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Land Cover Association of *Culex tarsalis* in Western Montana

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Land Cover Association of *Culex tarsalis* in Western Montana

Submitted in partial fulfillment of the requirements for graduation with honors from the
Department of Biology at Carroll College, Helena, MT

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Carroll College, Helena, MT

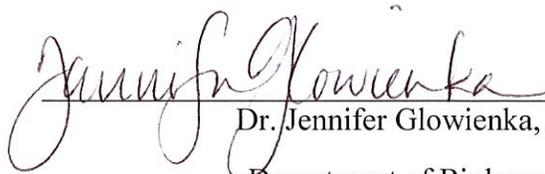
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Abstract

Culex tarsalis is the major vector of West Nile Virus (WNV) in Montana, so a higher concentration of *Cx. tarsalis* would most likely correspond to an area of high WNV incidence. Previous studies performed in the U.S. found that wetland land cover had a positive influence on *Cx. tarsalis*, while the studies on Normalized Difference Vegetation Index (NDVI) presented conflicting results. In this experiment I attempted to answer the question: Does land cover type and NDVI affect *Culex tarsalis* distribution in Western Montana? It was hypothesized that both land cover type and a high NDVI value would have a positive effect on *Cx. tarsalis* distribution. In order to test this hypothesis, mosquitoes were trapped in Western Montana and *Cx. tarsalis* individuals were sorted from other species of mosquitoes. The land cover type and an NDVI value was observed for each trap site. The presence and amount of *Cx. tarsalis* was recorded, and statistical analysis was performed to determine which land cover type and NDVI value had the largest influence on *Cx. tarsalis* distribution. I found that wetland land cover had the largest positive effect on *Cx. tarsalis*, while forest land cover had the largest negative effect on *Cx. tarsalis*. On average, the sites where *Cx. tarsalis* was present had a larger NDVI value associated with it. These results agree with the findings that other studies have found on *Cx. tarsalis* and land cover, while adding to *Cx. tarsalis* and NDVI studies.

Introduction

West Nile Virus (WNV, *Flaviviridae*, *Flavivirus*) is an arthropod-borne virus, which can cause flu-like symptoms, or in extreme cases, encephalitis or meningitis (Centers for Disease Control and Prevention, 2017). WNV is a single stranded RNA virus that was introduced into America in 1999, and has since spread across the country (Kramer et al., 2008; Komar, 2003). During the first sixteen years after its introduction, there have been 43,937 cases of WNV fever, of which 20,265 of those cases were neuroinvasive, and in total WNV has caused 1,911 deaths (Centers for Disease Control and Prevention, 2015).

The transmission of WNV cycles between birds and ornithophilic *Culex* mosquitoes (Worwa et al, 2015). Of the 66 mosquitoes that are recognized as viable vectors of WNV, the most prominent species in Montana is *Culex tarsalis* (Centers for Disease Control and Prevention, 2017; Montana DPHHS, 2016). The overall fitness of WNV in a mosquito host is described by dose-dependent susceptibility to infection, initial replication in midgut epithelium, release into the hemocoel, and replication in salivary glands, which then leads to transmission of the virus through salivation during a blood meal (Worwa et al, 2015).

Above-normal temperatures have been among the most consistent factors associated with WNV outbreaks (Hoover and Barker, 2016). With increased temperature, the gonotrophic period (GP) and the extrinsic incubation period (EIP) are shortened, which increases the efficiency of transmission (Reisen et al., 2006). A shorter GP corresponds to an increased

rate in mosquito-host interaction, and the rate at which the mosquito population increases (Reisen et al., 2006). A shortened EIP decreases the time period during which a mosquito is biologically infected, and when it can start transmitting the virus through taking in blood meals (Reisen et al., 2006).

Landscape factors are known to influence the distribution of *Cx. tarsalis* (Wimberly et al., 2008). Land cover and *Cx. tarsalis* studies have been conducted in states similar to Montana and parts of Montana, but not the state as a whole. In South Dakota, grassland and pasture were found to have a positive correlation to the presence of *Cx. tarsalis* (Chuang et al., 2011). In a study covering the Great Plains area (parts of Montana and Wyoming, North Dakota, South Dakota, and Nebraska), rural areas and irrigated land were found to have a positive correlation to the presence of *Cx. tarsalis* (Wimberly, 2008). Crop and grassland land cover are associated with *Cx. tarsalis* because it breeds in standing water that receives large amounts of sunlight (Bowden et al. 2011). Similarly, the findings produced by Wimberly et al. point towards a strong relationship between WNV incidence in *Cx. tarsalis* and rural land cover, with an increased probability of WNV if that land is regularly irrigated (Wimberly et al., 2008).

Normal Difference Vegetation Index (NDVI) is a measure of photosynthetic activity in plants. It is thought that NDVI can give insight to the presence of *Cx. tarsalis* because NDVI can reflect characteristics of vegetation that are influenced by landscape, temperature, and precipitation (DeGroot, 2008). DeGroot et al. (2008) found a negative

relationship between NDVI and human WNV disease cases in Iowa. In Colorado, Winters et al. (2008) found a positive relationship between NDVI and human WNV disease cases. Since the Winters and DeGroot studies reached opposing conclusions and because NDVI has yet to be used in *Cx. tarsalis* distribution mapping in Montana, the present study investigated the viability of NDVI as an indicator in Montana of *Cx. tarsalis* presence.

Hokit et al. (unpublished data) applied a vector habit suitability model (**Figure 1**) to attempt to predict *Cx. tarsalis* hotspots throughout the state of Montana. Included in this vector habit suitability mode are land cover, bird-host distribution, and climate patterns, for *Cx. tarsalis* (Hokit et al., unpublished data). The objective for this thesis is to test this vector habitat suitability model for accuracy to predict *Cx. tarsalis* hotspots throughout Montana. Testing for accuracy was carried out by randomly trapping near predicted *Cx. tarsalis* hotspots using the vector habit suitability model as described in Hokit et al. (unpublished data).

I hypothesized that both the land cover and NDVI analysis would be sufficient prediction tools for *Cx tarsalis* in Western Montana. With this refined model, a deeper understanding of *Cx tarsalis* distribution may be met, and preventative methods such as spraying may be able to lower *Cx tarsalis* numbers and WNV infection rates in Montana.

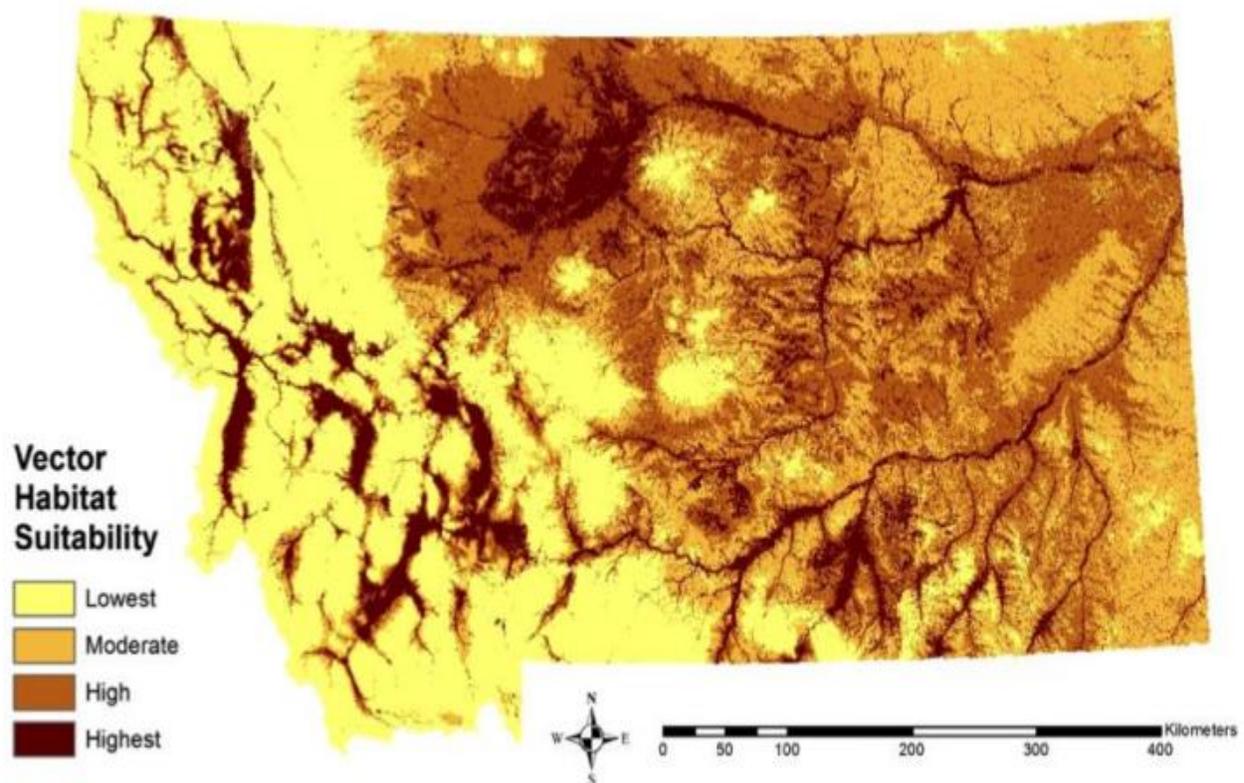


Figure 1: Vector Habitat Suitability for *Cx. tarsalis* density in Montana (Hokit et al., unpublished data)

Methods

Field Collection:

Trapping sites were randomly selected from areas where we had little or no data on the mosquito population, as well as sites that had yielded *Cx. tarsalis* in previous trapping years (i.e. control sites). Each trap site was given a number that corresponded to its longitudinal and latitudinal coordinates and its locality name. Each site was equipped with a CDC light trap and a source of CO₂, and each trap was allowed to run overnight. The source of CO₂ varied between a CO₂ tank or a cooler with holes drilled in the bottom and dry ice placed inside. Traps were collected the following morning and then frozen within the trapping day to preserve the sample. Each trap was placed in either a -20°C or -80°C freezer until no mosquito activity was observed.

Sorting:

Each collection was sorted in the Wiegand Undergraduate Research Laboratory at Carroll College by undergraduate research students. Small portions from 100-250 mosquitoes of each trap were placed in a petri dish and sorted under a dissecting microscope with an ice pack at the base of the petri dish. *Cx. tarsalis* were separated from the rest the trap, and were identified as the “target species.” The mosquitoes that were not part of the “target species” were then collected and packaged as “non-target species” for further identification at Montana State University. The “non-target species” and “target species” were quantified and were recorded under their trap-specific identification number.

Data Analysis:

Trap information was first uploaded into a GIS and organized spatially with their respective longitudinal and latitudinal coordinates. A land cover type was associated with each trap site using the GIS sample tool. The data was then classified into six categories: Agriculture, Developed, Forest, Grassland, Riparian/Wetland, and Shrub land (**Figure 2**). With the land cover and *Cx. tarsalis* data, a G-test analysis was performed to test for statistical associations between land cover type and the presence/absence of *Cx. tarsalis*. Each land cover category's observed *Cx. tarsalis* presence frequency was compared to the expected frequency of *Cx. tarsalis* by generating a G-statistic and comparing the difference with a null model that assumed no significant difference between the frequencies. Each collection site was also sampled for NDVI value, and a T-test was performed comparing the NDVI values between sites where *Cx. tarsalis* was present to sites where *Cx. tarsalis* was absent (**Figure 3**).

Results

The G-test yielded a p-value of $P < 0.0001$, suggesting that land cover has an effect on *Cx. tarsalis* distribution. A deviation from expected was calculated using the expected values from the G-test and statistical significance was found with forest land cover and riparian/wetland land cover (**Figure 3**). *Cx. tarsalis* was found more frequently than expected in wetlands and less frequently than expected in forests. The NDVI T-test yielded a p-value of 0.0025, suggesting that NDVI also has an effect on *Cx. tarsalis* distribution. The mean NDVI value of *Cx. tarsalis* present was 0.558, and the mean NDVI value of *Cx. tarsalis* absent was 0.611. The difference between the mean NDVI values suggest a negative correlation of NDVI to *Cx. tarsalis* presence at least in western Montana.

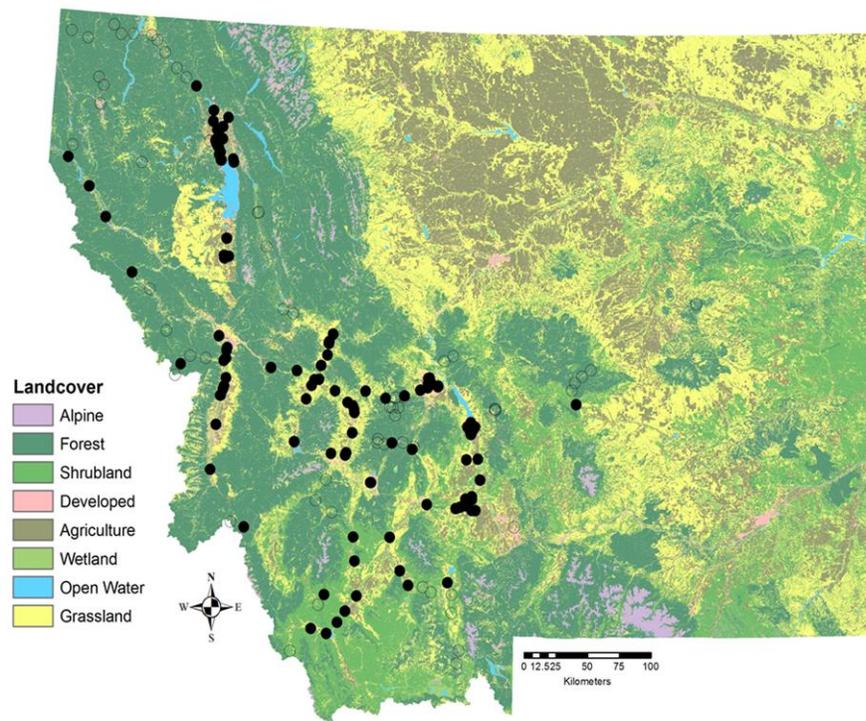


Figure 2: Trap sites from the summers of 2016 and 2017 and land cover of Western Montana

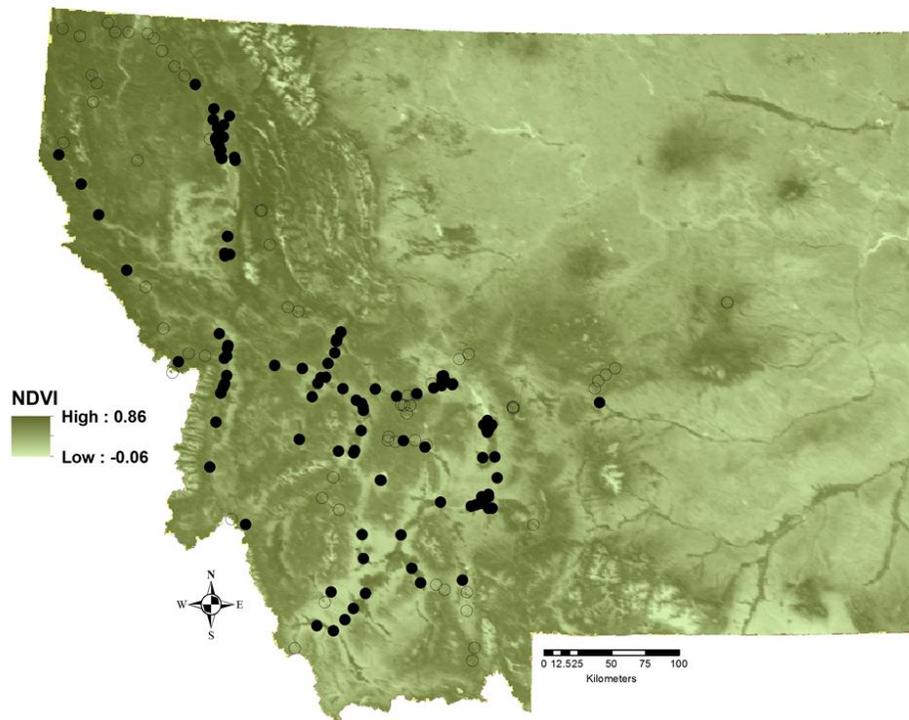


Figure 3: Trap sites from the summers of 2016 and 2017 and NDVI mapping of Western Montana

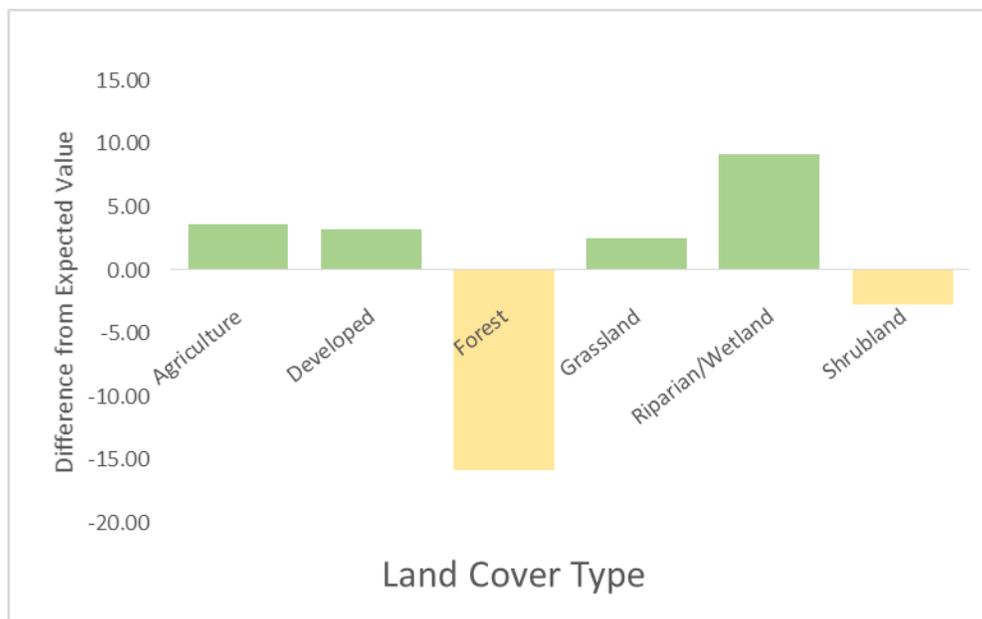


Figure 4: Deviation from expected values for each land cover type. A difference +/- 5 is deemed statistically significant.

Discussion

Analysis of land cover of trapping sites and the presence of *Cx. tarsalis* suggests that land cover has a statistically significant effect on *Cx. taraslis* distribution. More specifically, riparian/wetland land cover has a strong positive correlation with *Cx. tarsalis* distribution, while forest land cover has a negative correlation with *Cx. tarsalis* distribution. The most positive correlation was in riparian/wetland land cover, but a weaker positive correlation was found in agriculture land cover (difference of 3.59 from expected) and grassland land cover (difference of 2.52 from expected). Other studies have shown land cover to impact *Cx. tarsalis* distribution. Positive correlations have been shown between *Cx. tarsalis* distribution and grassland and pasture land cover (Chuang et al., 2011), wetland cover (Ezenwa et al., 2007), and rural and irrigated land cover (Wimberly et al., 2008). These analyses also agree with Bowden et al. (2011) where their findings suggest a negative correlation between *Cx. taraslis* distribution and forest land cover.

The NDVI data correlations also suggests that the amount of photosynthetic activity of vegetation could impact *Cx. taraslis* distribution in Western Montana. The T-test between the two sets of data in which *Cx. tarsalis* was present, and not present was significant suggesting NDVI could help map *Cx. taraslis* distribution. The difference in the mean NDVI values from the analysis suggests that *Cx. taraslis* distribution has an inverse correlation with NDVI. These findings agree with DeGroote et al. (2008), where an inverse relationship was found between NDVI and human WNV disease cases. However, these findings disagree with Winters et al. (2008), where a positive correlation was found between NDVI and human WNV disease incidence. Both of these studies

focus on human WNV incidence instead of *Cx. taraslis* distribution. However for transmission to occur there must be an adequate population of *Cx. taraslis* present. Ultimately these studies can provide insight into *Cx. taraslis* distribution. My results suggest that land cover and NDVI data are valuable mapping indicators in *Cx. taraslis* distribution. With these indicators and other ecological indicators, a precise map of *Cx. taraslis* distribution can be generated. A map of *Cx. taraslis* distribution can highlight *Cx. taraslis* hotspots throughout Western Montana and eventually the entire state. A more accurate identification of *Cx. taraslis* hot spots will allow for better location-specific preventative measures to be undertaken, like spraying small bodies of water and issuing local WNV public safety warnings.

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