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Remotely-Sensed Vegetation Indices Identify Mosquito Clusters of West Nile Virus Vectors in an Urban Landscape in the Northeastern United States

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ABSTRACT

Heterogeneity in urban landscapes can influence the effectiveness of mosquito-borne disease control. We used remotely sensed vegetation indices to discriminate among mosquito habitats within a densely populated urban environment in New Haven, CT. ASTER derived vegetation indices were identified for 16 sites where adult mosquitoes were trapped over the summer of 2004. Canonical correlation analysis showed a significant relationship between the environmental variables (normalized difference vegetation index, disease/water stress index and distance to water) and four local West Nile virus competent vectors (Cx. pipiens, Cx. restuans, Cx. salinarius, and Ae. vexans) (0.93, P = 0.03) explaining 86% of the variance in the environmental and mosquito measures. Sites were clustered based on these remotely sensed environmental variables. Three clusters were identified which provide insight into the distribution of West Nile virus vectors in an urban area. Identification of habitat differences of mosquitoes within the urban landscape has important implications for understanding West Nile virus transmission and for control of vector-competent mosquito species. **Key Words: Mosquito(es)—Statistical Analysis—vector-borne—West Nile.**

INTRODUCTION

SINCE ITS INTRODUCTION in 1999, West Nile virus (WNV) has spread rapidly throughout the western hemisphere and has become the most prevalent and widespread mosquito-borne pathogen in North America (Hayes et al. 2005). In the northeastern United States, WNV activity in mosquitoes and subsequent human disease cases typically occur in urban areas (Andreadis et al. 2004). *Culex* mosquitoes are the predominant vectors, but the species involved in enzootic transmission among wild birds and/or epidemic transmission to humans varies. *Cx. pipiens* and *Cx. restuans* are largely ornithophilic and are generally thought to play a larger role in the enzootic cycle (Kulasekera et al. 2001, Apperson et al. 2004, Turell et al. 2005, Molaei et al. 2006), while *Cx. salinarius,* a more catholic feeder that readily bites mammals, is more likely involved in transmission to humans (Andreadis et al. 2004, Molaei et al. 2006). *Aedes vexans* has additionally been incriminated as a probable "bridge vector" because of its local abundance, vector competence, aggressive mammalian biting behavior, and frequent infection with WNV (Turell et al. 2001, Andreadis et al. 2004, Molaei and Andreadis 2006). In this study, we examined how internally heterogeneous urban areas can result in spatial segregation of these two kinds of vectors, with potential implications to WNV transmission dynamics and control measures.

Efforts to control vector mosquitoes and prevent human cases in urban landscapes are challenging. Habitat preferences for larval devel-

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opment and distribution of adults of the aforementioned mosquito vectors are largely cryptic and difficult to identify and characterize in a way that is informative for control purposes. Adulticiding with chemical pesticides in densely populated urban areas frequently encounters significant opposition by the public and its effectiveness is often limited when it is not specifically targeted into areas with high vector abundance. More precise identification of WNV infection foci with correspondingly high abundance of mosquito vectors could help to increase efficacy. Field identification of foci using conventional arrays of CO_2-baited light and/or gravid mosquito traps is labor intensive, and the effective area surveyed is limited to a small area around the traps. The use of remotely sensed data provides a cost-effective alternative approach to mapping vector species distribution (Cline 1970, Hayes et al. 1985, Kerr and Ostrovsky 2003), which frequently corresponds to foci of virus activity (Barrera et al. 2002, Blackmore et al. 2003, Andreadis et al. 2004). We explored whether remotely sensed data could aid in detecting such foci by identifying environmental conditions suitable for WNV mosquito vectors in the urban landscape of New Haven, Connecticut.

A major challenge in using remote sensing in urban environments is the heterogeneity and scale of variables to be measured (Stefanov et al. 2001, Herold et al. 2003, Maktav et al. 2005), especially in disease applications (Rogers and Randolph 2003, Tatem and Hay 2004). Urban areas are comprised of a variety of land surfaces, but the spatial resolution of commonly used satellite imagery often precludes meaningful delineation of land surface types beyond a simple "urban" class. Some regions have land use classified maps, but these land use classifications may not be relevant for identifying vector distribution.

This study evaluated the use of high spatial resolution satellite imagery to discriminate regions of within-urban areas associated with greater abundance of four putative mosquito vectors of WNV in this region: *Cx. pipiens, Cx. restuans, Cx. salinarius,* and *Ae. vexans* (Andreadis et al. 2001, 2004, Turell et al. 2001, Ebel et al. 2005). Since our objective was to identify transmission foci, rather than the abundance of individual mosquito species, we examined the association between the whole vector community and a set of environmental parameters. Significant environmental parameters (normalized difference vegetation index [NDVI], disease water stress index [DWSI], and distance to water) were then used to identify clusters of trap sites with similar communities of vectors. WNV transmission to humans would be expected in areas where clusters with high abundance of enzootic and bridge vectors are in close proximity and should therefore be considered targets for control.

MATERIALS AND METHODS

Eighteen collection sites were selected in the city of New Haven, CT. A stratified random design was used to allocate the number of traps in proportion to the major urban landscape types (e.g., grass, impervious, residential), obtained from a statewide land use classification with 30 m^2 resolution (Civco et al. 1993). After the initial selection, some traps were relocated to facilitate access and two were eliminated due to security concerns. Traps were located within a 1.7-km^2 area within New Haven, with a mean distance of 763 m between traps (Fig. 1). Mosquito abundance was analyzed with respect to land use classes; we did not find significant predictors, however, these results are not presented as they are secondary to the analysis of scale and vector distribution.

From June 7 to October 14, 2004, CO_2-baited CDC miniature light traps were operated biweekly on one half of the collection sites, for four consecutive nights, resulting in each site being sampled every other week. Traps were set each night and retrieved the following morning. Mosquitoes were killed with triethylamine, and females were identified to species using the descriptive keys of Andreadis et al. (2005).

A 23 June 2003 ASTER scene was acquired for the study area. ASTER data have relatively high spatial resolution (15 × 15 m visible and near infrared bands and 30 × 30 m in the short wave infrared [SWIR]) and allow better differentiation of within-urban features (Yamaguchi et al. 1998). SWIR was resampled to 15 ×

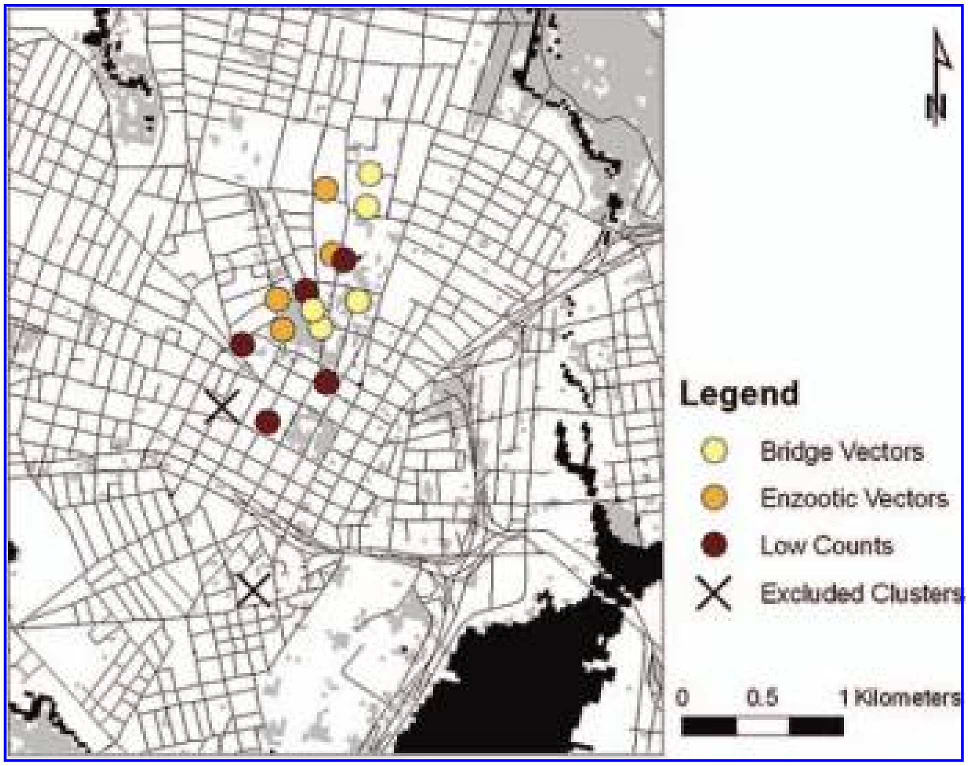

FIG. 1. Trapping sites for the sampling area in New Haven, CT. Traps within the groups resulting from this analysis are shown on a classified image where water is black and vegetation is shaded in gray. "Bridge vectors" indicates those traps included in the highly vegetated clusters where *Culex salinarious* was found in greatest abundance and *Cx. pipiens* and *Cx. restuans* in moderate abundances. "Enzootic vectors" indicates the more urban areas with high abundances of *Cx. pipiens* and *Cx. restuans*. "Low counts" indicates the highly urban areas where few mosquitoes were collected. "Excluded clusters" indicates the two "clusters" that included just one site each and were removed from analysis.

15 m to match the smaller pixels of the visible and near infrared. Two vegetation indices (VI) potentially indicative of mosquito habitat were derived from the imagery: NDVI and DWSI using ENVI v 4.2 (Research Systems 2005).

NDVI is a measure of the presence and condition of green vegetation and is calculated as a normalized ratio of the red and near infrared bands (Lillesand and Kiefer 1994):

$$NDVI = \frac{NIR - \mathrm{Re}d}{NIR + \mathrm{Re}d} = \frac{0.76\ to\ 0.86\ \mu m - 0.63\ to\ 0.69\ \mu m}{0.76\ to\ 0.86\ \mu m + 0.63\ to\ 0.69\ \mu m}$$

NDVI values range from −1 to 1, with bare soil and impervious surface having values near 0, and high values indicating increasing green biomass and photosynthetic activity (Bubier et al. 1997, Penuelas and Filella 1998). NDVI can be related to common ecological measures such as leaf area index (Baret and Guyot 1991) and net primary productivity (Kerr and Ostrovsky 2003).

DWSI is a measure of the internal water content of vegetation (Apan et al. 2004) and is calculated as

$$\mathrm{DWSI} = \frac{NIR + Green}{SWIR + \mathrm{Re}d} = \frac{0.76\ to\ 0.86\ \mu m + 0.52\ to\ 0.60\ \mu m}{1.60\ to\ 1.70\ \mu m + 0.63\ to\ 0.69\ \mu m}$$

It has the advantage of not being distorted by atmospheric water absorption (Penuelas et al. 1997, Penuelas and Filella 1998). Though primarily considered a measure of internal water, the inclusion of the SWIR may also measure ground water content that could be relevant for mosquito breeding.

The average values of the VIs were calculated within a 50-m buffer area at each trap site (Longley 1999), which represents approximately 35 pixels. Given the comparatively limited flight range of the most abundant mosquito, *Cx. pipiens*, these buffer areas were considered appropriate to capture the biologically relevant variables within their typical range of movement (Horsfall 1955). The buffer was generated using ArcGIS software (Environmental Systems Research Institute 2004). A map of the wetlands in Connecticut was obtained from the National Wetlands Inventory (U.S. Fish and Wildlife Service 2004). These maps have an estimated 90% accuracy for identifying wetlands greater than 3 acres, 70% accuracy for 1–3 acre wetlands, and approximately 25% accuracy for including wetlands less than 1 acre (Nichols 1994). The distance of the trap location to the nearest body of water, including both open water (lakes and rivers) and wetlands, was determined in ArcGIS using a spatial join of the points to the wetland inventory map.

Canonical correlation analysis was used to examine the association between the environmental variables (NDVI, DWSI, and distance to water) and the abundance of four putative mosquito vectors (*Cx. pipiens, Cx. restuans, Cx. salinarius,* and *Ae. vexans*) collected in the study area. The square of each correlation is interpreted as a coefficient of determination (R^2) in a multiple regression analysis (Gittins 1985). A redundancy analysis was also performed to evaluate within- and between-domain variance associated with a given canonical variate. This test is expected to provide a more accurate measure of the explanatory power of the canonical variates (Gittins 1985).

Clusters of sites with similar environmental characteristics were then generated using Wards linkage with Euclidean distance hierarchical cluster analysis. These clustering methods result in biologically relevant interpretations (McCune and Grace 2002). Visual identification of the optimal number of clusters was confirmed using the Calinski and Harabasz stopping rule (Calinski and Harabasz 1974, Stata Corp LP 2005b).

Statistical analyses were conducted using the "canon" and "cluster" commands in Stata 9.0 (Stata Corp LP 2005a). Kruskal-Wallis equality-of-populations rank test was used to distinguish mosquito abundance between the groups. Simpson's diversity index (1-D) (Simpson 1949) was calculated with a 95% confidence interval (Grundmann et al. 2001). Because all four species were found in these traps, 1-D was used in this study to assess species evenness within the groups (Magurran 1988). Count data were log transformed for the most abundant species (*Cx. pipiens, Cx. restuans,* and *Cx. salinarius*) and square root transformed for *Ae. vexans* to normalize the data. Environmental data were standardized by dividing over the sample standard deviation to account for differences in measurement scales.

RESULTS

Mosquitoes were collected over 495 trap nights. A total of 2787 female mosquitoes representing 19 species were captured, 91.7% of which included the four principal vectors of interest (*Cx. pipiens* = 63%, *Cx. restuans* = 24.1%, *Cx. salinarius* = 2.5%, and *Ae. vexans* = 2.0%) (Table 1). Using canonical correlation analysis, the community of these four species was related to the set of environmental variables ex-

TABLE 1. NUMBER OF MOSQUITOES COLLECTED BY SPECIES

	Total number	*Count per night*
Focal species		
Aedes vexans	56	1.76
Culex pipiens	1755	56.58
Culex restuans	673	21.41
Culex salinarius	71	2.37
Other species		
Anopheles barberi	2	0.06
Anopheles puncipennis	9	0.18
Anopheles quadrimaculatus	2	0.12
Anopheles walkeri	1	0.06
Coquillettidia perturbans	21	0.71
Culux territans	4	0.12
Ochlerotatus canadensis	1	0.03
Ochlerotatus trivittatus	4	0.14
Ochlerotatus cantator	27	0.49
Ochlerotatus intrudens	2	0.18
Ochlerotatus japonicus	59	1.40
Ochlerotatus stimulans	4	0.42
Ochlerotatus taeniorhynchus	1	0.41
Ochlerotatus triseriatus	15	0.44
Uranotaenia sapphirina	1	0.03

TABLE 2. CANONICAL CORRELATIONS FOR EACH OF THE CANONICAL VARIATES MEASURING THE RELATIONSHIP BETWEEN THE ENVIRONMENTAL VARIABLES AND THE MOSQUITO DATA

Canonical variates	*Canonical correlation*	*Canonical R^2*	*Statistical test*		
			F	*df*	p
1	0.928	0.86	2.43	12	0.03
2	0.306	0.09	0.20	6	0.97
3	0.134	0.02	0.10	2	0.90

tracted for the sites. The null hypothesis of no association between the environmental and mosquito data was rejected (Roy's Greatest Root, $p < 0.001$ and Wilks' Lambda, $p = 0.03$). The correlation between the first environmental canonical variate and the first mosquito variate was 0.93 ($p = 0.03$) (Table 2). Squaring the first canonical variate, 86% of the variation in the linear combination of species (W_1) was attributable to the variation in the linear combination of environmental measures (V_1). The next two canonical variates were not significantly correlated.

The first environmental canonical variate (V_1) accounted for 36% of the variance common to the environmental variables, while the first mosquito canonical variate (W_1) accounted for 33% of the variance of the species measured (Table 3). Strong correlations were found for NDVI and DWSI with V_1 (0.90 and 0.50, respectively). Thus, V_1 reflected some aspect of increasing measures of both NDVI and DWSI and, to a lesser degree, proximity to water. Because the correlations between W_1 and all of the mosquito species were relatively high, W_1 represented some aspect that was common to all species and was exemplified by *Cx. salinarius* (0.70).

A substantial part (31%) of the total variance of the mosquito abundance was accounted for by V_1. Abundance of the four mosquito species together increased with increasing NDVI and DWSI. *Cx. salinarius* was most strongly (0.75) associated with the linear representation of the environmental data (V_1), followed closely by *Ae. vexans* (0.71). These results indicate a linear relationship is a good fit to describe the association between the environmental measures and the mosquito abundance (Fig. 2).

Because of the strong correlation between NDVI, DWSI, and proximity to water and the abundance of the four WNV vector species, these environmental variables were used to identify clusters of similar habitat within the city of New Haven. Five clusters were created based on NDVI, DWSI, and proximity to water (Fig. 3). Clusters 2 and 4 included only one site and were excluded from further analysis. The remaining three clusters showed a gradient from highly vegetated residential areas (Cluster 1) to a more urban, less vegetated en-

TABLE 3. CORRELATIONS BETWEEN THE ENVIRONMENTAL AND MOSQUITO VARIABLES WITH THE CANONICAL VARIATES

	Canonical variates					
	Environmental variates			*Mosquito variates*		
	V_1	*V_2*	*V_3*	*W_1*	*W_2*	*W_3*
Environmental variables						
NDVI	0.90	−0.35	0.28	0.83	−0.11	0.04
DWSI	0.50	−0.04	−0.86	0.47	−0.01	−0.12
Distance to water	−0.15	−0.96	−0.24	−0.14	−0.29	−0.03
Variance extracted	0.361	0.349	0.292	0.310	0.032	0.006
Redundancy	0.310	0.031	0.006	0.266	0.003	~0
Mosquito variables						
Culex pipiens	0.44	0.07	0.67	0.41	0.02	0.09
Culex restuans	0.52	0.11	0.75	0.48	0.03	0.10
Culex salinarius	0.75	−0.57	0.20	0.70	−0.17	0.03
Aedes vexans	0.71	0.69	0.07	0.66	0.21	0.01
Variance extracted	0.383	0.205	0.264	0.331	0.019	0.005
Redundancy	0.29	0.019	0.005	0.285	0.002	~0

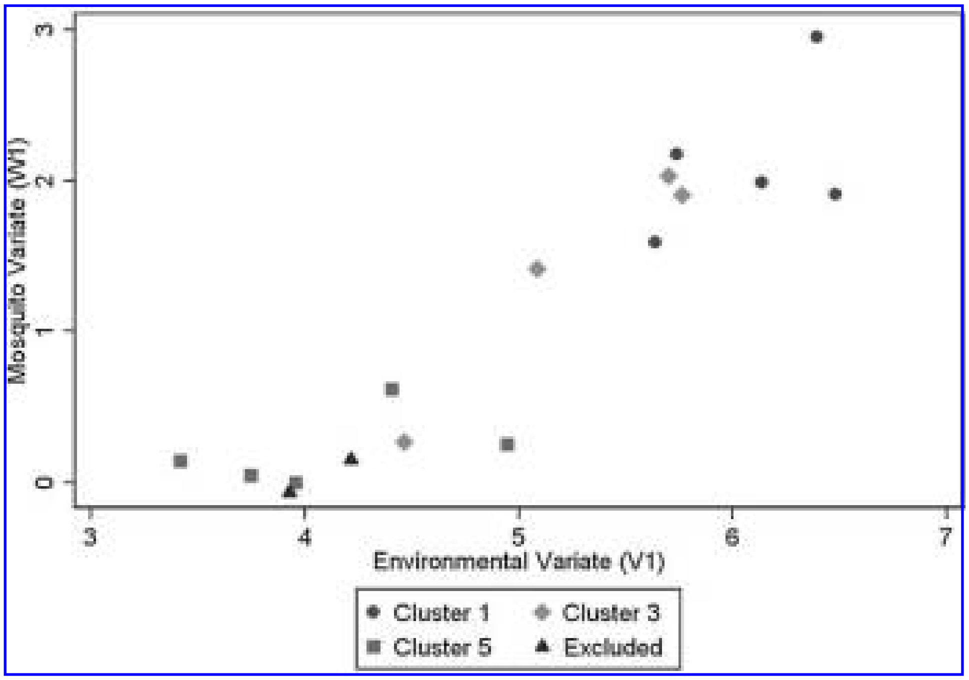

FIG. 2. Canonical analysis of abundance of four mosquito species with reference to the measured environmental variables (NDVI, DWSI, and distance to water) for the 16 sites in New Haven, CT (canonical correlation = 0.93, p = 0.03). This plot shows the positive linear relationship with the canonical correlates for the four mosquito species abundances and the measured environmental variables.

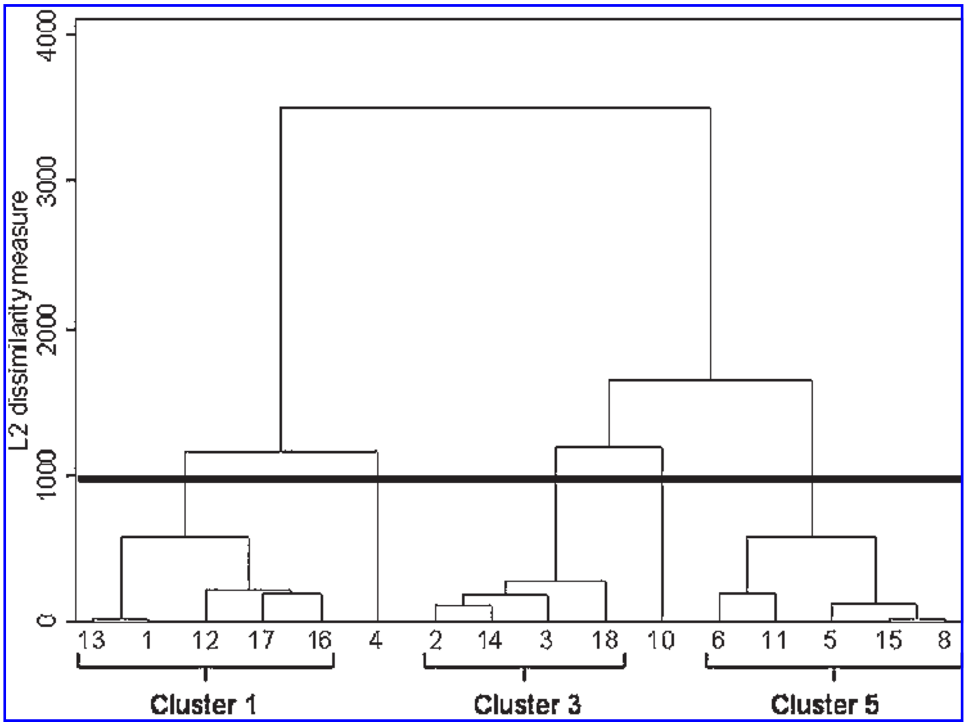

FIG. 3. Cluster analysis of the sites based on their dissimilarity of environmental variables. Sites that are closer together in the dendrogram are more similar. The horizontal bar indicates the cutoff into five groups: Cluster 1, with the highest vegetation values, Cluster 2, with moderate vegetation; Cluster 5, urban. The two “clusters” created by sites 4 and 10 were excluded from further analysis.

Table 4. Environmental Variables Used for the Clustering Algorithm

	Cluster 1 (n = 5)	*Cluster 3* (n = 4)	*Cluster 5* (n = 5)
NDVI	0.67 (0.02)	0.56 (0.10)	0.43 (0.12)
DWSI	6.12 (0.09)	5.73 (0.05)	5.22 (0.19)
Distance to water (km)	1.31 (0.17)	1.29 (0.13)	1.40 (0.04)

All data are presented as mean (SD).

vironment (Cluster 5) (Table 4). Distance to water was similar across the three clusters.

Dropping the two "clusters" that had only one member, abundance measures for all four species were significantly different among the three remaining clusters (Kruskal-Wallis, *Cx. pipiens* $p = 0.05$, *Cx. restuans* $p = 0.02$, *Cx. salinarius* $p = 0.02$, *Ae. vexans* $p = 0.04$) (Table 5). *Cx. salinarius* was found with greatest abundance in the more residential Cluster 1. *Cx. pipiens* and *Cx. restuans* followed a similar pattern of greater abundance in moderately vegetated areas (Cluster 3) with moderate abundance in the more residential Cluster 1. Very few mosquitoes of any species were found in the highly urban areas (Cluster 5).

The probability that any two individuals belonged to different species in Clusters 1 and 5 was high and not significantly different (Cluster 1: 1-D = 0.93, 95% CI 0.90–0.96 and Cluster 5: 1-D = 0.99, 95% CI 0.93–1). Cluster 3 was significantly less diverse than the other clusters (1-D = 0.69, 95% CI: 0.67–0.71) due to the dominance of *Cx. pipiens* and *Cx. restuans*.

DISCUSSION

This study showed spatial segregation between the presumed enzootic vectors (*Cx. pipiens* and *Cx. restuans*) and the presumed bridge vectors (*Cx. salinarius* and *Ae. vexans*) in a 1.7-km^2 urban setting in the northeastern United States. Canonical correlation analysis indicated a close association between the community of vectors and high resolution imagery derived vegetation indices (NDVI and DWSI) and distance to water. Thus, we used a clustering algorithm to generate groupings of traps based on the relevant environmental characteristics. Three clusters of sites were identified: one with high abundance of the primary enzootic vectors, *Cx. pipiens* and *Cx. restuans* (Cluster 3); one with high abundance of *Cx. salinarius*, the putative bridge vector, and relatively high evenness of all four species (Cluster 1); and one with lower abundance of all four vectors (Cluster 5). The spatial distribution of the three clusters is expected to affect WNV transmission dynamics and strategies for control.

Table 5. Mosquito Count per Night by Cluster

	Cluster 1 (n = 5)	*Cluster 3* (n = 4)	*Cluster 5* (n = 5)	p
Culex pipiens				
Mean (SD)	92.2 (55.37)	244.0 (188.67)	34.0 (27.61)	
Median	87	223.5	29	0.05
Culex restuans				
Mean (SD)	41.6 (22.94)	95.5 (67.89)	9.4 (7.40)	
Median	48	95.5	9	0.02
Culex salinarius				
Mean (SD)	8.4 (2.70)	5.3 (6.50)	1.2 (1.30)	
Median	8	2	1	0.02
Aedes vexans				
Mean (SD)	5.4 (4.28)	6.8 (6.24)	0.4 (0.55)	
Median	3	7	0	0.04

Kruskal-Wallis test for variance with unequal means was used to calculate differences between clusters.

WNV transmission

This study provides evidence that the enzootic transmission cycle among wild birds and epidemic transmission to humans might be spatially segregated within an urban area. The enzootic cluster (Cluster 3), dominated by *Cx. pipiens* and *Cx. restuans*, is characterized by moderate amounts of green vegetation. The species in this cluster have been shown to be ornithophilic (Magnarelli 1977, Kulasekera et al. 2001, Apperson et al. 2004, Turell et al. 2005, Molaei et al. 2006). Because of the ornithophilic nature of these species, areas with higher abundance may be indicative of areas where avian hosts are more likely to occur. The need of resting areas for mosquitoes and birds explains the absence of these species in Cluster 5. The higher abundance of these species in the canopy supports the role they have in maintaining enzootic transmission in birds (Anderson et al. 2004, Darbro and Harrington 2006, Andreadis and Armstrong 2007). Moderately vegetated areas may provide habitat where American robins, an important host species for *Cx. pipiens* in this region, congregate in urban areas (Apperson et al. 2004, Kilpatrick et al. 2006, Molaei et al. 2006).

The higher vegetation values in Cluster 1 were indicative of habitat corresponding to residential areas and the cluster had higher numbers of *Cx. salinarius*. This species is implicated as a bridge vector because of its indiscriminant feeding behaviors and the prevalence of WNV infection (Andreadis et al. 2004, Molaei et al. 2006). One might expect greater human WNV disease activity in the more residential areas where *Cx. salinarius* and humans are more abundant, and yet other vectors also occur with great enough abundance to maintain the enzootic cycle. A critical threshold of *Cx. pipiens* abundance may be necessary to maintain enzootic transmission of the virus while allowing for enough diversity of mosquito species that bridge vectors might be involved, as was present in Cluster 1. This was apparent in an outbreak of human WNV cases in greater New Haven in 2006 (Gerrish and Andreadis 2006) with cases occurring in residential areas.

Because of their known roles in WNV transmission, we have focused on *Culex* species in this investigation. The role of *Ae. vexans* in WNV transmission has not been as precisely defined. In this study, we found comparable numbers in both the enzootic and the bridge vector clusters with greater abundance in the more urban Cluster 3. A role for *Ae. vexans* in transmission to humans is supported by our data where it is more abundant in both urban and residential areas.

Urban land use heterogeneity

The scale of heterogeneity in urban land use often limits the ability to identify different habitat resources of urban species, particularly with respect to disease vectors (Rogers and Randolph 2003, Tatem and Hay 2004). Use of ASTER data in this study allowed for measurement of heterogeneity at a scale relevant to mosquito habitat. This is evident from the significant relationships observed between the mosquito and environmental canonical variates.

Remotely sensed vegetation indices are likely to be important for distinguishing within urban mosquito habitats, given the importance of vegetation as resting sites for mosquitoes and their hosts. Landsat derived NDVI was shown to be important for identifying a high-risk WNV habitat within a densely populated urban area in Queens, New York (Brownstein et al. 2002). To include both potential adult and larval development habitats in our analysis, we included NDVI and DWSI extracted at a smaller spatial scale (15 m^2 resolution ASTER imagery) as well as distance to water (30 m^2 derived with post-processing). The satellite derived vegetation indices are extracted at a biologically appropriate buffer size of 50 m. The selection of a biologically appropriate buffer size has been shown to be important in identifying habitat predictors of species distribution (Diuk-Wasser et al. 2006, 2007). The use of these additional vegetation indices at different spatial scales may contribute to our ability to correlate habitat with mosquito species abundance where others have not found a significant relationship (Drummond et al. 2006, Andreadis and Armstrong 2007). This additional information can then be used to create prediction maps for the abundance and distribution of mosquito vectors in urban landscapes.

Implications for control

The spatial segregation of enzootic and bridge vectors, in combination with the temporal detection of WNV in vector species (Andreadis et al. 2001, 2004, Andreadis and Armstrong 2007), could be used to optimize control efforts during the course of the season. Our findings and analyses suggest that early integrated control measures including source reduction, larviciding, and timely adulticiding when needed could be more effectively targeted to moderately vegetated areas (Cluster 3) where peridomestic populations of *Cx. pipiens* and *Cx. restuans* appear to be more concentrated and where enzootic amplification of WNV is thus more likely to occur given the limited flight ranges of these two species. Measures directed to mitigate human cases, on the other hand, could be focused in more highly vegetated residential regions (Cluster 1) where *Cx. salinarius* is more abundant.

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