Climatic, Ecological, and Socioeconomic Factors Associated with West Nile Virus Incidence in Atlanta, Georgia, U.S.A.

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Climatic, ecological, and socioeconomic factors associated with West Nile virus incidence in Atlanta, Georgia, U.S.A.

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ABSTRACT: The integrated effects of the many risk factors associated with West Nile virus (WNV) incidence are complex and not well understood. We studied an array of risk factors in and around Atlanta, GA, that have been shown to be linked with WNV in other locations. This array was comprehensive and included climate and meteorological metrics, vegetation characteristics, land use / land cover analyses, and socioeconomic factors. Data on mosquito abundance and WNV mosquito infection rates were obtained for 58 sites and covered 2009-2011, a period following the combined storm water – sewer overflow remediation in that city. Risk factors were compared to mosquito abundance and the WNV vector index (VI) using regression analyses individually and in combination. Lagged climate variables, including soil moisture and temperature, were significantly correlated (positively) with vector index as were forest patch size and percent pine composition of patches (both negatively). Socioeconomic factors that were most highly correlated (positively) with the VI included the proportion of low income households and homes built before 1960 and housing density. The model selected through stepwise regression that related risk factors to the VI included (in the order of decreasing influence) proportion of houses built before 1960, percent of pine in patches, and proportion of low income households. ***Journal of Vector Ecology*** **41 (2): 232-243. 2016.**

Keyword Index: West Nile virus, water quality, socioeconomic health risk, forest cover loss, urbanization, climate induced health risk.

INTRODUCTION

Accompanying inevitable increases in the U.S. population is an increase in the number projected to live in urban landscapes. An associated shift from forest to urban land use will have significant negative implications for water quality and hydrologic stability (Lockaby et al. 2013). In particular, the hydrologic and water quality changes that arise following forest conversion to urban land uses often have negative implications for human health (Patz et al. 2004). As examples, high concentrations of fecal coliform, *E. coli*, and other pathogenic organisms are often found in urban streams (Paul and Meyer 2001, Nagy et al. 2011, 2012, Lockaby et al. 2013). Additionally, in many portions of the United States, unstable hydrology associated with loss of forest cover and concurrent increases in impervious surface, cause combined sewer overflows (CSOs) to exceed capacity during storms and inject untreated sewage effluent directly into associated streams. As a consequence of the increased pollution and pooling of overflow waters, habitats for vectors such as *Culex sp*. mosquitoes may be enhanced, thereby stimulating West Nile virus (WNV) risk to humans (Chaves et. al 2009, 2011, Calhoun et al. 2007, Vazquez-Prokepec et al. 2010). However, WNV transmission can be strongly influenced by an array of other factors, including socioeconomic conditions, bird habitats, and climatic trends. A clear understanding of linkages among land use changes, climate, socioeconomic conditions, and occurrence of WNV remains elusive.

WNV has occurred in the 48 contiguous states, all of which have reported at least one neuroinvasive case. In addition to threatening lives, outbreaks of WNV in the United States are very costly in terms of both medical treatment and event control. As an example, a 2005 outbreak in Sacramento, CA generated costs of approximately $3 million, of which about $700 thousand was associated with vector control (emergency spraying) and $2.3 million was related to medical treatment and lost productivity of patients (Barber et al. 2010). Consequently, millions of dollars in cost savings to communities and individuals could accrue from improvements in understanding risk. Cumulative cost savings at the national level could extend into billions of dollars. Understanding the relationship between urban forest cover, climate, socioeconomics, and incidences of arbovirus can provide insights into developing management strategies that reduce risk to humans.

Our general hypothesis was that as forest cover decreases and impervious surfaces increase in watersheds, there will be a corresponding increased risk of WNV associated with urban land-use conversion and degradation of water quality in urban streams. Also, increased risk will be manifested to the greatest extent in low income neighborhoods following warm winters and mild springs. In addition, risk will decline in relation to larger forest patch sizes.

Despite the significant amount of research that has focused on linkages between environmental degradation and human health, the relationships among environmental factors, social context, and incidence of diseases, such as WNV infection, remain poorly understood (Vazquez-Prokopec et al. 2010). Prior research strongly suggests that forest cover, in general, affects the incidence of WNV and other arboviruses (Wilson 1994, Brown et al. 2008, LaBeaud et al. 2008). However, there are conflicting reports concerning whether the forest – risk relationship is positive or negative and almost no information is available regarding the specific forest attributes and mechanisms involved. In addition, variation in surface water hydrology due to changes in land uses may seriously affect human health across developed landscapes and create conditions of clear risk for human epidemics (Schwarzenbach et al. 2010), although this relationship is particularly unclear. As a result, our existing knowledge base offers little information that could aid in managing urban forests for risk reduction.

Often, environmental factors are only assessed at a very general level and solely from remotely sensed data. As a result, conflicting reports have emerged. In some cases, research suggests that health risks associated with arboviruses may be inversely related to the presence of forest or vegetation cover. LaBeaud et al. (2008) indicated that WNV risk in their Ohio study area declined as agricultural land, wetland, or forest increased. Also, Brown et al. (2008), working across the northeast U.S., found higher incidences of WNV within counties exhibiting greater urban and lowest forest cover. In contrast, in New York and Chicago the number of human WNV cases rose as vegetation cover increased (Brownstein et al. 2002 and Ruiz et al. 2004, respectively). Clearly, a better understanding of the relationships between land cover and WNV occurrence is needed.

There are indications that forest distribution, rather than its amount alone, is important. Vazquez-Prokopec et al. (2010) suggested that a positive relationship existed between numbers of infected mosquitoes and the range of percent tree cover within 1 km of sampling sites. Similarly, Rochlin et al. (2011) noted increases in WNV risk as road density and forest fragmentation increased. However, as with forest stand characteristics, we have little understanding of how distribution of urban tree cover relates to health risks. Several studies have focused on landscape factors that are associated with risk of WNV infection in Georgia. Gibbs et al. (2006) found that higher risk was associated with urban environments, moderate housing densities, and higher winter temperatures. In Atlanta, Vazquez-Prokopec et al. (2010) noted a close association between incidence of WNV in humans and proximity to CSOs, an effect related to poorly designed sewer systems and impacts of impervious surfaces on hydrology.

Socioeconomic characteristics, especially components relating to housing density, housing age, rent/ownership, landscaping, income, education, and race or ethnicity are often significantly correlated with WNV infection rates. Liu et al. (2011) found that West Nile virus prevalence in northern Virginia was associated with low to medium levels of impervious surface, some forest canopy, old houses, and low income. The authors suggest that the link to old houses was due to two factors: old sewer systems and mature trees for bird habitat. Exploring the factors that contribute to WNV, Ozdenerol et al. (2008) found that low income, minority neighborhoods with high renter occupation, old structures, and lots of vacant lots were associated with WNV prevalence. They suggest that poor storm drainage and the lack of landscape upkeep could lead to increased mosquito habitat and higher WNV prevalence. According to a statewide analysis in Georgia, the highest risk was in low housing densities with about one house per ten acres (Gibbs et al. 2006). Rochlin et al. (2011) found that WNV risk in Suffolk County, NY, was primarily associated with suburban middle class neighborhoods rather than with affluent outer suburbs or the poorer inner cities. This conclusion was similar to that of Ruiz et al. (2004), who identified the 'inner suburb" of mostly white residents, with homes from the 1940-50s, and fairly high income as being the most at-risk areas of these cities. The authors suggest that the sewer systems and the vegetative cover of mature trees and some open fields of these neighborhoods were potential reasons for the increased risk.

However, the findings regarding many of the socioeconomic variables are not consistent. For example, DeGroote et al. (2008) found that rural instead of urban or suburban areas of Iowa had the highest incidences of human diseases. Furthermore, Harrigan et al. (2010) found that low per capita income was primarily linked with WNV risk. Their study highlights the neglected swimming pools and habitats that could retain water and become mosquito breeding sites. In Florida, Hribar (2007) examined the presence of larvae of two potential vector mosquitoes in sixteen different habitat types including septic tanks, tires, buckets, ornamental ponds, flower pots, swimming pools, and hot tubs, among others. He found larvae of at least one species of *Culex* in over half of the habitats. Dowling et al. (2013) also focused on containers in Washington D.C. and found mosquito larvae in over a third of the 850 habitats sampled. This research suggests that the relationship between the socioeconomic variables and the natural environment may be specific to the regional context. Additionally, many of the studies relied solely on census track data for the demographic variables while others focused primarily on presence of water-holding containers. Analyses that utilize both census and field data are lacking.

The relationship of WNV dynamics with current and past meteorological conditions has been studied at a robust level. Invasion of WNV in North America was associated with above-average temperatures, with decreased or delayed viral activity during cool summers (Reisen et al. 2006). Warmer temperatures, elevated humidity, and heavy precipitation have been found to increase the relative rate of human WNV infection, independent of season (Soverow et al. 2009). Both higher temperatures and reduced rainfall together were strongly associated with WNV prevalence in mosquitoes and human WNV cases (Ruiz et al. 2010). Finally, Shaman et al. (2005) found that drought followed by wetting of the land surface was significantly associated with the spatiotemporal variability of human WNV cases. There is a general agreement in the literature that a meteorological situation of mild, wet winters and warm springs followed by intense summer drought are associated with WNV outbreaks in the eastern

U.S. (Chung et al. 2013). However, many questions remain regarding the influence of climate and projected climatic changes on WNV incidence.

The impact of climate change on the transmission of vector-borne diseases risk is a strongly debated topic (Kilpatrick and Randolph 2012, Chaves et al. 2012). The acceleration of the global burden of tropical diseases, such as malaria and dengue, due to climate change is arguable (Githeko et al. 2000, Campbell et al. 2015). The affect of climate change on mosquitoes may be diverse due to variation in the response of vectors to temperature (Ruybal et al. 2016), but some researchers claim that climate change will reduce the transmission rate in some tropical regions where the temperature will be too high, and the geographic shift in distribution will have little effect overall on disease transmission (Rogers and Randolph 2006). Although climate is a fundamental regulator of the incidence of vector-borne diseases, the significance of weather dynamics in comparison to other potential risk factors is uncertain (Naish et al. 2014).

Many diseases of tropical origin, including WNV, are sensitive to climate and are likely to change their distribution in the coming decades (Hoover and Barker 2016). However, our knowledge of the effects of climate on mosquito population dynamics is still limited, due to the contrasting results from different studies. A topic that has received less attention is the extended effect of lagged climate conditions on mosquito populations over a range of time. Most studies focus mainly on the interaction between climate variables and mosquito abundance at the same time or over a single point lag in time. This only considers the conditions at a certain time point prior to sampling. Single time lags might not capture meteorological effects on mosquito abundance if preceding conditions contributed to breeding and survival over weeks to months (Chuang et al. 2012). To develop a more robust inference, the association of vector abundance with leading climate variables under specific time interval lags requires evaluation.

MATERIALS AND METHODS

Study area

Metropolitan Atlanta was chosen as the study area because it is the largest metropolitan area in the southeastern U.S., surveillance has been conducted continuously by the Georgia Department of Health and its partners since 2001, and WNV is established in the area where it is mainly transmitted by *Culex quinquefasciatus* (Gibbs et al. 2006). In Atlanta, increased *Culex quinquefasciatus* abundance and WNV transmission were found to be associated with organic pollution caused by combined sewer overflow events (Vazquez-Prokopec et al. 2010). However, the City of Atlanta remediated the CSO system by separation of combined sewers into distinct sewer and stormwater lines and the construction of off-line storage facilities (Lund et al. 2014). These modifications were completed in 2008, and while streams associated with the CSO overflows still provided suitable habitat for immature mosquitoes, the remediation of the facilities successfully reduced the productivity of *Culex* species (Lund et al. 2014). In order to avoid the potentially confounding effect of CSO related increases in mosquito productivity and WNV transmission risk (Vazquez-Prokopec et al. 2010), our study period was restricted to 2009-2011, after the remediation of the combined sewer facilities. *Culex quinquefasciatus* is the main vector of WNV in the southeastern U.S. (Anderson et al. 2010, Eastwood et al. 2011) and is the most abundant mosquito species in Atlanta. Mosquitoes had been collected overnight using paired CO_2-baited CDC light traps (Silver 2008) and gravid traps (Reiter 1983), classified by species, pooled by date, location, species, and trap type, and tested for WNV infection at the University of Georgia (Vazquez-Prokopec et al. 2010). In order to characterize the distribution of WNV transmission risk in Atlanta after CSO remediation, mosquito trap locations were identified that had been used at least ten times between 2009 and 2011 in Fulton, DeKalb, and Cobb counties. Fifty-eight locations were identified, and study sites (Figure 1) were established in the 1 km radius area around these locations, based on the maximum flight distance of *Cx. quinquefasciatus* (Reisen et al. 1991). Additional studies with *Cx. quinquefasciatus* have reported maximum flight distances ranging from <1.0 to 2.1 km (Fussell 1964, Lindquist et al. 1967).

Mosquito data collection

To characterize the distribution of mosquito vectors, mosquito abundance was estimated across the study sites as the average number of *Culex quinquefasciatus* collected per trap night, also known as the catch per unit effort (Magori et al. 2011). Correcting for the number of trap nights was important because collections were done during a single night in Fulton and DeKalb counties, while Cobb County left gravid traps out for two consecutive nights. The average infection rate was also used to characterize the distribution of WNV levels in collected *Cx. quinquefasciatus* across the study sites calculated using maximum likelihood methods (Biggerstaff 2006), which provide confidence intervals. As opposed

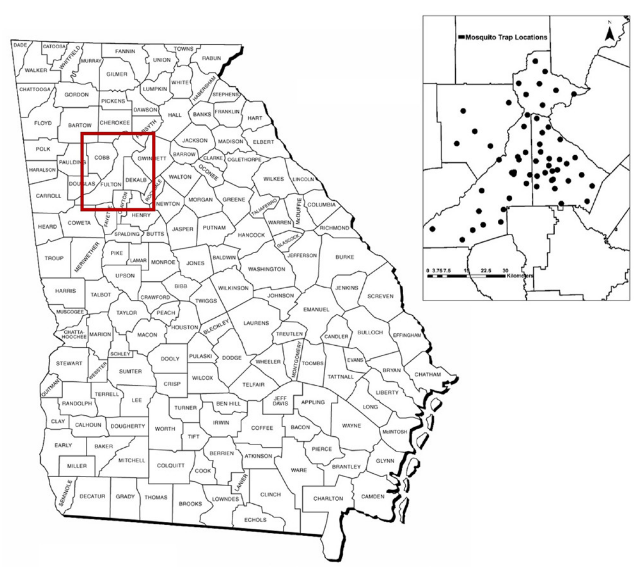

Figure 1. Map of study area showing distribution of mosquito trap sites in and around Atlanta.

to the simpler Minimum Infection Rate, these maximum likelihood estimates do not depend on the assumption of a single infected mosquito in a WNV-positive mosquito pool (Kwan et al. 2012). In order to characterize the distribution of WNV transmission risk, the vector index (VI) was estimated as the product of the *Cx. quinquefasciatus* abundance and the maximum likelihood estimate of the infection rate (Kwan et al. 2012, Kilpatrick and Pape 2013, Chung et al. 2013). It is calculated by multiplying the average number of mosquitoes collected per trap night, by the proportion infected with WNV*1000. The VI can be interpreted as the average number of WNV-positive *Cx. quinquefasciatus* collected in the traps per trap night (Chung et al. 2013), which correlates with the number of WNV-infectious females in search of blood meals at each particular location. Previous research has shown that the VI can be used as a reliable indicator of WNV transmission risk and identified a threshold of 0.5 for significant public health risk (Chung et al. 2013).

Landscape data collection

To identify landscape attributes of the sites, land use/land cover was classified within a 1 km radius of each site based on high resolution aerial imagery. One-meter resolution imagery for the year 2010 was obtained and object based image analysis (OBIA) performed for Cobb, Fulton, and DeKalb Counties. Four categories of land cover, water, forest, impervious and open/other were produced using the modified Anderson level I scheme and a land cover map was created for 1 km radius buffers around 58 sites. Percent impervious and forest cover were calculated for each study site based on this classification. Landscape attributes were measured for global spatial autocorrelation using Moran's I (Moran 1950). The forest category was later classified in terms of forest type, i.e., the two to three major species that dominated forest cover within each patch defined inside the 1 km buffer.

Socioeconomic data collection

To characterize socioeconomic conditions across study sites, 2010 census data by census tracts for Fulton, DeKalb, and Cobb counties were downloaded from www.census.gov. Those data were used to calculate a weighted average of the socio-economic variables that fell within the sites based on the degree of overlap with the 1 km radius buffers. Three income categories (low \$0-\$25,000, medium \$25,000-\$100,000, and high >\$100,000) and three housing age classes were defined (pre 1960s, 1960-1990, and newer than 1990). All vacant houses were placed into one of the following categories: for rent, for sale, sold but not occupied, seasonal use, and other. Housing density for each census tract was calculated by dividing the number of total households with the area of the census tract. Based on their association with WNV risk in the literature, we selected the proportion of low income populations (Harrigan et al. 2010, Rochlin et al. 2011), the proportion of houses built before the 1960s (Ruiz et al. 2004), the proportion of vacant houses (Reisen et al. 2008), and housing density (Trawinski and Mackay 2010, Harrigan et al. 2010) as potential socioeconomic attributes of interest.

Social context within the 1 km radius of all study sites zone was determined from the 2010 census data. Three data collection tracks were selected within each 1 km buffer, within three sectors of the buffer. The points were selected to be on a road as close to the center of the sectors as feasible. Data were collected along a 100 meter track. All visible items that could hold water were grouped into three categories and the number in each was recorded within approximately 25 m on either side of the track center. The categories were: (1) trash (tires, buckets, pots, plastic garbage bags, etc.), (2) ornamental (birdbaths, ponds, fountains, etc.), and (3) driveway/lot (boat, abandoned car, etc.). Geo-tagged photos were taken along each track to represent notable items that could hold water. Also, along the track, general characteristics of lawns and yards, land use type of the track, and characteristic housing types were recorded. Finally, abandoned tires found along transects were counted.

Climate data collection

To assess the effect of climate variation on VI data, the study was conducted in two parts. First, the potential relationship between lagged climate variables and mosquito infection rate was defined. For this part, the vector index was estimated using data collected from mosquito traps between 2002 and 2009 located in north-central Georgia. The interval lags for the climate variables showing the highest positive or negative correlations were then applied to the weather data near the 58 selected mosquito trap sites for the period 2009-2011. These analyses were done on two time scales, weekly and a four-week moving average. To perform the analysis in a monthly scale with sufficient data, a four-week moving average of mosquito infection rate and climate data were obtained.

For the first part of the analysis, mean weekly precipitation, temperature, potential evapotranspiration (PET), and available moisture in the surface layer from 2002 to 2009 were downloaded from National Weather Service, Climate Prediction Center (CPC) (http://www.cpc.ncep.noaa.gov/products/monitoring_and_data/drought.shtml).
Soil moisture was estimated by a one-layer hydrological model, in the upper 1-2 m of soil (Huang et al. 1996, Van den Dool et al. 2003). Potential evapotranspiration was computed from observed temperature using the Thornthwaite method (Thornthwaite 1948). Climate data were obtained for division 2 in the north-central part of Georgia. Figure 2 shows the weekly climate data over the period 2002 to 2009 for counties located in the study area. Mosquito data were obtained from 2002 to 2009 for the counties in division 2.

For the second part of the analysis, the temperature data near the 58 selected mosquito trap sites for the period 2009-2011 were obtained from the National Center for Environmental Information, National Oceanic and Atmospheric Administration, http://www.ncdc.noaa.gov/. The average 0 to 10 cm layer 1 soil moisture content data was obtained from NASA Goddard Earth Sciences (GES) Data and Information Services Center, GLDAS_NOAA, http://hiscentral.cuahsi.org/pub_network.aspx?n=262.

Forest patch and land use / land cover analyses

Ecological analyses were conducted within a 1 km radius of 58 mosquito trap sites to provide an evaluation of changing habitat availability for birds and *Culex* mosquitoes in the greater Atlanta, GA metropolitan area. The patch analysis links to the landscape evaluation by providing habitat structural information, thus yielding insights into habitat availability and suitability for birds. Landscape and forest patch metrics were averaged for each site and used as independent variables in the analyses of WNV VI.

Percent canopy cover within each 1 km radius site was divided into forest patches based on observed spatial continuity in forest distributions in order to account for differences in habitat structure. At each site, representative forest patches were selected for vegetation sampling. Three plots were established within each patch where overstory tree species, diameter at breast height (DBH), and heights from one of each overstory species present were estimated. Also, understory species composition, height, and percent area in shrubs were measured as well. Additionally, topographic positions were characterized as either riparian, slope, or upland. For each site, areas of patches and tree stem density per ha for each diameter class were averaged.

Linear regression models and correlation analyses were used to assess the impact of forest vegetation and land use data on VI. Linearity criteria were met for the forest patch size vs VI and for percent pine composition vs VI. A weighted approach was used to improve the fit of the regression analysis dealing with VI * percent impervious surface. Regression analysis indicated that patch size and percent of pine composition of patches were significantly and independently related to vector indices.

Socioeconomic data analysis

Data from three collection tracks including trash, ornamentals, driveways, and house/apartments were averaged for each 1 km mosquito trap buffer. The factors with low overall numbers such as number of abandoned buildings and number of boats were removed from the analysis. All VIF values for the remaining parameters were less than 2, which show there was no severe collinearity among the parameters. Weighting factors were used to adjust the independent variable in the low income * VI regression for unequal variances and were assessed in the pre-1960s housing * VI regression. In the latter, the weighting resulted in a poorer fit while the low income regression was improved.

Climate data analysis

To visualize the potential relationship between antecedent meteorological variables and mosquito infection rate time-series data, Cross Correlation Maps (CCMs) were applied. This graphical method was introduced by Curriero et al. (2005) who assessed the influence of preceding environmental conditions during a time lagged interval on the abundance of *Aedes sollicitans* species. Since then, this tool has been used to identify the timing and duration of potential meteorological effects on mosquito populations (Shone et al. 2006, Lebl et al. 2013). The CCMs were developed for both weekly and four weeks moving average data. The maximum time lag was set to 20 weeks. All analyses were performed in R statistical software (version 3.0.2, R Core Team 2013).

RESULTS AND DISCUSSION

Landscape and patch factors

Results of our landscape analysis showed significant negative correlations between VI and pine species cover as a percentage of total forest cover within a 1 km radius around trap sites, and average forest patch size (Figures 3 and 4). Urban forests with greater pine species composition had significantly decreased VI compared to stands dominated by deciduous overstory species (Figure 3).

Sites with larger forest patches also were linked with decreased VI (Figure 4), a relationship that may be driven by higher avian species diversity in the larger patches (Ezenwa et al. 2006), i.e., a possible example of the dilution effect (Swaddle and Calos 2008, Ostfeld 2011). The greater number of avian species may decrease the probability of contact between an infected mosquito and a competent host species. This type of relationship has been reported for WNV in Louisiana (Ezenwa et al. 2006) and Missouri (Allan et al. 2009). Percent pine cover and patch size were positively correlated according to the Pearson statistic ($R = 0.51$, $p <0.001$). However, each was shown to be independently correlated to VI using regression analysis. The correlation of VI and pine cover was unexpected and could relate to two factors, avian habitat or microenvironment. If pine forests were not an optimal corvid habitat, then the negative relationship with VI relationship would be understandable. However, Buler and Hamilton (2000) indicate that corvids are very active in Louisiana pine forests and represent the dominant predator of artificial nests there. Also, if pine forests in Atlanta exhibited greater diversity of non-passerine bird species compared to other forest types, this trait may account for the lower VI (Ezenwa et al. 2006). However, observations made by the Atlanta Audubon Society (Adam Beteul, Director of Conservation, personnel communication) indicate that bird species utilizing pine forests in Atlanta are generally similar to those that use hardwoods with some exceptions (e.g., brown-headed nuthatch (*Sitta puvilla*), more common in pine forests, while the white-breasted nuthatch (*Sitta carolinensis*) is more common in deciduous forests). Finally, the relationship may reflect the influence of the drier sites usually occupied by pine in the southeastern Piedmont physiographic region compared to those of deciduous species. The drier conditions could translate to less hospitable larval mosquito habitats. If the dry site theory regarding the pine near Atlanta is correct, such a relationship would not be expected in areas where pine commonly grow on wetter sites such as the southern coastal plain.

Many of the vegetation metrics measured (e.g., understory height, overstory heights, and diameters) did not show significant relationships with VI. While we cannot state that relationships may have emerged with a more intensive sampling scheme, the low intensity of sampling performed for these metrics was not sufficient to account for the large

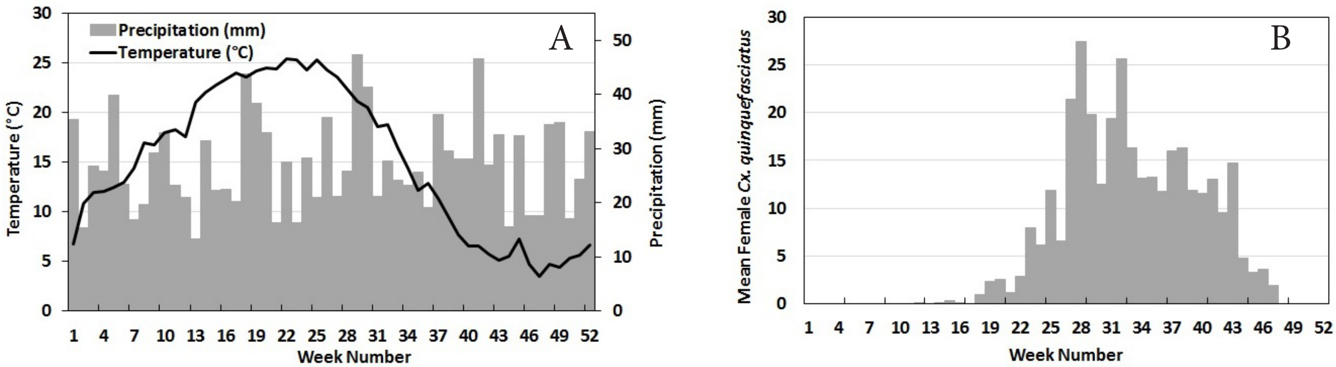

Figure 2. Average weekly temperature and precipitation (A) and number of female *Culex quinquefasciatus* over the period 2002 to 2009 for the north central portion of GA (B).

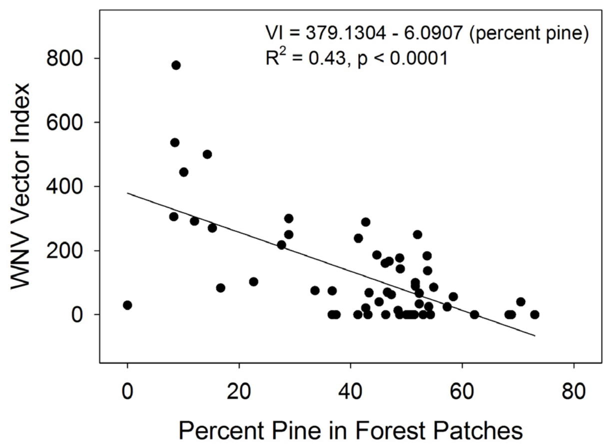

Figure 3. Relationship between WNV vector index and the proportion of pine comprising forest patches in study area.

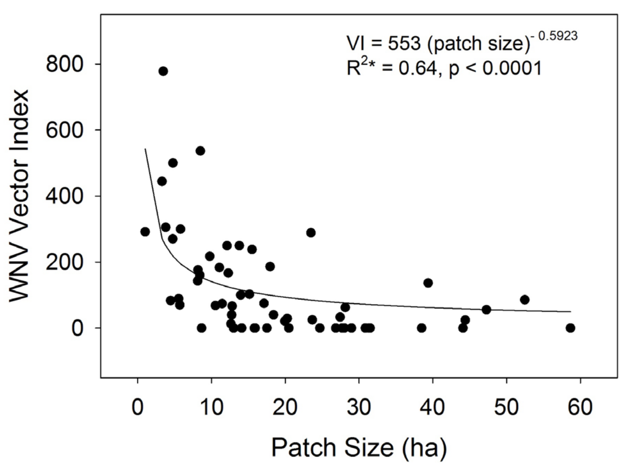

Figure 4. Relationship between WNV vector index and average size of urban forest patches in study area.

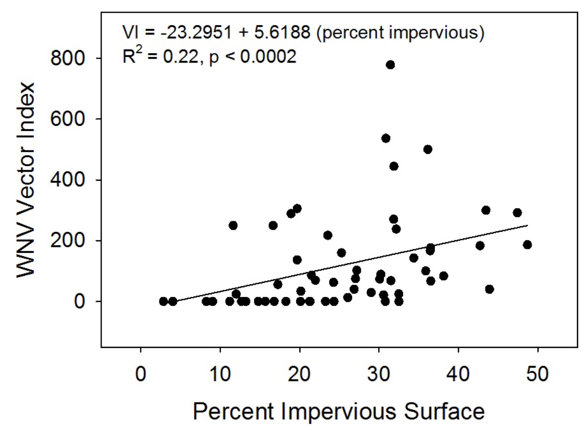

Figure 5. Relationship between WNV vector index and the percent impervious surfaces at each sampling site in study area.

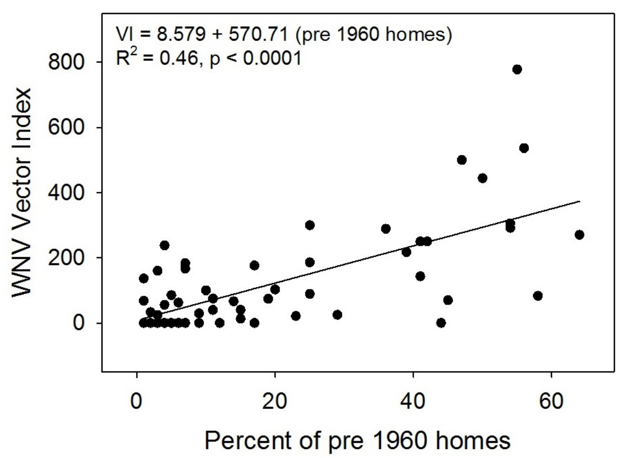

Figure 6. Relationship between WNV vector index and percent of homes surveyed that were built before 1960.

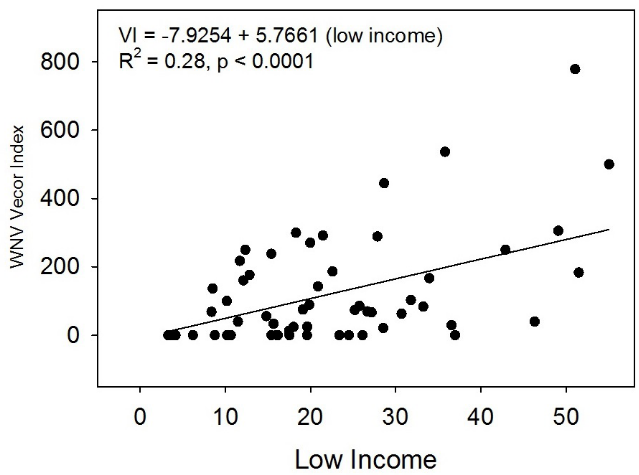

Figure 7. Relationship between WNV vector index and percent of low income households.

amount of variation that was encountered.

An additional landscape metric that was significantly and directly correlated with VI included the amount of impervious surface within a site (Figure 5). As impervious surface cover increased so did risk of WNV, further suggesting that maintenance of adequate forest cover and greenspace in urban areas may be essential to minimizing WNV transmission risk. Changes in hydrology and water quality associated with increasing levels of impervious surface and, perhaps, higher occurrence of stormwater catchments in watersheds, are well documented, as have been previously discussed. Briefly, these changes involve increased velocity and discharge of streams, higher flooding tendencies, and degraded water quality (Lockaby et al. 2013). Increased flooding and lower water quality are factors that could lead to improved breeding habitats for *Culex* ssp.

Our landscape analyses showed negative correlations between WNV risk and some forest characteristics and positive correlations with anthropogenic land-use activities related to urbanization. Certain forest metrics were associated with decreased VI, including percent pine species cover and larger forest patches. These data suggest that landscape planning directed at retention of larger forest patches, as well as more active management of forest species composition in urban patches, may be useful in minimizing risk of WNV transmission.

Socioeconomic factors

Of the socioeconomic variables developed from the census, three had correlations to the VI at the 0.05 level; housing density (R=0.53), the proportion of pre-1960 homes (R=0.68)-(Figure 6), and number of low income households (R=0.51)-(Figure 7). Correlations of data collected from the field included items significant at the 0.05 level; discarded tires (R=0.32) and number of parks and fields (R=-0.39). We also performed correlations between proportion of pre-1960 housing and low income households with a number of the other socioeconomic variables to investigate the context of the neighborhoods that are most associated with these metrics. Significant correlations included the following: with pre-1960 homes: containers R=0.37, p=0.004, potted plants R=0.45, p<0.0001, vacant lots R=0.50, p<0.0001, and total number of buildings R=0.39, p=0.002; with low income: tires R=0.47, p<0.0001). A socioeconomic description of the area with increased risk of WNV entails low income neighborhoods with many pre-1960 homes associated with containers, vacant lots, and overgrown yards.

Climatic factors

Based on the generated CCMs, the highest correlation was found for temperature and PET averaged from 20 weeks prior to trapping and extended into 8 or 6 weeks prior to sampling, with the Spearman's rank correlation value of 0.65 for both variables (Figure 8). Also, surface moisture averaged over 20 to 5 weeks prior to the capture event was negatively correlated with weekly VI, R=-0.56. Precipitation averaged over (t-20) to (t-16) weeks was negatively and weakly correlated with VI at week t, R= -0.09. Considering the seasonal behavior of *Culex* species and their activity, which peaks in summer/early fall, the identified interval lags indicate the preceding late winter and spring as the most relevant time periods. Warmer and drier late winters and early springs favor the development of *Culex* vectors and lead to higher infection rates in summer and early fall.

According to CCMs (Figure 8), VI had the highest correlation with temperature and PET averaged from 15 weeks prior to sampling and extended into 10 or 12 weeks prior to sampling with the Spearman's rank correlation value of 0.79 for both. Also, surface moisture averaged over 18 to 7 weeks prior to the capture event was negatively correlated with four weeks moving average VI, with a correlation coefficient value of -0.70. Averaged precipitation over (t-20) to (t-11) weeks were also negatively and weakly correlated with VI at week t. Counting back for these interval lags highlights the role of climate in spring on infection rate in summer. Warm and dry springs extend the duration of the mosquito season and vector activity. It also accelerates the development rate and survival of adult female mosquitoes (Patz et al. 2008, Morin and Comrie 2013). Also, vector breeding conditions are facilitated and the frequency of transmission events is increased due to dry conditions by gathering hosts and vectors around nutrient-rich water bodies (Shaman et al. 2005).

Climatic factors for Atlanta area and for 2009-2011

In order to apply the findings of the previous analysis to 58 sites and to combine it with the findings of landscape and socioeconomic analyses, results were summarized as follows. From the weekly scale analysis, the top two correlated variables with VI were temperature averaged over 20 to 6 weeks prior to sampling, R=0.65, and surface moisture averaged over 20 to 5 weeks prior the capture event, R=-0.56. From the four weeks moving average scale analysis, the top two correlated variables with VI at week t were averaged over (t-15) to (t-10) weeks and surface moisture averaged over (t-18) to (t-7) weeks prior to sampling with R=0.79 and -0.70, respectively. These interval lags were applied to the climate data for the 58 sites. For combining these parameters with other risk

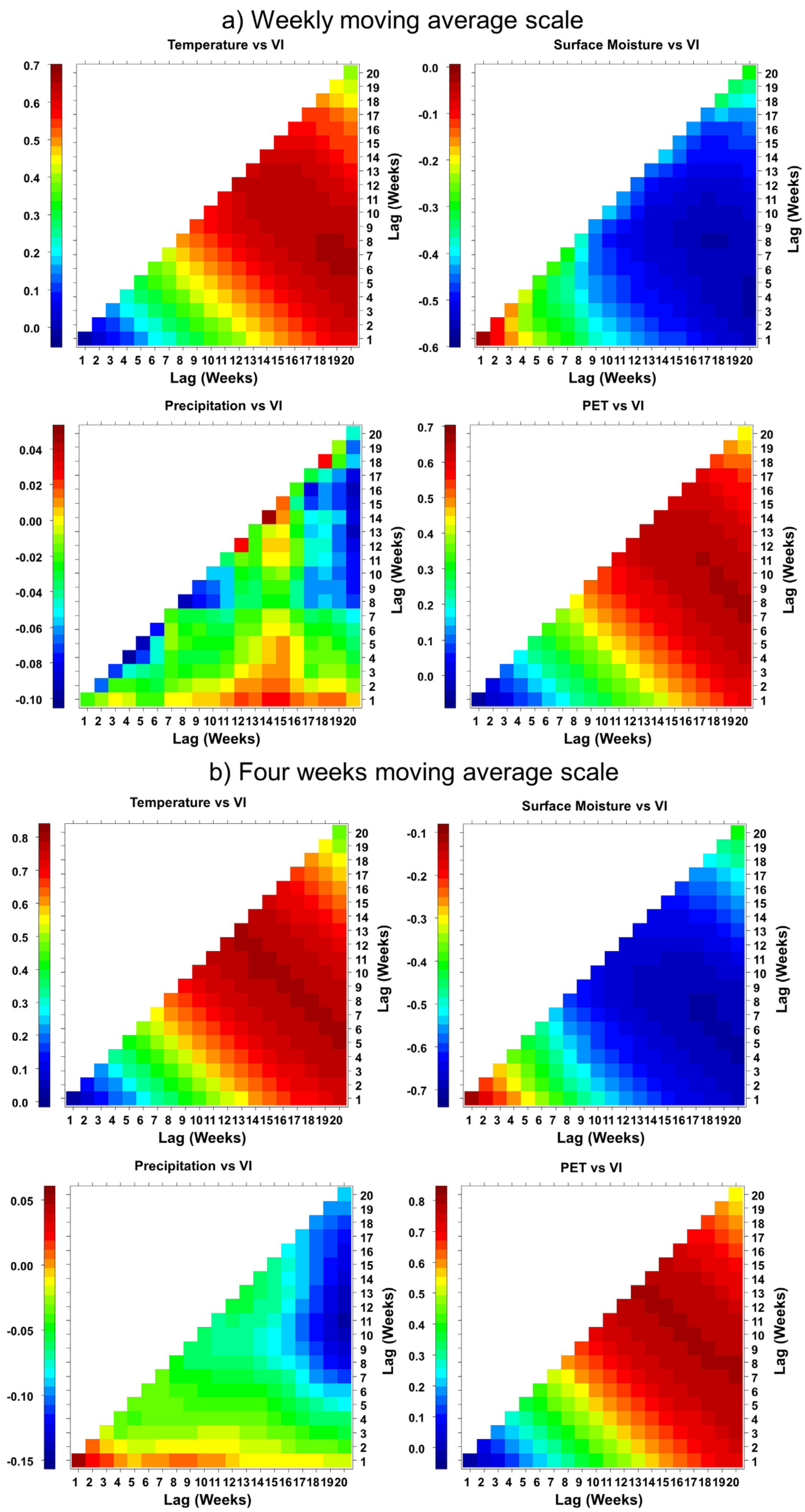

Figure 8. Cross correlation maps (CCMs of a) weekly and b) four weeks moving average Vector Index vs climate variables. Soil moisture refers to 0-2 m of soil. The red color in each CCM indicates the highest positive correlation between the climate variable and Vector Index, the blue color indicates the highest negative correlation.

Table 1. Independent variables included in stepwise regression analysis for prediction of WNV Vector Index. Soil moisture refers to 0-10 cm depth.

Variable	Correlation with VI (R)	P>F
Area of patch	-0.50	<0.0001
Percent pine	-0.65	<0.0001
Percent impervious surface	0.37	0.0030
Housing density	0.53	<0.0001
Percent low income	0.51	<0.0001
# parks and fields	-0.39	0.0020
# of discarded tires	0.32	0.0150
# houses (pre-1960)	0.68	<0.0001
Average weekly soil moisture summer 2009	0.40	0.0020
Average weekly soil moisture spring 2009	0.34	0.0094
Average weekly soil moisture winter 2009	0.39	0.0020
4-week moving average soil moisture winter 2009	0.40	0.0021
4-week moving average soil moisture summer 2009	0.37	0.0044
4-week moving average soil moisture spring 2009	0.33	0.0094
Average weekly soil moisture summer 2010	0.35	0.0087
Average weekly soil moisture winter 2011	0.39	0.0059
Average weekly soil moisture fall 2011	0.35	0.0087
4-week moving average soil moisture winter 2011	0.36	0.0059

factors, the season based interval lagged temperature and soil moisture were defined for each site and for years 2009-2011.

Integration of risk factors

Selected socioeconomic, climate, and forest vegetation metrics were combined using stepwise regression in order to determine the suite of risk factors that was most effective in predicting WNV VI. The metrics chosen were those exhibiting the strongest correlations with VI. Variables selected for inclusion in the stepwise analysis are listed in Table 1. In tandem with the percentage of pine in forest patches and the proportion of low income populations, the proportion of houses built prior to 1960 had the strongest relationship with WNV VI. Older homes may embody several conditions that relate to WNV risk, including older infrastructure (e.g., cracks in foundations and sidewalks that hold water, cracked septic tanks, and, if untended, overgrown yard). It is possible that sewer systems in some older parts of the city may have less integrity and leak to a greater extent than those in more modern sections of Atlanta. Water quality data collected from streams within each of the sites show significant, positive correlations between total nitrogen (R=0.50), sulfate (R=0.51), potassium (R=0.59), and calcium (R=0.54) concentrations and the proportion of houses built before 1960. The older housing correlation with total nitrogen in particular suggests that water quality may be degraded in areas with older infrastructure. Consequently, it is possible that *Culex* habitat quality may be enhanced in the same areas. None of the water quality metrics were significantly correlated with low income.

Pre-1960 homes and low income were also correlated with a number of items that help explain the increased risk of WNV in these neighborhoods, including the high number of water collecting sources for larvae (containers, potted plants, tires) and landscaping characteristics (overgrown yards, vacant lots, parks, and fields) that are conducive to mosquito habitat. The combined water quality and neighborhood characteristics associated with this era of housing culminate in enhanced risk for WNV. Surprisingly, none of the meteorological variables were included in the final model, indicating that, although climate is one of the main drivers of seasonal behavior of mosquitoes, other environmental factors played a more significant role in mosquito activities and their infection rate in this case.

Our results reinforce the conclusion that WNV risk must be examined in terms of many different factors that intertwine and change over different scales of time and space. We found significant relationships between WNV VI and variables that may or may not be related to VI in a causal manner (Table 1). In particular, we suggest that area of forest patches and percent impervious surface in patches represent metrics that relate to WNV VI in a clear and direct fashion. Among the 'best' model variables, reasons for a negative relationship between percent pine and VI are not obvious but warrant further study. The independent variables, housing density, low income households, and older housing reflect relationships that exist between VI and more specific underlying factors with which they are confounded, such as low water quality in older neighborhoods. We also suggest that identification of the underlying mechanisms associated with the social variables should be a priority for further research. Regarding meteorological factors, results indicate that appropriate modeling of 4-week moving averages of temperature and PET from the preceding winter and spring were effective in predicting *Culex* abundance. While the temperature indices were not highly correlated with VI, precedent soil moisture levels were significantly related. However, this does not imply that temperature is unimportant in relation to VI because soil moisture data reflect the integrated influence of both moisture and temperature (PET). We may speculate that if the timeline of the study had been longer, we may have noted more influence of the meteorological metrics in the 'best' model. Mesocosm approaches might be used to unravel some of the complexities of relationships between climate and mosquito abundance if the mesocosms adequately reflect habitats. It seems that interdisciplinary approaches are the only mode of investigation that has potential to clarify and enable accurate predictions of risk. Also, it is evident that creative, new methodologies, coupled with interdisciplinary expertise, are clearly needed to make progress in risk.

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