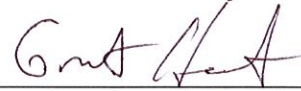


Influence of Avian Distributions on West Nile Virus Infection Rates

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

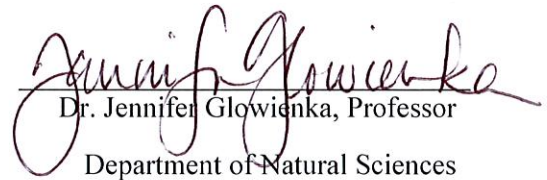
Kaitlin McHugh
Carroll College, Helena, MT
March 13, 2018

This thesis for honors recognition has been approved for the Department of Natural Sciences by:



Dr. Grant Hokit, Director

Department of Natural Sciences



Dr. Jennifer Glowienka, Professor

Department of Natural Sciences



Dr. Brandon Sheafor, Professor

Department of Natural Sciences

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Abstract

Evidence has shown that human infection rates for West Nile virus are largely dependent on viral amplification that occurs between competent avian host species and mosquito vectors. The threat of West Nile virus to humans is influenced by the diversity and competence of avian host species available to the mosquito vectors. This study used data collected through avian surveys in Montana to analyze the diversity and competence of avian species in those areas. These data were compared to existing GIS model predictions of avian diversity and avian viral competence in Montana to test the accuracy of the model. The GIS model diversity predictions were then compared to the infection rates for avian survey locations, sites that tested positive for West Nile virus, and sites where the primary vector species *C. tarsalis* was found. It was hypothesized that areas with low avian diversity and high viral competence of avian species would be associated with increased infection rates, whereas high diversity and low competence would be associated with lower infection rates. In this study, the GIS model appeared to be an accurate indicator of avian diversity, and in all cases an amplification effect was observed in which sites containing greater avian diversity appeared to have an increased risk of West Nile virus. However, it is still unclear how significant the role of avian viral competency is in viral amplification and dilution.

Introduction

In the past 20 years West Nile virus has contributed to nearly 2000 deaths and hundreds of thousands of human infections in the United States alone (Levine et al. 2017). While the annual number of human cases of West Nile virus in Montana is generally low, in some years the number of cases has risen into the hundreds, and at least 14 fatalities have been recorded since 1999 (Montana.gov 2017). West Nile virus is primarily transmitted in a cycle between birds and mosquitoes, but mammals may also be affected if fed upon by infected mosquitoes (Petersen et al. 2013). While there is evidence that West Nile virus may persist in overwintering female mosquitoes (Nelms et al. 2013), the virus is mainly carried between seasons by virally competent avian hosts (Kilpatrick et al. 2006). Viral amplification begins in the early summer as mosquitoes begin to feed first on infected avian hosts, and then on healthy hosts, transmitting the infection between them (Nelms et al. 2013).

Viral competence is highly variable between avian species, with passerine birds typically displaying higher viral competence than non-passerine birds (Komar et al. 2003). Thus, the composition of avian species in an area can play an important role in the amount of viral amplification that occurs. Keesing et al. (2006) described one type of dilution effect in which increased biodiversity reduces the number of encounters between vectors and competent hosts, decreasing the potential for transmitting disease. This phenomenon was observed by Swaddle and Calos (2008) when counties in the eastern United States with greater avian diversity were observed to be less likely to have reported human cases of West Nile virus than neighboring counties with lower avian diversity. Additionally, Ezenwa et al (2006) showed a strong correlation between a high abundance

of non-passerine avian species with reduced mosquito infection rates and reported human cases in Louisiana. However, if there is a high proportion of virally competent host species in an area an amplification effect may be observed instead, with higher species diversity corresponding to higher mosquito infection rates (Levine et al. 2017).

The threat of West Nile virus to humans is influenced by both the diversity and the viral competence of the avian host species available to the mosquito vectors. This study used data collected during avian surveys conducted throughout the summer of 2017 in two locations near Helena, Montana in order to analyze the diversity and viral competence of avian species in those areas. These data were compiled with data from Sevenmile Creek (Sater 2017) and Medicine Lake (Bernt 2013) and compared to the existing GIS model predictions of avian diversity and avian viral competence in Montana to test the accuracy of the model. After this comparison, the GIS model predictions of avian diversity and viral competence will be compared to the infection rates for avian survey locations and sites that tested positive for West Nile virus during the summer of 2017. I hypothesized that areas with low avian diversity and high viral competence of avian species would be associated with increased infection rates, whereas areas with high diversity and low viral competence would be associated with lower infection rates. If substantiated, these relationships could be used to help predict regions where the threat of West Nile virus infection may be elevated based on the diversity and competence of avian species in the area.

Methods

Avian Surveys

Avian surveys were conducted in the summer of 2017 at two mosquito trapping locations near Helena, Montana. These locations included the Helena Regulating Reservoir and the northern end of Lake Helena, and were chosen to correspond to regions with high expected proportions of *C. tarsalis* mosquitoes. All surveys were conducted between 7:00 am to 10:00 am and performed in fifteen-minute intervals at four defined points at each location. This resulted in a total of 20 point surveys per location. Avian species were identified by visual observation, by song, and by call, and the total number of birds of each species identified was recorded for each survey.

Data Analysis Part 1: Testing Avian Distributions

Data were compiled to find the relative abundance of each species at each site. From these relative abundances, three diversity indices were calculated for each location: the overall Shannon diversity index, the Shannon diversity index excluding known virally competent birds, and the index including only birds previously shown to be virally competent, the latter being a community competence index. For the community competence index, birds were separated into two groups: those with high competence and those with moderate competence. These viral competence values were taken from data collected by Kilpatrick et al. (2006) and Komar et al. (2003). The relative abundances of birds with high viral competence were multiplied by 1.6 and values for birds with moderate viral competence were multiplied by 0.5 as described by Hokit et al. (unpublished) based on known viral competencies of birds previously indicated to be highly or moderately competent (Komar et al. 2003; Kilpatrick et al 2006). The resulting

values were used to calculate the community competence to account for the effects of varied viral competency in different bird species.

Additionally, point surveys conducted at Medicine Lake (Bernt 2013) and Sevenmile Creek (Sater 2017) were similarly analyzed to find their respective relative abundances and Shannon diversity indices for further comparison. Linear regressions were performed for each of the three diversity indices calculated for each location and compared to the values generated from the GIS model to test if the relationships in diversity were proportional at each site between data collected from the field and data generated from the model.

Data Analysis Part 2: Testing West Nile Infection Rates

All six avian survey sites were analyzed by plotting the three diversity indices for each site against West Nile virus risk and generated by an infection risk model (Hokit et al, unpublished). Regression analysis was used to test for associations between the diversity indices and the model predictions. In addition, all sites where *C. tarsalis* were collected and all sites that tested positive for West Nile virus (Figure 1) were selected and the West Nile virus infection risks of these locations were plotted against each of the cumulative diversity indices predicted from the GIS model. The regressions were performed to determine if there was a statistically significant correlation between West Nile virus infection risk and avian diversity or avian viral competence.

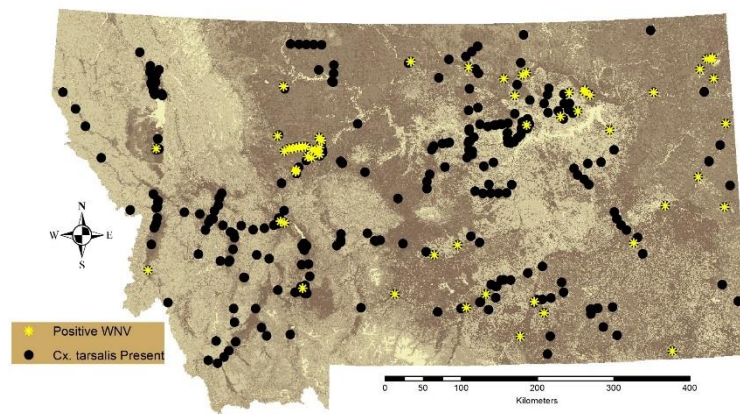


Figure 1: Image generated from GIS model displaying sites where *C. tarsalis* were captured and sites testing positive for West Nile virus.

Results

Avian surveys were conducted to generate data for comparison with GIS model predictions of avian diversity and avian viral competence. A total of 106 avian species and 10694 individual birds were counted during these surveys (Table 1). Note that several avian species were observed at multiple locations, so each of these species were counted only once in the total number of observed species.

Table 1: Avian survey data detailing the number of birds and number of species observed at each sampling location

Location	Number of Birds	Number of Species
Helena Regulating Reservoir (2017)	2375	31
Lake Helena (2017)	357	32
Sevenmile Creek (2017)	783	54
Medicine Lake Headquarters (2013)	1367	32
Medicine Lake Campground (2013)	697	26
Bridgerman's Point (2013)	5115	35
Total	10694	106

From the individual site data, values for the overall and virally competent Shannon diversity indices were calculated and the corresponding predicted values were extracted from the GIS model (Table 2).

Table 2: Comparison of the observed Shannon diversity indices and community competence (CC) indices at each survey location with the GIS-predicted Shannon diversity indices, community competence indices, and West Nile virus risk.

Location	Observed Shannon Index	Predicted Shannon Index	Observed CC Index	Predicted CC Index	Predicted WNV risk
Helena Regulating Reservoir (2017)	1.7387	6.98	0.5261	1.98	0.23
Lake Helena (2017)	2.8637	9.52	0.8869	2.92	0.73
Sevenmile Creek (2017)	3.4835	11.09	0.8257	3.53	0.51
Medicine Lake Headquarters (2013)	2.3045	8.1	0.5875	2.44	0.29
Medicine Lake Campground (2013)	2.4297	7.79	0.4325	3.18	0.65
Bridgerman's Point (2013)	1.8209	6.8	0.1829	2.15	0.56

To determine the accuracy of the GIS model predictions, the observed overall Shannon diversity indices were plotted against the predicted values from the GIS model to see if the two sets of values were proportional (Figure 2). A linear regression yielded a p-value of 0.002 and an R^2 of 0.964, indicating a strong, highly significant positive linear relationship between the two sets of data.

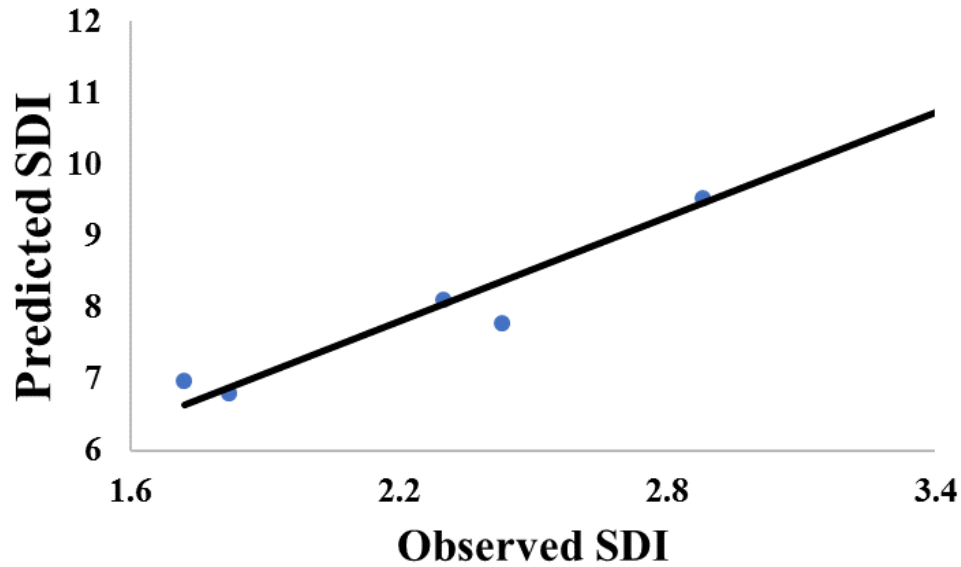


Figure 2. Plot of predicted Shannon diversity index (SDI) generated from the GIS model against the Shannon diversity index calculated from data collected in the field. For the regression, $p = 0.002$ and $R^2 = 0.964$.

Similarly, a positive relationship was also shown between cumulative and observed community competence indices, but the results were not statistically significant ($p=0.231$) and linearity was weak ($R^2 = 0.333$; Figure 3).

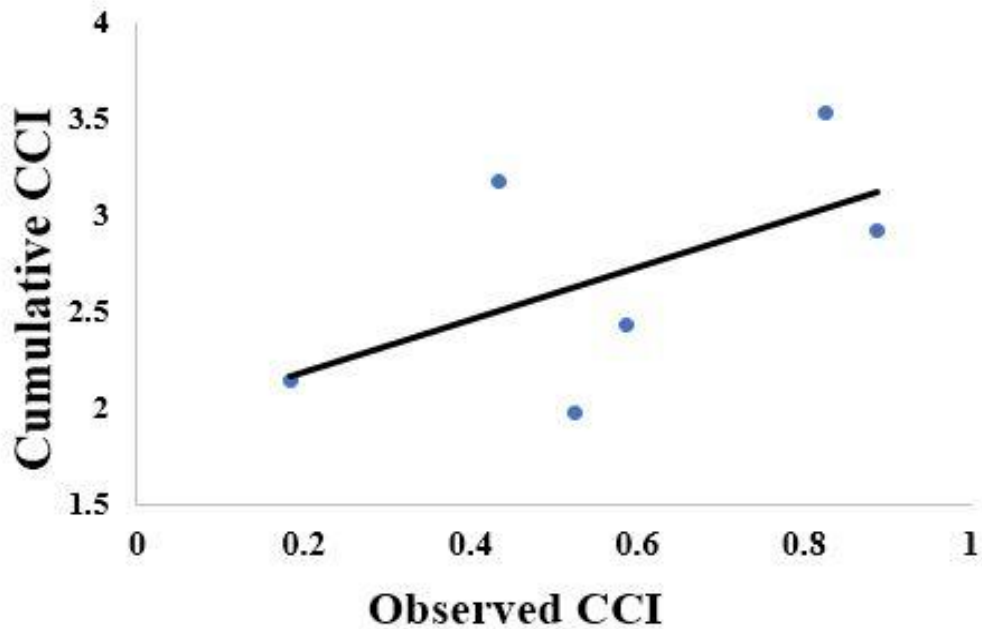


Figure 3. Plot of predicted community competence index (CCI) generated from the GIS model against the community competence index calculated from data collected in the field for virally competent bird species. For the regression, $p=0.231$ and $R^2 = 0.333$.

After testing the GIS model predictions against data collected in the field, I tested for relationships between the GIS predictions of overall diversity and diversity of virally competent species against the predicted West Nile virus risk for all locations where *C. tarsalis* were collected. Linear regressions were performed for these predicted diversity values against the West Nile virus infection rates (Figure 4). In all three cases, there was a statistically significant positive trend with p-values below 0.001. The R^2 values were 0.2695 for Figure 4A, 0.2017 for Figure 4B, and 0.2373 for Figure 4C.

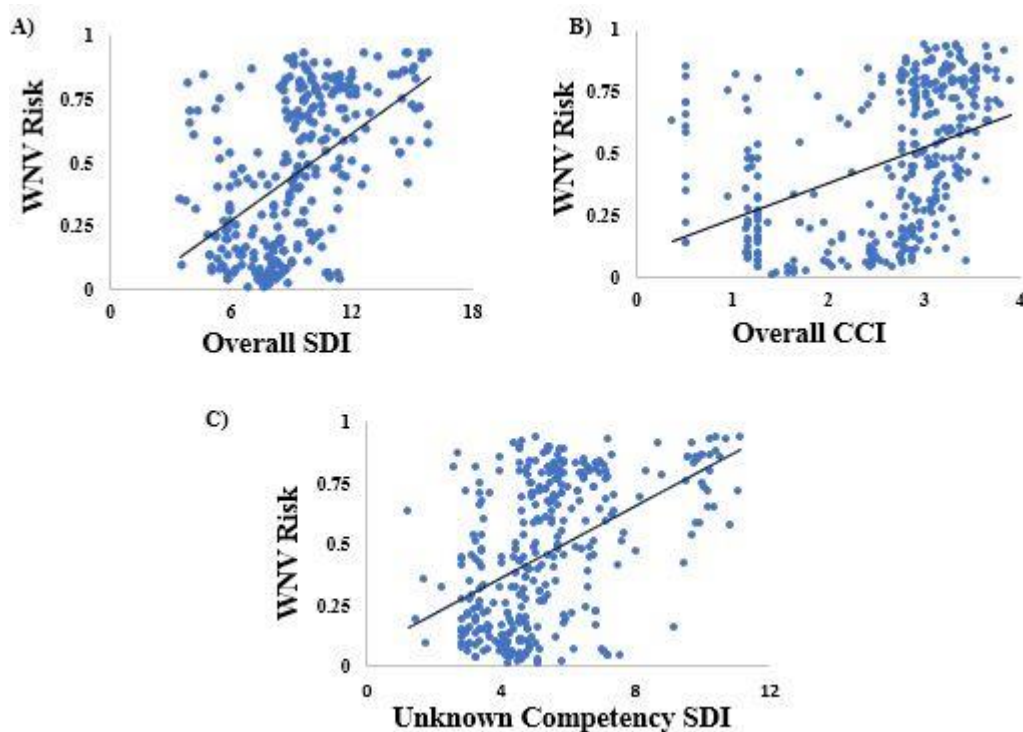


Figure 4: Graphs of the predicted West Nile virus (WNV) risk from the GIS model at all sites where *C. tarsalis* were collected against: **A)** the Shannon diversity index (SDI) for all birds identified at each trapping location, **B)** the community competence index (CCI), and **C)** the Shannon diversity index excluding virally competent birds

Similarly, linear regressions were conducted comparing the GIS predictions for overall Shannon diversity (Figure 5A), Shannon diversity of virally competent species

(Figure 5B), and Shannon diversity for species with unknown competence (Figure 5C) against West Nile virus risk. For Figure 5A the R^2 was 0.283, for Figure 5B the R^2 was 0.357, for Figure 5C the R^2 was 0.255, and in all three cases the p-value was less than 0.001. Once again, all three graphs exhibited weak linearity, but the positive correlation was highly statistically significant.

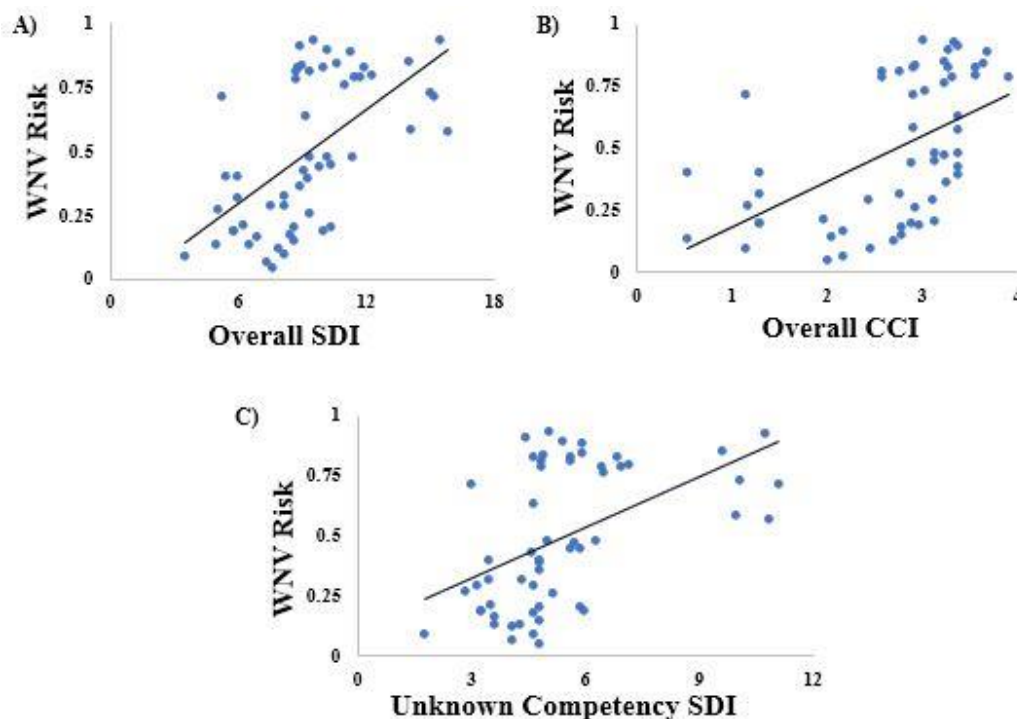


Figure 5: Graphs of the predicted West Nile virus risk from the GIS model at all sites that tested positive for West Nile virus against: **A)** the Shannon diversity index (SDI) for all birds identified at each trapping location, **B)** the community competence index (CCI), and **C)** the Shannon diversity index excluding virally competent birds

Discussion

This study aimed to test the accuracy of GIS model predictions of avian diversity, avian viral competence, and the hypothesis that increased avian diversity and decreased avian viral competence would be correlated to a decreased risk of West Nile virus infection. The generated results found a highly statistically significant positive linear

relationship between the GIS model predictions for avian diversity and data collected in the field, suggesting that the GIS model is a good indicator of the actual diversity of avian species in different regions of Montana. As would be expected with an accurate model, there was a positive correlation between the GIS model predictions of avian viral competence and data collected from the field; however, the relationship was not statistically significant. While this may suggest that the GIS model is not an accurate predictor for avian viral competence, it may be premature to draw this conclusion due to the small number of locations used in this study. It may be worthwhile to collect data from more field locations to determine if this would influence the results.

Comparisons of West Nile virus risk against the three diversity indices consistently yielded highly statistically significant positive correlations. This suggests an amplification effect, in which increased diversity of avian species may be an indicator for increased risk of West Nile virus. These results are consistent with those of Levine et al. (2017), in which increased host diversity was correlated with increases of both West Nile virus infection rates in mosquito vectors and West Nile virus antibodies in avian host blood samples. While these results seem to conflict with the dilution effects observed by Ezenwa et al. (2006) and Swaddle and Calos (2008), there may be a simple explanation for these differences. While neither Swaddle and Calos (2008) nor Levine et al. (2017) took viral competency into account in their studies, Levine et al. (2017) suggested that the identity (and viral competency) of avian species may play a greater role in West Nile virus risk than overall diversity. This observation would be consistent with the differences between the present study and the results of Ezenwa et al. (2006), as the majority of avian species present in Ezenwa et al. (2006) were non-passeriform species,

whereas in the present study the majority of birds (59.4% of recorded species and 54.4% of total birds) were passeriform species, and thus more likely to be virally competent (Komar et al. 2003). Of course, if this were the case it would be expected that the graphs comparing West Nile virus risk and avian diversity that excluded virally competent species in Figures 4C and 5C would exhibit negative correlations rather than the positive correlations seen. However, the viral competencies of most bird species observed in this study are unknown, making it impossible to isolate data from all the birds that are virally incompetent. It is possible that even by removing the known virally competent birds from the data set, a large proportion of the remaining birds may still have been virally competent. If this were the case, it is possible that the positive relationship observed may still be consistent with the hypothesis that a greater proportion of virally competent birds is more likely to result in an amplification effect whereas a greater proportion of virally incompetent birds is more likely to result in a dilution effect, but it is impossible to know without generating more data about the viral competency of observed species.

The results of this study appear to indicate that the existing GIS model is an accurate predictor of the true avian diversity in Montana, and that there is a correlation between increased avian diversity and increased risk of West Nile virus throughout the state. These observations suggest that avian diversity is a valuable layer to include in the GIS model predictions for West Nile virus risk. To strengthen the predictive power of the model, it may be valuable to collect data from more locations throughout the state of Montana to determine if this relationship holds true in specific regions. Additionally, it would be necessary to generate more data on the viral competency of bird species with unknown viral competencies to further investigate how viral competency may influence

amplification. Until such data is available, it is also possible that comparisons could be made to see if regions with higher proportions of non-passerine avian species are more likely to exhibit a dilution effect.

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