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An abstract of

A thesis submitted to the Faculty of the
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Abstract

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Background: Dengue, the most important mosquito borne viral disease in the world, is a major source of morbidity and mortality in tropical and temperate urban environments. While spatial clustering of the dengue vector *Aedes aegypti* has been previously studied, long term trends in clustering have not. This represents a promising area of study to help effectively target vector control in areas with limited resources.

Objective: This study longitudinally quantified the long term (over 3 years) spatial trends of Aedes aegypti clustering in the Maynas neighborhood of Iquitos, a city in the Peruvian Amazon, and determined the factors that influence a home's membership in a cluster of high mosquito abundance.

Methods: Spatial methods at the global (neighborhood) and local (household) level were applied to understand long term trends in adult and pupae clustering from 9 entomologic surveys spaced ~4 months. A GLM model was used to determine which household and environmental characteristics predicted proportion of time a household was a member of a cluster.

Results: While individual analysis of entomologic surveys did not indicate the occurrence of any apparent clustering, the proportion of time house was a cluster for adults as well as the proportion of time a house was a cluster for pupae ranged from 0-1, with some houses being members of clusters a high proportion of the time. Average kernel density across survey also showed a clear long term pattern of clustering. The best model predicting proportion of time house was a member of a cluster used household characteristics.

Discussion: Although *Ae aegypti* is highly heterogeneous and poorly predicted, overall there is a strong distribution pattern, Results from the model indicate household characteristics like water source and number of residents are good predictors of cluster membership. Future research should connect information on clusters to dengue infection, to determine if living in, or visiting a cluster raises risk of infection with dengue.

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LITERATURE REVIEW

Vector borne diseases account for significant morbidity and mortality worldwide. This is especially true for resource-poor countries, and many vector-borne diseases are also neglected tropical diseases (NTDs), which disproportionately affect poor and marginalized populations [1]. A vector is an animal or object which carries a pathogen from one host to another; typically an invertebrate arthropod [2]. For a vector borne disease to complete a cycle of transmission, a vector must feed on an infected and infectious host, undergo a pathogen incubation period, and transmit the pathogen to a new host. Seven of the 10 neglected tropical diseases targeted for control programs by WHO are VBDs [3]. Examples of the impact of vector borne diseases worldwide include Malaria, which affects roughly 247 million people each year, with nearly 1 million people dying from the disease, Chagas disease, which affects an estimated 7 to 8 million worldwide, the majority of which are concentrated in Latin America, and Lymphatic Filariasis, which affects an estimated 150 million people worldwide [3,4,5]. Emerging vector-borne diseases are also important; dengue, West Nile Virus, Chikungunya, and Lyme disease have all drastically expanded their distribution range in the last half century [6]. These diseases often have limited treatment options, and some have no cure.

Of all vector-borne diseases, dengue virus (DENV) is the most important mosquito-borne viral disease in the world [7]. An estimated 390 million cases of dengue virus infection occur annually throughout the tropical and subtropical world out of an estimated 3.97 billion people at risk [8]. Roughly 96 million of cases are apparent; 2.1

million of those cases are in the form of Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS), the more severe manifestations of the disease, [8,9,10]. In the last twenty years, dengue fever epidemics have increased in number and magnitude, due to the dramatic range expansion of *Ae. aegypti* and the global propagation and mixing of the four DENV viral strains [11]. Many factors have influenced the spread of dengue, among them population increases, travel, and urbanization [7].

Dengue is caused by 4 related but distinct viral strains or serotypes of the genus *Flavivirus*, family Flaviridae [11]. Manifestations of dengue infection can range from asymptomatic infection to nonspecific fever or classical Dengue Fever (DF) [12]. Symptoms of classic DF include severe headache and pain behind eyes, joint pain, muscle and bone pain, and rash [13]. Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS), the most serious manifestations, also include hemorrhagic manifestations, severe abdominal pain and persistent vomiting, as well as shock, and may be life threatening without medical treatment [13]. Untreated, a DHF or DSS infection has a case fatality rate of 30-40%, but with medical treatment the case fatality rate is less than 1% [13]. Infection confers serotype-specific partial immunity, but only to the strain of virus that caused the infection. A person who has had one strain of the virus is actually more susceptible to other strains[11]. This may be due in part to antibody-dependent enhancement, where antiviral antibodies actually enhance viral entry into a host cell [11]. Antibody-dependent enhancement occurs when, after having been previously infected with on serotype of dengue, a person becomes infected after some time with a different serotype [11]. This is caused by the highly cross-reactive nature of antibodies between serotypes [14]. This results in higher viral loads, and more serious manifestations of the disease. Because of this, a person may be infected with dengue up to 4 times.

DENV is transmitted primarily by the vector *Aedes aegypti,* a daytime biting and highly anthropophilic mosquito species. Incubation of the virus lasts 3 to 15 days in humans, after which symptoms such as acute fever, headache myalgia and fatigue manifest [12]. Mosquitoes become infective after they bite an infected human and undergo an extrinsic incubation period lasting from 7 to 14 days, depending on ambient temperature [15]. Currently, there is no effective treatment of dengue, and while there are vaccines being researched, it will be years before an effective one is found [9,16]. In the absence of other tools, vector control is the only effective method for preventing virus transmission [17].

The mosquito *Ae. aegypti* plays a prominent role in the transmission of dengue. *Ae. aegypti* is highly adapted to the human environment; it predominantly breeds in artificial containers such as plastic containers found in and around the home, prefers to rest in cool, dark areas including inside the home, and feeds almost exclusively on humans [7]. Outdoor passive rain-filled containers are the most productive sources of pupae [18]. Female *Ae. aegypti* seldom disperse beyond 100m, so adult mosquitoes are likely to stay in close proximity to the larval habitats from which they emerged [19,20,21].

In absence of other tools, vector control has shown the most promise in impacting transmission of dengue (Morrison and Scott 2010). Current vector control efforts are largely similar to the same methods that were used to eliminate Yellow Fever in Panama in 1904 [22]. These include container reduction, treatment of containers with larvicide to kill immatures, vector surveillance, and emergency application of ULV (ultra low volume) space sprays in the home. Vector control of *Ae. aegypti* faces many more challenges than control of Anopheline and Culex mosquitoes due to the ineffectiveness of larger insecticide spray campaigns that use trucks and aerial sprays, the emergence of insecticide resistance in adult *Ae aegypti*, and the challenges of reaching and covering all households at risk in an urban area [23]. Vector control must be done in the home for *Aedes* due to its adaptations to the human lifestyle. Other vector control methods that are currently being investigated for *Ae. aegypti* include genetically modified mosquitoes and insecticide treated materials, however these interventions have yet to prove effective [16]. Vector surveillance is an essential part of vector control and long term understanding of dengue transmission. For dengue, the goal of vector control is to lower the mosquito population down to levels where virus transmission cannot occur. However dengue presents a challenge in that this level is still unknown. There is no entomological measure to predict dengue risk like the entomological inoculation rate for malaria[15]. By improving surveillance, the connection between mosquito densities and transmission can be better understood.

Ae. aegypti abundance is spatially heterogeneous, and some areas are more likely to produce more mosquitoes than others [24,25]. Understanding the spatial and temporal distribution of *Ae. aegypti* is essential for controlling transmission of DENV, because knowledge about long-term vector presence and abundance can help inform local public health and vector control agencies on the locations of potential transmission hot-spots [24,26,27] . Additionally, by understanding the distribution of environmental variables and vectors, predictions about future vector abundance can be made [28]. However, effective predictions that accurately predict infestation are rare, due to the skill and understanding of ecological context necessary to create them [28].

One promising aspect of the application of spatial analysis methods to vector control is that of identifying hotspots of vector production that can be targeted for control. The majority of this research on identifying and targeting hotspots has been done on hotspots in connection to malaria, although research concerning the efficacy of spatial targeting of Chagas disease also exists [29,30,31]. Malaria researchers have identified that stable hotspots of asymptomatic parasitemia and unstable hotspots of febrile malaria prevalence exist, as well as stable hotspots in areas of low level transmission [32]. There is also evidence on a theoretical level that targeting hotspots of mosquito production could have significant impact on the cost effectiveness of vector control interventions [31]. Chagas researchers also show the theoretical promise of spatial targeting, both logistically and economically, in areas where full coverage cannot be achieved [30]. These findings would support the development of a vector control program that utilizes targeting of hotspots. However, it is important to note that many challenges to identifying hotspots exist, specifically the ambiguity in definition of what a hotspot is, with definitions ranging from an entire country to a household [24,31]. Additionally, while these theoretical studies about spatial targeting show promise, none have empirically demonstrated these results. While several studies have looked at dengue hotspots, none have attempted to spatially target vectors, beyond classic vector control of houses within 100 meters of a case.

While understanding spatial and temporal distributions of *Ae. aegypti* is important for effective disease control, current research on long-term spatial analysis of clustering of *Ae. aegypti* is limited; most studies are either concurrent with a dengue virus epidemic or cross-sectional [33,34]. Little information exists at fine spatial (e.g. household) and temporal scales (e.g. multiple time points longer than a year), however the limited analysis that has been done indicates that this is the appropriate scale at which to study *Aedes aegypti* spatial clustering [33]. Findings of *Ae. aegypti* cluster analysis have been mixed; Getis et al. found that adult *Ae. aegypti* clustered up to 30 meters while pupae did not cluster beyond the household, while Melo et al found that *Ae. aegypti* eggs clustered from 200 to 800 meters [24,35]. Additionally, *Aedes aegypti* abundance is highly variable over short time periods[24,25]. Koenraadt et al found that areas treated with insecticide spray to control *Ae. aegypti* recovered 50 percent after only one week [36]. One study looking at spatial stability of clusters found significant spatial correlation in rank order of mosquito abundance per trap, however only one year of data was analyzed [25]. Using a longer period of time for analysis may elucidate more stable trends in vector abundance. By analyzing entomologic data at fine spatial and temporal scales, long-term patterns of distribution can be better understood, which is essential for the development of interventions targeting transmission hot-spots.

In order to better predict and prevent future dengue outbreaks, it is important to understand whether consistent hotspots of *Aedes aegypti* infestation exist, and to identify what underlying factors are associated with their occurrence. Therefore, this thesis aims to understand long-term patterns vector distribution, as well as the underlying factors that account for these patterns, in a well-studied neighborhood in the city of Iquitos, Peru. This is achieved by first estimating the long-term (over 3 years) pattern of spatial clustering of *Aedes aegypti* abundance within two study neighborhoods in Iquitos, Peru.

Next, the major drivers of presence (or absence) of *Aedes aegypti* clusters, both within and between neighborhoods are defined. Last, using the information gathered, a predictive model of *Aedes aegypti* clustering is developed at the neighborhood level. Understanding temporal trends in vector abundance and clustering has the potential to help improve understanding of disease transmission and potentially inform local public health and vector control agencies where to target their vector control efforts.

ROLE

For this manuscript, the author was responsible for the data analysis, writing of all sections, and the development of the tables and figures. Data was collected by other researchers previously involved in the project.

RESEARCH MANUSCRIPT

PLoS Neglected Tropical Diseases

TITLE

Long-term trends in the spatial clustering of *Aedes aegypti* infestation within a tropical urban environment

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ABSTRACT

Background: Dengue, the most important mosquito borne viral disease in the world, is a major source of morbidity and mortality in tropical and temperate urban environments. While spatial clustering of the dengue vector *Aedes aegypti* has been previously studied, long term trends in clustering have not. This represents a promising area of study to help effectively target vector control in areas with limited resources.

Objective: This study longitudinally quantified the long term (over 3 years) spatial trends of Aedes aegypti clustering in the Maynas neighborhood of Iquitos, a city in the Peruvian

Amazon, and determined the factors that influence a home’s membership in a cluster of high mosquito abundance.

Methods: Spatial methods at the global (neighborhood) and local (household) level were applied to understand long term trends in adult and pupae clustering from 9 entomologic surveys spaced ~4 months. A GLM model was used to determine which household and environmental characteristics predicted proportion of time a household was a member of a cluster.

Results: While individual analysis of entomologic surveys did not indicate the occurrence of any apparent clustering, the proportion of time house was a cluster for adults as well as the proportion of time a house was a cluster for pupae ranged from 0-1, with some houses being members of clusters a high proportion of the time. Average kernel density across survey also showed a clear long term pattern of clustering. The best model predicting proportion of time house was a member of a cluster used household characteristics.

Discussion: Although *Ae aegypti* is highly heterogeneous and poorly predicted, overall there is a strong distribution pattern, Results from the model indicate household characteristics like water source and number of residents are good predictors of cluster membership. Future research should connect information on clusters to dengue infection, to determine if living in, or visiting a cluster raises risk of infection with dengue.

INTRODUCTION

Dengue virus (DENV) is the most important mosquito-borne viral disease in the world [7]. An estimated 390 million cases of dengue virus infection occur annually throughout the tropical and subtropical world out of an estimated 3.97 billion people at risk [8]. Roughly 96 million of cases are apparent; 2.1 million of those cases are in the form of Dengue Hemorrhagic Fever (DHF) or Dengue Shock Syndrome (DSS), the more severe manifestations of the disease, [8,9,10]. In the last twenty years, dengue fever epidemics have increased in number and magnitude, due to the dramatic range expansion of *Ae. aegypti* and the global propagation and mixing of the four DENV viral strains [11]. Many factors have influenced the spread of dengue, among them invasion of multiple serotypes, population increases, travel, and urbanization [7]. Currently, there is no effective treatment of dengue, and while there are vaccines being researched, it will be years before an effective one is found [9,16]. In the absence of other tools, vector control is the only effective method for preventing virus transmission [17].

DENV is transmitted primarily by the vector *Aedes aegypti,* a daytime biting and highly anthropophilic mosquito species. *Ae. aegypti* is highly adapted to the human environment; it breeds in artificial containers such as plastic containers found in and around the home, prefers to rest in cool, dark areas including inside the home and preferentially feeds from humans [7]. Outdoor passive rain-filled and often unattended containers are the most productive sources of pupae [18]. Female *Ae. aegypti* seldom disperse beyond 100m, so adult mosquitoes are likely to stay in close proximity to the larval habitats from which they emerged [19,20,21]. Empiric evidence from both entomologic field surveys and population genetics studies support the notion that *Ae.*

aegypti abundance is spatially heterogeneous, and that some areas are more likely to produce more mosquitoes than others [24]. Understanding the spatial and temporal distribution of *Ae*. *aegypti* is essential for controlling transmission of DENV, because knowledge about long-term vector presence and abundance has the potential to help inform local public health and vector control agencies on how to target their vector control efforts \ [24,26,27]. Additionally, by understanding the distribution of environmental variables and vectors, predictions about future vector abundance can be made [28].

While understanding spatial and temporal distributions of *Ae. aegypti* is important for effective disease control, current research on long-term spatial analysis of clustering of *Ae*. *aegypti* is limited; most studies are either concurrent with a dengue virus epidemic or cross-sectional[33,34]. Little information exists at fine spatial (e.g. household) and temporal scales (e.g. multiple time points longer than a year), however the limited analysis that has been done indicates that this is the appropriate scale at which to study *Aedes aegypti* spatial clustering [33]. Findings of *Ae. aegypti* cluster analysis have been mixed; Getis found that adult *Aedes aegypti* clustered up to 30 meters while pupae did not cluster beyond the household, while Melo et al found that *Ae. aegypti* eggs clustered from 200 to 800 meters [24,35]. Additionally, *Aedes aegypti* abundance is highly variable over short time periods[24,25]. Koenraadt et al found that areas treated with insecticide spray to control Ae. aegypti recovered in adult abundance 50 percent after only one week [36]. One study looking at spatial stability of clusters found significant spatial correlation in rank order of mosquito abundance per trap, however only one year of data was analyzed [25]. Using a longer period of time for analysis may elucidate more stable trends in abundance. This study aims to understand long-term patterns of vector distribution by estimating the long-term (over 3 years) pattern of spatial clustering of *Ae. aegypti* abundance within a well defined neighborhood of the city of Iquitos, Peru. Next, the major drivers of presence (or absence) of *Aedes aegypti* clusters were identified by developing a predictive model of *Aedes aegypti* clustering at the neighborhood level. Understanding vector presence and abundance will help inform local public health and vector control agencies decide where to target their vector control efforts.

MATERIALS AND METHODS

Data:

***Study area*:** This study was took place in the city of Iquitos, the largest urban center in the Peruvian Amazon. The location of the city can be seen in Figure 1. The city is surrounded on three sides by the Amazon, Nanay, Itaya rivers. The city is only accessible from other parts of the country by air or water. This results in a city of roughly 370,000 inhabitants that is geographically isolated [37]. Figure 1. The city is only accessible from other parts of the country by air or water. Human demography, *Ae. aegypti* entomological indices, and dengue epidemiology for Iquitos have been studied in detail [18,24,26,37,38,39]. Over the last 12 years, Iquitos has been a field site for various long-term epidemiological research studies through the University of California-Davis-Emory University entomology field station and the U.S. Naval Medical Research Unit (NAMRU-6). While a successful campaign by the Pan American Health Organization (PAHO) led to the elimination of *Aedes aegypti* in the Peruvian Amazon in the mid-twentieth century, a reduction in vector control programs that followed this success led to the reintroduction of *Ae. aegypti* in the 1960s and dengue fever in the 1980s [40].

Within Iquitos, Maynas was the neighborhood of focus. As seen in figure 1, the Maynas neighborhood is located in Punchana district in the north central part of the city [18,41]. The Maynas neighborhood is older, more urbanized, and in general wealthier than other neighborhoods in Iquitos, though economic prosperity in the neighborhood varies widely [24,39]. However, Maynas has also historically had significantly more *Aedes aegypti* than other neighborhoods in Iquitos. One reason for this is that problems with reliability of the water supply have been a problem in the past for this neighborhood, so residents have been more likely to store their water [24]. Source of water varies widely at the household level; some homes have piped water, while others have neither water nor sewer services [24]. In addition to high *Ae. Aegypti* densities, the Maynas neighborhood has also historically had one of the highest seroprevalence rates in the city [38]. This paper expands on a previous study by Getis [24] in the same neighborhood, and looks to see whether trends from this previous study hold true over longer time periods.

Data collection and management

Data for this study was collected using standardized entomological surveys performed ~ 4 months from 2009 to 2012 comprising 9 survey “circuits”. All houses (645 in Maynas) were painted with a unique house code [24]. Unoccupied or closed houses, as well as houses that did not give permission, schools, businesses and offices were not surveyed. An attempt to survey a house was made a minimum of 3 times per circuit.

Entomological data collection:

Immatures and adults were surveyed because the pupal index is highly correlated with adult production (Morrison et al 2010). The survey was conducted in teams of two, where one person conducted the demographic survey while the other looked for aspirated for adults. Adults were aspirated using a Prokopack mosquito aspirator (Vazquez-Prokopec et al. 2009). Survey teams were rotated daily to limit temporal and collector biases [41]. Adult aspirations were conducted in each room of the house as well as outside the house [41]. Upon completion of the demographic survey the other team member looked in potential *Ae. Aegypti* development sites for water, larvae and pupae. All containers found to be holding water were measured, classified, and scored for sunlight exposure, fill method (actively via faucet or passively by rain), and presence of a cover. If immatures were found, pupae were collected for processing at the field laboratory.

Survey data was imported into ArcGIS and georeferenced using the geographic coordinates of the unique house codes. The coordinate system and datum used were Universal Transverse Mercator and WGS -84. There were roughly 6,190 data points in the Maynas neighborhood. Each point was identified by coordinates, district, neighborhood, block number, location code as well as survey circuit and date. This study made use of the Geographic Information System(GIS) created by the Naval Medical Research Detachment (NMRD) and Amy Morrision for the city of Iquitos.

Analysis

Spatial Analysis

To estimate long-term patterns of *Aedes aegypti* adult and pupae, spatial distributions in Maynas spatial statistical analysis methods were applied. First, clustering in each circuit was estimated. Spatial analysis utilized ArcMap 10.1, and PPA, two recognized spatial analysis programs. The statistical package SAS was also used for regression. Pupae were analyzed as a measure of productivity, while adults were analyzed as a measure of entomologic risk.

Clustering

To assess clustering, first, a global weighted k-function was calculated with PPA. Global WK-function was used to detect global spatial clustering in individual-level data. Global WK-function is a second order analysis. There are multiple tests available to estimate this. L(d), the calculated K-function is compared to the expected K-function L(h), calculated using Monte Carlo randomizations of the data. Weighted k-function analysis was used to determine if weights or values at each point were clustered, dispersed, or random within the pattern of points.

Next, local Gi*(d), was calculated using PPA. This is also called hotspot analysis. Local clusters are clusters that occur at some locations. Local Gi*(d) was calculated for pupal and adult abundance. Local Gi*(d) identifies individual cluster members. Gi* takes each house as a center, and searches outwards from the center to find if nearby houses have more or less than expected mosquitoes. Clusters occur when greater than expected numbers of mosquitoes occur near each other. From this, houses which are members of clusters as well as houses which are not are identified, using a z-score of 2.575 as a cutoff for cluster membership, as per Getis et al 2003. This information is used to identify cluster membership for the model that is constructed. The distance at which the z-score peaked for each cluster member was also calculated.

Additionally, to estimate temporal patterns in clustering, clusters found using Getis Gi* were summed across circuits, then divided by number of times house was surveyed, as per Barrera (2011), to determine which areas have consistent clusters.

Kernel density

To assess trends over the entire collection period, kernel density estimates were first derived for each circuit using interpolation via a bandwidth of 100 meters [42]. This technique is dependent on the choice of bandwidth: a bandwidth that is too small will not offer much for interpolation if points are too far apart, while a bandwidth that is too large will include spurious data that is unrelated in estimates. This bandwidth was selected based on the median of the highest k-function values found at each circuit. To assess a pattern of high kernel density, all calculated kernel densities were averaged together into a new raster for adults and pupae. This analysis is a visual representation of the combined kernel densities from each circuit.

Modeling

A generalized linear model (GLM) was constructed to study cluster membership. The GLM takes into account correlated data structures, which is appropriate in this case because longitudinal and clustered data is being used. Using multi model selection, the best model was defined. Two models were constructed; one to predict the proportion of time a house was a cluster for pupae, and another for adults.

Environmental and Household Covariates

Environmental and household characteristics used to predict outcome included NDVI, temperature, rainfall, number of household residents, water source, and number of containers. Normalized Difference Vegetation Index (NDVI) was also calculated for each block over the study area, and an average NDVI value for each city block was calculated. Average daily temperature and rainfall over the collection period were calculated for each circuit. Results from the demographic survey were used to calculate average number of household residents, proportion of time households used rainwater and/or faucet, and average number of water-holding containers.

The full model was

Model: $y = \text{Intercept} + \beta_1 * \text{NumberContainers} + \beta_2 * \text{Temperature} + \beta_3 * \text{NDVI} + \beta_4 * \text{NumResidents} + \beta_5 * \text{WaterSourceRain} + \beta_6 * \text{WaterSourceFaucet}$

RESULTS

Survey Results and Neighborhood Characteristics

Over the course of all 9 circuits, 3,731 house entomological surveys were performed. A total of 645 unique houses were surveyed, with an average of 415 (standard deviation, SD, 40) houses surveyed in each circuit. Seventy-seven percent of buildings surveyed were residential. Surveys lasted on average 70 days, but to reach as many houses as possible some surveys lasted longer. After full survey of Maynas households, the collection team would return to try to sample closed houses, which is why some survey circuits were longer. On average, each house was surveyed 5.8 (SD 2.7) times. The average number of people living in a house was 6 (SD 3.1). As previously mentioned, water source has historically been unstable in the Maynas neighborhood, and percentage of households using two of the most important water sources (faucet and rain water), ranged from 91 to 95 percent for faucet, and 2 to 7 percent for rain water. Use of these two water sources was not mutually exclusive, so a third measure, number of water holding containers per household, was also important, and households had an average of 2.8 (SD 2) containers. Almost all (98.9%) of houses had at least 1 container throughout the study period. Temperature, another significant influence on mosquito development, was fairly stable throughout the circuits, ranging from 26.2 to 27.5 degrees.

On average, 0.9 (SD 4.6) adults were found per house, as well as 0.8 (SD 11.2) pupae, however there was considerable range in these numbers (see Figures 2 and 3). The number of adults collected per house ranged from 0 to 163, and the number of pupae collected per house ranged from 0 to 433. An average of 69.8 (SD 12.9) percent of houses per survey had no adults, while 96.6 (SD 1.5) percent of houses had no pupae. There was no obvious pattern to both pupal and adult prevalence. As seen in Figure 4 there was a weak negative correlation (-0.43) between total number of adult females and pupae found per circuit, while in Figure 5 there was a weak positive correlation (0.33) between number of adult females and number of pupae captured per house.

Global statistics

K Function

Clustering occurred during all survey circuits for both pupae and adult presence. For presence of pupae, on average clustering occurred up to 288.9 (SD 82.1, Median 250, LQ250-UQ300) meters. Clustering peaked for pupae on average at 133.3 (SD 70.7, Median 100, LQ100-UQ100) meters. Alternatively, for presence of adults average maximum distance of clustering was 255.6 (SD 30, Median 250, LQ250-UQ250) meters. Clustering peaked for adults on average and median distance 100 (SD 25, Median 100, LQ100-UQ100) meters. Weighted K functions were also performed for abundance of adults and pupae, and can be seen in Table 3. Clustering of abundance was much less apparent, with number pupae only clustering in 1 circuit (5), from 250 to 300 meters, peaking at 250 meters. For number of adults, clustering was found during 3 survey circuits (3, 4, and 8). In circuit 3, clustering of number of adults occurred at 150 meters, while for circuits 4 and 8 clustering of number of adults occurred at 50 meters.

Local Statistics

Clusters

Hotspot analysis maps for pupae and adults can be found in Figures 6 and 7. On average, 11.3 percent of households were a member of a cluster for pupae, and 14 percent were a member for adults. There was no obvious consistent temporal pattern to both pupae and adult clusters. The proportion of vectors found within cluster households also did not appear to follow a consistent temporal trend. On average, 72.2 (SD 15.3) percent of all pupae and 43.4 (SD 12.5) percent of adults were found in households that were a member of a cluster.

In addition to whether or not a household was a cluster, the distance at which the z-score of the household peaked was also recorded, as seen in Figures 8 and 9. Average peak distance of clusters of adults was 47.75 (SD 10.9) meters, while average peak distance of clusters pupae was 42.2 (SD 7.3) meters (seen in Figures 10 and 11). Neither the average peak distance of pupae nor adults followed the expected seasonal trend, though pupae and adult values were highly correlated (0.78) with each other.

To see if some areas were consistently a member of a cluster, proportion of time house was a member of a cluster was calculated. Proportion of circuits a house was a cluster can be seen for pupae in Figure 12, and for adults in Figure 13. There is a clear spatial pattern of areas that are members of a cluster a high proportion of the time, with the majority of high proportion (0.668-1.000) households for both adults and pupae being located in two adjacent blocks.

Kernel Density Estimation

Kernel density estimation utilized a bandwidth of 100 meters for both adults and pupae, based on the median peak distance of clustering from the K-function analysis. Output from Adults and pupae can be viewed in Figures 14 and 15. Output of the kernel density estimation closely mirrored output of the Gi*(d), however it also provided an estimation of vector density. Values varied widely between circuits. For adults, values ranged from 0.003 adults per meter squared during circuit 1 (March 2009), compared to up to 0.03 adults per meter square during circuit 4 (February 2010). Values for pupae ranged from 0.004 pupae per square meter in circuit 5 (July 2010) up to 0.042 pupae per square meter during circuit 3 (November 2009). For averaged kernel densities, shown in Figures 16 and 17, the densities of adults and pupae clearly highlighted two different blocks in the Maynas neighborhood. These two blocks are the same adjacent blocks with high values found by calculating the proportion of time a house was a cluster.

Model

For both pupae and adults, 4 models were compared: the full model, an environmental model (NDVI and temperature), a household model (number of residents, use of rain water, and use of faucet water), and an entomological model (number of containers). The results of the full model can be seen in Table 4. The best model, determined using Akaike’s Information Criterion (AIC), was the household model, seen in table 5. The number of residents and the use of water from faucet were both positively and significantly associated with both the number of pupae and adults (Table 5). Houses which collected rainwater for consumption were not statistically associated with *Ae. aegypti* hotspots, but their inclusion increases the model's fit to the data (Table 5).

NDVI and average temperature were not significant for both pupae and adult outcomes in both the full model as well as the environmental model. Number of containers was significant for both pupae and adult outcomes at the 0.01 level in the entomological model, but not the full model.

DISCUSSION

The goal of this study was to understand long term trends in *Aedes aegypti* spatial distribution and clustering. There was a clear pattern of clustering over the three year time period studied. At the global or neighborhood level, data from the Maynas neighborhood showed consistent trends in both the distance up to which clustering occurred, as well as the distance at which clustering peaked. At the local level, while significant clusters existed, little to no pattern of cluster membership of households was initially found by looking at individual circuits. The temporal distribution of pupae and adults was highly irregular. However, by looking at the proportion of survey circuits a house was a cluster over the study period, a clear pattern of cluster location could be found for both pupae and adults. The longitudinal aspect of this analysis is a significant improvement over previous studies. The high temporal variability and instability of survey results from circuit to circuit, means that is essential to analyze data longitudinally to identify key premises for *Aedes aegypti* production.

It was essential to perform analysis for both adult females and pupae. Total numbers of pupae and adult females found per survey were only weakly correlated. Pupal and adult surveys operate on different assumptions. While it is easy to tell where pupae were produced because they are found within the same container that they were laid in, pupae are only a proxy for the actual number of females that will emerge and be able to transmit the virus. Female adult collections cannot be used on their own either; while the current aspiration techniques used are the most effective for finding adult females, there is no gold standard for collection of adults. Adult aspirations still only find a portion of all adult females in the home, and thus do not represent the complete population of biting females.

Despite the heterogeneity between adult female and pupae spatial distributions, consistent temporal patterns in clustering and cluster locations were found between adult females and pupae for most of the analysis. This is evidenced by the similar households that were a member of a cluster a high proportion of the time for both pupae and adults. These households should be identified as key premises for mosquito infestation. It can also be seen in the similar results of the k-function analysis, specifically the maximum distance households were clustered to, as well as the average distance at which clustering peaked for both pupae and adults.

Some findings from spatial analysis were surprising. Both clustering and size of clusters did not follow an expected seasonal trend. This may be because cluster size is independent of seasonality. However this also may be because Iquitos does not have conventional seasons, but is rather characterized by a “wet” and “dry” season. It may be worthwhile to use height of the nearby rivers as an indicator of Iquitos’ seasons.

Relatively few studies have calculated the spatial extent of clusters, due to the time and resource intensity of data collection, and the distance of clustering is likely to change dependant on different environmental conditions and household characteristics. Getis et al, looking at clustering, also found significant temporal variability short-term between two surveys, but did not look at long term trends. Getis also found that clustering disappeared after 30 meters, which is different from what was found in this study. Barrera found hotspots of infestation of adults that showed spatial stability over time, but looked on a coarser scale, and for less time.

This study is an improvement over previous studies in that it has studied clustering longitudinally, over a longer period of time than others. These results have significant implications for vector control. By looking at areas that are consistently a member of a cluster for pupae and adults, vector control can be targeted. The presence of areas that produce a significant portion of all mosquitoes, or key premises, may indicate that focal control of persistent clusters may be warranted. This relates to work by Chadee, identifying the role of key premises of *Aedes aegypti* production in serving as sources for introduction and re-infestation [43].

The results of the GLM models showed that household variables were most important for predicting a household's membership in a cluster a high proportion of the time. Human density has already been connected with production of *Aedes aegypti* [44]. It was surprising that use of faucet water had a positive effect on cluster membership, as it was originally predicted to have a negative effect on membership. Use of a faucet should indicate that residents do not need to store their water, cutting down on the number of water containers in which *Aedes* can lay eggs. This indicates that it may be more appropriate to investigate the water source stability as a predictor of cluster membership instead.

While this study had rich household data, some environmental data was only available at coarse spatial or temporal scales, which may account for why it was not significant in any of the models. NDVI was limited in spatial and temporal scales, and could only be calculated once at the block level. Previous studies have found NDVI significantly associated with *Ae. aegypti* abundance, so it is likely that obtaining better data would improve the model as well, though Iquitos' proximity to the equator may render this irrelevant [45,46,47]. Additionally, temperature data was available only at the city level, measured several kilometers from the study site. As the Maynas neighborhood is in the "downtown" area of Iquitos, it is possible that the neighborhood exists in a heat island. This has been hypothesized for other insects and vectors of disease [48,49]. Rainfall data was also only available at the city level. As ambient temperature and rainfall are significantly connected to speed of mosquito development and extrinsic incubation period length for dengue, it is worth exploring whether improving the spatial and temporal scale of environmental data may improve models predicting *Ae. aegypti* abundance and household membership in a cluster.

While there were many promising findings from this study, these results are limited due to the size of the study area, so results will not be generalizable to other areas. While the trends present in the Maynas neighborhood are significant, considerable differences exist in infestation levels even within Iquitos, let alone globally. It is essential that this type of analysis is repeated in other environments as well to understand if global trends in long term clustering occur in other areas as well. Within Iquitos, another neighborhood, Tupac Amaru, has been surveyed alongside Maynas. Maynas is different in terms of many neighborhood and household characteristics, and has historically experienced lower infestation than Maynas [24]. It would be worthwhile to repeat the analyses on this neighborhood, to see if similar trends are found.

Currently, dengue research is moving towards studying the ecology of the disease system over individual aspects of transmission in depth. In this case we have only looked at the dengue vector, but it is essential to connect this research to infection data. Understanding hotspots of *Aedes aegypti* clustering over time may help understanding of virus transmission, by showing which locations put individuals at the highest risk for infection. Based on results of this study we propose to connect infection data to see whether people living in *Aedes aegypti* hotspots have higher infection prevalence.

It is also becoming increasingly clear that dengue infection does not necessarily occur only in the home. People who visit a high mosquito abundance area are also likely at increased risk for infection. This connects to new research being done by Stoddard et al. on the impact of human movement on dengue infection risk, aiming to understand how a person's interactions in space put them at increased risk for dengue [37].Their research indicates human movement is significant in propagating dengue transmission. We also propose to connect human movement data to see whether visitation to areas within *Aedes aegypti* hotspots is significantly connected to infection.

REFERENCES

1. Gratz NG (1999) Emerging and resurging vector-borne diseases. Annual Reviews of Entomology 44: 51-75.
2. (2002) Vector-Borne Diseases. In: Breslow L, editor. Encyclopedia of Public Health. New York: Macmillan Reference USA.
3. Eisen L, Eisen RJ (2011) Using Geographic Information Systems and Decision Support Systems for the Prediction, Prevention, and Control of Vector-Borne Diseases. Annual Reviews of Entomology 56: 41-61.
4. Organization WH (2013) Chagas Disease (American Trypanosomiasis).
5. Organization WH (2013) Lymphatic Filariasis- Epidemiology.
6. Gubler D (2008) The Global Thread of Emergent/Reemergent Vector-Borne Diseases. In: Lemon SM, Sparling PF, Hamburg MA, Relman DA, Choffnes ER et al., editors. Vector-Borne Diseases: Understanding the Environmental, Human Health, and Ecological Connections, Workshop Summary (Forum on Microbial Threats) Washington, D.C.: Institute of Medicine. pp. 43-64.
7. Halstead S (2008) Dengue virus-mosquito interactions. Annual Reviews of Entomology 53: 273-291.
8. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, et al. (2013) The global distribution and burden of dengue. Nature.
9. Kyle J, Harris E (2008) Global spread and persistance of dengue. Annual Reviews of Microbiology 62: 71-92.
10. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, et al. (2012) Refining the Global Spatial Limits of Dengue Virus Transmission by Evidence-Based Consensus. PLoS Neglected Tropical Diseases 6: e1760.
11. Rothman AL (2011) Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. Nature Reviews 11: 532-541.
12. Mathew A, Rothman AL (2008) Understanding the contribution of cellular immunity to dengue disease pathogenesis Immunological Reviews 225: 300-313.
13. Control CfD (2012) Symptoms and Treatment: Dengue.
14. Dejnirattisai W, Jumnainsong A, Onsirisakul N, Fitton P, Vasanawathana S, et al. (2010) Cross-Reacting Antibodies Enhance Dengue Virus Infection in Humans. Science 328: 745-748.
15. Scott TW, Morrison AC (2010) Longitudinal FIeld Studies Will Guide a Paradigm in Dengue Prevention. In: Atkinson P, editor. Vector Biology, Ecology, and Control: Springer.
16. Vazquez-Prokopec GM (2011) Dengue control: the challenge ahead. Future Microbiology 6: 251-253.
17. Reiter P, Gubler D (1997) Surveillance and control of urban dengue vectors. In: Gubler D, Kuno G, editors. Dengue and dengue hemorrhagic fever. Wallingford, UK: CAB International.
18. Morrison A, Astete H, Gray K, Getis A, Focks D, et al. (2004) Spatial and Temporal abundance patterns of Aedes aegypti producing containers in Iquitos, Peru. Journal of Medical Entomology 41: 1123-1142.

19. Harrington L, Scott TW, Lerdthusnee K, al. e (2005) Dispersal of the dengue vector Aedes aegypti within and betweeen rural communities. American Journal of Tropical Medicine and Hygiene 72: 209-220.
20. Morlan H, Hayes R (1958) Urban Dispersal and activity of Aedes aegypti. Mosquito News 18: 137-144.
21. Edman J, Scott T, Costero A, Morrison A, Harrington L, et al. (1998) Aedes aegypti (Diptera: Culicidae) movement influenced by availability of oviposition sites. Journal of Medical Entomology 35: 578-583.
22. Gubler D (1998) Resurgent Vector-Borne Diseases as a Global Health Problem. Emerging Infectious Diseases 4: 442-450.
23. Esu E, Lenhart A, Smith L, Horstick O (2010) Effectiveness of peridomestic space spraying with insecticide on dengue transmission; systematic review. Tropical Medicine and International Health 15: 619-631.
24. Getis A, Morrison AC, Gray K, Scott TW (2003) Characteristics of the spatial pattern of the dengue vector Aedes aegypti, in Iquitos, Peru. American Journal of Tropical Medicine and Hygiene 69: 494-578.
25. Barrera R (2011) Spatial Stability of Adult *Aedes aegypti* Populations. American Journal of Tropical Medicine and Hygiene 85: 1087-1092.
26. Wong J, Stoddard ST, Astete H, Morrison AC, Scott TW (2010) Oviposition Site Selection by the Dengue Vector Aedes aegypti and Its Implications for Dengue Control. PLoS Neglected Tropical Diseases 5: e1015.
27. Eisen L, Lozano-Fuentes S (2009) Use of Mapping and Spatial and Space-Time Modeling Approaches in Operational Control of Aedes aegypti and Dengue. PLoS Neglected Tropical Diseases 3: e411.
28. Reisen B (2010) Landscape Epidemiology of Vector Borne Diseases. Annual Reviews of Entomology 55: 461-483.
29. Carter R, Mendis KN, Roberts D (2000) Spatial targeting of interventions against malaria. Bulletin of the World Health Organization 78: 1401-1411.
30. Vazquez-Prokopec GM, Spillman C, Zaidenberg M, Gurtler RE, Kitron U (2012) Spatial Heterogeneity and Risk Maps of Community Infestation by Triatoma infestans in Rural Northwestern Argenitna. PLoS Neglected Tropical Diseases 6: e1788.
31. Bousema T, Griffin J, Sauerwein R, Smith D, Churcher T, et al. (2012) Hitting Hotspots: Spatial Targeting of Malaria for Control and Elimination. PLoS Med 9.
32. Bejon P, Williams TN, Liljander A, Noor AM, Wambua J, et al. (2010) Stable and Unstable Malaria Hotspots in Longitudinal Cohort Studies in Kenya. PLoS Medicine 7: e1000304: 1000301-1000314.
33. Yoon I-K, Getis A, Alstadt J, Rothman A, D T, et al. (2012) Fine Scale Spatiotemporal Clustering of Dengue Virus Transmission in Children and Aedes aegypti in Rural Thai Villages. PLoS Neglected Tropical Diseases 6: e1730.
34. Vazquez-Prokopec GM, Kitron U, Montgomery B, Horne P, Ritchie SA (2010) Quantifying the Spatial Dimension of Dengue Virus Epidemic Spread Within an Urban Environment. PLoS Neglected Tropical Diseases 4: e920.
35. Melo DPOd, Scherrer LR, Eiras AE (2012) Dengue Fever Occurrence and Vector Detection by Larval Survey, Ovitrap and MosquiTRAP: A Space-Time Clusters Analysis. PLoS ONE 7: e42125.

36. Koenraadt C, Aldstadt J, Kijchalao U, Kengluecha A, Jones J, et al. (2007) Spatial and temporal patterns in the recovery of Aedes aegypti (Diptera: Culicidae) populations after insecticide treatment. Journal of Medical Entomology 44: 65-71.
37. Stoddard ST, Forshey BM, Morrison AC, Paz-Soldan VA, Vazquez-Prokopec GM, et al. (2012) House-to-house human movement drives dengue virus transmission. Proceedings of the National Academy of Science 110: 1-6.
38. Morrison A, Minnick S, Rocha C, Forshey B, Stoddard S, et al. (2010) Epidemiology of dengue virus in Iquitos, Peru 1995 to 2005: Interepidemic and epidemic patterns of transmission. PloS Neglected Tropical Diseases 4: e670.
39. Liebman K, Stoddard S, Morrison A, Rocha C, Minnick S, et al. (2012) Spatial dimensions of dengue virus transmission across interepidemic and epidemic periods in Iqutios, Peru (1999-2003). PloS Neglected Tropical Diseases 6: e1472.
40. Gubler D (1989) Aedes aegypti and the Aedes-borne disease control in the 1990s: top down or bottom up. American Journal of Tropical Medicine and Hygiene 40: 571-578.
41. Morrison A, Astete H, Chapilliquen F, Raimirez-Prada C, Diaz G, et al. (2004) Evaluation of a sampling methodology for rapid assessment of Aedes aegypti infestation levels in Iquitos, Peru. Journal of Medical Entomology 41: 502-510.
42. Waller L, Gotway C (2004) Spatial Statistics for Public Health: John Wiley & Sons.
43. Chadee DD (2004) Key Premises, a guide to Aedes aegypti (Diptera: Culicidae) surveillance and control. Bulletin of Entomological Research 94: 201-207.
44. Padmanabha H, Durham D, Correa F, Duik-Wasser M, Galvani A (2012) The interactive roles of Aedes aegypti super-production and human density in dengue transmission. PLoS Neglected Tropical Diseases 6: e1799.
45. Arboleda S, Jaramillo-O N, Peterson AT (2012) Spatial and Temporal Dynamics of Aedes aegypti Larval Sites in Bello, Colombia. Journal of Vector Ecology 37: 37-48.
46. Gleiser RM, Zalazar LP (2010) Distribution of mosquitoes in relation to urban landscape characteristics. Bulletin of Entomological Research 100: 153-158.
47. Moloney JM, Skelly C, Weinstein P, Maguire M, Ritchie S (1998) Domestic Aedes aegypti breeding site surveillance: limitations of remote sensing as a predictive surveillance tool. American Journal of Tropical Medicine and Hygiene 59: 261-264.
48. Paz S, Albersheim I (2008) Influence of Warming Tendency on Culex pipiens Population Abundance and on the Probability of West Nile Fever Outbreaks (Israeli Case Study: 2001-2005). EcoHealth 5.
49. Bertolotti L, Kitron U, Walker E, Ruiz MO, Brawn JD, et al. (2008) Fine-scale genetic variation and evolution of West Nile Virus transmission "hot spot" in suburban Chicago, USA. Virology 374: 381-389.

TABLES

Circuit	Survey Dates		Houses Surveyed (N)	Adults		Immatures	Water Supply		Environment
	Start	End		All Adults (N)	Adult Females (N)	Pupae (N)	Faucet (%)	Rain Water (%)	Average Daily temperature (°C)
1	3/4/09	4/3/09	412	117	77	532	95%	7%	26.58
2	6/26/09	8/6/09	424	184	72	214	91%	3%	26.57
3	11/13/09	12/14/09	319	287	128	988	96%	3%	27.5
4	2/25/10	4/29/10	431	646	285	268	91%	4%	27.25
5	7/2/10	9/13/10	411	675	329	98	93%	4%	26.15
6	10/6/10	2/23/11	426	620	338	177	92%	2%	26.98
7	2/23/11	5/17/11	401	164	70	285	94%	3%	26.54
8	7/4/11	9/12/11	458	298	140	204	93%	3%	26.45
9	10/24/11	2/1/12	449	490	246	167	94%	4%	26.86

Table 1. Maynas neighborhood characteristics by survey circuit

Circuit	Pupae			Adults		
	Clustering Start	Clustering End	Clustering Peak	Clustering Start	Clustering End	Clustering Peak
1	0	250	100	0	250	100
2	0	250	100	0	200	100
3	0	300	100	0	300	50
4	0	300	200	0	300	150
5	0	500	300	0	250	100
6	0	250	100	0	250	100
7	0	250	100	0	250	100
8	0	250	100	0	250	100
9	0	250	100	0	250	100
Average	0.0	288.9	133.3	0.0	255.6	100.0

Table 2. K-function output, Pupae and Adults. Testing to see if presence of pupae and adults was clustered. 500 meters, roughly half the study area, was used as a cutoff distance. 99 permutations using Monte Carlo randomization were used for calculating the confidence envelope. Clustering occurs if values of L(d) exceed the expectation under Complete Spatial Randomness (CSR) for a value at a given distance. The distance up to which clustering occurred (Clustering End), as well as the distance at which L(d) peaked (Clustering Peak), were reported by survey circuit.

	Pupae			Adults		
	D Start	D End	D Max	D Start	D End	D Max
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	150	150	150
4	-	-	-	50	50	50
5	250	300	250	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	-	-	50	50	50
9	-	-	-	-	-	-

Table 3. Weighted K function, Pupae and Adults. Testing to see if abundance of pupae and adults was clustered. 500 meters, roughly half the study area, was used as a cutoff distance. 99 permutations using Monte Carlo randomization were used for calculating the confidence envelope

	Pupae				Adults			
	Estimate	**Std. error**	**z value**	**Pr(>|z|)**	**Estimate**	**Std. error**	**z value**	**Pr(>|z|)**
Variable								
Intercept	1.0327	0.8657	1.42	0.2329	1.1413	1.1111	1.06	0.3043
Temperature	-0.0375	0.0324	1.34	0.2464	-0.0413	0.0416	0.99	0.3198
NDVI	-0.1487	0.1217	1.49	0.2216	-0.1216	0.1562	0.61	0.4361
Number of Residents	0.0064	0.0017	13.88	0.0002***	0.0084	0.0022	14.35	0.0002***
Water Source Rainwater	0.0502	0.0249	4.07	0.0438*	0.0681	0.0320	4.54	0.0331*
Water Source Faucet	0.0445	0.0430	1.07	0.3011	0.0541	0.0552	0.96	0.3272
Number of Containers	0.0032	0.0032	1.02	0.3118	0.0041	0.0041	1.00	0.3169

Table 4. Results of the Linear GLM Models testing the different factors influencing the proportion of circuits a household was a cluster member for larvae and pupae. Statistical significance: .0.1; *0.05; **0.01; ***0.001.

	Pupae				Adults			
	Estimate	Std. error	z value	Pr(>|z|)	Estimate	Std. error	z value	Pr(>|z|)
Variable								
Intercept	0.0081	0.0246	0.11	0.7417	0.0200	0.0315	0.40	0.5247
Number of Residents	0.0071	0.0017	18.54	<.0001***	0.0092	0.0021	18.82	<.0001***
Water Source Faucet	0.0499	0.0247	4.07	0.0436*	0.0691	0.0317	4.75	0.0293*
Water Source Rainwater	0.0512	0.0419	1.49	0.2217	0.0637	0.0538	1.40	0.2361

Table 5. Results of the best Linear GLM Model, using household variables, testing the different factors influencing the proportion of circuits a household was a cluster member for larvae and pupae. Statistical significance: .0.1; *0.05; **0.01; ***0.001.

FIGURES

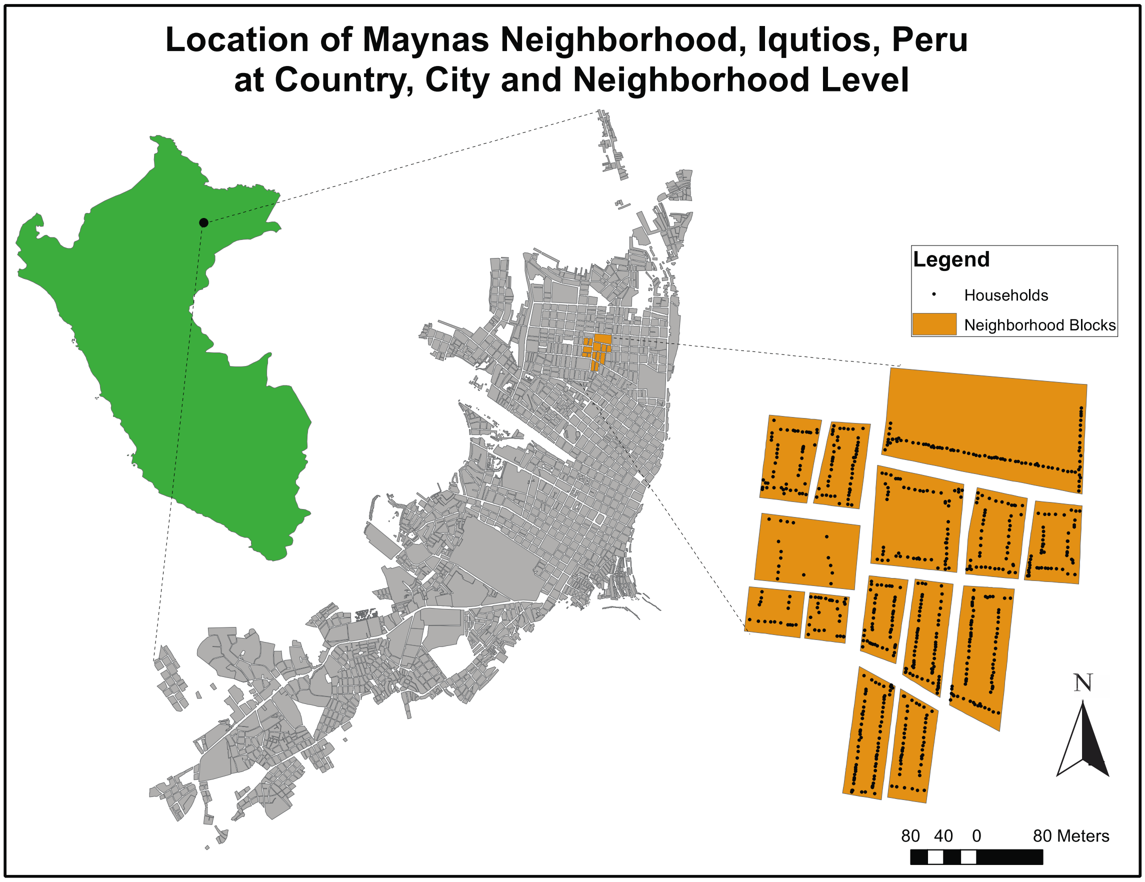

Figure 1. Map of Maynas Neighborhood of Iquitos, Peru.

Mar 2009 Jun 2009 Nov 2009

Feb 2010 July 2010 Oct 2010

Feb 2011 Jul 2011 Oct 2011

Figure 2. Adult Abundance by Survey Circuit**.** Maps of adult abundance during each survey circuit. Numbers of adults collected were partitioned into groups of 0, 1, 2, 3-5 6-10 and >10 to account for the few number of observations at higher numbers.

Figure 3. Pupal Abundance by Survey Circuit. Maps of pupae abundance during each survey circuit. Numbers of pupae collected were partitioned into groups of 0, 1, 2, 3-5 6-10 and >10 to account for the few number of observations at higher numbers.

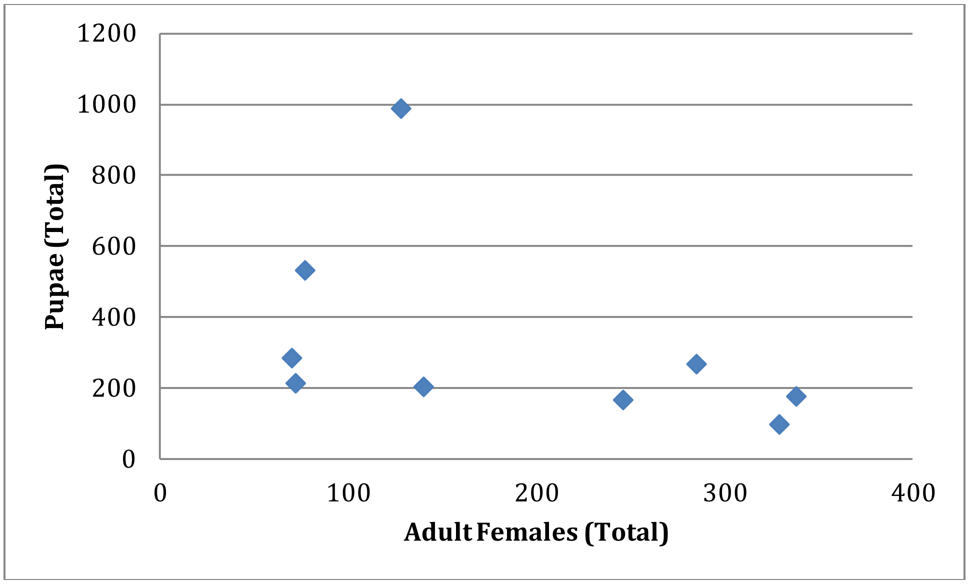

Figure 4. Ratio of total number of adult females to total number of pupae found per circuit. There is moderate negative correlation (-0.43) between number of adult females and number of pupae captured per circuit.

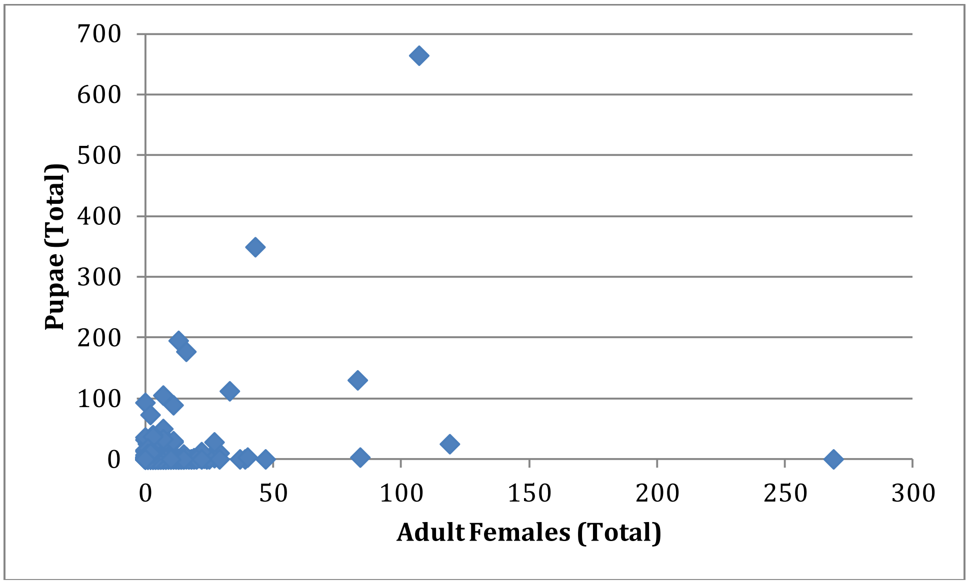

Figure 5. Ratio of total number of adult females to total number of pupae found per household. As seen in the graph, there is only a weak positive correlation (0.33) between number of adult females and number of pupae captured per house.

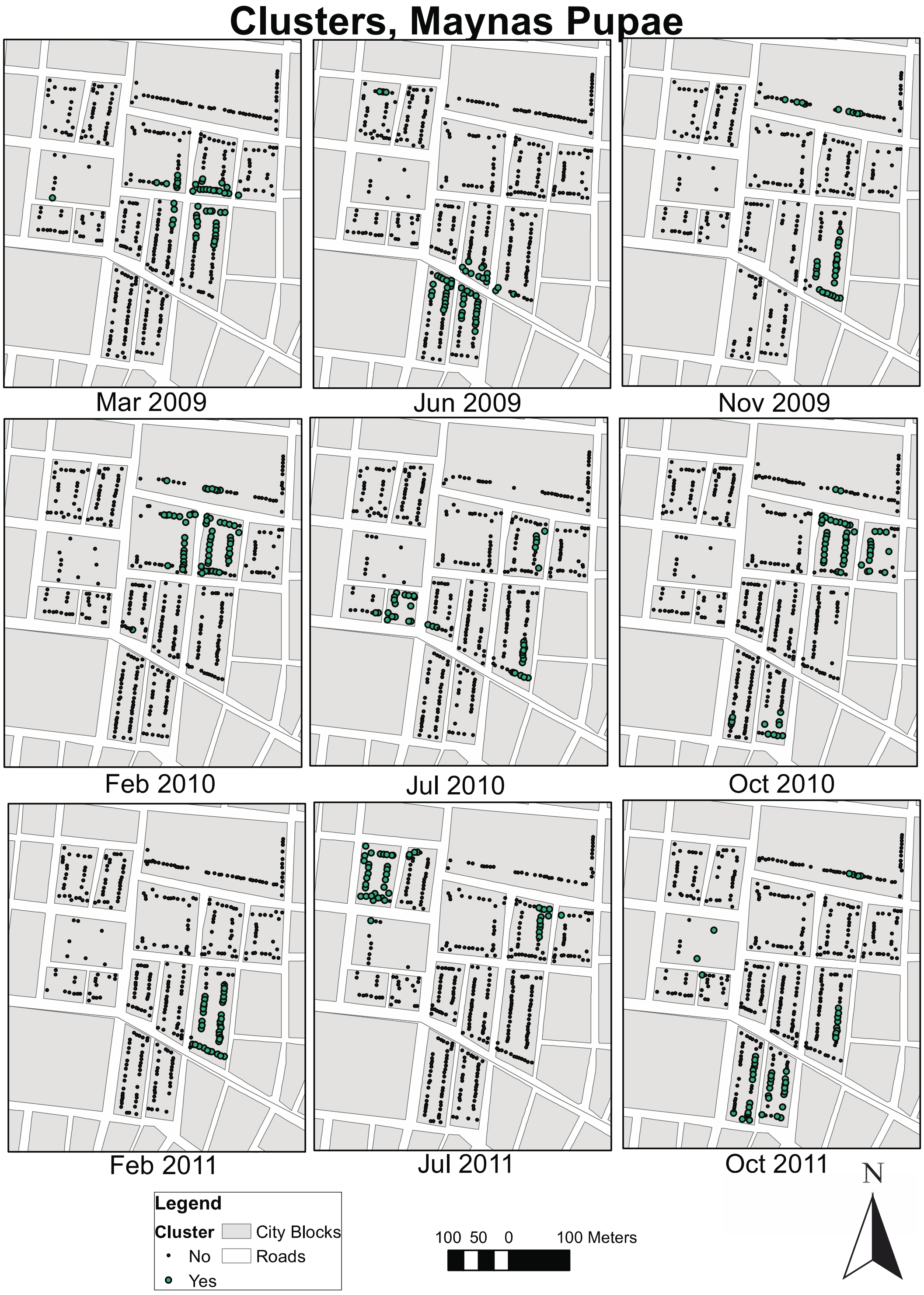

Figure 6. Pupae Abundance Cluster Members (Gi(d) > 2.575; P<0.05) by Survey Circuit. A z-score of 2.575 was used as a cutoff for cluster membership.

Figure 7. Adult Abundance Cluster Members (Gi(d) > 2.575; P<0.05) by Survey Circuit. A z-score of 2.575 was used as a cutoff for cluster membership.

Figure 8. Distance at which Z-score peaked for Pupae Gi*(d) tests by circuit. Households for which no distance resulted in a Z-score over the cutoff of 2.575 were labeled as not significant (NS)

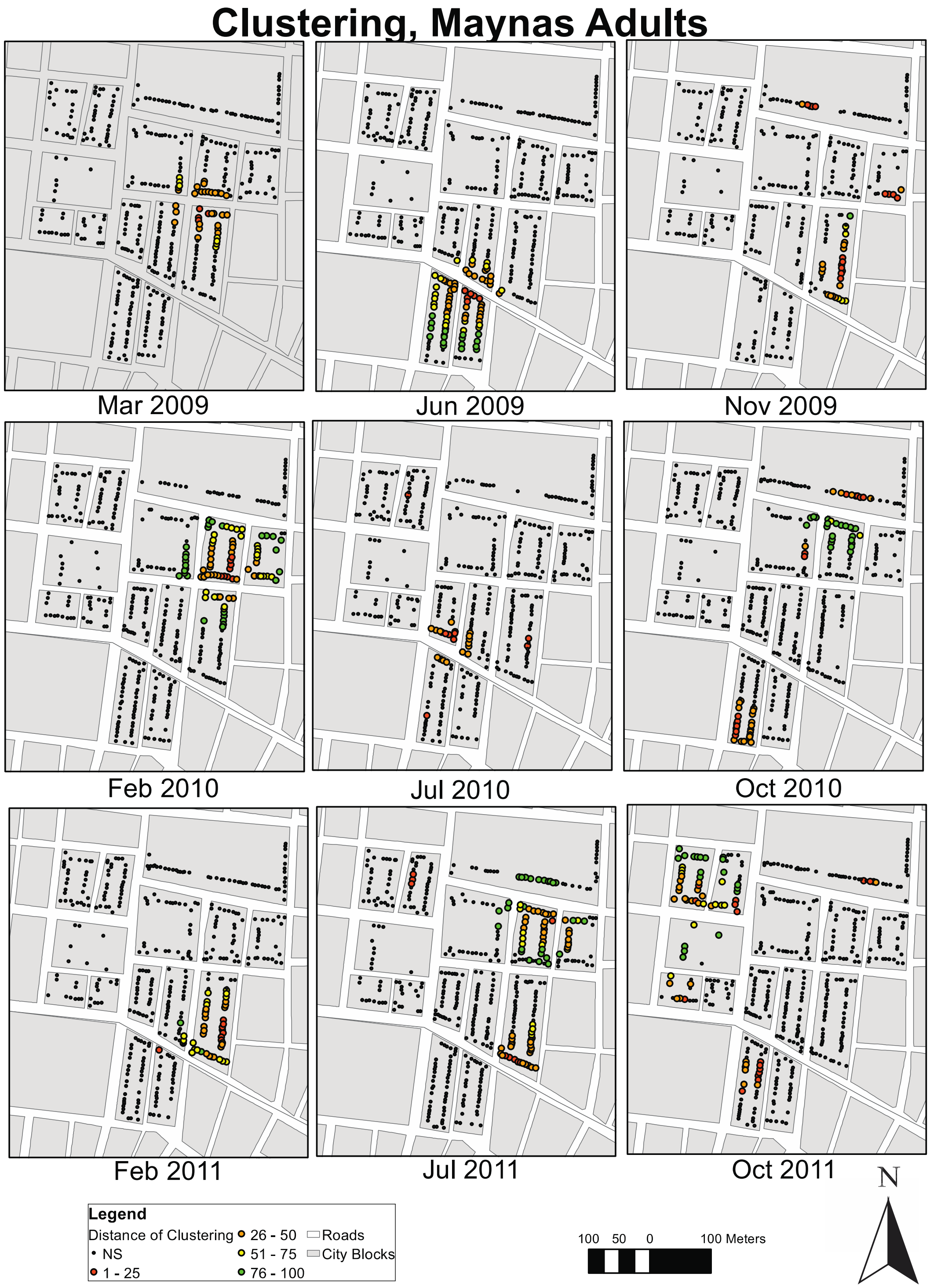

Figure 9. Distance at which Z-score peaked for Adult Gi*(d) tests by circuit. Households for which no distance resulted in a Z-score over the cutoff of 2.575 were labeled as not significant (NS)

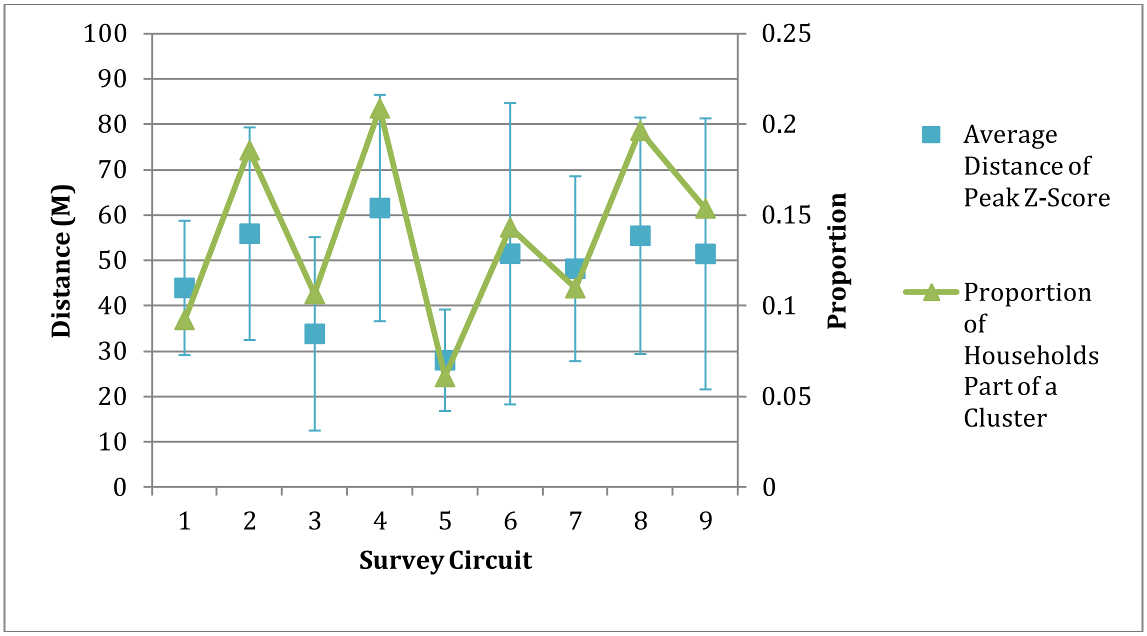

Figure 10. Average distance at which the Z-score peaked for Gi*(d) tests compared with proportion of households part of a cluster for pupae over study period.

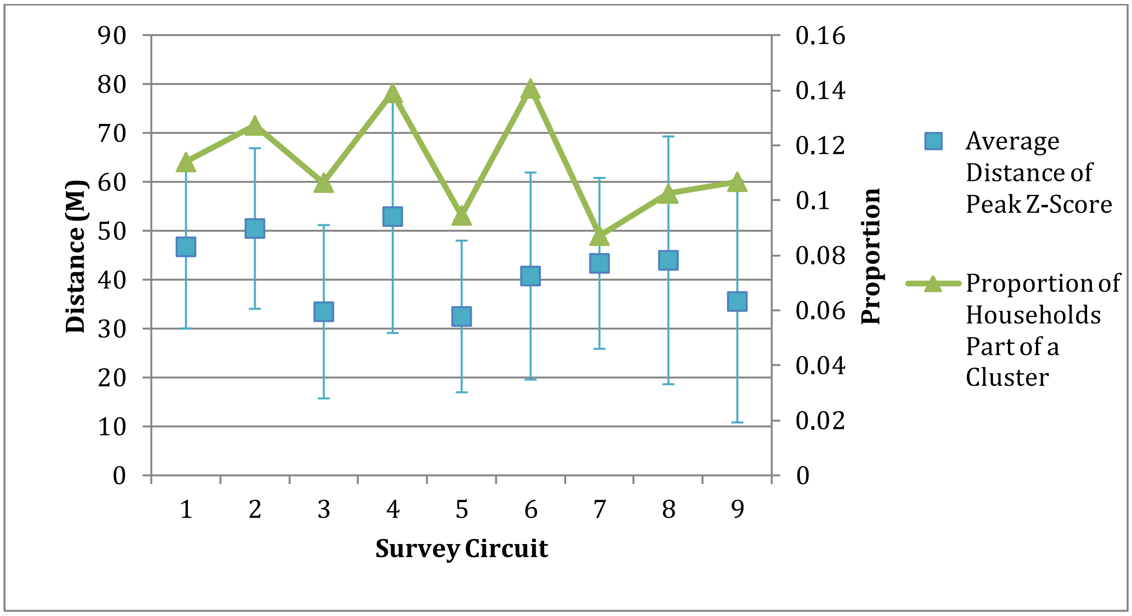

Figure 11. Average distance at which the Z-score peaked for Gi*(d) tests compared with proportion of households part of a cluster for adults over study period

Figure 12. Proportion of Time House is a Cluster Member, Maynas Pupae. Most houses are never a cluster member, with very few being a member a high proportion of the time

Figure 13. Proportion of Time House is a Cluster, Maynas Adults. Most houses are never a cluster member, with very few being a member a high proportion of the time

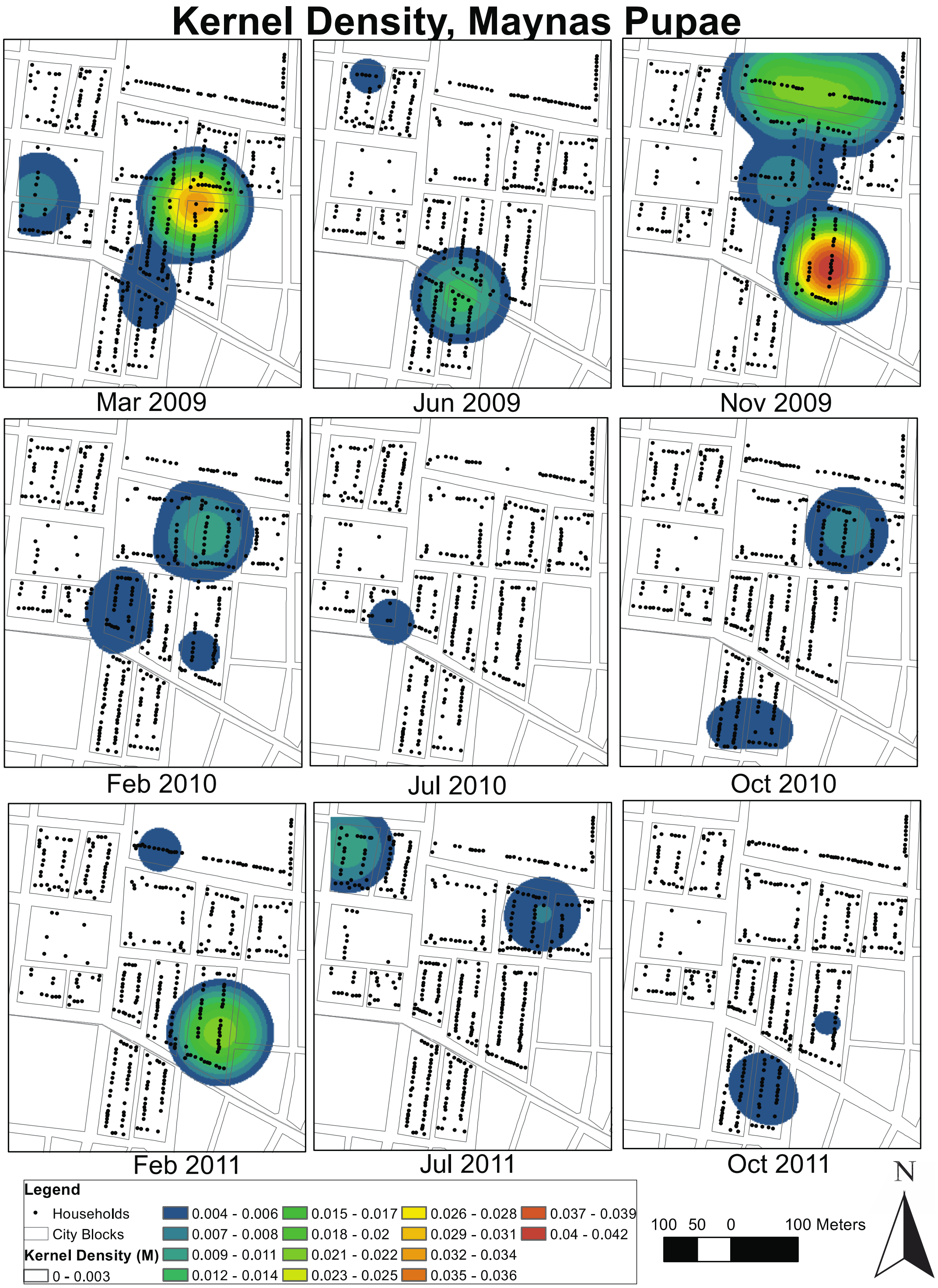

Figure 14. Kernel Density by Survey Circuit, Maynas Pupae Density is represented as expected number of pupae per square meter

Figure 15. Kernel Density by Survey Circuit, Maynas Adults. Density is represented as expected number of adults per square meter

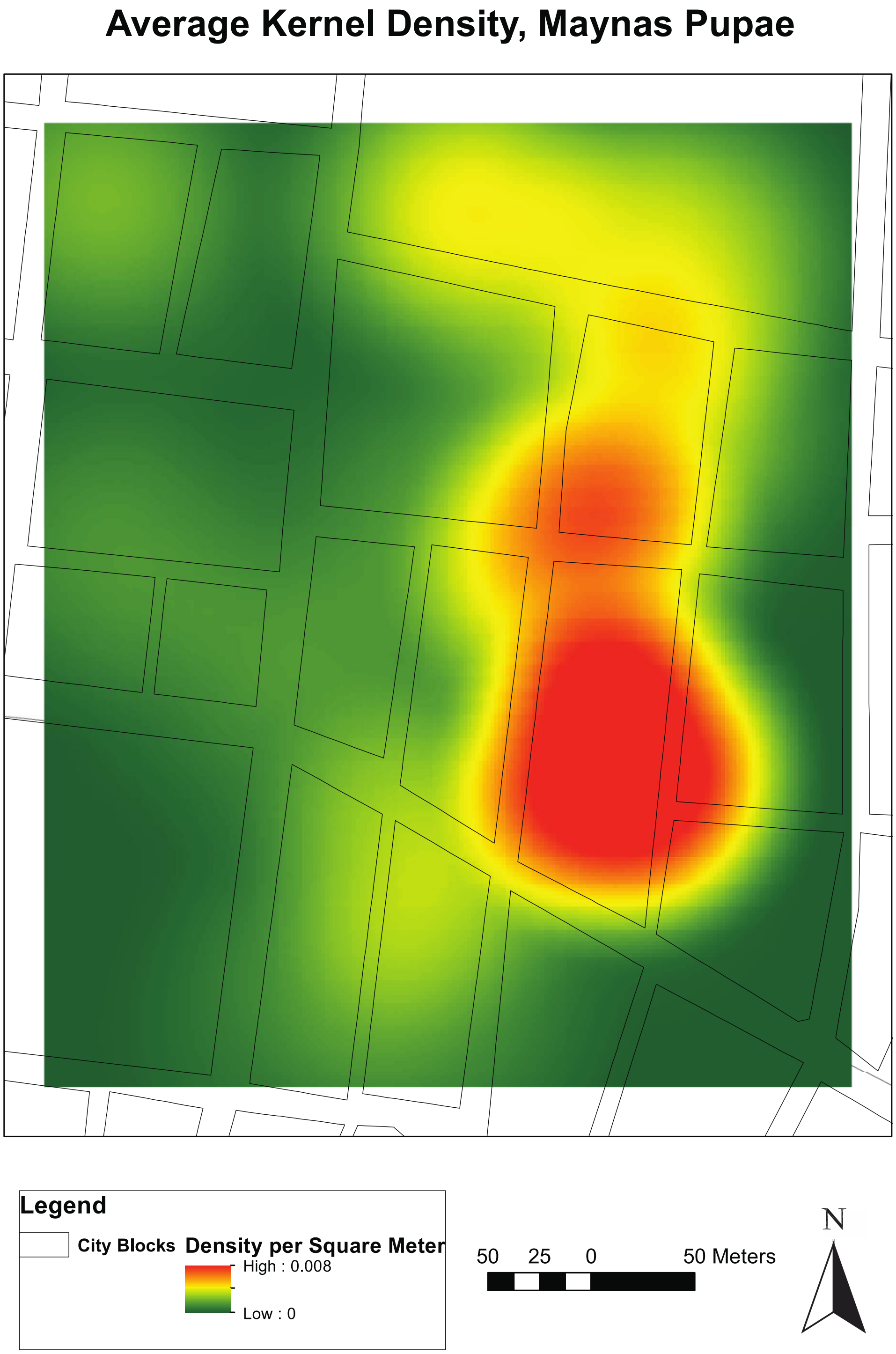

Figure 16. Average Kernel Density, Maynas Pupae. Average density across all circuits, represented in number of pupae per square meter

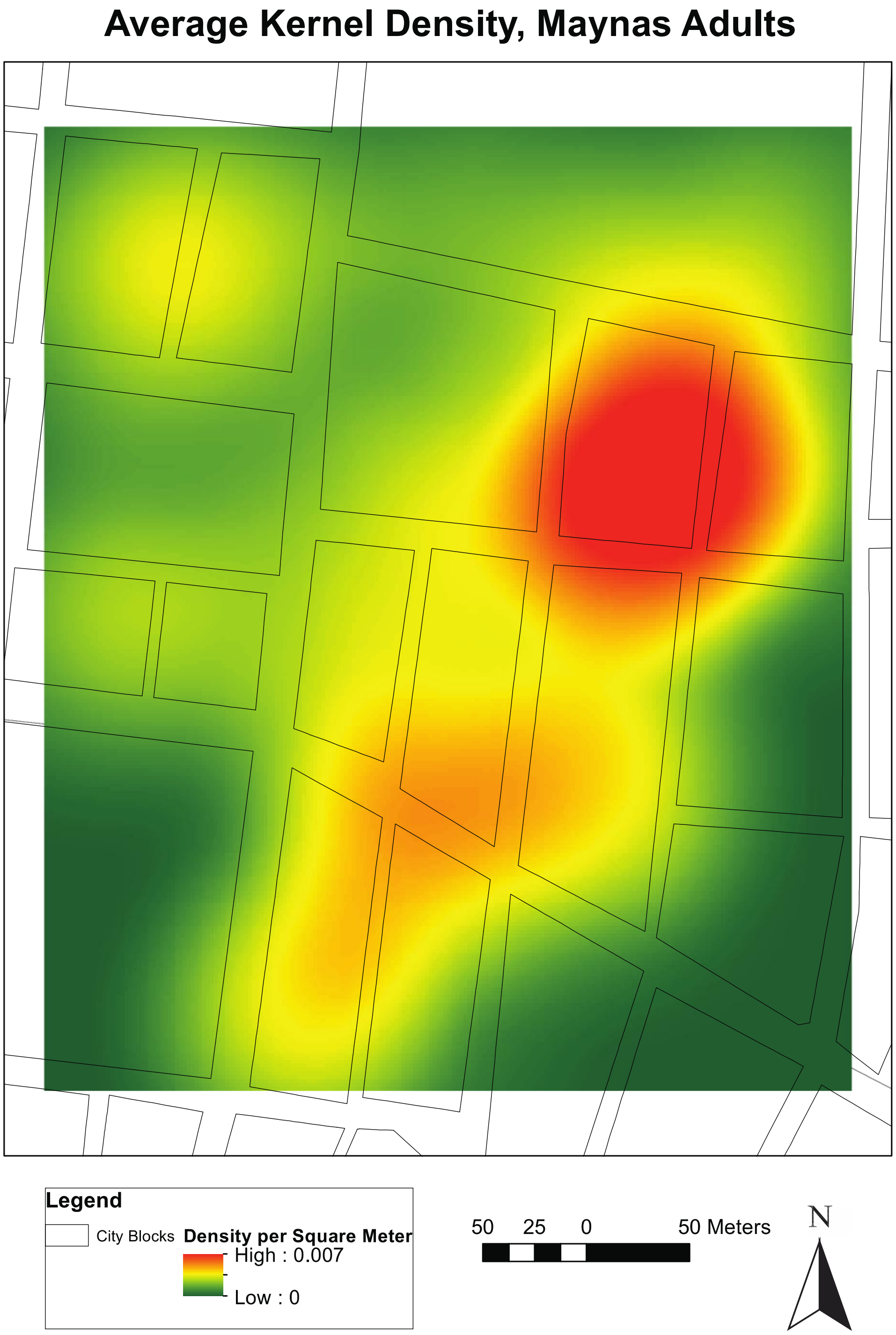

Figure 17. Average Kernel Density, Maynas Adults. Average density across all circuits, represented in number of adults per square meter