TROPICAL RESOURCES

The Bulletin of the Yale Tropical Resources Institute

In this issue

Please access the 2011 Bulletin at environment.yale.edu/tri

in order to view maps, graphs, photographs, and figures in color.

All figures used in these articles are the authors' own unless otherwise indicated.

II. HUMAN HEALTH

Characterizing the Urban Environment of Dengue Mosquitoes in Patillas, Puerto Rico

Eliza Little, MESc 2011

Abstract

Aedes aegypti is a mosquito of particular importance because it is implicated in dengue transmission in tropical urban areas around the world. More than half of the world's population now lives in urban areas with continued urban growth expected. Much has been done to curb *Ae. aegypti* populations through integrative vector control. However, the efficacy of vector control methods may be undermined by the presence of alternative, competent species. In Puerto Rico a native tree dwelling mosquito, *Ae. mediovittatus*, has been shown to be a competent dengue vector in laboratory settings and its habitat has been found to overlap with *Ae. aegypti*. GIS and remote sensing have proven to be effective in identifying suitable mosquito habitats in ex-urban settings. This research discusses their utility of analyzing heterogeneous urban landscapes and the features present therein that drive the abundance and distribution of these important *Aedes* species. Analysis suggests that the use of high-resolution remote sensing can be used to determine the environmental drivers of the distribution of *Ae. aegypti* and *Ae. mediovittatus*. Although these species tend to segregate into different habitats, their habitats do overlap. Further exploration of areas of overlap is necessary to determine the involvement of *Ae. mediovittatus* in this dengue disease system.

Introduction

This project is inspired by the observations that the variability of environmental characteristics across a landscape affects the distribution of species and their interactions, and that the ineluctable growth of urban ecosystems provides new nodes for disease ecology.

Eliza Little graduated from McGill University with a B.A. in Environmental Science in 2005. She worked as a field biologist for 3 years before returning to do a Joint Masters at Yale between the Schools of Epidemiology & Public Health and Forestry & Environmental Studies. There she specialized in disease ecology, studying epidemiology, urban ecology, GIS, and remote sensing. She will continue studying disease ecology next year on a Fulbright Research grant in Bangalore, India.

The World Health Organization (WHO) estimates that more than 2.5 billion people in the world are at risk for dengue fever (WHO 2009), representing a thirty-fold increase in the last 50 years (CDC 2009). The emergence of dengue as a significant disease coincides with rapid urbanization over the same time period. Currently, dengue is prevalent in 100 countries with an estimated 50-100 million cases of dengue fever per year (WHO 2009), with most of these cases occurring in Asia, Africa and Latin America. Between 2000 and 2030, Asia's urban population will increase from 1.36 billion to 2.64 billion, Africa's from 294 million to 742 million, and that of Latin America and the Caribbean from 394 million to 609 million. As a result of these shifts, developing countries will have 80 per cent of the world's
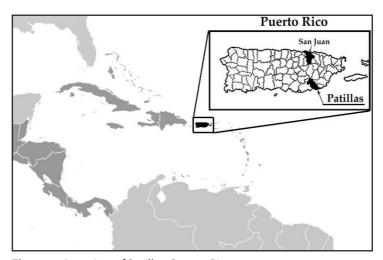
Figure 1. Location of Patillas, Puerto Rico

urban population in 2030 (UNFPA 2007). In developing countries, where urbanization may outpace the government's capacity to provide essential services such as running water and sanitation, the risk of dengue transmission may increase.

This research combines satellite remote sensing with geospatial analytical tools that map the environmental drivers which determine the spatial distribution of dengue mosquitoes across an urban area. Broadly, the main goal of this research is to characterize the environment within urban areas in Patillas, Puerto Rico, and determine the distribution and abundance of dengue mosquitoes. Specifically, we aim to define the environmental determinants of *Ae. aegypti* and *Ae. mediovittatus* and what environmental conditions promote their co-occurrence. We hypothesize that *Ae. aegypti* will be more closely associated with urban areas while *Ae. mediovittatus* will be more likely to occur in forested areas, and that their co-occurrence will occur at the edges of both habitat types or in urban areas with a high tree canopy.

Dengue transmission mechanisms and spatial characteristics

Dengue is a viral fever transmitted by the bites of infected mosquitoes (the vector) main-

ly of the genus *Aedes*, species *aegypti* (Hotez et al. 2008; WHO 2009). Unfortunately, there is currently no vaccine for the treatment of dengue. Best practices for dengue prevention are integrated vector control methods including reduction of vector breeding containers and community education and empowerment (Gubler 2006). It is difficult to sustain community par-

Photograph 1. View through town to Lake Patillas. This picture illustrates the typical architecture style of the area and that much of the area is forested.

ticipation strategies integral to the success of these programs especially in inter-epidemic years (Lloyd 2003).

Aedes aegypti has emerged as the most important dengue vector worldwide due to its behavioral and ecological traits. Not only is *Ae. aegypti* susceptible to dengue viruses, it relies on blood meals, is highly anthropophilic, and has a high frequency of biting multiple hosts over the course of its lifetime (Harrington et al. 2001). *Aedes mediovittatus*, also called the Caribbean treehole mosquito, is associated with arboreal vegetation (Cox et al. 2007; Smith et al. 2009) similar to the more widely distributed species *Ae. albopicuts* (Honorio et al. 2009). *Ae. mediovittatus* has been implicated in the transmission of dengue based on the observations that it readily feeds on humans, and supports dengue virus replication and vertical transmission through generations (Gubler et al. 1985).

Mosquitoes are sensitive to ecological processes that determine their distribution in the environment. Accordingly, the ecology of *Aedes* species should inform vector control

Table 1. *Aedes* species by BG observation.

Presence of *Aedes* species	Frequency	Percent
Neither species	403	32%
Ae. mediovittatus	241	19%
Ae. aegypti	334	26%
Both species	288	23%

measures in order to adequately direct resources to the areas of greatest need (Wilson 2002). The spatial overlap of *Ae. mediovittatus*, *Ae. aegypti*, dengue viruses, and humans in urban environments has not been determined. Based on the known geographic range of both mosquito species, it is reasonable that these two species overlap spatially. And, because *Ae. mediovittatus* bites humans, it is likely that this mosquito species can become infected with dengue viruses (Freier and Rosen 1988). Although the role of *Ae. mediovittatus* remains unknown, it should not be ruled out as an important vector in the transmission dynamics of dengue in Puerto Rico, especially in urban areas with high canopy cover.

The recent launches of satellites capable of recording high-resolution remote sensed
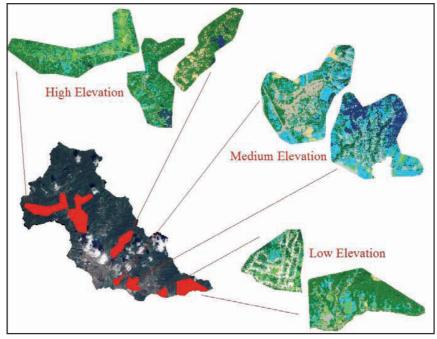

Figure 2. Maximum Likelihood Classification by location.

Table 2. Results of multinomial logistic regression using metrics generated with FRAGSTATS.

	Relative Risk	Standard Error	z-score	P-value	95% CI
Presence of *Ae. Mediovittatus*					
Trees	1.22	0.15	1.65	0.099	(0.96, 1.55)
House Density	0.82	0.13	-1.22	0.223	(0.60, 1.13)
Forest Area (Avg)	1.12	0.15	0.85	0.398	(0.86, 1.47)
Forest (NP)	1.65	0.21	3.95	< 0.001	(1.29, 2.12)
Presence of *Ae. aegypti*					
Trees	1.57	0.23	3.1	0.002	(1.18, 2.10)
House Density	2.54	0.39	6.16	< 0.001	(1.89, 3.42)
Forest Area (Avg)	0.36	0.14	-2.59	0.01	(0.17, 0.78)
Forest (NP)	1.52	0.22	2.9	0.004	(1.15, 2.02)
Presence of both *Aedes* species					
Trees	2.6	0.54	4.65	< 0.001	(1.174, 3.91)
House Density	2.34	0.39	5.26	< 0.001	(1.01, 1.02)
Forest Area (Avg)	0.26	0.09	-3.74	< 0.001	(0.13, 0.53)
Forest (NP)	2.58	0.4	6.07	< 0.001	(1.90, 3.50)

imagery have made it possible to assess the utility of such imagery to study the environmental drivers of *Aedes* species. Unfortunately, the use of high resolution remote sensing for determining the spatial extent of mosquito vectors is lacking, especially in urban areas (Chowell et al. 2008, Troyo et al. 2009). Remote sensing can be used to inform our understanding of the dynamics of disease vectors, and the structured methodology of using such technology may provide a mechanism for targeting control programs to areas of greatest risk (Chowell et al. 2008).

Study location

The study was conducted from May to August 2010 in the municipality of Patillas in South Eastern Puerto Rico (Figure 1). Patillas is 125 km² ranging in terrain from coastal to mountainous with peaks of 900 m. According to the US census 2000, the total population of Patillas is 20,152 with a density of 164 people per square kilometer with a mean age of 32 years old and a median household income of USD 12,021. The selection of Patillas as a study site is based on the availability of preliminary

data indicating presence of both *Ae. aegypti* and *Ae. mediovittatus*. Dengue is endemic in Puerto Rico with epidemics occurring every 2 to 3 years since before 1985 (Gubler et al. 1985) and surveillance in Patillas has shown that dengue is endemic to this area (Barrera et al. 2008).

Methodology

BG-Sentinel traps were used to capture adult mosquitoes between May and August of 2010. Trap sites were selected by locating one house per 100m² sampling grid. The value of the sampling grid is based on the maximum flight range of *Ae. aegypti*. Trap placement was restricted to areas in close proximity to buildings to only include areas that were inhabited and where dengue transmission was likely to occur.

We used the WorldView2 satellite sensor, which was recently launched on October 8, 2009. WorldView2's features include high spatial resolution of 1.82 m (2 meter output pixels) and an 8-band multispectral image. Based on the maximum flight range of *Ae. aegypti*, the spatial resolution provided by WorldView2 is important for analyzing the fine-

scale environmental drivers of these mosquito species. The WorldView2 image was acquired on March 25, 2010 and processed using ENVI 4.7 and 4.8. Supervised classification using a maximum likelihood algorithm was used for the classification of vegetation into 4 different categories. Overall the classifications had excellent accuracy ranging from 80% to 99%, with associated kappa coefficient (.73 to .9) (Figure 2).

Further processing of classification to generate predictive variables was accomplished using a combination of Arc GIS 10, FRAGSTATS, and Geospatial Modelling Environment (GME).

Findings

The presence of *Aedes* species by observation is represented in Table 1. A total of 1,266 observations and 22 different variables from 243 different landscape-patches were analyzed to assess what environmental drivers influence the distribution and abundance of *Aedes* species. The model with the lowest Akaike Information Criterion (AIC) had predictive value (chi square = 183, 9 df, p<0.0001). The model included the following predictive variables: proportional area of the forest class (trees), house density, the average forest-patch area, and the number of forest-patches within the landscape-patch (Table 2).

This model indicates that the relative risk of the presence of *Ae. mediovittatus* compared to the base outcome of either species present is significantly predicted by the number of forest patches. The presence of *Ae. aegypti* or the presence of both *Aedes* species present compared to the base outcome of neither species present increases with the increasing proportional area of the forest class (trees), house density, and the number of forest patches, but decreases with increasing average forest area. Surprisingly, the cohesion metric did not surface as a main predictor of *Aedes* species. The FRAGSTATS generated metrics of average area of forest class-

Photograph 2. A Patillas resident's backyard showing the high tree canopy and many potential containers to collect water suitable for immature development of dengue mosquitoes– including tarps, chairs, grill, and buckets.

patches and the total number of those forest class-patches at the scale of 100 m may do a better job of explaining the patchiness of the forest class than a cohesive metric that would indicate the connectivity between patches.

Analysis

Mosquito abundance was collected from 243 BG traps over 5/6 sampling periods, for a total of 1266 observations. Female *Ae. aegypti* and *Ae. mediovittatus* numbers were converted into categorical values. For *Aedes aegypti*, the mean number of female mosquitoes was found and a categorical variable of high (above the mean) or low (below the mean) was used for the analysis. For *Ae. mediovittatus*, a categorical value of presence versus absence was used for the analysis due to the high percentage of traps that did not capture any *Ae. mediovittatus* females.

Landscape metrics were calculated within a 100 m radius of the BG trap used to trap the mosquitoes. For this research, the circular area around each BG trap is identified as a landscape-

patch. In all, there are 243 landscape-patches corresponding to 243 BG traps.

A pixel count for each class—soil, grass, scrub, tree, and urban—was calculated for each landscape-patch. These pixel counts were then converted into the proportional area each class represented within the landscape-patch by dividing by the total pixels of that landscape-patch. The proportional area gives an overall value for each class within the landscape-patch but it does not give any information as to the distribution or form that the area takes.

A class-patch, forest or urban, is defined as a contiguous grouping of like-neighbored pixels. For each landscape-patch, the total number of class-patches, the class area distribution statistics, and a cohesion metric were calculated using FRAGSTATS (McGarigal 2002) for the two focal classes (forest and urban). Class metrics represent aggregate metrics for each focal class analyzed. The number of patches is a simple measure of the extent of fragmentation of the class-patch type but does not give a measure of area or distribution of class-patches. The class-patch area distribution is characterized by the mean, area-weighted mean, median, range, standard deviation, and coefficient of variation for each focal class.

Preexisting data included a GIS file of all structures within Patillas. Within Arc GIS these structure points were subjected to kernel density, then focal statistics were calculated for all pixels to count the total kernel density within a 100 m radius, and finally the extraction of these values to the BGs resulted in a structure density within each landscape-patch.

All predictive variables were normalized by subtracting the mean and dividing by the standard deviation. A multinomial logistic regression analysis run in Stata 10 was used to determine the environmental drivers of the presence of *Ae. mediovittatus*, *Ae. aegypti*, or both species compared to the base outcome of neither species present. A multi-model approach was used to select the model with the lowest Akaike Information Criterion (AIC), which selects the most parsimonious model that fits the data.

The final multinomial logistic regression models for *Ae. aegypti* and *Ae. mediovittatus* suggest that the two mosquito species segregate into different habitats within this area of Puerto Rico. *Ae. aegypti* favors areas that are dense in structures and trees high in the number of patches but low in forested area, and *Ae. mediovittatus* prefers areas that have a high number of tree patches.

Conclusion

It seems that these two *Aedes* species do segregate in heterogeneous areas with *Ae. aegypti* more abundant in urban areas and *Ae. mediovittatus* in forested areas. Coexistence occurs in areas with a high proportion of forest-patches that are broken into many forest class-patches and high housing density. However, the mosquito trapping collected many more *Ae. aegypti* than *Ae. mediovittatus*, which may have skewed the model predicting both mosquito species to the criteria that influence *Ae. aegypti*. Using the information from this model, areas with high tree patchiness could be identified and a focused trapping schedule for these areas may provide for a greater number of *Ae. mediovittatus* to be collected as necessary to assess if these mosquitoes carry dengue viruses. Using high-resolution remote sensed imagery to classify heterogeneous urban environments to generate an array of landscape metrics provides useful information to determine the distribution and abundance of *Aedes* mosquito species. A multinomial logistic regression analysis can be used to assess predictive variables of the occurrence of these species, which in turn could be used to focus intervention efforts on areas of greatest risk.

Acknowledgements

References

Barrera, R., et al. 2008. Unusual productivity of *Aedes aegypti* in septic tanks and its implications for dengue control. Medical and Veterinary Entomolgy, 22(1):62-69.

CDC (Centers for Disease Control and Prevention). 2009. Dengue: Entomology and Ecology. <http://www.cdc.gov/dengue/entomologyEcology/index.html>.

Chowell, G., et al. 2008. Spatial and temporal dynamics of dengue fever in Peru: 1994-2006. Epidemiology and Infection, 136(12):1667-77.

Cox, J., et al. 2007. Habitat segregation of dengue vectors along an urban environmental gradient. American Journal of Tropical Medicine and Hygiene, 76(5):820-6.

Freier, J. E., and L. Rosen. 1988. Vertical transmission of dengue viruses by *Aedes mediovittatus*. American Journal of Tropical Medicine and Hygiene, 39(2):218-22.

Gubler, D. J., et al. 1985. *Aedes* (Gymnometopa) *mediovittatus* (Diptera: Culicidae), a potential maintenance vector of dengue viruses in Puerto Rico. J Med Entomol 22(5):469-75.

Gubler, D.J. 2006. Dengue/dengue haemorrhagic fever: history and current status, in New Treatment Strategies for Dengue and Other Flaviviral Diseases: Novartis Foundation Symposium 277:3-16; discussion 16-22, 71-3, 251-3 (eds G. Bock and J. Goode), John Wiley & Sons, Ltd, Chichester, UK.

Harrington, L. C., et al. 2001. Why do female *Aedes aegypti* (Diptera: Culicidae) feed preferentially and frequently on human blood? Journal of Medical Entomology, 38(3):411-22.

Honorio, N. A., et al. 2009. Temporal Distribution of Aedes aegypti in Different Districts of Rio De Janeiro, Brazil, Measured by Two Types of Traps. Journal of Medical Entomology, 46(5): 1001-1014.

Hotez, P. J., et al. 2008. The Neglected Tropical Diseases of Latin America and the Caribbean: A Review of Disease Burden and Distribution and a Roadmap for Control and Elimination. Plos Neglected Tropical Diseases 2(9):e300.

Lloyd, L. 2003. Best Practices for dengue prevention and control in the Americas. Environmental Health Project Brief. USAID, Washington, DC. <http://pdf.usaid.gov/pdf_docs/Pnadb073.pdf>

McGarigal, K., et al. 2002. FRAGSTATS: Spatial Pattern Analysis Program for Categorical Maps. University of Massachusetts, Amherst.

Smith, J., et al. 2009. Seasonal and habitat effects on dengue and West Nile virus vectors in San Juan, Puerto Rico. Journal of the American Mosquito Control Association, 25(1):38-46.

Troyo, A., et al. 2009. Urban structure and dengue incidence in Puntarenas, Costa Rica. Singapore Journal of Tropical Geography, 30(2):265-282.

UNFPA (United Nations Population Fund). 2007. State of World Population: Unleashing the Potential of Ubran Growth. < http://www.unfpa.org/swp/2007/english/print/introduction.html>.

WHO (World Health Organization). 2009. Dengue/dengue haemorrhagic fever. <http://www.who.int/csr/disease/dengue/en/>

Wilson, M.E. and H. Chen. 2002. Dengue in the Americas. In World Health Organization Dengue Bulletin, 26: 44-61.