


# Lower socioeconomic status neighborhoods in Puerto Rico have more diverse mosquito communities and higher *Aedes aegypti* abundance

Nicole A. Scavo <sup>1,\*</sup>, Roberto Barrera,<sup>2</sup> Limarie J. Reyes-Torres<sup>1</sup> and Donald A. Yee<sup>1</sup>

<sup>1</sup>School of Biological, Environmental, and Earth Sciences, University of Southern Mississippi, Hattiesburg, MS 39406, USA and <sup>2</sup>Dengue Branch, Division of Vector-Borne and Infectious Diseases, Centers for Disease Control and Prevention, San Juan, Puerto Rico

\*Corresponding author. E-mail: nicole.scavo@tam.u.edu

Submitted: 17 August 2020; Received (in revised form): 25 February 2021. Accepted: 28 February 2021

## Abstract

Mosquito community dynamics in urban areas are influenced by an array of both social and ecological factors. Human socioeconomic factors (SEF) can be related to mosquito abundance and diversity as urban mosquito development sites are modified by varying human activity, e.g., level of abandoned structures or amount of accumulated trash. The goal of this study was to investigate the relationships among mosquito diversity, populations of *Aedes aegypti*, and SEF in a tropical urban setting. Mosquitoes were collected using BG Sentinel 2 traps and CDC light traps during three periods between late 2018 and early 2019 in San Juan, Puerto Rico, and were identified to species. SEFs (i.e. median household income, population density, college-level educational attainment, unemployment, health insurance coverage, percentage of households below the poverty line, amount of trash and level of abandoned homes) were measured using foot surveys and U.S. Census data. We found 19 species with the two most abundant species being *Culex quinquefasciatus* ( $n = 10\,641$ , 87.6%) and *Ae. aegypti* ( $n = 1558$ , 12.8%). We found a positive association between *Ae. aegypti* abundance and mosquito diversity, which were both negatively related to SES and ecological factors. Specifically, lower socioeconomic status neighborhoods had both more *Ae. aegypti* and more diverse communities, due to more favorable development habitat, indicating that control efforts should be focused in these areas.

**Key words:** socioeconomics, community ecology, mosquitoes, vectors, *Aedes aegypti*

## Introduction

Both biotic and abiotic factors combine to explain the distribution of species on a global scale. At a broader scale, abiotic factors, such as elevation and latitude, play an important role in explaining the distribution of species (Pavoine and Bonsall 2011; Bertuzzo et al. 2016). Evolutionary processes, like genetic drift and colonization, and biological interactions, such as competition, are important at regional and local scales (Pavoine and

Bonsall 2011). Species distribution patterns in cities are often affected by these same variables in addition to others that are unique to urban landscapes, such as time since urbanization, urban patch area, isolation from source populations, pollution, and surrounding land use types (Jones and Leather 2012). Intermediate urbanization has been shown to support the highest diversity across a number of taxa (e.g. birds, butterflies, plants) (Blair 1999; McKinney 2008). The effect of time since urbanization on diversity has been less studied (McKinney 2008),

though time has been shown to have some unique effects. For instance, leaf litter arthropod diversity declined over time (i.e. years) regardless where a site fell on an urban-rural gradient (Van Nuland and Whitlow 2014). However, for many taxa, we lack detailed explorations for how cities affect patterns of species diversity.

About 55% of the world's population currently lives in urban centers, a figure that is projected to increase to 68% by the year 2050 (United Nations 2018). As cities grow and expand into natural areas, understanding ecological questions, such as species distribution patterns, becomes increasingly important for human quality of life, the conservation of biodiversity, and the control of vector and pest species. Insects are a good system in which to study these urban species distributions and biodiversity patterns due to their ubiquity, diversity, and relative ease of sampling (McIntyre 2000). Species richness in native bees, butterflies, carabid beetles and elaterids beetles is inversely correlated to urbanization (Jones and Leather 2012). However, mosquitoes, which are of high importance as vectors of many human pathogens, are not as well studied within an urban context. In the studies that have been conducted, mosquito diversity shows a similar pattern to other insect groups, with diversity measures increasing with distance from city center (Cox et al. 2007; Johnston et al. 2014; Ferraguti et al. 2016; Wise de Valdez 2017). Additionally, a study in Chicago, USA, showed more heterogeneous urban environments supported higher mosquito diversity (Chaves et al. 2011). Mosquito diversity has been linked to vector abundance in several studies.

Vector abundance, defined as the number of individuals in one vector species, has been shown to be inversely correlated to mosquito biodiversity in Thailand (Thongsripong et al. 2013) and the USA (Chaves et al. 2011) but has not been studied enough to show a consistent relationship. In many cases, low diversity corresponds to areas with high levels of urbanization (measured as human population or other classical metrics, Thongsripong et al. 2013; Ferraguti et al. 2016). Vector abundance studies have also taken SES variables, such as income and education level, into account when analyzing their distribution (Keating et al. 2003; Little et al. 2017), and poverty and infrastructural decay have been linked to higher mosquito production in two US cities (LaDeau et al. 2013). Little et al. (2017) found that more potential oviposition containers and containers with larvae present were found in low socioeconomic status (SES) neighborhoods compared to middle or high SES neighborhoods. Another study found a higher prevalence of disused containers (e.g. trash) in low SES neighborhoods (Dowling et al. 2013), a factor that likely leads to more mosquito habitat as the containers are unmanaged. Both *Ae. aegypti* and *Ae. albopictus* had higher relative abundance in low compared with high SES neighborhoods in Panama City (Whiteman et al. 2019).

Unmanaged containers, combined with poor drainage or frequent flooding, have the potential to increase larval development sites in cities. These conditions are more often present in poorly planned, low SES neighborhoods. For example, our lowest two SES sites (Martin Peña and Cataño) flooded on multiple occasions during our sampling periods, while other sites did not. Keating et al. (2003) investigated well-drained versus undrained urban areas and found more suitable mosquito habitat in poorly drained areas indicating poorly planned areas built in flood plains may be more prone to produce large mosquito populations. Yee et al. (2019) found that flooding was linked to higher nitrogen levels in *Ae. aegypti* in Puerto Rico; however, this did not lead to higher level of arbovirus (i.e. Zika) in the

mosquito. Although *Ae. aegypti* larvae are not found in open water habitats, they may be affected by the nutrients in flood water that stay in containers after flood waters recede. Given the relative paucity of studies, more clarity is needed on the relationships among mosquito vector species, mosquito diversity, and SES and ecological variables.

This study examined how mosquito diversity changed across an urban landscape and analyzed the relationships among diversity, community composition, and the abundance of the main disease vector, *Ae. aegypti* (yellow fever mosquito), in the San Juan Metropolitan Area, Puerto Rico, USA. *Aedes aegypti* was chosen as the focus species of this study as it the primary mosquito vector on the island of Puerto Rico, transmitting dengue, Zika, and chikungunya (Sharp et al. 2013, 2014). Moreover, the Center for Disease Control (CDC) identifies the Zika virus the top mosquito-borne disease on the island (2020). Given the importance of *Ae. aegypti* as a vector of human pathogens, we focused on this species in this study. We hypothesized that diversity measures would vary across neighborhoods due to differences in environmental and social factors, and that the combination of all three factors will be correlated with the abundance of *Ae. aegypti*. We predicted that species richness, Shannon index of diversity ( $H'$ ), and community composition would vary among neighborhoods and that these differences will be correlated with environmental (e.g. level of abandonment) and human metrics (e.g. median household income). That is, low SES neighborhoods will have higher human population density and trash abundance, leading to higher presence of container habitats and thus higher *Ae. aegypti* abundance. We also predicted that diversity measures would be inversely related to *Ae. aegypti* abundance (e.g. higher diversity of mosquitoes would lead to lower abundance of *Ae. aegypti*) given that diversity measures (e.g. evenness) are lower at high densities of one species (in this case, *Ae. aegypti*). Lastly, we predicted that low SES neighborhoods would have the highest *Ae. aegypti* abundance and lowest mosquito diversity, whereas middle SES neighborhoods would have the highest diversity and lowest *Ae. aegypti* abundance. We also predict that high SES neighborhoods will have intermediate diversity and *Ae. aegypti* abundance. To our knowledge, this is the first study to investigate the relationships among *Ae. aegypti*, mosquito diversity, and socioeconomic factors (SEFs) in a tropical urban setting. Knowledge of the distribution patterns of vector species in cities will allow managers to better use resources to target key areas of mosquito production and reduce the risk of vector-borne disease.

## Materials and methods

### Site selection

Puerto Rico is part of the Greater Antilles archipelago located between the Caribbean Sea and the North Atlantic Ocean. It covers roughly 9104 km<sup>2</sup> and has a population of over three million people (US Census Bureau 2018). San Juan (18°27' N, 66°05' W), its capital and largest city, has a population of approximately 350,000 (US Census Bureau 2018). The San Juan Metropolitan Area has a subtropical, maritime climate with the rainy season occurring in May or October, making mosquitoes most abundant during this time of year. The elevation rises and the level of urbanization falls moving south from the Atlantic Ocean. The municipalities that make up the area present a mosaic of highly urbanized areas, wetlands, urban forest fragments, and managed green spaces (Smith et al. 2009).

Sampling took place in eight neighborhoods across three municipalities (San Juan, Cataño, and Carolina) (Fig. 1). Neighborhoods were chosen *a priori*, based on past work in the area (Yee et al. 2019) and were then placed along a gradient of SES and ecological factors using a cluster analysis. For our purposes, we refer to a neighborhood as an area where sampling took place that had relatively constant social and environmental conditions. Although the names represent a location on the map, they are meant to represent human communities, not a political unit such as a county or a municipality.

One gated community, Villa Venecia, was sampled. This neighborhood was physically close to another, Vistamar, leading to the two sites having different SES but similar surrounding habitat ecological characteristics (e.g. close to salt water) (Fig. 1). Torrecilla was unique in its proximity to mangrove forests and saltwater habitats. Cataño and Martin Peña were characterized by closely placed housing and semi-frequent flooding from canals within the communities. El Comandante and Puerto Nuevo were unique based on their proximity to a large, forested park.

### Human and landscape variables

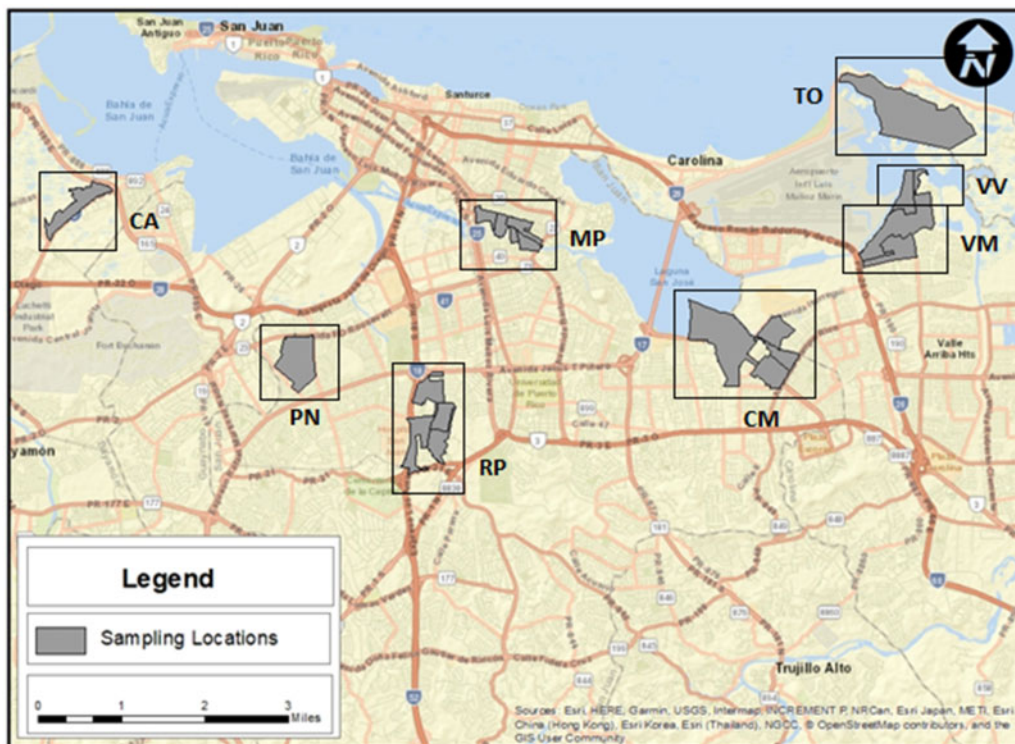
The heterogeneity of the neighborhoods was quantified by taking human and landscape variables into account. Median household income, population density, college-level educational attainment, unemployment, health insurance coverage, and percentage of households below the poverty line were used as human variables (i.e. SES) and were based on US Census data (2018) (Table 1). Median household income, educational attainment and level of abandonment were chosen as variables for our study based on their importance shown in previous mosquito studies (Dowling et al. 2013; LaDeau et al. 2013, 2015; Little

et al. 2017). Park size and amount of litter/trash were used as they have been shown to effect mosquito abundance or diversity (Medeiros-Sousa et al. 2017; Little et al. 2017). Additionally, distance to the nearest water body seems to affect mosquito community composition (Zittra et al. 2017). Water body presence was included due its role as mosquito habitat and as a proxy for likelihood of flooding. Foot surveys ( $n = 103$ ) were conducted along 50-m transects to assess levels of abandonment (e.g. number of abandoned buildings), type of spaces (e.g. water body, park), and incidence of litter. These surveys were conducted over October 2018, January 2019 and May 2019 in each neighborhood with locations being randomly selected based on trap locations.

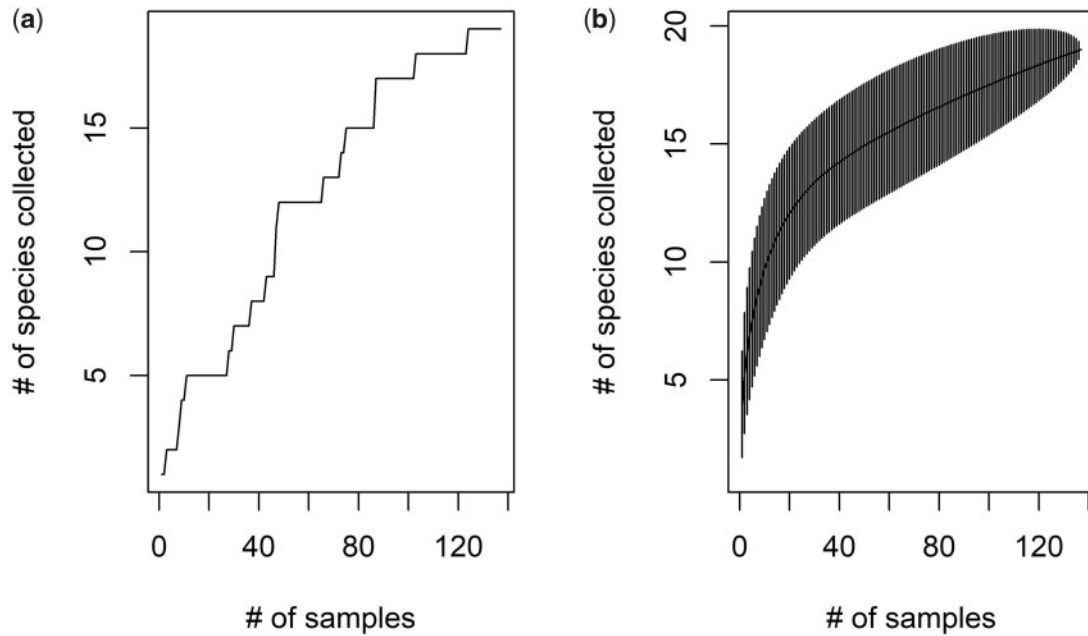
### Mosquito sampling

Adult sampling took place during the rainy season (October 2018 and May 2019) and the dry season (January 2019). Adult *Ae. aegypti* were collected using BG Sentinel 2 traps (Biogents, Regensburg, Germany) baited with scented BG lures (Biogents, Regensburg, Germany) set for 48 h. These traps are designed to attract anthropophilic mosquitoes, especially host seeking females. During each sampling event, six traps per neighborhood ( $n = 144$ ) were placed within residences, either outdoors or indoors, which were chosen based on willingness and availability of residents to participate. Traps were placed at residences at least 200 m apart from one another.

CDC light traps (Bioquip, Rancho Dominguez, CA) baited with CO<sub>2</sub> were used in tandem with BG Sentinel 2 traps in four residences per neighborhood ( $n = 96$ ). These traps were used to sample the wider mosquito community as they are designed to attract any insect that cues on light. At residences with both trap types, traps were placed at least 10m apart to avoid



**Figure 1:** Map of sampling locations around the San Juan Metropolitan area. Neighborhood designations are as follows: CM, El Comandante; CA, Cataño; MP, Martin Peña; PN, Puerto Nuevo; RP, Río Piedras; TO, Torrecilla; VM, Vistamar; VV, Villa Venecia.



**Figure 2:** Species accumulation curves. a, Species accumulation curve from collected samples and b, Species accumulation curve using rarefaction (permutations=10,000).

**Table 1:** Socio-economic status and ecological variables by neighborhood in the San Juan area

Variable	VV	RP	VM	PN	CM	TO	CA	MP
SES	High	Medium	Medium	Medium	Medium	Low	Low	Low
Mean # of abandoned homes	0.308	0.080	0.308	0.461	0.154	0.538	0.769	0.769
Mean # of parks	0.000	0.167	0.154	0.000	0.308	0.077	0.000	0.308
Mean # of freshwater bodies	0.000	0.000	0.153	0.000	0.077	0.000	0.231	0.385
Mean # of litter items	4.1	5.1	15.3	20.8	40.7	19.4	22.5	41.5
Human population density per mi <sup>2</sup>	641	630	1001	629	955	1433	1509	681
Proportion unemployment	0.167	0.332	0.293	0.306	0.421	0.586	0.180	0.447
Proportion with college education	0.614	0.687	0.519	0.386	0.353	0.204	0.227	0.164
Proportion below poverty	0.147	0.246	0.246	0.445	0.481	0.536	0.599	0.626
No health insurance	73	70	162	143	163	256	118	114
Median household income	65,000	15,000	25,000	10,000	10,000	10,000	10,000	10,000

Number of abandoned homes, parks, freshwater bodies, and litter items are mean values calculated from foot surveys ( $n = 103$ ) in October 2018, January 2019 and May 2019. The remainder of the variables are mean values calculated from the US Census data (2018) ( $n = 21$ ). CM, El Comandante; CA, Cataño; MP, Martín Peña; PN, Puerto Nuevo; RP, Río Piedras; TO, Torrecilla; VM, Vistamar, VV, Villa Venecia. Sites are placed along an SES-ecological gradient with group placements below site.

competition between traps. Small coolers (Igloo thermos,  $\frac{1}{2}$  gal.) filled with 1–2 lbs. of dry ice were placed next to light traps with a plastic tube directing the released  $\text{CO}_2$  towards the entrance of the trap. The addition of  $\text{CO}_2$  would likely increase the likelihood of attracting host-seeking female mosquitoes. Light traps were hung about shoulder height (1.5 m) and left for 48 h. Mosquitoes collected in traps were frozen at  $-20^\circ\text{C}$  and later identified to species using *The Mosquitoes of Puerto Rico* (Tulloch 1937) and *Key to the Mosquito Genera of Puerto Rico* (Barrera, unpublished) based on morphological characters. Both females and males were included in all analyses.

### Statistical analysis

Sampling effort was assessed by comparing the collected species accumulation curve from samples to a species accumulation curve

through rarefaction with 10,000 permutations. The  $\alpha$ , or site, diversity of the mosquito communities was analyzed using species richness ( $S$ ), defined as the number of mosquito species collected, evenness ( $J$ ), and the Shannon–Wiener diversity index ( $H'$ ).  $H'$  is calculated using the following equation:

$$H' = - \sum p_i \ln p_i$$

where  $p_i$  is the proportional abundance of a given species (Magurran 1988). Evenness was calculated using the following equation:

$$J = H' / \ln S$$

where  $H'$  is the Shannon diversity index for that sample and  $S$  is the species richness. These indices were chosen due to their



common use in ecology for analyzing patterns in biodiversity and were calculated at the neighborhood level (i.e. all species summed across all traps) (Magurran 1988).

Species richness data did not meet the assumptions of normality and was therefore analyzed using a Kruskal–Wallis Rank Sum test using data combined from BG Sentinel traps and CDC light traps to make a comparison among neighborhoods. A Dunn test was used to assess differences among communities based on the results of the Kruskal–Wallis test (Dunn 1961). Given that opportunistic larval sampling data was included in determining species richness, it was not included in the following permutational multivariate analysis of variance (perMANOVA), which only included adult data. Overall abundance,  $H'$ , and  $J$  were assessed using a perMANOVA due to the assumption of normality not being met. The perMANOVA test is a permutational version of a classical MANOVA and tests the null hypothesis ‘of equivalency of the centroids among groups’ (Anderson and Walsh 2013). A Bray–Curtis dissimilarity matrix and 10,000 permutations was used to carry out this analysis with data collected from combined BG Sentinel and CDC light traps. Individual Kruskal–Wallis tests were conducted on the overall abundance,  $H'$ , and  $J$  to determine their contribution to the results of the perMANOVA. Given that multiple tests were run on the same data, a Bonferroni correction was used to adjust the number of comparisons (i.e.  $\alpha = 0.0125$ ). However, given the controversy of using such diversity indices, that is their emphasis on uncertainty, their differential weighting of proportional data, and their vagueness (Strong 2016), a multivariate approach to analyze  $\beta$  diversity via community composition was also used. Beta diversity was considered relevant to assess if community composition varied among neighborhoods with similar values of diversity indices. Given that some mosquitoes are more important vectors than others, composition as well as diversity is relevant to framing results in a public health context. An analysis of similarities using a Bray–Curtis dissimilarity matrix with 10,000 permutations was conducted. This analysis tests the null hypothesis of no differences between sites by running a permutative analogue of a one-way analysis of variance using a rank similarity matrix (Clarke 1993).

*Aedes aegypti* abundance did not meet the assumption of normality, consequently two Kruskal–Wallis tests were used, one to compare abundance among neighborhoods and one among sampling dates. A Dunn test was then used to determine which neighborhoods varied significantly from the others. The diversity measures ( $S$ ,  $J$ ,  $H'$ ) were related to *Ae. aegypti* abundance using multiple linear regressions.

SES and ecological factors were analyzed using a principle component analysis (PCA) incorporating the landscape and human metrics: median household income, population density, educational attainment, employment levels, health insurance coverage, percentage of households below the poverty line, level of home abandonment, incidence of litter, and number of parks and water bodies (Table 2). Data was standardized by subtracting the mean and dividing by the standard deviation to account for the high range in degrees of magnitude. A single link agglomerative cluster analysis was used to place the neighborhoods in groups along a gradient of SES and ecological factors. Agglomerative cluster analyses produce larger and larger clusters from discontinuous data until all objects are placed into a single, all-inclusive group (Borcard et al. 2011). When run with a single linkage method, this can lead to inefficient groups but it still can place groups along a clear gradient (Borcard et al. 2011). The Calinski–Harabasz metric is a validation method that shows the quality of a cluster analysis sum of squares between

**Table 2:** PCA loadings for social and environmental variables for axes 1–3

Variable	PC 1	PC 2	PC 3
Abandoned homes	0.300	−0.298	0.413
Park presence	0.176	0.640	−0.096
Water	0.271	0.193	0.504
Litter	0.370	0.312	0.074
Human population density	0.233	−0.492	−0.121
Unemployment	0.274	0.174	−0.492
College Education	−0.424	0.148	−0.089
% below poverty	0.424	−0.063	0.101
No health insurance	0.258	−0.247	−0.506
Income	−0.336	−0.089	0.174

and within clusters (Ünlü and Xanthopoulos 2019) and was used to determine the ideal number of groups. Cluster groups and PCA axes were used as proxies for drawing conclusions about the variation in SES and ecological factors in across our sites. This heterogeneity was then related to diversity measures using a linear model with PCA axes that had an eigen value  $\geq 1$  as independent variables.

Lastly, a canonical correspondence analysis (CCA) was used to relate the five most common mosquito species, the neighborhoods, and the SES and ecological variables. Multiple CCA models were run and the best fit was chosen by comparing variance inflation factors (VIF). VIF are a measure of the proportion by which variance of a regression coefficient is inflated by the presence of other variables and are used to change models to reduce the effect of collinearity (Borcard et al. 2011). The model with the lowest VIF scores was chosen, though no specific threshold was used. Statistical workflow and output are summarized in Table 3. Additionally, species correlations were run among the five most abundant species. All analyses were run in R studio (version 1.1.414).

## Results

We collected 12,154 mosquitoes over the three sampling periods (Table 4). *Culex quinquefasciatus* ( $n = 10,641$ , 87.6%) was the most abundant species followed by *Ae. aegypti* ( $n = 1,558$ , 12.8%). Nineteen species were collected in total, with species in the genera *Aedes* and *Culex* being the most common (Table 4). The rarefied species accumulation curve (Fig. 2) showed richness starting to level off at 120 samples indicating that our sampling effort was robust but still may have missed rare species. Species richness varied significantly among neighborhoods ( $H = 33.67$ ,  $df = 7$ ,  $P < 0.001$ ) (Fig. 3). Torrecilla and Cataño had the highest species richness, with the lowest species richness in Martín Peña, Puerto Nuevo, Río Piedras, Villa Venecia and El Comandante, with Vistamar intermediate to these groups.

The perMANOVA showed significant differences in mosquito abundance,  $H'$ , and  $J$  among neighborhoods ( $F_{7,113} = 2.84$ ,  $P = 0.001$ ). When broken down by individual Kruskal–Wallis tests, abundance contributed significantly to these results ( $H = 27.53$ ,  $df = 7$ ,  $P < 0.001$ ), whereas Shannon diversity contributed but not significantly ( $H = 13.30$ ,  $df = 7$ ,  $P = 0.065$ ). Evenness did not contribute to the results ( $H = 6.97$ ,  $df = 7$ ,  $P = 0.432$ ). The multivariate analysis also demonstrated differences in diversity among neighborhoods with the results of the ANOSIM test showing that community composition varied significantly based on neighborhood ( $R = 0.163$ ,  $P < 0.001$ ), i.e. that there was

**Table 3:** Summary of order, input, and output of statistical test used in analysis

Order	Statistical test	Input	Outcome
1	Species Accumulation Curve	Mosquito diversity data	Species accumulation curves Sufficient sampling effort
2	Hierarchical agglomerative clustering analysis + Calinski-Harabasz metric	SES + ecological data	Dendrogram Sites placed along a gradient and into groups based on SES + ecological data
3	PCA	SES + ecological data	Reduced SES + ecological data in the form of PCA loadings
4	Kruskal-Wallis test + Dunn test	Mosquito diversity data	Significant differences between sites ( <i>Ae. aegypti</i> abundance, S)
5	perMANOVA	SES + ecological groupings Mosquito diversity data SES + ecological groupings	Significant differences between sites (J, H', total overall abundance)
6	Linear Models	Mosquito diversity data PCA loadings	No significant relationships
7	CCA	Mosquito diversity data SES + ecological data	CCA loadings and ordination plot with correlations shown between SES, ecological, and species variables

**Table 4:** Abundance of female and male mosquitoes caught using BG Sentinel traps and CO<sub>2</sub> baited CDC light traps in October 2018, January 2019, and May 2019

Species	Abundance (576 trap days)
<i>Aedes aegypti</i>	1,558
<i>Ae. mediovitatus</i>	1
<i>Ae. taeniorhynchus</i>	13
<i>Ae. tortilis</i>	45
<i>Anopheles albimanus</i>	1
<i>An. grabhamii</i>	12
<i>An. vestitpennis</i>	2
<i>Culex antillumagnorum</i>	1
<i>Cx. atratus</i>	16
<i>Cx. erraticus</i>	14
<i>Cx. iolambdis</i>	14
<i>Cx. nigripalpus</i>	109
<i>Cx. peccator</i>	95
<i>Cx. quinquefasciatus</i>	10,614
<i>Cx. sardinae</i>	1
<i>Cx. taeniopus</i>	6
<i>Deinocerites magnus</i>	9
<i>Toxorhynchites</i> sp.	1
<i>Uranotaenia. lowii</i>	2
Total	12,154

more variation between neighborhood groups than within neighborhood groups.

*Aedes aegypti* abundance varied significantly by neighborhood ( $H=26.93$ ,  $df=7$ ,  $P<0.001$ ) but not by time of year ( $H=2.00$ ,  $df=2$ ,  $P=0.368$ ). Cataño, Torrecilla and Martín Peña had the most *Ae. aegypti*, whereas Río Piedras and Villa Venecia had the fewest; other sites were intermediate. All diversity metrics ( $H'$ ,  $J$ ,  $S$ ) showed a significant relationship to *Ae. aegypti* abundance ( $F_{3,4}=9.68$ ,  $P=0.026$ ,  $R^2=0.78$ ). Torrecilla and Cataño both had the highest species richness and the highest *Ae. aegypti* abundance, whereas, Río Piedras and Villa Venecia had the lowest values for both measures.

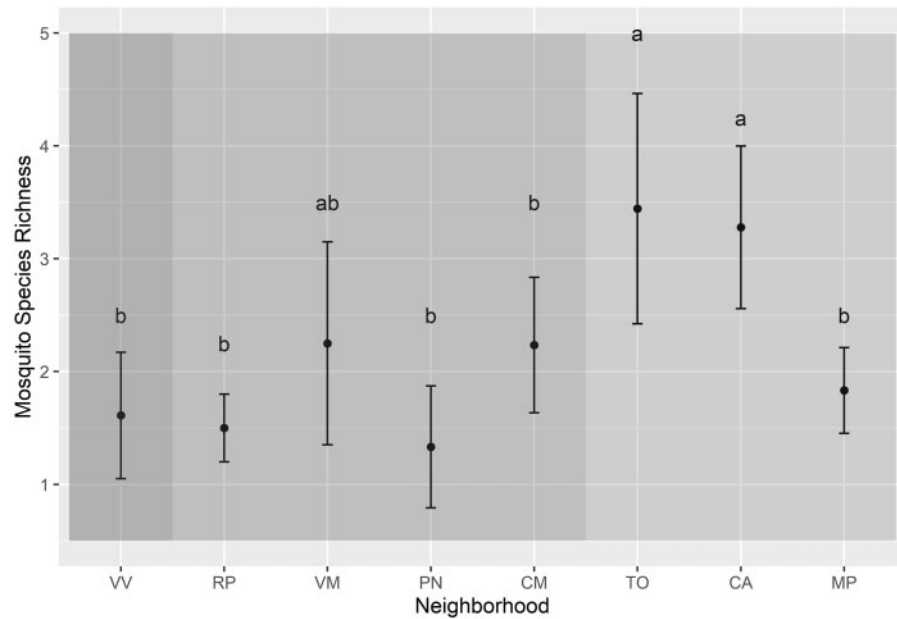
Results from the PCA showed that the neighborhoods sampled represented of the gradient of socioeconomic and

environmental San Juan Metropolitan Area (Fig. 4). The first three axes were included in further linear regression analyses as they all had eigen values of  $\geq 1$ . Together these axes explained 86.4% of the variance present in the landscape. Evenness showed marginal positive relationship to PC 2 ( $t=2.58$ ,  $P=0.061$ ), and overall the model was not significant ( $F_{3,4}=2.40$ ,  $P=0.21$ ,  $R^2=0.37$ ). Values of  $S$  ( $F_{3,4}=0.48$ ,  $P=0.71$ ,  $R^2=-0.28$ ) and  $H'$  ( $F_{3,4}=0.1.01$ ,  $P=0.48$ ,  $R^2=0.00$ ) showed no relationship to any axes. The low agglomerative coefficient of the cluster analysis (0.314) showed inefficient groupings, again indicating heterogeneity among the neighborhoods sampled. Neighborhoods were placed into five groups by the Calinski-Harabasz metric. However, the groupings did place the neighborhoods on a gradient of the socio-economic and environmental factors being considered which aid in the interpretation of results (Fig. 5).

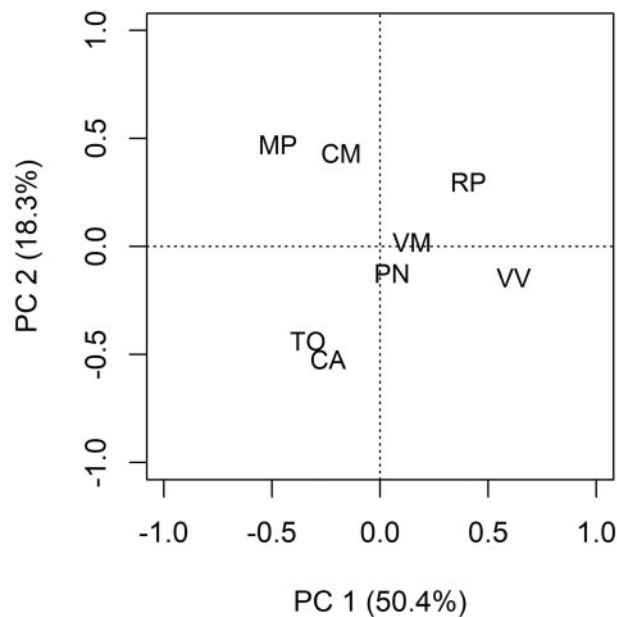
The CCA model including abandonment, human density, unemployment, litter and income was the best fit according to VIF and were therefore included in the analysis where other environmental variables were excluded. These factors explained 43.5% of the variance present in the mosquito community. *Aedes aegypti* presence, income, and litter presence were negatively associated to axis 1 compared to human population density and unemployment which had a positive association (Fig. 6). Torrecilla and Cataño were the only neighborhoods positively associated with axis 1, indicating their positive relationship to human population density and unemployment (Fig. 6). *Culex quinquefasciatus*, a vector of West Nile virus, was aligned with Torrecilla and Cataño as well. *Culex peccator* was strongly associated with income. No strong correlations were found among species, except between *Ae. tortilis* and *Cx. nigripalpus* (+0.968).

## Discussion

This is the first study conducted in San Juan examining how SES and ecological variables affect the abundance of *Ae. aegypti* and communities of mosquitoes. Our study focused on mosquito communities in urban environments with a more in-depth examination of *Ae. aegypti*, the primary arbovirus disease



**Figure 3:** Mosquito species richness by neighborhood. Plot of means  $\pm$  2 standard errors. Kruskal-Wallis Rank Sum test ( $H = 33.67$ ,  $df = 7$ ,  $P < 0.001$ ). Sites are arranged along the SEF gradient with the highest on the left-hand side. Boxes indicate SES (dark gray, high; medium gray, medium; light gray, light gray). Neighborhood designations are as follows: CM, El Comandante; CA, Cataño; MP, Martín Peña; PN, Puerto Nuevo; RP, Río Piedras; TO, Torrecilla; VM, Vistamar; VV, Villa Venecia.



**Figure 4:** First two axes of Principle Component Analysis on socio-economic and environmental variables. Neighborhood designations are as follows: CM, El Comandante; CA, Cataño; MP, Martín Peña; PN, Puerto Nuevo; RP, Río Piedras; TO, Torrecilla; VM, Vistamar; VV, Villa Venecia.

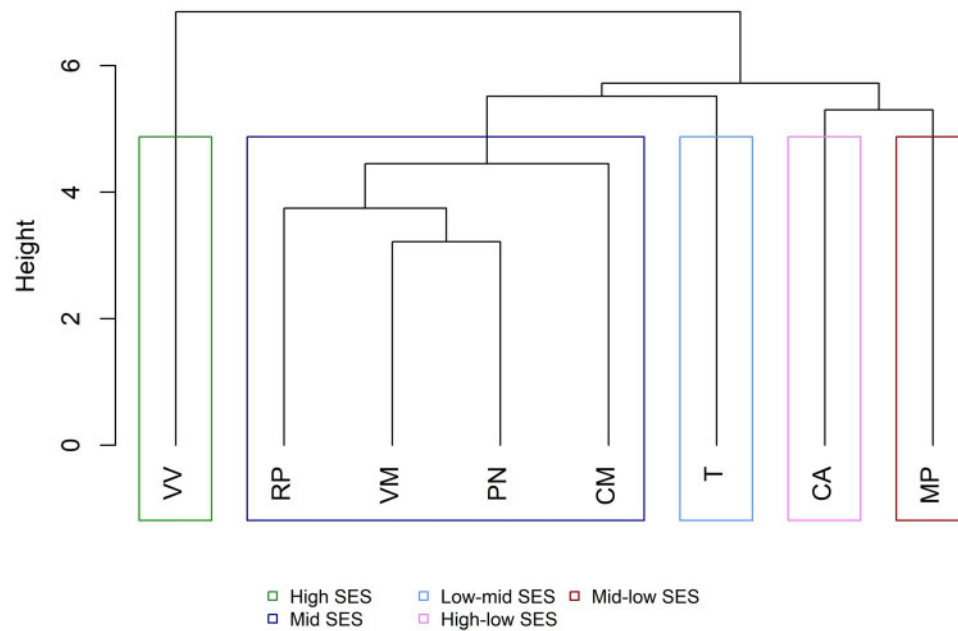
vector in Puerto Rico. We hypothesized that diversity measures would vary across neighborhoods because of differences in environmental and social factors, and that each of the factors would be correlated with the abundance of *Ae. aegypti*. This hypothesis was supported as mosquito diversity metrics and community structure were shown to vary across the heterogeneous environment of the San Juan Metropolitan Area. Both diversity metrics and SES and ecological factors were related to the abundance of *Ae. aegypti*, that is higher abundance was correlated

with low SES and higher diversity, which supported our initial hypothesis.

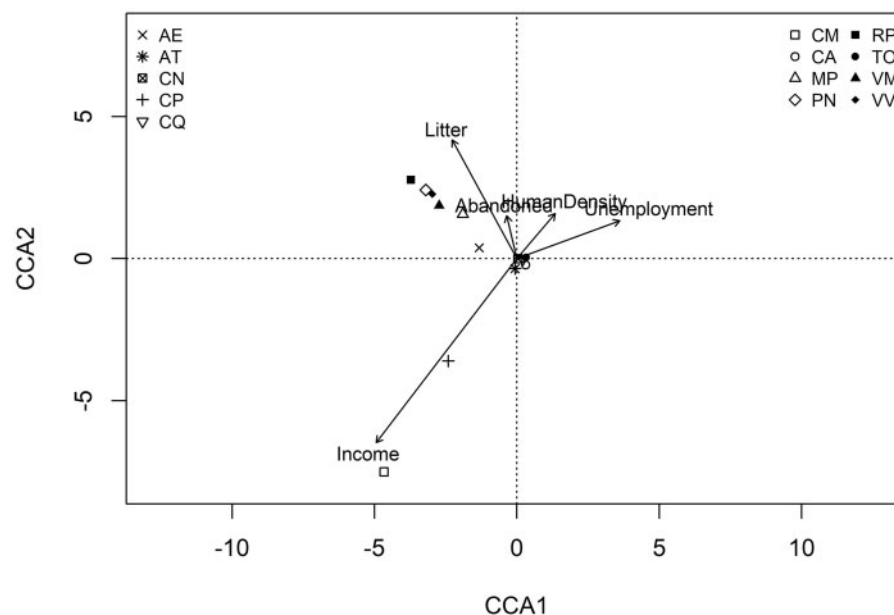
We found that mosquito community diversity varied with the SES of a neighborhood. Half of the neighborhoods in this study fell into the middle SES category, a group that was characterized with higher educational attainment and income (e.g. Río Piedras), and was generally associated with lower mosquito species richness. Conversely, the neighborhoods lowest along the SES gradient (e.g. Cataño) showed the highest mosquito species richness and were distinguished by high human population density, the presence of abandoned houses, and residents without health insurance.

The relationship between mosquito diversity and population density is less-well explored. Two studies showed a decrease in mosquito diversity when moving from rural to urban areas (Johnston et al. 2014; Ferraguti et al., 2016). However, one of these (Ferraguti et al. 2016) showed a decrease in mosquito species richness with increasing human population density. Our results show the opposite—mosquito richness increased as human population density increased. However, our study investigated mosquito diversity in an urban only-context, whereas Ferraguti et al. (2016) conducted their study along an urban–rural gradient. Abandoned building presence has been shown to predict both larvae habitat availability and the abundance of adult mosquitoes in urban areas (Little et al. 2017), and abandoned houses may provide habitat to a wider variety of mosquitoes due to a likelihood of having more microhabitats created by overgrown vegetation.

When looking at the scale of neighborhoods within the San Juan Metropolitan Area, our sites can be seen as heterogeneous in terms of socioeconomics and geography. Although finer spatial scales are likely to play an important role in mosquito distributions, environmental parameters at a larger scale, such as geography and land cover type, affect species richness and the structure of mosquito communities (Claflin and Webb 2017; Zittra et al. 2017). Additionally, heterogeneity measured on a larger scale (km) has been shown to influence mosquito species richness in urban areas (Chaves et al. 2011). A rural study found



**Figure 5:** Dendrogram placing sites into five groups along a gradient of socio-economic and environmental factors. Groups were determined by the Calinski–Harabasz metric. Neighborhood designations are as follows: CM, El Comandante; CA, Cataño; MP, Martín Peña; PN, Puerto Nuevo; RP, Río Piedras; TO-Torrecilla; VM, Vistamar; VV, Villa Venecia.



**Figure 6:** Canonical Correspondence Analysis. The plotted socio-environmental variables explained 43.5% of the variance in the mosquito community. AE- *Aedes aegypti*, AT- *Ae. tortilis*, CN- *Culex nigripalpus*, CP- *Cx. peccator*, CQ-*Cx. quiuefasciatus*. Neighborhood designations are: CM-El Comandante, CA-Cataño, MP-Martín Peña, PN-Puerto Nuevo, RP-Río Piedras, TO-Torrecilla, VM-Vistamar, VV-Villa Venecia.

that finer scales (10–100 m) are important for determining distributions of host-seeking mosquitoes, which generally stay within that range for to find their resources (Reiskind et al. 2017). Our study indicated neighborhood scale (km) variables explain some of the variance in mosquito community diversity, such as  $H'$  and  $J$ , in San Juan, Puerto Rico. For example,  $H'$  was affected at the km scale (park presence) and the m scale (litter presence). This supports the idea that both neighborhood level variables and microhabitat variables are important in explaining the diversity of mosquitoes in urban environments.

Villa Venecia and Río Piedras, the two highest neighborhoods along the SES and ecological gradient, had the lowest *Ae. aegypti* abundance. In turn, Cataño, Martín Peña, and Torrecilla, the three neighborhoods on the low end of the SES and ecological gradient had the highest *Ae. aegypti* abundance. These patterns led us to conclude that SES shows an inverse relationship with *Ae. aegypti* abundance. This relationship is consistent with some studies focusing on a related species, *Ae. albopictus* (Little et al. 2017) and malaria vectors in the *Anopheles* genus (Keating et al. 2003). However, a recent review showed *Aedes* occurrence



is not consistently related to SES variables on a global scale (Whiteman et al. 2020), a result likely due to idiosyncrasies linked to individual study locations. *Aedes aegypti* presence was most closely related to amount of litter and median household income of a neighborhood. It may seem surprising that *Ae. aegypti* presence was negatively related to human population density, however, the species has been linked to high housing density as opposed to population density itself (Cox et al. 2007). A study in Buenos Aires drew similar conclusions with *Ae. aegypti* oviposition, with it being more closely associated with high housing density (i.e. less urbanized areas) rather than high apartment density or high human population density (i.e. more urbanized sites) (Carbajo et al. 2006). Differing levels of resting sites, oviposition sites, blood sources, and nectar available at different levels of housing densities may explain this relationship (Carbajo et al. 2006). It is also important to note that our lowest SES locations often displayed the highest percentage of abandoned buildings, whose unkept locations would lead to more resting sites and plants that could be sugar sources, and thus may provide further support to the notion that high housing density, and not humans *per se* is an important factoring explaining *Ae. aegypti* abundance.

The differential distribution of *Ae. aegypti* in San Juan could lead to a variable distribution of the arboviruses it can pass onto hosts and therefore human health risk, especially in neighborhoods like Cataño, Torecilla, and Martín Peña, where we found the most *Ae. aegypti*. Dengue incidence in Puerto Rico has been positively correlated with female *Ae. aegypti* abundance (Barrera et al. 2011), which suggests that dengue infection risk may vary with SES alongside *Ae. aegypti* abundance. Moreover, Waterman et al. (1985) found the low socioeconomic status was associated with dengue incidence. However, Yee et al. (2019) found that female *Ae. aegypti* in Martín Peña were linked to higher nitrogen levels with lower Zika infection rates, suggesting that there is not always a positive relationship between *Ae. aegypti* abundance and arbovirus prevalence. A knowledge gap in mosquito control has been reported between high and low SES residents (Syed et al. 2010; Dowling et al. 2013), another factor that could heighten inequalities in human disease risk in the area.

We found that *Ae. aegypti* abundance had a positive relationship to overall mosquito diversity. These results are inconsistent with other studies of mosquito vector abundance and diversity measures. A study in Chicago, Illinois, found that *Culex pipiens* abundance was negatively related to mosquito species richness and the  $H'$  (Chaves et al. 2011). Thongsripong et al. (2013) also found an inverse relationship between vector species (i.e. *Ae. aegypti* and *Cx. quinquefasciatus*) and diversity in urban areas in Thailand. A similar pattern may be exhibited by *Ae. aegypti* in San Juan but was not detected in this study due to a relatively small dataset. Chaves et al. (2011) proposed mechanisms of this relationship that are specific to *Cx. pipiens* biology, indicating that their results may not be in accordance with other species. The authors of the Thailand study propose several mechanisms, such as competition between *Ae. aegypti* and *Ae. albopictus* and pesticide resistance (Thongsripong et al. 2013), that could explain that relationship between vector species abundance and mosquito diversity. Given that *Ae. albopictus* is not present in Puerto Rico, the pattern may not exist with *Ae. aegypti* in San Juan due to different dynamics. Moreover, islands are recognized to have lower species richness than mainland communities (Kier et al. 2009), which could explain why the results of our study differ from other non-island studies.

Other variables that may have influenced patterns for *Ae. aegypti* were left out of this study due to issues with data

availability. Precipitation and temperature, which have been positively linked with *Ae. aegypti* abundance in Puerto Rico (Barrera et al. 2011), were not evaluated as the only publicly available weather data is collected at one location, the Muñoz-Marin International Airport. Given the scale of our study (i.e. neighborhood level), city level weather data would not allow a comparison among neighborhoods. Land cover was also left out of the analysis due to the lack of availability of landscape cover data after Hurricane Maria hit Puerto Rico in September 2017. Using pre-Hurricane Maria data was deemed inappropriate given the severity of land cover change Hurricane Maria inflicted on Puerto Rico (Hu and Smith 2018). Thus, it remains possible that other factors not measured here influenced the distribution of *Ae. aegypti* and mosquito biodiversity, in general, in the San Juan Metropolitan Area. Additionally, the mosquito community was treated as a whole, as opposed to two different communities based on larval habitat, which could have influenced the community results given the differing ecology of open-water versus container mosquitoes (Laird 1988).

In conclusion, SES is linked to both *Ae. aegypti* abundance and mosquito biodiversity in the urban landscape of the San Juan Metropolitan Area. *Aedes aegypti* abundance showed a positive relationship to mosquito biodiversity measures. This study indicates that neighborhoods of low SES should be a target for mosquito control with a focus placed on populations developing in trash containers. For similar studies in the future, a survey of residents' mosquito knowledge and control practices would be beneficial to add to analyses and would give more insight for control purposes.

## Acknowledgements

We thank the various students who helped contribute to this research effort: N. Mackey, C. Dean, K. Noa, C. Folks, D. Sweet, and T. Sánchez. We also thank G. Felix, J. Zimmerman, el Verde Field Station and its staff, and the residents of the communities where sampling took place. A special thank you to the Center for Disease Control Dengue Branch for providing equipment.

## Funding

This research was supported by a grant to D.A. Yee from the National Science Foundation (DEB-1806122).

## Data availability

Data is available on Dryad under the name Mosquito Community Composition in San Juan, Puerto Rico (doi:10.5061/dryad.5x69p8d2k).

Conflict of interest statement. None declared.

## References

- Anderson, M., and Walsh, D. (2013) 'PERMANOVA, ANOSIM, and the Mantel Test in the Face of Heterogeneous Dispersions: What Null Hypothesis Are You Testing?', *Ecological Monographs*, **83**: 557–74.
- Barrera, R., Amador, M., and MacKay, A. (2011) 'Population Dynamics of *Aedes aegypti* and Dengue as Influenced by Weather and Human Behavior in San Juan, Puerto Rico', *PLoS Neglected Tropical Diseases*, **5**: e1378.
- Unpublished. Key to the Mosquito Genera of Puerto Rico.

- Bertuzzo, E. et al. (2016) 'Geomorphologic Controls on Elevational Gradients of Species Richness', *Proceedings of the National Academy of Sciences of the United States of America*, **113**: 1737–42.
- Blair, R. (1999) 'Birds and Butterflies along an Urban Gradient: Surrogate Taxa for Assessing Biodiversity?', *Ecol. Appl.*, **9**: 164–70.
- Borcard, D., Gillet, F., and Legendre, P. (2011) *Numerical Ecology* with R. New York: Springer-Science + Business Media, LLC.
- Carbajo, A., Curto, S., and Schweigmann, N. (2006) 'Spatial Distribution Pattern of Oviposition in the Mosquito *Aedes aegypti* in Relation to Urbanization in Buenos Aires: Southern Fringe Bionomics of an Introduced Vectors', *Medical and Veterinary Entomology*, **20**: 209–18.
- Chaves, L. et al. (2011) 'Climatic Variability and Landscape Heterogeneity Impact Urban Mosquito Diversity and Vector Abundance and Infection', *Ecosphere*, **2**: art70.
- Center of Disease Control and Prevention. (2020) *Vector-Borne Disease Profile (2004-2018)*. Puerto Rico: CDC. <<https://www.cdc.gov/nceid/dvbd/vital-signs/puerto-rico.html>>. Last accessed August 16, 2020.
- Claffin, S., and Webb, C. (2017) 'Surrounding Land Use Significantly Influences Adult Mosquito Abundance and Species Richness in Urban Mangroves', *Wetlands Ecology and Management*, **25**: 331–44.
- Clarke, K. (1993) 'Non-Parametric Multivariate Analyses of Changes in Community Structure', *Austral Ecology*, **18**: 117–43.
- Cox, J. et al. (2007) 'Habitat Segregation of Dengue Vectors along an Urban Environmental Gradient', *The American Journal of Tropical Medicine and Hygiene*, **76**: 820–6.
- Dowling, Z. et al. (2013) 'Socio-Economic Status Affects Mosquito (Diptera:Culicidae) Larval Habitat Type Availability and Infestation Level', *Journal of Medical Entomology*, **50**: 764–72.
- Dunn, O. (1961) 'Multiple Comparisons among Means', *Journal of the American Statistical Association*, **56**: 52–64.
- Ferraguti, M. et al. (2016) 'Effects of Landscape Anthropization on Mosquito Community Composition and Abundance', *Scientific Reports*, **6**: 29002.
- Johnston, E. et al. (2014) 'Mosquito Communities with Trap Height and Urban-Rural Gradient in Adelaide, South Australia: Implications for Disease Vector Surveillance', *Journal of Vector Ecology*, **36**: 48–55.
- Jones, E., and Leather, S. (2012) 'Invertebrates in Urban Areas: A Review', *European Journal of Entomology*, **109**: 463–78.
- Hu, T., and Smith, R. (2018) 'The Impact of Hurricane Maria on the Vegetation of Dominica and Puerto Rico Using Multispectral Remote Sensing', *Remote Sensing*, **10**: 827.
- Keating, J. et al. (2003) 'A Geographic Sampling Strategy for Studying Relationships between Human Activity and Malaria Vectors in Urban Africa', *The American Journal of Tropical Medicine and Hygiene*, **68**: 357–65.
- Kier, G. et al. (2009) 'A Global Assessment of Endemism and Species Richness across Island and Mainland Regions', *Proceedings of the National Academy of Sciences of the United States of America*, **106**: 9322–7.
- Laird, M. (1988) *The Natural History of Larval Mosquito Habitats*. San Diego, CA: Academic.
- LaDeau, S. et al. (2015) 'The Ecological Foundations of Transmission Potential and Vector-Borne Disease in Urban Landscapes', *Functional Ecology*, **29**: 889–901.
- et al. (2013) 'Higher Mosquito Production in Low-Income Neighborhoods of Baltimore and Washington, DC: Understanding Ecological Drivers and Mosquito-Borne Disease Risk in Temperate Cities', *International Journal of Environmental Research and Public Health*, **10**: 1505–26.
- Little, E. et al. (2017) 'Socio-Ecological Mechanisms Supporting High Densities of *Aedes albopictus* (Diptera: Culicidae) in Baltimore, MD', *Journal of Medical Entomology*, **54**: 1183–92.
- Magurran, A. (1988) *Ecological Diversity and Its Measurement*. Netherlands: Springer Netherlands.
- McIntyre, N. (2000) 'Ecology of Urban Arthropods: A Review and Call to Action', *Annals of the Entomological Society of America*, **93**: 825–35.
- McKinney, M. (2008) 'Effects of Urbanization on Species Richness: A Review of Plants and Animals', *Urban Ecosystems*, **11**: 161–76.
- Medeiros-Sousa, A. et al. (2017) 'Mosquitoes in Urban Green Spaces: Using an Island Biogeographic Approach to Identify Drivers of Species Richness and Composition', *Scientific Reports*, **7**: 17826.
- Pavoine, S., and Bonsall, M. (2011) 'Measuring Biodiversity to Explain Community Assembly: A Unified Approach', *Biological Reviews of the Cambridge Philosophical Society*, **86**: 792–812.
- Reiskind, M. et al. (2017) 'Mosquitoes of Field & Forest: The Scale of Habitat Segregation in a Diverse Mosquito Assemblage', *Medical and Veterinary Entomology*, **31**: 44–54.
- Sharp, T. et al. (2013) 'Virus-Specific Differences in Rates of Disease during the 2010 Dengue Epidemic in Puerto Rico', *PLoS Neglected Tropical Diseases*, **7**: e2159.
- et al. (2014) 'Chikungunya Cases Identified through Passive Surveillance and Household investigations - Puerto Rico, May 5 - August 12', *MMWR Morb. Mortal. Wkly. Rep.*, **63**: 2024.
- Smith, J., Amador, M., and Barrera, R. (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**: 38–46.
- Strong, W. (2016) 'Biased Richness and Evenness Relationships within Shannon-Wiener Index Values', *Ecological Indicators*, **67**: 703–13.
- Syed, M. et al. (2010) 'Knowledge, Attitudes and Practices regarding Dengue Fever among Adults of High and Low Socioeconomic Groups', *Journal of Pakistan Medical Association*, **60**: 243–7.
- Thongsripong, P. et al. (2013) 'Mosquito Vector Diversity across Habitats in Central Thailand Endemic for Dengue and Other Arthropod-Borne Diseases', *PLoS Neglected Tropical Diseases*, **7**: e2507.
- Tulloch, G. S. (1937) The mosquitoes of Puerto Rico. The Journal of Agriculture of the University of Puerto Rico. A Tropical Rainforest: A Study of Irradiation and Ecology at El Verde, Puerto Rico E-159-E-167.
- United Nations. (2018) *World Urbanization Prospects: 2018 Revision*. <<https://population.un.org/wup/Publications/Files/WUP2018-Report.pdf>>. Last accessed August 16, 2020.
- United States Census Bureau (2018) *Puerto Rican Commonwealth Population Totals and Components of Change: 2010-2018*. <<https://www.census.gov/data/tables/time-series/demo/popest/2010s-total-puerto-rico.html>>. Last accessed August 16, 2020.
- Ünlü, R., and Xanthopoulos, P. (2019) 'Estimating the Number of Clusters in a Dataset via Consensus Clustering', *Expert Systems with Applications*, **125**: 33–9.
- Van Nuland, M., and Whitlow, W. (2014) 'Temporal Effects of Biodiversity and Composition of Arthropods Communities along and Urban-Rural Gradient', *Urban Ecosystems*, **17**: 1047–60.
- Waterman, S. H. et al. (1985) 'Dengue Transmission in Two Puerto Rican Communities in 1982', *The American Journal of Tropical Medicine and Hygiene*, **34**: 625–32.
- Whiteman, A. et al. (2019) '*Aedes* Mosquito Infestation in Socioeconomically Contrasting Neighborhoods of Panama City', *EcoHealth*, **16**: 210–21.

- et al. (2020) Do socioeconomic factors drive *Aedes* mosquito vectors and their arboviral diseases? A systematic review of dengue, chikungunya, yellow fever, and Zika virus. *One Health*, **11**: 100188.
- Wise De Valdez, M. (2017) 'Mosquitoes Species Distribution across Urban, Suburban, Semi-Rural Residences in San Antonio, Texas', *Journal of Vector Ecology : Journal of the Society for Vector Ecology*, **42**: 184–8.
- Yee, S. et al. (2019) 'Linking Water Quality to *Aedes aegypti* and Zika in Flood-Prone Neighborhoods', *EcoHealth*, **16**: 191–209.
- Zittra, C. et al. (2017) 'Landscape Structure Affects Distribution of Potential Disease Vectors (Diptera: Culicidae)', *Parasites & Vectors*, **10**: 205.