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Linking Water Quality to *Aedes aegypti* and Zika in Flood-Prone Neighborhoods

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Abstract

The ability of ecosystems to regulate water quality and flood events has been linked to health outcomes, including mosquito-borne illnesses. In the San Juan Bay Estuary watershed of Puerto Rico, habitat alterations and land-use development have disrupted watershed hydrology, exacerbating wastewater discharges and subjecting some neighborhoods to frequent flooding events. In 2016, the mosquito-borne illness Zika became a new cause for concern. We hypothesized that nutrient-enriched flood water could provide pulses of supplemental nutrients to local mosquito populations. We conducted a field study in six neighborhoods adjacent to the estuary to assess whether environmental variability of nutrient inputs could be linked to breeding habitat containers, *Aedes aegypti* larvae and adults, and the acquisition of Zika virus by adult mosquitoes. The most frequently flooded neighborhood had consistently higher levels of nitrogen in estuary water, leaf detritus, containers, and adult mosquitoes compared to other neighborhoods. Adult body nitrogen was significantly related to both nitrogen content of containers and leaf detritus from the local trapping area. Disseminated Zika concentration in adult *Ae. aegypti* tended to decrease as body carbon and nitrogen increased. Our study provides preliminary evidence that environmental variability in nutrient inputs can influence viral acquisition by mosquito vectors. This suggests that management actions to reduce flooding and improve water quality should go hand-in-hand with more traditional vector control methods, such as aerial spraying, to help control spread of vector-borne diseases.

Keywords

Aedes aegypti; Nitrogen; Carbon; Stable isotopes; Zika

Introduction

Decision makers are increasingly framing environmental management issues within the context of benefits to the health and well-being of stakeholders (Rhodus et al. 2013; Korfmacher et al. 2015; Oosterbroek et al. 2016). Land-use change, climate change, and point and nonpoint source runoff alter the state of ecosystems and their services, including disservices, leading to subsequent effects on human health (Myers et al. 2013). The ability of ecosystems to regulate water quality and flood events has been linked to decreases in a number of health outcomes, including infectious and parasitic diseases, mental disorders, and respiratory illnesses (Hinga and Batchelor 2005; Ivers and Ryan 2006; Oosterbroek et al. 2016; De Jesus Crespo and Fulford 2017). In coastal communities, greenspaces such as wetlands may play a role in mitigating mosquito-borne illnesses by moderating flooding events or regulating water quality (Carver et al. 2015; De Jesus Crespo et al. 2018). With growing concerns about the health risks of aerial spraying for mosquitoes, alternative management strategies that leverage these natural ecosystem services may provide more sustainable approaches to managing mosquito vectors.

Estuary management programs, in particular, have been linking actions to improve the habitat and water quality of the estuary to goals of improving human health and public safety (SJBEP 2000). For example, the San Juan Bay estuary, Puerto Rico, comprises a connected system of bays, lagoons, and canals in a highly urbanized watershed of over half a million residents, with socioeconomic conditions varying widely across neighborhoods (Azar and Rain 2007). Habitat alterations and land-use development have disrupted the natural flow of water throughout the estuary system, subjecting some neighborhoods to frequent flooding events and exacerbating the effects of wastewater discharges, including untreated sewage and stormwater runoff into areas of the bay (SJBEP 2000). A recent Health Impact Assessment identified vector-borne illnesses, including dengue, as a major health concern for flood-impacted communities neighboring the estuary (Sheffield et al. 2014; Korfmacher et al. 2015), and supported efforts to reduce flooding and debris that create mosquito breeding sites (Ahern et al. 2005; Pires and Gleiser 2010).

Most recently, the mosquito-borne illness Zika has become a cause for concern. Zika virus (ZIKV) is a flavivirus, closely related to other arboviruses like dengue, West Nile, and yellow fever, and was originally isolated in Uganda, Africa (Dick et al. 1952; Musso and Gubler 2016). ZIKV was first detected in the Americas in Brazil in 2015 (Zanluca et al. 2015) and spread explosively throughout the Americas in 2016 (WHO 2016a). The first confirmed case of locally acquired Zika infection in Puerto Rico was reported in December 2015 (WHO 2016b), and Zika cases continued to increase rapidly, peaking in August 2016 with as many as 2500 confirmed cases per week (Puerto Rico Department of Health 2017). In North America, *Aedes aegypti* and *Ae. albopictus* are the only mosquitoes known to be competent vectors of ZIKV (Chouin-Carneiro et al. 2016), with only *Ae. aegypti* known to have an established distribution in Puerto Rico (Sharp et al. 2013).

Like other arboviruses, transmission of Zika virus is influenced by a complex array of interactions among humans, wildlife, mosquito vectors, and the environment (Thomas et al. 2017). Mosquitoes become exposed to virus when they take a blood meal from an infected

person (Gratz 2004), or wildlife that may serve as pathogen reservoirs (Gonzalez-Salazar et al. 2017). The virus then replicates in the mosquito's midgut and salivary glands, and after an incubation period, may be spread when the infectious mosquito takes its next blood meal (Musso and Gubler 2016). The capacity of a mosquito vector to spread a virus depends on factors such as the probability of acquiring and transmitting the pathogen, the proportion of vectors acquiring the pathogen, and the density of vectors per human host (Anderson and Rico-Hesse 2006; Klempner et al. 2007). Environmental factors, such as larval nutrition (Nasci and Mitchell 1994), larval density (Alto et al. 2005; 2008a; Reiskind and Lounibos 2009), adult nutrition (Canyon et al. 1999), adult body size (Scott et al. 2000; Alto et al. 2008b), and seasonality and climate (Almeida et al. 2005), have all been shown to affect virus acquisition for mosquitoes. Moreover, a handful of studies have directly linked water quality factors, such as nutrient enhancement through fertilizer runoff (Mutero et al. 2004) or water chemistry in septic systems (Burke et al. 2010), to the abundance of mosquito larvae and adults. To our knowledge, however, no studies have assessed whether local variability in water quality can be linked to variability in the acquisition of virus by adult mosquitoes. As a result, the degree to which proposed actions in the San Juan Bay watershed to reduce sewage discharges, improve stormwater drainage, and restore natural flow could mitigate vector-borne illnesses (Sheffield et al. 2014; Korfmacher et al. 2015) is challenging to ascertain.

We hypothesized that variability in point and nonpoint source runoff creates variability in estuary water quality (Fig. 1). During extreme rainfall events, larval habitat containers, such as buckets, food containers, or discarded vehicle tires, may experience inputs of flood waters as rivers, canals, or lagoons overflow (Hashizume et al. 2012). Normally, larval containers are filled through precipitation events, and allochthonous inputs from leaf or animal detritus are important sources of nutrients (Kling et al. 2007). In areas where waterbodies are subjected to runoff or wastewater discharges, flood water could provide pulses of supplemental nutrients to local mosquito populations, dependent on the frequency of flooding. In larval containers, nutrients such as nitrogen are often limiting, such that the growth, survival, and mass of larvae, and consequently of adults, are crucially affected by allochthonous inputs (Carpenter 1983; Yee and Juliano 2006). Nutrition and mass subsequently affect the acquisition and multi-plication rates of virus within adult mosquitoes (Nasci and Mitchell 1994; Canyon et al. 1999; Scott et al. 2000; Alto et al. 2008b) and ultimately, along with the abundance of infected mosquitoes (Klempner et al. 2007), transmit to humans.

We conducted a field study in neighborhoods bordering water bodies within the San Juan Bay estuary system in June 2016, when confirmed Zika cases in Puerto Rico were approximately 672 per week and were rapidly increasing (Puerto Rico Department of Health 2016). In each neighborhood, we assessed the particulate nitrogen and carbon content of estuary water, leaf detritus, water from larval containers, *Ae. aegypti* larvae within containers, and local adult *Ae. aegypti* populations, to determine the degree to which environmental variability in nutrients is carried through to adult mosquitoes. Sampling locations were chosen to cover a range of variability in water quality, observed in periodic water quality monitoring data (SJBEP 2016), in order to maximize our potential to detect differences. Finally, we tested adults for ZIKV to determine whether adult nutrition affects

acquisition of Zika virus. By simultaneously collecting field data on water quality, leaf detritus, larval habitat, mosquito larvae, mosquito adults, and virus, our study is one of the first to attempt to connect environmental variability to the capacity of mosquito vectors to spread disease.

Study Area

The San Juan Bay Estuary (SJBE) is an interconnected system of channels and lagoons, many of which have been severely altered by human activity (Brandeis et al. 2014). The SJBE is the only tropical estuary in the US National Estuary Program, and is the most highly urbanized, with approximately 82% of the watershed developed. The remaining watershed is dominated by moist forest and wetlands, including the most extensive contiguous mangrove forest in Puerto Rico (Brandeis et al. 2014). We selected neighborhoods adjacent to six waterbodies: La Malaria channel, Martin Peña channel, Suarez channel, Torrecilla Lagoon, Piñones Lagoon, and Rio Piedras (Fig. 2). Waterbodies were chosen to reflect a range of water quality parameters, including salinity (0.17–34.03 PSS; SJBE 2016), nitrogen (0.23–35.6 Kjeldahl nitrogen mg/L; SJBE 2016), and carbon (0.122–7.475 TOC mg/L; SJBE 2016). Cumulative Zika cases up to June 2016 were comparable across the four municipios (i.e., county-equivalent areas) covered by the study area (Puerto Rico Department of Health 2016; Cataño municipio, 175 cases per 100,000 inhabitants; San Juan municipio, 293 cases per 100,000 inhabitants; Carolina municipio 156 cases per 100,000 inhabitants; Loiza municipio: 136 cases per 100,000 inhabitants).

We focused our most intensive sampling efforts on the Martin Peña neighborhood, with the five other waterbodies included for comparison. The Martin Peña neighborhood comprises eight distinct adjoining communities, but is referred to as a single neighborhood as the sampling unit for the purpose of this study. Decades of unregulated dumping and construction into the severely blocked Martin Peña Channel, along with deficient sewage infrastructure and direct discharges of untreated residential wastewater, has led to frequent flood events during which residents are exposed to polluted surface waters in their homes and roadways (Korfmacher et al. 2015). As many as 70% of residents report flooding in or near their home in a given year, with some residents reporting their homes flooded upwards of 20 times per year (Sheffield et al. 2014). Flood waters often reach heights greater than 30 cm, sufficient to cover accumulated trash from illegal dumping or other containers. The Martin Peña neighborhood experienced such a flood event two weeks prior to our June 2016 sampling. Sampled neighborhoods from other waterbodies were also within established flood zones (FEMA 2009; Fig. 2), though it is unknown whether they had experienced flooding recent to our field sampling.

Study Design

A sampling site within each neighborhood was defined by the placement of an adult mosquito trap within 50–150 m of the adjacent waterbody and any sampled larval container habitats within 150 m of that trap (Fig. 2). All trap locations were adjacent to water sources within the estuarine watershed, with similar vegetation and canopy cover. With the exception of a state forest location near Piñones Lagoon (PN1), all trap locations were within the San Juan metropolitan area in neighborhoods with dense housing. Across the six neighborhoods,

we placed a total of 12 adult mosquito traps, with between 1 and 5 traps placed per neighborhood. We searched for water-filled containers with larvae in the 150 m radial area surrounding each trap, with between 1 and 4 larval containers located and sampled per trap (Table 1). Container sampling was highly dependent on our ability to find containers in accessible areas (e.g., not in a private yard), and we cannot assume potential sources for adults were exhaustively sampled. However, adult *Ae. aegypti* typically disperse less than 150 m (Harrington et al. 2005), so larval containers at each sampling site were assumed to be a representative source of adult mosquitoes in that area. Sites were sampled on June 7 and 8, 2016, with approximately half of the sites sampled each day.

To collect adult mosquitoes, we used BG-Sentinel traps (Biogents AG, Regensburg, Germany), baited with a human scent lure (BG-Lure; Biogents AG). BG-Sentinel traps are effective at capturing host-seeking adult *Ae. aegypti* (Maciel-de-Freitas et al. 2006), which are likely to have taken a blood meal and thus been exposed to virus. A battery-operated fan prevents adults from escaping after they have entered the trap. Efforts were made to centrally locate each trap among paired sampled containers, dependent on the ability to place traps in a secure location, such as the gated yard of a homeowner, to minimize disturbance. Traps were left out for a 48-h period before adults were collected. One trap malfunctioned (LM1; Fig. 2) and therefore did not capture any adults. Collected adults were identified to sex and species, with 0–30 female *Ae. aegypti* collected per trap (Table 1). Only adult female *Ae. aegypti* were used for subsequent analysis. Adults were frozen and transported to the respective laboratories for nutrient and ZIKV analysis.

For each trap location, we collected three 50 mL samples of surface water from each waterbody, within the shortest direct distance of the approximate center of that site. Water samples were filtered on site through 2.5-cm glass fiber filters (Whatman GF/F), with filters immediately placed on ice and then in a drying oven within a few hours of collection each day. Because water samples are highly dynamic, varying daily with recent precipitation, runoff, or waterflow, we also collected three leaf samples from the dominant vegetation (i.e., typically mangrove) directly adjacent to the water sampling location as a long-term, more stable proxy of local waterbody nutrients. Leaves were placed in a drying oven within a few hours of collection. For a few locations (RP2, LM1, SU1), leaf or water samples could not be collected due to accessibility issues. All samples were dried at 65°C for approximately 48 h before being transported to the continental USA.

We similarly filtered three 50 mL water samples from any accessible larval container habitats that could be located at each sampling site. Containers included discarded plastic food containers, buckets, rubber boots, plant trays, and discarded vehicle tires, and had to contain live *Ae. aegypti* larvae to be included. Subsamples of approximately 5–10 *Ae. aegypti* larvae were collected from each container and placed on a filter in the drying oven at the end of each collection day. In addition to scattered containers, we also sampled water and larvae from tires from two tire shops within the Martin Peña neighborhoods (Table 1). Because tire shops experience frequent turnover, we treated them as separate sampling locations from scattered discarded containers which, based on visual inspection, likely spent a much longer time in the environment and had a greater likelihood of having received environmental inputs. For example, although used tires at the shop near MP3 (Tire 1; Fig. 2)

had been flooded with water approximately 0.5 meters high in the week prior to our study, they had already been removed for recycling, with the more recent tires that we sampled having not been exposed to flood water (personal communication with shop employee).

Nutrient Analysis

Filtered contents from waterbody and container water, leaf samples, larvae samples, and adults were analyzed for particulate nitrogen (N) and carbon (C) content. Due to low body weight, 5–10 larvae were analyzed as a single sample. After removing heads for ZIKV analysis, individual adult mosquitoes were analyzed separately.

We hypothesize that nutrient content (%N, %C, C:N) of larval container habitats and subsequent adult mosquitoes would be similar to nutrient content of adjacent waterbodies, either directly via flood water or indirectly through detritus, such that containers or adults from the same trap location are more similar to one another than those from other locations. Additionally, nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) stable isotopes were used to track the unique source locations of nutrients. Stable isotopes provide a record of assimilation, processing, and recycling of nutrients, such that samples collected from different locations can have unique isotopic signatures, particularly from anthropogenic sources such as fertilizer or sewage discharges (Oczkowski et al. 2009, 2011). Similar isotope values between waterbodies and larval containers or adults would provide further support that local waterbodies were sources for nutrients, above and beyond any similarities in nutrient content.

Carbon and nitrogen stable isotopes were measured using an Elementar Vario Micro elemental analyzer connected to a continuous-flow Isoprime 100 isotope ratio mass spectrometer (Elementar Americas, Mt. Laurel, NJ) at the Environmental Protection Agency in Narragansett, Rhode Island. Replicate analyses of isotopic standard reference materials USGS 40 ($\delta^{13}\text{C} = -26.39\%$; $\delta^{15}\text{N} = -4.52\%$) and USGS 41 ($\delta^{13}\text{C} = 37.63\%$; $\delta^{15}\text{N} = 47.57\%$) were used to normalize isotopic values of working standards (blue mussel homogenate) to the air ($\delta^{15}\text{N}$) and Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) scales (Paul et al. 2007). Isotope values are expressed in δ notation following the formula $\delta X (\%) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where X is ^{13}C or ^{15}N and R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ isotopic ratio, respectively. Working standards were analyzed after every 24 samples to monitor instrument performance and check data normalization. The precision of the laboratory standards was better than $\pm 0.3\%$ for C and N. A few samples with peaks less than 0.5 were excluded from analysis to improve accuracy, except where removal would result in the complete elimination of a sampling site (RP1). Analyses in which RP1 was included were consistent with those where it was removed.

The sample particulate N and C content was calculated from area/mass relationships, determined during stable isotope analysis, for replicate standards of known N and C (Oczkowski et al. 2009, 2011). The relationship between the N and C contents of the standards and their analytical peak areas were used to determine the N and C contents of the samples via the peak areas of the samples. For adult mosquitoes, larvae, and leaf detritus, sample N or C was divided by total sample mass to determine the %N or %C. For particulate

matter, N and C content was expressed in mg/L by dividing the mg of N and C by the volume of water filtered (usually 50 mL).

ZIKV Processing

To quantify the level of infection with ZIKV, heads from individual adult females were analyzed to assess disseminated virus rather than viruses that might be within a blood meal in the midgut. Total RNA in individual mosquito heads was isolated using TRI-reagent (Molecular Research Center, Inc., Cincinnati, OH, USA). Complementary DNA (cDNA) was synthesized using the iSCRIPT cDNA Synthesis Kit (Bio-Rad Laboratories, Inc.), and RT-qPCR assays were performed in a CFX96 Real-Time System (Bio-Rad Laboratories, Inc.) using iTaq Universal Probe Supermix (Bio-Rad Laboratories, Inc.). The RT-qPCR procedure was performed twice for each sample. Primers and a probe specific to the Envelope gene of ZIKV were designed and synthesized according to the previous work (Acharya et al. 2016). Due to methodological and cost restrictions, we were limited to testing two batches of 57 individual female adults for ZIKV, for a total of 114 tested adults. After 40 PCR cycles, zero or trace amounts of ZIKV near or below threshold detection limits were considered as zeros (< 1.2 ZIKV E copies/ng total RNA), with values greater than 18 ZIKV E copies/ng total RNA considered as true positives for Zika.

In anticipation of a second Zika outbreak in summer 2017, we repeated the adult mosquito trapping in June 2017 (Appendix A) with the intention of comparing the relationships between adult body nutrients and ZIKV from 2016 to 2017. However, by then, confirmed Zika cases in humans had dropped to less than ten per week across all of Puerto Rico and were continuing to decline (Puerto Rico Department of Health 2017). We detected no virus in trapped adult *Ae. aegypti* from June 2017 (Appendix A). Therefore, here we focus our analysis solely on June 2016 data.

Statistical Analysis

To assess potential linkages between environmental variability in nutrients and ZIKV in adult mosquitoes (Fig. 1), we first assessed whether there was significant natural spatial variability in nutrients from either estuary water or potential leaf detritus, in body nutrients of larval or adult mosquitoes, and in ZIKV concentration. Second, we used multiple regressions to explore whether nutrient variability and ZIKV concentration in mosquitoes could be explained by nutrient variability in the local environment.

We used nested Multivariate Analysis of Variance (MANOVA) to test if trap locations or neighborhoods differed significantly in nutrients, including N, C, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and the ratio of C to N, for waterbody water samples, leaf detritus samples, container water samples, larvae samples, or adult samples. Adult mass was included as an additional dependent variable along with nutrient variables in the adult MANOVA. Trap locations were nested within each neighborhood. If MANOVAs were significant, we followed up with univariate ANOVAs to determine which nutrient variables were most likely contributing to any significant differences. Due to unbalanced sampling design, with a variable number of replicates per location, we used Type II Sums of Squares to assess significance. The sampling units for each trap location were the individual 50 mL waterbody water samples

(1–3 per location), individual leaves (1–3 per location), the average of the replicate water samples from each container (1–5 per location), the combined larval value per container (1–5 per location), or individual adults (2–30 per location). Data were tested for normality and Box–Cox-transformed as needed to normalize data prior to analysis. Significant differences between pairs of trap locations or neighborhoods were assessed using Tukey-adjusted least squares means. MANOVAs were conducted in R (www.r-project.org) using the “stats” and “lsmeans” libraries.

To test whether ZIKV concentration differed significantly across locations or neighborhoods, we used general linear models (GLMs) with a Tweedie distribution because ZIKV concentration data were not normal due to a high frequency of zeroes in non-infected mosquitoes. The Tweedie distributions describe a family of distributions characterized by a power parameter, p , which includes the special cases of Poisson or gamma distribution for $P = 1$ or $P = 2$, respectively. For $1 < P < 2$, the distribution is a compound Poisson-gamma distribution, which allows for bimodal distributions where variables are described by different underlying processes, namely a discrete Poisson distribution (e.g., the number of mosquitoes positive for ZIKV) with a continuous gamma distribution (e.g., ZIKV concentration levels within positive mosquitoes). The Tweedie distribution is a good choice for bimodal or zero-inflated data that contain a large number of zeros (Shono 2008). We first estimated the power parameter, p , of the Tweedie function for the ZIKV concentration data using maximum likelihood and then used this fitted distribution, with a log-link power function, in the GLM. The sampling unit was individual adult mosquitoes (2–30 per location), with trap locations nested within neighborhoods. Analyses were conducted in R (www.r-project.org) using the package “tweedie” within “statmod.” Results were consistent with a standard logistic regression analysis on the fraction of Zika-positive adults per trap (GLM with a binomial distribution, not shown).

We used a series of multiple regressions to determine whether differences in container nutrients, larval nutrients, adult nutrients, adult mass, or ZIKV concentration could be explained by differences in the nutrient concentrations from potential nutrient sources. To minimize the number of possible explanatory variables under consideration, we limited fixed effects in each regression to the most proximate potential source for nutrients (Fig. 1): (1) waterbody nutrients or leaf detritus nutrients were considered as explanatory variables for container nutrient content, (2) container nutrients were considered as explanatory variables for larval nutrient content, (3) larval nutrients were considered as explanatory variables for adult nutrient content, (4) adult nutrient content was considered as explanatory variables for adult mass, and (5) adult nutrient content or mass was considered as explanatory variables for ZIKV concentration. Because trapped adults did not directly originate from sampled larvae, we also included the average container nutrients, leaf detrital nutrients, and waterbody nutrients within each neighborhood as potential predictors of adult nutrient content.

In addition to nutrient sources, we considered the influence of flooding and container availability (i.e., type and number) on the dependent variables in regressions. Sampled containers were categorized as tires, 5-gallon buckets, small miscellaneous containers (e.g., boots, food tubs), and large miscellaneous containers (e.g., boat, plastic bin). Because we

could not definitively know whether individual sampled containers had flooded recently, we instead used the Flood Hazard Composite Index (NOAA 2018) as a proxy for the likelihood of neighborhood flooding (Table 1). The Composite Index counts the number out of 10 flood hazard data sets, including FEMA flood zones, shallow coastal flooding, and storm surge, that intersect each pixel, which we then averaged to estimate a single value for a 150-m radius around each trap location. Because inputs of containers into nutrients are idiosyncratic and potential inputs from flooding would depend on waterbody nutrients, we did not expect that nutrient content would be significantly related to container type or flood risk (e.g., smaller containers have lower nitrogen; flooded sites have higher nitrogen). Indeed, preliminary analysis with these variables as fixed effects in regressions confirmed no significant relationships between container type or flood risk and nutrient content of containers, larvae, or adults. However, frequent flooding or container type could impact the variability in nutrient content across samples. For example, recently flooded containers may be similar to one another; small containers may be more variable than larger ones; adults emerging from sites with numerous container habitats may be more variable than adults originating from sites with few. Moreover, tires and recently flooded sites in Martin Peña were more heavily sampled than other sites or types of containers, contributing to heteroscedasticity in fitted models. Hence, we tested whether homogeneity of residuals significantly improved when model variance was allowed to vary with either container type, container count around each adult trapping site, or flood risk. Variance structure was modeled in R using “*varPower*” for continuous effects (flood risk, container count) and “*varIdent*” for categorical effects (i.e., container type).

Because there were multiple replicates (e.g., containers, adults per trap) within each sampling site, we accounted for repeated measures in regression analysis by treating trap location as a random effect. Replicates (e.g., waterbody water samples) were averaged to produce a single value to be paired with each sample (e.g., container) at each trap location. For sites MP3 and MP5, including nearby tire piles in container and larval averages as potential sources for adult nutrients did not change results (not shown). Locations with incomplete data (e.g., no leaf samples due to inaccessibility) were dropped from regressions. All variables were Box–Cox-transformed to adhere to assumptions of normality and minimize nonlinear relationships that can arise from non-normal data. Variables were then standardized (mean-centered and scaled by standard deviation) to facilitate the comparison of model coefficients for variables of different magnitude, such that mean relationships with each variable can be interpreted by setting other fixed effects in the fitted regression equation to their standardized mean of zero. Highly correlated variables (Pearson correlation coefficient, $r > 0.5$) were prohibited from simultaneously being allowed as predictor variables to prevent issues with collinearity (e.g., C or N with C:N ratio). Regressions were conducted in R using procedure “lme” within package “nlme” for nutrients and mass, assuming a normal distribution, and with procedure “glmer” within package “lme4” for ZIKV, assuming a Tweedie distribution. For regression analyses, we followed a basic mixed-effects modeling protocol (Zuur et al. 2009; Lima 2017): i) fit the full model with all fixed effects and random effect (location) using maximum likelihood (ML), ii) test for heteroscedasticity and the significance of variability weights (container type, container count, or flood risk) in improving homogeneity of residuals to optimize the variability

structure in subsequent models, iii) test the significance of fixed effects using ML estimation and likelihood ratio tests, and iv) estimate the final optimal model coefficients using restricted maximum likelihood (REML) estimation, which are unbiased compared to ML, but are meaningless for comparing fixed effects. Goodness of fit for fixed effects in the final model were calculated using “r.squaredGLMM” of the package “MuMIN” in R; regression lines and confidence intervals were plotted using “visreg.”

Results

Nutrient Availability at Trapping Locations

Trap locations and neighborhoods differed significantly in nutrients in both estuarine water and leaf detritus, establishing that there was a range of potential availability of nitrogen and carbon to larval containers from different trap locations and neighborhoods (MANOVA; Table 2; Fig. 3a, b). Likewise, nutrient content of larval container water also differed significantly among neighborhoods, although containers within a neighborhood were similar to one another (MANOVA; Table 2; Fig. 3c). Despite significant neighborhood differences in container water nutrients, *Ae. aegypti* larvae inhabiting those containers were not significantly different in nutrient content between neighborhoods or trap locations (MANOVA; Table 2; Fig. 3d). However, earlier instar larvae grown on mixed resources tend to be most similar in nutrient content, with differential effects of nutrient availability not appearing until later developmental stages (D. Yee, unpublished laboratory data for different life stages grown on mixed resources: first instars: variance in %N = 0.11; fourth instars: variance in %N = 0.50; adults: variance in %N = 2.5). Indeed, similar to container habitats, adult *Ae. aegypti* did differ significantly across neighborhoods in nitrogen and C:N ratios (MANOVA; Table 2; Fig. 3e). Adults collected from different trapping locations also differed significantly in mass, with the largest adults collected from Martin Peña (MP5), Piñones (PN1), and Torrecilla (TO1).

Water samples from the neighborhood adjacent to the Martin Peña channel, particularly MP4 and MP5, had the highest concentrations of nitrogen and carbon in particulate matter compared to other locations (Fig. 3a). Martin Peña sites were also among locations with the highest concentrations of nitrogen and carbon in leaf detritus samples. Container water in the Martin Peña neighborhood also was significantly higher in N and C than most other neighborhoods, and adults were among the highest in N (Fig. 3e). Rio Piedras and Suarez Channel neighborhoods also had high levels of N in adults, larvae, and nearby leaf detritus. Adults collected from Piñones and Torrecilla had the lowest %N values (Fig. 3e), comparable to lower values for nearby estuarine water, leaf samples, and containers (Fig. 3a, b, c).

Relationships Between Nutrient Content and Nutrient Sources

Particulate nitrogen and carbon content of larval container water was significantly related to the nitrogen and carbon content of leaf detritus at sampling locations (Table 3; Fig. 4a). Larval nutrient content also tended to increase with increasing nitrogen content of water from their natal container habitats (Table 3; Fig. 4b). Adult nitrogen content was significantly related to the mean nitrogen content of containers, as well as nitrogen content

of leaf detritus at the sampling location (Table 3; Fig. 4c). Differences in adult mass across locations were not significantly explained by adult nutrient content (Table 3).

Trap locations covered a range of stable isotope signatures ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) for both estuarine water and leaf detrital samples (Fig. 5a, b), with significant differences across locations and neighborhoods that could be used to track potential sources of inputs to containers (MANOVA; Table 2). However, we detected no corresponding significant differences in stable isotopes across neighborhoods or locations for container habitats, larvae, or adult *Ae. aegypti* (MANOVA; Table 2), although container water $\delta^{13}\text{C}$ was significantly positively correlated with nearby estuary $\delta^{13}\text{C}$ (Table 3). Within containers, larval stable isotopes were strongly correlated with the stable isotope signatures of their container water (Table 3; Fig. 5c, d), indicating that stable isotopes can effectively be used to link larval nutrient content to its most direct nutrient source. These differences did not appear to persist to adults, however, as stable isotopes in adults were not significantly related to those of potential estuary, leaf, container, or larval sources (Table 3).

In general, the explanatory power of regression models between nutrient content and nutrient sources did not depend on flood risk at the trap location, the number of sampled containers, or the variability in types of sampled containers (Table 3). There was significantly higher variability in nitrogen concentration in container water from tires than other types of containers, and accounting for this variance structure improved model fit between leaf and container nitrogen. Similarly, large containers from Piñones had significantly higher variability in $\delta^{13}\text{C}$. Variability in adult nutrient content tended to decrease with the number of sampled containers (%C, $\delta^{15}\text{N}$) and increase at locations with higher flood risk (%C, $\delta^{13}\text{C}$). Accounting for these higher variances, however, did not improve model fit with source nutrients (Table 3).

Zika Concentration in *Ae. aegypti*

In total, 130 adult *Ae. aegypti* were collected from 11 functioning traps at the 12 sampled trap locations (Table 1). Adult density per trap differed significantly across neighborhoods (Poisson GLM; Chisq = 48.74; $df = 4$; $P < 0.001$), with the Torrecilla trap TO1 having the highest density, followed by the traps in the Martin Peña neighborhood, and the Suárez channel traps having the lowest density per trap. Adult density per trap was somewhat higher the first sampling night than the second (mean of 13.2 adults per trap, first night; mean of 9.3 adults per trap, second night).

Eighteen of the 114 tested adult *Ae. aegypti* were infected with ZIKV in amounts ranging from 18 to 100 copies/ng total RNA, a rate of 15.7 infected (Table 1). Traps from San Juan municipio (MP, RP) had the lowest percentage of infected adult mosquitoes, about 9.7%, despite being the municipio with the highest density of reported human cases (293.2 per 100,000 people; Puerto Rico Department of Health 2016). Adult mosquitoes collected from Carolina municipio (SU) and Loiza municipio (PN, TO) were 40% or 24.3% positive for Zika, respectively, where the density of human cases was somewhat lower (Carolina: 156.9 cases per 100,000 persons; Loiza: 136.4 cases per 100,000 persons; Puerto Rico Department of Health 2016). No adults were collected from Cataño (LM) due to a trap malfunction.

ZIKV concentration in adult mosquitoes differed significantly across neighborhoods (Tweedie GLM; $df=4$, 104; F value = 27.67; $P < 0.001$) but were consistent across locations within neighborhoods (Tweedie GLM; $df=5$, 104; F value = 0.56; $P = 0.727$). Adult mosquitoes trapped adjacent to Rio Piedras, Torrecilla Lagoon, and Suárez Channel had significantly higher ZIKV concentration than individuals trapped near Martin Peña or Piñones Lagoon (Fig. 3f). Differences were both due to differences between neighborhoods in adults being positive or negative for zero (binomial GLM; $F = 19.4$; $P < 0.001$), as well as differences among locations in ZIKV concentration only within Zika-positive mosquitoes (normal GLM; $F = 3.80$; $P = 0.037$).

ZIKV concentration increased significantly with both adult C and N (Table 3; Fig. 4d). ZIKV concentration was also positively related to individual adult mass, but this variable did not explain significant variability when C and N were also included in the final model (Table 3). To confirm trap locations with Zika-negative mosquitoes were not dominating the observed relationships between C, N, and ZIKV, possibly due to an unmeasured location variable, we ran regressions again but limiting data to locations where Zika-positive mosquitoes were trapped. Regression results were consistent, with ZIKV concentration increasing significantly with both N and C (samples = 48; $2.95 + 0.17\%N$ Adult + $0.16\%C$ Adult; N: $P < 0.001$; C: $P < 0.001$).

Discussion

Although flooding events are widely perceived as creating aquatic breeding sites for mosquito larvae (Ahern et al. 2005; Pires and Gleiser 2010), the role of floods as a potential source of nutrients and the consequences for disease outbreaks is largely underdeveloped. Our study found significant differences across neighborhoods in the nutrient content of larval habitat containers and ZIKV in adult *Ae. aegypti*, which may in part reflect significant environmental variability in estuary water from flooding events and leaf detritus as potential inputs to containers.

Containers can be highly variable in a number of factors, including type, size, detritus inputs, or age, that contribute to high variability in nutrient content (e.g., Murrell et al. 2011; Yee et al. 2015a, b). Despite this, we found significant differences in container nutrient content among neighborhoods, which was significantly correlated with the nitrogen and carbon content of leaf detritus in the local area. Moreover, containers tended to have similar $\delta^{13}C$ signatures as waterbodies in the local area. The lack of a significant relationship between container water and estuary water nutrient content (N or C) broadly across sampling locations may not be too surprising given the dynamic nature of water quality with precipitation events, wind, and runoff. Furthermore, even containers right next to each other may or may not receive flood water, depending on their specific height, orientation, and other factors, and over time, flooded containers may begin to diverge from one another through differential evaporation or additional allochthonous inputs. In this sense, leaf detritus may simply be a better proxy than estuarine water for longer-term trends in local environmental inputs.

Martin Peña was the only neighborhood known to have flooded in the weeks prior to sampling, and known to experience frequent, periodic flooding. Containers from neighborhoods adjacent to the Martin Peña channel had higher nitrogen and carbon content than most other neighborhoods, consistent with the higher nitrogen content in water from the channel and in leaf detritus in the local environment. Moreover, adult *Ae. aegypti* collected from the Martin Peña neighborhood had higher body nitrogen content relative to neighborhoods adjacent to other waterbodies. Adult *Ae. aegypti* nutrient content in Martin Peña was also remarkably consistent from 2016 to 2017, compared to other neighborhoods, which were more variable over time (Appendix A). These data provide tantalizing evidence for linkages among inputs from the surrounding environment, containers, and mosquitoes produced from those environments.

Nutrient content of larval *Ae. aegypti* was tightly linked to the nutrient content of their container habitats, particularly $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Adult *Ae. aegypti* nitrogen content was also positively related to both the nitrogen content of typical containers in each sampling location, and the nitrogen content of leaf detritus in each area. The relatively small amount of variability explained may indicate other unmeasured environmental factors, such as animal detritus, which are influencing the availability of nutrients to larval mosquitoes (Kling et al. 2007). Moreover, our inability to exhaustively sample larval containers in each area, nor conclusively determine whether adults trapped in a particular location originated from the sampled larval containers, also likely contributes to unexplained variability. Indeed, “hidden” containers such as underground septic systems can provide a significant source of adult *Ae. aegypti* in Puerto Rico (Barrera et al. 2008). Furthermore, while larval body nutrients were closely linked to the current nutrient content of container water, adult body nitrogen may be more closely related to the nutrient content of container water 7–10 days prior when they were developing as larvae. More extensive sampling, including changes in container nutrients over time, is needed to more dynamically link environmental variability in nutrients to that of mosquitoes.

In addition to neighborhood differences in environmental and mosquito nutrient content, we also found neighborhood differences in ZIKV concentration. At the time of sampling, the neighborhoods adjacent to Rio Piedras, Torrecilla Lagoon, and Suárez channel had the highest levels of ZIKV concentration in adult *Ae. aegypti*, with no infected adults collected near Martin Peña channel or Piñones lagoon. Higher levels of nitrogen and carbon content in adult bodies tended to be associated with higher ZIKV concentration. Furthermore, the higher ZIKV concentrations could not merely be attributed to a body size effect (i.e., bigger mosquitoes take a greater blood meal and consume more virus from infected hosts) because %N and %C, scaled per unit body mass, explained greater variability than adult body size. Moreover, in our field data, adult mass was not significantly related to adult body nutrients. Nitrogen has generally been associated with increased severity of disease in other systems, often by providing abundant building blocks needed for pathogen replication, but underlying mechanisms and results are inconsistent depending on the specific form of nitrogen and immunity-related responses (Dordas 2008). Laboratory studies, particularly those discerning viral uptake, dissemination, and infectiousness, are needed to tease apart the underlying mechanisms by which nitrogen or carbon may be impacting viral concentrations in mosquito vectors.

Our results are consistent with previous laboratory studies that have found that larval nutrition, adult nutrition, and body size can affect the ability of mosquitoes to acquire dengue or Ross River virus (Nasci and Mitchell 1994; Alto et al. 2008b), and with modeling studies indicating that environmental variability in nitrogen may influence dengue cases (De Jesus Crespo et al. 2018). Our study, however, is one of the first field studies to identify a pathway by which environmental variability in potential nutrient inputs into container habitats can affect nutrient content in mosquito larvae and adults, leading to variable viral acquisition in adult mosquitoes.

It is important to note that higher levels of viral titer in individual adult mosquitoes do not necessarily confer higher rates of viral infection in humans. Our study focused on dissemination of the pathogen but not the interaction with vector density (Anderson and Rico-Hesse 2006, Klemperer et al. 2007). Indeed, two of the neighborhoods with the highest ZIKV concentrations, Rio Piedras and Suárez channel, also had the lowest densities of mosquitoes caught per trap. Additionally, the lack of Zika-positive mosquitoes from Martin Peña is somewhat surprising, given the relatively high nitrogen levels in adults at this location, as well as the high density of human cases in the San Juan municipio (Puerto Rico Department of Health 2016). Although our study focused on nutrient availability from the local environment, other factors can influence the probability that a vector acquires a pathogen, including host density, pathogen density in hosts, and climatic or behavioral factors that influence host-seeking or biting behavior (LaDeau et al. 2015). More extensive sampling would be needed to more accurately assess mosquito density, host density, biting behavior, and other factors across the different neighborhoods, to be able to rigorously understand the role environmental nutrient variability plays on vectorial capacity.

Social and infrastructural factors can also buffer or enhance the susceptibility of people to health outcomes (Myers et al. 2013; Oosterbroek et al. 2016). Age, housing density, and access to air-conditioning are all factors that can influence transmission of dengue virus to humans (Méndez-Lázaro et al. 2014; Barrera et al. 2011; De Jesus Crespo et al. 2018). Even less is known about the complex suite of ecological and social factors influencing Zika transmission (Thomas et al. 2017). Indeed, our sampling was a single snapshot at a time when the spread of Zika was accelerating through the San Juan metropolitan area and throughout Puerto Rico. It is possible that the neighborhood differences in ZIKV infection in adult mosquitoes reflect local acquisition patterns from the human population, such that certain neighborhoods simply had not been affected yet. However, because the neighborhoods where mosquitoes were strongly positive for ZIKV (Torrecilla, Rio Piedras, Suárez channel) are adjacent to the two neighborhoods where mosquitoes were negative for ZIKV (Martin Peña, Piñones), we did not see a strong spatial pattern (e.g., radiating from a source point of local human acquisition) that would support this. Furthermore, cumulative Zika cases by June 2016 were reasonably similar across the three municipalities covered by the study area (Puerto Rico Department of Health 2016). More extensive temporal sampling is needed to tease apart the suite of ecological and social factors affecting the spread of the virus, and what role environmental variability in water and detrital quality might play in that spread.

Our study provides preliminary evidence that environmental variability in nutrient inputs can influence the acquisition of virus by mosquito vectors. In addition to the potential influence on viral titer, variability in leaf detrital inputs has been demonstrated to influence survival, growth, and competition among mosquito larvae (Yee et al. 2007; Muturi et al. 2012). This suggests a mechanism by which urban greenspaces, in addition to temperature regulation or biological control (De Jesus Crespo et al. 2018), may influence vector-borne illnesses through detrital resource inputs. Moreover, flooding events, in addition to providing pools of short-term breeding habitat (Ahern et al. 2005; Pires and Gleiser 2010), can also provide a pulse of nutrients to existing larval containers depending on the water quality of nearby waterbodies. This suggests that management actions to reduce flooding and improve water quality should go hand-in-hand to help control spread of vector-borne diseases. As such, environmental restoration of ecosystem services in the urban landscape could be part of a sustainable solution to help reduce vector disease transmission by mitigating flooding events and regulating water quality (Carver et al. 2015; De Jesus Crespo et al. 2018).

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Appendix A

In June 2017, we sampled adult mosquito populations at many of the same sampling locations to compare to the June 2016 field collection (Table 4). As in 2016, adult mosquitoes were collected using BG-Sentinel traps (Biogents AG, Regensburg, Germany) baited with a human scent lure (BG-Lure; Biogents AG). Traps were left out for a 48-h period before adults were collected. Collected adults were identified to sex and species, with 0–32 female *Ae. aegypti* collected per trap (Table 4). Only adult female *Ae. aegypti* were used for subsequent analysis.

For the 2017 sampling, individual adult heads were preserved in 1 mL RNAlater (Ambion, Austin, TX, USA) within 6 h of field collection. Heads and bodies were then frozen for transport to the continental USA. Adult mosquitoes were processed for particulate nutrient content (%N, %C, C:N) and stable isotopes (d¹⁵N, d¹³C) as described in the main text. We used nested Multivariate Analysis of Variance (MANOVA) to test whether trap locations, neighborhoods, or years (2016 vs 2017) differed significantly in nutrients, including N, C, δ¹³C, δ¹⁵N, the ratio of C to N, or adult mass.

In addition to ZIKV, we extended the viral analysis to include chikungunya and dengue using the 2017 collected mosquitoes. Following the removal of the RNAlater by gentle pipetting, 200 μ L of DMEM media (Gibco, no glucose, no glutamine, no phenol red; Thermo Fisher Scientific, Waltham, MA) was added to each mosquito head in RNase-free tubes. Individual mosquito tissues were homogenized using sterile, RNase-free disposable tissue grinders, centrifuged for 5 min in the cold, and the supernatant was removed for subsequent RNA extraction. RNA extractions were performed immediately following tissue homogenization using the QIAamp Viral RNA kit (Qiagen, Germantown, MD) according to manufacturer's instructions with minor modifications by adding additional washes with Buffer AW1 and AW2 (Mbanzulu et al. 2017). The purified RNA was analyzed for RNA integrity and concentration using the BioAnalyzer RNA 6000 Nano Reagent kit (Agilent, Santa Clara, CA) and stored at -80°C . Real-time reverse transcription-PCR (RT-qPCR) was performed for dengue, chikungunya, and ZIKV using a multiplex viral kit (MyBioSource, San Diego, CA). All of the above procedures were performed aseptically using RNase-free materials.

In total, 144 adult female mosquitoes were collected in traps (Table 4). Of the 144 adults tested for virus, not a single individual was confirmed to have ZIKV, chikungunya, or dengue. Results were consistent with public health data that, in contrast to concerns of an anticipated second outbreak of Zika, rates of arboviral cases in Puerto Rico were quite low in June 2017, with less than 10 confirmed cases across all of Puerto Rico (Puerto Rico Department of Health 2017). Adult nutrient content differed significantly from 2016 to 2017, but the effect differed among neighborhoods (Neighborhood $\times$ Year; Table 5). Adult mosquitoes from Martin Pena were remarkably consistent in both %N and %C between 2016 and 2017, despite a general pattern of lower %C and higher %N in 2017 for most other neighborhoods. Adult mass tended to be lower in Martin Pena, consistently in both 2016 and 2017. There were no significant differences in stable isotopes across trap locations, neighborhoods, or years (Fig. 6).

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Figure 1.

Causal flow diagram illustrating how nitrogen and carbon inputs into mosquito larval containers through flooding events detrital inputs could influence viral load in adult mosquitoes and ultimately disease transmission to humans. Solid paths were tested with data; dashed paths were inferred.

Figure 2.

Water, larval habitats, and adult mosquitoes were sampled from FEMA flood zones (gray shaded areas) in and around San Juan, Puerto Rico. Mosquito trap locations (stars) and estuary sampling locations (solid circles) were sited within each of six sampled neighborhoods. Larval habitat containers were located and sampled within 150 meters of traps. Two tire piles (concentric circles) were also sampled for comparison to dispersed containers.

Figure 3.

Mean particulate carbon and nitrogen, $\pm$ SE, across trapping locations for, **a** estuary water samples (log-scale) and **b** leaf samples, and across neighborhoods for **c** larval containers, **d** larval *Ae. aegypti*, and **e** adult *Ae. aegypti*. Symbol sizes indicate mean individual adult mass in **e**. Mean ZIKV concentration, $\pm$ SE, per individual adult *Ae. aegypti* at each trap location is also given (**f**). Symbol shapes and colors visually distinguish different neighborhoods (e.g., MP black circles; PN dark gray triangles).

Figure 4.

Relationships between source nutrients and particulate carbon or nitrogen for **a** containers, **b** larval, or **c** adult *Ae. aegypti*, and **d** ZIKV concentration, as determined by multiple regressions (see Table 3). Adults from each trap were paired with the average leaf nitrogen value for that trapping location (**c**). For regressions with more than one significant fixed effect (**c**, **d**), mean regression lines were plotted by fixing the second variable in the fitted regression equation (Table 3) to its mean value for data either above (black lines; black circles) or below (gray lines; gray circles) the standardized mean of zero (representing 4.7 mg N/L container in **c** or 49.2% C adult in **d**). Gray ribbons around regression lines indicate 95% confidence intervals.

Figure 5.

Nitrogen and carbon stable isotope values, $\pm$ SE, in each neighborhood for estuary, leaf, and larval container samples (**a**, **b**), as well as for larvae within each container (**c**, **d**) and corresponding fitted regression lines (see Table 3). Gray ribbons around regression lines indicate 95% confidence intervals.

Figure 6. Mean particulate carbon and nitrogen values, $\pm$ SE, across neighborhoods for adult *Ae. Aegypti* in 2016 and 2017. Symbol sizes indicate mean individual adult mass.

Table 1.

Number of Estuary Water Samples and Mosquito Larvae Containers Sampled Corresponding to Each Trap Location, as Well as the Number of Adult *Aedes aegypti*.

Neighborhood	Trap location	Flood composite index	Estuary water samples	Leaf samples	Larval container samples	Female <i>Ae. aegypti</i> per trap	Fraction <i>Ae. aegypti</i> infected with ZIKV
<i>Martin Peña Channel</i>	MP1	3.25	3	4	1 Tire	18	0/18
	MP2	7.21	2 ^a	3	3 Tires, 1 bucket	13	0/13
	MP3	7.65	3	4	1 Tire, 1 bucket, 1 small tub	0	–
	MP4	6.14	3	3	1 Tire, 1 small tub	16 ^c	0/15
	MP5	2.50	3	4	2 Buckets	27 ^c	0/12
<i>Pinones Lagoon</i>	PN1	8.90	3	5	1 Large boat, 1 large bin	7	0/7
<i>Rio Piedras</i>	RP1	3.00	3 ^b	3	1 Tire	10	6/10
	RP2	1.03	0	0	0	4	1/4
<i>La Malaria channel</i>	LM1	3.18	3	0	1 Tire, 1 small boot, 1 small pan	<i>Trap malfunction</i>	
<i>Suarez channel</i>	SU1	2.48	1 ^a	0	1 small bin	2	1/2
<i>Torreçilla Lagoon</i>	SU2	3.00	3	4	0	3	1/3
	TO1	5.21	3	4	0	30	9/30
<i>Tire Pile 1</i>	–	7.71	–	–	5 Tires	–	–
<i>Tire Pile 2</i>	–	2.93	–	–	5 Tires	–	–

^a Additional samples with peak areas of < 0.5 nA were removed from analysis.

^b All samples had peak areas < 0.5 nA, but were included in analysis to prevent total loss of data.

^c Not all females were tested for virus due to methodological limitations.

MANOVA Results, Followed by Univariate Tests, Evaluating Whether Particulate Carbon and Nitrogen Variables Differed Among Trap Locations or Between Neighborhoods in San Juan, Puerto Rico, 2016.

Table 2.

Factor	Estuary water		Leaf sample		Larval container		Larval <i>Ae. aegypti</i>		Adult <i>Ae. aegypti</i>	
	df	Pillai	df	Pillai	df	Pillai	df	Pillai	df	Pillai
Trap location	5, 19	1.77 ***	4, 25	1.06 *	4, 18	1.08	4, 17	0.83	5, 102	0.45 *
Neighborhood	5, 19	2.05 ***	4, 25	1.97 ***	6, 18	1.86 *	6, 17	1.35	4, 102	0.46 **
	Variable	F value	Variable	F value	Variable	F value	Variable	F value	Variable	F value
Trap location	N mg/L	3.05 *	N%	0.16	N mg/L	0.46	N%	1.36	N%	0.48
Neighborhood		8.56 ***		4.22 ***		2.79 *		0.93		7.28 ***
Trap location	C mg/L	2.76 *	C%	3.65 *	C mg/L	0.44	C%	1.22	C%	1.69
Neighborhood		9.11 ***		27.69 ***		2.02		0.92		1.23
Trap location	$\delta^{15}\text{N}$	2.32 @	$\delta^{15}\text{N}$	6.48 **	$\delta^{15}\text{N}$	1.72	$\delta^{15}\text{N}$	1.19	$\delta^{15}\text{N}$	1.03
Neighborhood		2.48 @		8.02 ***		2.38 @		1.44		0.70
Trap location	$\delta^{13}\text{C}$	3.21 *	$\delta^{13}\text{C}$	0.49	$\delta^{13}\text{C}$	0.80	$\delta^{13}\text{C}$	0.75	$\delta^{13}\text{C}$	1.70
Neighborhood		2.77 *		4.30 **		1.01		0.62		0.78
Trap location	C:N	3.10 *	C:N	0.08	C:N	3.55 *	C:N	1.06	C:N	0.66
Neighborhood		9.02 ***		1.63		6.12 **		1.74		6.35 ***
Trap location		—	—	—	—	—	—	—	Mass	6.30 ***
Neighborhood		—	—	—	—	—	—	—		3.28 *

Significance indicated in bold, where

*** $P < 0.001$;

** 0.001 $P < 0.01$;

* 0.01 $P < 0.05$;

@ 0.05 $P < 0.1$.

Table 3.

Significant Coefficients from Regression Analyses Comparing Relationships Between Most Proximate Source Nitrogen or Carbon Variables on Container, Larval, or Adult *Ae. aegypti* Nitrogen or Carbon, Adult Mass, and ZIKV Concentration.

Variable	N	Variance structure		Intercept	Fixed-effects variables		Fixed effect R ²
		Flood	Container type		Estuary	Leaf	
N mg/L	15	NS	T>B>S>L ^{**}	-0.11 ^{NS}	NS	0.61 × %N^{**}	0.16
C mg/L	15	NS	NS	0.00 ^{NS}	NS	0.49 × %C[*]	0.23
δ ¹⁵ N	15	NS	NS	0.00 ^{NS}	NS	NS	0.00
δ ¹³ C	15	NS	L>S>T>B [*]	0.02 ^{NS}	0.36 × δ¹³C[*]	NS	0.25
Larvae							
		Flood	Container type	Container			
%N	28	NS	NS	0.06 ^{NS}	0.33 × mgN/L[@]		0.10
%C	28	NS	NS	0.05 ^{NS}	NS		0.00
δ ¹⁵ N	28	NS	NS	0.00 ^{NS}	0.34 × δ¹⁵N[@]		0.11
δ ¹³ C	28	NS	NS	0.04 ^{NS}	0.75 × δ¹³C^{***}		0.52
Adult							
		Flood	Container count	Estuary		Leaf	Larvae
%N	74	NS	NS	0.00 ^{NS}	NS	0.38 × %N^{***}	0.18
%C	74	NS	-0.37 ^{**}	0.05 ^{NS}	NS	NS	0.00
δ ¹⁵ N	74	0.38[*]	-0.34 ^{**}	0.02 ^{NS}	NS	NS	0.00
δ ¹³ C	74	0.45[*]	NS	-0.09 ^{NS}	NS	NS	0.00
Mass (mg)		Flood	Container count	Adult N		Adult C	Adult C/N
		NS	NS	-0.05 ^{NS}	NS	NS	-
		NS	NS	-0.05 ^{NS}	-	-	NS
ZIKV							
		Flood	Container count	Adult N		Adult C	Adult Mass
		NS	NS	-2.72 ^{NS}	0.19 × %N^{***}	0.14 × %C^{**}	NS
		NS	NS	-2.74 ^{NS}	-	-	0.16 × mg^{**}

Dashed lines (–) indicate predictor variables not included in multiple regressions due to high covariance with other factors. Model variables are transformed to adhere to normality and standardized. Significance indicated in bold, where

Containers were classified as tires (T), buckets (B), small containers (S), or large containers (L).

$P < 0.1$.

$P < 0.05$;

$P < 0.01$;

$P < 0.001$;

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Table 4.

Number of Adult Female *Ae. aegypti* Trapped and Processed in 2017, and Results of Viral Analysis.

Neighborhood	Trap location	Female <i>Ae. aegypti</i> per trap	<i>Ae. aegypti</i> infected with ZIKV	<i>Ae. aegypti</i> infected with chikungunya	<i>Ae. aegypti</i> infected with dengue
<i>Martin Peña Channel</i>	MP1	0	–	–	–
	MP2a	9	0	0	0
	MP2b	5	0	0	0
	MP3	7	0	0	0
	MP4a	4	0	0	0
	MP4b	4	0	0	0
<i>Pinones Lagoon</i>	MP5	0	–	–	–
	MP6a	7	0	0	0
	MP6b	0	–	–	–
	PN1	NS	NS	NS	NS
	RP1	6	0	0	0
	RP2	0	–	–	–
<i>La Malaria Channel</i>	LM1a	16	0	0	0
	LM1b	9	0	0	0
	SU1	7	0	0	0
	SU2a	2	0	0	0
<i>Suarez Channel</i>	SU2b	2	0	0	0
	SU2c	5	0	0	0
	SU2d	8	0	0	0
	TO1a	21	0	0	0
<i>Torrecilla Lagoon</i>	TO1b	32	0	0	0

Traps were placed at the same locations as mapped in Figure 2, with more than one trap placed in some locations (e.g., front yard and back yard; labeled a–d). One new location, MP6, situated roughly halfway between the two fire locations (Fig. 2), was sampled in 2017 but not 2016.

NS: Location not sampled in 2017.

Table 5.

MANOVA Results, Followed by Univariate Tests, Evaluating Whether Particulate Carbon and Nitrogen Variables Differed Among Trap Locations or Between Neighborhoods from 2016 to 2017 in San Juan, Puerto Rico.

Factor	Adult <i>Ae. aegypti</i>	
	df	Pillai
Trap location	15,224	0.47[@]
Neighborhood	6224	0.37^{***}
Year	1224	0.17^{***}
Neighborhood × Year	3224	0.16^{**}
Variable	F value	
Trap location	N%	1.87[*]
Neighborhood		5.1^{***}
Year		4.98[*]
Neighborhood × Year		5.46^{**}
Trap location	C%	1.82[*]
Neighborhood		3^{**}
Year		25.35^{***}
Neighborhood × Year		2.91[*]
Trap location	d15N	0.52
Neighborhood		1.46
Year		0.01
Neighborhood × Year		0.39
Trap location	d13C	1.14
Neighborhood		2.05[@]
Year		0.58
Neighborhood × Year		1.04
Trap location	C:N	0.71
Neighborhood		3.19^{**}
Year		20.51^{***}
Neighborhood × Year		5.8^{***}
Trap location	Mass	2.44^{**}
Neighborhood		1.67
Year		4.83[*]
Neighborhood × Year		1.1

Significance indicated in bold, where

^{***} $P < 0.001$;

^{**} 0.001 $P < 0.01$;

^{*} 0.01 $P < 0.05$;

[@] 0.05 $P < 0.1$.

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