FACTORS IMPACTING THE URBAN TRANSMISSION POTENTIAL OF MOSQUITO-BORNE ARBOVIRUSES IN NEW ORLEANS, LOUISIANA

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If you want to find the secrets of the universe, think in terms of energy, frequency and vibration.
- Nikola Tesla

Dans les champs de l'observation le hasard ne favorise que les esprits préparés.
In the fields of observation chance favors only the prepared mind.
- Louis Pasteur

. . . the road goes on forever, I've got one more silver dollar, But I'm not gonna let 'em catch me, no, Not gonna let 'em catch the Midnight Rider. . .
- Gregg Allman

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II. Abstract

Urban epidemics of mosquito-borne diseases are driven by a close association between mosquito vectors and artificial container habitats near residences. In New Orleans, Louisiana abundant populations of the mosquito species *Aedes (Stegomyia) aegypti* (Linnaeus) and *Aedes (Stegomyia) albopictus* (Skuse) (Diptera: Culicidae), are potentially capable of supporting local transmission of viruses such as dengue, chikungunya and Zika introduced by infected individuals. Container-inhabiting *Aedes* mosquito populations are focally abundant, unevenly distributed across the urban landscape. Accurate estimations of mosquito abundance are important in determining transmission potential and for focusing disease surveillance and control measures. Mosquito collection methods target specific life stages and each has inherit biases. This study characterized common and productive container habitats in residential neighborhoods, evaluated field techniques for measuring vector mosquito abundance, and evaluated mosquitoes for host bloodmeal sources. Knowledge, attitude and practice surveys were conducted in three urban neighborhoods to identify behavioral and residential predictors of potential *Aedes aegypti* and *Ae. albopictus* container habitats. Container habitats were systematically evaluated for immature mosquito abundance. Habitat abundance was compared between neighborhoods and for associations with recent precipitation. In a separate study, collections of adult mosquito were compared using various standard adult mosquito collection traps. Finally, molecular identification techniques were used to determine adult mosquito bloodmeal sources, to human feeding rates during two time periods. These studies will allow public health authorities to develop a comprehensive understanding of the local transmission risk of recently-emerging arboviruses such as Zika, a broad knowledge base of species behavior and can be used to produce tailored educational campaign targeting in advance of future epidemics.

III. Background and Significance

The mosquito species *Aedes* (*Stegomyia*) *aegypti* (L.) (Diptera: Culicidae) likely arrived in New Orleans and other port cities throughout the Eastern United States on trade ships sailing from West Africa between the fifteenth and eighteenth centuries (1). Between 1795 and 1905 major epidemics of yellow fever along the east coast and in southern states caused thousands of deaths including over 26,000 in New Orleans, Louisiana from 1839-1860. In 1905, an epidemic in the city was the last recorded in the United States (2-3). Essential in bringing the epidemic to a halt was the recognition of *Aedes aegypti* mosquitoes as vectors of disease and the importance of mosquito population habitat suppression (2). This species of mosquito lives alongside human dwellings, oviposits in household water containers, and will readily enter homes to feed frequently and preferentially on humans. Females require a blood meal for the development of eggs, and often take multiple blood meals for each batch, increasing the potential for virus transmission. Because of the global emergence of arboviruses including dengue, chikungunya and Zika, *Aedes aegypti* remains an important vector of human disease.

Various models have indicated a high likelihood of chikungunya and Zika virus introduction into New Orleans and subsequent local transmission potential (4-5). Autochthonous dengue transmission has been recently detected in Houston, Texas (6), Key West (7) and along the Texas, Mexico border (8-9). Local transmission of chikungunya and Zika viruses has occurred in Florida and Texas (10) following the return of infected travelers. The Gulf Coast of the Southern U.S. is particularly at risk for introduction of these arboviruses. The warm climate, high humidity and rainfall in these sub-tropical regions is beneficial to the mosquito lifecycle by positively impacting the availability of local mosquito habitat and population abundance. The area is also characterized by frequent travel including international air travel, cruise ship arrivals and in some areas, pedestrians arriving by rail, bus or vehicles. Frequent travel to areas where arboviral epidemics are presently occurring could result in travel-related introductions. Finally, local epidemics could originate from high human exposure to vectors. Contact between human and mosquito vectors is often modulated by socioeconomic factors and poverty has been associated with indicators of elevated exposure (11). Areas with high rates of poverty are often characterized by poor quality of infrastructure, lack of window screening, low rates of air conditioning usage, and an increased exposure to climate and environmental hazards (12). Large-scale epidemics are not expected however, as lifestyle and housing in U.S. will limit arboviral transmission (13).

Decision-making for vector and epidemic control strategies includes the biology and behavior of the mosquito vector; feeding behaviors, estimations of vector density/ abundance, survivorship and vector competence (14). The suppression of mosquito populations integrates physical, biological and chemical control activities including source reduction, the physical elimination of larval habitats (15).

The goal of this dissertation is to identify behavioral and residential predictors of the presence of artificial container habitats and immature mosquito abundance and to examine how knowledge and attitudes shape protective behaviors among New Orleans residents. Various estimates of vector abundance will be compared and mosquitoes will be evaluated to determine human-vector contact and therefore the potential for arboviral transmission.

IV. Literature Review

Early Aedes population surveillance and control efforts in New Orleans

Louisiana historically continued to suffer from heavy infestations of *Ae. aegypti* even following yellow fever control efforts. Surveys by Louisiana State Board of Health entomologists from 1929-1943 list *Ae. aegypti* as abundant in 50/64 parishes and common in the remainder of the state (16). Surveillance and mapping of infested areas in New Orleans was prepared by personnel from Malaria Control in War Areas in 1944. In 1945, a dengue epidemic was reported in St. James parish and *Ae. aegypti* was found at 100% of properties inspected but diminution of the species was beginning to observed statewide. Embarking on *Ae. aegypti* eradication program subsequent surveys were conducted by the Communicable Disease Center (CDC), Louisiana State Board of Health and Department of Defense (3). In New Orleans the percentage of water-holding containers infested with immature mosquitoes (larvae or pupae), or container indices, were as high as 10.5 in 1944, 8.8 in 1945, 7.4 in 1956 and then declined to 0 in 1958. In 1961 the last naturally-occurring population was documented in a shipping dock area by the Division of Foreign Quarantine.

The decline of urban *Ae. aegypti* in New Orleans was concurrent with increasing household use of persistent insecticides. In addition beginning in 1965, following the formation of the New Orleans Mosquito Control Board, area-wide applications of adulticides commenced. Initially focused on the management of salt-marsh mosquitoes, activities of the Board shifted when detectable *Ae. aegypti* populations reappeared in 1972 (17). At the time it was assumed *Ae. aegypti* populations were introduced annually through shipping activities, a genetic analysis of sub-populations supported a disappearance and re-infestion through multiple re-introductions (18). However, it is also likely that vector was not entirely absent from the city since adequate surveillance efforts were not in place to detect the species presence. In 1979, a survey of artificial habitats determined that infestation rates were considered adequate to support dengue transmission (19). Frequent containers containing immature mosquitoes included drink bottles, birdbaths, wheelbarrows, 5-gallon buckets, trash containers and tires. An average of 48.5 positive containers were found per city block, 43.7% of premises accessed were positive for mosquito larvae and a Breteau index (number positive containers per 100 premises inspected) of 85 was observed.

In 1985 an additional invasive container-inhabiting species, the Asian Tiger mosquito *Aedes albopictus* (Skuse), was discovered in Houston, Texas and was quickly identified in New Orleans by 1986. Within the first decade, the presence of this species was documented across 15 states expanding along major transportation routes, likely through the movement of scrap tires (20). In Florida, following the introduction of *Ae. albopictus* declines in *Ae. aegypti* populations were observed (21). Initial studies into the decline of *Ae. aegypti* examined cross-species mating effects. Although reproductive isolation exists between these two species (22), insemination occurs and competitive displacement of *Ae. aegypti* by *Ae. albopictus* may be impacted by mating interference; cross-insemination by males or satyrization (23-24). These impacts may play a role in the initial population decline and bidirectional cross-mating has been observed in natural populations, but at levels so low (<5%) that would not entirely explain the decline. A potential strategy to evade this interference is females producing offspring from multiple males or polyandry, this has been demonstrated in field-collected *Ae. aegypti* populations in New Orleans (25).

Following this initial population replacement, factors during other life stages may impact localized abundance. While eggs of both these species can survive drying for long periods of time, *Ae. aegypti* eggs are more desiccation-resistant and hot, dry climates favor their survivorship relative to *Ae. albopictus* (26). The eggs of *Ae. albopictus* can undergo diapause or overwinter, allowing them to tolerate cooler temperatures and occupy a greater geographic range (27)(Figure 1.1). Competition studies in the larval stage have demonstrated that larval *Ae. albopictus* have a strong competitive advantage over *Ae. aegypti*, especially in low-nutrient, natural resource environments (28). Seasonal fluctuations in environmental resources due to the changing weather patterns can increase larval competition for resources as well, and this has been observed in *Ae. albopictus* collections from tire piles in New Orleans (29). These competition factors can contribute to reduced adult mosquito fitness and habitat segregation. Urbanization in Florida is correlated with oviposition traps containing a greater number *Ae. aegypti* eggs and in rural, suburban, and vegetated urban areas had a greater number of *Ae. albopictus* eggs (30). This distribution is also present in New Orleans, the highest proportion of *Ae. agypti* eggs among ovitrap collections is in the areas of highest housing and population density, particularly Mid-City, Broadmoor (Uptown), Marigny and Bywater (31).

Measurements of Mosquito Abundance

Accurate estimations of mosquito vector abundance are important in determining transmission potential and to focus disease surveillance and control measures. Traditional measures of abundance include container indices, direct observations of water-holding containers for the presence of immature mosquito life stages to determine if *Aedes* population levels have reached densities high enough to put communities at risk of disease transmission (32). Several indicies are used:

House index (HI): percentage of houses infested with larvae and/or pupae

Container index (CI): percentage of water-holding containers inspected, infested with larvae or pupae

Breteau index (BI): number of positive containers per 100 houses inspected

Recent studies have demonstrated an association between container indicies and the prevalence of human dengue infections in Peru (33) and Cuba (34). The number of pupae/ person is considered a more reliable estimate for potential for dengue transmission in Puerto Rico (35). Since the flight range of this species is small, these indicies may serve to identify areas of high density or latent potential for mosquito population increase given suitable conditions. Factors that may impact development and emergence from larval environments include temperature, habitat and resource depletion in the container environment effect larval development and survival rates (36). Population development rates can be estimated using complex Dynamic Life Table Models which parameterize yearly temperature, seasonal temperature variation and numbers and types of habitat sites required to host local population of *Aedes aegypti* (37). These models consider all life stage survival and development rates (egg, larvae, pupae and adult) and adult female oviposition rates.

Direct observations and collections of adult mosquitoes is also used for population estimation and frequently to test for the presence of arboviruses. Historically, adult mosquitoes were collected using mechanical aspirators either by collecting those resting indoors or human landing using the observer to attract host-seeking mosquitoes, the abundance was expressed over a fixed period of time (mosquitoes per minute). This method worked well even in low-population settings but was time-consuming, needing to be performed systematically and potentially exposed collectors. These collections have been gradually replaced by stand-alone, battery-operated adult traps including the Centers for Disease Control and Prevention light trap (CDC LT) and BG Sentinel™ trap (BGS)(BioGents AG, Regensburg, Germany). Both of these traps target host-seeking mosquitoes attracted to specific environmental cues; the CDC-LT uses dry ice to produce CO_2 and a small light while the BGS trap utilizes a human scent lure cartridge, relying on air currents produced in the trap along with the contrast in color near the trap entrance. Because the presence of adult *Ae. aegypti* is highly variable across space and time, trap placement is especially important. More abundant collections were correlated with trap placement in shaded areas rather than in full sun (38) and in highly vegetated areas (39). The CDC-LT is a reliable tool for the collection of many mosquito species including *Culex* species for West Nile virus surveillance, however it does not specially target the collection of *Aedes aegypti* or *Ae. aelbopictus*. In New Jersey, BGS collections contained 3 times as many *Ae. albopictus* than CDC-LT collections (40), however, the BGS traps *Ae. albopictus* adult collections did not correlate with larval-based indices (41).

Finally, another collection method takes advantage of the behavior of *Aedes* to oviposit in artificial containers. Ovitraps are small black glass or plastic containers, containing water or an infusion of plant materials and utilizing a substrate (such as seed germination paper, felt or wood) to collect eggs (42). Ovitraps are placed at fixed sites, representative of the habitat types present in the community and the substrate and water in the traps is changed weekly to prevent adult mosquito emergence. These traps are relatively easy and inexpensive to use. Species presence can be described as the percentage of positive ovitraps (with eggs) at a set geographic location and may also be used to detect population fluctuations. A small number of ovitraps have been used to reliably estimate mosquito population abundance in urban neighborhoods (43).

Human-mosquito interactions, vector exposure and mosquito abundance factors

Southeastern U.S. cities like Jacksonville and New Orleans have a strong potential for travel-related virus introductions of imported arboviruses but due to somewhat cooler winters the seasonal abundance of adult *Ae. aegypti* modulates the potential risk of year-round local transmission (5) (Figure 1.2). In this study, *Ae. aegypti* potential abundance was estimated using a simulated adult cycle, the DyMSiM (Dynamic Mosquito Simulation Model) (44), the Skeeter Buster model (45), and meteorological data from the North American Land Data Assimilation Phase2. The model predicts meteorologically suitable conditions for moderate to high *Ae. aegypti* population abundance in New Orleans from May through November, and simulated adult *Ae. aegypti* abundance is similar to observed ovitrap accumulations (6). However, the generation of field data sets for vector abundance and spatial risk models based on vector populations may still be uninformative in the absence of knowledge of the permissiveness of homes, potential for human exposure and pathogen availability (46-47). Travel-related introductions of dengue, chikungunya and Zika may not be transferred if infected individuals are protected from mosquito exposure.

Vector-human contact is modulated by socioeconomic and behavioral factors. Evidence suggests that impoverished communities in the Southeastern U.S. are at higher risk of introduction of infectious diseases (11) as compared with other communities since the warm climate, high humidity and rainfall support vector populations and the high rates of urban poverty increase potential exposure and limit healthcare access. Areas with high rates of poverty are often characterized by poor quality of infrastructure, lack of window screening, low rates of air conditioning usage, and an increased exposure to climate and environmental hazards (12). Mosquito abundance has been found to be associated with socio-economic level; in Baltimore and Washington D.C. *Aedes* pupae were more likely to be sampled in below median income neighborhoods (32). However, these containers were more likely to be associated with resident-occupied properties rather than abandoned properties and lots. While having more potential water-holding containers associated with areas of urban decay, they were found to be mostly dry, indicating that perhaps these non-managed container habitats are impacted more by seasonal fluctuations of rainfall, as opposed to those within residential environments.

The artificial container habitats of *Ae.aegypti* are frequently small (17), the bottle cap (or teaspoon) is commonly referred to as the smallest habitat utilized where the life cycle is able to be completed before complete evaporation. A study in New Jersey, simulated predicted evaporation rates of a bottle cap using a maximum of 2.8 hours exposed to sunlight at temperatures of 19 Celsius, average fall temperatures in New Jersey (48). This was compared to temperature-dependent developmental rates for *Aedes albopictus* and *Ae. aegypti*. (Figure 1.3). The model suggests that under minimal direct sunlight exposure, both *Ae. aegypti* and *Ae. albopictus* could develop within a bottle cap before complete evaporation. Additional field studies in New Jersey demonstrated that in sampled containers the effects of evaporation were increased larval density, and decreased developmental rates. In a mid-Western field trial of tire habitats, high temperature and drying were also associated with a greater per capita mortality rate. The tire water volume fluctuated between 25% and 90% of maximum volume (due to precipitation) and drying time in autumn was nearly twice that of summer (49). The study found significant temperature, precipitation, and temperature-precipitation effects for adult production.

The rate of evaporation of water depends on the water temperature, air temperature, air humidity and air velocity above the water surface (surface area). The evaporation rate can be lowered by reducing the size (surface area), decreasing water or air temperature or increasing the relative humidity. This helps to explain the frequency of small containers as mosquito habitats; the small surface areas influences the rate of evaporation in these environments. While the Southeastern U.S. experiences much higher average temperatures than the studies in New Jersey, additional factors such as humidity or heavy vegetation which produces shade could mitigate and slow down water evaporation rates. In the deserts of Arizona along the U.S. Mexico border, *Ae. aegypti* was positively associated with highly vegetated areas (50) but its presence was highly variable across space and time. Higher temperatures accelerate the mosquito life cycle, with certain temperature limits (36) but survival has been observed up to 35C (95F) (51). Summer and autumn temperatures in New Orleans frequently rise above 32C (89.6F), allowing the life cycle (from egg to emergent adult) to be completed in a 7 day period (Figure 1.3) and faster than the rate of evaporation in the smallest environment.

It is possible that even in cooler months, despite lower vector abundance, human exposure could be elevated, as residents change their behaviors; abstain from air conditioning usage instead relying on keeping doors and windows open or engage more frequently in outdoor activities. Reduced frequency of air conditioning usage is also exhibited among persons living in public housing in Florida (52), which may play a role in increasing risk of arbovirus transmission. A randomized population-based evaluation of knowledge, attitudes, and behaviors toward dengue was conducted in Key West, Florida following an outbreak there in 2009-2010.

Residents of public housing recalled fewer outreach materials, were less likely to correctly identify how dengue transmission occurs, where mosquitoes lay their eggs, and were less likely to perform dengue prevention practices such as removing standing water.

Vector biology and blood-feeding behaviors

Vector abundance and ability to make contact with human hosts are important components of transmission potential. A tool to determine contact rates of vector mosquitoes and human hosts is through bloodmeal identification. One method uses a multiplexed polymerase chain reaction (PCR), to target mitochondrial cytochrome b (Cytb) fragments from mammalian hosts (53). Using this method in Thailand, bloodmeal sources were frequently identified as human among *Ae. aegypti* (99%) and *Ae. albopictus* (100%) adults collected in homes and vegetation (54). Harrington postulated a selective advantage for *Ae aegypti*'s frequent feeding on human blood; the unique isoleucine concentration of human blood is associated with increases in fitness and synthesis of energy reserves (55). Longitudinal studies of in Thailand and Puerto Rico estimate on average *Ae. aegypti* took 0.76 and 0.63 human bloodmeals per day, respectively (56) which aids in the potential for arbovirus transmission. Studies also found high rates of human bloodmeal sources in *Ae. albopictus*, but often other animals as well. In Brazil *Ae. albopictus* bloodmeal sources were frequently determined to be from humans and cattle (57), and in Hawaii humans, dogs and cattle (58). In Rome Province, Italy, human blood index varied from 79-96% in urban and 23-55% rural areas, in rural areas, horses and bovines were more common than humans (59). In New Jersey, *Ae. albopictus* blood meals were less often derived from humans (58.2%) but were also associated with domesticated pets (23.0% cats and 14.6% dogs) (60). It was also found conversely that *Ae. albopictus* fed from humans significantly more often in suburban than in urban areas while cat-derived blood meals were greater in urban habitats. Identifying host bloodmeal origin and its relationship to host availability can assist in the understanding of the potential role *Ae. aegypti* and *Ae. albopictus* may have in arbovirus transmission to humans in New Orleans.

Transmission potential is also dependent on vector competence (vector efficiency), the ability of the insect to become infected and transmit the pathogen. In addition to extrinsic factors such as human contact rates and mosquito population density, intrinsic factors include genetic (inherited) traits, and ability for the mosquito to become infected after ingestion of the infective bloodmeal (61). A study of chikungunya virus transmission demonstrated a strong three-way combination of mosquito population (genetic variability), virus strain and ambient temperature (62). Transmission events are a complex interplay between vectors, pathogens and suitable environmental conditions.

Mosquito Control Efforts

The local abundance of mosquitoes is also impacted by the presence of organized mosquito control programs. These programs often use an integrated mosquito management (IMM) approach; conducting surveillance for mosquito vector species, using the most appropriate control for the situation, and evaluating the effectiveness of implemented control methods on vector populations (63). Control can be physical such as source reduction (eliminating, removing or modifying larval habitats), biological (use of predators or pathogens) or chemical (use of EPA-approved insecticides). These programs also utilize education and community outreach, informing those at risk to adopt personal protective measures and perform source reduction. The New Orleans Mosquito & Termite Board actively engages in vector mosquito surveillance (including adult collection of *Aedes* mosquitoes using BGS traps), public education, community outreach and container source reduction (such as the removal of tires) and area-wide application of insecticides.

Study area

The U.S. Census 2010 data estimates the population of the Greater New Orleans area as 1,262,888. Within Orleans Parish, which encompasses the City of New Orleans, the population is 435,536. The City is divided into planning districts (Figure 1.4), and within those further subdivided into neighborhoods (64). Following the devastating impacts of Hurricane Katrina and subsequent levee breaches of 2005, greater than half (40 of 72 neighborhoods) have recovered over 90 percent of the population in 2016, then they had before the levees failed (Figure 1.5). Housing in New Orleans is frequently characterized by neighborhoods containing historic architecture constructed over a period spanning almost three hundred years. Nearly half of the homes were constructed prior to World War II (65). Specific grid collection areas are in the Bywater, Mid-City and East Carrollton (Uptown) neighborhoods (Figure 1.5). The Mid-City neighborhood is much larger in size, population and average number of household members and has a higher proportion of renters than the other two neighborhoods (Table 1.1).

V. Research Questions

Some previous surveys have found an association between mosquito abundance, substandard housing and socioeconomic status. Abundance of *Aedes* species from container assessments and adult mosquito collections, will be accessed for associations with knowledge, attitude and practices (KAP) of residents and residential characteristics within three distinct urban New Orleans neighborhoods.

<u>Specific Aim 1:</u> Identify behavioral and residential predictors of immature and adult *Aedes* mosquito abundance.

- Hypothesis 1A: Protective residential behaviors will be associated with lower mosquito habitat abundance.
- Hypothesis 1B: Container indices will be similar between neighborhoods and associated with periods of recent precipitation.
- Hypothesis 1C: Water-holding and mosquito positive containers are more likely to be filled naturally by rainfall events (precipitation) than managed and filled by residents.

Accurate estimations of mosquito vector abundance are important in determining transmission potential and to focus disease surveillance and control measures. *Aedes* mosquito populations are not evenly distributed across the urban landscape, and each collection method targets specific life stages and has inherit biases.

Specific Aim 2: To explore variability between different methods of estimation of *Aedes* population abundance.

- Hypothesis 2A: The BG Sentinel trap will more frequently and consistently collect *Aedes aegypti* and *Ae. albopictus* than the CDC light trap.
- Hypothesis 2B: Ovitrap collections were more sensitive of *Ae. aegypti* and *Ae. albopictus* presence than adult mosquito collection methods but were poor indicators of mosquito abundance.

Vector abundance is only one component of transmission potential, local *Ae. aegypti* and *Ae. albopictus* populations must also be competent vectors for the arbovirus and have frequent contact with human hosts.

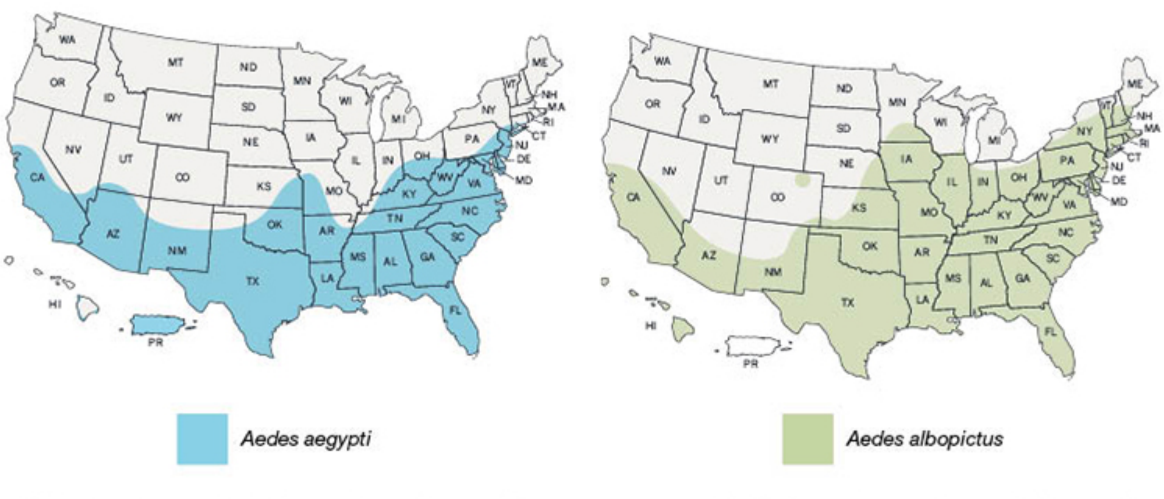
Specific Aim 3: To describe impact of *Ae. aegypti* vector host-seeking behavior on the local transmission potential for Zika virus in New Orleans, LA.

- Hypothesis 3A: Field-collected *Ae. aegypti* will demonstrate a low rate of human feeding and frequently feed on non-human mammalian hosts.

- Hypotheses 3B: Human feeding rates will have no association with population density.

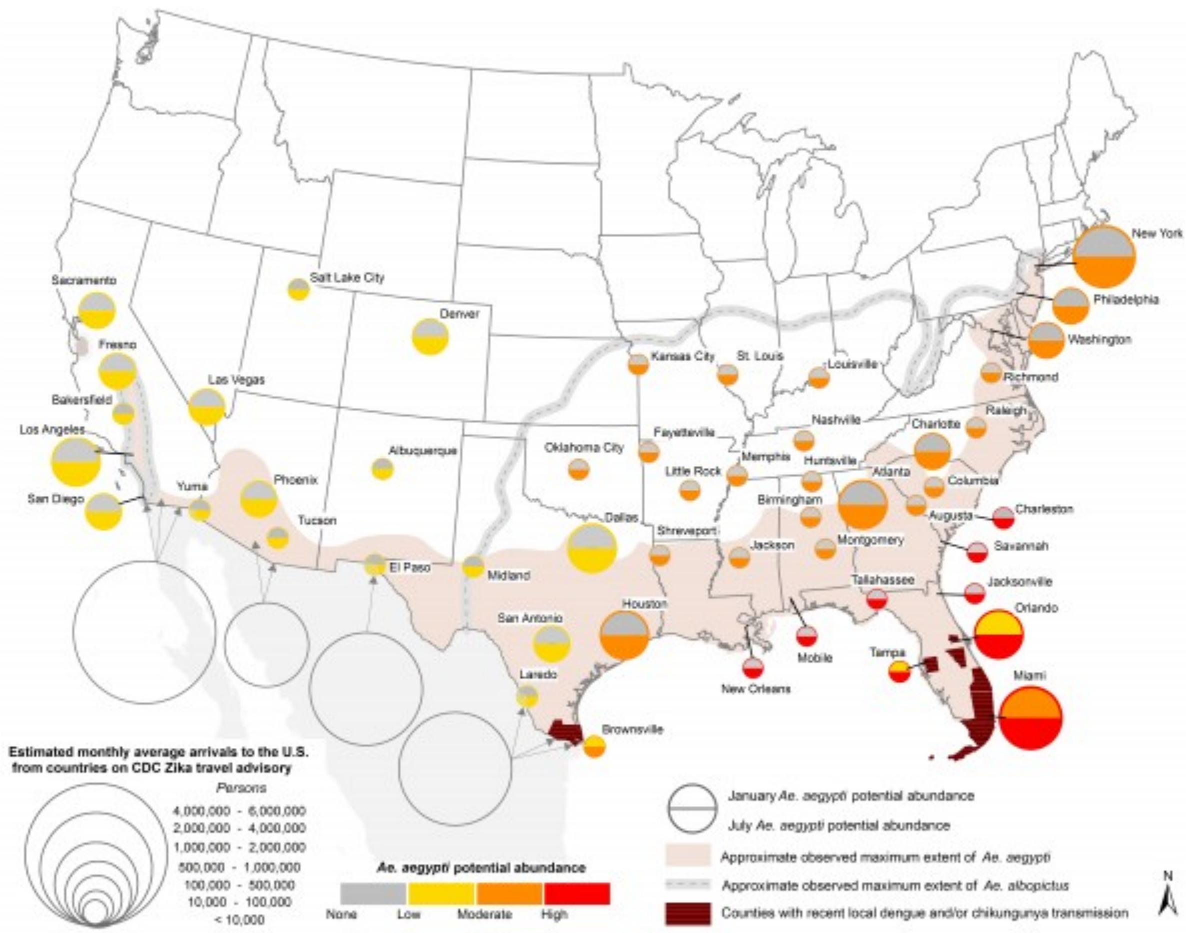
Figures and Tables

Figure 1.1 Estimated range of Aedes albopictus and Aedes aegypti in the United States, 2016.



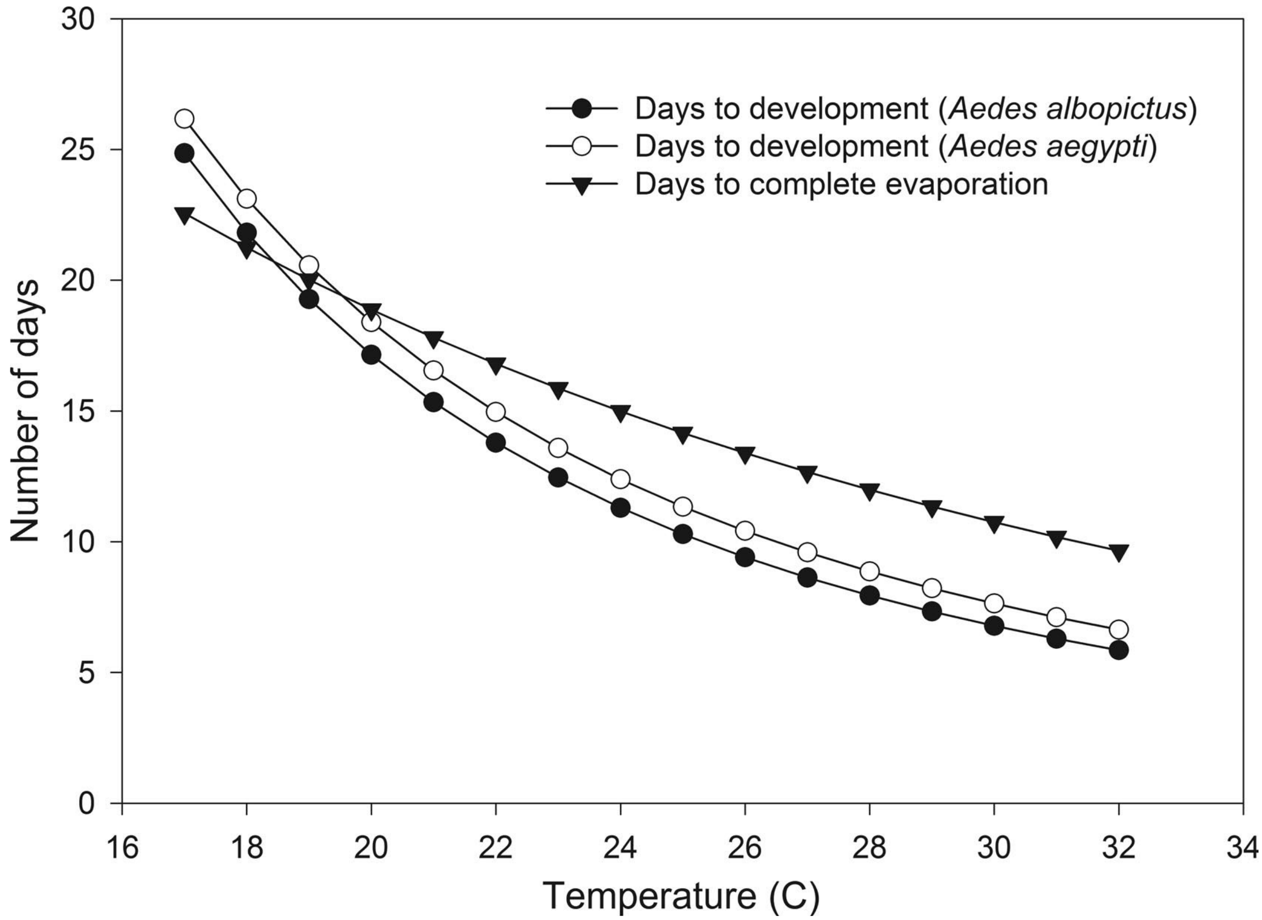
Source: Centers for Disease Control and Prevention, 2016.

Figure 1.2 United States map showing 1) *Ae. aegypti* potential abundance for Jan/July (colored circles), 2) approximate maximum known range of *Ae. aegypti* (shaded regions) and *Ae. albopictus* (gray dashed lines), and 3) monthly average number arrivals to the U.S. by air and land from countries on the CDC Zika travel advisory.



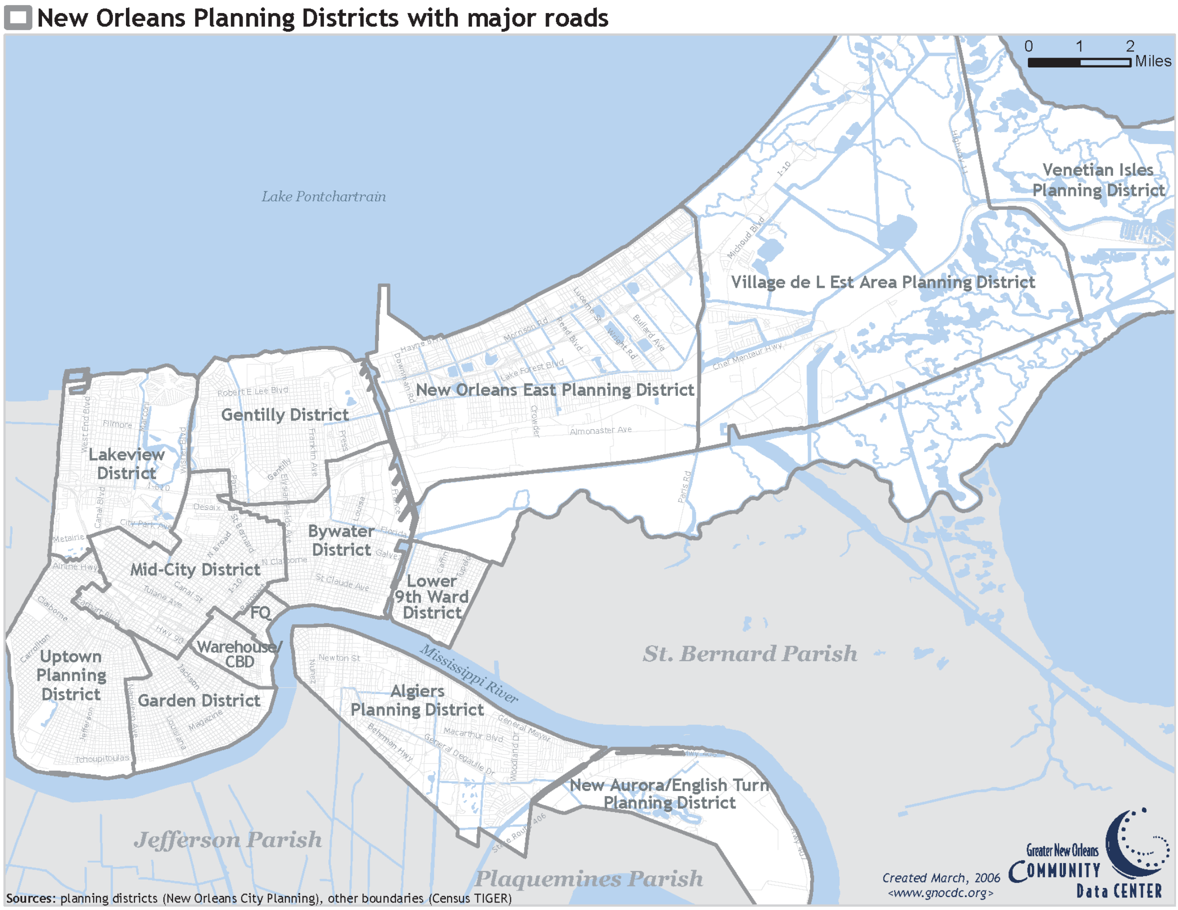
Source: Monaghan AJ, Morin CW, Steinhoff DF, Wilhelmi O, Hayden M, Quattrochi DA, Reiskind M, Lloyd AL, Smith K, Schmidt CA, Scalf PE, Ernst K. On the Seasonal Occurrence and Abundance of the Zika Virus Vector Mosquito Aedes Aegypti in the Contiguous United States. PLOS Currents Outbreaks, 2016.

Figure 1.3 Predicted evaporation rates of a bottle cap, commonly referred to as the smallest habitat utilized by container-dwelling mosquitoes, using a maximum of 2.8 hours exposed to sunlight at temperatures 19 Celsius (average fall temperatures in New Jersey) and temperature-dependent developmental rates (days) for *Aedes albopictus* and *Ae. aegypti*.



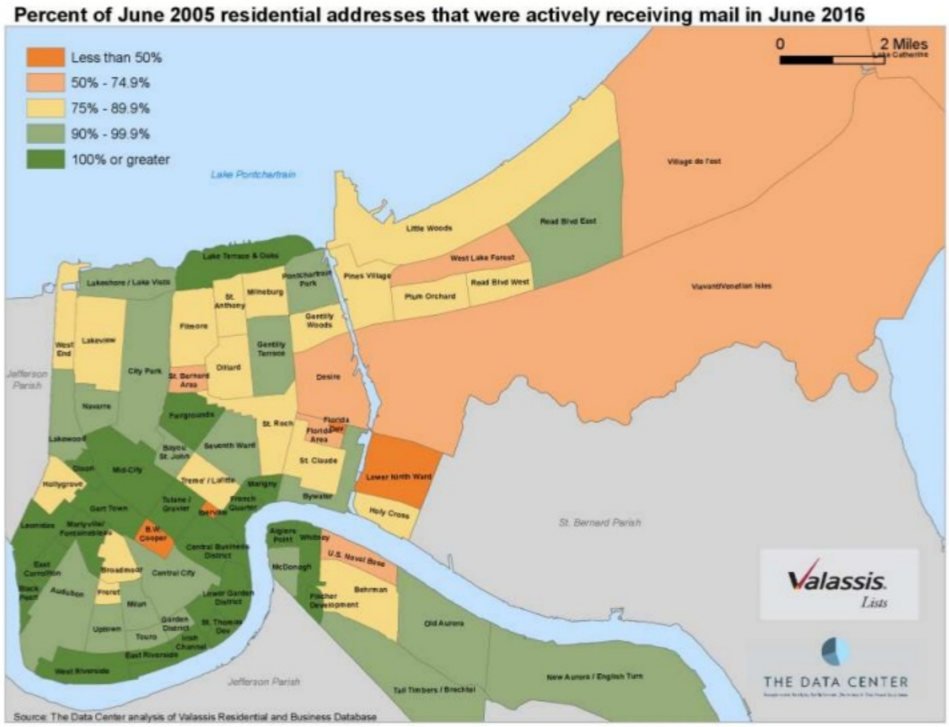
Source: Bartlett-Healy, K, S Healy & GC Hamilton. 2011. A Model to Predict Evaporation Rates in Habitats Used by Container-Dwelling Mosquitoes. Journal of Medical Entomology; 48(3): 712-716.

Figure 1.4 City of New Orleans map by Planning Districts



Source: The New Orleans Data Center, 2006.

Figure 1.5 Estimated percent occupied residences using active mail delivery in the City of New Orleans neighborhoods, 2016.



Source: The New Orleans Data Center, 2016.

Table 1.1 Demographic information in selected neighborhoods in New Orleans, data derived from U.S. Census 2010 estimates

Neighborhood	Population	Households	Household Average	Female	Male	Owner	Renter
East Carrollton	4,253	2,084	2.04	49.9%	50.1%	39.1%	60.9%
Mid-City	14,633	5,258	2.78	40.6%	59.4%	24.0%	76.0%
Bywater	6,820	2,713	2.51	51.8%	48.2%	46.5%	53.5%
Average	8,569	3,352	2.45	47.4%	52.6%	36.5%	63.5%

Source: The New Orleans Data Center, 2016.

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VI. Methods and Materials

Knowledge, attitude, and practices questionnaires and environmental surveys

Survey materials were submitted for expedited review and approved by the Tulane University Biomedical Institutional Review Board (IRB) (Project Title: [782121-2] Arbovirus KAP and Exposure Survey, Principal Investigator: Sarah Michaels, Review Type: Expedited Review, Approval Date: October 21, 2015, Continuing Review/Progress Report: October 21, 2016, Current Status: Open for Enrollment). In place of written consent, a study flyer was created (Appendix A) and verbal consent was accepted. Additional study materials include an interviewer-administered Questionnaire (Appendix B) of household demographics, behavioral practices, recent medical history and knowledge and attitudes regarding mosquitoes and arboviruses, and an Environmental Inspection (Appendix C) to record observations regarding the home and yard and assess the property for any water-holding containers.

The administration of the questionnaire lasted approximately 10-15 minutes. Either concurrently or afterwards, the research team conducted an environmental survey of the property, identifying potential mosquito habitats. Descriptions of water-holding containers were recorded and if immature mosquitoes were present, samples were collected. Container descriptions recorded included the container size, material (plastic, metal, ceramic), color and a general brief categorical description (plant saucer, bucket, pet dishes, bird bath, etc.). The observation of the presence of mosquitoes including presence of larvae, pupae and pupal exuvia (indicating pupal emergence) was also recorded for each container. Immature mosquito samples were removed from the container using a turkey baster (ECKO, Rosemont, IL) and were placed in Whirl-pak water collection bags (Nasco, Fort Atkinson, WI) for transport. When samples were returned to the insectary, they were transferred to larval rearing chambers (BioQuip, Rancho Dominguez, CA), and reared to adulthood for identification (80F, day/ night cycle: 18hrs/ 6 hrs). On a subset of properties and if the participants agreed, the study team would additionally place mosquito collection traps (BG Sentinel, BGS) on the property overnight. The study team returned the following day to collect the adult specimens and trap. Their removal only takes a few minutes and did not add any additional effort by the study participants. Mosquito adults were identified to species using the Louisiana Mosquito Control Training Manual.

Collection grid areas were established in the New Orleans neighborhoods of Bywater, Mid-City and Uptown, areas with high population density and abundant vector populations. The specific blocks selected were based on previous work in the area, presence of abundant artificial container habitats such as tire shops and cemeteries, resident-reported mosquito activity and safety/ accessibility. These neighborhoods are comparable in housing type and presence of single and multifamily residences, however the Mid-City neighborhood is much larger in size, population and average number of household members and has a higher proportion of renters than the other two neighborhoods. A specific area within Mid-City was be targeted. Based on the population in these selected areas of the city (1) and given a 95% confidence interval, an adequate sample size was determined to be approximately 350 surveys.

Each occupied residence within a specific square city block of a grid area was visited in a systematic fashion, working through 1 block at a time in attempt to make contact with residents on multiple days and times. If the resident was not at home, a door hanger was left to allow them to indicate willingness to participate or to refuse. The doorhangers were placed back on the door by the resident to schedule a time for a follow-up visit or allow them to contact the study staff by phone or email. Residents were able to enroll in either or both the questionnaire and environmental inspection. If multiple separate households existed within the same property, only one was enrolled in the study and the shared outdoor area was surveyed. Participant household addresses were recorded but the information was kept separate from questionnaire responses as instructed by the Tulane IRB.

Surveys were collected from October-December in 2015 and 2016. Survey data was entered in Microsoft Excel (2013) and analyzed by SAS software Version 9.3 (SAS Institute Inc., Cary, NC, USA) for descriptive statistics, chi-square analysis and ANOVA with a pairwise t-test and STATA 14.2 (Stata Corporation, College Station, TX) for logistic regression. Daily precipitation and temperature dataset was obtained from the NOAA National Climatic Data Center (Global Historical Climatology Network - Version 3).

Mosquito collections

Adult mosquito collections were performed using traps which target the collection of host-seeking females. These traps included the Centers for Disease Control & Prevention light trap (CDC-LT) baited with dry ice (CO_2) and the BG-Sentinel Trap (BGS - Biogents, Regensburg, Germany) baited with the BG-Lure (ammonia, lactic and caproic acid). Trap locations were identified with stable large numbers of *Aedes aegypti* and *Ae. albopictus*, in a transect along the Mississippi River and in the interior section of the city, referred to as Mid-City. Traps were placed overnight in shaded and vegetated areas at each site from late afternoon through the following morning. Trap positions were approximately 50 feet apart and positions were changed weekly. Mosquitoes collected were held at -20°C until identification. All samples were identified to species using the Louisiana Mosquito Control Training Manual. At the same site, ovitraps collections were performed to assess egg populations. These ovitraps were small black plastic cups lined with seed germination paper and filled with tap water. Ovitraps were placed in areas of vegetation, and reset weekly by removing and replacing water and paper. Following removal, the seed germination paper was dried for 24 hours and then hatched in dechlorinated tap water in larval rearing trays (BioQuip, Rancho Dominguez, CA) at standard insectary conditions (80F, day/ night cycle: 18hrs/ 6 hrs). Specimens were reared to 3^{rd} – 4^{th} instar larvae or adulthood for identification. Data was managed and summary statistics were conducted in MS Excel (2013), data was analyzed by SAS software Version 9.3 (SAS Institute Inc., Cary, NC, USA).

Laboratory bloodmeal analysis

This study contains two collection periods – August-October 2006 and May-September 2016. The 2006 adult mosquito collections were made with diurnal dry-ice baited CDC miniature light traps (CDC-LT) and Nasci aspirators (NA) in areas moderately to heavily flooded. In 2016, surveillance was conducted using BG-Sentinel Traps Version 2 (Biogents, Regensburg, Germany) (BGS2) in combination with the BG-Lure cartridge at 37 sites around the city of New Orleans between May 27th and September 30th, 2016. Site locations were based on: (1) travel-related Zika cases (2) areas with historically abundant *Aedes aegypti* populations and (3) ports and areas frequented by tourists. Traps were placed overnight, afternoon through the following morning weekly. Collected mosquitoes were held at -20°C until identification. All samples were separated and recorded by species, sex, date, and location.

Visibly blood-engorged mosquitoes separated from other collections and analyzed individually. Abdomens were removed and DNA was extracted using the QIAGEN DNA mini-kit (QIAGEN Inc., Valencia, CA), following manufacturer's instructions for extracting total DNA from insects. Extracted DNA was quantified the using the NanoDrop Spectrophotomer

(Thermo Fisher Scientific, Wilmington, DE). A PCR was conducted on the extracted DNA using a Taq PCR core kit, per manufacturer's instructions (QIAGEN Inc., Valencia, CA). The multiplexed primer sequences were Human 741F, UNFOR403, and UNREV1025, based on mitochondrial cytochrome b found in mammals (2). Positive female *Ae. aegypti* controls were selected from colony material and fed with human donor blood or bovine blood. Samples were visualized on a 1% agarose gel. Those that were then confirmed mammal positive and human negative will be sent for DNA sequencing (Tulane Sequencing Services, New Orleans, LA) and resulting sequences are to be identified via NCBI BLAST.

Limitations

It is likely that the container indices will be strongly correlated with the time at which they are observed, making it difficult to draw inter-neighborhood comparisons over time and potentially to relate these observations to household characteristics. Fluctuations in water-holding container abundance and presence of immature mosquitoes is strongly related to weather (including precipitation, temperature and evaporation rate) and seasonality. Since neighborhood comparisons are intended, the sample size calculation of 350 surveys city-wide may be an underestimation. Recruitment may also be variable across neighborhoods.

Adult mosquito collection could also be impacted by weather events or trap failures. Since the operation of the adult traps are dependent on an external electricity source in the form of a rechargeable battery, in operation could result because of lack of a full charge or loose wire, causing a failure in the trap fan or light bulb. Ovitraps can also fail (not result in egg collection) due to tipping over or the drying out completely. Lack of data which results when these events occur, by bias data analysis. Heavy precipitation and high winds also impact the abundance of mosquitoes present in the local environment as they will often take shelter and may not engage in typical host-seeking behaviors.

The bloodmeal analysis results may have been impacted by the relatively low number of blood-engorged *Ae. aegypti* collected and able to be analyzed. These low numbers make it problematic to infer relationships to host abundance. However, since other studies have demonstrated a near 100% rate of *Ae.aegypti* feeding on humans, any evidence to demonstrate their ability to feed on other non-human mammals would be noteworthy.

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VII. Main Result Summary - Three-Manuscripts

Manuscript 1.

The Effect of Knowledge, Attitudes and Behaviors on the Abundance of *Aedes* Mosquito Populations in New Orleans, Louisiana

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Abstract

Background: Large urban populations of *Aedes aegypti* and *Ae. albopictus* are present in New Orleans, Louisiana. The potential for introduction by a viremic individual of an *Aedes* mosquito vectored arbovirus like Zika or chikungunya is of great concern. To understand local transmission potential, measures of vector abundance were collected.

Methods: This study identified frequent larval development container habitats on residential properties and assessed the knowledge, attitudes and practices of New Orleans residents regarding mosquitoes and arboviruses. A total of 298 households were enrolled in 3 urban neighborhoods (Uptown, Bywater and Mid-City) in October-December 2015 and 2016.

Results: The mean number of containers was significantly different between neighborhoods ($p=0.03$) and the number in the Bywater (2.2) was significantly greater than Uptown (1.3) ($p<0.05$). Significant associations were also found between home ownership and the number of water-holding containers present; renters were significantly more likely to have at least 1 water-

holding container the in yard (Chi-square $p<0.01$). Women were also significantly more concerned than men about getting infected with an arbovirus (Chi-Square, $p<0.001$). The water-holding containers that were managed (pet dishes, bird baths) (26.0%) were less common than those filled by rainfall (plants, buckets, coolers). The presence of water-holding containers was also significantly associated with recent rainfall events (>0.5 inch within a 48 hour period prior to the assessment) (Chi-Square, $p<0.05$). Additional behavioral and residential determinants of the residential presence of mosquito container habitats were assessed using logistic regression. ***Conclusions:*** It is essential to identify and eliminate residential mosquito habitats for container-inhabiting *Aedes* on a community-wide level. The results from these surveys will be used to produce targeted educational outreach materials to encourage and focus interventions. The long-term control of arboviral diseases is only possible through an integrated public health approach and sustainable vector control strategies including the education and involvement of residents.

Introduction

Accurate estimations of mosquito vector abundance are important in determining transmission potential and to focus disease surveillance and control measures of *Aedes* mosquito vectored arboviruses. Traditional measures of mosquito abundance include container indices; direct observations of water-holding containers for the presence of immature mosquito life stages to determine if *Aedes* population levels have reached densities high enough to put communities at risk of disease transmission (1).

Recent studies have demonstrated an association between container indicies and the prevalence of human dengue infections in Peru (2) and Cuba (3). The number of pupae/ person is considered a reliable estimate for the potential of dengue transmission in Puerto Rico (4). Since the flight range of *Aedes aegypti* and *Ae. albopictus* is small (<150ft), these indices may serve to identify areas of high density or latent potential for elevated mosquito populations. While these indicies are representative of the potential for adult mosquito production, additional factors may impact development and emergence from the larval environment.

Small artificial containers frequently serve as highly productive mosquito habitats; the small surface areas decreases the rate of evaporation in these environments. While the Southeastern U.S. experiences high average temperatures, additional factors such as humidity or shade supplied from heavy vegetation could mitigate and slow down water evaporation rates. In the deserts of Arizona along the U.S. Mexico border, *Ae. aegypti* was positively associated with highly vegetated areas (5) but its presence was highly variable across space and time. Higher temperatures also accelerate the completion of the mosquito life cycle. Summer and autumn temperatures in New Orleans frequently rise above 32C (89.6F), allowing the life cycle (from egg to emergent adult) to be completed in a 7 day period and faster than the rate of evaporation in the smallest environment (6).

Following a dengue outbreak in Key West, Florida 2009-2010, a randomized population-based survey was conducted. Analysis was performed to determine whether knowledge, attitudes, and behaviors exhibited by public housing residents differed from the non–public housing in the study population (7). Residents of public housing were significantly less likely to recall outreach materials, correctly identify how dengue transmission occurs and where mosquitoes lay their eggs. These residents were also less likely to perform dengue prevention practices such as removing standing water.

In Trenton, New Jersey, during the peak mosquito season (July-August), the abundance of immature *Ae. albopictus* was significantly higher in corrugated extension spouts than open containers (buckets, plant saucers, and tires) (8). Of the 20,039 water-holding containers examined, only 1.2% contained immature *Ae. albopictus* (5.3% if only key containers were counted). Standard larval-based indices did not correlate with adult catches using BG-Sentinel traps. In neighborhood studies in Baltimore, MD and Washington, DC, *Aedes* pupal density was greater in container habitats in below-median income neighborhoods. However, density was not correlated with resident-reported mosquito nuisance (8). In additional study in the same areas, *Aedes albopictus* larvae were 5.61 times more abundant in parcels in higher socio-economic, low-decay blocks (9). Containers associated with residences were more likely to hold water and contain immature mosquitoes and most discarded containers were dry. The conclusions in this study is that containers switch from rain-fed unmanaged containers early in the season to shaded or watered (managed) container habitats by mid-season (9).

Methods

Survey materials were approved by the Tulane University Biomedical Institutional Review Board (IRB) (Arbovirus KAP and Exposure Survey - 782121-2). In place of written consent, a study flyer was distributed and verbal consent was accepted. Study materials included an interviewer-administered Questionnaire (household demographics, behavioral practices, recent medical history and knowledge and attitudes regarding mosquitoes and arboviruses) and an Environmental Inspection (record observations regarding the home and yard and assess the property for any water-holding containers). Categorical descriptions of water-holding containers, material and locations were recorded and if immature mosquitoes were present, samples were collected. Container descriptions recorded included the container size, material, color and a brief categorical description. The presence of mosquitoes including larvae, pupae and pupal exuvia (indicating pupal emergence) was also recorded for each container. Immature mosquito samples were removed from the container using a turkey baster and were placed in Whirl-pak (Nasco, Fort Atkinson, WI) water collection bags for transport. When samples were returned to the insectary, they were transferred to larval rearing chambers (BioQuip, Rancho Dominguez, CA), and reared to adulthood for identification (80F, day/ night cycle: 18hrs/ 6 hrs).

Collection grid areas of 6-10 city blocks were established in the Bywater, Mid-City and Uptown neighborhoods (Figure 1). The specific blocks selected were based on previous work in the area (Michaels, unpublished data), presence of abundant artificial container habitats such as tire shops and cemeteries, resident-reported mosquito activity and safety/ accessibility. Neighborhoods are comparable in housing type and presence of single and multifamily residences, however the Mid-City neighborhood is much larger in size, population and average number of household members and has a higher proportion of renters than the other two neighborhoods. A specific area within Mid-City was targeted. Based on the population in these selected areas of the city (1) and given a 95% confidence interval, an adequate sample size was determined to be 350 surveys.

Trained personnel visited each occupied residence within a specific city block of a grid area in a systematic fashion, working through 1 block at a time in an attempt to make contact with residents on multiple days and times (up to 3 visits). If the resident was not at home, a door hanger was left to allow them to indicate willingness to participate or to refusal and to schedule an appointment by phone or email. Residents were able to enroll in either or both the questionnaire and environmental inspection. If multiple separate households existed within the same property, only one household was enrolled in the study and the shared outdoor area was surveyed. Participant household addresses were recorded but the information was kept separate from questionnaire responses as instructed by the Tulane IRB.

Household questionnaire and environmental survey data was entered in Microsoft Excel (2013) and analyzed by SAS software Version 9.3 (SAS Institute Inc., Cary, NC, USA) for descriptive statistics, chi-square analysis and ANOVA and STATA 14.2 (Stata Corporation, College Station, TX) for logistic regression. Daily precipitation and temperature dataset was obtained from the National Oceanic and Atmospheric Administration, National Climatic Data Center (Global Historical Climatology Network - Version 3) (12-13).

Results

Enrollment & demographics: Surveys were collected from October-December in 2015 and 2016. A total of 298 residents were enrolled; 94 in November & December 2015 and 204 in October & November 2016 (Refusal rate 13.3%). In Uptown and Mid-City, 111 surveys were collected in each neighborhood and a total of 50 in the Bywater overall. Respondents were 45.7% male and 54.2% female, 47.2% were renters and 52.8% owners of the property assessed. Sixty-nine percent were 1-2 person households (69.1%, range 1-9 persons, median 2). About half of the participants were under 40, (48.3% Ages 18-40), 35.8% were 40-64 years and 15.9% over age 65 years. Respondents were generally well-educated; 43.0% had graduate degrees, nearly half had attended at least some college classes or had completed an undergraduate degree (46.6%). Over 70% (71.1%) of respondents had central air conditioning, the majority of the remainder had window units (23.6%) covering at least some of the living and sleeping space of the home, and 4.9% had both central and window unit air conditioning. Only 1 respondent had no air conditioning at all. (Table 1).

Knowledge and behavioral responses: Residents reported being bitten by mosquitoes frequently (43.3%) (Table 2), and sometimes taking precautions to avoid mosquito bites (36.3%), including applying repellent on themselves or members of their family frequently (31.0%) or sometimes (29.2%). However, most residents do not make any pesticide applications to the yard to prevent mosquitoes (74.3%). Residents are split on frequency that windows are left open in the home, most reported rarely or never leaving the windows in the home open (58.6%); however a third (36.8%) reported leaving them open frequently and 4.6% daily. Mosquitoes are likely to be found inside the house sometimes (56.5%). Most residents believe that the chance of being infected with a mosquito-borne disease is low (60.9%) and are not concerned (41.6%).

Residents indicated that mosquitoes were a problem in their yard (57.5%) and that they limited the time spent outside (54.6%) (Table 3). However, when asked how many days a week they spent time outside in the evening, 31.6% of respondents said every day and only 18.8% never, which included time spent near the home and also engaging in other outdoor activities away from the home. Most are involved with gardening or yard work (63.2%). Many households have pets (65.6%), of these cats (66.0%) and dogs (56.5%) were the most common.

The majority of respondents believe that the city (76.7%) is responsible for mosquito control and also believe homeowners (57.8%) share some of that responsibility. When asked what they do when mosquitoes are found in the yard, many empty containers (65.5%).

Nearly every participant thought that mosquitoes spread disease and correctly identified West Nile virus as a disease vectored by mosquitoes (both 97.2%). However, fewer had heard of other domestically-transmitted arboviruses; St. Louis encephalitis (SLE) (47.2%) and eastern equine encephalitis (EEE) (35.5%). Nearly all participants were familiar with malaria (95.7%) and many with dengue (68.1%) but far less with chikungunya virus (28.0%). Since this survey was written prior to the emergence of Zika virus in the Americas, there was not a specific knowledge question directed at the disease, however many homeowners mentioned it during the interview.

Container assessments: Property inspections yielded a mean of 1.3 water-holding containers/ residence (SD 2.8, Range 0-24). Containers were likely to be small or medium (79.1%) in size. Container types recorded included flower pots, planters & plant saucers (71), buckets (33), coolers (22), pet dish/bowls (17), fountains (12), bird baths (11), watering cans (11), trash cans (9), rain barrels (8), tires (6), fence posts (4) and wheelbarrows (3) (n=289). Overall, 60 of the 289 water-holding containers assessed contained mosquito larvae (Container Index – 20.7) and 17 pupae (Pupal Index 5.8). The mosquito larvae collected were identified as *Aedes aegypti* (85.9%), *Culex quinquefasciatus* (11.3%) and *Ae. albopictus* (3.3%).

Containers that were managed to continuously have water present (pet dishes, bird baths, rain barrels, watering cans) (26.0%) were less common than those filled by rainfall (planters, buckets, coolers, trash cans). Precipitation data (NOAA National Climatic Data Center, 12-13) was compared to assessment dates to determine if recent rainfall events impacted these observations; recent precipitation was defined as >0.5 inch within the 48 hour period prior inspection. These events were uncommon, affecting only 31/ 273 observations, however the presence of one or more water-holding containers was 5.2 times more likely than finding no containers after a recent rainfall event (Chi-square, p=0.02).

Neighborhood was also strongly associated with the mean number of containers (p=0.03), the mean number of containers in the Bywater neighborhood (2.2 containers/ house) was significantly different and greater than the mean number of containers in the Uptown neighborhood (0.8 containers/ house) but not significantly different from Mid-City (1.4 containers/ house) (ANOVA, $p<0.05$) (Figure 2).

Data analysis: Significant associations were also found between home ownership and the presence of water-holding containers; renters were more likely to have at least 1 container the in yard (Chi-square $p<0.01$). Women were also significantly more concerned than men about getting infected with an arbovirus (Chi-Square, $p<0.001$).

Descriptive and chi-square statistics were used to summarize household survey data to evaluate differences in bivariate outcomes and to select demographic characteristics. Logistic regression was used to test whether additional behavioral and residential determinants of the presence of mosquito container habitats existed (Table 4). Gender was selected because of its strong association with increased concern of becoming infected with a mosquito-borne disease. Those that indicated a likelihood of participation in yardwork or gardening, thought that homeowners were responsible for mosquito control or responded to mosquitoes in the yard by emptying containers. The residences likely to be positive for water-holding containers were those assessed within 48 hours of at least a 0.5 inch precipitation event (OR 3.02, 95% CI 1.27–7.15) and those that were renters rather than owners of the property (OR 2.16, 95% CI 1.24–3.77).

Discussion

Given the high potential risk of introduction of Zika and chikungunya viruses into New Orleans (14-15), the high container index (20.7) and presence of *Ae. aegypti* vector populations in the cooler months of the year (October-December) arboviral transmission is of great concern. Anthropogenically-driven factors including urbanization, human movement and poverty influence mosquito distribution and abundance, potential for human contact, and the presence of arboviruses (16). During the cooler months, it is possible that human exposure could actually be elevated over the warm summer months, as residents change their behaviors; abstaining from air conditioning usage and instead relying on keeping windows open or engaging in more frequent outdoor activities. However, this increase in exposure may be mediated by climatological conditions including temperature which may decrease the likelihood of mosquito-mediated arboviral transmission.

Bottle caps or approximately a teaspoon of water is commonly references as the smallest environment where *Aedes* mosquitoes can complete their life cycle prior to evaporation. Environmental modeling in New Jersey demonstrated that when containers are small, *Aedes albopictus* and *Ae. aegypti* could develop within a bottle cap before complete evaporation (17). These containers were exposed to typical environmental conditions, including a range of temperatures but with minimal direct sunlight. The rate of evaporation of water depends on the water temperature, air temperature, air humidity and air velocity above the water surface (surface area). The frequency of small containers as mosquito habitats is likely explained by the small surface area and its influence on the rate of evaporation in these environments. While the Southeastern U.S. experiences much higher average temperatures than the studies in New Jersey, additional factors such as humidity or heavy vegetation which produces shade could mitigate water evaporation rates. Precipitation data in 2015-2016 (Figure 3), also demonstrated large rain events exceeding 4 inches in a 24-hr period in the Fall (August-October) months. In Trenton, New Jersey, during the peak mosquito season (July-August), only 1.2% contained immature *Ae. albopictus* (5.3% if only key containers were counted). During this study (October-December), the high container index of 20.7 well exceeds measures of abundance in NJ, indices should be compared with additional time frames and to adult mosquito collections to determine if the counts are correlated and when the peak of the season occurs in New Orleans. The presence of immature mosquito larvae may represent a latent population risk of emergence but if containers are flooded or weather does not favor survival, risk or abundance may be overstated.

In Florida, residents of public housing were less likely to perform dengue prevention practices such as removing standing water (7) and in Baltimore, MD and Washington, DC, *Aedes* pupal density were greater in container habitats in below-median income neighborhoods (8). While this study did not test the association between socio-economic levels, a difference in container abundance was demonstrated between neighborhoods and between renters and owners. Area with a greater abundance of rental properties would likely be associated with elevated presence of containers. For renters, the responsibility for care and maintenance of the outdoor spaces may be shared between tenants and owner or multiple owners and so may be less under the control of the individual to alter. This is similar as to what was observed in public housing in Florida, where much of the outdoor space was shared.

Knowledge of mosquito-borne disease was high, and most residents perceived mosquitoes to be a problem in the yard and report getting bitten frequently. Most reported the use of repellents frequently or sometimes, however when asked specifically, many residents mentioned using naturally-derived and unregistered repellents. These repellents often have not been evaluated for efficacy and could lead to a false sense of protection. Since questions about outdoor exposure did not specify the location around the home, it is unclear how much exposure happens in the residential outdoor environment or in other outdoor locations, or the types of activities participated in (running, walking versus sitting). Additional localized exposure should be elucidated for future exposure studies. Furthermore, while most residents report mosquitoes inside the house and many frequently leave windows in the home open, it is unclear if these events are linked temporally or if housing construction impacts the ability of mosquitoes to enter homes. The assumption is that in New Orleans, *Aedes aegypti* and *Ae. albopictus* mosquito habitats, resting locations and feeding occurs outdoors.

This study identified that that the most common and productive urban habitats for *Ae. aegypti* in New Orleans are associated with containers allowed to be filled with rainwater. In the cooler months, large, frequent rain events and potentially lower evaporation rates allow create conditions which support a greater abundance of these habitats. Citizens are knowledgeable regarding mosquito-borne diseases but this knowledge does not translate to reduced abundance of potential habitats around the home. This indicates the opportunity to educate and empower individuals to monitor the outdoor environment more effectively. Given the abundance of competent vector populations, the long-term control of arboviral diseases in New Orleans is only possible through an integrated public health approach, rapid case identification, and vector control.

Conclusions

Large urban populations of *Aedes aegypti* and *Ae. albopictus* are present in New Orleans, Louisiana. The potential for introduction by a viremic individual of mosquito vectored arbovirus like Zika or chikungunya is of great concern. This study identified some potential barriers in prevention and control that may impact a public health response. Results from these surveys have been used to produce educational materials tailored to the New Orleans environment and specific containers likely to contain mosquito larvae. To understand local transmission potential, additional measures of vector abundance and continuous vector surveillance should be conducted.

Acknowledgments

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Figure 1. City planning districts in New Orleans. Sample collection areas occurred in Uptown, Mid-City and Bywater areas.

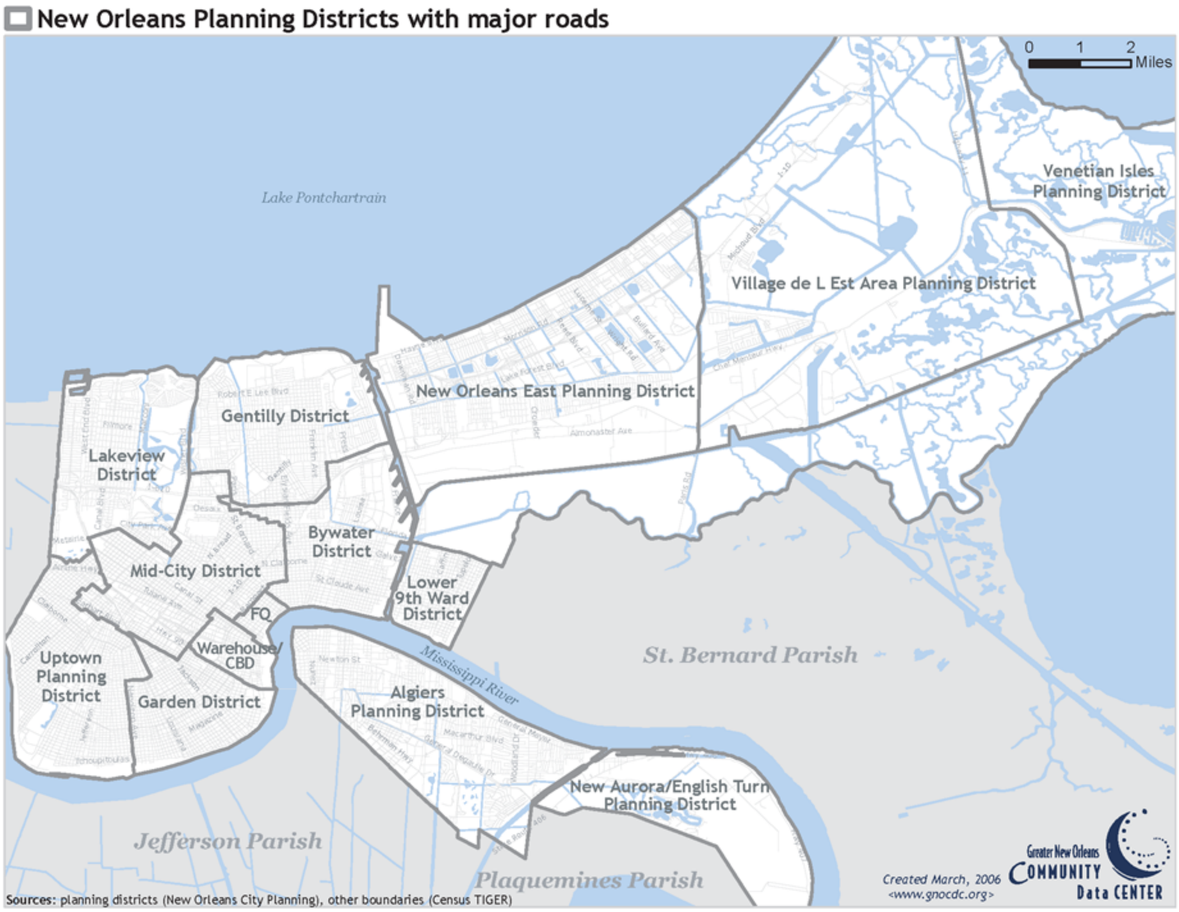


Table 1: Demographic and household information from Arbovirus KAP and Exposure Survey in New Orleans, 2015 and 2016.

Variable	Category	N	Percent
Age	18-20	3	1.1
	21-30	48	17.4
	31-40	80	29.0
	41-50	46	16.7
	51-34	56	20.3
	65+	43	15.6
Gender	Male	130	45.8
	Female	154	54.2
Education	High School	29	10.5
	College	129	46.6
	Graduate	119	43.0
Household Size	1	65	24.2
	2	121	45.0
	3	31	11.5
	4	38	14.1
	5+	14	5.2
Home Ownership	Rent	134	47.2
	Own	150	52.8
Air Conditioning	Central	202	71.1
	Window	67	23.6
	Both	14	4.9
	None	1	0.4

Table 2: Self-reported behaviors and practices concerning mosquito exposure from Arbovirus KAP and Exposure Survey in New Orleans, 2015 and 2016.

Variable	Frequently		Sometimes		Rarely		Never	
How often do you get bitten?	**122**	**43.3**	81	28.7	63	22.3	16	5.7
Take any precaution to avoid bites?	88	31.3	**102**	**36.3**	33	11.7	58	20.6
Use repellents on self or family?	**87**	**31.0**	**82**	**29.2**	44	15.7	68	24.2
Use pesticides/ herbicides in yard?	13	4.7	28	10.1	30	10.9	**205**	**74.3**

How often do you leave windows open?	**Daily**		**Frequently**		**Rarely**		**Never**	
	13	4.6	103	36.8	9	3.2	155	55.4
How often mosquitoes found inside home?	**Frequently**		**Sometimes**		**Always**		**Never**	
	25	8.8	161	56.5	31	10.9	67	23.5
How likely are you to get a virus spread by mosquitoes?	**Very likely**		**Somewhat**		**Not likely**		**Never**	
	19	6.8	72	25.8	170	60.9	18	6.5
Does this make you concerned?	**Very**		**Somewhat**		**Not**		**Don't know**	
	51	18.2	99	35.2	117	41.6	14	5.0

Table 3: Self-reported knowledge and behaviors concerning mosquito exposure from Arbovirus KAP and Exposure Survey in New Orleans, 2015 and 2016.

Variable	Yes		No	
	N	Percent	N	Percent
Are mosquitoes a problem in yard?	162	57.5	120	42.6
Mosquitoes limit time spent outside?	155	54.6	129	45.4
Do you do any gardening/ yardwork?	180	63.2	104	36.5
Have any pets?	187	65.6	97	34.0
Dog	161	56.5	124	43.5
Cat	188	66.0	97	34.0
Who is responsible for mosquito control?				
Homeowners	163	57.8	119	42.2
Neighborhoods	91	32.27	191	67.7
City	216	76.6	66	23.4
Don't know	38	13.48	244	86.5
What do you do when you find mosquitoes in yard?				
Empty containers	185	65.6	97	34.4
Use repellent	137	48.8	144	51.3
Call 311	13	4.6	269	95.4
Nothing	49	17.4	233	82.6
Do you think that mosquitoes spread disease?	273	97.2	8	2.9
Have you heard of: West Nile virus	274	97.2	8	2.8
St. Louis encephalitis	133	47.2	149	52.8
Eastern Equine encephalitis	100	35.5	182	64.5
chikungunya	79	28.0	203	72.0
dengue	192	68.1	90	31.9
malaria	270	95.7	12	4.3

Figure 2. The mean number of water-holding containers per household observed during environmental inspections by neighborhood in New Orleans, 2015 & 2016.

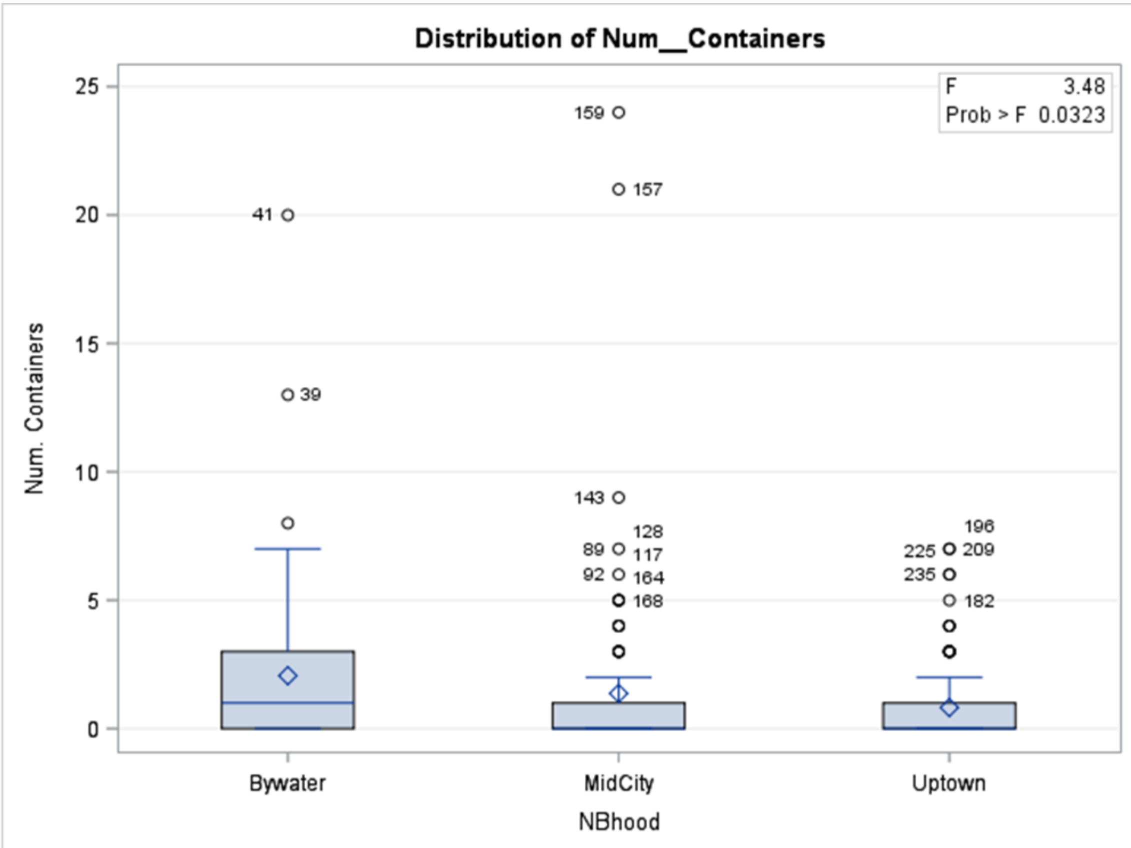
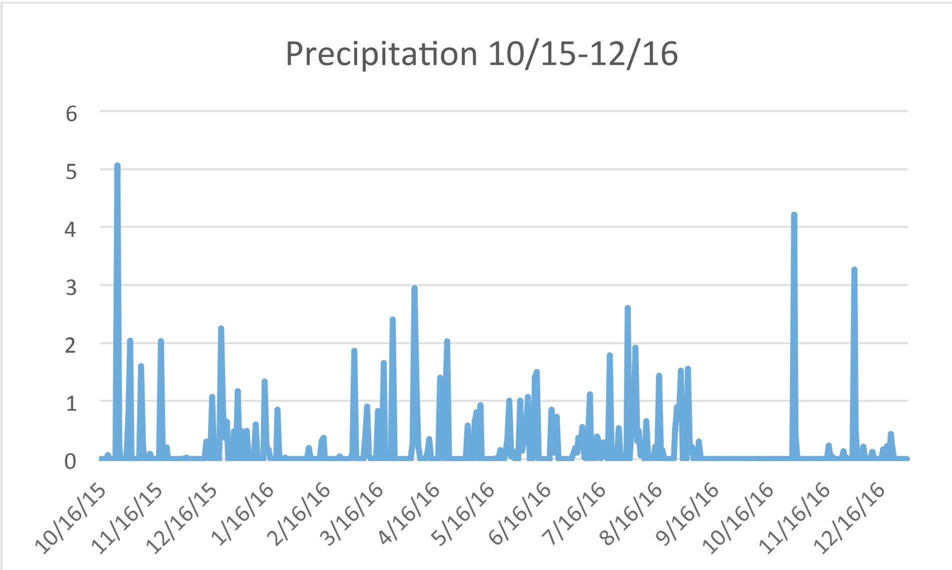


Table 4: Logistic regression predicting the determinants of the presence of water holding containers (n=256) (p=0.0002).

	Covariates	**Odds Ratio**	**Std. Err.**	**z**	**P>z**	**[95% Conf.**	**Interval]**
Neighborhood	Bywater (Reference)	1.00					
	Mid-City	0.52	0.19	-1.74	0.08	0.25	1.08
	Uptown	**0.30**	0.12	-3.11	**0.00**	0.14	0.64
Gender	Male (Reference)	1.00					
	Female	1.46	0.40	1.38	0.17	0.85	2.51
Ownership	Rent (Reference)	1.00					
	Own	**2.16**	0.61	2.71	**0.01**	1.24	3.77
Garden/ Yardwork	No (Reference)	1.00					
	Yes	1.29	0.37	0.87	0.38	0.73	2.26
MC Responsibility Homeowners	No (Reference)	1.00					
	Yes	1.11	0.31	0.37	0.71	0.64	1.91
Precipitation >0.5in within 48 hrs	No (Reference)	1.00					
	Yes	**3.02**	1.33	2.51	**0.01**	1.27	7.15
Pseudo R2 = 0.0824							

Figure 2. Precipitation in New Orleans October 2015 – December 2016.



Data source: Menne, M.J., Durre, I., Vose, R.S., Gleason, B.E. and T.G. Houston. 2012. An Overview of the Global Historical Climatology Network-Daily Database. J. Atmos. Oceanic Technol., 29, 897-910. doi:10.1175/JTECH-D-11-00103.1.

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Manuscript 2.

Title: Accurately Capturing Mosquito Population Density for the Estimation of Arboviral Transmission Risk

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Abstract

Accurate estimations of mosquito vector abundance are important in determining transmission potential and to focus disease surveillance and control measures. Abundant populations of both *Aedes aegypti* and *Ae. albopictus* co-exist in urban New Orleans. These species are efficient in utilizing artificial container habitats and are aggressive pests and important arbovirus vectors of dengue, chikungunya and Zika viruses. Mosquito populations however are not evenly distributed across the urban landscape, *Ae. aegypti* is correlated with urbanization and *Ae. albopictus* with suburban, rural and vegetated urban habitats (such as large parks). Surveillance for these species include collecting the eggs laid in ovitraps and adult mosquito collection traps including the CDC-LT (Center for Disease Control & Prevention light trap) and Biogents Sentinel (BGS1) traps. Studies were conducted in order to determine which

adult trap would be optimal at collecting *Aedes* species and if the adult collections were an accurate representation of the species composition and abundance as compared with ovitrap collection at the same location, From June-October 2013, paired collections were made at locations with abundant *Aedes* and trap positions were changed weekly. Trap failures were substantially higher with CDC-LT and the BGS1 collected a larger average number of both species. These collections correlated with species abundance as demonstrated by ovitrap collection data.

Background

Accurate estimates of mosquito abundance, documentation of species presence and detection of arboviruses can be accomplished through the collection of adult vector mosquitoes. Historically, collections were performed by landing or aspirator collections. While this method works well it is time-consuming, needs to be performed systematically and potentially exposes collectors to pathogens carried by mosquitoes. Gradually, these collections have been replaced by stand-alone, battery-operated adult traps including the Centers for Disease Control and Prevention light trap (CDC-LT) (John W. Hock Company, Gainesville, FL) (1) and BG Sentinel^{TM} trap (BGS trap, BioGents AG, Regensburg, Germany). Both of these traps target host-seeking mosquitoes attracted to specific environmental cues; the CDC-LT uses dry ice to produce CO_2 and a small light to attract mosquitoes while the BGS1 trap utilizes an octenol (1-octen-3-ol) lure sachet (AgriSense, Pontypridd, United Kingdom) and a mesh BG lure containing ammonia, lactic acid, and fatty acids (Biogents AG) mimicking the scent of human skin/ sweat. The BGS1 relies on air currents produced in the trap to move the plume of chemical attractant into the air column, through the top cover of the trap. The trap also utilizes visual cues of a black/ white contrast in color near the trap entrance. While the CDC-LT is the gold standard collecting tool for a variety of host-seeking mosquito populations, while the BGS1 was specifically designed for the collection of *Aedes aegypti* mosquitoes. As both trap collections remain alive until removal, either can be evaluated for the presence of arboviruses. The CDC-LT is a reliable tool for collection of *Culex* species for West Nile virus surveillance.

Various field trials have compared *Aedes aegypti* and *Ae. albopictus* (here referred to collectively as *Aedes*) collections methods, these have represented a variety of climates and conditions but none have been conducted in an area where both species are both locally abundant. The only previous studies in urban New Orleans in 2005-2006 determined that the CDC-LT was superior for *Aedes* collections than human landing and Nasci aspirator vegetation collections (D. Wesson unpublished data). In Central Florida, the BGS1 was superior to landing counts, aspirator collections and gravid trap collections using an oak-leaf infusion for collecting *Aedes albopictus* (2). The BGS1 was also found to be the most sensitive trap type to measure abundance and spread into new locations (3). In New Jersey, the BGS1 collected 3 times as many *Ae. albopictus* than the CDC-LT (4). However, the same group found that the larval-based indices did not correlate with adult collections using BGS traps for *Ae. albopictus* (5). Because the presence of adult *Aedes* is highly variable across space and time, trap placement is especially important. More abundant collections were correlated with trap placement in shaded areas rather than in full sun in New Jersey for *Ae. albopictus* (6) and in highly vegetated areas in Arizona for *Ae. aegypti* (7).

Methods

The City of New Orleans Mosquito and Termite Control Board (NOMTCB) and Tulane University School of Public Health and Tropical Medicine monitored 18 trap locations throughout the city weekly from June-October 2013. Trapping locations were in a transect along the Mississippi River bisecting a number of neighborhoods and in Mid-City, in areas with large, stable populations of *Ae. aegypti* and *Ae. albopictus* mosquitoes (Figure 1). Trapping locations included residential properties and municipal buildings, in shaded, vegetated and secure locations.

Paired, dry-ice baited CDC-LT and BG-Lure® and octenol lure-baited BGS1 were set overnight, mid-afternoon until late the following morning (2pm-11am). Trap positions were 20-30 ft apart, the positions of the traps were rotated each week. BGS1 traps were placed on the ground while CDC-LT traps were hung at eye-level. CDC-LT were hung at approximately 5ft, usually on a low tree branch with the collection position (trap entrance) approximately 4ft above the ground. Both traps were run as per manufacture instructions and with a rechargeable battery (BGS1-12v, CDC-LT-6v). At the same time and location, pairs of black plastic ovitraps (20 oz., Berry Plastics, Evansville, IN) lined with seed germination paper (Anchor Paper, St. Paul, MN) and filled with dechlorinated tap water were placed to monitor egg-laying populations. Ovitraps were placed in areas of vegetation, and reset weekly by removing and replacing water and paper. Following removal, the seed germination paper was dried for 24 hours and then hatched in dechlorinated tap water in larval rearing trays (BioQuip, Rancho Dominguez, CA) at standard insectary conditions (80F, day/ night cycle: 18hrs/ 6 hrs). Specimens were reared to 3rd – 4th instar larvae or adulthood for identification. Adult mosquitoes were placed in -20C for storage and identified to species using the Louisiana Mosquito Control Association Manual.

Data was managed and summary statistics were conducted in MS Excel (2013), data was analyzed by SAS software Version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

From June 6th – October 8th, 2013 a total of 102 paired BGS1 and CDC-LT collections were made. These contained a total of 1,524 adult *Aedes* mosquitoes, of which nearly twice as many total *Ae. aegypti* were collected than *Ae. albopictus* (1.86 ratio of *Ae. aegypti: Ae. albopictus*) (Table 1). The mean *Ae. aegypti* collected by BGS1 was 10.9 mosquitoes/ trap night (SD: 18.3) and CDC-LT 4.1 mosquitoes/ trap night (SD: 9.2) and the mean *Ae. albopictus* collected by BGS1 was 6.7 mosquitoes/ trap night (SD: 13.4) and CDC-LT 1.3 mosquitoes/ trap night (2.1). The BGS1 collections were also more likely to contain at least one mosquito for each species. It collected *Ae. albopictus* approximately 1.4x and *Ae. aegypti* 2.0x as often as the CDC-LT. The BGS1 also collects a nearly 50% ratio of male: female mosquitoes (53.6% female *Ae. aegypti* and 58.7% female *Ae. albopictus*) while the CDC-LT captures a much higher percentage of females (73.0% female *Ae. aegypti* and 78.4% female *Ae. albopictus*).

A total of 47/ 204 (18.1%) trap collections contained neither *Aedes* species, 16 of these were collected by BGS1 and 31 by CDC-LT. Trap failures occurred in CDC-LTs significantly more frequently than BGS1 (OR 2.35, 95% CI (1.19, 4.63) (p=0.02), this included the fan or light being not being operational when collected. Positive ovitrap collections of *Ae. aegypti* tended to predict the presence of adult *Ae.aegypti* using CDC-LT (p = .0536) and are significantly associated with BGS1 collections of *Ae. aegypti* (p = .0051) but not with *Ae. albopictus*. Ovitraps were a more sensitive indicator of *Aedes* presence then adult traps.

Discussion

Initial analysis of adult collection data indicates that in an environment where the two species co-occur, despite a higher abundance of *Ae. aegypti* there was a preference for the BGS1 to collect *Ae. albopictus* relative to the CDC-LT. This data may have been impacted by a higher rate of trap failures of the CDC-LT to collect either species or by the sex ratio differences in trap collection, as the BGS1 tended to collect a higher proportion of males. It is also possible that the traps were also impacted by height differences, both *Aedes* species tend to prefer staying lower to the ground, however *Ae. albopictus* is known to utilize tree holes for egg-laying and has is frequently collected to heights of 1m (2). Since traps were used with only a single attractant (CO_2 only, lures only) this may also demonstrate different receptivity or response to environmental cues and influences between species.

The BGS1 trap was originally designed for the capture of *Ae. aegypti* and has recently been demonstrated for *Ae. albopictus* collection (8). The sex ratio observation of nearly 50% male:female collection has been observed in other studies for *Ae. albopictus* in Maryland (9). Taking this into account, the larger total number of mosquitoes collected by the trap would allow for the understanding of temporal and spatial population dynamics and evaluate the effectiveness of control interventions (10-11). Given the mean of 10.9 mosquitoes/ trap *Ae. aegypti* collected in this study, a reduction of a minimum of 3.27 mosquito/ trap (or 30%) would be significant (at an 80% detection rate and approximately 50 samples collected before and following the intervention). Despite these advantages in collection, the utilization of the trap in surveillance programs has been hampered by durability, expense and has undergone several design alterations (12-13).

The ovitrap data collected were used as a representation of the relative abundance of each species to compare species composition in the adult traps. The species composition was as expected from previous ovitrap studies (14) and ranged from 50/50 to 90/10 *albopictus/ aegypti* (Figure 2). The local environment (ratio of *aegypti/albopictus* ovitrap collections), was predictive of the species composition in the adult traps by location. The use of ovitraps however can be resource intensive when there are not trained personnel and facilities for rearing mosquitoes.

Conclusions

It is important to understand the inherent bias of different adult mosquito collection traps especially if the resulting data is to be used to monitor for risk of mosquito-borne pathogens and to target and evaluate control measures. This has become especially important in light of the emerging epidemics of Zika and chikungunya viruses (15-16) and the desire to describe the presence and abundance of local mosquito vector populations. Ovitraps were shown to be the most sensitive indicator of *Ae. aegypti* and *Ae. albopictus* presence and adult mosquito trapping was representative of the local species composition. The BG Sentinel trap (BGS1) collected a larger average number of both species and would be a better choice to detect changes in the mosquito populations due to temporal trends or following interventions.

Acknowledgments

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Figure 1. Trap locations for BGS1 and CDC-LT comparison study in New Orleans, LA, June-October 2013.

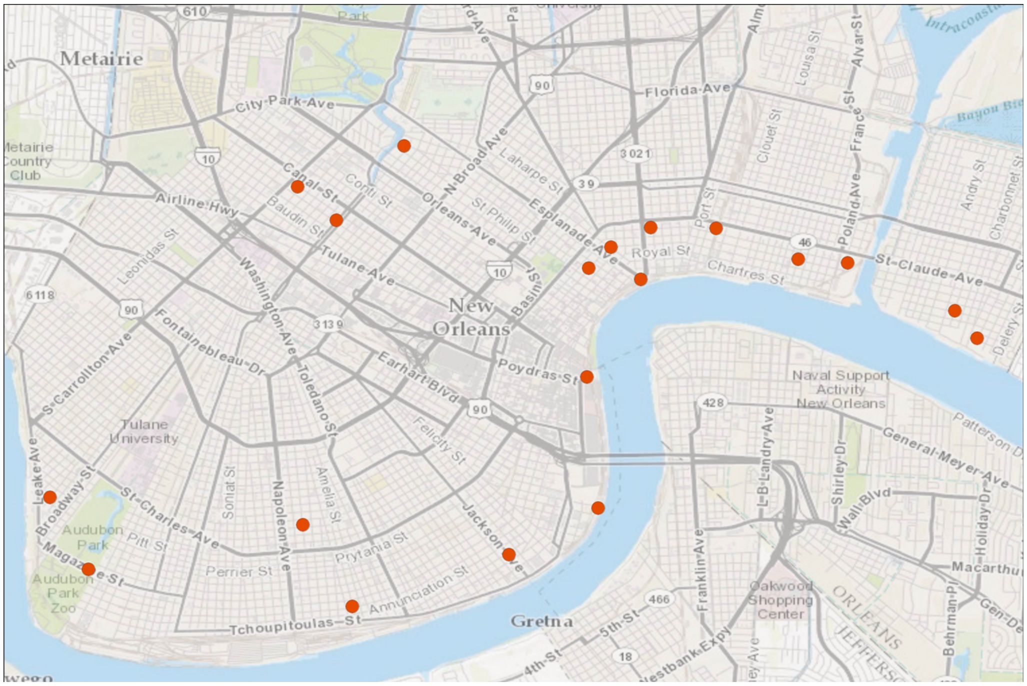
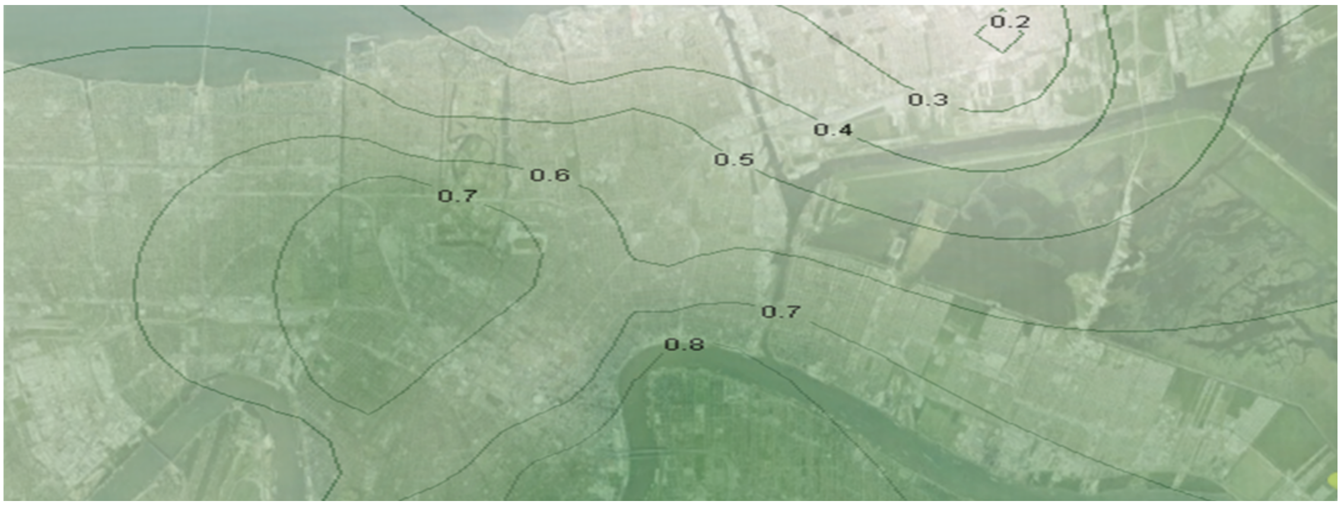


Table 1. Trap comparison study data comparing CDC-LT and BGS1 in New Orleans, LA May-October 2013.

		Aedes aegypti			***Ae. albopictus***				**Ratio**
Trap type	**N**	**Total**	**Mean**	**StdDev**	**Total**	**Mean**	**StdDev**	**Total**	***aegypti: albopictus***
BGS1	102	1109	10.9	18.3	686	6.7	13.4	1795	1.62
CDC-LT	102	415	4.1	9.2	134	1.3	2.1	549	3.10
Total	204	1524	7.5	14.9	820	4.0	9.9	2344	1.86

	Aedes aegypti				***Ae. albopictus***			
Trap Type	Females	Males	Total	% Female	Females	Males	Total	% Female
BGS1	594	515	1109	53.6	403	283	686	58.7
CDC-LT	303	112	415	73.0	105	29	134	78.4

Figure 2: Ovitrap collection in New Orleans, LA June-October 2013. Numbers represent ratio of *Ae.aegypti*: total *Aedes* collected, darker areas represent higher ratios of *Ae. aegypti*, lighter areas (lower ratios) are indicative of higher numbers of *Ae. albopictus*. (Credit: Justin Davis, PhD, University of South Dakota)



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Manuscript 3.

Title: Host-seeking Behaviors of *Aedes aegypti* in the Outdoor Residential Environment

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Abstract

New Orleans has abundant urban populations of *Aedes aegypti* and *Ae. albopictus*. The purpose of this study is to determine the feeding behaviors of *Ae. aegypti* and *Ae. albopictus* and the relationship human feeding rates have with host availability at two specific time points. The first collection period was August-October 2006 during the post-disaster and recovery period following Hurricane Katrina. The second period was May-September 2016, in areas with historically abundant *Ae. aegypti* populations along the Mississippi River. Despite the higher availability of human hosts in 2016, among *Ae. aegypti* bloodmeals identified, a very low human feeding rate was observed. Having a better understanding of where and when vector/ human interactions occur is informative and can assist in the allocation and prioritization of arboviral disease prevention messaging.

Background

Vector abundance and ability to make contact with human hosts are important components of transmission potential. Bloodmeal identification through a multiplexed polymerase chain reaction (PCR), to target mitochondrial cytochrome b (Cytb) fragments from mammalian hosts is used to determine contact rates of vector mosquitoes and human hosts (1). Analysis of *Ae. aegypti* adult female collections in Thailand demonstrated that 99% of single-host bloodmeals had fed exclusively on humans and a low frequency of feeding on other hosts including bovine, swine, cat, rat and chicken (2). Harrington postulated a selective advantage for frequent feeding on human blood; the unique isoleucine concentration of human blood is associated with increases in fitness and synthesis of energy reserves (3). In longitudinal studies in Thailand and Puerto Rico, human feeding rates were above 90% and multiple bloodmeal events were observed (42% in Thailand, 32% in Puerto Rico) (4). The majority of bloodfed mosquitoes were collected inside homes in this study but bloodfeeding frequency was independent of collection site (indoor vs outdoor). In Rome, Italy, high rates of human-feeding was found among *Ae. albopictus* collections in urban and rural locations; human-feeding was observed in 79-96% at urban sites and 23-55% in rural sites (5). In New Jersey, *Ae. albopictus* blood meals were less often derived from humans (58.2%) but were also associated with domesticated pets (23.0% cats and 14.6% dogs) (6). It was also found conversely that *Ae. albopictus* fed from humans significantly more often in suburban than in urban areas while cat-derived blood meals were greater in urban habitats, indicating some differences in residential exposure. Identifying host bloodmeal origin as it relates to host availability would assist in the understanding of whether this behavior is a result of human host preference.

The purpose of this analysis is to determine the feeding habits of *Ae. aegypti* and *Ae. albopictus* (referred to here collectively as *Aedes*) in New Orleans, and the relationship human feeding rates have with host availability at two specific time points. The first collection period is in 2006 during the post-disaster and recovery period following Hurricane Katrina. Trap locations were in neighborhood areas that had been flooded and were at varied degrees of human repopulation. During the second time period in 2016, collections were made in areas with historically abundant *Ae. aegypti* populations, areas frequented by tourists and visitors and along the Mississippi River port. This comparison is intended to determine if changed population dynamics between 2006-2016 impacted the frequency of human bloodfeeding of *Aedes* mosquitoes and what implications this may have on the potential of *Ae. aegypti* as an arbovirus vector and the subsequent risk of arbovirus transmission in New Orleans.

Methods

This study contains two collection periods: August-October 2006 and May-September 2016. The 2006 adult mosquito collections were made with diurnal dry-ice baited CDC miniature light traps (CDC-LT) and Nasci aspirators (NA) in areas moderately to heavily flooded. Collections yielded 4,027 *Ae. aegypti* and 1,573 *Ae. albopictus* adults of which 162 were fully or partially blood-engorged females (2.9%) (88 *Ae. aegypti*: 22 CDC-LT, 67 NA; 74 *Ae. albopictus*: 2 CO2, 72 NA). In 2016, surveillance was conducted using BG-Sentinel Traps Version 2 (Biogents, Regensburg, Germany) (BGS2) in combination with the BG-Lure cartridge at 37 sites around the city of New Orleans between May 27th and September 30th, 2016. A total of 5,309

Aedes mosquitoes were collected including 1,903 female *Ae. aegypti* and 1,195 female *Ae. albopictus*, 17* *Ae. aegypti* (<1%) were blood-engorged.

Collected mosquitoes were held at -20°C until identification. All samples were separated and recorded by species, sex, date, and location. Positive female *Ae. aegypti* controls were selected from colony material and fed with human donor or bovine blood. Mosquito abdomens were removed and DNA was extracted using the DNAzol BD reagent (Molecular Research Center, Inc) or the QIAGEN DNA mini-kit (QIAGEN Inc., Valencia, CA), following manufacturer's instructions for extracting total DNA from insects.

The PCR was performed using a Taq PCR core kit (QIAGEN Inc., Valencia, CA) or Invitrogen SuperMix (ThermoFisher Scientific) per manufacturer's instructions. Extracted DNA was quantified the using the NanoDrop Spectrophotomer (Thermo Fisher Scientific, Wilmington, DE). A multiplex PCR with human-specific forward primers (334bp) and additional mammalian primers (633 bp) (Human 741F, UNFOR403, and UNREV1025) based on mitochondrial cytochrome b found in mammals was used (2). Samples were visualized on a 1% agarose gel. For samples confirmed mammalian positive and human negative, PCR products were cleaned using the PureLink PCR Purification Kit (ThermoFisher Scientific) samples were submitted for DNA sequencing (Tulane Sequencing Services, New Orleans, LA). Resulting sequences were identified using NCBI BLAST.

Results

For the 2006 samples, mammalian blood-sources were detected in 67 of 162 (41.4%) by the multiplex PCR method. 17 (25.4%) of these bloodmeals were from a human origin.

Preliminary sequencing results provide evidence of host feeding on rodents, felines and canines. Of 2016 the blood-fed *Aedes aegypti* analyzed, 14/17 (88%) had fed on non-human mammals only, 3 had fed exclusively on humans (17.6%).*

Discussion

The sample size used in this analysis is small but results are suggestive that despite the abundance of *Aedes* species collected, human feeding rates were low. The availability of humans may impact the frequency of human-derived bloodmeals, traps that were placed in public areas close to an outdoor reading area of a library and near a recreational space collected a higher frequency of human bloodmeals. Using different adult mosquito collection techniques may also had an impact on the frequency of blood-engorged mosquitoes collected. The CDC-LT and BGS2 both collect host-seeking mosquitoes, reducing the likelihood that a full bloodmeal was previously taken. Dawn and dusk vegetation sweeps using the Nasci aspirator collected the highest numbers of engorged mosquitoes, likely because they were resting in the vegetation to digest bloodmeals. *Aedes aegypti* however is known to take frequent small bloodmeals, reducing the likelihood of capturing engorged females. It was also observed while not appearing visually engorged, PCR detection of bloodmeal was still possible in some specimens. Additional trap types like the Gravid *Aedes* traps (GAT) may increase the collection of blooded *Aedes aegypti* but once the bloodmeal is digested and eggs have formed, the host DNA is no longer detectable.

The New Orleans population estimates in 2006 indicate that areas heavily flooded were largely uninhabited by humans and animal rescue operations also removed thousands of domestic animals. The presence of non-human mammalian bloodmeals may also be suggestive of a host preference other than humans.

Blood meal analysis could be an additional tool to better understand the relationship between host abundance and human exposure and the environments that are most at risk for disease transmission.

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VIII. Conclusions and Recommendations

Large urban populations of *Aedes aegypti* and *Ae. albopictus* are present in New Orleans, Louisiana and the potential for introduction of Zika or chikungunya viruses by a viremic individual is of great concern. To understand local transmission potential, estimates of vector abundance should be conducted and factors impacting the availability of urban container habitats should be evaluated.

Results of this study suggest that residents are knowledgeable regarding mosquito-borne diseases but that knowledge does not translate to reduced abundance of potential habitats around the home. The mean number of water-holding containers is strongly associated with specific neighborhoods and is more likely to be present at rental properties than those occupied by owners. Container habitats are largely unmaintained by residents but are instead filled by rainfall and therefore are impacted by precipitation events especially temporally, when evaporation rates may be lower. The abundance of immature mosquitoes in these environments suggest that the population of *Aedes aegypti* is high enough to support transmission of arboviruses if introduced.

When conducting measures of adult mosquito abundance it is important to understand the inherent bias of different adult mosquito collection traps. Adult mosquito trapping by BGS1 and CDC-LT was found to representative of the *Aedes aegypti* and *Ae. albopictus* species composition as compared to ovitrap collections but sex ratio and species differences suggest a difference in responses to environmental cues used in the design of these traps. They may also be impacted by the height differences in trap design. In environments where populations may be lower, ovitraps were shown to be the most sensitive indicator of *Aedes* presence. The BG Sentinel trap (BGS1) collected a larger average number of both species than the CDC-LT, had less trap failure events and collected a greater relative collection of males. The BGS1 collection data is sensitive enough to be used to detect changes in the mosquito populations due to temporal trends or following interventions.

Along with vector abundance, the ability of vectors to make contact with human hosts is an important component of transmission potential. Residential surveys revealed that residents believe mosquitoes to be a problem in their local residential environment and that mosquitoes did regularly enter homes. Residents engaged in personal protective behaviors like use of repellents but did not always use products with proven efficacy. Bloodmeal analysis of collected mosquitoes demonstrated that while *Ae. aegypti* fed frequently on human hosts in the outdoor environment, they also fed frequently on non-human mammmals.

Given the abundance of competent vector populations, the long-term control of arboviral diseases in New Orleans is only possible through an integrated public health approach, using rapid case identification, and effective vector control. Results from these surveys can been used to create effective outreach programs and to produce educational materials tailored to the New Orleans environment. This study identified some potential barriers in prevention and control but indicates the opportunity to educate and empower individuals to engage in behaviors to reduce mosquito exposure and disease transmission.

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X. Appendices

A. New Orleans Mosquito-borne Virus Survey – Study Flier

B. Household Questionnaire

C. Environmental Questionnaire

Appendix A: Study Flyer

Arbovirus Knowledge, Attitudes, & Practice (KAP) and Exposure Survey

Tulane University 

Principal Investigator: Sarah Michaels, MSPH, PhD student, smichael@tulane.edu
Co-Investigators: James Welty, MPH, PhD student & Rindcy Davis, MPH, PhD student
Faculty Advisor: Dawn M. Wesson, PhD

Sponsor: Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana

Purpose: The purpose of this research study is to better understand what people living in New Orleans think about mosquitoes and to assess their awareness of the diseases mosquitoes can carry. We expect to enroll 350 residents in the study from certain neighborhoods in the city (Uptown, Mid-City & Bywater).

Study Description: The following describes what will happen if you agree to be in the study:

Our research team is going door to door in certain neighborhoods in the city. If you agree to participate, a member of the team will explain the study to you. If you choose to participate, there will be a brief questionnaire which should take approximately 15 minutes to complete. The research team will also look around the outside of your yard for potential mosquito habitats and collect larvae from water-holding containers. This portion should also take about 15 minutes and can be done at the same time as the survey. We may also ask if we can place mosquito traps in your yard overnight to collect adult mosquitoes. You may participate in any part of the study and you can quit the study at any time.

Risks: The risks to you from enrolling in this study are small. You may find that certain questions make you uncomfortable. You do not have to answer any questions that you do not want to answer. At any point, you can choose to withdraw from the study.

Benefits: There are no direct benefits for study participants but you may learn more about mosquito-borne disease prevention and control strategies. The environmental survey and collection of mosquitoes may decrease mosquito exposure in your yard.

Voluntary Participation: Being part of this research is voluntary. You may choose to be part of it or not. If you choose to be part of it now, but later change your mind, you may withdraw from the study at any time.

Costs/Payment: There are no costs involved in this study.

Questions: If you have any questions about your rights as a research subject, please call the IRB Compliance Officer at (504) 988-3229. If you have questions about the study, please contact Sarah Michaels (504-352-0919), Clint Welty (575-740-4192), or Rindcy Davis (214-288-0624).

Appendix B: Household Questionnaire

New Orleans Mosquito-borne Virus Survey

HOUSEHOLD QUESTIONNAIRE

Invitation: Hi, my name is ______ I am part of a research team from Tulane University School of Public Health & Tropical Medicine. We are conducting a survey on behaviors regarding mosquitoes, risks for mosquito-borne disease and locations where mosquitoes breed in residential areas. Would you have some time to talk now or should we come back at a later, more convenient time?

VISIT	Date	Time	Result of Visit
1st	____________	____________	
2nd	____________	____________	
3rd	____________	____________	

We would like to ask you some questions about your home, members of your household, pets and thoughts on mosquitoes, mosquito-borne diseases and what you think can be done to control mosquitoes on a personal and community level. We would also like to take a look around the outside of your home for standing water and mosquitoes. We may also ask if we can leave a mosquito trap in your yard overnight. All survey results will be kept confidential.

Do you have any questions at this time? Would you like to continue with the survey?

Agree to Participate ☐ **Over the age 18** ☐

No, **refused to participate** in all parts of survey ☐

Questionnaire only ☐ Environmental Assessment only ☐

A.1	Date of visit	|____|____|-|____|____|-|____|____|____|____| *(Day)* - *(Month)* - *(Year)*

	C. DEMOGRAPHIC DATA					
B.1	Age	1 - ☐ 18-20	2 - ☐ 21-30	3 - ☐ 31-40	4 - ☐ 41-50	5 - ☐ 51-64	6 – ☐ 65+
B.2	Gender	1 - ☐ Male	2 - ☐ Female	0 - ☐ Other			
B.3	Occupation						
B.4	Education level	5 - ☐ Graduate 4 - ☐ Undergraduate 3 - ☐ High School 2 - ☐ Middle School 1- ☐ Primary 0 - ☐ None					
B.5	Type of housing	1 - ☐ House (Single family)	2 - ☐ House (Multi-family)	3 - ☐ Apart ment (single unit)	4 - ☐ Apartment complex (multi-unit)	5 - ☐ Communal	0 - ☐ Other
B.6	Do you rent or own your home?	1 - ☐ Rent	2 - ☐ Own	0 - ☐ Neither			

	C. Household Characteristics									
C.1	Household Income	1 - ☐ Under 30,000	2 – ☐ 30,000-60,000	3 - ☐ Greater than 60,000						
C.2	Number of Household members	1 - ☐ **1**	2 - ☐ **2**	3 - ☐ **3**	4 – ☐ **4**	5 - ☐ **5**	6 - ☐ **6**	7 – ☐ **7**	8 - ☐ **8**	9 – ☐ **9 +**
C.3	Last night, how many people slept in the home?	____________ person(s)								

C.4	Type of A/C	1 – ☐ Central Air	2 – ☐ Window Unit	3 – ☐ Both	0 – ☐ None	
C.5	Do you have any pets?	1 – ☐ Yes		0 – ☐ No		
C.6	Types of pets (please check all that apply below)			Other animals you have seen around your home since June 1st? (check all that apply below)		
	☐ Dogs			☐ Racoons		
	☐ Cats			☐ Squirrels		
	☐ Birds			☐ Opossums		
	☐ Rabbits			☐ Rabbits		
	☐ Chickens			☐ Rodents		
	☐ Other ________________			☐ Other ______________________		
C.7	How often do you find mosquitoes in your home?	1 – ☐ Always	2 - ☐ Frequently	3 – ☐ Sometimes	0 – ☐ Never	
	D. Environmental Characteristics					
D.1	Do you keep windows in the home open?	1 – ☐ Yes, Every day	2 – ☐ Yes, Frequently	2 – ☐ Yes, Rarely	0 – ☐ No	
D.2	If yes, how frequently do you open the windows?	1 – ☐ All day	2 – ☐ At certain times	0 – ☐ Never		
D.3	(If answered at certain times), what times during the day do you keep the windows open?	1 – ☐ Morning	2 – ☐ Afternoon	0 – ☐ Evening		
D4	During the warmer months, how many nights a week do you	______________ day (s)				

	spend outside?				
D.5	Do spend time outside for work?	1 – ☐ Yes		0 – ☐ No	
D.6	If yes, how many hours per week?	____________ hours			
D.7	Do you garden or do yard work?	1 – ☐ Yes		0 – ☐ No	
D.8	Do mosquitoes limit the time you spend outside?	1 – ☐ Yes		0 – ☐ No	
D.9	How often do you get bitten by mosquitoes?	1 – ☐ Frequently	2 - ☐ Sometimes	3 – ☐ Rarely	0 – ☐ Never
D.10	Do you take precautions to avoid mosquito bites?	1 – ☐ Frequently	2 - ☐ Sometimes	3 – ☐ Rarely	0 – ☐ Never
D.11	If yes, what precautions do you take to avoid mosquito bites?				
D.12	Do you use repellents on yourself or members of your family?	1 – ☐ Frequently	2 - ☐ Sometimes	3 – ☐ Rarely	0 – ☐ Never
D.13	Do you use Pesticides/Herbicides in the yard?	1 – ☐ Frequently	2 - ☐ Sometimes	3 – ☐ Rarely	0 – ☐ Never
D.14	Besides Pesticides/Herbicides, do you use anything else to prevent mosquitoes in your yard?	1 – ☐ Yes		0 – ☐ No	
D.15	If yes, please list what you use in your yard.				

E. Recent Travel History

E.1	Have you traveled outside of the U.S recently?	1 – ☐ Yes, within the past year		0 – ☐ No	
E.2	If yes, list the location(s)				
E.3	Was it a cruise?	1 – ☐ Yes		0 – ☐ No	
E4	Were you exposed to mosquitoes during your recent travel?	1 – ☐ Yes		0 – ☐ No	
E.5	Prior to travel, did you consult a travel clinic or health information regarding mosquito exposure?	1 – ☐ Yes		0 – ☐ No	
F. Medical History					
F.1	Have you had fever, body aches, muscle aches within the past 2 to 3 months?	1 – ☐ Yes		0 – ☐ No	
F.2	If yes, did you seek medical care?	1 – ☐ Yes		0 – ☐ No	
F.3	Did you receive a diagnosis?	1 – ☐ Yes		0 – ☐ No	
F.4	Was a blood sample taken?	1 – ☐ Yes		0 – ☐ No	
F.5	Did you have laboratory work done?	1 – ☐ Yes		0 – ☐ No	
F.6	Were you prescribed any medications?	1 – ☐ Yes		0 – ☐ No	
F.7	Do you seek medical treatment when you have a mild illness?	1 – ☐ Alway	2 - ☐ Sometim	3 – ☐ Rarely	0 – ☐ Never
F.8	Do you seek medical treatment when members of your household have a mild illness?	1 – ☐ Yes		0 – ☐ No	
F.9	Where do you go to receive treatment?	______________________________			
G. Beliefs & Attitudes toward Arboviruses					

G.1	Are mosquitoes a problem in your yard?	1 – ☐ Yes		0 – ☐ No	
G.2	Who do you think is responsible for mosquito control? (check all that apply)	1 – ☐ Homeowners	2 – ☐ Neighborhoods	3 – ☐ City/Health Department	4 – ☐ No one/don't know
G.3	What do you do when you find mosquitoes in your yard?	1 – ☐ Remove/empty containers	2– ☐ Use repellent	3 – ☐ Report to city/ 311	4 – ☐ Nothing/don't know
G.4	Do you think mosquitoes spread disease	1 – ☐ Yes		0 – ☐ No	
G.5	Have you heard of the following? (check all that apply)				
	1 – ☐ West Nile virus 2 – ☐ St. Louis encephalitis 3 – ☐ Eastern Equine Encephalitis EEE	4 – ☐ chikungunya 5 – ☐ dengue fever 6 – ☐ malaria			
G.6	How likely are you to get a virus spread by mosquitoes?	1 – ☐ Very likely	2 – ☐ Somewhat likely	3 - ☐ Not likely	0- ☐ Never
G.7	Does this make you concerned?	1 – ☐ Very concerned	2 - ☐ Somewhat concerned	3 – ☐ Not concerned	4 - ☐ Never thought about it/ Don't know
G.8	Have you ever gotten any information about mosquito control?	1 – ☐ Yes		0 – ☐ No	
G.9	If yes, where did you find the information about mosquito control?				
G.10	Would you like additional information regarding mosquitoes?	1 – ☐ Yes		0 – ☐ No	

COMMENTS:____

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Appendix C: Environmental Survey

	C. Household Description			
C.1	Assess general structural integrity of Residence (1-3):	1 - ☐ Poor condition; many holes, missing siding, rotting wood	2 - ☐ Decent condition; some holes but generally intact	3 - ☐ Good or great condition
C.2	Does the home have air conditioning?	1 – ☐ Yes	0 – ☐ No	
C.3	If yes, is the AC a central unit, window unit, or both?	1 – ☐ Central	2 – ☐ Window	3– ☐ Both
C.5	Do the windows have screens?	1 – ☐ Yes	0 – ☐ No	
C.6	If yes, condition of the windows	1 – ☐ All screens intact (no holes)	2– ☐ Some screens have holes	3 – ☐ Many screens have holes
	Notes: ________________________________			
C.7	Does the home have screens on its doors?	1 – ☐ Yes	0 – ☐ No	
C.8	If yes, condition of the door screens:	1 – ☐ All screens intact (No holes)	2 – ☐ Some screens have holes	3 – ☐ Many screens have holes
	Notes: ________________________________			
D.	**Environmental Characteristics**			

D.1	Does the property have (check if present):			
	Kennel/ dog or cat house			
	Chicken Coop			
	Containers			
	Water leak			
	Waste tires			
	Debris/ Trash			
D.2	Does the property have a maintained lawn?	1 – ☐ Yes	0 – ☐ No	
D.3	How well maintained is the Landscaping?	1 – ☐ poorly maintained, over-run with weeds	2 – ☐ well maintained, few weeds	3 – ☐ Lawn recently mowed, less than ankle deep
D.4	Does the property have a swimming pool, fountain or pond?	1 – ☐ Yes	0 – ☐ No	
D.5	Is it maintained?	1 – ☐ Yes	0 – ☐ No	
D.6	Are there domestic animals present?	1 – ☐ Yes	0 – ☐ No	
D.7	If yes, What type? (check what applies)	Number		
	1 – ☐ Dogs			
	2 – ☐ Cats			
	3 – ☐ Chickens			
	4 – ☐ Other: ____________________			

D.8	Are there wild animals present?	1 – ☐ Yes	0 – ☐ No
D.9	If yes, list animals:		Number
D.10	Is there any evidence of rodent activity?	1 – ☐ Yes	0 – ☐ No
Note location of water holding containers here (label with container #)			

House #	Container #	Container Type	Size	Larvae	Pupae	Skins	Description

COMMENTS:____

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