THE EPIDEMIOLOGY AND ENTOMOLOGICAL INTERACTIONS ASSOCIATED WITH DENGUE TRANSMISSION IN ANG MO KIO GRC, CENTRAL SINGAPORE

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAIʻI AT MĀNOA IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
MICROBIOLOGY
DECEMBER 2013

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Keywords: *Aedes*, *Aedes aegypti*, *Aedes albopictus*, dengue, transmission, virus

DEDICATION PAGE

I dedicate this dissertation to my field research assistant, editor, and amazing husband, Joshua Lee Green; to my daughter, Lyra Lane Green, who gave me strength; and to my always encouraging mother, who filled out my first college application and never let me give up. I could not have accomplished this without each of you by my side, thank you.

ACKNOWLEDGMENTS

My deepest appreciation goes to Dr. Duane Gubler, for believing in me and providing the opportunity to work in Singapore. I promised not to let you down. The project took longer than expected but I hope you are happy with the results. Thank you for being a great mentor and an inspiration to me in all aspects of my life.

I would also like to thank the IGERT team: Dr. Shannon Bennett, Dr. Bruce Wilcox, Dr. Durrell Kapan, and Mr. Ron Paik, who sent me on an amazing journey that, changed my outlook on life, myself, and the world and made me a better human being.

Thank you to my PhD Dissertation Committee Members: Dr. Shannon Bennett, Dr. Brett Ellis, Dr. Hongwei Li, Dr. Paul Patek, and Dr. Alan Katz. Your support and feedback throughout the dissertation process has been conducive to my success.

And last but definitely not least, thank you Brett, for being a great friend, an awesome mentor, my hardest critic, and the best drinking buddy. I hope distance doesn't keep our families from spending time together.

ABSTRACT

Dengue is arguably the most important arboviral disease of humans, having increased dramatically in geographic range and prevalence over the last 25 years. Dengue virus has two main vectors, *Aedes aegypti* and *Aedes albopictus*. For decades both vectors have also been increasing their geographic range on regional and global scales. This study took place in Singapore, where dengue fever is a major public health threat despite a successful vector control program. Similar to other hyperendemic countries, local dengue transmission dynamics in Singapore are not well understood: where dengue transmission is occurring, the relative contribution of the two dengue vectors, and the ability to correlate traditional vector surveillance methods to transmission risk remains controversial. In collaboration with the Program of Infectious Diseases at Duke-NUS Graduate Medical School, Singapore Ministry of Health, Singapore National Environmental Agency, and Ang Mo Kio Town Council an adult *Aedes* female fixed position vector surveillance program was established that detailed temporal and spatial *Ae. aegypti* and *Ae. albopictus* distribution and abundance in Ang Mo Kio, Central Singapore. This surveillance method yielded similar results to standard surveillance techniques over a range of habitats and time points. Furthermore, sensitivity of the adult surveillance method presented here is uniquely increased by placing traps on the second floor of Housing Development Board (HDB), government subsidized multistory residential buildings, as opposed to ground level; average *Ae. aegypti* catch rate of the ground floor was 0.09 and average *Ae. aegypti* catch rate of the second floor was 0.42. Starting on the second floor a very strong inverse relationship between *Ae. aegypti* catch rate and floor height (Pearson linear correlation r=-0.91, t=-4.47, df=4, p=0.01) was also identified. In addition, intensive entomological investigations, in focal areas with varying levels of *Aedes* abundance, identified by the fixed position surveillance system, uncovered details about mosquito ecology and "hotspots" at a local scale that can improve our understanding of dengue transmission dynamics. Dengue transmission is believed to primarily occur in residential units but host seeking *Ae. aegypti* and *Ae. albopcitus* were collected at similar frequencies in congregation areas on the ground floors of, HDBs and at greater abundance than inside residential units. Improving knowledge on the focal nature of dengue transmission is critical to designing more

targeted and cost-effective surveillance and control strategies in the future, both in Singapore and urban areas elsewhere.

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CHAPTER 1

Project Background

Abstract

Despite the existence of one of the most effective and successful vector control programs worldwide dengue has resurged in Singapore in recent decades. Similar to other hyperendemic countries, dengue transmission dynamics in Singapore are not well understood: where dengue transmission is occurring, the relative contribution of the two dengue vectors, and the ability to correlate traditional surveillance methods to transmission risk remains controversial. Identifying areas with increased risk of human-mosquito exposure by detailing *Ae. aegypti* and *Ae. albopictus* distribution and abundance can help direct vector control efforts and ultimately reduce dengue transmission.

Specific Aims

The overall goal of this project was to develop a detailed understanding of the underlying epidemiological and entomological interactions that influence dengue transmission in a complex urban environment in Singapore. Our central hypothesis was that vector abundance and dengue incidence will be correlated with each other and with the density of humans in space and time, and that the distribution of vectors and humans will in turn be determined by urban features of the landscape. By overlaying spatial and temporal entomological and epidemiological data with urban environmental data we identified ecological correlates that help to elucidate dengue transmission dynamics and provide a more accurate assessment of disease risk. Vector borne disease transmission dynamics involves a complex interaction of multiple agents that are spatially and temporally heterogeneous. Elucidating these dynamic interactions on a local scale is of fundamental importance in understanding dengue transmission dynamics. The primary objective of this proposal was to decipher the relationship between human-vector exposure and circulating dengue virus within Ang Mo Kio district, Central Singapore. Achieving this objective has contributed to acquiring the necessary tools to help direct vector control efforts to priority areas that can have the greatest effect on reducing transmission.

Aim 1

An adult mosquito surveillance program was developed and the MosquiTRAP was evaluated under local entomological conditions.

1.1 The most efficient number of fixed MosquiTRAPs required for adult mosquito surveillance in central Singapore was determined.

1.2 The MosquiTRAP was compared to other entomological tools (BG sentinel, indoor aspirations, ovitraps, and larval surveys) in areas with high, medium, and low mosquito abundances.

Aim 2

Population-level spatiotemporal dynamics of dengue vectors and the spatial distribution of humans in central Singapore was detailed.

2.1 The spatiotemporal distribution/abundance of adult *Ae. aegypti*, *Ae. albopictus* vectors and their infection status was monitored.

2.2 The spatial distribution of human density using residential and commercial development was determined.

Aim 3

Epidemiological and entomological factors that are associated with dengue transmission risk at a local level were identified.

3.1 Multiple data layers were analyzed to determine relationships between: human density, distribution and abundance of dengue vectors, land use, and infected mosquitoes.

3.2 The interactions of humans and mosquitoes were described relative to the two dengue vectors and the different environments at a local level.

Introduction

Dengue was first documented in Singapore in the early 1900s with dengue hemorrhagic fever (DHF) emerging 60 years later [1]. In response to the rising incidence of DHF the Singapore government conducted a series of entomological studies from 1966 to 1968 [1-5]. The main objective was to investigate the ecology of *Ae. aegypti* and *Ae. albopictus* in the urban areas of Singapore and to evaluate their respective roles in the epidemiology of

DHF. Both species were found throughout urban areas of Singapore. Historically, *Ae. aegypti* larval habitats were found mainly indoors at 73% while only 50% of *Ae. albopictus* larval habitats were indoors. The premise index ranged from 2.3-29.6%. Chan et al. [2] stated that the indices they used (premise index, larval density index, receptacle index, and infested receptacle index) were biased against *Ae. albopictus* due to the fact that *Ae. albopictus* is often found breeding in open areas. They concluded that both vectors were transmitting dengue virus (DENV) in Singapore even though the dengue incidence seemed to correlate closer with the distribution of *Ae. aegypti* than with *Ae. albopictus* [5]. The results of the studies were used to develop a national vector control program incorporating larval reduction, health education, and law enforcement that was put into effect in 1969. The program was successful in maintaining a very low premise index of around 2% and decreased dengue incidence for 15 years [6]. Despite the reduction of vectors in residential areas dengue reemerged in the late 1980s (Figure 1.1). The country is now hyperendemic and within the last decade dengue epidemics are the largest recorded in Singapore history.

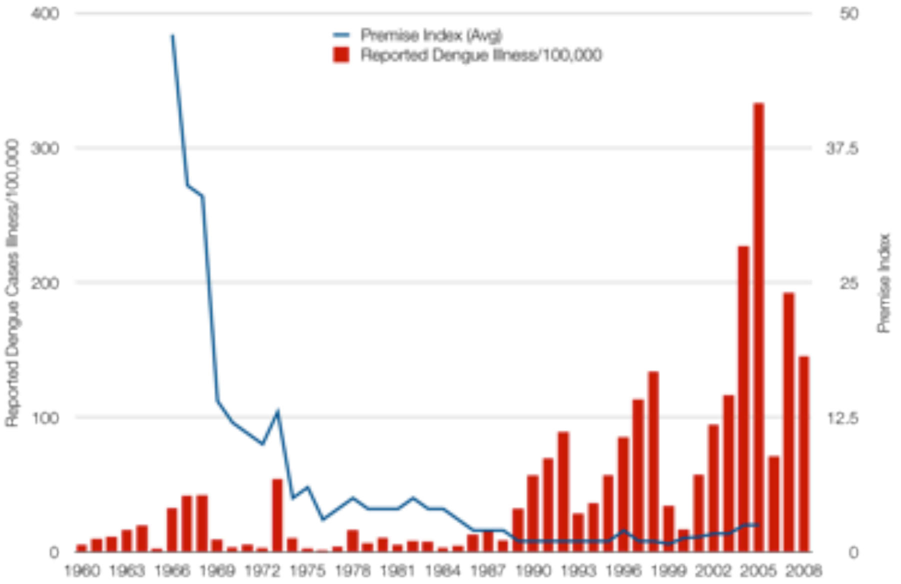


Figure 1.1 Dengue incidence in Singapore and average national premise index from 1960-2008.

Dengue Transmission

Despite the existence of one of the most effective and successful vector control programs worldwide dengue is still a major public health problem in Singapore. Several factors are believed to have contributed to the recent resurgence of dengue in Singapore; lowered herd immunity, more clinically overt infection as a consequence of adult infection, a shift in the surveillance emphasis of the vector control program, and increased contribution of virus transmission outside the home. Researchers are uncertain as to where the majority of dengue transmission occurs and the relative contribution of the two dengue vectors. Generally, dengue cases cluster geographically by residence, wherein households are believed to be the primary arena of transmission with the endophagic mosquito *Ae. aegypti* as the primary vector. In Singapore a low percentage of cases cluster geographically; there is an increase in adult incidence rates; and the existence of cases that link via molecular epidemiology but lack residential association. These epidemiological findings suggest a possible increase in the relative contribution of transmission occurring in public areas.

Increase in Adult Incidence Rates

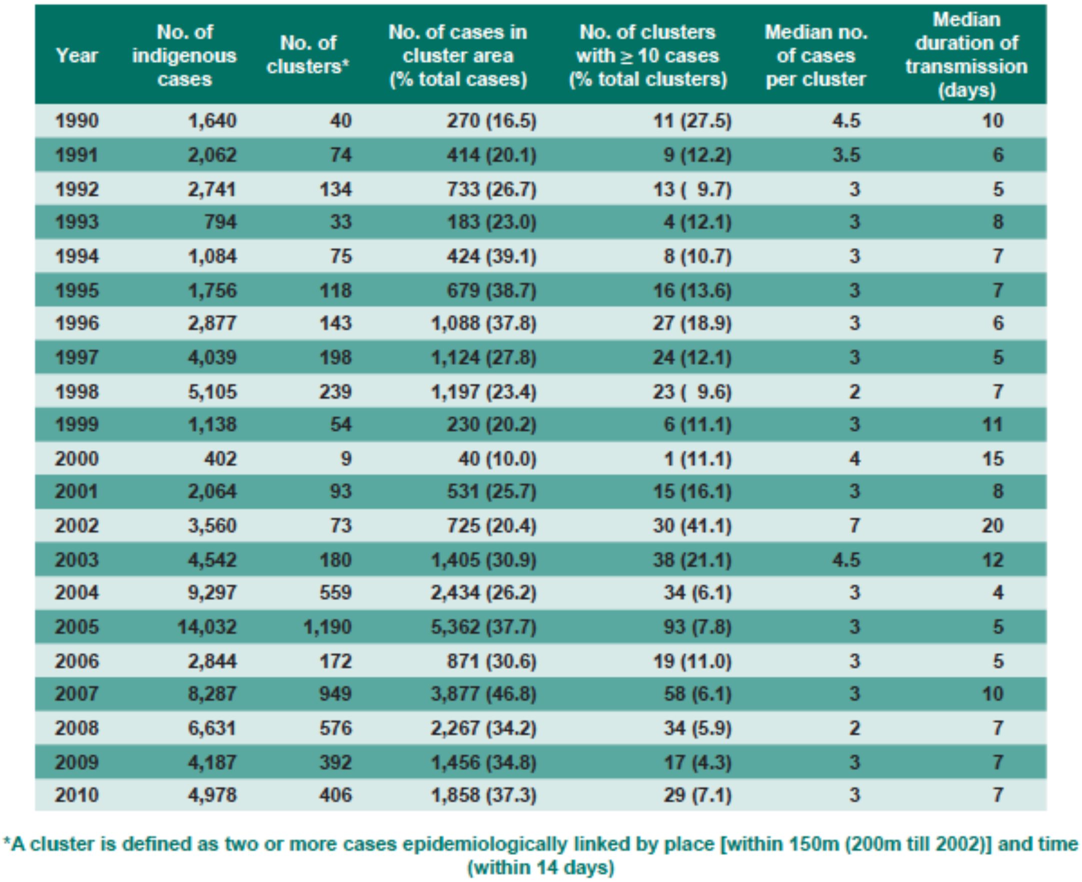
Traditionally, in dengue endemic countries of Southeast Asia, children bear the burden of the disease [7]. Over the last four decades in Singapore there has been an increase in the age specific incidence rate of dengue cases. Ooi et al. [8] showed that between 1977 and 2000 the proportion of cases under 15 decreased linearly at a rate of 1.2% each year. The highest age specific incidence rates for 2010 were in 35-44 year olds [9]. In Singapore adults of working age are likely to spend most of their time away from home. In other endemic countries young children, who tend to spend most of their time at home usually have the highest incidence rates. Moreover females who are more likely to care for children at home have a lower incidence of dengue then males: the male to female incidence ratio in Singapore is 1.6:1 [9]. These data suggests that the risk of acquiring dengue may increase with time spent away from home. A decrease in herd immunity can also lead to higher age specific incidence rates. A combination of lower herd immunity and extradomiciliary transmission may contribute to the observed trends.

Low Percentage of Case Clusters

The household has typically been considered the primary location for dengue transmission due to the spatiotemporal clustering of cases around residences [10, 11]. In Singapore greater than 50% of cases are sporadic, i.e., not belonging to a cluster. The yearly percent of dengue cases that clustered geographically between 1990-2010 ranged from 10-46.8% and the median number of cases per cluster was two to seven with an average of three cases per cluster (Table 1.1) [9]. The high number of sporadic cases may be the result of individuals becoming infected away from the home without further transmission at the residence. The lack of case clusters and the small size of the clusters suggests that exposure (time spent in contact) at the home is not sufficient to account for a large portion of transmission. The apparent limited domiciliary transmission may be due to the high success of Singapore's vector control efforts in maintaining a low premise index.

Molecular Epidemiology Linked Cases Lack Geographic Association

Recent evidence produced by the Early Dengue Infection and Outcome Study (EDEN) found that 75% of cases that were linked via molecular epidemiology could not be clustered by home address [12]. This suggests that individuals are spatially and temporally linked in areas other than their residence where human-mosquito exposures are high enough to support transmission.



Year	No. of indigenous cases	No. of clusters*	No. of cases in cluster area (% total cases)	No. of clusters with ≥ 10 cases (% total clusters)	Median no. of cases per cluster	Median duration of transmission (days)
1990	1,640	40	270 (16.5)	11 (27.5)	4.5	10
1991	2,062	74	414 (20.1)	9 (12.2)	3.5	6
1992	2,741	134	733 (26.7)	13 (9.7)	3	5
1993	794	33	183 (23.0)	4 (12.1)	3	8
1994	1,084	75	424 (39.1)	8 (10.7)	3	7
1995	1,756	118	679 (38.7)	16 (13.6)	3	7
1996	2,877	143	1,088 (37.8)	27 (18.9)	3	6
1997	4,039	198	1,124 (27.8)	24 (12.1)	3	5
1998	5,105	239	1,197 (23.4)	23 (9.6)	2	7
1999	1,138	54	230 (20.2)	6 (11.1)	3	11
2000	402	9	40 (10.0)	1 (11.1)	4	15
2001	2,064	93	531 (25.7)	15 (16.1)	3	8
2002	3,560	73	725 (20.4)	30 (41.1)	7	20
2003	4,542	180	1,405 (30.9)	38 (21.1)	4.5	12
2004	9,297	559	2,434 (26.2)	34 (6.1)	3	4
2005	14,032	1,190	5,362 (37.7)	93 (7.8)	3	5
2006	2,844	172	871 (30.6)	19 (11.0)	3	5
2007	8,287	949	3,877 (46.8)	58 (6.1)	3	10
2008	6,631	576	2,267 (34.2)	34 (5.9)	2	7
2009	4,187	392	1,456 (34.8)	17 (4.3)	3	7
2010	4,978	406	1,858 (37.3)	29 (7.1)	3	7

*A cluster is defined as two or more cases epidemiologically linked by place [within 150m (200m till 2002)] and time (within 14 days)

Table 1.1 Description of dengue incidence and case clusters from 1990-2010. MOH 2011 annual communicable disease report, vector-borne/zoonotic diseases section (Table 2.8)[9].

Recent Entomology

The MOH conducted a retrospective study investigating the 2005 dengue outbreak in Singapore that included an entomological component. When comparing the 1966 and 2005 data, drastic differences can be seen between the percentage of outdoor breeding habitats and premise index range. The 2005 study found that 50% of all mosquito larval habitats were located outdoors and the premise index ranged from 0.33-2.28% [13]. In 1966 the premise index ranged from 2.3-29.6% and 73% of *Ae. aegypti* and 50% of *Ae. albopictus* of larval habitats were found indoors [2]. An increase in the proportion of breeding outdoors could potentially increase the proportion of outdoor human-mosquito exposure. Both studies found that *Ae. aegypti* distribution showed a greater correlation with dengue cases than *Ae. albopictus* (Figure 1.2), therefore it is commonly believed that *Ae. aegypti* is the primary vector.

At present there are two factors that may be inflating this association: geo-referencing cases by home address, and the premise index. Geo-referencing cases to home addresses automatically increases the likelihood of association with *Ae. aegypti* regardless of the transmission location. The 1966 survey suggested that the premise index was biased against *Ae. albopictus* and we also believe that it may not be an appropriate index for Singapore due to its vertical nature. It was developed for landed houses in 1922 to eliminate *Ae. aegypti* and reduce the incidence of yellow fever [14]. Therefore it may not provide a valid description of *Aedes* mosquito dynamics within high-rise buildings and it is not an index of disease risk since it does not correlate with dengue incidence. Also it is not a standardized measurement: it may or may not include the surrounding area of a residence, and it does not account for mosquito abundance.

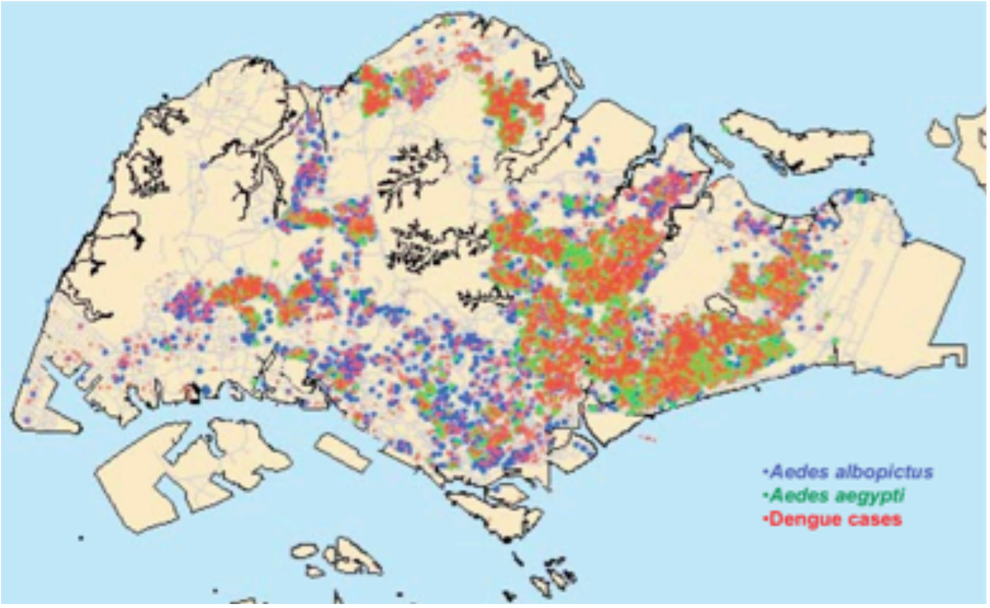


Figure 1.2 Geographic description of *Ae. aegypti* and *Ae. albopictus* spatial distribution and dengue incidence for 2005 [13].

Vector Competence and Capacity

Vector competence studies comparing the oral infectivity of *Ae. aegypti* and *Ae. albopictus* to dengue virus have been inconsistent [15-18]. Regardless, two out of the three studies in Singapore comparing the infection rates of *Ae. aegypti* and *Ae. albopictus* showed similarly rates of infection [5, 19, 20]. Since both species are efficient at transmitting dengue virus the most important parameter in comparing their vector capacities may be human-mosquito exposure.

Adult Mosquito Monitoring

Monitoring adult insects populations using sticky traps has been recommended in the agricultural industry for at least 50 years, but was not widely adopted until the 1980's [21]. In agriculture the intended purpose of this tool is quite similar to the needs of entomological disease research: 1) detect the introduction of pests, 2) monitor changes in population densities; 3) assess the length and level of suppression resulting from pest management activities. The use of these monitoring devices for disease vectors (e.g. mosquito, sandflies, blackflies, tsetse, etc.) began appearing in the literature sporadically in the 1990's, and more frequent evaluations for container habitat mosquitoes began within the last 6 years [22-25]. The adult sticky trap is based upon the design of the most sensitive method of detection, the ovitrap, with the oviposition substrate replaced by a sticky card. The MosquiTRAP (Figure 1.3) is a sticky trap that has been designed to maximize the potential for a mosquito to visit the trap and come into contact with the sticky card. The MosquiTRAP is generally used with an attractant (e.g. Atr*Aedes*: a synthetic ovipositon attractant developed from grass infusion). In disease control and prevention strategies, such as flooding an area with a high number of traps, sticky traps (and other lethal/autocidal traps) have an advantage in comparison to ovitraps because they have the potential to remove an infected mosquito from the population.

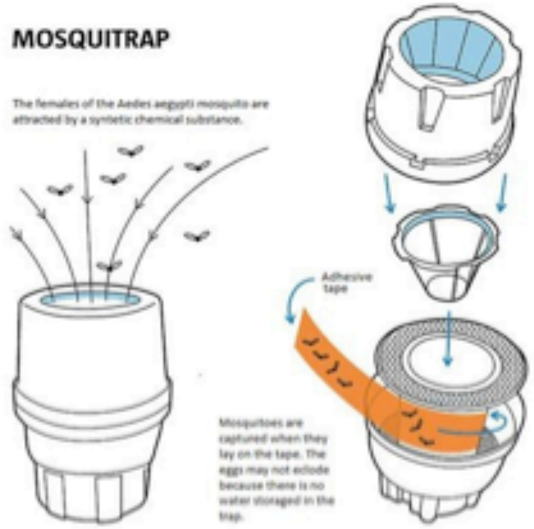


Figure 1.3 Description of MosquiTRAP components and function [26].

The evaluation studies published on MosquiTRAP (and related sticky traps) have demonstrated particular effectiveness for outdoor monitoring [25, 27, 28], the detection of *Ae. aegypti* and *Ae. albopictus* [25, 29-31], and comparable or better efficiency than backpack aspiration methods [28, 32, 33]. One report also suggests its ability to detect the presence of the disease vectors when larval surveillance was unsuccessful [27].

Evaluation of the sensitivity of sticky traps in comparison to ovitraps has been mixed and includes reports of similar sensitivity [25, 31, 34], greater sensitivity and precision at some densities [29], and less sensitivity [27, 29, 35, 36]. Correlations between adults collected from sticky traps and oviposition monitoring [27, 29, 35], as well as climate variables are also mixed [27, 29, 35].

Most importantly, results thus far have demonstrated that adult mosquito monitoring studies have been able to detect difference in densities temporally and spatially [27, 29, 31, 32, 35, 37]. In a few instances high adult densities have coincided with dengue transmission [30, 38]. Studies have also been conducted to detect virus and to understand the dispersal patterns of vectors [34, 39-42]. Overall the results suggest fixed position sticky traps have the potential to be an efficient, cost-effective, and sustainable surveillance method.

Spatial Surveillance and Control

Geographic information systems and spatial analysis capabilities are important in understanding the distribution of mosquitoes, designing optimal sampling strategies, and investigating epidemiological and ecological interactions. Singapore has unique capacities to employ this technology because of its strong infrastructure and availability of geospatial datasets. Most of the studies utilizing these capabilities for monitoring entomological parameters and guiding control activities have been directed towards other vector borne disease such as malaria [43-47]. Of the examples for dengue, currently under varying levels of development or operability, most have been developed in Brazil including MI-Dengue, which measures adult mosquitoes using the MosquiTRAP [30]. MI-Dengue utilizes fixed position MosquiTRAP monitoring that is connected to a geospatial information system, used to calculate entomological risk and guide municipal actions [30].

Relevant Studies

The first study to characterize the spatiotemporal pattern of a dengue epidemic was in 1998. Morrison et al. [48] analyzed the spatial clustering for all cases, georeferenced by their residential address, and the spatiotemporal attributes of the Florida, Puerto Rico epidemic in 1991-1992. There was significant case clustering within households over short periods of time (≤ 3 days) but in general the spatial pattern of cases was similar to the population distribution as a whole. They were unable to identify spatial cluster at a larger scale. They suggested that the reason for the rapid and explosive spread of the virus might be due to the high mobility of the Florida citizens. In addition to this, rapid spread and limited spatiotemporal patterns around the residences could also be observed if transmission was occurring in high density public areas.

Heterogeneity in host-vector contact and exposure is fundamental in shaping population-level disease dynamics [49, 50]. Heterogeneities in human-mosquito exposure have recently been incorporated into dengue disease transmission models. Favier et al. [51] analyzed the Easter Island epidemic in 2002 to determine if human and mosquito spatial structure had an influence on dengue epidemiology. They modeled the epidemic using a version of the classic susceptible infected recovered (SIR) model, which assumes homogeneous mixing and then adapted the model to include theoretical heterogeneous contact rates (intra-household, inter-household, inter-areas). Only when including heterogeneity in contact rates were they able to reproduce the epidemiological curve. The homogenous mixing model was unable to describe epidemics on a local scale even in a relatively small, localized and initially immunologically naïve population. This study showed that human population structure and heterogeneity in human-mosquito exposure are determinants for the evolution of an epidemic [51].

Studies are now starting to spatially analyze empirical data to identify hot spot areas and independent variables associated with dengue risk. Honorio et al. [52] modeled vector density, using the MosquiTRAP, and the seroprevalence of dengue in three neighborhoods with different socioeconomic profiles in Rio De Janeiro, Brazil. After spatially analyzing recent dengue infections and *Ae. aegypti* distribution they found that the neighborhood with the highest rate of recent infection to dengue also had the lowest mosquito abundance. This observation was attributed to the conditions of the area, which were best for human mosquito exposure. They also found that seroprevalant hot spots were located nearest to commercial areas. Stoddard et al. [53] created an exposure model for dengue transmission that takes into account human spatial and temporal distributions. By integrating empirical data into the model they were able to identify locations of where individuals frequented, the duration, and the risk of exposure to host seeking females. They determined that certain areas such as a local clinic contributed more to disease transmission than areas with higher vector abundances.

It has been proposed that only a small portion of a population contributes significantly to the spread of many infectious diseases, including sexually transmitted and vector-borne diseases. Empirical and theoretical studies on HIV/AIDS have shown that human contact networks influence the spread of disease wherein a few individuals with high sexual contacts rates contributed disproportionately to disease transmission [54, 55]. Woolhouse et al. [56] analyzed the host to vector and host to host ratios of 10 data sets consisting of vector borne and sexually transmitted diseases and found that distributions of transmission rates were aggregated; most households or individuals experienced low to zero transmission while some experience very high rates. All data sets analyzed complied with the 20/80 rule of transmission: 20% of the population contributes to at least 80% of net transmission potential. The 20/80 rule may also be relevant for locations of transmission where 20% of locations contribute to 80% of disease transmission [57]. In highly mobile communities such as Singapore where outdoor transmission seems to be epidemiologically significant, certain locations may be providing very high degrees of exposure and contributing disproportionately to disease transmission.

Vector borne disease transmission dynamics involve a complex interaction of multiple agents that are spatially and temporally heterogeneous. Modern entomological surveillance methods and advanced analytical tools, which can account for these dynamic interactions, are fundamental to developing the foundation for understanding dengue transmission dynamics [58]. This study provided insight into the relative contribution of the two local dengue vectors and contributed to developing the necessary tools to help direct vector control efforts to priority areas that can have the most effect on reducing transmission.

CHAPTER 2

Evaluation of a fixed position adult *Aedes aegypti* surveillance system (MI-Dengue) in Singapore

Abstract

MI-Dengue, an adult female *Aedes* surveillance system that incorporates geospatial information technology to stratify entomological risk, was evaluated in Ang Mo Kio, Central Singapore. The MosquiTRAP was compared to standard vector surveillance strategies and evaluated under a range of different urban habitats. Under local entomological conditions, the concentration of MosquiTRAPs for efficient *Ae. aegypti* surveillance was determined for horizontal and vertical space. The MI-Dengue was sufficient at differentiating areas with significantly higher *Ae. aegypti* abundances, which can be targeted by vector control activities possibly reducing dengue transmission.

Introduction

In the twenty first century vector surveillance and control continue to be the only available methods for primary dengue prevention. The most efficient entomological surveillance methods employ geographic information systems and spatial analysis to monitor mosquito distributions, identify high-risk areas, and investigate epidemiological and ecological interactions [13, 59-61]. MI-Dengue is one of few modern systems with integrated monitoring for guided dengue vector control activities [30]. The surveillance system incorporates a geospatial information technology to stratify entomological risk areas and direct municipal actions. Adult mosquito abundances are monitored with fixed position sticky traps, MosquiTRAPs, which are designed to maximize mosquito capture and operational efficiency [30, 59, 61]. Entomological risk is calculated from the mean female *Aedes* index (MFAI) and the temporal mean female *Aedes* index (MFAIt) and categorized as one of three levels for a given area: non-critical, dengue alert, or critical focal control areas [30, 62].

The MI-Dengue system has previously been shown to reduce dengue and to be more cost effective than traditional larval surveillance [30, 63]. The MosquiTRAP is typically more sensitive than larval surveillance [27, 64] and equal [32] or less sensitive than oviposition trap surveillance [65]. Dengue clusters also correlate more significantly with the fixed position adult mosquito captures in comparison to oviposition trap or larval surveillance [61]. Evidence suggests that MI-Dengue is an efficient, cost-effective, and sustainable surveillance method. However, this system has been evaluated in limited geographic regions and populations, and dengue transmission arenas can vary greatly, particularly with respect to the distribution and density of mosquitoes and susceptible humans, in ways that could affect the role of adult mosquito surveillance in control programs.

To evaluate the utility of a fixed position adult mosquito surveillance system in a contrasting geographic setting with a different control program, we chose Singapore because of long-standing dengue endemicity, existing infrastructure and in-depth historic and ongoing dengue surveillance data and control programs. The current vector surveillance and control program in Singapore is an advanced system that utilizes geographic information systems, molecular dengue diagnostics, and larval risk estimates (i.e. *Aedes* premise index) conducted extensively year-round [13]. The integrated program includes larval source reduction, focal response to dengue case clusters, health education, and law enforcement, and has been in operation since 1969. The program was successful in maintaining a very low premise index of around 2% and decreased symptomatic dengue incidence for 15 years [6]. However, despite the low premise index dengue reemerged in the late 1980s with 2013 potentially having the largest epidemic within the last decade.

Traditional larval surveillance has been based upon the premise index, and related indices, which originally were developed from surveys of homes during yellow fever control efforts in the 1920s [14]. These immature mosquito surveillance methods have not changed dramatically in the last 50 years and may not provide an accurate description of mosquito dynamics and dengue risk in urban areas, especially those with significant vertical structure such as high-rise buildings. The aim of the current study was to evaluate a modern fixed position adult mosquito surveillance system (i.e. MI-Dengue) in comparison to traditional risk measures in Central Singapore. Studies included the optimization of surveillance trap density, operational evaluation of vertically distributed mosquitoes, and comparison of entomological indices including oviposition traps, larval surveillance, adult mosquito aspiration, BG Sentinel traps, and MosquiTRAPs.

Methods

Study Site

The study was conducted for 14 months, August 2010 to October 2011, in Ang Mo Kio GRC (Group Representation Constituency), Central Singapore, and included five subdivisions: Teck Ghee, Kebun Baru, Cheng San, Jalan Kayu, and Yio Chu Kang. The population in Ang Mo Kio GRC was approximately 179,297 at a density of 13,792 persons per sq km [66]. The study area was 6.21 sq km and included six single family home communities, 430 government subsidized housing buildings containing 49,423 units, 10 private condominiums, at least 20 schools, two large parks, two mass rapid transit hubs, one hospital, one business sector, and more than eight shopping areas of varied size. The area is characterized by a sea of multi-storied government subsidized housing buildings, commonly referred to as HDBs (Housing Development Board) that contain commercial shops or a void deck on the ground floor. The average HDB in the study site was 12 stories and included 117 units, with open corridors and a void deck.

MI-Dengue and the MosquiTRAP

MI-Dengue is a modern entomological surveillance system designed to monitor adult female mosquitoes using fixed position MosquiTRAPs [30]. The MosquiTRAP, an adult sticky trap, is based on the principle of oviposition traps and captures adult female mosquitoes attracted to a potential oviposition site. The MosquiTRAP is baited with an artificial attractant, Atr*Aedes*: a synthetic oviposition attractant developed from volatile chemicals associated with immature mosquito sites [67]. Following capture on a sticky card, mosquitoes are identified to species in the field, and immediately entered into the surveillance system using mobile phone technology. Traps are checked weekly and mosquitoes are brought to the laboratory for confirmation and/or further analysis.

Horizontal and Vertical Trap Density

A study to determine the optimal density of fixed traps for horizontal and vertical surveillance was conducted over nine weeks, August 16, 2010 to October 15, 2010. The Yio Chu Kang subdivision of Ang Mo Kio was stratified by use: three residential, one commercial/mixed-use area, one business sector). Residential areas ranged from 0.25-0.34 sq km, mixed-use was 0.29 sq km, and the business area was 0.95 sq km.

All five areas were evaluated using horizontal trap densities of 12, 24, and 48 MosquiTRAPs per 0.9 sq km. Each trap density was investigated for a period of three weeks: 12 traps /0.9sq km (weeks one-three); 24 traps /0.9sq km (weeks four-six); 48 traps /0.9sq km (weeks seven-nine). The selected trap densities were based on a pre-determined density widely used in Brazil (16 traps per sq km) and the belief that lower mosquito densities in Singapore may necessitate higher trap densities [30]. A 50-meter grid with numbered grid boxes was used to help facilitate trap placement in horizontal space. Grid boxes were chosen at random using a random number generator but final trap placement within the grid boxes was based on, the most favorable environment for trap productivity (e.g. security of location, exposure, vegetation, etc.) and upon available permission (e.g. government agency, school principle, landowner, business manager, etc.). If permission was not granted an adjacent location was chosen.

Vertical MosquiTRAP capture rates were investigated in the three residential areas concurrent with horizontal trapping. Three vertical strategies were performed for a period of three weeks and rotated between residential areas. These strategies were designed with operational efficiency in mind and included: ground level only; ground, middle, and top floors; and every other floor. The every other floor strategy started at the second floor, which is the first floor containing residential units, the ground floor was not included. Buildings were selected according to horizontal grid sampling. All vertical trap placements in residential areas were along outdoor corridors on selected floors or the open void decks on the ground floor. Buildings with trapping regimes covering every other floor typically received 5-6 traps (e.g. 10-12 story buildings).

Fixed Position Trap Surveillance

Following the optimization of trap densities, a total of 247 MosquiTRAPs were placed systematically throughout the Ang Mo Kio study site along a grid at a density of 36 traps per 900 sq m (Figure 1). If a fixed position trap was located at an HDB building it was placed outdoors on the second floor. Placement of fixed position MosquiTRAPs occurred from May 16, 2011 to July 13, 2011. MosquiTRAPs were monitored weekly and the attractant, Atr*Aedes*, and sticky card were replaced monthly. Each MosquiTRAP contained water treated with *Bacillus thuringienis* ssp. *israelensis* (BTI) to prevent unintentional larval development.

Twelve fixed position oviposition traps were also monitored during this same period. Oviposition traps were placed in representative areas around the study site: five in commercial/mixed-use areas, five in HDBs, one in a single family home community, and one in a manufacturing area. Oviposition traps were serviced and grass infusion replaced twice weekly. All eggs were transported to an insectary at Duke-NUS Graduate School for species identification.

Figure 2.1 Boundaries of the study site in Ang Mo Kio, Central Singapore are highlighted in red. MosquiTRAP locations (red dots), at a density of 36 traps per 0.9 sq km, were determined using a grid system. The study site was approximately 6.21 sq km.

Focal Investigations

Intensive focal investigations are described in detail in Chapter 4. Briefly, focal investigations were conducted periodically over 2-3 week periods and within a 300 meter radius of the fixed position surveillance MosquiTRAPs. Investigations were intended to collect all mosquito stages and followed a standard protocol that included: horizontal placement of eight oviposition traps, six BG Sentinel traps, and 18 MosquiTRAPs; vertical trap concentration was based on vertical area, one oviposition trap per 2 sq m, two MosquiTRAPs per sq m, and one BG Sentinel per 4 sq m; indoor aspiration and larval survey, targeting 20% of residences and/or commercial shops; and weekly outdoor larval surveys. The location of focal investigations were based on fixed position MosquiTRAP indices, mean female *Aedes* index (MFAIt), which was calculated as the average number of *Aedes* captured over three weeks [30]. Six areas underwent focal investigation and included two with high MFAIt, two with moderate MFAIt, and two with low *Ae. aegypti* MFAIt..

National Environment Agency (NEA) Surveillance Data

The NEA provided additional larval surveillance data for the study site. NEA field officers collected the surveillance data independently. The provided data included the location of the larval habitat (anonymized to block), number of immatures, and species identification. Approximately one month is needed for NEA field officers to comb the Ang Mo Kio study site for larval habitats.

Data Analysis

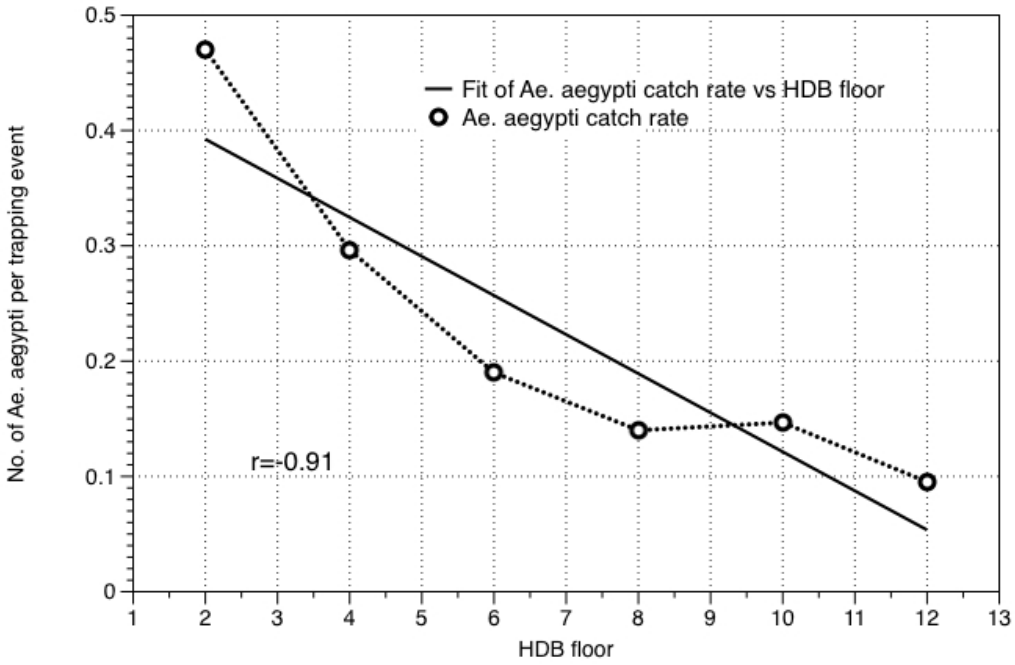
Statistical analyses were performed in R version 2.15.2 (2012, The R Foundation for Statistical Computing, http://www.R-project.org) and IBM SPSS Statistics version 19.0.0. Selection of the optimal density of traps per 900 sq km was determined according to the density at which the average weekly capture no longer significantly increased. To investigate the distribution of *Ae. aegypti* in HDBs the data was fit to a negative binomial generalized linear model and odds ratios were calculated for capture rates by floor. The sensitivity of each HDB floor was calculated by dividing the number of times an *Ae. aegypti* mosquito was captured on the study floor by the number of times an *Ae.aegypti* mosquito was captured on any floor. Sensitivity was calculated based weekly *Ae. aegypti* captures and *Ae. aegypti* captures over three week intervals. Linear correlation (Pearson) was used to identify relationships between: mosquito catch rate and HDB floor; weekly fixed position oviposition trap and MosquiTRAP catch rate; monthly fixed position MosquiTRAP catch rate and monthly NEA larval surveillance data; and MosquiTRAP catch rate in comparison to other methods. A negative binomial generalized linear model and post hoc test (Tukey Contrasts) was used to determine if MFAIt could be used to differentiate between areas with high and low *Ae. aegypti* abundances.

Results

Surveillance results were analyzed in order to: 1) Determine an optimal horizontal and vertical density of fixed position traps; 2) Monitor differences in capture rates (CR) according to fixed position sticky traps within the study area; 3) Validate if fixed position trap CR were representative of low or high mosquito abundances; and 4) Compare surveillance methods.

Horizontal and Vertical Trap Density

Vertical trap sampling indicated that the ground level collected the lowest average *Aedes* per trap (i.e. MosquiTRAP) per week in comparison to higher locations (ground=0.09 adults per week per trap, ground/middle/top=0.15, every other floor=0.27). This result differed by species, with fewer *Ae. albopictus* collected higher than ground level compared to *Ae. aegypti*, 10 versus 151 respectively. The vertical distribution of *Ae. aegypti* within an HDB was fit to a generalized linear model to calculate odds ratios for abundance by floor (Table 2.1). When analyzing the every other floor sampling strategy, the likelihood of collecting *Ae. aegypti* on the second floor (CR=0.51 *Ae. aegypti* per week per trap) was significantly higher than the sixth (CR=0.28), eight (CR=0.16), tenth (CR=0.15), and twelfth (CR=0.09) floors but not the fourth floor (CR=0.30) (Table 2.1). The trend over all floors however was an inverse relationship between *Ae. aegypti* catch rate and floor height (Pearson linear correlation r=-0.91, t=-4.47, df=4, p=0.01) (Table 2.1).



Floor placement	Odds ratio	95% CI	Wald Chi Square	df	P-Value
2nd vs 12th	3.22	1.83 - 5.68	16.58	1	<0.0001
2nd vs 10th	2.76	1.56 - 4.74	12.54	1	<0.0001
2nd vs 8th	2.76	1.56 - 4.74	12.54	1	<0.0001
2nd vs 6th	2.35	1.32 - 4.16	9.62	1	0.002
2nd vs 4th	1.68	0.92 - 3.74	2.77	1	0.096

Table 2.1 Linear correlation analysis (Pearson) was performed on HDB floors and *Ae. aegypti* catch rate (top). The vertical distribution of *Ae. aegypti* in HDBs. Generalized linear models were used to calculate odds ratios for the total number of *Ae. aegypti* collected on the 2nd floor compared to all other floors during the every other floor vertical trap placement strategy (bottom).

The second floor was determined to be the most sensitive floor for capturing *Ae. aegypti* mosquitoes. The sensitivity of the second floor, based on three-week intervals was 77%; the second floor was positive for *Ae. aegypti* mosquitoes 20 times during the three week intervals and the number of times any floor was positive for *Ae. aegypti* was 26. The sensitivities of the remaining floors were: fourth floor 58%, sixth floor 44%, eighth floor 28%, tenth floor 33%, and twelfth floor 29%. If sensitivity was calculated based on weekly *Ae. aegypit* captures the sensitivity of each floor decreased: second floor 52%, fourth floor 35%, sixth floor 23%, eighth floor 15%, tenth floor 22%, and twelfth floor 10%. It was decided that placing traps on the second floor of HDBs was the most operationally efficient strategy for fixed position surveillance and that the second floor could be used in lieu of the ground floor when trap placement was at HDBs.

During horizontal optimization of trap densities an average of 0.25 adult mosquitoes were captured per trap (i.e. MosquiTRAP) per week in the commercial/mixed-use area, 0.12 adults per trap per week in the business area, and 0.09 adults per trap per week in the residential areas. The ground floor of HDB in residential areas, which is most often a void deck, is a poor location for MosquiTRAPs and oviposition, due to high human traffic and no vegetation. To calculate the optimal density of horizontal MosquiTRAP the second floor was used due to the unproductiveness of the ground floor traps.

Mosquito capture rates from MosquiTRAPs increased when trap densities were increased starting from a low density of 12 traps per 0.9 sq km (commercial/mixed-use= 0.25 adult per trap per week, business sector=0.05, second floor of HDB=0.42) to moderate density of 24 traps per 0.9sq km (commercial/mixed-use= 0.29, business sector=0.23, second floor of HDB=0.5) (Figure 2.2). However, the same rate increase was not observed when trap concentrations increased from a moderate to a high density of 48 traps per 0.9 sq km (commercial/mixed-use= 0.21, business sector=0.08, second floor of HDB=0.42). Results suggest a decrease in average capture rates above approximately 27-32 traps per 0.9 sq km and to avoid under sampling, 36 traps per 0.9sq km was chosen to be an appropriate number for fixed position adult surveillance (see Figure 2.1).

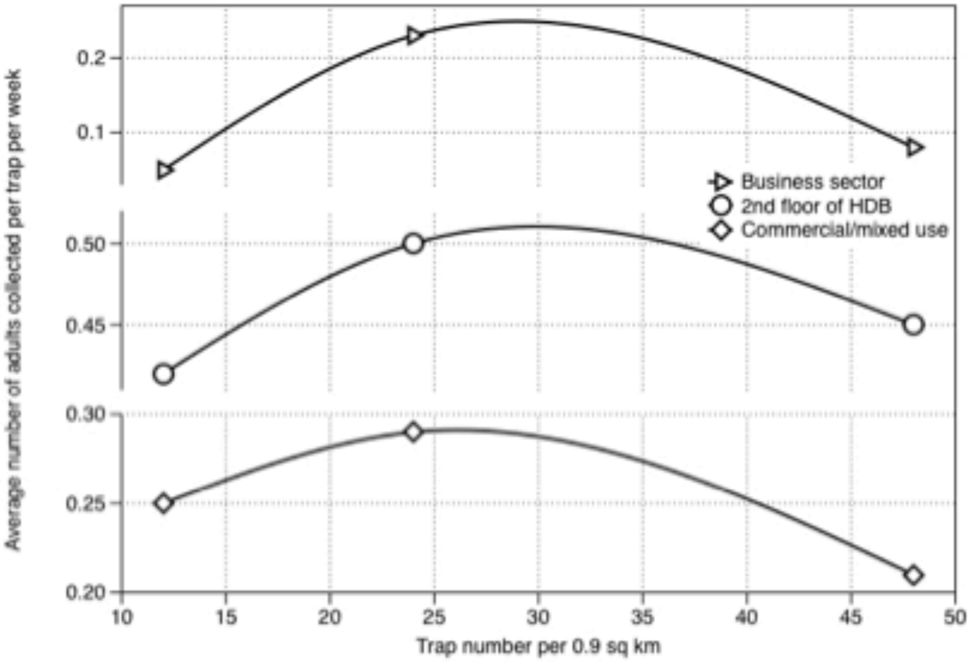


Figure 2.2 Displays the average number of adults collected per trap per week with increasing concentrations of horizontally placed MosquiTRAPs in: the business area, the 2nd floor residential, and the commercial/mixed-use area.

Fixed Position Trap Surveillance

MosquiTRAPs were serviced weekly from May 23, 2010 to October 31, 2010, totaling 23 weeks and 5,318 successful sampling events. The fixed position results are summarized in Table 2.2. A total of 2,467 *Aedes* mosquitoes were collected, 937 (38%) were *Ae. aegypti* and 1,458 (59%) were *Ae. albopictus*. The remaining 72 *Aedes* spp mosquitoes were damaged and the species could not be determined. The average catch rate for *Aedes* mosquitoes in Ang Mo Kio was 0.46 mosquitoes per trapping event. The average rates for *Ae. aegypti* and *Ae. albopictus* were 0.18 and 0.28, respectively. Oviposition traps were serviced twice weekly, totaling 543 successful collections. The traps collected 20,500 eggs, yielding an average catch rate of 37.84 eggs per collection. Out of a total of 4,920 eggs hatched, 1,930 (39%) were *Ae. aegypti* and 2,990 (61%) were *Ae. albopictus*. A linear correlation analysis (Pearson) found weekly oviposition trap catch rates were significantly correlated with weekly MosquiTRAPs *Aedes* catch rates (r=0.47, t=2.44, df=21, p=0.024) (Figure 2.3).

Trap type	Max no. deployed [a]	Total collections	Total specimens (avg. catch rate)	Total *Ae. aegypti* (avg. catch rate)	Total *Ae. albopictus* (avg. catch rate)
MT	247	5,318	2,467 (0.46)	937 (0.18)	1,458 (0.28)
OT	12	543	20,5000 (37.84)	1,930 (7.44) [b]	2,990 (11.55) [b]

Table 2.2 Fixed position *Aedes* adult surveillance overview.
[a]Maximum number refers to the total number deployed once placement was completed.
[b]Egg hatch rate averaged 45% and only weeks 1-11 are available.

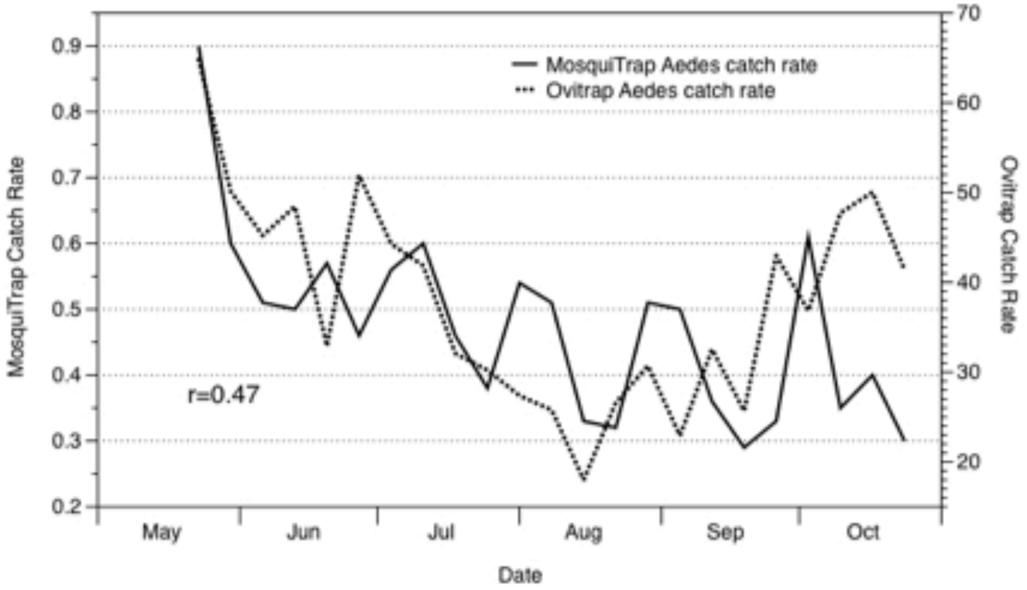


Figure 2.3. Linear correlation analysis (Pearson) was performed on weekly *Aedes* catch rates by fixed position MosquiTRAPs and ovitraps for the duration of surveillance.

NEA Larval Surveillance Data

The monthly number of *Aedes* larval habitats identified by NEA field officers within the study site significantly correlated with the monthly total number of *Aedes* mosquitoes collected by MosquiTRAPs (r=0.93, t=4.50, df=3, p=0.020) (Figure 2.4).

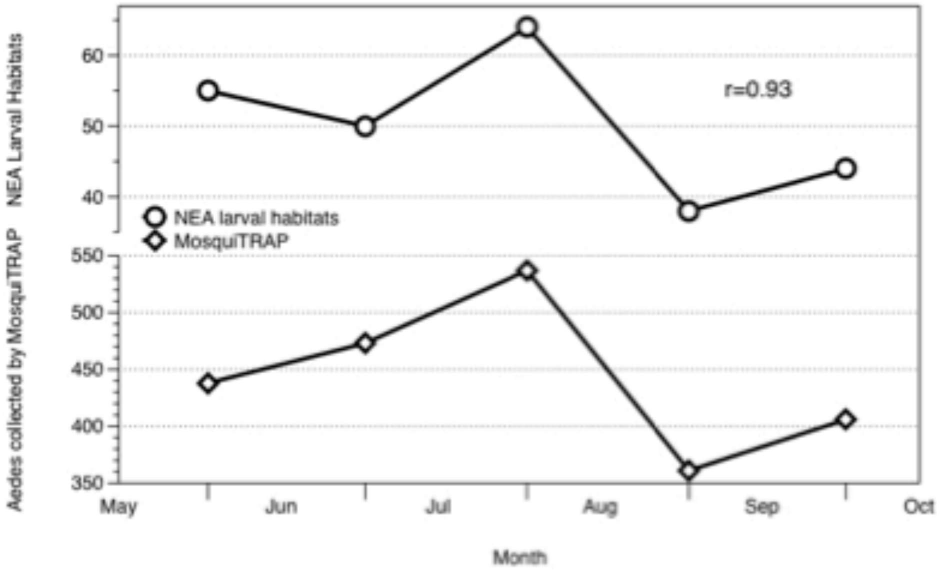
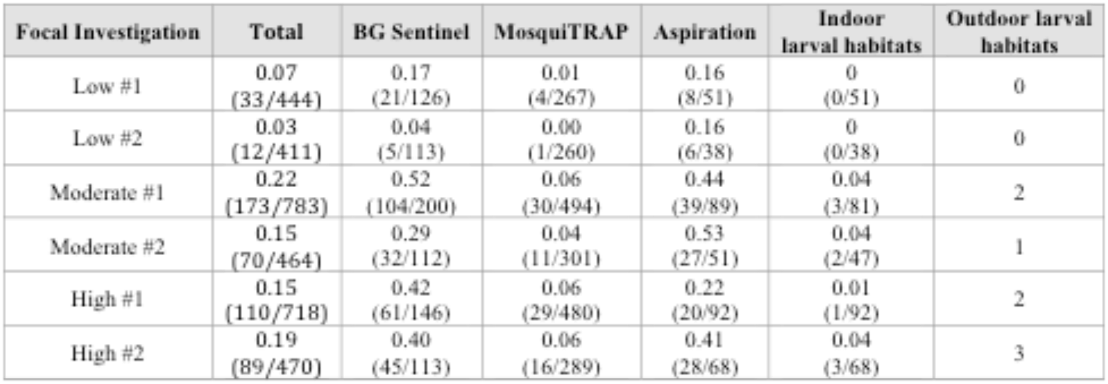


Figure 2.4 Linear correlation analysis (Pearson) was performed on monthly totals of *Aedes* collected by fixed position MosquiTRAPs and the monthly number of larval habitats identified by NEA field officers.

Focal Investigations

Focal investigations took place between June 27, 2010 and October 17, 2010, each ranging from 2-3 weeks. Focal investigation locations were chosen based on fixed position MosquiTRAP *Ae. aegypti* MFAIt. MFAIt was calculated weekly for each fixed position MosquiTRAP. Six locations were chosen: Low 1 (MFAIt=0) and Low 2 (MFAIt=0.3); Moderate 1 (MFAIt=0.7) and Moderate 2 (MFAIt=1); and High 1 (MFAIt=2) and High 2 (MFAIt=2.3). The MFAIt for *Ae. albopictus* was also calculated but locations were based on *Ae. aegypti* rates alone.

Focal investigation results are summarized in Table 2.3. Focal investigations Low #1 and Low #2 collected the least number of adult *Ae. aegypti* mosquitoes, 0.07 and 0.03 *Ae. aegypti* per trapping event. Focal investigations Moderate #1, Moderate #2, High #1, and High #2 had similar total *Ae. aegypti* catch rates ranging from 0.15 to 0.22 *Ae. aegypti* per trapping event. The number of *Ae. aegypti* collected per positive trapping event for focal investigations Low #1 and Low #2 were 0.19 and 0.06, Moderate #1 and Moderate #2 were 0.37 and 0.22, and High #1 and High #2 were 0.58 and 0.38.



Focal Investigation	Total	BG Sentinel	MosquiTRAP	Aspiration	Indoor larval habitats	Outdoor larval habitats
Low #1	0.07 (33/444)	0.17 (21/126)	0.01 (4/267)	0.16 (8/51)	0 (0/51)	0
Low #2	0.03 (12/411)	0.04 (5/113)	0.00 (1/260)	0.16 (6/38)	0 (0/38)	0
Moderate #1	0.22 (173/783)	0.52 (104/200)	0.06 (30/494)	0.44 (39/89)	0.04 (3/81)	2
Moderate #2	0.15 (70/464)	0.29 (32/112)	0.04 (11/301)	0.53 (27/51)	0.04 (2/47)	1
High #1	0.15 (110/718)	0.42 (61/146)	0.06 (29/480)	0.22 (20/92)	0.01 (1/92)	2
High #2	0.19 (89/470)	0.40 (45/113)	0.06 (16/289)	0.41 (28/68)	0.04 (3/68)	3

Table 2.3 *Ae. aegypti* catch rates for the different trapping methods were calculated for each focal investigation. Specimens collected per trapping events are in parentheses.

To determine if the mean number of *Ae. aegypti* collected were significantly different between focal investigations, the data was fit to a negative binomial generalized linear model with a multiple comparison of means post hoc test (Tukey Contrasts). The mean number of total adult *Ae. aegypti* collected by BG Sentinels, MosquiTRAPs, and aspirations combined were significantly greater in focal investigations Moderate #1 (p=<0.001), Moderate #2 (p=0.055), High #1 (p=0.021), and High #2 (p=0.002) in comparison to focal investigations Low #1. Focal investigations Moderate #1 (p=<0.001), Moderate #2 (p=<0.001), High #1 (p=<0.001), and High #2 (p=<0.001) were also significantly greater than focal investigation Low #2 (Figure 2.5).

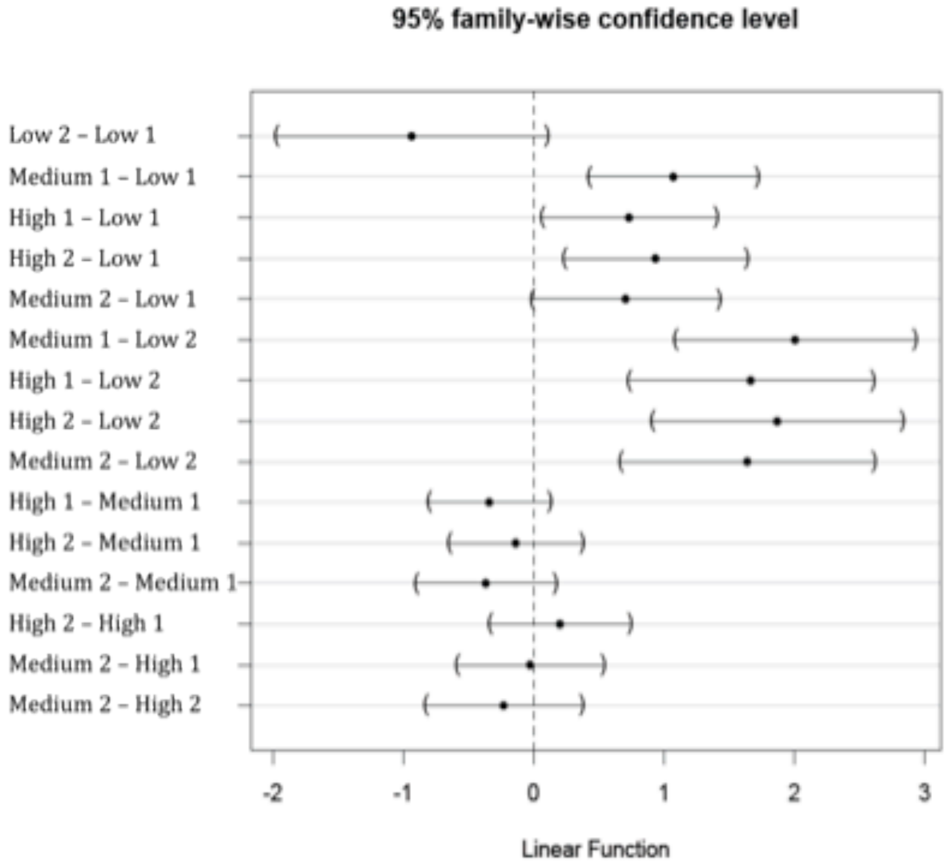


Figure 2.5 Total adult *Ae. aegypti* data (BG Sentinel, MosquiTRAP, and aspiration) was fitted to a negative binomial generalized linear model with multiple comparisons of means (Tukey Contrasts) to discern a significant difference in *Ae. aegypti* abundance among focal investigations.

The mean numbers of *Ae. aegypti* mosquitoes collected by the different trap types (BG Sentinel, MosquiTRAPs, aspiration, and outdoor and indoor larval surveys) were also compared between focal investigations (Table 2.3). The mean numbers of *Ae. aegypti* collected by BG Sentinels were significantly greater in focal investigations Moderate #1 (p=<0.001), Moderate #2 (p=0.003), High #1 (p=<0.001), and High #2 (p=<0.001) than focal investigation Low #2. The mean number of *Ae. aegypti* collected by BG Sentinels were also significantly greater in focal investigation Moderate #1 (p=<0.001), High #1 (p=0.027), and High #2 (p=0.064) than focal investigation Low #1. Focal investigation Moderate #2 was not significantly different than Low #1. The mean number of *Ae. aegypti* collected by MosquiTRAPs and aspirations were not significantly different between focal investigations. No outdoor larval habitats were found in focal investigations Low #1 and Low #2. One outdoor larval habitat was found in focal investigation Moderate #2, two in Moderate #1 and High #1, and three in High #2. The percentage of residences and or commercial shops positive for larval habitats in focal investigations Moderate #1 and Moderate # 2 were 4% (11% and 6% including eggs, that is, positive for eggs or larvae). Only 1% (4% including eggs) of residence and or commercial shops in focal investigation High #1 and 4% (13% including eggs) in High #2 were positive for larval habitats. There were no residences or commercial shops positive for larval habitats in focal investigations Low #1 and Low #2.

Correlation of MosquiTRAP to Other Entomological Tools

Focal investigations took place for a total of 13 weeks. Linear correlation analyses were performed on the weekly catch rates of each trap during focal investigations (Figure 2.6). The weekly *Ae. aegypti* catch rate of MosquiTRAPs was significantly correlated with the weekly *Ae. aegypti* catch rate of BG Sentinel (r=0.62, t=2.72, df=12, p-value=0.02) and weekly number of *Ae. aegypti* collected by aspirations (r=0.63, t=2.88, df=12, p-value=0.01) (Figure 2.6). The total number of *Aedes* collected by MosquiTRAPs significantly correlated with the total number of eggs collected by ovitraps (r=0.55, t=2.28, df=12, p=0.04).

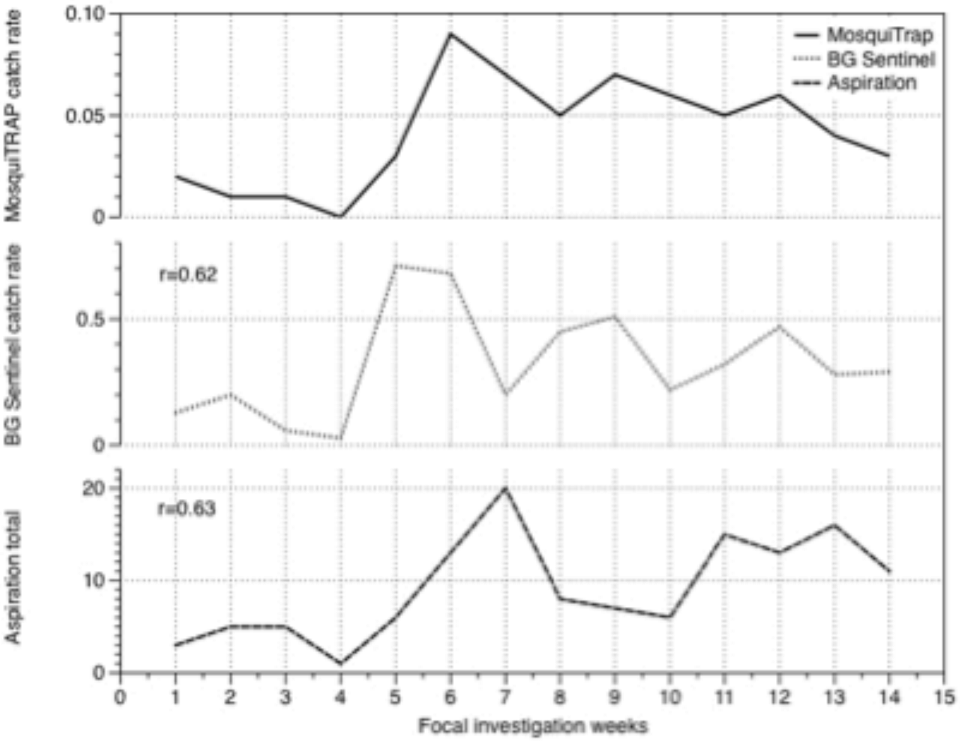


Figure 2.6. Linear correlation analyses (to determine Pearson's r) were performed on the weekly *Ae. aegypti* catch rates collected by MosquiTRAPS and the weekly *Ae. aegypti* catch rates collected by BG Sentinels and the total number of *Ae. aegypti* collected by indoor aspirations, during focal investigations.

Discussion

Singapore has been described as a vertical city: government subsidized housing can reach twenty-eight stories and eighty-two percent of the population reside in these high-rise buildings [66]. An effective fixed position mosquito surveillance system would include both Singapore's horizontal and vertical environments. The current study evaluated MI-Dengue, an adult *Aedes* surveillance system, in Ang Mo Kio, Singapore using both horizontal and vertical trap positioning strategies. Results showed that distributing surveillance traps in the vertical dimension (on the second floor) gave the highest sensitivity for detecting mosquitoes in a given building. For long-term surveillance placing one MosquiTRAP on the second floor of high-rise buildings is very cost effective, less labor intensive, less intrusive to residents and therefore more successful, when compared to immature surveillance. Overall, five days and two field officers were required to service all surveillance MosquiTRAPs within the 6sq km study area. Results of the MosquiTRAP adult oviposition trap was ground-truthed against standard vector surveillance strategies and was found to be consistent with these strategies across a range of different urban habitats that varied in usage and human densities (e.g., residential versus commercial). MosquiTRAP fixed position surveillance significantly correlated with larval and oviposition surveillance. MosquiTRAPs also significantly correlated with BG Sentinel traps and mosquito aspiration. Due to limitations in trap placement comparing trap sensitivity was not feasible.

MI-Dengue was able to identify areas with low *Ae. aegypti* abundances from areas with significantly higher abundances. The areas surrounding MosquiTRAPs with a moderate or high MFAIt had similar *Ae. aegypti* abundances. This lack of differentiation between areas could be due the MFAIt cut off points; a greater difference in MFAIt may be able to predict areas with moderate and high number of *Ae. aegypti*.

Vector control indices have historically focused on juvenile stages regardless of their lack of association and predictive value of disease occurrence [14, 68]. Adult *Ae. aegypti* density is more closely associated with disease incidence and is therefore a better estimate of disease risk. An established fixed position vector surveillance program would have the ability to differentiate areas with the highest *Ae. aegypti* abundances, hot spots, and direct vector control activities. These areas should be further investigated using host seeking traps that will enable stratifying human-mosquito exposure and identifying areas of increased dengue transmission risk. This paper adds to the mounting evidence illustrating that MI-Dengue is an efficient, cost-effective, and sustainable adult female *Aedes* surveillance method and a valuable addition to dengue research and prevention strategies.

CHAPTER 3

Spatial and temporal distribution and abundance of adults *Aedes* mosquitoes and dengue incidence in Ang Mo Kio, Central Singapore

Abstract

Dengue is still a significant public health threat in Singapore despite a successful vector control program. A fixed position adult female *Aedes* surveillance system, MI-Dengue, was used to identify spatial and temporal patterns in *Ae. aegypti* and *Ae. albopictus* distribution, abundance, and infection status, in Ang Mo Kio, Central Singapore. This allowed the identification of consistently hot locations. *Ae. aegypti* abundance and distribution was found to have a positive linear correlation with HDB characteristics, suggesting that *Ae. aegypti* density was highest in large HDBs. Dengue cases referenced by home address were evaluated temporally and spatially with *Ae. aegypti* and *Ae. albopictus* catch rates. Weekly *Ae. albopictus* catch rates correlated more with dengue incidence compared to *Ae. aegypti*. *Ae. aegypti* and *Ae. albopictus* catch rates at traps near dengue cases were higher than average. This study provides evidence in favor of an adaptive adult *Aedes* population management program that directs vector control activities potentially decreasing dengue incidence.

Introduction

Understanding mosquito population spatial dynamics, spatial variation in host-vector exposure, and how landscape factors influence local mosquito abundance and distribution is essential for reducing mosquito populations and disease incidence. Vector surveillance programs that monitor entomological parameters through geographic information systems and spatial analysis have become an important tool in elucidating the heterogeneity of mosquito population dynamics and epidemiological and ecological interactions. Recent advancements in entomological methodologies have enabled the incorporation of large-scale adult mosquito monitoring into vector surveillance programs. Historical methods of immature surveillance have been shown to be more labor intensive, have similar or less sensitivity, and are difficult to correlate with dengue risk [14, 68].

MI-Dengue, an established vector surveillance program in Brazil, utilizes fixed position adult sticky traps, MosquiTRAPs, integrated into a geospatial information system, to calculate entomological risk and guide municipal actions [30]. MI-Dengue and similar adult monitoring systems are capable of detailing the dynamics of *Aedes* populations, identifying hot spots or clusters of relatively high mosquito abundances, and locating areas with the highest rates of human-mosquito exposure at varying temporal and spatial scales [61, 69, 70]. A recent study showed that adult mosquito clusters correlated better with dengue incidence clusters than larval clusters and were temporally more precise in predicting dengue incidence then clusters identified by oviposition [61].

Identifying consistent hot spots of vector abundance has the potential to improve vector control outcomes and decrease disease incidence. When evaluating areas for clustering or hot spots, the sampling scale has been found to be very influential [69, 71, 72]. Barrera [69] investigated the spatial stability of *Ae. aegypti* abundance in two neighborhoods in Puerto Rico. Global spatial dependency was not detected but local spatial clustering at the scale of 130 meters was significant with hot spots exhibiting stability over varying time periods [69]. Theoretically, an adaptive population management program that targets areas that are consistently "hot" would likely reduce local dengue transmission [69].

Heterogeneity in human-mosquito exposure is fundamental in shaping population-level disease dynamics and should be incorporated into vector surveillance programs [48, 49]. Human-mosquito exposure has recently been included in dengue disease transmission models [50, 53, 73, 74]. Stoddard et al. [53] created an exposure model for dengue transmission that takes into account human spatial and temporal distributions. By integrating empirical data into the model they were able to identify locations where individuals frequented, the duration, and the risk of exposure to host seeking females. They determined that certain areas such as a local clinic contributed more to disease transmission than areas with higher vector abundances. Vanwambeke et al. identified areas of increased risk of dengue transmission on Oahu by modeling the distributions of both humans and mosquitoes in recreational and residential areas. They proposed the inclusion of a spatially explicit host-vector parameter to further improve dengue transmission mechanistic models [73].

Despite the existence of one of the most successful vector control programs, dengue continues to be a major public health problem in Singapore. The country is hyperendemic and within the last decade dengue epidemics are the largest recorded in Singapore history

[6]. As of August 2013, the biggest epidemic is currently taking place with over 15,000 cases and five deaths, thus far [75].

The current study utilizes the MI-Dengue system to detail the temporal and spatial dynamics of adult *Ae. aegypti* and *Ae. albopictus*, to distinguish consistent mosquito abundance hot spots, and to identify environmental variables that affect *Aedes* distribution and abundance in Ang Mo Kio, central Singapore. The identification of hot spots has the potential to improve vector control actives by directing efforts to high-risk areas and possibly reduce dengue transmission.

Methods

Study Site

This study was conducted for 24 sampling weeks, May 2011 to November 2011, in Ang Mo Kio GRC (Group Representation Constituency), central Singapore. The study site was previously described in Chapter 2. Briefly, the study area was 6.21 sq km, with a population of approximately 179,297. The area is characterized by a sea of multi-storied government subsidized housing buildings, commonly referred to as HDB (i.e. Housing Development Board) that contain commercial shops, a void deck, or housing units on the ground floor.

Fixed Position Adult Mosquito Surveillance

Aedes adult female populations were monitored using fixed position MosquiTRAPs. The MosquiTRAP, an adult sticky trap, is based on the principle of oviposition traps and captures adult female mosquitoes upon investigation of oviposition sites. The MosquiTRAP is baited with an artificial attractant, Atr*Aedes*: a synthetic oviposition attractant developed from volatile chemicals associated with immature mosquito sites [67]. Mosquitoes were identified to species in the field following capture on the sticky card and placed into labeled eppendorf tubes. Mosquitoes were stored in a liquid nitrogen dry shipper prior to biweekly transport to the Duke-NUS Graduate Medical School insectary, where they remained at -80°C until species confirmation and further testing. MosquiTRAPs were monitored weekly and the attractant, Atr*Aedes*, and sticky card were replaced monthly. Non-functioning MosquiTRAPs were replaced within 24 hours of discovery. Under multiple occurrences of tampering, MosquiTRAPs were moved to a neighboring site. Each MosquiTRAP contained water treated with *Bacillus thuringienis* ssp. *israelensis* (BTI) to prevent unintentional larval development.

A total of 247 MosquiTRAPs were placed systematically throughout the Ang Mo Kio study site along a grid at a density of 36 traps per 900sq m. If a fixed position trap was located at an HDB building it was placed along the corridor on the second floor. Independent variables assessing human density in residential and non-residential areas that potentially influenced the abundance and distribution of *Ae. aegypti* and *Ae. albopictus* were measured at each trap location. HDB characteristics: number of units, number of units along corridor, number of three bedroom or less units, number of four bedroom or more units, the number of commercial shops located on the ground floor, and the age of the HDB were measured for each trap placed inside a HDB. Independent variables measured at outdoor trap locations included: the quantity of outdoors seating; and the number of HDB, HDB with shop houses, landed houses, private condos, parks, playgrounds, food courts, and businesses within sight and 50 meters.

Virus Detection and Isolation

Female *Aedes* mosquitoes collected during fixed position surveillance were screened for the presence of flaviviruses using RT-PCR and mosquito inoculation. A variable number of either *Ae. aegypti* or *Ae. albopictus*, collected from individual trapping events were homogenized on ice in 400 ul of 10% FBS DMEM media using a plastic pestle. Mosquitoes were screened in pools, created by combining 10ul of homogenate from 15 eppendorf tubes. RNA was extracted from each pool using the Omega E.Z.N.A Viral RNA Kit (catalogue no. R6874-02). The extracted RNA was screened for the presence of flaviviruses by RT-PCR with a pan-flavivirus probe developed by researchers at Duke-NUS PEID and Quanta Biosciences, One-Step qRT-PCR Kit (catalogue no. 101414-172). The PCR products were purified with Qiagen MinElute PCR Purification Kit (catalogue no. 28004) and sequenced. Pools with positive sequencing results were dissected and individual samples were screened for the presence of flaviviruses using the same protocol. Homogenate from the positive sample was centrifuged at 10,000 rpm for 10 minutes at 4°C before inoculation into four days old *Ae. albopictus* males. Mosquitoes were held at 28°C and 60-70% humidity for one week after inoculation and tested for disseminated virus by RT-PCR using the CDC Multiplex protocol [76].

Dengue Incidence

The Communicable Disease Division of the Singapore Ministry of Health (MOH) supplied clinical and laboratory confirmed dengue case incidence data for individuals who resided, worked, or studied in Ang Mo Kio GRC during the study period. The dengue case data provided by MOH included: home address (anonymized), date of healthcare visit, age, and school name or work address.

Data Analysis

Statistical analyses were performed in R version 3.0.1 (2013, The R Foundation for Statistical Computing, http://www.R-project.org) and SADIEShell version 1.22 (2011, Spatial Analysis by Distance Indices, http://home.cogeco.ca/~sadiespatial/index.html). SADIE was used to investigate *Ae. aegypti* and *Ae. albopictus* global and local spatial patterns. SADIE, a software program developed specifically for spatial analysis on ecological data, was used to calculate the overall index of aggregation (Ia), local indices for clustering (v), and overall (X) and local spatial association (chi-p) [77, 78]. Spatial patterns were evaluated for the entire data set and at four-week intervals. Local cluster indices and local spatial associations were used to create contour maps of the study area to visualize fine scale changes in *Ae. aegypti* and *Ae. albopictus* spatial patterns. Catch rates were transformed into integers, by adding one, multiplying by 10, and rounding up, to adjust for uneven sampling, i.e. non-functioning traps.

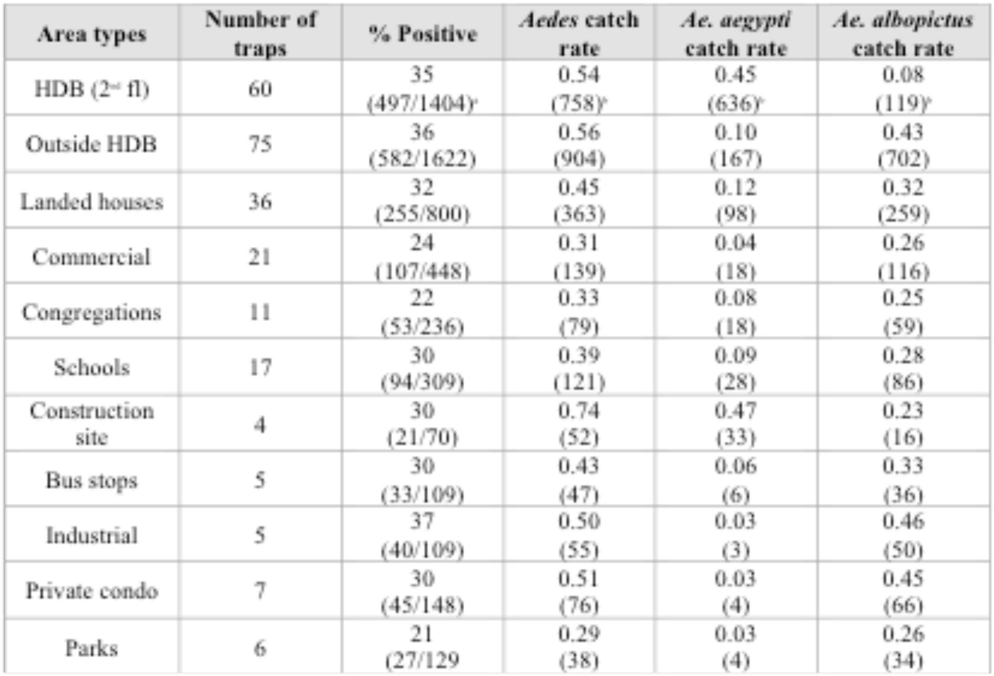
The Kruskal-Wallis test was used to investigate variation in *Ae. aegypti* and *Ae. albopictus* catch rates among MosquiTRAP locations grouped into area types. To explore the relationship between independent variables and *Ae. aegypti* and *Ae. albopictus* distribution and abundance the data was divided into two datasets, indoor and outdoor MosquiTRAPs. Mantel tests were performed on each data set to test for spatial autocorrelation by distance. Log and square root transformations were attempted. If transformation was unsuccessful regression analysis was performed using generalized linear models. Transformed datasets were analyzed using linear regression analysis.

Principle Component Analysis (PCA) and linear correlation analyses (Pearson) were performed on the indoor dataset to identify variable contribution and relationships between independent variables to improve the linear regression model.

A linear correlation analysis (Pearson) was performed on weekly dengue case incidence and weekly *Aedes* catch rates. Cross-correlation function estimation was used to determine if time lags improved correlation. Temporal Mean Female *Aedes* Index, average number of female *Aedes* collected over three weeks, was measured for traps within 60 meter of case reference locations before and after healthcare visit.

Results

A total of 247 MosquiTRAPs were placed throughout Ang Mo Kio, Singapore and sampled weekly from May 19, 2011 to November 03, 2011. The number of *Aedes* mosquitoes collected was 2,632 with a total catch rate (CR) of 0.49 *Aedes* mosquitoes per trapping event. Of those, 1015 were *Ae. aegypti* (CR=0.19) and 1543 were *Ae. albopictus* (CR=0.29). Traps were placed in 11 different area types listed in Table 3.1.



Area types	Number of traps	% Positive	*Aedes* catch rate	*Ae. aegypti* catch rate	*Ae. albopictus* catch rate
HDB (2nd fl)	60	35 (497/1404)^a	0.54 (758)^b	0.45 (636)^b	0.08 (119)^b
Outside HDB	75	36 (582/1622)	0.56 (904)	0.10 (167)	0.43 (702)
Landed houses	36	32 (255/800)	0.45 (363)	0.12 (98)	0.32 (259)
Commercial	21	24 (107/448)	0.31 (139)	0.04 (18)	0.26 (116)
Congregations	11	22 (53/236)	0.33 (79)	0.08 (18)	0.25 (59)
Schools	17	30 (94/309)	0.39 (121)	0.09 (28)	0.28 (86)
Construction site	4	30 (21/70)	0.74 (52)	0.47 (33)	0.23 (16)
Bus stops	5	30 (33/109)	0.43 (47)	0.06 (6)	0.33 (36)
Industrial	5	37 (40/109)	0.50 (55)	0.03 (3)	0.46 (50)
Private condo	7	30 (45/148)	0.51 (76)	0.03 (4)	0.45 (66)
Parks	6	21 (27/129	0.29 (38)	0.03 (4)	0.26 (34)

Table 3.1 MosquiTRAP locations for fixed position monitoring grouped by area type with the number of locations/MosquiTRAPs within each. Area type productivity was determined by percent positive of traps, *Aedes* catch rate, and species-specific catch rates.
[a] Total number of traps positive divided by total number of trapping events.
[b] Total number collected.

The spatial distribution and abundance of *Ae. aegypti* and *Ae. albopictus* was very heterogeneous between and within area types. The percent positive among the different area types was very similar, ranging from 21%-37%, but the number of *Ae. aegypti* and *Ae. albopictus* collected during trapping events was significantly different. A Kruskal-Wallis test revealed a significant difference in average catch rate of *Ae. aegypti* (chi-squared = 86.6593, df = 10, p-value = 2.455e-14), and *Ae. albopictus* (chi-squared = 86.5914, df = 10, p-value = 2.532e-14) among area types. The area type that had the highest abundance of *Ae. aegypti* was inside HDBs. MosquiTRAPs placed on the second floor of HDBs (n=636) collected almost four times as many *Ae. aegypti* as MosquiTRAPs placed outside HDBs (n=167). The commercial area, which was expected to have high *Ae. aegypti* catch rate, had one of the lowest (CR=0.04) *Ae. albopictus* catch rates were more evenly distributed among area types except for inside HDBs. Outside HDBs (CR=0.43), industrial (CR=0.46), and private condos (CR=0.45) had the highest catch rates.

Ae. aegypti catch rates from the 247 locations were organized into a five number summary (maximum, upper quartile, mean, lower quartile, and minimum), two were in the sample maximum (CR=1.1-1.25) and 16 locations were in the upper quartile (CR=0.75-1.08). Fifteen of these locations were located inside HDBs, two at construction sites, and one outside an HDB. *Ae. albopictus* catch rates organized into a five number summary consisted of three locations in the sample maximum (CR=1.33-1.55) and six locations in the upper quartile (CR=0.95-1.13). Three of these locations were outside HDBs, two were in the private condos, one was in the industrial, one was in public congregation, and two were in the landed houses. *Ae. aegypti* and *Ae. albopictus* catch rate distributions can be seen in figure 3.1A and 3.1B.

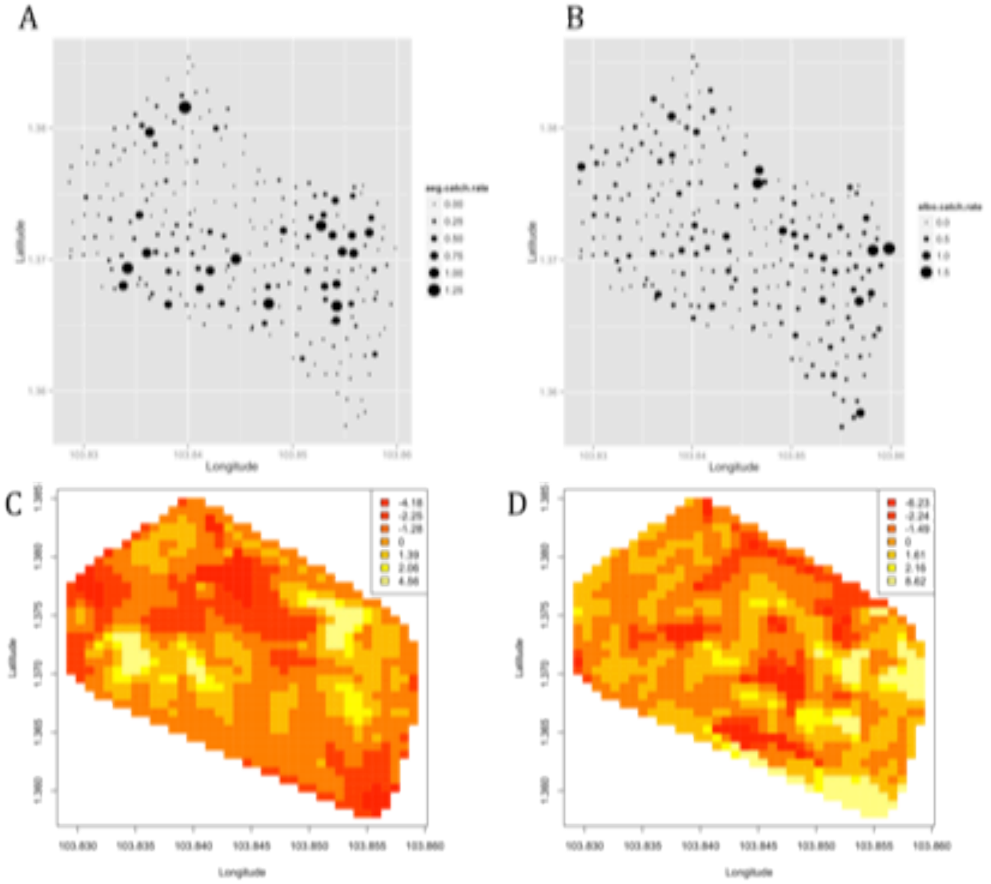


Figure 3.1 Distribution, abundance and spatial patterns of *Aedes* mosquitoes determined by MosquiTRAP catch rates, A) *Ae. aegypti* and B) *Ae. albopcitus*, and patch and gap clustering indices, C) *Ae. aegypti* and D) *Ae. albopcitus*.

Aedes *Spatial Patterns*

The index of aggregation (Ia) calculated for the overall spatial patterns of *Ae. aegypti* (Ia=1.33, p=0.06) and *Ae. albopictus* (Ia=1.33, p=0.06) were not more aggregated than expected based on random permutation. A cluster index was given for each location and used to create contour maps (figure 3.1C and 3.1D). The color arrangement for each contour map was determined independently using individual cluster index distributions: maximum, 95th percentile, patch mean, zero, gap mean, 5th percentile, and the minimum. The yellow areas had significantly high cluster indices indicating *Aedes* mosquito patches or hot spots and the red areas had significantly low cluster indices indicating gaps where very few *Aedes* were collected. In figure 3.1C, thirteen MosquiTRAPs had significantly high *Ae. aegypti* cluster indices (v=2.06 to 4.56) and twenty MosquiTRAPs significantly low *Ae. aegypti* cluster indices (v=-2.25 to -4.18). In figure 3.1D, twenty-three MosquiTRAPs had significantly high *Ae. albopictus* cluster indices (v=2.16 to 8.62) and twenty-five MosquiTRAPs had significantly low *Ae. albopictus* cluster indices (v=-2.24 to -6.23). Overall no spatial association was detected in the aggregate data between *Ae. aegypti* and *Ae. albopictus* (X=-0.10, p=0.9) though significant local association (chi_p=2.0 to 3.13, p<0.05) and disassociation (chi_p=-4.55 to -2.07, p<0.05) were seen in five and eight MosquiTRAPs, respectively.

Ae. aegypti and *Ae. albopictus* population dynamics were examined by conducting cluster analyses at four-week intervals, Figure 3.2. The index of aggregation for *Ae. albopictus* was significant during weeks 1-4 (Ia=1.56 p=0.01), 5-8 (Ia=1.40 p=0.05), 9-12 (Ia=1.44 p=0.03), and 21-24 (Ia=1.912 p=0.007). The index of aggregation for *Ae. aegypti* was significant only during weeks 5-8 (Ia=1.56 p=0.006). Local cluster indices indicated several significant hot spot locations during every interval for each species. A spatial association analysis was performed on consecutive four-week intervals to identify MosquiTRAPs that were consistently “hot” or “cold”. In figure 3.2A and 3.2B, the 36 circled MosquiTRAPs had significant local spatial association (p<0.05), either hot or cold spots, for two consecutive four-week intervals, the three squared MosquiTRAPs were consistent for three consecutive intervals, the triangle is a MosquiTRAPs that was consistently hot for four consecutive intervals, and the diamond is a MosquiTRAPs that was consistently hot for five consecutive intervals. Out of the 36 MosquiTRAPs that were consistent for eight weeks 21 belonged to hot spots and 15 belonged to cold spots and two of the three MosquiTRAPs consistent for 12 weeks belonged to hot spots. Table 3.2, details the overall spatial association for *Ae. aegypti* and *Ae. albopictus* between consecutive four-week intervals. Significant spatial association was seen in each pair of discrete time points.

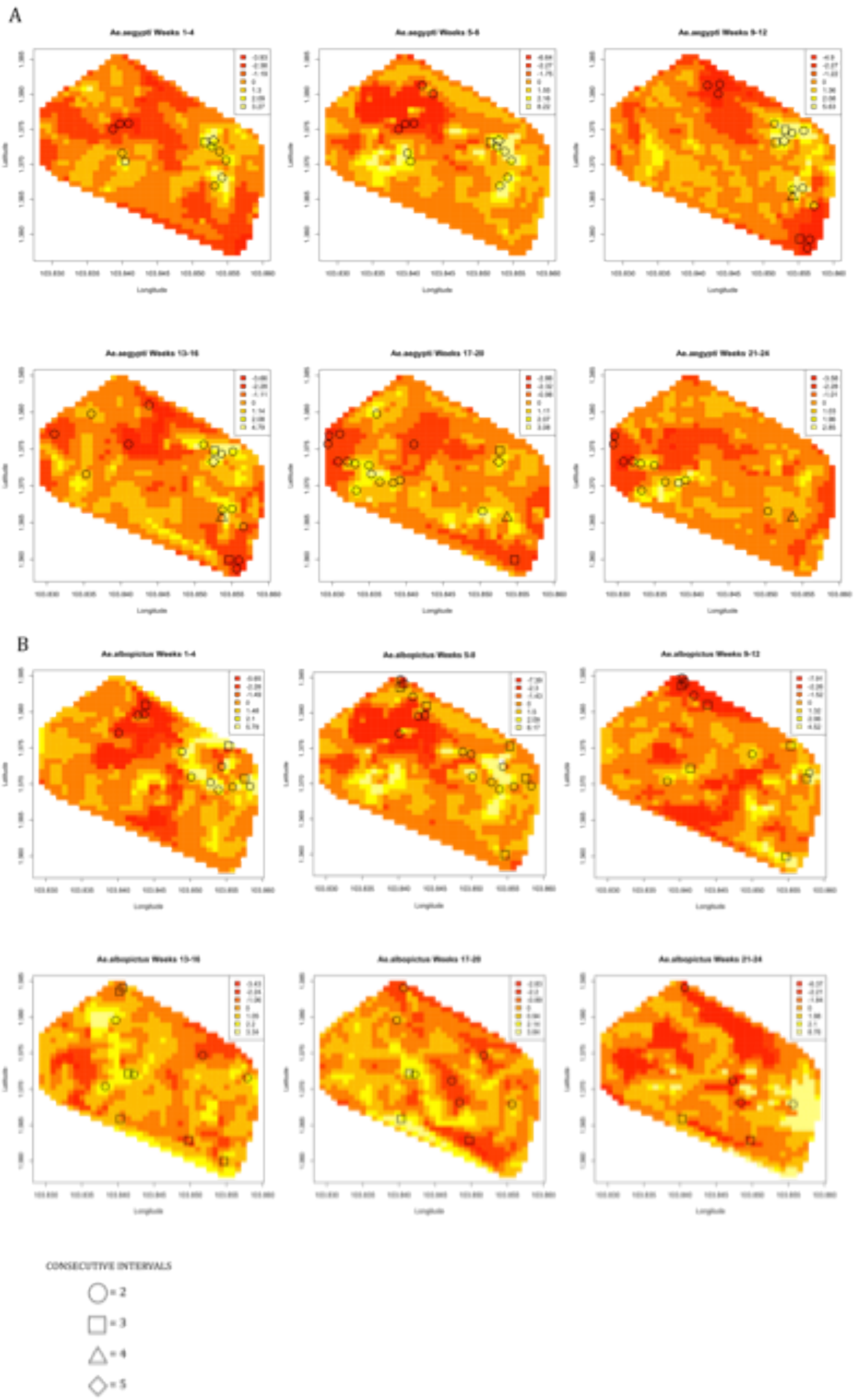
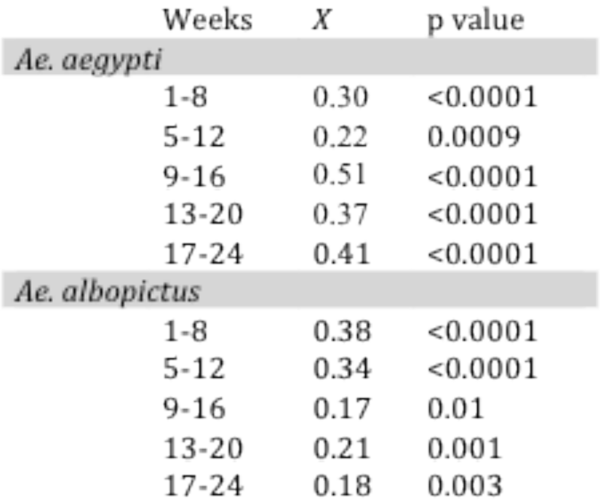


Figure 3.2 Cluster analysis details temporal distributions of *Ae. aegypti* (A) and *Ae. albopictus* (B) at four-week intervals.



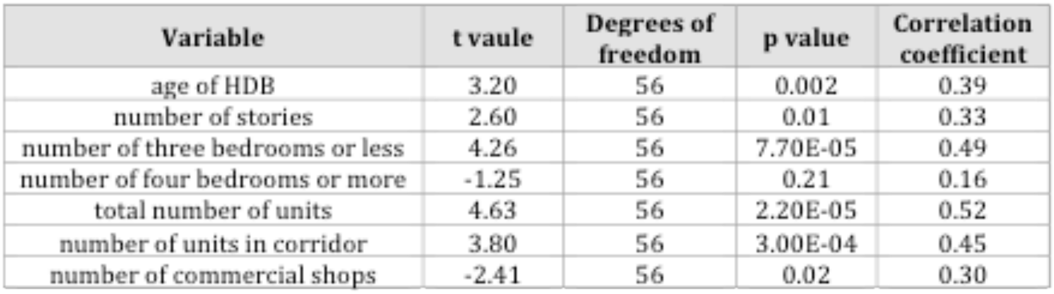
Weeks	X	p value
Ae. aegypti		
1-8	0.30	<0.0001
5-12	0.22	0.0009
9-16	0.51	<0.0001
13-20	0.37	<0.0001
17-24	0.41	<0.0001
Ae. albopictus		
1-8	0.38	<0.0001
5-12	0.34	<0.0001
9-16	0.17	0.01
13-20	0.21	0.001
17-24	0.18	0.003

Table 3.2 Overall spatial association (X) detected in *Ae. aegypti* and *Ae. albopictus* populations between consecutive four-week intervals within the Ang Mo Kio study site.

Independent Variables Affecting Aedes *Structure*

Relationships among independent variables measured at each trap location and *Aedes* distribution and abundance were evaluated using regression analysis. Outdoor and indoor MosquiTRAPs were separated into two datasets. *Ae. aegypti* counts of the indoor dataset and *Ae. albopictus* counts of the outdoor dataset were transformed using a square root transformation. *Ae. aegypti* counts, of the outdoor dataset was zero inflated and did not approach normality. The Mantel test determined that indoor *Ae. aegypti* (r=-0.01, p=0.61), outdoor *Ae. aegypti* (r=-0.04, p=0.93), and outdoor *Ae. albopictus* (r=0.03, p=0.09) did not exhibit significant spatial autocorrelation by distance.

Analysis of *Ae. aegypti* collected outdoors was approached using generalized linear models. A generalized linear model with a negative binomial distribution and three independent variables best fit the data determined by AIC values. The three independent variables that accounted for the most variability were, the number of HDB (t=2.10, df=186, p=0.03), landed houses (t=1.77, df=186, p=0.08), and food courts (t=-1.97, df=186, p=0.05) within sight and 50m. A linear regression analysis of *Ae. albopictus* collected outdoors found two individual variables significant, the number of HDBs (t=2.29, df=185, p=0.02) and playgrounds (t=1.98, df=185, p=0.05) within sight and 50m. After improving the fit of the model, determined by AIC value and R-squared, by combining the two variables, both variables, the number of HDBs (t=1.81, df=184, p=0.07) and the number of playgrounds (t=1.30, df=184, p=0.19) lost significance. A linear correlation analysis (Pearson) performed on *Ae. aegypti* collected indoors found six independent variables significantly influencing abundance and distribution of *Ae. aegypti* inside HDBs. A PCA was performed and the number of units with three bedrooms or less (57%) and the number of units in the corridor (56.9%) contained the majority of the variability seen in principal component one. A biplot revealed that the numbers of units in a corridor and the number of three bedrooms or less occupied the same Euclidian space and therefore were considered related variables. A linear regression analysis that best fit the data found significant positive relationships between *Ae. aegypti* collected inside HDBs and the number of three bedroom or less units (t=4.245, df=54, p=8.64e-5), age of the HDBs (t=2.22, df=55, p=0.03), and total number of units (t=4.30, df=55, p=7.06e-5) (Table 3.3).



Variable	t vaule	Degrees of freedom	p value	Correlation coefficient
age of HDB	3.20	56	0.002	0.39
number of stories	2.60	56	0.01	0.33
number of three bedrooms or less	4.26	56	7.70E-05	0.49
number of four bedrooms or more	-1.25	56	0.21	0.16
total number of units	4.63	56	2.20E-05	0.52
number of units in corridor	3.80	56	3.00E-04	0.45
number of commercial shops	-2.41	56	0.02	0.30

Table 3.3 Linear correlation analyses revealing the relationships between HDB characteristics and *Ae. aegypti* collected indoors.

Flavivirus Surveillance

One of the 144 pools of female *Aedes* mosquitoes tested positive for the presence of flaviviruses. A single trapping event out of the fifteen that made up the pool was identified as positive. A DENV-2 strain was isolated from the mosquito homogenate by mosquito inoculation. The infected *Ae. albopictus* mosquito was collected by a MosquiTRAP placed near commercial shops on the bottom floor of a HDB with a catch rate of 0.42 *Ae. albopictus* per trapping event. The HDB had a commercial bottom with 13 shops including a hawker (food court).

Dengue Incidence

Seventy-eight dengue cases that resided, studied, or worked in the study site, were reported to the MOH during the time of the study. Figure 3.3, details the weekly dengue incidence and mosquito catch rate during the 24 study weeks. A linear correlation analysis (Pearson) identified a significant moderate correlation between *Ae. albopictus* weekly catch rate and weekly dengue incidence (r=0.45, t=2.37, df=22, p=0.02) and a moderate but not significant correlation between *Ae. aegypti* and dengue incidence (r=0.34, t=1.68, df=22, p=0.10). A strong significant correlation between *Ae. aegypti* and *Ae. albopictus* catch rates (r=0.78, t=5.87, df=22, p=6.6e-06) was also revealed. The cross correlation function (CCF) was used to identify lags in weekly *Aedes* catch rate that increased correlation with dengue incidence (Figure 3.4). The most dominate cross correlation between *Ae. albopictus* and dengue incidence was positive at -1 weeks suggesting that an above average increase in *Ae. albopictus* lead to an above average number of cases one week later (r=0.44). The most dominate cross correlation between *Ae. aegypti* and weekly dengue incidence was also positive but at positive five weeks suggesting that cases and *Ae. aegypti* catch rates were positively correlated with cases leading catch rates by five weeks (r=0.45). Weekly dengue incidence correlation with total *Aedes* weekly catch rates also improved with a lag, the correlation estimate was strongest at -1 week and positive (r=0.43).

A contour map of *Aedes* catch rates produced, using cluster index results from a SADIE analysis was overlaid with dengue case reference locations and the upper quartile and maximum catch rate locations for *Ae. aegypti* and *Ae. albopictus* (Figure 3.5). Dengue cases were indexed to 63 different locations around Ang Mo Kio, 52 locations had a MosquiTRAP placed within 60 meters. The average catch rates of these traps were above the overall average for *Ae. aegypti* (CR=0.28), *Ae. albopictus* (CR=0.39), and *Aedes* (CR=0.69). The MFAIt was calculated for each trap three weeks before the case was reported and three weeks after. The average *Ae. aegypti* MFAIt before (MFAIt=0.30) and after (MFAIt=0.30) dengue case reporting and the average *Ae. albopictus* MFAIt before (MFAIt=0.46) and after (MFAIt=0.42) were also higher than the overall average catch rates.

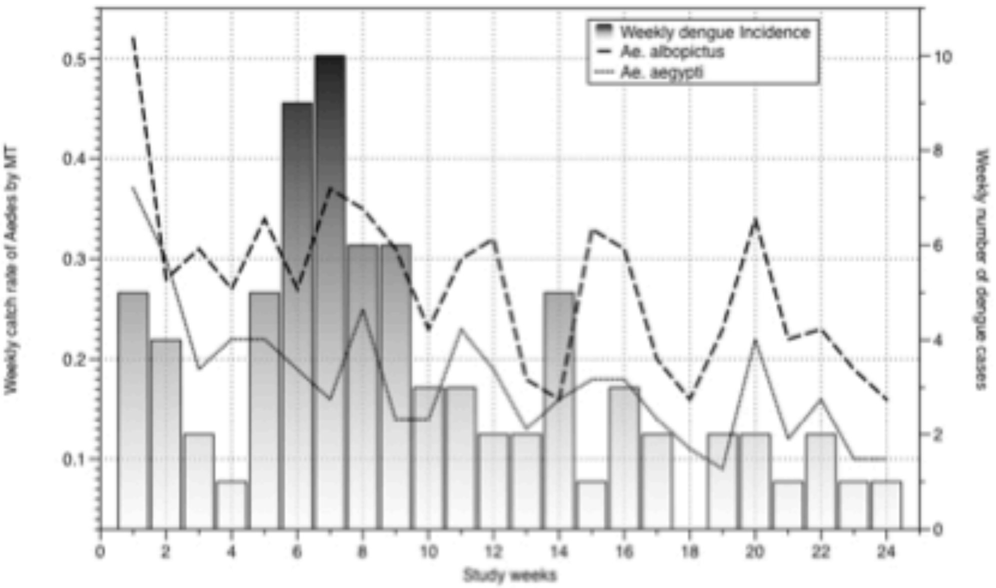


Figure 3.3 Weekly dengue incidence and weekly *Ae. aegypti* and *Ae. albopictus* catch rates during the 24 study weeks.

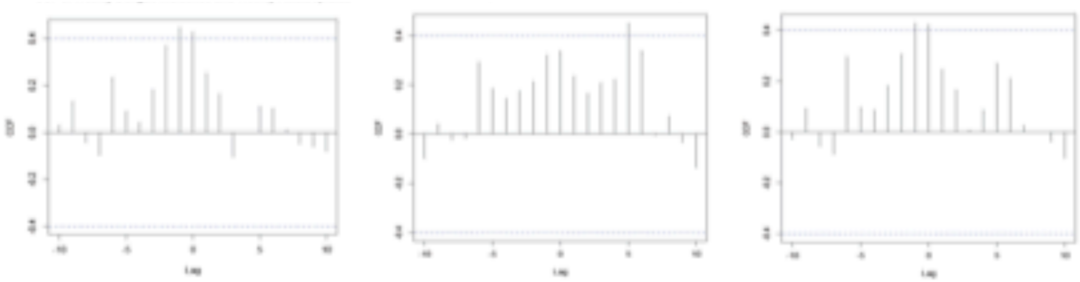


Figure 3.4. Cross correlation function analysis displaying correlation estimates at different time lags for A) *Ae. aegypti* B) *Ae. albopictus* C) *Aedes*

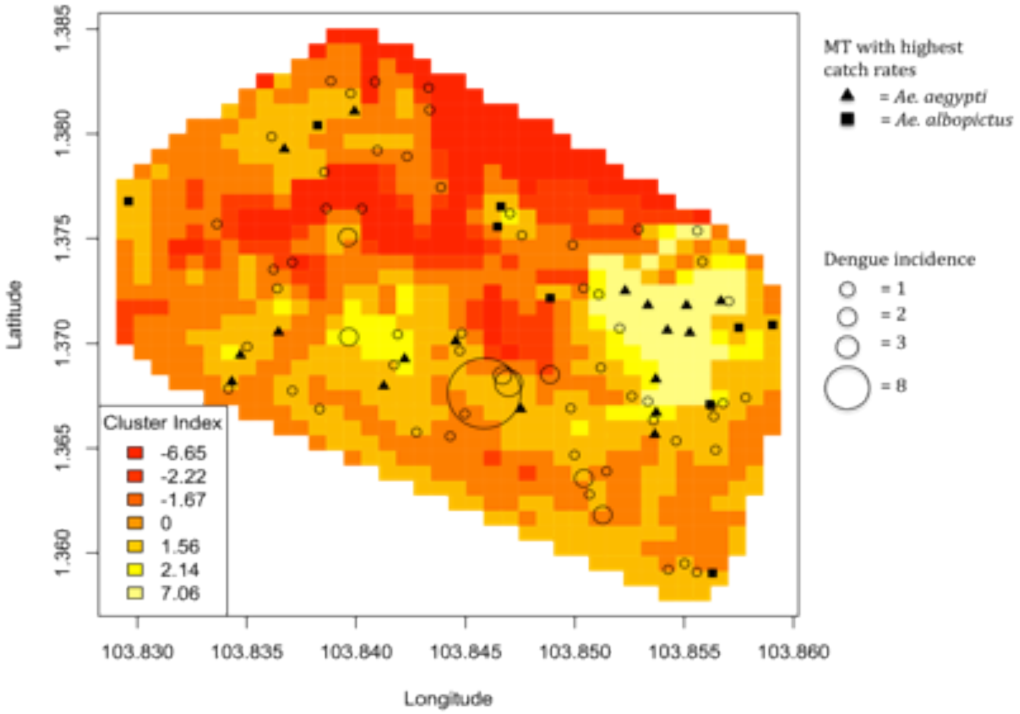


Figure 3.5 *Aedes* spatial patterns determined by patch and gap clustering, and upper and maximum quartile locations, overlaid with dengue case incidence at reference locations.

Discussion

The overall (global) index of aggregation of *Ae. aegypti* and *Ae. albopictus* over the 24 study weeks was not significant, indicating that high densities of mosquitoes were not isolated to a few areas. Overall aggregation was seen during the four-week interval analysis. This indicates that not only spatial scale but also temporal scale is important when identifying areas that may be contributing to dengue transmission. MosquitTRAPs with cluster indices within the 95th percentile suggests that the distributions of *Aedes* near these locations were non-random. The temporal analysis of dengue spatial patterns revealed multiple clusters or hot spots that were consistently hot for two or more four-week intervals. The MosquiTRAP that was a consistent hot spot for 20 weeks was located on the second floor of an HDB. The *Ae. aegypti* catch rate at this location was only 0.54 *Ae. aegypti* per trapping event. This location did not even belong to the upper quartile but it had a consistent higher than average catch rate and nearby locations also had higher than average *Ae. aegypti* average catch rates. The MosquiTRAP that was consistently hot for 16 weeks was also located on the second floor of an HDB. This location was part of the upper quartile with an *Ae. aegypti* catch rate of 0.75. An adaptive population management program has the potential of directing vector control efforts to such areas and possibly reducing dengue transmission risk near these hot spots. Overall spatial association between adjacent intervals for both species was highly significant also indicating stability in *Aedes* abundance.

Ae. aegypti spatial distribution and abundance was significantly influenced by HDB characteristics. The largest HDBs, determined by the total number of units, tended to have longer corridors and more three bedroom or less units. Observationally, three bedroom or less units usually belonged to lower income families. *Ae. aegypti* abundance was significantly greater in larger HDBs i.e., the highest density of people. The presence of a commercial bottom did not influence *Ae. aegypti* abundance. Though there are not higher mosquito abundances near commercial bottoms they may provide a place for increased risk of exposure. A HDB with a commercial bottom is usually a high congregation area with large outdoor hawkers (food stalls), fruit and vegetable markets, a variety of different types of shops, and sitting areas. *Ae. aegypti* catch rates were highest inside HDBs suggesting that it is the primary breeding area not necessarily the primary location of transmission by *Ae. aegypti*. It is responsible to assume transmission is likely occurring inside and around the HDBs. *Ae. aegypti* have been shown to leave the indoor premises during feeding hours if no one is home only to return in the evening to roost . HDBs with commercial bottoms, such as the HDB where DENV2 was isolated, are busy congregation centers within the area type that collected the most *Ae. aegypti*. The use of host seeking traps inside HDBs, within the commercial bottom, and other outdoor areas surrounding HDBs would provide insight into transmission risk.

Dengue incidence data was referenced to home, work address, or school address. It is very tempting to infer transmission at these locations but of course that is not necessarily true. If multiple cases occur at a location within a discrete period of time it is safer to assume local transmission. MFAIt for traps within 60m of case reference locations were higher than average with seven belonging in the upper and maximum quartiles of *Ae. aegypti* catch rates and five separate MosquiTRAPs belonging to the upper and maximum quartiles of *Ae. albopictus* catch rates. Unfortunately, a trap was not located within 60m of the HDB that contained eight reported dengue cases. The large cluster in figure 5 contains five of the 18 MosquiTRAPs belong in the upper and maximum *Ae. aegypti* quartile. Fifteen dengue cases occurred in and around the perimeter of the *Ae. aegypti* cluster. It is interesting that few cases resided inside the cluster with the majority scattered along the perimeter.

Reported cases of dengue incidence within Ang Mo Kio GRC had a stronger correlation to *Ae. albopictus* catch rates than *Ae. aegypti*. Introducing a time lag suggested that an increase in *Ae. albopictus* catch rates one week earlier improved the correlation with increased dengue incidence. The strongest correlation between *Ae. aegypti* and dengue incidence was at positive five weeks suggesting that an increase in dengue incidence lead an increase in *Ae. aegypti* catch rate. One possible explanation is that *Ae. albopictus* relies more heavily on natural habitats that are influenced by rainfall, which usually coincides with the dengue season.

Vector borne disease transmission dynamics involve a complex interaction of multiple agents that are spatially and temporally heterogeneous. We hope that this study contributes to building a foundation for understanding these dynamic interactions and to developing the necessary tools to help direct vector control efforts to priority areas that can have the most effect on reducing transmission.

CHAPTER 4

Fine scale *Ae. aegypti* and *Ae. albopictus* distribution and dengue virus transmission implications in Ang Mo Kio, Central Singapore

Abstract

Intensive entomological research was conducted in six locations within Ang Mo Kio, Central Singapore. A variety of entomological trapping methodology: aspiration, BG Sentinels, oviposition traps, MosquiTRAPs, and larval surveys, were used to investigate each life stage of *Aedes* mosquitoes. *Ae. aegypti* catch rates from host seeking traps were significantly higher outdoors, on the ground floor of HDBs, than inside residential units and were similar to *Ae. albopictus* catch rates. The results indicate that congregation areas on the ground floor of HDBs, where human-mosquito exposure is high, are potential areas of dengue transmission and that *Ae. albopictus* may play an important role in the circulation of dengue viruses.

Introduction

Dengue was first documented in Singapore in the early 1900s with dengue hemorrhagic fever (DHF) emerging 60 years later [1]. In response to the rising incidence of DHF the Singapore government conducted a series of entomological studies from 1966 to 1968 [1-5]. The main objective was to investigate the ecology of *Ae. aegypti* and *Ae. albopictus* in the urban areas of Singapore and to evaluate their respective roles in the epidemiology of DHF. The results of the studies were used to develop a national vector control program incorporating larval reduction, health education, and law enforcement that was put into effect in 1969. The program was successful in maintaining a very low premise index of around 2% and decreased dengue incidence for 15 years [6]. Despite the reduction of vectors, dengue reemerged in the late 1980s. The country is now hyperendemic and within the last decade dengue epidemics are the largest recorded in Singapore history. The Singapore Ministry of Health conducted a retrospective study investigating the 2005 dengue outbreak that included an entomological component based on larval habitats. The most important differences seen between the 1966 and 2005 studies were the percentage of outdoor breeding habitats and the premise index. In 2005, 50% of all mosquito larval habitats were located outdoors and the premise index ranged from 2.28-0.33% [13]. In

1966, the premise index ranged from 2.3-29.6% and 73% of *Ae. aegypti* and 50% of *Ae. albopictus* of larval habitats were found indoors [2]. An increase in outdoor larval habitats could potentially influence dengue transmission outside of the home by increasing human-mosquito exposure outdoors.

A thorough entomological study of *Ae. aegypti* and *Ae. albopictus* that includes host-seeking behavior has not been conducted since the introduction of the vector control program. Singapore's environment has changed considerably since the late 1960s, which has likely influenced *Ae. aegypti* and *Ae. albopictus* ecology. In 1968, the population of Singapore was 2 million with half living in the central area slums, in huts in the rural area, or in squatter settlements on the urban fringe. Only a third of the population lived in multistory housing estates [1]. In 2010, 82% of the population resided in government subsidized multistory housing [66].

Generally, dengue cases cluster geographically by residence, wherein households are believed to be the primary arena of transmission with the endophagic mosquito, *Ae. aegypti*, as the primary vector. In Singapore a low percentage of cases cluster geographically [9]; there is an increase in adult incidence rates [8]; and the existence of cases that link via molecular epidemiology but lack residential association [12]. These epidemiological findings suggest a possible increase in the relative contribution of transmission occurring outside of the home.

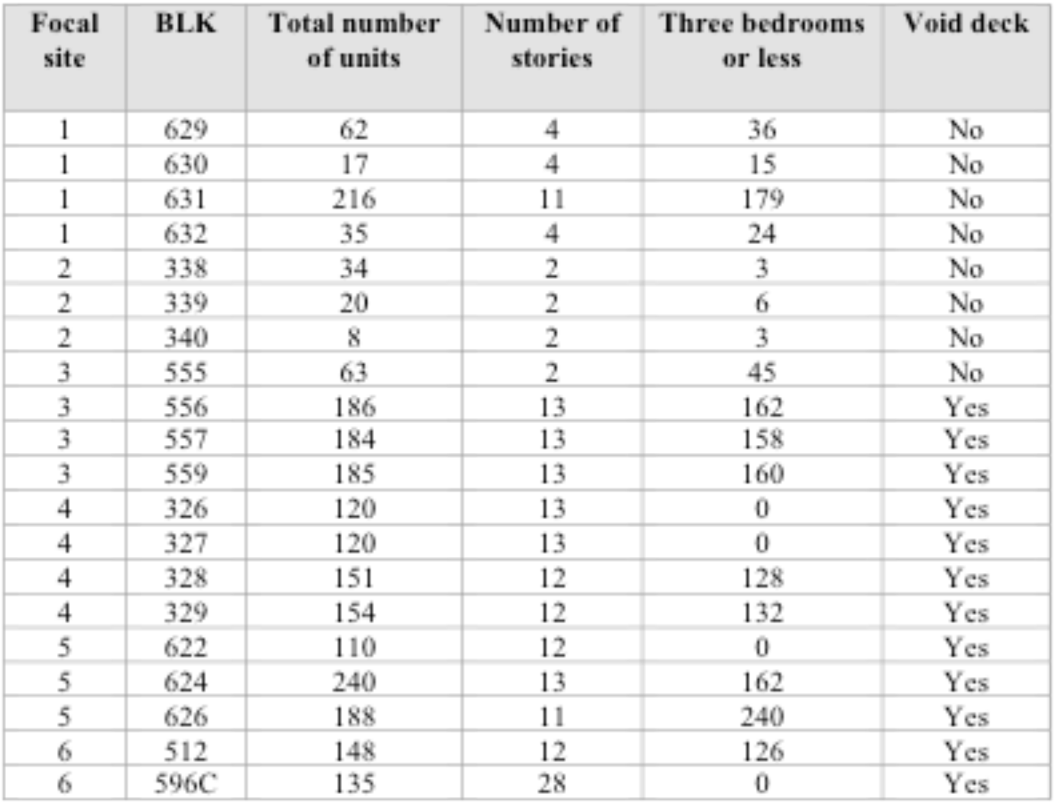
The primary location of dengue transmission and the relative contribution of the two dengue vectors is still controversial. Both *Ae. aegypti* and *Ae. albopictus* are efficient at transmitting dengue virus and similar infection rates have been documented in Singapore [19, 20]. The most important parameter in comparing their vector capacities may be human-vector exposure. The objective of this investigation is to contribute to the understanding of dengue transmission and improve vector control efforts by detailing *Ae. aegypti* and *Ae. albopictus* host seeking and oviposition behavior in an urban environment in Singapore.

Methods

Study Sites

Intensive focal entomological investigation took place in six locations within Ang Mo Kio, central Singapore (Figure 4.1). Observations from a fixed position surveillance program within the Ang Mo Kio study site facilitated focal study site selection. The Ang Mo Kio study site and the fixed position surveillance program are described in Chapter 2. Focal study site locations were chosen based on the mean female *Aedes* index (MFAI), calculated as the average number of *Aedes* captured over three weeks, from fixed position surveillance MosquiTRAPs [30]. The six focal study sites encompassed two high *Ae. aegypti* MFAI MosquiTRAPs (focal study sites #4 and #5), two moderate (focal study sites #3 and #6), and two low (focal study sites #1 and #2). Intensive focal investigations were conducted for 2-3 week periods in a 300-meter radius surrounding the chosen fixed position surveillance MosquiTRAP. The six focal study sites included a commercial area (focal study site #2), three mixed commercial residential areas (focal study sites #1, #3, and #6), and two residential areas (focal study sites #4 and #5). The number of government subsidized housing buildings, Housing Development Board (HDB), and their characteristics varied among the different focal study sites (Table 4.1).

Figure 4.1. Focal study site locations within the Ang Mo Kio, Central Singapore.



Focal site	BLK	Total number of units	Number of stories	Three bedrooms or less	Void deck
1	629	62	4	36	No
1	630	17	4	15	No
1	631	216	11	179	No
1	632	35	4	24	No
2	338	34	2	3	No
2	339	20	2	6	No
2	340	8	2	3	No
3	555	63	2	45	No
3	556	186	13	162	Yes
3	557	184	13	158	Yes
3	559	185	13	160	Yes
4	326	120	13	0	Yes
4	327	120	13	0	Yes
4	328	151	12	128	Yes
4	329	154	12	132	Yes
5	622	110	12	0	Yes
5	624	240	13	162	Yes
5	626	188	11	240	Yes
6	512	148	12	126	Yes
6	596C	135	28	0	Yes

Table 4.1. Characterization of HDBs located within the six focal investigation sites.

Entomological Collections

A grid facilitated the distribution of horizontal sampling. To ensure similar trapping effort (i.e. same number of traps per square kilometer) for vertical space, HDBs were placed on a two dimensional plane and the approximate area was calculated. Focal investigations followed a standard protocol that included: horizontal placement of nine oviposition traps, six BG Sentinel traps, and 18 MosquiTRAPs; vertical placement of one oviposition trap per 2 sq m, two MosquiTRAPs per sq m, and one BG Sentinel per 4 sq m; indoor aspiration and larval survey; and weekly outdoor larval surveys. Entomological traps (oviposition, MosquiTRAPs, and indoor BG Sentinels) were serviced six days a week except for outdoor BG Sentinels, which were serviced daily.

Weekly outdoor larval surveys were conducted in public space at ground level and along HDB corridors. Indoor larval surveys and aspirations were performed concurrently within residences and commercial properties. All juvenile mosquitoes were removed from the habitats and reared to adult for species identification. The same field team performed all larval surveys and aspirations. On average, 23% of residential and commercial properties were sampled.

Oviposition traps were placed outdoors at a concentration of one trap per 50 m and in HDB corridors. Oviposition traps in HDB corridors were placed on every other floor starting with the third floor. All oviposition traps were lined with seed germination paper and filled with 10% grass infusion that was replaced weekly. Grass infusion was prepared by placing dried cut grass and tap water in sealed barrels seven days before use. Seed germination paper positive for eggs were stored in zip-lock bags for 2-4 days following collection and dried for 12 hours before transport to Duke-NUS Graduate Medical School for counting, hatching, and species identification.

BG Sentinel traps were placed in ground floor common areas and residential units. Six BG Sentinels were placed in common areas that were chosen based on suitability, permission, and security. The outdoor BG Sentinels were housed in metal cages, chained to permanent fixes, and ran off 12V power supplies. Indoor BG Sentinel traps were rotated every three days among participating residential units that were selected using a random number generator. BG Sentinels were serviced using hand held aspirators.

MosquiTRAPs were placed outdoors at a concentration of two traps per 50m and in corridors of HDBs. MosquiTRAPs in corridors were placed on every other floor starting with the second floor. The MosquiTRAPs were baited with an artificial attractant, Atr*Aedes*: a synthetic oviposition attractant developed from volatile chemicals associated with immature mosquito sites [67]. MosquiTRAPs also contained water treated with *Bacillus thuringienis* ssp. *israelensis* (BTI) to prevent unintentional larval development.

Flavivirus Detection

Captured adult mosquitoes were identified in the field, separated by species, placed in labeled eppendorf tubes, and stored in a liquid nitrogen dry shipper prior to biweekly transport to Duke-NUS Graduate School insectary, where they were stored at -80°C until further testing. Adult female *Aedes* were separated out and individual head tissues were examined for flavivirus infection by immunofluorescence assay (IFA).

Data Analysis

Statistical analyses were performed in R version 3.0.1 (2013, The R Foundation for Statistical Computing, http://www.R-project.org). A two sample Student's t Test identified differences in the weekly average *Ae. aegypti* and *Ae. albopictus* catch rates among the different entomological trapping methods. Linear correlation analysis (Pearson) and the Mann-Whiteny U test were used to evaluate the relationship between indoor and outdoor entomological collections and HDB characteristics and *Aedes* catch rates. Linear correlation analysis was also used to investigate the distribution of *Ae. aegypti* within an HDB building.

Results

During the 14 week duration of the entomological study, 940 adult *Aedes* mosquitoes (487 *Ae. aegypti* and 434 *Ae. albopictus*), and 12,210 mosquito eggs were collected. In addition, 17 larval habitats were sampled. Figure 4.2 displays the number of *Ae. aegypti* and *Ae. albopictus* collected weekly from the six focal study sites for each entomological collection method. The number of *Ae. aegypti* collected by aspiration was significantly higher than *Ae. albopictus* (t=4.28, df=5, p=0.007) and the number of *Ae. albopictus* collected by MosquiTRAPs was significantly higher than *Ae. aegypti* (t=2.69, df=8, p=0.02). BG Sentinels collected similar amounts of *Ae. aegypti* and *Ae. albopictus*.

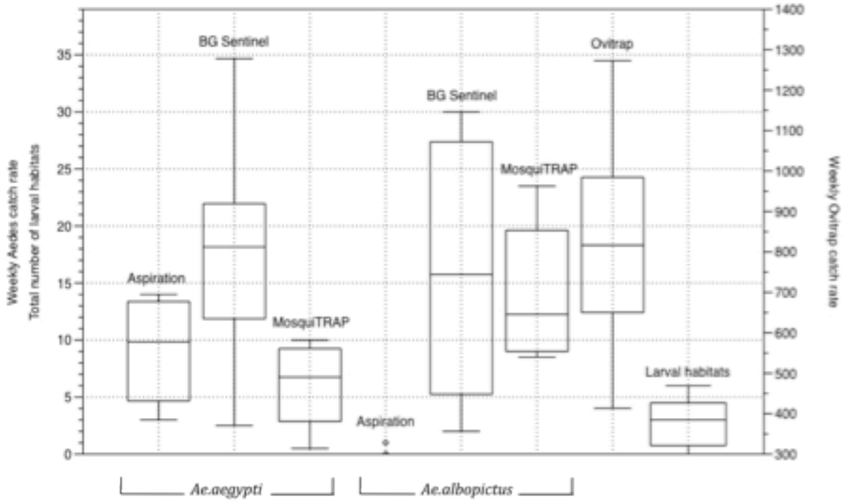


Figure 4.2 Box plot displaying the average weekly *Ae. aegypti* and *Ae. albopictus* catch rates for aspiration, BG Sentinel, and MosquiTRAP entomological trapping methods, the weekly oviposition catch rate, and the total number of larval habitats for the six focal investigations separated into quartiles.

<u>Aedes *Distribution*</u>

Fine scale *Ae. aegypti* and *Ae. albopictus* distributions were determined by examining indoor and outdoor trap productivity (Figure 4.3). Ovitraps were more successful outdoors with the median catch rate 2.5 times that of the indoor catch rate (W=4, p=0.02) (Figure 4.3A). Of the eggs hatched from oviposition traps located indoors, 68% were *Ae. aegypti*, 18% were *Ae. albopictus* and 13% were mixed species. Of the eggs hatched from ovipositon traps placed outdoors, 6% were *Ae. aegypti*, 92% were *Ae. albopictus*, and 2% were mixed species. This suggests that *Ae. aegypti* primarily utilizes larval habitats inside or along the corridors of HDB while *Ae. albopictus* primarily utilizes larval habitats outdoors.

Very few larval habitats were identified that contained larvae and or pupae suggesting that temporary larval habitats and or cryptic habitats may be the primary producers of *Ae. aegypti* and *Ae. albopictus* mosquitoes. The overall percentage of residential units positive for larvae was 2%, with a range of 0% to 4% between the focal investigations. This data is consistent with reports from the national vector control program [9]. Sixteen larval habitats were identified inside HDBs or along the corridors and three larval habitats were found outdoors. Fourteen of the habitats located indoors contained all *Ae. aegypti* larvae and two habitats contained both species (Figure 4.3B). The mixed species larval habitats occurred on the second and third floors of HDBs. Fifty-three larval habitats containing only eggs were identified inside HDB residential units (n=16) and along HDB corridors (n=37). The three outdoor larval habitats contained all *Ae. albopictus* larvae. This is consistent with ovipostion trap results and the general beliefs of *Ae. aegypti* and *Ae. albopictus* oviposition behavior [79].

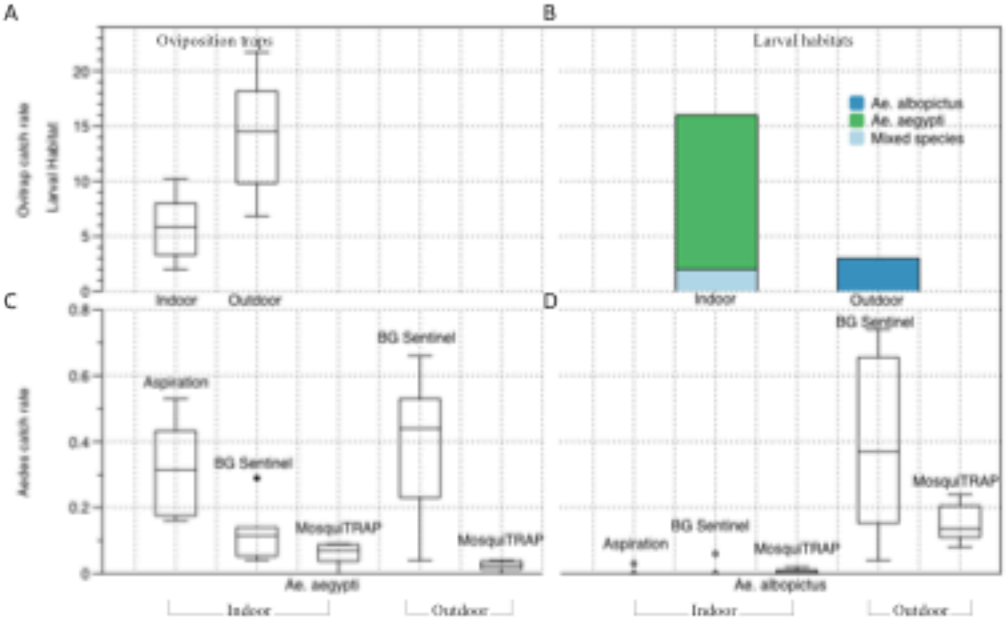


Figure 4.3 Indoor and outdoor distribution of mosquitoes characterized by, A) oviposition trap catch rates, B) *Ae. aegypti*, *Ae. albopictus*, and mixed species larval habitats, adult C) *Ae. aegypti* and D) *Ae. albopictus* catch rates using BG Sentinels, MosquiTRAPs and aspiration collection methods.

Indoors, aspiration had the highest *Ae. aegypti* catch rates compared to the other two adult trapping methods (Figure 4.3C). The daily average aspiration catch rates from inside HDB residential units and shop houses ranged from 0.16 to 0.53 *Ae. aegypti* per aspiration with 14% to 26% percent of aspirated HDB units positive for *Ae. aegypti*. The median daily *Ae. aegypti* catch rate for BG Sentinels placed inside residential units was 0.11 *Ae. aegypti* per trapping event, with an outlier focal study site averaging a daily catch rate of 0.3 *Ae. aegypti* per trapping event. Significantly more *Ae. aegypti* were collected by host seeking trap outdoors compared to inside residential units (W=32, p=0.03). Outdoors, BG Sentinels had a median daily catch rate of 0.44 *Ae. aegypti* per trapping event, four times that of BG Sentinels placed indoors. MosquiTRAPs were more productive for *Ae. aegypti* indoors than outdoors but this difference was not statistically significant.

Utilizing the three adult entomological trapping methods (aspiration, BG Sentinel, and MosquiTRAP) a total of twelve *Ae. albopictus* mosquitoes were collected indoors during the six focal investigations (Figure 4.3D). Consistent with oviposition traps and larval surveys, MosquiTRAPs placed outdoors collected significantly more *Ae. albopictus* than *Ae. aegypti* with the median catch rate over five times higher (W=0, p=0.005). BG Sentinels placed outdoors had similar *Ae. albopictus* and *Ae. aegypti* catch rates.

Linear correlation analysis (Pearson) was used to identify the relationship between weekly indoor and outdoor catch rates during the 14 weeks of the study. Figure 4.4 displays the linear relationship between *Ae. aegypti* catch rates of MosquiTRAP and BG Sentinel placed outdoors and indoors. The *Ae. aegypti* catch rates for BG Sentinels and MosquiTRAPs placed outdoors and indoors, were each correlated (r=0.54, t=2.22, df=12, p=0.05 and r=0.55, t=2.30, df=12, p=0.04, respectively). Combined BG Sentinel and MosquiTRAP catch rates for traps placed outdoors and indoors generated the highest correlation coefficient (r=0.67, t=3.16, df=12, p=0.008).

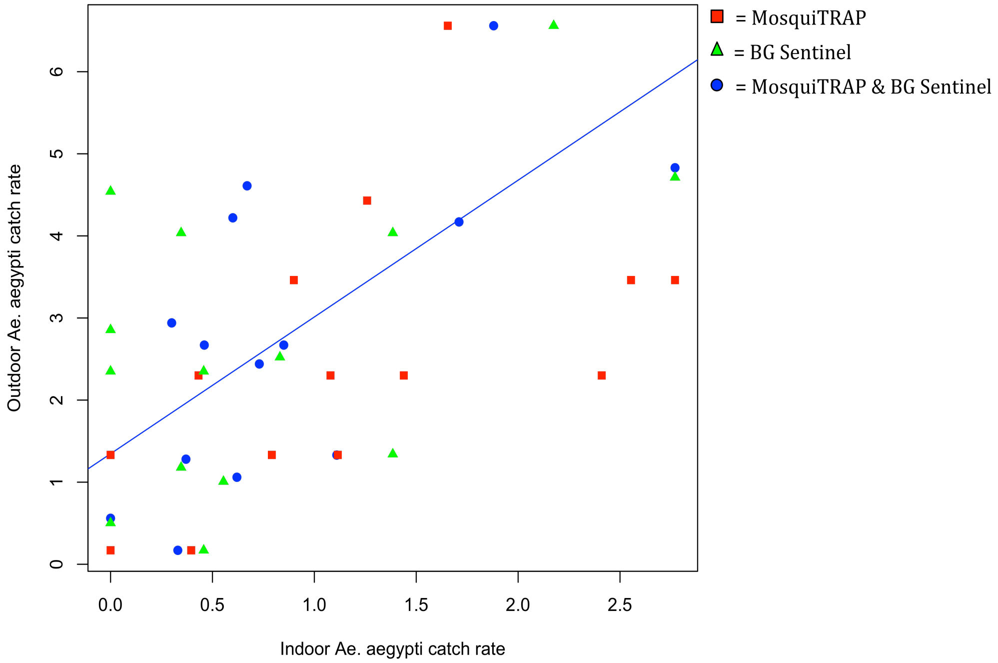


Figure 4.4 Linear correlations between *Ae. aegypti* adult catch rates collected indoors and outdoors by BG Sentinels and MosquiTRAPs.

Ae. aegypti *Distribution Within HDB*

The distribution of *Ae. aegypti* within HDBs has previously been shown to have a negative linear relationship with HDB height (Chapter 2). A Linear correlation analysis (Pearson) was used to investigate the relationship between HDB floor height and *Ae. aegypti* catch rates from aspiration, BG Sentinel, and MosquiTRAP. Aspiration *Ae. aegypti* catch rates had a very strong significant negative correlation with floor height (r=-0.88, t=-5.32, df=8, p=0.0007) (Figure 4.5). BG Sentinel and MosquiTRAP *Ae. aegypti* catch rates also had a strong negative linear correlation with floor height but they were not significant (r=-0.48, t=-1.65, df=9, p=0.13) and (r=-0.54, t=-1.72, df=7, p=0.13). The significance level may have been affected by a few *Ae. aegypti* hot spots that increased catch rates at higher floors.

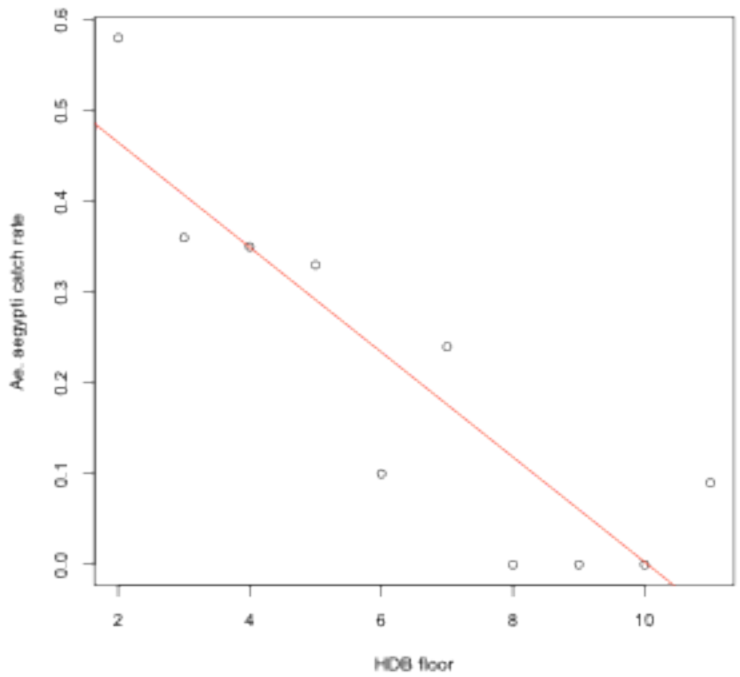
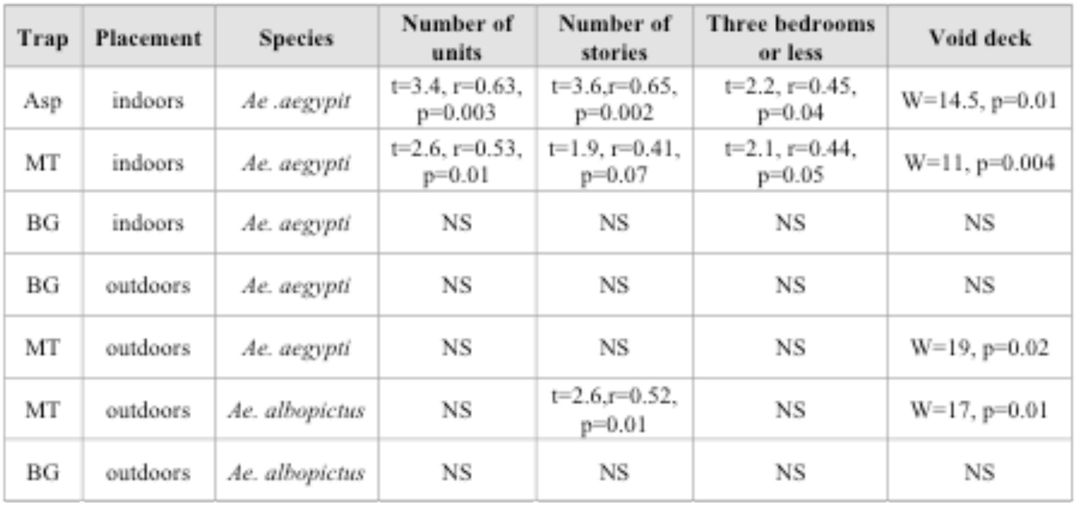


Figure 4.5. Distribution of *Ae. aegypti* within an HDB building determined by aspiration.

__HDB Characteristics Affecting__ __Aedes__ *__Populations__*

A linear correlation analysis (Pearson) was used to examine the relationship between HDB characteristics, described in Table 4.1, and *Aedes* catch rates (Table 4.2). MosquiTRAP and aspiration *Ae. aegypti* catch rates were found to significantly correlate with the number of units in an HDB, the number of three bedroom or less units, and the number of stories, consistent with previous findings (Chapter 3). The number of stories was also found to correlate with outdoor MosquiTRAP *Ae. albopictus* catch rates. The presence of a void deck positively correlated with outdoor MosquiTRAP *Ae. aegypti* and *Ae. albopictus* catch rates, aspiration *Ae. aegypti* catch rates, and indoor MosquiTRAP *Ae. aegypti* catch rates. A significant relationship was not detected between BG Sentinel *Ae. aegypti* and *Ae. albopictus* catch rates and the HDB characteristics measured.



Trap	Placement	Species	Number of units	Number of stories	Three bedrooms or less	Void deck
Asp	indoors	*Ae .aegypit*	t=3.4, r=0.63, p=0.003	t=3.6,r=0.65, p=0.002	t=2.2, r=0.45, p=0.04	W=14.5, p=0.01
MT	indoors	*Ae. aegypti*	t=2.6, r=0.53, p=0.01	t=1.9, r=0.41, p=0.07	t=2.1, r=0.44, p=0.05	W=11, p=0.004
BG	indoors	*Ae. aegypti*	NS	NS	NS	NS
BG	outdoors	*Ae. aegypti*	NS	NS	NS	NS
MT	outdoors	*Ae. aegypti*	NS	NS	NS	W=19, p=0.02
MT	outdoors	*Ae. albopictus*	NS	t=2.6,r=0.52, p=0.01	NS	W=17, p=0.01
BG	outdoors	*Ae. albopictus*	NS	NS	NS	NS

Table 4.2 Linear correlation analysis (Pearson) and Mann-Whitney U test determined the affects certain HDB characteristics had on *Aedes* catch rates for different entomological traps.

Identification of Hot Spots

Entomological traps with catch rates above the 95th percentile are displayed in Figure 4.6. Focal study sites #1 and #2, which had low *Ae. aegypti* MFAI, had two MosquiTRAPs and two BG Sentinel traps with significantly high *Ae. albopictus* catch rates. Focal study site #1 also had an ovitrap with a significantly high catch rate. Focal study sites #3 - #6 (moderate to high *Ae. aegypti* MFAI) contained the greatest number of hot traps. Eight HDB residential units were considered hot due to significantly high aspiration *Ae. aegypti* catch rates. Five of the residential units were located on the second floor, one was a HDB shop house, and the remaining two were located on the third and seventh floors. Fourteen BG Sentinel traps were identified as hot; twelve were located on the ground floor and two were inside HDB residential units on the fourth and fifth floors. Nineteen MosquiTRAPs were identified as hot; ten were located on the ground floor, four on the second floor, three on the fifth floor, and two were on the eleventh floor. All five hot ovitraps were located on the ground floor. HDB BLK 624 was the hottest block with seven traps collecting significantly high *Ae. aegypti* catch rates. HDB BLKs 596C and 556 each contained three traps with significantly high *Ae. aegypti* catch rates. During the study period the home addresses of two dengue patients were referenced to HDB BLKs 596C and 556.

Figure 4.6 Focal sites displaying the locations of mosquito traps that had significantly high catch rates.

Flavivirus Detection

Adult *Aedes* females were screened for the presence of flaviviruses using immunofluorescene assay. Dengue virus particles were not detected in the 349 *Ae. aegypti*, 401 *Ae. albopictus*, and 9 *Aedes* female mosquitoes tested.

Discussion

Dengue transmission dynamics in Singapore with respect to where people are exposed and acquiring infection and the role of the two dengue vectors, are still unclear. A significantly greater number of *Ae. aegypti* were collected by host seeking traps on the ground floor (n=227, CR=0.41), in the void decks and commercial areas of the HDB buildings, rather than inside residential units (n=41, CR=0.16). These data are not consistent with other studies. Most evidence suggests *Ae. aegypti* primarily seeks hosts inside residences [80-82]. This may be unique to Singapore due to its distinct residential environment. The majority of the population lives in multistory residential buildings with many containing congregation areas on the ground floors. These areas can have a considerable amount of human traffic during *Aedes* primary feeding times resulting in high levels of human-mosquito exposure. This may also explain some of the inconsistency between previous studies: *Ae. aegypti* are mainly found in residential areas [9], but the lack of case clusters [9], increase in adult incidence [8], and cases that link via molecular epidemiology but lack residential association [12], suggest non-residential transmission. A primary location of dengue virus transmission may be in the residential areas but not inside residential units.

Ae. aegypti abundance is positively correlated with HDB characteristics, indicating the larger the HDB the more *Ae. aegypti*. This may be due to more residential units providing more potential larval habitats. Results also suggests that an increase in the number of *Ae. aegypti* within a building is associated with an increase in the number outdoors including the number of host seeking *Ae. aegypti* on the ground floors.

Overall *Ae. albopictus* primarily ovipositions and seeks blood meals outdoors; very few *Ae. albopictus* were collected inside HDB residential units or along HDB corridors. Host seeking traps placed outdoors collected similar amounts of *Ae. albopictus* and *Ae. aegypti* suggesting that *Ae. albopictus* likely plays important role in dengue virus transmission. The results of this study show that dengue transmission is likely occurring outside of residential units within residential areas and that *Ae. albopictus* is probably playing an important role in dengue transmission dynamics.

CHAPTER 5

Conclusions and Implications

Vector borne disease transmission dynamics involves a complex interaction of multiple agents that are spatially and temporally heterogeneous. Awareness of these dynamic interactions on a local scale is of fundamental importance in understanding dengue transmission dynamics. The overall goal of this dissertation was to develop a detailed understanding of the underlying epidemiological and entomological interactions that influence dengue transmission in a complex urban environment in Singapore. To achieve this goal a geospatial *Aedes* adult female fixed position surveillance system was evaluated and adapted to local conditions (Chapter 2). This surveillance system produced similar results to standard surveillance tools relative to which it has been reported to be more efficient, sensitive and cost effective [27, 30]. The *Aedes* surveillance system identified spatial and temporal patterns in *Ae. aegypti* and *Ae. albopictus* distribution and abundances and allowed for evaluation of the relationship between environmental variables and *Aedes* densities (Chapter 3). *Aedes* adult female surveillance systems also provide the opportunity for concurrent dengue virus surveillance. Dengue virus was isolated from *Ae. albopictus* collected by a MosquiTRAP on the ground floor of an HDB. The *Aedes* surveillance system was able to differentiate areas with significantly higher abundances of *Ae. aegypti*. Areas with varying amounts of *Ae. aegypti* abundance were investigated using a variety of entomological trapping methods (Chapter 4). Through these intensive entomological investigations we were able to identify areas with high levels of human-mosquito exposure. The ground level of HDBs, void decks and commercial bottoms, had the highest *Ae. aegypti* and *Ae. albopictus* catch rates from host seeking traps. *Ae. aegypti* primarily ovipositioned inside residential units and along the corridors of HDBs but *Ae. aegypti* catch rates outdoors were significantly greater than inside residential units.

The results of this study and others suggest that fixed position sticky traps have the potential to be an efficient, cost-effective, and sustainable surveillance method. Identification of consistent hot spots of vector abundance has the potential to improve vector control outcomes and decrease disease incidence. This study identified locations with high human-mosquito exposure that were previously not considered as potential areas for increased risk of dengue transmission. BG Sentinels could be placed on the ground floors of HDBs within dengue cluster areas to eliminate host seeking *Aedes* females and possibly remove infected mosquitoes from the population. As a preventative measure BG Sentinels could also be placed in areas that are consistently hot along with other vector control activities to reduce the potential of dengue transmission. We hope this dissertation substantiates the importance of adult *Aedes* surveillance and increases current knowledge on dengue transmission dynamics in urban environments.

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