

Fall 12-2019

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Mosquito Diversity and Positive Pools for the West Nile Virus in Forested versus Non-forested
Areas

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Introduction:

Mosquitoes are vectors for many different arboviruses including the West Nile Virus. The West Nile Virus (WNV) is a neurotropic pathogen that can cause fever and encephalitis. The leading cause of mosquito vector borne disease in the United States is due to WNV (CDC, 2018). WNV was introduced to the United States in 1999 with the first human cases observed in New York (Turell et al., 2001). Only three years later, WNV activity had spread to nearly all of the United States and infected specifically humans and horses (Granwehr et al., 2004). The primary reservoir for the West Nile Virus is birds. The transmission of WNV occurs from birds to mosquitoes to humans and horses (Swetnam et al., 2018). The species of mosquito primarily responsible for the transmission of WNV is the *Culex* species (Kilpatrick et al., 2005). This study will specifically deal with *Culex* species positive for the West Nile virus and the preference toward forested versus non-forested areas. It will also include another variable of greater mosquito diversity in forested versus non-forested areas.

Previous studies have shown that it is possible for new viruses to be introduced to the United States and become established (Moreno-Madrinan & Turell, 2018). This precedent has been set by the West Nile virus. Moreno-Madrinan & Turell suggest that socioeconomic status has the potential to resist anthroponotic viruses but not zoonotic viruses. The same study defines zoonotic viruses as those that transmit between non-human animals and humans and anthroponotic as a disease where humans are the primary host. The West Nile virus is a zoonotic virus; therefore, despite higher socioeconomic standards, countries like the United States are still at risk from the WNV (Moreno-Madrinan & Turell, 2018). WNV is even more threatening due to its ability to be maintained in nature by transmission between mosquitoes and avian hosts (Vazquez et al., 2016). The importance of this experiment is to investigate if forested areas

versus non-forested areas present more mosquito diversity or have more positive cases of West Nile virus. This study will provide insight on the type of habitat that may be more at risk for infection as well as the diversity of mosquito pools in the area.

In humans, it is unlikely to see symptoms as the viremia level is extremely low (Colpitts et al., 2012). Eight out of ten people infected with WNV are asymptomatic; however, some do experience fever, joint pains, vomiting, diarrhea and other mild symptoms. Though it is very rare, it is possible for those infected to experience more severe symptoms. Out of the 20% of people who experience symptoms, less than 1% experience high fever, vision loss, coma, tremors, and paralysis (CDC, 2018). Horses have more severe symptoms of the encephalitis, typically seeing high fever, impaired coordination, paralysis, and even death (Rios et al., 2010). Since the symptoms in horses are so severe, a vaccine has been created against WNV. However, there is no vaccination for humans due to the fact the disease is asymptomatic. This study will deal specifically with *Culex* species positive for WNV. The season that the mosquito is most active is summer to fall in the US and, due to climate change, there are emerging vectors of the disease still to be determined. One study has shown climate change has the potential to expand the transmission of dengue through *Ae. aegypti*. vectors (Butterworth et al., 2017). However, the same study mentions that more research should be done in this area.

The research questions for this experiment are based off of the findings of the 2017, 2018, and 2019 Wood County, Ohio, mosquito surveillance and *Culex* species data collected in different trap types by Dr. Daniel Pavuk's research laboratory in the Department of Biological Sciences at Bowling Green State University. The variables consist of a location ID, trap type, county, agency, collection date, week number, species collected, number in pool, and Polymerase chain reaction (PCR) results as West Nile vectors. The Ohio Department of Health

(ODH) Zoonotic Disease Program at the Bureau of Infectious Diseases identified all mosquito species except for *Culex*. ODH used rapid PCR in this case to amplify West Nile Virus RNA from *Culex* mosquitoes. There are two questions posed during the experiment. Firstly, is mosquito diversity greater in forested versus non-forested areas? The hypothesis for this question is that there will be a difference in diversity in mosquito species in habitats considered forested versus non-forested environments. This is because it is known that mosquitoes tend to favor forests, wetlands, marshes, and urban locations (Hay et al., 1998). Secondly, are there greater numbers of mosquitoes testing positive for the West Nile Virus in forested versus non-forested environments? It is hypothesized that this question will have the same answer; that there will be a difference in the number of mosquitoes testing positive for the West Nile Virus in habitats with trees than without.

Materials and Methods:

This experiment used the data collected from Dr. Daniel Pavuk's research laboratory at the Department of Biological Sciences at Bowling Green State University and data obtained from the Ohio Department of Health Zoonotic Disease Program to answer two research questions: the first being, is there a difference between mosquito diversity in forested versus non-forested areas; the second being, is there a difference between mosquitoes positive for the West Nile virus in forested areas versus non-forested areas. The *Culex* species were collected in Wood County, Ohio, and confirmed at Bowling Green State University. PCR was used to identify if the mosquitoes were positive or negative for the West Nile Virus. This data is used by the Ohio Department of Health to keep track of the location of positive pools. When positive pools are identified, the GPS coordinates for the sampling site are sent to the Centers for Disease Control. Since the data collection portion of the research has already been done, the primary

goal of this experiment is to analyze the data. This will be done by specifically looking at the portions of the collected data dealing with location ID, date collected, species type, and positive vectors for West Nile virus. The location ID is separated into a four-digit state ID being 8710 for Ohio and then is followed by a dash and three digits representing different sampling sites. There were nine sampling sites in the year 2017 and eleven in 2018. This experiment will have dependent variables being the number of positive mosquito pools and the mosquito species diversity. It will have independent variables of habitat type of forested and non-forested. The number of positive mosquito pools as well as mosquito species diversity are dependent on the habitat type. In order to determine if there is a correlation, used the Shannon diversity index and then ran regression and correlation analyses on the relationships of these variables. I also ran a t-test to determine significance of the difference of numbers of positive mosquito pools and mosquito species diversity. In order to obtain data on the West Nile Virus prevalence, I calculated a relative risk (RR) ratio to determine whether mosquitoes were more at risk to be positive for WNV in forested versus non-forested areas.

Results:

I used the results from the 2018 “Not Tested” portion of the mosquito pools in Northwest Ohio to determine species diversity. First, I separated each entry by location site. There were 11 locations ranging from 001-011. There was a total of 7281 individual mosquitoes collected for that year. Locations 001, 002, 003, 004, and 008 were determined to be forested areas and locations 005, 006, 007, 009, 010, and 011 were determined to be non-forested areas. I then separated each location site by species and conducted a diversity test with Shannon’s diversity index calculation $H' = -\sum p_i \ln p_i$. I determined the species diversity in each location site. Our location sites ranged from 0.223 to 1.804. Location sites with a larger amount of individuals had

a higher Shannon Diversity Index value. The t-test results determined that forested locations had a significantly higher mean number of species than non-forested sites with a $P = 0.005$, $t = 3.26$, and $df = 9$. We also determined from a t-test that forested locations had a significantly greater Shannon diversity index value than non-forested sites with a $P = 0.002$, $t = 3.75$, and $df = 9$. The results of the West Nile virus prevalence were found to be consistently the same for all location sites meaning this study found no difference in positive mosquito pools in forested and non-forested areas. I then used the data from 2017 and 2018 to represent a cohort and create a 2x2 relative risk table. The variables of the table were positive mosquitoes in forested areas (a), positive mosquitoes in non-forested areas (b), negative mosquitoes in forested areas (c), and negative mosquitoes in non-forested areas (d). I then used the formula $RR = (a/(a+b))/(c/(c+d))$. I found that the relative risk for 2017 and 2018 were 0.842 and 0.832 respectively. In 2019, there were no mosquitos positive for the West Nile Virus. This data suggests that there is actually a decreased risk between mosquitoes being positive for the WNV in forested areas than non-forested areas.

Discussion:

The Shannon diversity index value in our experiments ranged from 0.223 to 1.804. These values are not considered to be high, however, they do fall within the range of normal. In communities, there is typically a species that is more abundant than others with hundreds of individuals and a few rarer species with only about one to two individuals. A common Shannon Diversity index score would be around 1 to 2.5. Our lower values were due to sites with an extremely low amount of individuals. The results were uneven in terms of relative abundance. Site size ranged from 3 to 2019 individuals. The p-value for the Shannon Diversity Index came back to be $P = 0.002$ meaning there is a significant difference between forested versus non-

forested areas. Species numbers per site ranged from 2-25 with the forested sites having a significantly higher mean number of species with $P = 0.005$. In 2017 and 2018, every site consistently had some positive pools for WNV. In order to determine the risk of one mosquito habitat having more than the other I conducted a relative risk ratio. The results showed that for 2017 the RR value was 0.842 and in 2018 the value was 0.832. In 2019, there were no positive mosquito pools at all meaning the RR value would be 0. It is possible that in 2019 there were no mosquitoes positive for WNV due to the movement of avian hosts. Studies have shown that the migration of bird hosts have been implicit in the spread of pathogens (Swetnam et al., 2018). Other reasons could be due to the amount of positive vectors in the area, weather, or climate. A similar study found contradictory results using habitat preference of mosquitoes transmitting Rift Valley fever. That same study found that preferred habitats were concrete tanks, water springs, and swamps (Ondiba et al., 2019). This seems to suggest that the preferred mosquito habitat for *Culex sp.* Positive for the WNV would be forested, however, my study found that to be false. Previous studies have shown vector mosquitoes are able to thrive in non-forested environments (Wilke et al., 2019). Therefore, the result of my RR values could have occurred due to the *Culex* species preference to breed in human containers such as tires, bird baths, and buckets.

The null hypothesis for the first research question in my experiment that there is no difference between mosquito diversity in forested versus non-forested areas can be rejected. Whereas the null hypothesis for the second research question in my experiment being there is no difference in the numbers of mosquitoes testing positive for the WNV in habitats with trees compared to habitats without trees cannot be accepted or rejected. I was not able to run a statistical analysis on this data because confirmed numbers were not received from ODH Biologists. However, the results of the relative risk ratio have indicated that mosquitoes are

more at risk to be positive for the WNV in non-forested than forested areas. My experiment also found that there is a significant difference between the Shannon diversity index values for mosquito diversity in forested areas than non-forested areas. More research should be done in this area to investigate mosquito diversity preference to forested versus non-forested locations on a larger scale than Northwestern Ohio. In order to determine the difference in habitat preference for mosquitoes positive for the WNV, more data should be collected from many different location sites.

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Location ID	Location	City	Forested
001	515 Sand Ridge Rd	Bowling Green, OH	Yes
002	15325 Sand Ridge Rd	Bowling Green, OH	Yes
003	15575 Poe Rd	Bowling Green, OH	Yes
004	115 Bierley Ave	Pemberville, OH	Yes
005	406 Forest Dr	Rossford, OH	No
006	10440 Neiderhouse Rd	Perrysburg, OH	No
007	City Park	North Baltimore, OH	No
008	Mary Jane Thurston Park	Grand Rapids, OH	Yes
009	City Admin Building	Walbridge, OH	No
010	City Park	Wayne City, OH	No
011	Wayne Admin Bld	Wayne, OH	No

Figure 1. Table of mosquito surveillance locations for the years 2017, 2018, and 2019 from Dr. Daniel Pavuk's research laboratory located in the Department of Biological Sciences at Bowling Green State University.

Location 001			
Species	Number of individuals	p_i	$p_i \ln p_i$
<i>Aedes cantator</i>	5	0.00338524	-0.019256
<i>Aedes hendersoni</i>	14	0.009478673	-0.044158
<i>Aedes japonicus</i>	144	0.097494922	-0.226964
<i>Aedes sp.</i>	26	0.01760325	-0.071111
<i>Aedes sticticus</i>	69	0.046716317	-0.143123
<i>Aedes stimulans</i>	8	0.005416385	-0.028265
<i>Aedes triseriatus</i>	20	0.013540961	-0.058254
<i>Aedes trivittatus</i>	916	0.620176032	-0.296290
<i>Aedes vexans</i>	194	0.131347326	-0.266623
<i>Anopheles barberi</i>	1	0.000677048	-0.004941
<i>Anopheles punctipennis</i>	2	0.001354096	-0.008943
<i>Anopheles quadrimaculatus</i>	8	0.005416385	-0.028265
<i>Culex sp.</i>	57	0.03859174	-0.125605
<i>Culiseta melanura</i>	6	0.004062288	-0.022367
<i>Psorophora ferox</i>	2	0.001354096	-0.008943
<i>Uranotaenia sapphirina</i>	5	0.00338524	-0.019256
Total:	1477	1	-1.372365
Number of species (S) = 16			
Number of individuals of all species (N) =		1477	

Figure 2. Total number of species, individuals, and Shannon Diversity Index value for Location 001 in 2018.

Location 002			
Species	Number of individuals	p_i	$p_i \ln p_i$
<i>Aedes aurifer</i>	12	0.005943536	-0.03046
<i>Aedes canadensis</i>	27	0.013372957	-0.0577
<i>Aedes cantator</i>	6	0.002971768	-0.01729
<i>Aedes excrucians</i>	1	0.000495295	-0.00377
<i>Aedes flavescens</i>	3	0.001485884	-0.00968
<i>Aedes grossbecki</i>	6	0.002971768	-0.01729
<i>Aedes hendersoni</i>	3	0.001485884	-0.00968
<i>Aedes implicatus</i>	2	0.000990589	-0.00685
<i>Aedes japonicus</i>	72	0.035661218	-0.11888
<i>Aedes mitchellae</i>	1	0.000495295	-0.00377
<i>Aedes sollicitans</i>	11	0.005448242	-0.0284
<i>Aedes sp.</i>	72	0.035661218	-0.11888
<i>Aedes sticticus</i>	148	0.073303616	-0.19155
<i>Aedes stimulans</i>	1	0.000495295	-0.00377
<i>Aedes triseriatus</i>	50	0.024764735	-0.09159
<i>Aedes trivittatus</i>	970	0.480435859	-0.35219
<i>Adese vexans</i>	425	0.210500248	-0.32802
<i>Anopheles barberi</i>	1	0.000495295	-0.00377
<i>Anopheles punctipennis</i>	54	0.026745914	-0.09686
<i>Anopheles quadrimaculatus</i>	8	0.003962358	-0.02192
<i>Coquillettidia perturbans</i>	1	0.000495295	-0.00377
<i>Culex sp.</i>	3	0.001485884	-0.00968
<i>Psorophora ferox</i>	136	0.067360079	-0.18172
<i>Toxorhynchites sp.</i>	1	0.000495295	-0.00377
<i>Uranotaenia sapphirina</i>	5	0.002476474	-0.01486
Total:	2019	1	-1.7261
Number of species (S) = 25			
Number of individuals of all species (N) =		2019	

Figure 3. Total number of species, individuals, and Shannon Diversity Index value for Location 002 in 2018.

Location 003			
Species	Number of individuals	p_i	$p_i \ln p_i$
<i>Aedes cantator</i>	394	0.25751634	-0.34937
<i>Aedes dorsalis</i>	1	0.000653595	-0.00479
<i>Aedes grossbecki</i>	41	0.026797386	-0.09699
<i>Aedes japonicus</i>	21	0.01372549	-0.05886
<i>Aedes sollicitans</i>	6	0.003921569	-0.02173
<i>Aedes sp.</i>	56	0.036601307	-0.12107
<i>Aedes sticticus</i>	32	0.020915033	-0.08088
<i>Aedes triseriatus</i>	20	0.013071895	-0.0567
<i>Aedes trivittatus</i>	226	0.147712418	-0.2825
<i>Aedes vexans</i>	620	0.405228758	-0.36604
<i>Anopheles barberi</i>	2	0.00130719	-0.00868
<i>Anopheles punctipennis</i>	45	0.029411765	-0.10372
<i>Anopheles quadrimaculatus</i>	13	0.008496732	-0.04051
<i>Coquillettidia perturbans</i>	9	0.005882353	-0.03021
<i>Culex sp.</i>	19	0.012418301	-0.0545
<i>Culiseta melanura</i>	6	0.003921569	-0.02173
<i>Psorophora ferox</i>	6	0.003921569	-0.02173
<i>Psorophora horrida</i>	3	0.001960784	-0.01222
<i>Uranotaenia sapphirina</i>	10	0.006535948	-0.03288
Total:	1530	1	-1.76511
Number of species (S) = 19			
Number of individuals of all species (N) =		1530	

Figure 4. Total number of species, individuals, and Shannon Diversity Index value for Location 003 in 2018.

Location 004			
Species	Number of individuals	p_i	$p_i \ln p_i$
<i>Aedes canadensis</i>	4	0.034188034	-0.11541
<i>Aedes japonicus</i>	14	0.11965812	-0.25405
<i>Aedes sollicitans</i>	8	0.068376068	-0.18343
<i>Aedes sp.</i>	7	0.05982906	-0.16849
<i>Aedes triseriatus</i>	13	0.111111111	-0.24414
<i>Aedes trivittatus</i>	44	0.376068376	-0.36779
<i>Adese vexans</i>	23	0.196581197	-0.31977
<i>Anopheles punctipennis</i>	2	0.017094017	-0.06956
<i>Anopheles quadrimaculatus</i>	1	0.008547009	-0.0407
<i>Uranotaenia sapphirina</i>	1	0.008547009	-0.0407
Total:	117	1	-1.80405
Number of species (S) = 10			
Number of individuals of all species (N) = 117			

Figure 5. Total number of species, individuals, and Shannon Diversity Index value for Location 004 in 2018.

Location 005			
Species	Number of individuals	p_i	$p_i \ln p_i$
<i>Aedes canadensis</i>	13	0.017105263	-0.06959
<i>Aedes cantator</i>	86	0.113157895	-0.24657
<i>Aedes grossbecki</i>	2	0.002631579	-0.01563
<i>Aedes japonicus</i>	434	0.571052632	-0.31995
<i>Aedes sollicitans</i>	2	0.002631579	-0.01563
<i>Aedes sp.</i>	3	0.003947368	-0.02185
<i>Aedes sticticus</i>	8	0.010526316	-0.04794
<i>Aedes triseriatus</i>	2	0.002631579	-0.01563
<i>Aedes trivittatus</i>	8	0.010526316	-0.04794
<i>Aedes vexans</i>	161	0.211842105	-0.32876
<i>Anopheles barberi</i>	2	0.002631579	-0.01563
<i>Anopheles punctipennis</i>	9	0.011842105	-0.05253
<i>Anopheles quadrimaculatus</i>	22	0.028947368	-0.10254
<i>Coquillettia perturbans</i>	4	0.005263158	-0.02762
<i>Culex sp.</i>	4	0.005263158	-0.02762
Total:	760	1	-1.35542
Number of species (S) = 15			
Number of individuals of all species (N) =		760	

Figure 6. Total number of species, individuals, and Shannon Diversity Index value for Location 005 in 2018.

Location 006			
Species	Number of individuals	p_i	$p_i \ln p_i$
<i>Aedes canadensis</i>	5	0.027624309	-0.09915
<i>Aedes cantator</i>	22	0.121546961	-0.25615
<i>Aedes japonicus</i>	4	0.022099448	-0.08425
<i>Aedes sollicitans</i>	2	0.011049724	-0.04978
<i>Aedes stimulans</i>	1	0.005524862	-0.02872
<i>Aedes triseriatus</i>	1	0.005524862	-0.02872
<i>Aedes trivittatus</i>	20	0.110497238	-0.2434
<i>Aedes vexans</i>	125	0.690607735	-0.25565
<i>Anopheles quadrimaculatus</i>	1	0.005524862	-0.02872
Total:	181	1	-1.07454
Number of species (S) = 9			
Number of individuals of all species (N) = 181			

Figure 7. Total number of species, individuals, and Shannon Diversity Index value for Location 006 in 2018.

Location 007			
Species	Number of individuals	p_i	$p_i \ln p_i$
<i>Aedes japonicus</i>	8	0.533333333	-0.33526
<i>Aedes trivittatus</i>	1	0.066666667	-0.18054
<i>Aedes vexans</i>	3	0.2	-0.32189
<i>Anopheles quadrimaculatus</i>	3	0.2	-0.32189
Total:	15	1	-1.15957
Number of species (S) = 4			
Number of individuals of all species (N) = 15			

Figure 8. Total number of species, individuals, and Shannon Diversity Index value for Location 007 in 2018.

Location 008			
Species	Number of individuals	p_i	$p_i \ln p_i$
<i>Aedes aurifer</i>	1	0.000863558	-0.0060919
<i>Aedes cantator</i>	16	0.013816926	-0.0591622
<i>Aedes japonicus</i>	31	0.026770294	-0.0969208
<i>Aedes sp.</i>	90	0.077720207	-0.1985472
<i>Aedes sticticus</i>	96	0.082901554	-0.2064333
<i>Aedes stimulans</i>	1	0.000863558	-0.0060919
<i>Aedes triseriatus</i>	8	0.006908463	-0.0343697
<i>Aedes trivittatus</i>	451	0.389464594	-0.3672582
<i>Adese vexans</i>	369	0.31865285	-0.3644283
<i>Anopheles punctipennis</i>	11	0.009499136	-0.0442333
<i>Anopheles quadrimaculatus</i>	39	0.033678756	-0.1142009
<i>Coquillettidia perturbans</i>	5	0.004317789	-0.0235104
<i>Psorophora ferox</i>	40	0.034542314	-0.1162546
Total:	1158	1	-1.6375026
Number of species (S) = 13			
Number of individuals of all species (N) = 1158			

Figure 9. Total number of species, individuals, and Shannon Diversity Index value for Location 008 in 2018.

Location 009			
Species	Number of individuals	p_i	$p_i \ln p_i$
<i>Anopheles punctipennis</i>	2	0.666666667	-0.27031
<i>Anopheles quadrimaculatus</i>	1	0.333333333	-0.3662
Total:	3	1	-0.63651
Number of species (S) = 2			
Number of individuals of all species (N) = 3			

Figure 10. Total number of species, individuals, and Shannon Diversity Index value for Location 009 in 2018.

Location 010			
Species	Number of individuals	p_i	$p_i \ln p_i$
<i>Aedes japonicus</i>	1	0.25	-0.34657
<i>Aedes triseriatus</i>	2	0.5	-0.34657
<i>Culex sp.</i>	1	0.25	-0.34657
Total:		4	1
Number of species (S) = 3			
Number of individuals of all species (N) =			4

Figure 11. Total number of species, individuals, and Shannon Diversity Index value for Location 010 in 2018.

Location 011			
Species	Number of individuals	p_i	$p_i \ln p_i$
<i>Aedes japonicus</i>	16	0.941176471	-0.05706
<i>Aedes vexans</i>	1	0.058823529	-0.16666
Total:		17	1
Number of species (S) = 2			
Number of individuals of all species (N) =			17

Figure 12. Total number of species, individuals, and Shannon Diversity Index value for Location 011 in 2018.

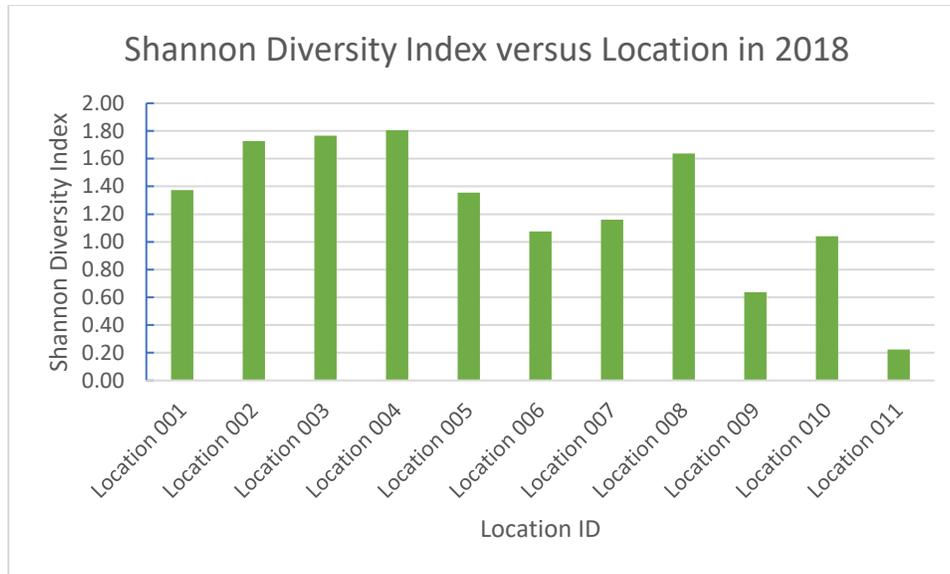


Figure 13. Shannon Diversity Index values for each location site based on data collected from Dr. Daniel Pavuk’s research laboratory located in the Department of Biological Sciences at Bowling Green State University and The Ohio Department of Health Zoonotic Disease Program at the Bureau of Infectious Diseases in 2018.

Site	Positive	Negative
Location 001	74	104
Location 002	217	100
Location 003	100	64
Location 004	83	4
Location 008	99	1
Forested Total	573	273
Location 005	350	52
Location 006	92	2
Location 007	13	85
Location 009	124	2
Location 010	0	0
Location 011	0	0
Non-forested Total	579	141

Figure 14. Mosquitoes positive and negative for the WNV based on location sites in 2017.

Site	Positive	Negative
Location 001	35	225
Location 002	31	445
Location 003	50	191
Location 004	175	105
Location 008	34	266
Forested Total	325	1232
Location 005	282	532
Location 006	141	658
Location 007	50	157
Location 009	115	195
Location 010	0	82
Location 011	34	233
Non-forested Total	622	1857

Figure 15. Mosquitoes positive and negative for the WNV based on location sites in 2018.

	Positive	Negative
Forested	573	273
Non-forested	579	141
Relative Risk	0.842	

Figure 16. Relative Risk ratio for mosquitoes positive and negative for the West Nile Virus in forested versus non-forested areas in 2017.

	Positive	Negative
Forested	325	1232
Non-forested	622	1857
Relative Risk	0.832	

Figure 17. Relative Risk ratio for mosquitoes positive and negative for the West Nile Virus in forested versus non-forested areas in 2018.