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Mapping the abundance of Culex tarsalis to environmental variables in Montana

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Mapping the abundance of *Culex tarsalis* to environmental variables in Montana

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Abstract

In Montana, West Nile Virus (WNV) was first recorded in 2002, and by 2003 there were 222 reported cases statewide. Currently, WNV still causes high fever, vomiting, muscle weakness, convulsions and paralysis in over 1,000 Americans each year. The primary vector of WNV differs spatially across the United States, with *Culex pipiens* as the primary vector in the east and *Culex tarsalis* contributing to the majority of human cases west of the Mississippi River. Different primary vectors suggest that geographical barriers of *Cx. pipiens* and *Cx. tarsalis* may be related to ecological factors. In the present study, mosquitoes were collected from three different locations in Montana using CDC light traps. Elevation, trap distance to water and canopy closure were measured at each location. Total mosquito abundance and *Cx. tarsalis* abundance were compared with each environmental characteristic independently. Although no correlations were linked to total mosquito abundance, *Cx. tarsalis* displayed preferential habitat patterns. Elevation and location were shown to impact *Cx. tarsalis* presence. Knowledge of environmental conditions conducive to the primary western vector can be used to target primary prevention efforts, decrease incidence of human WNV cases and advance subsequent undergraduate research of WNV ecology at Carroll College.

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INTRODUCTION

Since the first reported case of West Nile Virus (WNV) in Uganda in 1937, this mosquito-borne pathogen drifted west and reached the United States in 1999 (Pongsiri et al. 2009). In Montana, the disease was first recorded in June 2002, and by August 2003 there were 222 reported human cases statewide (CDC, 2010). Twelve years after its introduction into the United States, the virus causes high fever, vomiting, muscle weakness, convulsions and paralysis in over 1,000 Americans each year (CDC 2010, Kent et al. 2009). Although humans can only contract WNV from mosquito bites and blood transfusions, the persistence of WNV can be attributed to mosquitoes feeding on avian reservoir hosts that amplify the virus (CDC/Disease Conditions 2011, Kent et al. 2009).

Three species of mosquito are responsible for the majority of North American WNV transmission to humans: *Culex pipiens, Culex quinquefasciatus* and *Culex tarsalis* (Murray et al. 2010). *Cx. pipiens* is the major vector for WNV in the U.S east of the Mississippi River (Venkatesan and Rasgon 2010). *Cx. tarsalis*, the dominant West Nile Virus vector west of the Mississippi, has been linked to severe WNV outbreaks, especially in rural and suburban communities (CDC/Disease Conditions 2011. Hamer et al. 2011). Although the etiology of the disease is consistent across the nation, different primary vectors suggest that geographical barriers of *Cx. pipiens* and *Cx. tarsalis* exist, and may be related to ecological factors. In the northeastern part of the nation, the presence of *Cx. pipiens* has been correlated with low elevation, urbanization, proximity to water and high temperature (CDC/Disease Conditions 2011, Murray et al 2010, Hamer et al. 2011). Although the presence of *Cx. tarsalis* has been mapped to water during the larval stage of the life cycle, habitat characteristics during later life-stages continue to be investigated (Juliano 2010).

What is known about the preferential habitats of *Cx. tarsalis* is this species favors western North America and is rarely found east of the Mississippi river (Crans and McCuiston 1997). What remains to be analyzed are the vegetation types and the specific elevation range in Montana that these primary vectors seem to prefer for their choice of habitat.

In the present study, the objective was to determine if a relationship exists between *Cx. tarsalis* presence and canopy closure. Elevation and distance to water were also considered for correlation with *Cx. tarsalis* presence. The abundance of *Cx. tarsalis* was analyzed at three different locations across Montana during peak transmission. Instead of measuring the incidence of WNV in each location the number of susceptible vectors was targeted. This susceptible vector model may eliminate unrecognized infections and erroneous laboratory results that can arise in RNA virus extraction and amplification.

Cx. pipiens, the primary vector east of the Mississippi, is more abundant in regions labeled 'urban' than in deciduous, evergreen and mixed forests (Hamer et al. 2011, Brown et al. 2008). Based on this evidence, I hypothesized that *Cx. tarsalis* would show a similar habitat preference, translating to lower *Cx. tarsalis* abundance in areas with heavier canopy closure. By studying the relationship between the primary vector and environmental conditions conducive to WNV vectors, a habitat model can be created. Increasing our knowledge of the preferred habitat of *Cx. tarsalis* will lead to increased knowledge in the spatial epidemiology of WNV, including the risk factors for human infection. This working model of *Cx. tarsalis* habitat will help us better understand patterns in WNV to improve control strategies and primary prevention efforts. This state-wide analysis and description of *Cx. tarsalis* behavior is a working part of the WNV research program at Carroll College and provides useful insight on the patterns of WNV disease in Montana.

MATERIALS & METHODS:

SAMPLE COLLECTION

Mosquito samples were captured using Center for Disease Control and Prevention (CDC) light traps, with CO_2 or dry ice as the attractant (Figure 1). Traps were uniformly hung approximately three feet from the ground and were run overnight for at least nine hours. Traps were tested and assessed the morning of collection to ensure that they were working and the source of CO_2 was sufficient for the night. Trap locations were omitted from analysis if CO_2 ran out or the battery died. After collection, samples were immediately stored in a -20° C freezer for at least two days before analysis. Sorting consisted of analyzing mosquitoes using stereomicroscopes to count the number of *Cx. tarsalis* trapped at each location and the total number of mosquitoes trapped. *Cx. tarsalis* were identified based on the following anatomical features: median banding on the proboscis, a blunted abdomen, wide basal and apical bandings on each tarsal and the presence of thin scales on the dorsal wing surface (Figure 2).

Figure 1: CDC Light Trap hood, mesh and container.

Figure 2: Identifying Features of *Cx. tarsalis.* Median proboscis band (top left), apical and basal leg bands (bottom left), rounded abdomen (top right), thin wing scales (bottom right).

TRAPPING LOCATIONS

Starting in June, four traps were stationed at three different locations across mid-western Montana–Helena, Helmville and Ninepipe—one night per week for nine weeks (Figure 3). Of the four traps at each site, two traps were situated in open areas with little to no canopy closure and two in areas with varying amounts of canopy closure. The density of closure was initially estimated using Google Earth aerial images. Canopy closure was confirmed using ‘spherical densitometry’ (Jennings et al. 1998) with the following adaptation: a flat mirror was used with a 15 x 15 grid. Canopy closure was recorded as a percent using the equation C=Nc / Nt (Jennings et al. 1998), where Nc is the number of grid points covered by canopy and Nt is the total number of points on the grid n=225. Elevation and geographic coordinates were recorded at all sites using the eTrex Summit HC from Garmen and reported using Universal Transverse Mercator grid-12 map with the NAD27 datum. Distance from trap to water was measured with a Nikon LR550 laser range finder. Water sources were identified as streams, lakes, rivers or standing water.

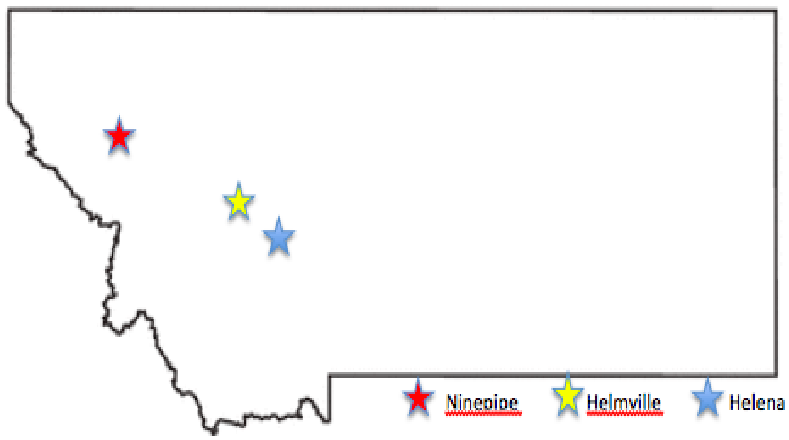


Figure 3: Trapping Locations Across Montana

DATA ANALYSIS

A simple regression analysis was completed for each dependent variable (total mosquito abundance and *Cx. tarsalis* abundance) across two independent variables (elevation and distance to water), for a total of four regression analyses. These tests were used to determine if elevation or distance to water had a significant effect on the presence of mosquitoes, and more specifically the presence of *Cx. tarsalis*.

After elevation and distance to water were confirmed as significant or revoked as negligible, a Multi-Factor ANCOVA was used to test for the effects of canopy closure and trapping location on the abundance of mosquitoes and *Cx.*

tarsalis. For the ANCOVA analysis, independent variables that were significant from the regression analysis were used as covariates.

RESULTS

Distance to Water:

Regression analysis suggests that no correlation exists for mosquito abundance (p=0.847) or *Cx. tarsalis* abundance (p=0.242) with the distance to water. Since regression analysis identified distance to water as having no effect on either of the dependent variables, this variable was not included in further data analyses.

Elevation:

Regression analysis suggested no correlation exists (p=0.922) between total mosquito abundance and elevation (Figure 4). However, regression suggests elevation has a significant impact on *Cx. tarsalis* abundance (p=0.017). *Cx. tarsalis* abundance was shown to be inversely proportional with elevation, as suggested by the linear equation y= -0.103x + 134.3 (Figure 5).

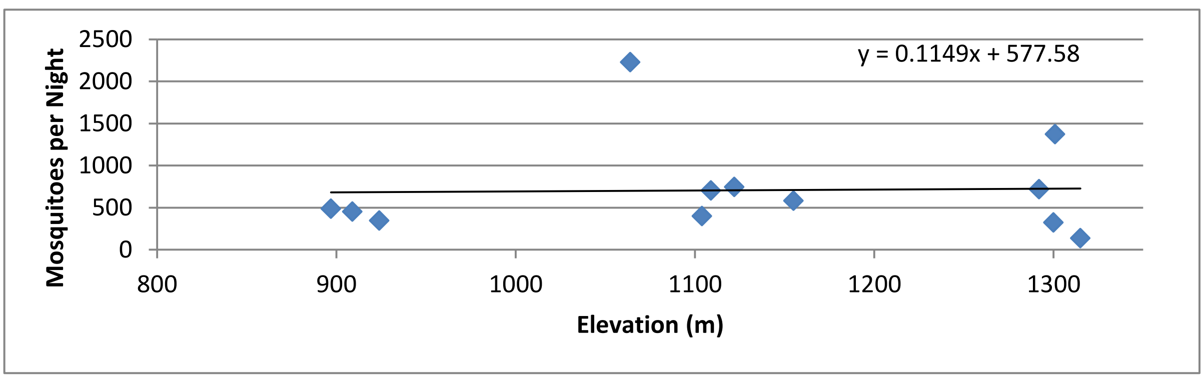


Figure 4: Scatter plot showing elevation has no effect on total mosquito abundance.

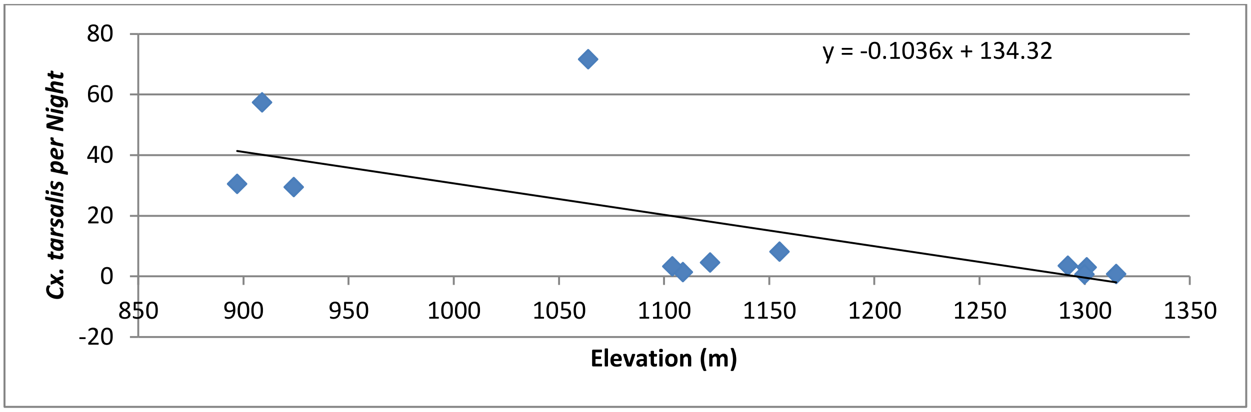
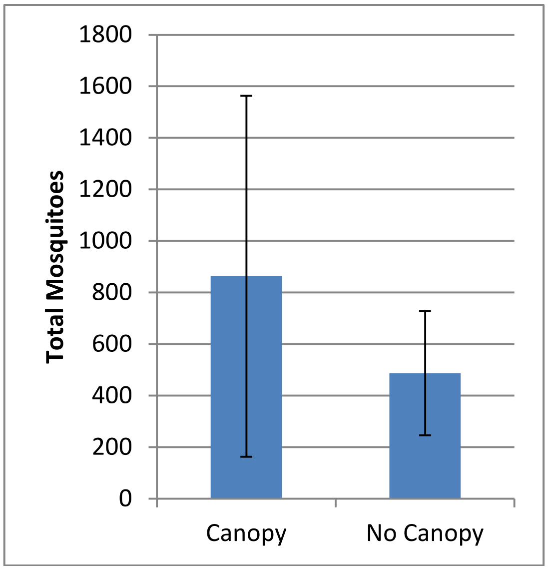


Figure 5: Scatter plot showing the negative correlation between elevation and *Cx. tarsalis* abundance.

Canopy Closure:

To test for the effects of canopy closure on the abundance of *Cx. tarsalis* and total mosquitoes, a Multifactor ANOVA was used. The mean values of total mosquitoes trapped per night and *Cx. tarsalis* trapped per night were both substantially higher for sites that had at least 84% canopy closure. On average 863 mosquitoes were caught per night in traps that were under canopy closure, while only 488 were caught at sites with no closure. As for *Cx. tarsalis*, 24.9 were caught on average by traps that were covered by canopy, compared with an average of 7.9 *Cx. tarsalis* caught at sites without a source of cover. However, once standard deviation is accounted for in the data set, the variance is too high to confirm that canopy cover alone impacts mosquito and *Cx. tarsalis* numbers (Figures 6 & 7, respectively). After accounting for changes in elevation the multifactor ANCOVA suggested there was still no significant association between canopy cover and mosquito abundance and *Cx. tarsalis* abundance (Table 1).



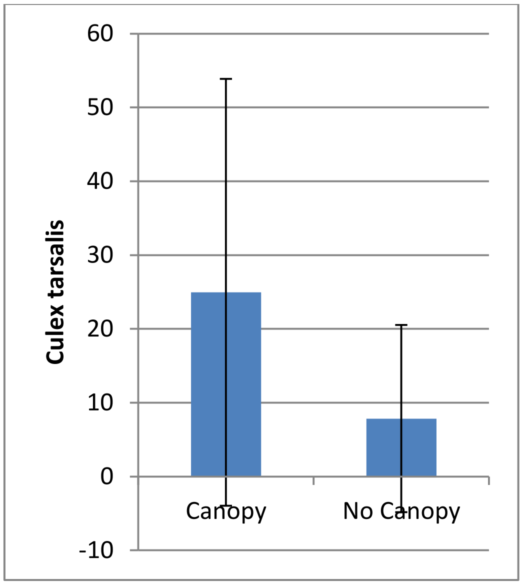


Figure 6**:** Effect of Canopy Closure on Total Mosquito Abundance

Figure 7**:** Effect of Canopy Closure on *Cx. tarsalis* Abundace

Table 1: Multifactor ANCOVA testing for the effects of canopy and location on *Cx. tarsalis* and total mosquito abundance.

Dependent Variable	**Factor**	**Error**	**F- value**	**p-level**
tarsalis	canopy	99.9	0.238	0.646
tarsalis	location	99.9	8.92	0.022
tarsalis	interaction	99.9	0.334	0.730
Total mos.	canopy	5.02E^5	0.666	0.446
Total mos.	location	5.02E^5	0.035	0.965
Total mos.	interaction	5.02E^5	0.082	0.922

Location:

In order to test for the effects of trapping location on *Cx. tarsalis* and total mosquito abundance a multifactor ANCOVA was used. In this analysis, elevation was accounted for as a covariate to account for its effects on abundance.

Although no correlation was found between total mosquito abundance and location (p=0.956; Figure 8), location had a significant effect on *Cx. tarsalis* abundance (p=0.022; Table 1). In order to pinpoint which trapping site possessed the highest amounts of *Cx. tarsalis* descriptive statistics were graphed for each individual location (Figure 9) and indicate that Ninepipe had the highest *Cx tarsalis* abundance.

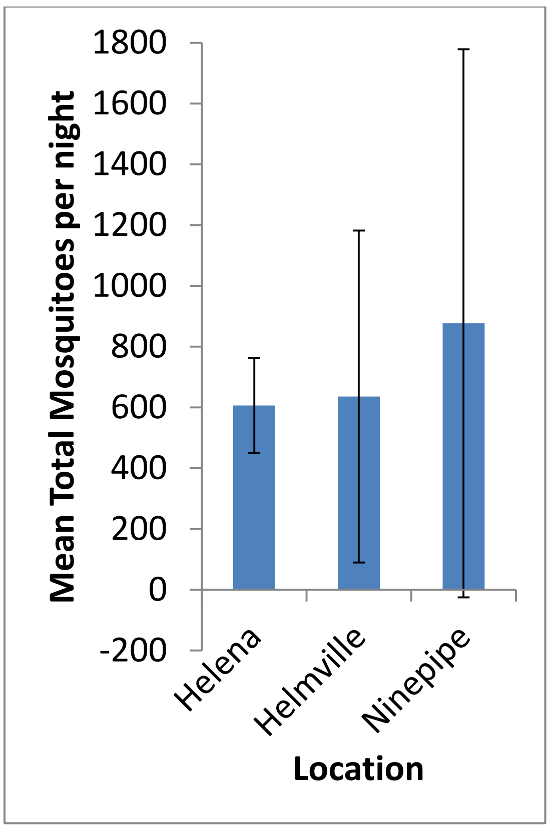


Figure 8: Effect of Location on Total Mosquito Abundance

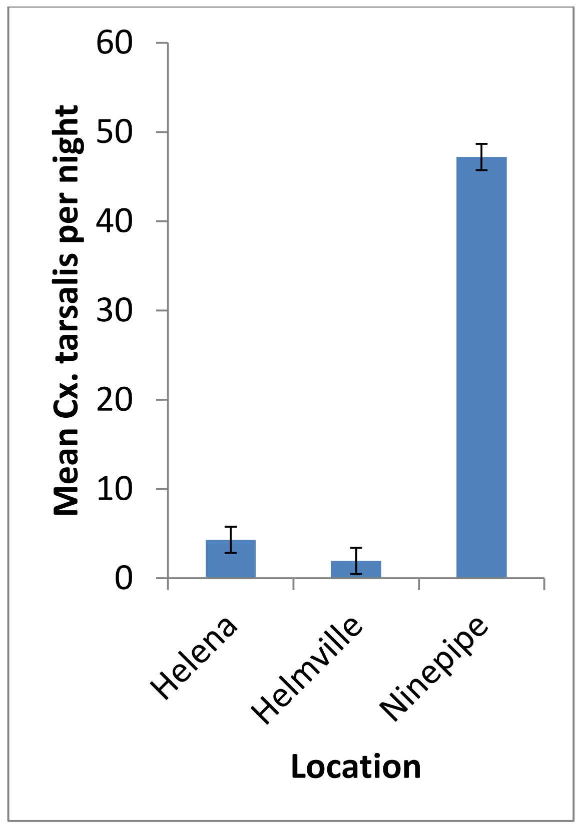


Figure 9: Effect of Location on *Cx. tarsalis* Abundance

Discussion / Conclusions

In 2008 elevation gradients were examined in Colorado in search of areas that have a large abundance of *Cx. tarsalis* (Eisen et al. 2008). Eisen et al. (2008) suggested that *Cx. tarsalis* were abundant in plains regions (<1,600 m), and collected at low abundances in foothills and low montane areas (1,610–1,730 m). These findings are consistent with the current findings in Montana where over 90% of *Cx. tarsalis* collected were at elevations below 1200 meters. These data suggest elevation plays a pivotal role in habitat preference for *Cx. tarsalis* in the western United States.

The primary purpose of the present research was to investigate the effect of canopy closure on *Cx. tarsalis* presence. Twice as many mosquitoes and over three times more *Cx. tarsalis* were collected in traps covered by at least 84% canopy. Although there appears to be a correlation between canopy closure and *Cx. tarsalis* abundance, large variance in the data prevented a statistically significant conclusion. However, this does not indicate a correlation does not exist. The large variance in the data could be the result of a small sample size. Only three locations were used to assess canopy closure. Additional study sites may yield data resulting in lower variance. Dense canopy closure has been linked to decreased abundance of *Cx. pipiens* in the eastern U.S (Brown et al. 2008); however, little research has been done on the impact it has on *Cx. tarsalis*. Future research should increase the number and diversity of trapping sites in order to better test the relationship between canopy closure and the presence of *Cx. tarsalis*.

In the present study the multifactor ANCOVA analysis accounted for the effect of elevation and still showed a positive association between location and the number of *Cx. tarsalis*. Upon in-depth analysis of each location independently, a mean of over 45 *Cx. tarsalis* per night during nine weeks of collection was measured at Ninepipe, which proved to be significantly more than Helena and Helmville, each yielding less than seven *Cx. tarsalis* per night. These data suggest that there are other environmental characteristics, besides elevation, that are conducive to the presence of *Cx. tarsalis.* Even with elevation included as a covariate, *Cx. tarsalis* display significant location patterns in Montana. Research on other factors that are influencing the presence of the primary vector should be investigated, especially at the Ninepipe location. In 2002 Hak Lee et al. suggested a positive correlation between the American Robin (*Turdus migratorius)*, American Crow (*Corvus brachyrhynchos)* and *Culex* mosquitoes. Although these specific species are not the most abundant avian hosts in Ninepipe, the location is a National Wildlife Reservoir, which may help explain the high abundance of *Cx. tarsalis.* Other environmental factors like specific vegetation type or proximity to xeric habitat may also impact the presence of *Cx. tarsalis* and should be investigated when creating an all-encompassing risk map for the western U.S.

During the summers of 2010 and 2011 a total of two counties, Sheridan and Blaine, revealed positive human tests for WNV in Montana (CDC, 2011). Though the number of human cases has declined since 2007, primary prevention efforts should focus on creating risk maps indicating where greater numbers of *Cx. tarsalis* are likely to be present in the state. In this study, location was shown to have a significant impact on *Cx. tarsalis* abundance suggesting that environmental factors influence preferential habitat selection by *Cx. tarsalis*. Future research should focus on pinpointing these factors by increasing sample sizes, accounting for the effect of elevation and testing other environmental variables.

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