV is an RNA arbovirus (arthropod-borne virus), that is an enveloped, single-stranded strain involved in an enzootic cycle of transmission between avian hosts and varying species of *Culex* mosquitos as well has humans (Worwa et al., 2015). The virus is inherited as autosomal dominant trait which can be compromised by inheritance via the male parent (Danforth et al., 2016). The overall fitness of the virus within the vector can be characterized by the concept of dose-dependent susceptibility to infection, which includes the initial viral replication, dissemination and then infection with replication (Worwa et al., 2015).

Transmission occurs via three mechanisms: 1) horizontal enzootic transmission, 2) vertical transmission via the mother mosquito to her progeny, and 3) females that are fated for diapause during overwintering (Danforth et al., 2016). Due to the limited alternate replication between the host and the vector, genetic variability is limited in the arbovirus (Worwa et al., 2015). Vertical transmission rates can be affected by rearing temperature, species of the vector, competency of the vector, the specific viral strain and abiotic factors as well as viral load as the egg matures (Nelms et al., 2013). In addition, larvae that inherit the virus through vertical transmission are not compromised due to differences in pupation rates and their ability to

become competent vectors (Dodson et al., 2012). Pupation rates have only shown an effect on the phenotypic inheritance of the mosquito, and have not altered the arbovirus transmission rate (Dodson et al., 2012). The phenotypic variances occur during varying ecological conditions including pH changes in the water and soil, emergent vegetation, temperature, and wind velocities (Dodson et al., 2012). In order to effectively pass the virus horizontally or vertically, the vector must pass through varying physical and physiological barriers, inclusive of extrinsic incubation periods, and environmental temperature (Kenny and Brault, 2014). Passing these barriers allows the virus to continually be carried by the infected mosquito and thus potentially be passed on to its host (Kenny and Brault, 2014).

Abiotic factors as well as intrinsic factors affect the competency of the vector (Kramer and Ciota, 2015). Abiotic interactions include: temperature, rainfall, land use, nutrition, population density and the vector-host contact (Kramer and Ciota, 2015). The virus requires a certain infection threshold in order to successfully infect its vector (Kramer and Ciota, 2015). These threshold variances vary between the virus and the species of the vector. The extrinsic incubation period affects the ability of the virus to be viable as well. This period is defined as the time between the ingestion of the infected blood by the vector to the transmission of the virus (Danforth et al., 2015). The temperature at which extrinsic incubation occurs is also a factor that determines the transmissibility of the virus to the host (Kramer and Ciota, 2015). Under laboratory conditions, the temperature threshold for viral replication in *C. tarsalis* ranges between 14°C and 35°C (Paz, 2015). This range of temperature allows for ultimate vector competence.

Higher than normal temperatures are linked to the outbreaks of WNV (Hoover and Barker, 2016). Changes within the weather patterns directly affect the transmission rates of WNV between the pathogen, host and vector (Hoover and Barker, 2016). These changes can also indirectly affect transmission rates via the changes in the ecosystem, such as the temperature of the water in which the mosquitos breed (Hoover and Barker, 2016). The rates of transmission increase with temperatures that are greater than 22°C (Danforth et al., 2016). Viral amplification can occur during warmer winters and springs (Paz, 2015). An increase in temperature accelerates larval development and decreases the generation time of the vector (Hoover and Barker, 2016). In North America, temperature is the main component that determines the magnitude and the timing of the increasing infection rate (Paz, 2015).

Progenies that are vertically infected may increase viral amplification in the summer months when horizontal transmission is also at its highest (Nelms et al., 2013). In addition, during the larval stage to the pupal stage, the virus can be degraded (Nelms et al., 2013). Although it remains unclear at what point the viral degradation begins, it is clear that it can be lost at some point during larval development (Nelms et al., 2013). Degradation most likely occurs due to environmental changes such as drastic temperature fluctuations (Nelms et al., 2013). In naturally occurring mosquito populations it is likely that temporal fluctuations influence vector competence (Richards et al., 2010). Most of these findings were investigated under ideal laboratory conditions and *in vitro* measures, thus it is important to recognize that measures also need to be investigated *in vivo* and not only under ideal laboratory conditions (Worwa et al., 2015).

The effects of the ecological conditions on *C. tarsalis* are not well understood in the state of Montana and due to this limited knowledge, Montana residents, as well as wildlife, face the threat of WNV becoming an emergent problem. Although *Culex* species have high transmission rates for WNV and other arboviruses, *C. tarsalis* is one of the main vector species in the state of Montana in the transmission of WNV. Several investigations in Chicago, Los Angeles, and Sacramento tested the transmission rates and the needs of the virus (e. g. shallow, standing waters, and consistent hot weather), while only a few investigations have been performed in Montana (Wimberly et al., 2014). It has been hypothesized that these environmental factors highly influence the risk of vector-borne and enzootic diseases of WNV (Wimberly et al., 2014).

The goal of this study was to test if pH changes, varying temperatures, presence of emergent vegetation, and varying wetlands affect the survival and reproduction abilities of *C. tarsalis* in Montana. Since each species has a fundamental niche, this project aimed to test the hypothesis that varying ecological conditions, such as an increase in temperature, presence of emergent vegetation and shallow, standing water, would increase the ability of *C. tarsalis* to effectively survive. These observations, in turn, may aid in the formulation of a predictive model that could alert potentially affected entities.

METHODS

Field Collection:

Study sites were mostly predetermined using Google Earth, augmented by incidental encounters with wetlands in the field, and included kettle ponds, stock ponds, and intermittent streams,

each with varying amounts of water and vegetation present. Each site was given a site number, locality name and a CO_2 trap that was set up near the body of water and left for collection overnight. Dry ice was placed in a cooler with holes in substitution of using a CO_2 tank. When traps were collected in the morning, independent variables were measured (Table 1). Collected mosquitos were placed in a cooler with dry ice to preserve the competency of the vector thus allowing the mosquitos to remain competent carriers of the virus.

Table 1. Independent variables that were measured during field collection.

Category	**Measurement**
soil, water	pH*
soil, water, air	temperature (°C)*
GPS (decimal degrees)	-
elevation	-
emergent vegetation	type, % of coverage
body of water	length and width
inlet or outlet	presence or absence
other animals	presence or absence
human modifications	presence or absence

*A Vernier LabQuest 2.0 reader with a Vernier pH reader and thermometer were used to measure.

Sorting:

Samples were stored at -20°C or -80°C until no mosquito movement was observed (approximately two to four hours). Each sample was then sorted in the Wiegand Undergraduate Research Laboratory at Carroll College (biosafety level II) in Helena, Montana. Portions of each sample were placed in a petri dish on an ice pack and sorted under a microscope. *C. tarsalis* mosquitos were separated and identified as "target" species. whereas all other mosquitos were identified as "non-target" species. The numbers of target mosquitos and non-target mosquitos were recorded and all mosquitos were placed in a -20°C freezer until extraction (target species) or further identification at Montana State University (non-target species).

Extraction:

C. tarsalis mosquitos were placed into labeled 1.5mL microcentrifuge tubes, not exceeding 50 mosquitos per tube. If the total number of *C. tarsalis* exceeded 50 mosquitos from a given site, they were split into groups of 50 mosquitos or less and labeled in alphabetical order. Samples were then homogenized and had their RNA extracted using a QIAGEN miniprep kit for fibrous tissue (Qiagen Inc.). The only exceptions made were the use of metal BBs in homogenization, as well as the addition of 1,000μl of RNAlater and 500μl of buffer RLT for samples of more than 10 mosquitos or the addition of 600μl of RNAlater and 300μl of buffer RLT for samples of less than

10 mosquitos. Extraction was completed by the QIAcube in increments of up to 12 samples under Protocol 372.

Polymerase Chain Reaction:

A TaqMan real-time polymerase chain reaction (RT-PCR) was used to identify if WNV was present. A rapid detection of the WNV protocol for TaqMan RT-PCR was followed with the primer sets and their respective probes in Table 3 (Lanciotti et al., 2000). Primers and probes were diluted according to the respective amount (nm) that was in the stock solutions. Samples were loaded into PCR plates (2.5μl) with their respective master mix (17.5μl; Table 2) and analyzed using the iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) following the WNV protocol (Lanciotti et al, 2000). A positive sample from previous weeks of research was used as a control while RNase free water was used as a negative control.

Table 2. The amount of each component of master mix needed to fill one well in the PCR plate.

Amount (μl)	**Component**
9.5	RNase free water
1	Working probe
1	Working forward primer
1	Working reverse primer
5	4x TaqMan

Table 3. Primers and probes with their respective sequences (Lanciotti et al., 2000).

Probe Set	Primer Set	Primer Sequence
WNV 3	3PRIME Reverse	CTAGGGCCGCGTGGG
	3PRIME Forward	CAGACCACGCTACGGCG
WNV ENV	ENV Forward	TCAGCGATCTCTCCACCAAA
	ENV Reverse	GGGTCAGCACGTTTGTCATTG

Analyzing PCR Graphs:

Samples positive for WNV expressed the same curvature as the positive control, whereas samples negative for WNV expressed a linear line similar to the negative control. Positive samples were reported to the Department of Public Health and Human Services in Helena and confirmed by state testing. These samples were stored at -80°C and used as positive controls in the following weeks. Negative samples were discarded.

Analyzing Raw Data: Preliminary Screening

Field data were compiled into EXCEL to determine significant factors influencing the distribution of *C. tarsalis*. Regression analysis and ANOVA tests were performed to identify statistical significance between continuous data (regression analysis) and the dependent variables as well as discrete data (ANOVA) and dependent variables. Dependent variables were identified as: 1) the total number of mosquitos present in each trap, 2) the abundance of *C. tarsalis* present in

each trap, and 3) the percentage of *C. tarsalis* present in each trap. Continuous data were identified as elevation, air temperature (°C), heating degree days (HDD, the number of degrees that the average temperature is above 14°C), water temperature (°C), water pH, soil temperature (°C), site length, site width, average normalized difference of vegetation index (NDVI), June NDVI, May, July, and August precipitation and bird diversity. Discrete data were identified as land cover (e.g. floodplain riparian, introduced vegetation agriculture, lowland prairie grassland, shrub land, steppe and savanna systems and wetland riparian water), depth of the water at the site, emergent vegetation, the percentage of the site with emergent vegetation, habitat type (e.g. intermittent creek, kettle pond, oxbow, stock pond and wetland) and water permanence (e.g. permanent or temporary).

Accounting for Normality

Raw data that suggested statistical significance were tested for parametric assumptions. Skewness and kurtosis were determined using the descriptive statistics tool in EXCEL, skewness and kurtosis were determined. By taking the standard error of skewness, the Z statistic could be found, allowing significant skewness to be determined (greater than two or less than negative two; Brown, 2016). The same principle was applied to kurtosis. If significant skewness was apparent, then the natural log of the data was calculated and skewness and kurtosis were tested again. If the natural log did not correct for significant skewness the $\log_{10}$, and the square-root of the data were used.

Testing Transformed Data

After correcting for skewness, the raw data that showed statistical significance were analyzed again using regression and ANVOA.

RESULTS

Of the 42 regression tests on the raw data, only 11 showed statistical significance, or close to statistical significance (Appendix, Tables 6 and 8). In addition, of the 18 ANOVA tests, nine independent variables suggested statistical significance or close to statistical significance (Appendix, Tables 7 and 9). After correcting for parametric assumptions, the number of significant results decreased (Tables 4 and 5). Only one regression analysis and one ANOVA analysis suggested statistical significance (Appendix, Tables 10 and 11). A significant negative correlation was found between the *C. tarsalis* abundance and May precipitation ($p = 0.0486$), as well as land cover ($p = 0.0146$; Figs. 1 and 2). Sixteen mosquito pools were tested for WNV and PCR analysis suggested that no sample was positive for WNV (Appendix, Table 12).

Table 4. Normalized regression data suggesting statistical significance ($p \leq 0.05$).

Dependent Variable	**Independent Variable**	**Coefficient**	**Degrees of Freedom**	**T- statistic**	**P-Value**
C. tarsalis abs	May precipitation	-65.729371	14	-2.160267	0.04857847

Table 5. Normalized ANOVA data suggesting statistical significance ($p \leq 0.05$).

Dependent Variable	Independent Variable	P-Value
C. tarsalis abs	land cover	0.015

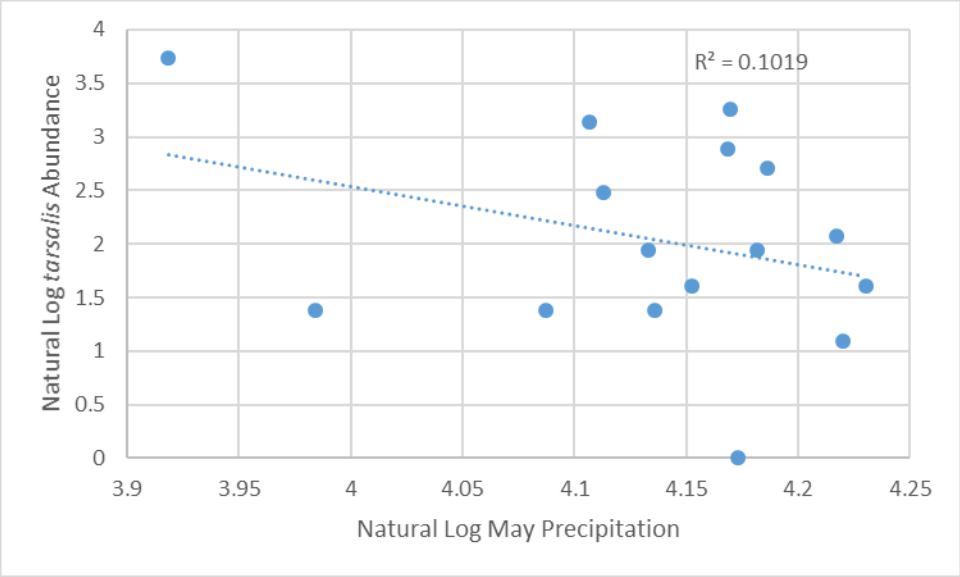

Figure 1. Regression analysis of significant normalized data ($p \leq 0.05$). The natural log of May precipitation vs the natural log of *C. tarsalis* abundance suggests a negative relationship and R^2=0.102.

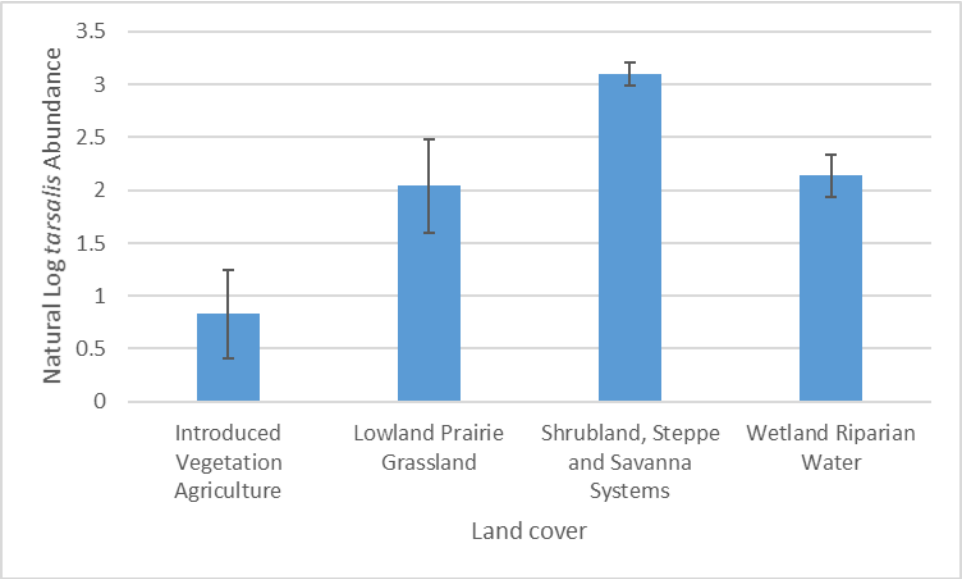

Figure 2. ANOVA analysis of the average of significant normalized data for the natural log of *C. tarsalis* abundance and land cover ($p \leq 0.05$).

DISCUSSION

The goal of this study was to determine what ecological factors affect the distribution of *C. tarsalis* in Montana. I hypothesized that long, consistent hot weather (temperatures around or above 33°C), the presence of emergent vegetation and shallow, standing water would be ecological factors aiding in population growth of *C. tarsalis* in Montana (Hokit et al., in review). Certain ecological effects, such as warmer winters and warmer springs can increase the presence and transmission of WNV from *C. tarsalis* (Paz, 2015). My results suggest that there is a correlation between two ecological factors and *C. tarsalis* abundance. Differences in land cover and early summer precipitation rates correlate to abundance rates of *C. tarsalis*, thus supporting my hypothesis. The main component in North America that determines the rate and magnitude of infection rate is temperature (Paz, 2015). It has been generally found across the US that temperature has risen and precipitation rates have increased (Paz, 2015). A similar correlation

was found in Canada, where the main predictors of WNV incidences were an increase in temperature overall and a decrease in rainfall into July (Paz, 2015). In European countries, it has been found that an increase in temperature, heat wave frequency and intensity have contributed to outbreaks of WNV (Paz, 2015). My study did not find that temperature and heating degree days were significant factors. This could have been due to the limited number of collection days, and the limited area that I was able to cover.

The negative trend correlating to May precipitation and the abundance of *C. tarsalis* mosquitos indicates that as May precipitation increases there is a decrease in the abundance of *C. tarsalis* mosquitos. In other studies, precipitation had both positive and negative correlations which were dependent upon the geographic area and the time at which the data were collected (Wimberly et al., 2014). This could be due to the relationship between early season with high precipitation correlating to low numbers of tarsalis. Data for temperature and precipitation had been applied from the years 1981 to 2010 across the United States and significant differences were found between eastern and western Colorado (Wimberly et al., 2014). Eastern Colorado had a strong positive correlation with moist springs and dry summers and in western Colorado a much weaker correlation was found that had dry conditions in both the spring and summer (Wimberly et al., 2014). Kenney and Brault (2014) also found a positive association between drought and an increase in mosquito infection rates in 2010.

Precipitation has been found to impact the presence and abundance of *C. tarsalis* by varying degrees. Storm water management in urban areas during times of drought supplies a regular supply of water from irrigation systems (Hoover and Barker, 2015). Irrigation runoff and pooled water in fields supply a mixture of flood water and organic matter suitable to mosquitos

to development (Hoover and Barker, 2015). Agricultural and urban irrigation contribute to the confusion surrounding the role of precipitation in the abundance of *C. tarsalis*. In scenarios involving irrigation and dry seasons, there can be an increase in the number of mosquitos, because floodwater provides a suitable habitat for mosquitos to thrive, with an increase in larval breeding sites consisting of fewer competitors and mosquito predators (Paz, 2015). These situations provide a more suitable habitat for floodwater mosquitos (e.g. collections of water that are temporary, and usually found in pastures or on the side of the road) and not *C. tarsalis*, thus suggesting an increase in the total number of mosquitos present, but not necessarily the number of *C. tarsalis* present.

The statistically significant effect of land cover on the abundance of *C. tarsalis* ($p = 0.015$) is similar to a study by Hoover and Barker (2015) that also suggest that land cover influence is as water important as water distribution is affected by vegetation (or the lack of vegetation) and land usage. Type of land cover was an important factor in determining *C. tarsalis* habitat types (Hokit et al., in review). Comparable with the results of this study, Hokit et al (in review) found that wetlands were also associated as suitable habitats of *C. tarsalis*. Although my study found that shrub land, steppe and savanna systems had a higher rate of *C. tarsalis*, wetlands also had a higher abundance of *C. tarsalis* compared to introduced vegetation agriculture.

My conclusion that land cover is associated with *C. tarsalis* habitat suitability agrees with Bowden et al. (2011) who concluded that the extent to which habitats are suitable differ at the regional level, but that urban land is positively associated with WNV disease. They also found that *C. tarsalis* was positively associated with agricultural land (Bowden et al., 2011). My

study suggested that shrub land, steppe and savanna systems were more determinative of *C. tarsalis* abundance. However, my study was limited to public lands, mostly in the Charles M. Russel wildlife area, which is not highly composed of agricultural aspects. This narrow scope of study limited the potential for agricultural lands to express significance.

The present study was completed during the summer of 2016 (June to August) thus limiting the number of data collection sites. In addition, restricting the study to a small portion of eastern Montana could significantly skew the results and not provide a comprehensive list of significant contributing factors. Other investigations have suggested that temperature is one of the main factors influencing the abundance of *C. tarsalis* and its ability to remain a competent vector of WNV (Paz, 2015; Wimberly 2015). Also, Danforth et al. (2016) found that temperature played a main role in the transmission of WNV and suggested that temperature fluctuations affected the extrinsic incubation periods. The present study did not suggest a correlation with temperature as a key factor. The narrowly focused geographic region did not provide extensive variation in temperature which could result in an inability to detect trends associated with temperature. Further investigations should be completed to fully understand the ecological factors, especially temperature, that affect *C. tarsalis* mosquitos.

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APPENDIX

Table 6. Regression analysis of raw data returned p-values suggesting statistical significance ($p \leq 0.05$). For completeness, data around 0.05 were accounted for, up to $p \leq 0.1$.

Dependent Variable	**Independent Variable**	**P-Value**
C. tarsalis abs	elevation	0.031
C. tarsalis abs	air temperature (°C)	0.016
C. tarsalis abs	HDD	0.101
C. tarsalis abs	May precipitation	0.056
C. tarsalis abs	August precipitation	0.173
% *C. tarsalis*	June NDVI	0.149
% *C. tarsalis*	bird diversity	0.122
total mosquitos	air temperature (°C)	0.016
total mosquitos	water temperature (°C)	0.021
total mosquitos	soil temperature (°C)	0.013
total mosquitos	June NDVI	1.07e-10

Table 7. ANOVA analysis of raw data returned p-values suggesting statistical significance ($p \leq 0.05$). For completeness, data around 0.05 were accounted for, up to $p \leq 0.1$.

Dependent Variable	**Independent Variable**	**P-Value**
C. tarsalis abs	land cover	0.158
C. tarsalis abs	emergent vegetation	0.021
C. tarsalis abs	habitat type	0.046
% *C. tarsalis*	land cover	0.097
% *C. tarsalis*	water permanence	0.074
total mosquitos	land cover	0.113
total mosquitos	habitat type	6.59e-05
total mosquitos	water permanence	0.163

Table 8. Regression analysis of each independent variable. Statistical significance was measured by $p \leq 0.05$ and $p \leq 0.1$.

Dependent Variable	Independent Variable	Coefficient	Test statistic	P-Value
% *C. tarsalis*	elevation	-0.002250138	-0.124354007	0.902802836
total mosquitos	elevation	-0.188339115	-0.265217345	0.794707236
C. tarsalis abs	elevation	0.018759574	2.403022867	0.030690702
% *C. tarsalis*	air temperature	-0.529770334	-0.37115709	0.716076848
total mosquitos	air temperature	124.9877512	2.750257645	0.015637071
C. tarsalis abs	air temperature	-1.189839607	-1.795412191	0.094197645
total mosquitos	HDD	4.461688681	0.882888395	0.3922048
C. tarsalis abs	HDD	-0.107413804	-1.752915788	0.101475918
% *C. tarsalis*	HDD	-0.079660856	-0.611284433	0.550808593
total mosquitos	water temperature	166.029806	2.607915972	0.020656541
C. tarsalis abs	water temperature	-0.487304842	-0.485883249	0.63456658
% *C. tarsalis*	water temperature	-0.376898778	-0.191230007	0.851090986
total mosquitos	water pH	62.12005635	0.433636984	0.673757516
C. tarsalis abs	water pH	-1.670607037	-0.625868561	0.545420058
% *C. tarsalis*	water pH	-6.991171782	-1.346379038	0.207903745
total mosquitos	soil temperature	178.287014	2.965168103	0.012855938
C. tarsalis abs	soil temperature	-0.149028278	-0.151498819	0.882324988

% *C. tarsalis*	soil temperature	-0.042412517	-0.092387363	0.928051398
total mosquitos	site length	0.185318315	0.06735293	0.947253191
C. tarsalis abs	site length	-0.009841501	-0.275152297	0.787218379
% *C. tarsalis*	site length	-0.058469295	-0.857115334	0.405814603
total mosquitos	site width	-5.268259138	-0.989389684	0.339264984
C. tarsalis abs	site width	-0.049372908	-0.699763406	0.495538958
% *C. tarsalis*	site width	0.03025488	0.21636275	0.831825899
total mosquitos	average NDVI	-3245.818762	-0.841440725	0.414243239
C. tarsalis abs	average NDVI	-30.49745532	-0.599411613	0.558474859
% *C. tarsalis*	average NDVI	-122.4885335	-1.288400413	0.218492965
total mosquitos	June NDVI	1.158027345	16.86540545	1.06933E-10
C. tarsalis abs	June NDVI	-45.51619595	-1.091466319	0.293496006
% *C. tarsalis*	June NDVI	-119.7875345	-1.526181915	0.149235523
total mosquitos	May precipitation	1.935720041	0.042684719	0.966555669
C. tarsalis abs	May precipitation	-1.076674237	-2.08546532	0.055812454
% *C. tarsalis*	May precipitation	0.127739692	0.110804042	0.913344549
total mosquitos	July precipitation	38.78544263	0.57858241	0.572062058
C. tarsalis abs	July precipitation	0.07555021	0.085484463	0.933086737
% *C. tarsalis*	July precipitation	0.664172346	0.387075262	0.704520747
total mosquitos	bird diversity	112.4680421	1.108386036	0.286373838

C. tarsalis abs	bird diversity	-1.733174926	-1.334138171	0.203455239
% *C. tarsalis*	bird diversity	-4.054330651	-1.645726351	0.12207644
total mosquitos	August precipitation	-0.273119141	-0.004218581	0.996693593
C. tarsalis abs	August precipitation	-1.130207515	-1.434588517	0.173364416
% *C. tarsalis*	August precipitation	0.056428874	0.034274517	0.973142128

Table 9. Complete raw data ANOVA tests of each individual variable. Statistical significance was measured by $p \leq 0.05$ and $p \leq 0.1$.

Dependent Variable	Independent Variable	P-Value
% *C. tarsalis*	land cover	0.097348038
total mosquitos	land cover	0.113065626
C. tarsalis abs	land cover	0.158223823
% *C. tarsalis*	depth of water	0.433807251
total mosquitos	depth of water	0.080172084
C. tarsalis abs	depth of water	0.281478841
total mosquitos	emergent vegetation	0.366747172
C. tarsalis abs	emergent vegetation	0.020591662
% *C. tarsalis*	emergent vegetation	0.660831122
total mosquitos	% site emergent vegetation	0.876050686
C. tarsalis abs	% site emergent vegetation	0.7397304
% *C. tarsalis*	% site emergent vegetation	0.931687646
total mosquitos	habitat type	6.59406E-05
C. tarsalis abs	habitat type	0.045929283
% *C. tarsalis*	habitat type	0.85769105
total mosquitos	water permanence	0.163930392
C. tarsalis abs	water permanence	0.44400989
% *C. tarsalis*	water permanence	0.074075029

Table 10. Normalized regression of raw data that showed statistical significance. Statistical significance was measured by $p \leq 0.05$.

Dependent	Independent	Coefficient	Degrees of Freedom	Test statistic	P-Value
C. tarsalis abs	elevation	0.001221644	14	1.661559783	0.118820837
total mosquitos	air temperature	-1.189839607	14	-1.795412191	0.094197645
C. tarsalis abs	air temperature	-1.189839607	14	-1.795412191	0.094197645
C. tarsalis abs	HDD	-0.107413804	14	-1.752915788	0.101475918
total mosquitos	water temperature	0.100157527	14	0.733370038	0.475434877
total mosquitos	soil temperature	0.14979661	11	1.211601872	0.253516979
total mosquitos	June NDVI	-1.359777818	14	-0.227896035	0.823021849
% *C. tarsalis*	June NDVI	-4.259211195	14	-0.62140421	0.544319904
C. tarsalis abs	May precipitation	-65.72937104	14	-2.160267017	0.048578466
% *C. tarsalis*	bird diversity	-0.306276694	14	-1.4951241	0.157079244
C. tarsalis abs	August precipitation	-1.130207515	14	-1.434588517	0.173364416
% *C. tarsalis*	August precipitation	-1.892345498	14	-0.455948439	0.655419413

Table 11. Normalized ANOVA of raw data that showed statistical significance. Statistical significance was measured by $p \leq 0.05$ and $p \leq 0.1$.

Dependent Variable	**Independent Variable**	**P-value**
% *C. tarsalis*	land cover	0.204036428
total mosquitos	land cover	0.303480612
C. tarsalis abs	land cover	0.014603408
total mosquitos	depth of water	0.581424366
C. tarsalis abs	emergent vegetation 1	0.686726155
total mosquitos	habitat type	0.892407326
C. tarsalis abs	habitat type	0.911816483
total mosquitos	water permanence	0.946138719
% *C. tarsalis*	water permanence	0.973821661

Table 12. The total number of *C. tarsalis* present in each trap and their respective outcomes of WNV.

Site number	**Number of mosquitos**	***C. tarsalis***	**% *C. tarsalis* in trap**	**WNV Test**
16-001	31	26	83.87	negative
16-002	78	23	29.49	negative
16-003	26	7	26.92	negative
16-004	689	12	1.74	negative
16-005	2300	5	0.22	negative
16-006	3000	7	0.23	negative
16-007	638	4	0.63	negative
16-008	22	4	18.18	negative
16-009	101	1	0.99	negative
16-010	541	18	3.33	negative
16-011	752	15	1.99	negative
16-012	481	3	0.62	negative
16-013	253	8	3.16	negative
16-014	333	5	1.50	negative
16-015	84	4	4.76	negative
16-016	436	42	9.63	negative