Observations and Mechanistic Modelling of the Role of *Aedes* Mosquitoes in Dengue Transmission in Singapore

by

Osama M.E. Seidahmed
B. S. Zoology, University of Khartoum, 2001
M. S. Zoology, University of Khartoum, 2006

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Signature of Author: .... Signature redacted ....
Department of Civil and Environmental Engineering
August 18, 2017

Certified by: .... Signature redacted ....
Elfatih A. B. Eltahir
Breene M. Kerr Professor of Hydrology and Climate
Thesis Supervisor

Accepted by: .... Signature redacted ....
Jesse Kroll
Professor of Civil and Environmental Engineering
Chair, Graduate Program Committee

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Abstract

Dengue is the most prevalent human arboviral disease in the world with an estimate of 390 million cases per year. The disease is mainly transmitted by two mosquitoes: *Aedes aegypti* and *Ae. albopictus*. Despite the continuous efforts to curb the spread of the disease in Singapore, there is an upsurge in dengue epidemics. In this thesis, the role of *Aedes* mosquitoes in shaping the spatiotemporal patterns of dengue transmission in Singapore is investigated. Field surveys are carried out to study the effect of monsoons on the disease vectors; the impact of urban housing on spatial patterns of dengue transmission is explored; and mechanistic models are developed to simulate the seasonality of dengue occurrence in the city.

The role of monsoons in shaping the seasonal pattern of dengue is investigated. Singapore has no pronounced dry season, while dengue cases decrease every year after a very wet monsoon. A preliminary survey documents that *Aedes* mosquitoes breed in drains close to houses. A hypothesis that the very wet monsoon results in a strong reduction of outdoor breeding of *Aedes* is tested using field data. A one-year entomological survey confirms the hypothesis and shows that a monsoonal sequence of flushing-drying conditions in the drains shapes the seasonal abundance of *Aedes* in Singapore. This finding can be used to optimize vector control efforts and to better understand dengue transmission in the context of climate change.

The influence of urban housing on the distribution of dengue at neighborhood and country scales is studied. Dengue incidence, vector abundance, and drainage network density are found to be higher in a subarea of low-rise housing in Geylang. Further, a holistic analysis at the country-scale confirms the role of urban housing in shaping spatial patterns of dengue. Dengue incidence is found to be proportional to the fraction of the area (or population) of low-rise housing. This finding suggests that affording public housing in agglomerations of high-rise buildings would have a positive impact on dengue control if this urban housing develops at the expense of low-rise areas.

A mechanistic model is developed to investigate the role of drainage networks in the outdoor breeding of *Aedes* and transmission of dengue virus. The HYDRology, Entomology and DEngue Transmission Simulator (HYDREDETS) consists of three coupled dynamic models, with an explicit representation of the spatial domain. The model is calibrated using field observations to study rainfall-effects on flushing/drying of aquatic stages and re-inoculation by dormant eggs. Dengue vertical transmission and the role of human movements are incorporated in the model. HYDREDETS is capable of simulating patterns of dengue transmission in Singapore, however with reduced seasonality. This finding supports the hypothesis that dengue seasonality in Singapore can be attributed to a significant degree, to flushing-drying sequence in outdoor breeding habitats.

Based on the conclusions of this thesis, spatiotemporal patterns of dengue in Singapore are shaped by climate and urban ecology which influence the ecology of *Ae. aegypti* and *Ae. albopictus*. HYDREDETS is a new modeling tool tailored to the study of vector-borne diseases related to urban hydrology. Dengue vector control can be optimized using these new tools and the improved understanding of the disease ecology.

Thesis Supervisor: Elfatih A.B. Eltahir
Title: Breene M. Kerr Professor of Hydrology and Climate

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"Enlightenment must come little by little - otherwise it would overwhelm."
- Idries Shah

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Chapter 1: Introduction

1.1 Arthropod-borne viral (Arboviral) diseases

Arthropod-borne viral (arboviral) diseases are a subset of viral diseases that involve an arthropod in their transmission to humans and other vertebrates. Mosquitoes, Ticks, sand flies and biting midges are the primary vectors for more than 135 arboviruses infectious to humans. Among these vectors are *Aedes* mosquitoes (belonging to the genus: *Aedes*, family: Culicidae) which are exceptionally capable of transmitting a broad spectrum of arboviral diseases including yellow fever, dengue, Zika virus, Chikungunya, West Nile virus, Rift Valley fever and Eastern equine encephalitis. All these important diseases are emerging and resurging at great rates worldwide to cause significant morbidity and mortality to humans and livestock [1].

The reemergence of arboviral diseases is characterized by an increase in incidence in vulnerable populations (i.e. in endemic areas) or expansion of their geographical range to infect naive populations. The unprecedented growth of the human population since World War II has largely contributed to the re/emergence of the arboviral diseases. This population growth has several societal and environmental consequences that increase human-vector contact and provide suitable breeding habitats for the vectors, such as urbanization, deforestation, inadequate water supply, insufficient urban water and sewer systems, and poor housing conditions. Also, the introduction of new arbovirus strains and vector populations into new areas has been associated with a tremendous advancement in transportation technology that increased international travel and trade [2,3].

Most of the emerging arboviruses -- notably are RNA viruses -- belong to three families: *Flaviviridae* (e.g., yellow fever, dengue, Zika and West Nile Virus), *Bunyaviridae* (e.g., Rift Valley Fever and Crimean–Congo hemorrhagic fever) and *Togaviridae* (e.g., Chikungunya and Eastern equine encephalitis virus). Human Arboviral cases are usually categorized into asymptomatic -- which are common in endemic areas -- and symptomatic infections with apparent disease signs. Arboviral infections show similarities in their clinical symptoms, such as fever, headache, rash, myalgia, arthritis, conjunctivitis and retro-orbital pain. Symptomatic patients could vary in their illness between being febrile, hemorrhagic and fatal neurological cases. The clinical manifestation of a patient is influenced mainly by the virus type, pathogenicity of the virus strain, gender and age [3,4].

A common pattern in the evolution history of arboviruses is that they originated from a sylvatic cycle (or enzootic). Hence, arboviruses had been maintained in enzootic cycles between the insect vectors and wild animals in forests (e.g. rodents, birds and non-human primates). Humans and domestic animals were dead-ends for incidences of spillover before these viruses were successfully maintained in a domestic cycle. The evolution of arboviruses has also involved genetic mutations and changes in host-ranges for both the virus and the vector. For instance, in Venezuelan Equine Encephalitis Virus (VEEV), while genetic mutations have increased the disease viremia (i.e. virus load in the host blood) resulting in epizootic strains (evolved from enzootic progenitors), some outbreaks had involved adaptation of VEEV to epizootic mosquitoes [4].

Unsurprisingly, Arboviral diseases are sensitive to the climate. Climatic variables define not only the spatiotemporal distribution of the arthropod vectors but also their vectorial capacity, dispersal and the evolution of the arbovirus. At a macro-scale, temperature or other climatic variables could be essential to the transmission of the disease in geographical zones such as the tropics and temperate regions [5]. However, other abiotic and biotic factors are involved in the disease dynamics at a micro-scale. For example, the degree of urbanization and pertinent community practices towards drinking water at a neighborhood could be significant in determining the risk of dengue, Chikungunya or Zika virus [6].

Furthermore, climate change is anticipated to impact transmission of vector-borne diseases [7,8]. This effect entails not only expansion in the geographic limits of many diseases vectors but also shifts in transmission seasons and interannual variability of outbreaks [9]. However, the use of standalone projections of extreme weather events to predict the emergence or persistence of arboviral diseases may result in inaccurate outcomes. For example, high temperatures could have a paradoxical effect on the transmission cycle, shortening the development time of the vector within breeding habitat or for the virus in the arthropod while at the same time decrease the vector survival or the man-vector contact. Hence, observational and modelling approaches are needed to prioritize the factors that will be crucial for transmission of arboviral diseases under climate change scenarios.

This knowledge gap about the interaction of the climatic and non-climatic factors affects not only our understanding of the epidemiology of arboviral diseases but also the accuracy of forecasting and surveillance systems that aim to prevent and control their outbreaks [10]. The anticipation of outbreaks can help in implementing timely interventions to prevent or at least reduce the magnitude of such outbreaks. For instance, action plans can be prepared in advance, and interventions measures such as insecticides or vaccines can be deployed on time to high-risk areas. Furthermore, unraveling mechanism(s) of spatiotemporal patterns of disease transmission can facilitate framing better adaptation and mitigation policies for the health impacts of climate change [8,11].

1.2 Dengue

Dengue is the most prevalent human arboviral disease in the world according to the World Health Organization (WHO). The disease is reported in 128 countries [12], with an estimate of 390 million cases that occur annually (**Figure 1.1a**). In endemic areas, dengue transmission continues around the year with seasonal and interannual patterns [13,14]. The probability of infection is higher in tropical areas placing almost 3.9 billion people at a direct risk of infection (**Figure 1.1b**). About 70% of these dengue infections occur in Asia, for example, India alone bears nearly half of the cases (**Figure 1.1c**). Besides, Africa and Latin America constitute 14% and 16% of this global burden, respectively [15]. The global distribution of dengue reflects the roles of climate and demographics of the world in the mosquito habituation and virus transmission.

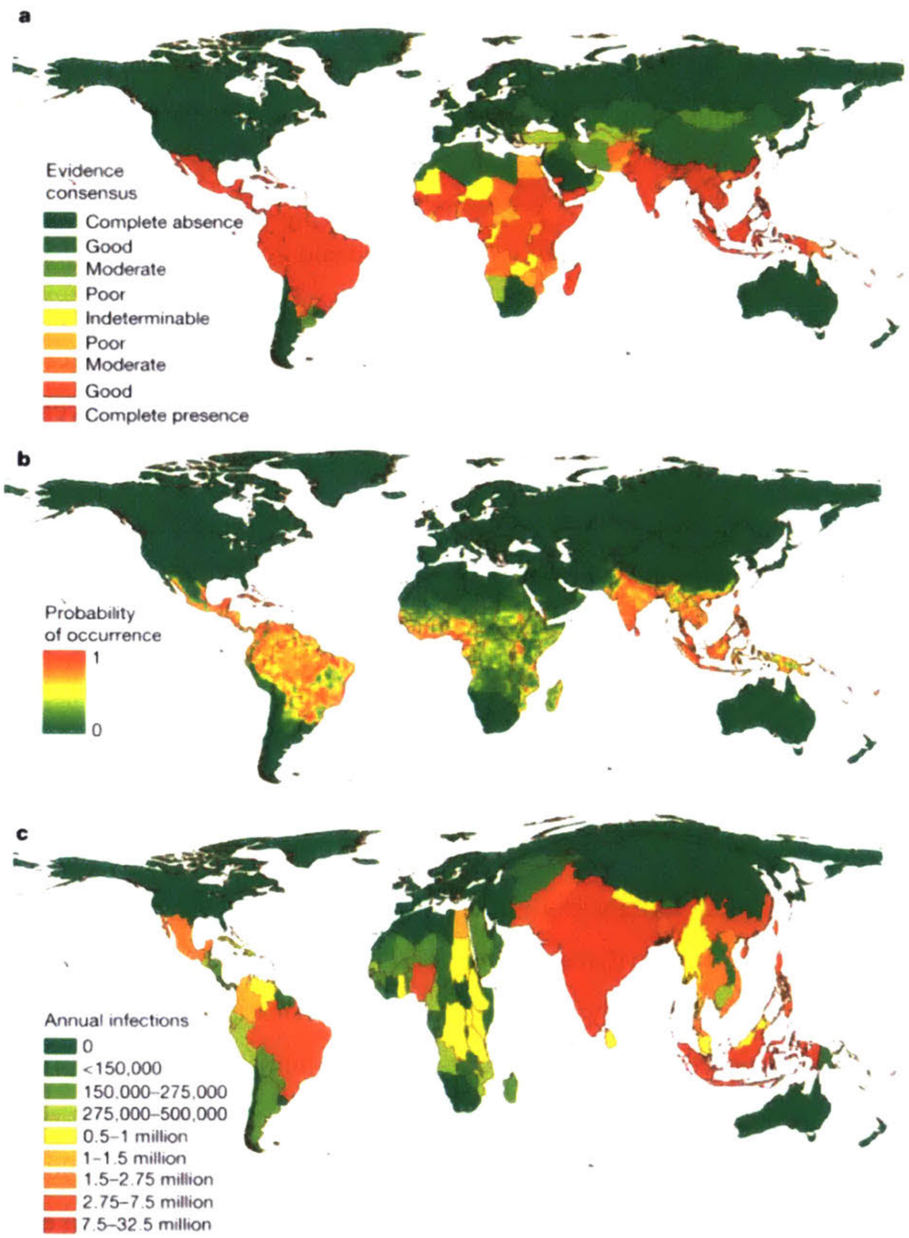

Figure 1.1 a. National and subnational evidence consensus on complete absence (green) through to complete presence (red) of dengue [12]. **b**. Probability of dengue occurrence at 5 km × 5 km spatial resolution of the mean predicted map (area under the receiver operator curve of 0.81 (±0.02 s.d., $n = 336$)) from 336 boosted regression tree models. Areas with a high probability of dengue occurrence are shown in red and areas with a low probability in green. **c.** Cartogram of the annual number of infections for all ages as a proportion of national or subnational (China) geographical area.
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During the last fifty years, dengue incidence has escalated 30-fold, placing half of the world population at risk [9]. Endemic territories in Asia, Latin America, and Africa are substantially burdened by the economic impact of dengue. In Americas, the annual cost of dengue illness is about $2.1 billion (in 2010 US dollars). In Puerto Rico, half of the costs of dengue treatment (annually averaged $38.7 million) were paid by the family of the patient while government spending was only 24%. Indirect costs of dengue included absence from a school or work for ~ 7 days [16]. Nevertheless, the disease cost is higher in developed countries. For example, in Singapore, this ranged between $0.85 and $1.15 billion in 2000-2009 [17,18]. About 1,300 disability-adjusted life years (DALYs) per million people are attributed to dengue in endemic regions [19]. The economic growth of many endemic countries particularly in Southeast Asia and Brazil has been associated with an increase in dengue epidemics instead of a reduction disease transmission [20].

The first major epidemics of dengue were documented in the 1950s in Philippines and Thailand. Consequently, Southeast Asian strains of the dengue virus have spread to other regions and are still playing a significant role in the resurgence of dengue on a global scale [21,22]. Origin of the dengue virus dates back to 1500-2000 years ago when an unknown African progenitor had been introduced to Asia [23]. While sylvatic serotypes of the dengue virus have circulated in the rainforests of Southeast Asia, four domestic serotypes (i.e., DEN-1, DEN-2, DEN-3, and DEN-4) of the flavivirus have independently evolved to adapt to human cycle [4,22]. These domestic strains, which are phylogenetically related, have diverged from the sylvatic progenitors in Asia and West Africa during the past 2000 years. The divergence is attributed to evolutionary changes in the host (i.e., from non-human primates to humans) and vectors (i.e. from arboreal *Aedes* mosquitoes to peridomestic species) [4].

Among a group of arboreal and domestic species of *Aedes* mosquitoes that can transmit dengue virus to the primates [24–26], *Aedes aegypti* is the most efficient biological vector of dengue in humans besides *Aedes albopictus* [27]. While these two species show a sympatric presence in several endemic areas, *Ae. albopictus* plays a secondary role in dengue transmission [27]. Also, *Aedes polynesians* is a local vector for dengue and Chikungunya in the Pacific islands [22].

Although the existence of forest reservoirs is suggested in the circulation of sylvatic strains, no animal reservoir for dengue virus is involved in human epidemics [28]. The *Aedes niveus* group transmit the sylvatic strains in the Malay peninsula. Immunological investigations have shown that monkeys belonging to *Macaca* and *Presbytis* are the reservoir and primary hosts for the enzootic disease in these rainforests [29].

A lifelong immunity to a specific strain is acquired after the recovery from a dengue infection. However, a previous infection only confers a temporary cross-immunity to other strains. Hence, in endemic areas, people could get up to four diseases along the life course. A past dengue infection (with a heterotypic strain) could increase the risk of severe dengue cases. A phenomenon related to dengue severity is the antibody-dependent enhancement (ADE) where a subsequent infection results in massive anamnestic antibody response and high antibody titer instead of neutralizing the virus. This immunological response happens because the remained antibodies bind to the virus and enhance the infection of monocytes [22,30–32]. Other factors that influence the risk of dengue infection and disease severity include the type of the virus strain, age and genetic background of the patients [22].

Most of the dengue cases are mild manifesting a high fever that onset for few days before the patients recover. However, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are life-threatening complications that occur in severe cases. DHF cases are clinically diagnosed by their hemorrhagic manifestations besides plasma leakage, and thrombocytopenia. Also, children under ten years are more vulnerable to DSS, which is characterized by a circulatory collapse and bleeding. Poor management of DHF and DSS cases can result in the death of the patients. The probability of death is 50 times higher in DSS compared to non-severe dengue cases. In some epidemics, type of the dengue strain is risk factor for DSS. Also, incidence rates of DHF and DSS are shown to increase during epidemic years in endemic countries [33–35].

Few sporadic outbreaks and no more than two strains had been co-circulated in endemic areas before the World War II. The smaller urban populations and limited means of transportation have underlined the "silent" dengue situation in the tropics at that time. Subsequently, soldiers of Japanese and Allied forces during the war were mainly affected by dengue, and this had resulted in the current hyperendemic situation of dengue in Southeast Asia. In Latin America, a successful vector control program of *Ae. aegypti* --in response to epidemics of yellow fever -- had resulted in the elimination of the species from 23 countries by 1950s. However, a reinvasion occurred after the program was terminated in the 1970s and introduction of the dengue virus from Asia resulted in the current hyperendemic situation in Brazil and other American countries. Urbanization and global trends of trade and human mobility are influencing the pandemics of dengue. For example, some air passengers have amplified from 0.069 to 3.3 billion between 1950 and 2014, respectively. Imported cases represent an integral part of dengue dynamics and evolution of epidemics [36].

No chemotherapy is yet available to cure dengue infections, and the treatment protocol for inpatients focuses on monitoring patients (with a hematocrit) for fluid volume to avoid severe complications. Hence, fluid resuscitation is often used in the treatment of DHF/DSS cases. Proper management of DHF/DSS cases could decrease the fatality rates of these cases to less than 1% [22,34]. A recent WHO management protocol for DHF/DSS patients includes intravenous dehydration to respond to the plasma leakage. This intervention was shown effective to lower the case fatality in severe cases rate by > 99% [34]. The WHO protocol for the treatment also emphasizes the importance of identifying the severity of dengue cases as early as possible using classification guidelines. Initially, dengue patients are diagnosed as undifferentiated fever, DF or DHF cases. However, out of four subsequent grades for DHF cases, DSS is identified as grade III or IV. The treatment of severe cases should occur in health care facility [34].

Despite high expectations for dengue vaccine trials [37–39] or field releases of a genetically modified mosquito particularly cytoplasmic incompatibility technique using Wolbachia bacteria [40–42], vector control remains the mainstay in dengue control strategies. In 2012, the World Health Organization (WHO) adopted a global strategy for dengue prevention and control for 2012-2020. The WHO strategy aims to reduce dengue mortality and morbidity in 2020 at least 50% and 25% compared to a baseline in 2010, respectively. To achieve these goals, WHO recommends three main interventions: 1) early detection and appropriate management of dengue cases through reorientation and rehabilitation of the primary healthcare levels, 2) improved prediction and efficient management of dengue outbreaks using entomological and epidemiological surveillance, 3) adoption of Integrated Vector Management (IVM) that should be tailored to the spatiotemporal characteristics of dengue transmission at the local levels and with emphasis on practical urban and household water management [43].

1.3 Problem statement

Singapore is an hyperendemic country for dengue fever. Among six endemic countries in the Asian sub-region of the WHO Western Pacific Region (WHO WPR), Singapore bears the largest burden of dengue [44–46]. A total of 78,092 dengue cases were reported between 2010 and 2016 in Singapore [47]. The two mosquitoes *Ae. aegypti* and *Ae. albopictus* coexist and circulate the four virus strains of dengue in the city. Breeding habitats of dengue vectors in Singapore are grouped according to their location into: i) indoor containers: such as domestic receptacles; and ii) outdoor habitats: such as discarded receptacles [48].

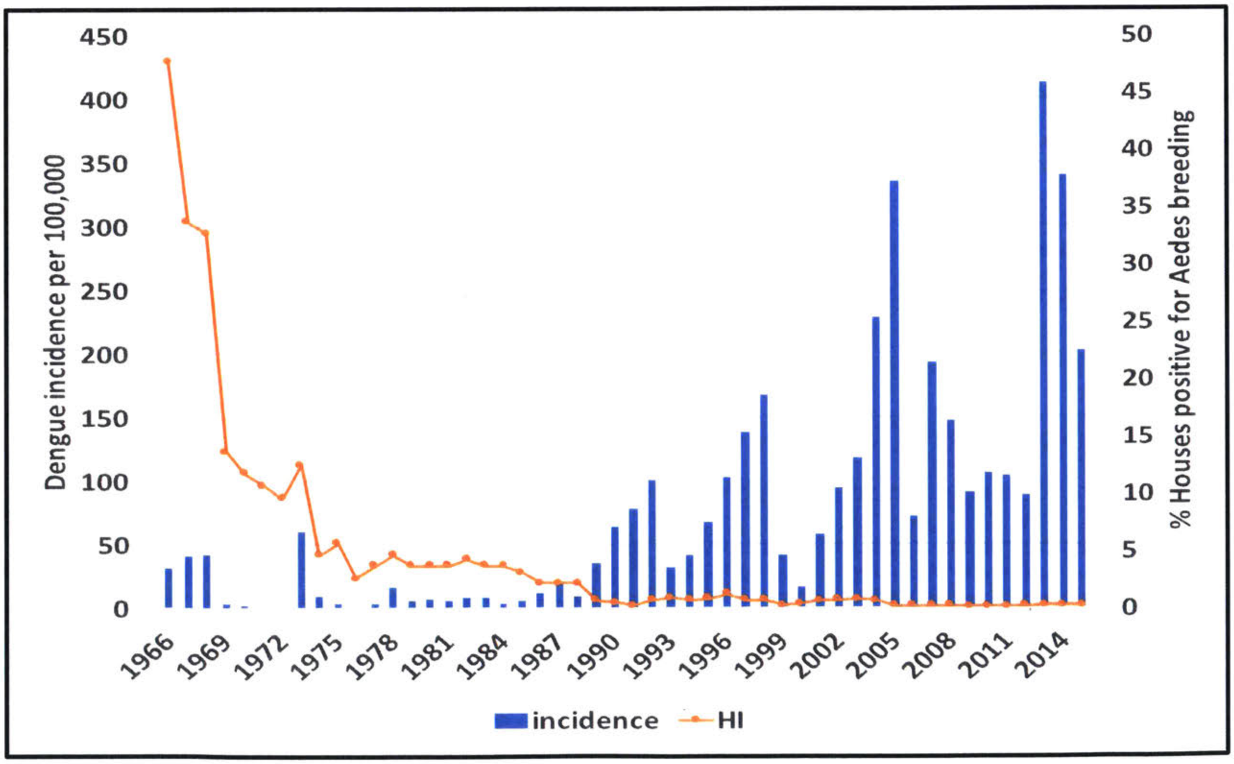


Figure 1.2 Dengue incidence per 100,000 and % positive Houses for breeding of *Aedes* mosquito in Singapore (1966-2015).

One of the entomological indicators used extensively in dengue vector surveillance is the house or premise index (HI) [49]. HI is the percent of positive premises for indoor breeding of *Aedes* mosquito. **Figure 1.2** shows a dramatic decline in HI in Singapore from around 48% in the 1960s to < 2% in 2000s. Since the 1970s, the vector control program has been focusing on the removal of positive breeding habitats inside houses [50,51]. This reduction had resulted in low incidence rates in the 1970s and 1980s. However, a resurgence occurred by 1990s and increase in frequency and magnitude of outbreaks have been witnessed during the last two decades. The largest epidemics had occurred in 1997-1998, 2004-2005, 2007-2008, and 2013-2014 [52–54]. The interepidemic period in Singapore ranges between two and six years.

Several factors have been proposed to explain the upsurge of dengue in Singapore. Explanations proposed include a decrease in herd immunity of the human population (i.e. following the initial suppression of the vector in 1970s and 1980s), shift to outdoor transmission in public venues, improvements in diagnosis and reporting of dengue cases, and reorientation of the vector surveillance to retrospectively target spots of dengue cases and clusters [50].

Nevertheless, serological surveys in Singapore have revealed that households attached to homes (e.g., retirees and homemakers) are more exposed to dengue infections compared to other groups (e.g., students) [45]. Also, no changes in clinical diagnosis or reporting during the last fifteen years in Singapore had happened (i.e. that increased detection of dengue cases). Moreover, efforts were scaled up to curb breeding of *Aedes* in schools and other public avenues, no longer constitute the primary breeding source [55].

Figure 1.3 Breeding of the dengue vector *Ae. aegypti* outdoor in drains close to the houses, Geylang neighborhood, Singapore (July 2013). Left: inspection of a perimeter drain using larval dippers; Right: 4th instar larvae of *Ae. aegypti.*

A preliminary survey in 2013 has revealed a breeding of *Ae. aegypti* in the drainage system and other outdoor receptacles close to the houses (**Figure 1.3**). This observation could have a substantial impact on our understanding of dengue transmission in Singapore particularly of the current upsurge of the disease in the city. For example, urban drains and outdoor breeding habitats are highly vulnerable to monsoons, and the interaction of climate and urbanization could shape the spatiotemporal patterns of dengue transmission in Singapore. Hence, we carried out longitudinal field surveys combined with experimental modelling to better understand the effects of these ecological factors on the role of *Aedes aegypti* and *Aedes albopictus* on dengue transmission.

1.4 Research Objectives

1.4.1 General Objective

To better understand ecological factors that underlie the spatiotemporal patterns of dengue transmission in Singapore.

1.4.2 Specific Objectives

- To investigate the effect of wet/dry seasons on the outdoor breeding ecology of *Ae. aegypti* and *Ae. albopictus*
- To explore the effect of urban ecology on *Aedes* and dengue distributions in Singapore.
- To model the effect of wet/dry seasons on the seasonality of dengue transmission of *Ae. aegypti* and *Ae. albopictus* in Singapore.

1.5 Biosafety and ethics statement

This study received a risk assessment approval from the Institutional Biosafety Committee (IBC) of Singapore-MIT Alliance for Research and Technology (SMART). The research was not conducted in any private residences, and no human samples were collected. Protective measures such as long sleeve shirts and trousers were used by the investigators during the field surveys to reduce the potential of mosquito bites.

5.11.6 Introduction to the thesis

In this thesis, dengue in Singapore is selected as a study case to investigate the effects of climate and urbanization on the epidemiology of dengue. Between 2012-2016, we carried out a field survey in Singapore. Entomological, hydrological, meteorological and disease datasets were collected during this period. Also, a mechanistic model coupling urban hydrology, entomology, and epidemiology of dengue were developed and tested.

I review the literature in **Chapter 2**, document observations of the field survey in **Chapter 3** and **Chapter 4**. Development and simulations of the mechanistic model are described in **Chapter 5** and **Chapter 6**. Finally, conclusions in **Chapter 7** include summary of results and major contributions of this study, remarks on future research, policy implications and recommendations to optimize dengue control in urban settings.

Chapter 2: Literature Review

2.1 Dengue ecology

The virus-mosquito-human triad provides a framework to understand the transmission of dengue. Dengue viruses need an extrinsic incubation period (EIP) in vector mosquitoes to replicate, before being transmitted to humans. In general, interactions of the triad elements with each other and with their shared environment shape the epidemiology of dengue.

2.1.1 Dengue vectors

Worldwide, two *Aedes* species are capable of transmitting dengue in urban settings: 1) the African yellow fever mosquito: *Aedes aegypti* (Linnaeus, 1762), and 2) the Asian tiger mosquito: *Aedes albopictus* (Skuse, 1894). These mosquitoes are considered "host-specific" because evidence has shown that endemic/epidemic strains of the dengue virus infect these two species compared to other sylvatic strains of the virus [4].

In the past, outbreaks of yellow fever in the Americas were a result of the dispersal of *Ae. aegypti* associated with the slave trade in the 17th and 18th centuries. For instance, *Ae. aegypti* was once observed as north far as the Boston area [24,56]. Epidemics of yellow fever had recurred in several ports along the eastern coast of the U.S. till 1822 [57]. On the other hand, a wider geographical distribution of *Ae. albopictus* has been documented during the last three decades [58]. Hence, *Ae. albopictus* is now found on every inhabited continent, from Australia to the Americas. This expansion is partially attributed to globalization (particularly the trade of used tires); as well as acclimatization of *Ae. albopictus* to the cooler climate of the temperate zone [27,59].

In an extensive compilation of records of mosquitos' presence, Kraemer and others have updated maps for the world distribution of *Ae. aegypti* and *Ae. albopictus* [60] (see **Figure 2.1** and **Figure 2.2**). A total of 42,067 records of "unique occurrence" for *Ae. aegypti* and *Ae. albopictus* were used to validate a Boosted Regression Trees (BRT) model. Accordingly, *Ae. aegypti* shows a predominance in the tropics: Africa, Brazil, India and southeast Asia, while only two countries in Europe are probably infested (Spain and Greece). Similarly, *Ae. aegypti* shows a wide range in Australia compared to *Ae. albopictus* (only at the east cost). In the contrary, *Ae. albopictus* prevailed wider in the subtropics and temperate areas in "southern Europe, northern China, southern Brazil, northern U.S. and Japan". The uncertainty of the distribution of *Ae. albopictus* in Africa is high, likely due to underreporting [60].

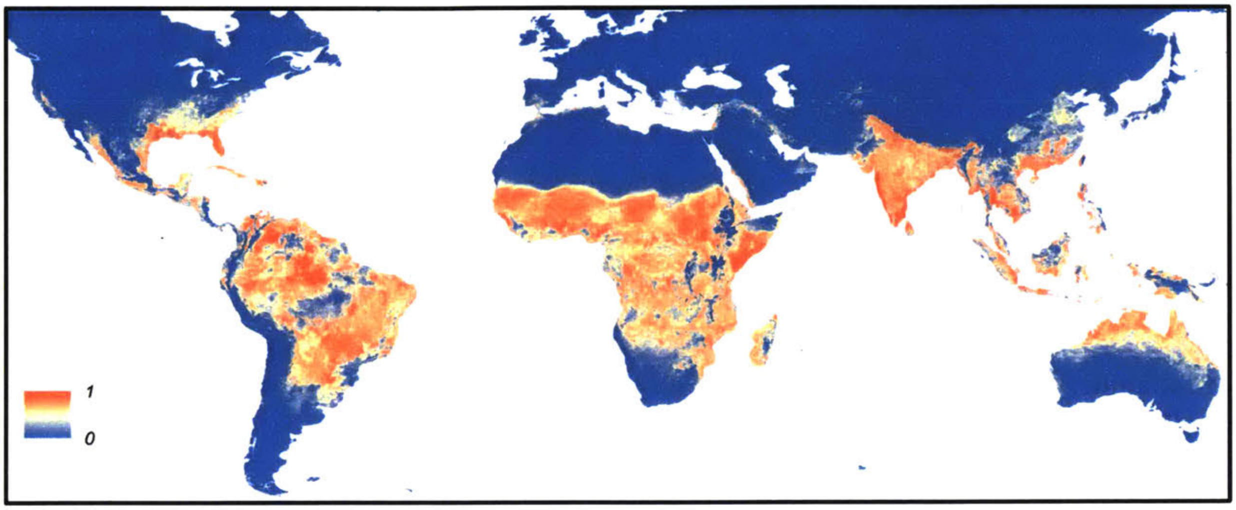


Figure 2.1 Global map of the predicted distribution of *Ae. aegypti*. The map depicts the probability of occurrence (from 0 blue to 1 red) at a spatial resolution of 5 km × 5 km (After Kraemer et al., 2015 [60]).

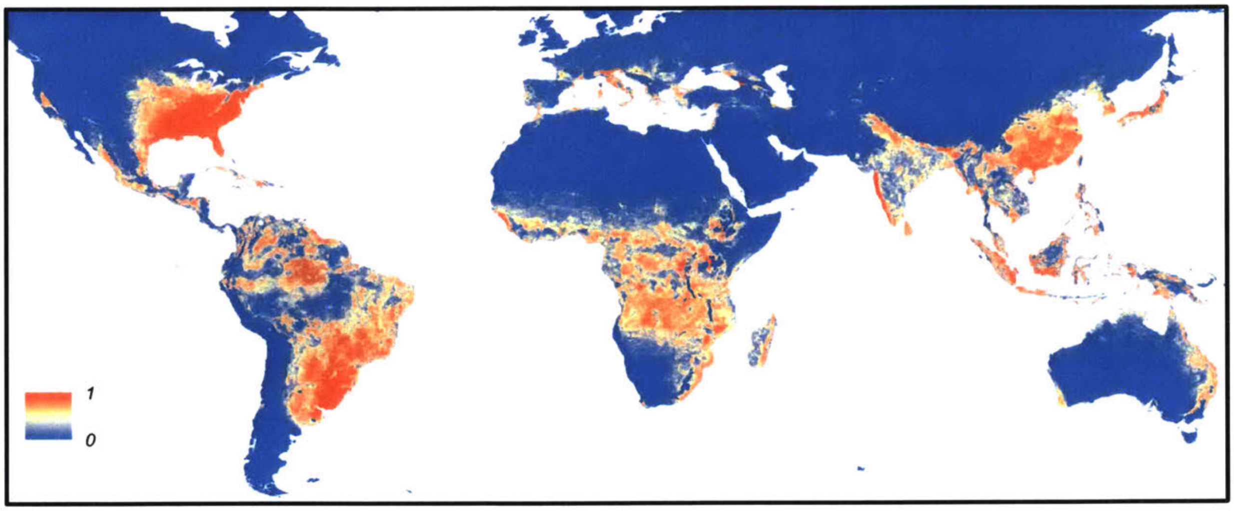


Figure 2.2 Global map of the predicted distribution of *Ae. albopictus.* The map depicts the probability of occurrence (from 0 blue to 1 red) at a spatial resolution of 5 km × 5 km (After Kraemer et al., 2015 [60]).

Despite the wider distribution of *Ae. albopictus*, *Ae. aegypti* is usually incriminated in severe outbreaks of dengue. Experimental works found that the dissemination rate of the dengue virus from the midgut of *Ae. albopictus* to other body tissues is less than that rate for *Ae. aegypti*. Also, *Ae. aegypti* is a domiciliary species (i.e. associated with urban habitats), and so it feeds more frequently on humans (i.e. anthropophilic). In contrast, *Ae. albopictus* is a sylvan mosquito (i.e. more commonly found in forests). Thus, the species feeds on various animal hosts including humans (i.e. partial zoophilic) [58]. Although the opportunistic feeding behavior renders *Ae. albopictus* with a better vectorial competence for the transmission of arboviral diseases that co-circulate in both the jungle and urban cycles (e.g., Chikungunya) [61], this feature undermines the species capacity to transmit host specific arbovirus such as dengue.

Females of these two species are aggressive biters that make multiple attempts, on the same or different hosts, until they get the necessary amount of blood for a gonotrophic cycle [62,63]. Similarly, both *Aedes* species prefer to lay their eggs in small containers that have a solid bottom to ensure the eggs hatch [24]. Also, *Ae. aegypti* and *Ae. albopictus* have a physiological capacity to become dormant at some life stages. Dormancy is defined as "an arrest in development that occurs at specific stage" during the lifecycle of the insect [64]. Some *Aedes* mosquitoes, such as

Ae. aegypti and *Ae. albopictus*, utilize dormancy as a "quiescence" survivorship strategy to evade unfavorable environmental conditions or as a "diapause" synchronization strategy for the seasonal species abundance [64]. The diapause of eggs is a seasonal survival strategy used by *Aedes* mosquitoes particularly observed in the overwintering of *Ae. albopictus* in temperate areas [65,66]. The main difference between diapause and quiescence is that the earlier one is hormonally programmed before oviposition (i.e. eggs will not hatch even if placed under favorable conditions till they take the necessary development time). While seasonal shifts in temperature and length of the day hours can trigger the hatch of diapause eggs, a return of wet conditions can illicit the hatching of quiescent eggs [64,67]. In the tropics, the quiescence or desiccation-resistance of the eggs is suggestively an evolutionary advantage for *Ae. aegypti* to over-compete *Ae. albopictus* and better adapt to the climate variability and climate change [68,69].

2.1.2 Transmission cycle

Four Flavivirus strains (genus: Flavivirus, family: Flaviviridae) are responsible for dengue infections. Remarkably, the dengue virus is the only arbovirus that has successfully maintained an entire anthroponotic transmission cycle with no need for a re-introduction from an enzootic cycle [22,32]. While the highly domiciliary mosquito *Aedes aegypti* represents the primary vector, the outdoor mosquito *Ae. albopictus* could serve as a secondary vector. Circulation of two or more serotypes of dengue virus together could increase the risk of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [9]. The virus takes between 4-7 days in the infected person before amplifying the adequate viremia load to infect a blood-feeding mosquito. There, the dengue virus will need about 5 to 12 days, depending on temperature, to develop in the mosquito before being transmitted to another human [70] (**Figure 2.3**).

Modes of Transmission. There are two primary means of dengue transmission:

1. Horizontal mode: which involves acquiring a viral load (viremia) from an infected human while taking a blood meal by an *Aedes* female. Then, development of the arbovirus in the alimentary tract of the mosquito, migration, and replication of the virus in the salivary glands of

the infected female, and lastly infecting the human or animal host during a second blood meal [9].

2. Vertical mode: which is also known as transovarial transmission. In this route, dengue virus is transmitted from infected mosquito females to their eggs inside the ovaries. Both *Ae. aegypti* and *Ae. albopictus* are capable of following this route and delivering the infection vertically down to the third generation of their offspring [71–73]. Dormant infected eggs can maintain the dengue virus in endemic areas during unfavorable conditions. Further details about the vertical mode of transmission are provided in **Chapter 5**.

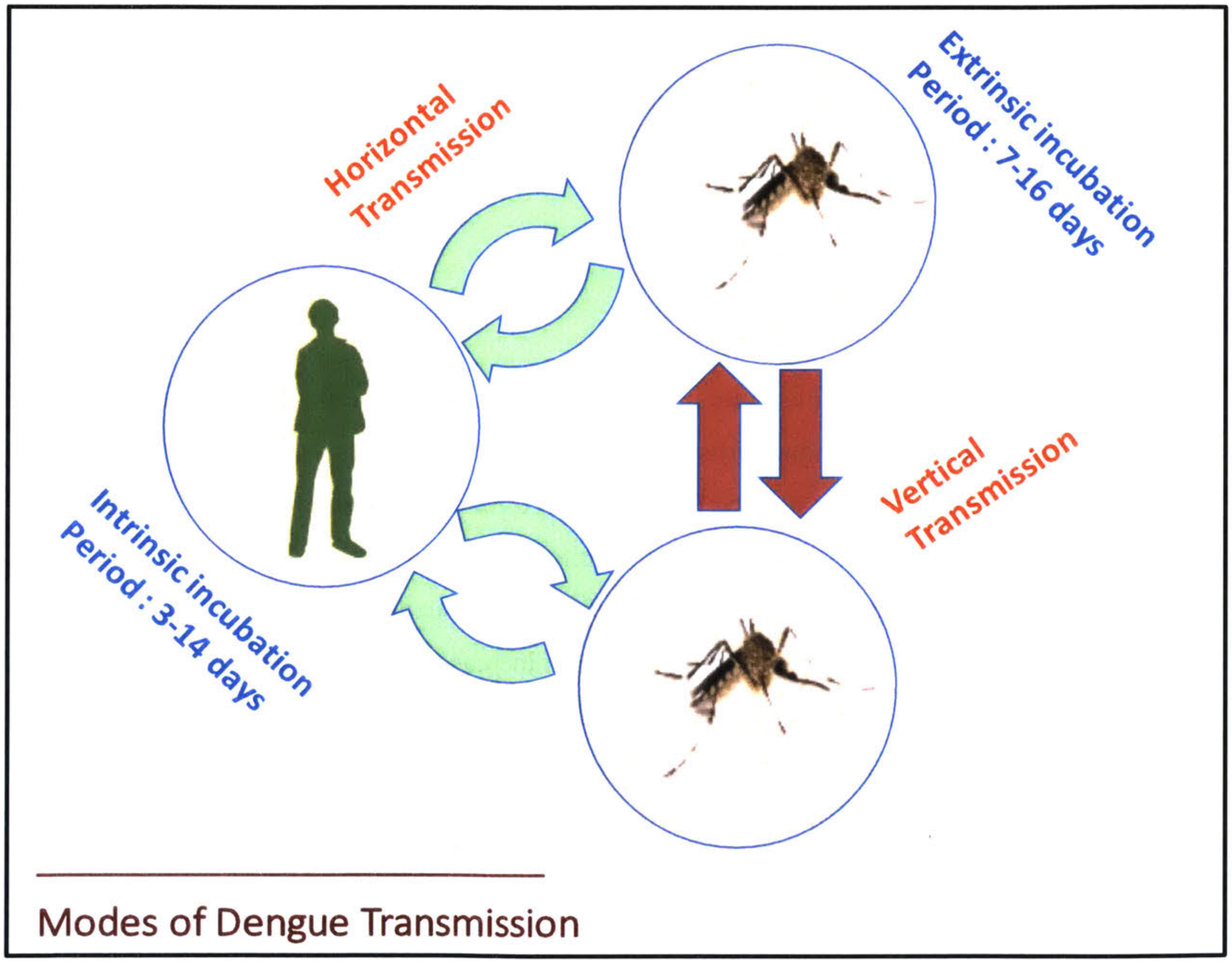


Figure 2.3 Dengue transmission cycle. There are two modes of dengue transmission: 1) horizontal route which involves infection from a human to mosquito or mosquito to human; and 2) vertical route that happens when an infected female passes the virus to its offspring. The duration of intrinsic incubation period (IIP) in infected human takes 3-14 extrinsic incubation period (EIP).

2.2 Measurements of transmission and disease

2.2.1 Basic reproductive number and vectorial capacity

In general, the transmission efficiency of vector-borne diseases is measured by two closely related parameters: basic reproductive number and vectorial capacity.

The basic reproductive number (R_0) is defined as "number of secondary cases produced by a single infected individual (index case) during their infectious period in an entirely susceptible population" [74,75]. Hence, R_0 depends on the actual prevalence rate in the population. Mathematically, R_0 can be expressed as:

$$R_0 = \frac{ma^2bp^n}{-r\ln p}$$

Where: m = ratio of female mosquitoes to humans; a: average daily biting rate of female mosquitoes; b: probability a human is infected after being bitten by an infectious mosquito; p: probability of mosquito survivorship per day; n: incubation period of the virus in the mosquito (days); r: rate of recovery in the human cases; $1/-\ln(p)$: average mosquito life expectancy.

In endemic areas, the fraction of susceptible population is less than one due to the presence of a herd immunity. Therefore, disease ecologists use effective reproductive ratio (R_e) to account for the resistant (immune) individuals in the community:

$$R_e = \frac{R_0S}{N}$$

where: S= number of susceptible individuals; N= total population

A related measurement to R_0 is the vectorial capacity (C). It is defined as "the daily rate at which future inoculations arise from a currently infective case". In contrast to R_0, C is independent of the disease prevalence [75–77]. Likewise, C is calculated by the following equation:

$$C = \frac{ma^2bp^n}{\ln p}$$

Hence, the transmission dynamics of dengue are a function of survivorship, density, and the biting rate of *Aedes* mosquito; and also the virus EIP in the mosquito [11,78,79].

2.2.2 Measures of human morbidity.

Measures of human morbidity are often used in epidemiological studies and disease surveillance and control. Among these measures are: incidence proportion (or attack rate), secondary attack rate, incidence rate, and prevalence. Calculations of these parameters are straightforward using human disease data, as followings:

$$1.\ \text{Incidence porportion} = \frac{\text{Number of new cases during fixed period}}{\text{Size of population at start period}}$$

$$2.\ \text{Secondary attack rate} = \frac{\text{Number of cases among contacts of primary cases}}{\text{Total number of contacts}} \times 100\%$$

$$3.\ \text{Incidence rate} = \frac{\text{Number of new cases during fixed period}}{\text{Time each person observed totaled for all persons}}$$

$$4.\ \text{prevelance} = \frac{\text{All new and pre-existing cases during a given period}}{\text{population during the same period}} \times 100\%$$

In endemic areas, prevalence = incidence x duration of the infection. While incidence rate is more relevant to investigate dengue in endemic settings, attack rate is appropriate in measuring transmission during outbreak periods [80].

Human morbidity measures are useful in calculations of basic reproductive number (R_0), and effective reproductive ratio (R_e) described earlier. For example, seroprevalence rate using IgM/IgG tests are used to verify the herd immunity of dengue in endemic areas [81].

2.2.3 Measures of vertical transmission

Vertical transmission is detected in field and experimental studies using molecular and biochemical tests. Four measurements of vertical transmission were previously used to verify the vertical route [73]:

1. Minimum infection rate (MIR): which is frequently reported in field studies, is the proportion of mosquito pools that are positive with dengue virus per tested specimens:

$$\text{Minimum Infection Rate (MIR)} = \frac{\text{number of positive pools}}{\text{total specimens tested}} \times 1000$$

2. vertical transmission rate (VTR): defined as the proportion of infected parent mosquitoes that produce at least one infected offspring.

$$\text{Vertical Transmission Rate (VTR)} = \frac{\text{number females producing infected offspring}}{\text{total number of infected females}}$$

3. Filial infection rate (FIR): which is defined as the proportion of infected progeny produced from infected parents, given that vertical transmission has occurred.

$$\text{Filial Infection Rate (FIR)} \\ = \frac{\text{number infected emerged females}}{\text{total number of emerged females from infected females}}$$

4. Vertical Infection Rate (VIR): defined the prevalence of vertical infection rate of the mosquito population; it is also the product of VTR x FIR

$$\text{Vertical Infection Rate (VIR)} = \frac{\text{number infected emerged females}}{\text{total number emerged from infected females}}$$

2.3 Spatial and temporal patterns of dengue

2.3.1 Seasonality

Seasonality is a cyclic temporal pattern of peaks and troughs of disease incidence that occurs in "stereotyped calendar periods" [10]. Drivers of seasonality are different and depend on transmission routes of infectious diseases. In many infectious diseases, immunity competence of hosts can fluctuate between the seasons increasing susceptibility to infections. For instance, increase of bacterial meningitis during the dry season is attributed to a reduction in mucosal integrity. The disease seasonality can also result from societal or behavioral changes. For example, school calendar has been shown to influence seasonal outbreaks of measles and other related childhood infections in Europe and the United States [82].

Nevertheless, climate can impact seasonality directly such as in winter peaks of gastroenteritis and flu. Also, climatic factors can indirectly trigger seasonality such as in vector-borne diseases because the insect vectors are poikilothermic and mostly have aquatic phase during their development [82]. Hence, temperature, rainfall, and other climatic factors influence the seasonal abundance of the vector. For example, dengue exhibits seasonal patterns in many endemic countries that show associations with temperature, relative humidity and monsoons [83–86]. For example, in Brazil, dengue incidence has a peak during the humid, rainy season (December to May), following a low transmission pattern during the dry season (June to November) [87]. However, seasonal patterns of dengue can result from a mixture of climatic and non-climatic factors.

2.3.2 Interannual variability

Interannual variability is characterized by an oscillation in the magnitude of a disease from year to year. This phenomenon is well documented in vector borne diseases [88]. Notably, epidemics of dengue in endemic areas show cyclic patterns of occurrence every 3-7 years [89–92].

One explanation for this variability is its forcing by multiyear periods of floods and droughts, i.e. interannual weather variation such as ENSO. El Niño Southern Oscillation (ENSO) was documented to influence dengue transmission in endemic areas [83,88,90,93,94]. ENSO is "the fluctuation of atmospheric pressure and sea surface temperature in the equatorial Pacific Ocean" [94]. Consecutive episodes of dryness (El Niño) and wetness (La Niña) periods alternate every 3-4 years between the eastern and western regions of the Pacific Ocean. Usually, variability of ENSO is measured using the Oceanic Niño Index (ONI). ONI is the moving average of 3-months anomalies of the sea surface temperature (SST) at the 3.4 regions of El Niño in the Pacific Ocean (5°N-5°S, 120°-170°W) [94].

The teleconnection between El Niño and dengue epidemics has been investigated in several studies [94,95]. Synchrony in dengue epidemics that occurred during the period 1998-1999 in Southeast Asia was correlated to elevated temperature and strong El Niño episode at that time. Average anomalies of +1.5 °C and - 53 mm have characterized provinces of outgoing traveling waves of dengue compared to provinces without waves. In contrast, regions of incoming waves of multiannual epidemics have shown lower precipitation anomalies averaging- 39.5 mm but with no significant difference in temperature compared to other areas. However, the non-stationarity of the relationship between interannual cycles of dengue epidemics and ENSO is suggestively a threshold-dependent. A region-scale increase in temperature could accelerate development of the *Aedes* mosquito populations and virus transmission in several sites. However, a pandemic of dengue – like the 1997/8 -- could result in depletion of susceptible reservoir of human population and decrease the risk of dengue epidemics in the coming years [95].

2.3.3 Impact of climate change on dengue

According to the atmosphere–ocean general circulation models (GCMs), greenhouse gas emissions would result in an increase in the mean global temperature between1.1 to 6.4 °C

[96,97]. Such global warming scenarios will have impacts, both direct and indirect (through its effects on the hydrological cycle), on dengue. They may change the distribution of dengue as well as its transmission season and intensity.

Several statistical models predict an expansion in the endemic map of dengue [98,99]. In a pioneering work, Patz and others [99] used projected temperature output from three GCMs to predict where dengue epidemics would increase by 2050. They found that temperate regions would have the largest change in dengue epidemic potential when the temperature increases by 1.2 °C. In another work, Hales and others used the projected vapor pressure as a predictor for the distribution of dengue in 2085. They found that 15-25% of the projected global population (i.e. 1.5-2 billion people) would be at risk of dengue transmission if greenhouse emissions increase annually by 1% [98]. In another study, researchers included projections for the economic development (in terms of GDP per capita) and population growth to investigate the impact of climate change on dengue distribution in the world by 2050. They found a small counter effect for the economic growth curbing dengue transmission risk by 0.12 billion people. They also projected this risk will increase in case that the GDP per capita has remained constant, 56% of the world population instead of 52% of the population in the previous scenario [96].

On the contrary, mechanistic population models show little expansion in dengue distribution. Instead, they find shifts in time and duration of the transmission season. In a recent work, the impact of four scenarios of climate change (A1fi higher and B1 lower emission scenarios) on dengue transmission by *Ae. albopictus* in U.S. was studied. The study used the outputs of four atmosphere-ocean general circulation models (GCMs): NCAR/CCSM3, UKMO/HadCM3, NOAA/GFDL CM2.1 and DOE/NCAR PCM. Projections were enforced using CO2 emission scenarios described in the he IPCC Special Report on Emission Scenarios [100]. The downscaled temperature outputs of the climate models in Atlanta, Chicago and Lubbock, were used to force a population model. Accordingly, while the transmission season of dengue will lengthen in the mid-century (2035–65) and end of the century (2069–99), a shift in the peak season of mosquito and disease, from summer to spring and fall, is predicted [101].

In Australia, Kearney and others [69] used a mechanistic model to study the effect of dryness on dengue. They focused on the atmospheric water balance of breeding containers. They found both water availability and egg desiccation resistance of *Ae. aegypti* delineate dengue transmission in the arid northern part of the country, but this transmission is constrained by cold weather in the southern part of Australia. Since rainfall is projected to decrease on the continent by 2050 (if moderate mitigations are applied), the impact would only be indirect via the human adaptation to store rainwater in potential breeding containers [69]. Considering global urbanization rates which put pressure drainage and water supply systems, particularly in developing countries, dengue control will be more difficult in such congested population centers.

In fact, it is not only climate change that will impact dengue and other arboviral diseases, but in a broader sense, global change- which also entails changes in land use, urbanization, and human disasters- could affect the incidence of this group of infectious diseases [7,8,102].

2.3.4 Spatial patterns of dengue

The locations of both humans and mosquitoes are important in dengue epidemiology. First, dengue transmission depends on the direct contact of these two dynamic populations. Several studies have shown that dengue transmission is sensitive to spatial scales (intra-households, inter-house and inter-areas) [103–108]. An interesting observation of dengue and other vector borne diseases is that transmission probability decreases inversely with the distance -- and contact -- between the humans and mosquitoes [104,109]. During the outbreak of DENV-2 in Cairns (north Queensland, Australian), 68% of the dengue cases had clustered within 800 meters close to the house of index case [103]. When the distance at a local level allows the contact of susceptible and infected individuals, aggregation of new cases -- the phenomenon of clustering – happens resulting in an outbreak. In Delhi (India), dengue cases "decreased with the distance" to the nearest forest. This spatial pattern was attributed to the fact that forest works as a resting place to *Ae. aegypti* [104]. However, heterogeneities in spatial scale and modes of mobility of humans and mosquitoes influence the spatiotemporal patterns of dengue at local as well as regional and global scales [110,111].

Second, characteristics of landscape of a location shape the vector microhabitat and indirectly influences dengue transmission. For instance, larvae of *Ae. aegypti* in Buenos Aires was more abundant in neighborhoods having intense vegetation [112]. The presence of a canopy can reduce the effect of extreme climatic conditions (e.g., temperature and humidity) at the microhabitat. In Cairns (Australia), the spatial orientation of the city along an NW-SE axis had affected the distribution of dengue cases in 2003. The prevailing NW wind from the SE quadrant of the index case was suggested to have impacted dispersal of *Ae. aegypti* increasing dengue transmission in the city [103].

Careful attention should be paid to spatial variability in dengue vector control. The response of a mosquito population can vary significantly with location, leading to a sampling bias. Hence, a collection of accurate data such as temperature and rainfall is needed when addressing the spatial ecology of dengue [89]. A better understanding of the spatial variability of dengue can improve the disease surveillance by identifying hotspots and areas at high-risk and hence decision making about the mobilization of dengue control resources particularly in underdeveloped countries [15]. One of the new development considering spatial variability in the control of dengue and other vector-borne diseases is the Spatial Decision Support Systems (SDSSs) [113]. Geographical Information System (GIS) and space-time modelling tools -- such as ArcGIS, QGIS -- are employed to analyze collected data and investigate impact of spatial variability on dengue transmission [114,115]. SDSSs were used in Mexico and Australia to assist dengue control programs in their operations [114,116]. In Chetumal and Merida (Mexico), Google Earth was combined with two free GIS software (i.e., HealthMapper and SIGEpi) to map dengue risk in these two cities [116]. In Cairns, classified clusters of dengue fever were used to optimize vector control activities such as Indoor Residual Spraying (IRS) [114].

2.4 Drivers of spatiotemporal patterns of dengue

In general, spatiotemporal patterns of vector borne diseases are driven by several factors that interact in space and time (e.g., seasonal or interannual). These factors could influence the vectorial capacity or bionomics of the mosquito (e.g. cyclic shifts in mosquito abundance, survivorship or biting), the virus (e.g. cyclic decrease in EIP or virus incubation period in the host), or the host (e.g. changes in human/animal behavior, susceptibility to the disease or population changes) [11]. All these cyclic drivers are regulated by climatic and non-climatic factors [10,117,118].

2.4.1 Climatic factors

Temperature: Temperature influences temporal patterns of dengue via its differential effects on the virus, mosquito, and human. First, as temperature increases, the EIP of the virus in the mosquito decreases [119,120]. For instance, increasing insectary temperature for *Ae. aegypti* from 17 °C to 35 °C could reduce the EIP of the dengue virus from 16 to 7 days, respectively[121]. However, recent work has shown that a wide temperature range of 18 °C around a daily temperature mean of 20 °C has shortened the EIP of the dengue virus to ~19 days compared to a narrow range of 8 °C fluctuating around the 30 °C that increased the needed time for *Ae. aegypti* to be infectious to ~ 29 days [122]. **Figure 2.4** shows the effects of the temperature on the longevity of adult mosquito and EIP. The Figure indicates that for ecosystems having a daily average temperature < 16 °C or > 37 °C, no autochthonous transmission could occur because adults of *Ae. aegypti* survive less than the duration needed for dengue virus development. The Figure also shows that in endemic areas where a daily temperature ranges 25 - 30 °C -- such as Singapore -- are continuously suitable for the disease transmission, ceteris paribus.

Second, the reproduction and development rates of mosquitoes are influenced by temperature. Principally, most of the insect population models follow an enzyme kinetic approach that is based on temperature to simulate the life stages [123–126]. The increase in temperature accelerates the development of the aquatic stages of the mosquito and shortens the adult gonotrophic cycle. For example, the duration of the aquatic cycle of *Ae. albopictus* in the Indian Ocean can reach a minimum of 8.8 days when the temperature is 30 °C [127]. Nonetheless, there are upper/lower temperature limits above and below which the activity of the mosquito halts and survivorship of aquatic and adult stages may be reduced [122]. Another temperature-dependent process is the feeding frequency of the vector on humans. An increase of 2-3 °C in temperature enhances the frequency of the biting rate of *Ae. aegypti* female [30]. Furthermore, flight performance of *Ae. aegypti* showed an optimum range between 15-30 °C, while this activity was much affected when the temperature exceeded 35 °C or below 10 °C or when relative humidity is $\leq$ 30% at 32 °C [128].

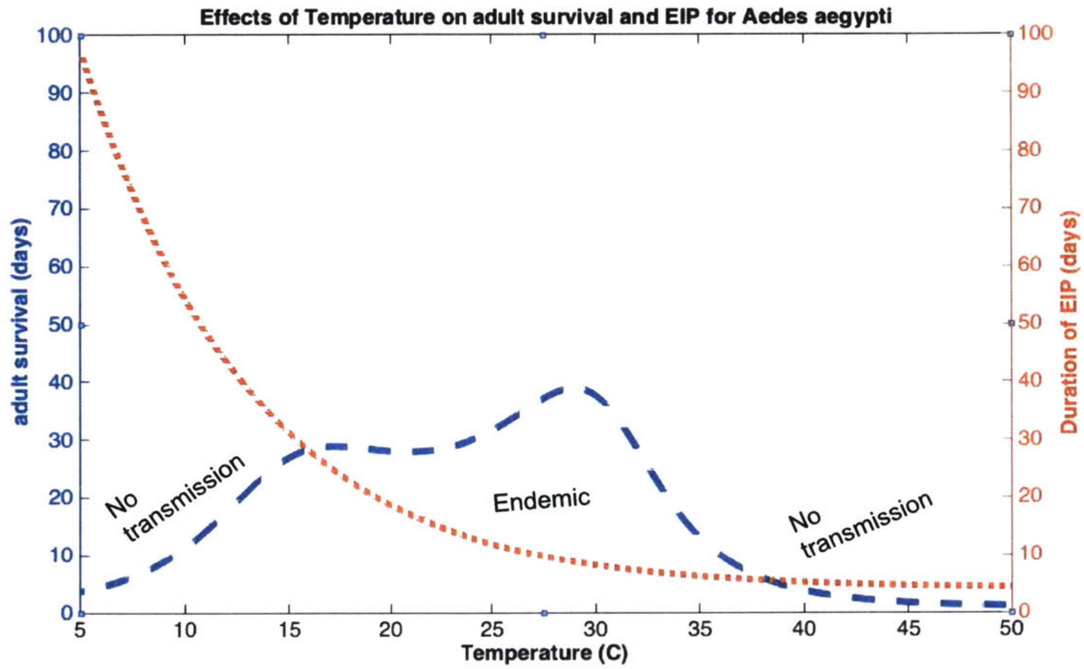


Figure 2.4 Effects of daily temperature on adult survivorship and extrinsic incubation period (EIP) of dengue virus. Adult survival and EIP were calculated using empirical temperature-dependent models, described in [120] and [124], respectively.

Rainfall: Rainfall influences dengue, primarily by generating wet conditions that favor the survival of the mosquito vector. First, a significant amount of rain is needed to create a natural breeding habitat or to feed an artificial one [129]. However, wetness is not a sufficient condition for a mosquito to lay its eggs at a site. In fact, mosquitoes are selective in laying their eggs in an appropriate breeding habitat having both a minimum mortality risk (e.g. predation or parasitism of offspring) and maximum nutritional benefits (i.e. food quality) for their offspring.
Second, hatching of dormant eggs is triggered by rainfall intensity [129,130]. In a field experiment in Florida, eggs of *Ae. albopictus* showed more mortality compared to *Ae. aegypti* when they were unflooded for 30 days. This difference in the desiccation-resistance between the two species implies that the local population of *Ae. aegypti* has an evolutionary advantage to escape mortality related to the seasonal dry conditions during winter or interannual droughts periods. However, the investigators rejected this advantage in places where the temperature > 26°C and relative humidity > 95% [68]. In addition, rainfall intensity may have adverse effects on larvae by pushing them down the water column or washing them out farther from the

breeding site [129]. In an experimental study, investigators found *Ae. aegypti* better resist flushing compared to Culex spp. While an increase in temperature caused more flushing, a 50% shorter artificial raining had caused flushing of fourth instars of *Ae. aegypti* [129]. In Nepal, inverse relationships between rainfall and abundance of both the newly introduced *Ae. aegypti* and the indigenous population *Ae. albopictus* were attributed to more indoor breeding during the dry season compared to the wet one [131].

Third, duration of rainfall affects air humidity, which in turn influences the survival and dispersal of adult mosquitoes. In Thailand, the increase in dengue and Chikungunya cases, which occurs during the hot, rainy season, is not attributed to an increase in mosquito breeding. Instead, humidity is believed to increase the survival and feeding activity of the vector [30]. Another field study in Bangkok revealed that females of the dengue vector would need an additional two days during the dry, cool season to take their first blood meal compared to 3 days during the wet season [30].

Humidity: Conversely, air humidity indirectly affects dengue transmission by inducing behavioral responses to dry periods both in humans and mosquitoes. In many Southeast Asian population centers, people store drinking water in containers during the dry season, which can be inhabited by *Ae. aegypti* [132]. For example, in the Indian subcontinent, dengue peaks happen during the hot, dry season because *Ae. aegypti* breeds in the tanks of air conditioners [30]. Using coupled statistical models, absolute humidity in Singapore was shown to give a better predictive power for weekly dengue incidence compared to other climatic variables. Absolute humidity is the mass of water vapor in a unit volume of air but without consideration of the temperature [133].

Interestingly, a direct correlation between humidity and dengue vector is demonstrated in several studies [134–136]. While a low humidity (RH=34%) negatively affected fecundity and survival of experimented females of *Ae. aegypti*, stress conditions that also included low sugar concentration (3%) had delayed oviposition of gravid females 1-4 days [137]. In Nepal, a positive correlation was shown between this climatic factor and the abundance of *Ae. albopictus* in a narrow range of a high relative humidity (between 80%-90%), compared to a negative association found for the population of *Ae. aegypti* [134]. Previous studies have also shown that eggs of *Ae. aegypti* and *Ae. albopictus* hatch better at high humidity levels [134–136].

Wind: Both *Ae. aegypti* and *Ae. albopictus* fly low and short distances to stay close to human habitats (i.e. ~ 200 meters) [138,139]. Although few studies have addressed the effect of the wind, it plays a significant role in the transmission of dengue through its direct effect on the vectors and a long one on rainfall. Also, wind radically enhances dispersal of adult mosquitoes. For example, in a release trial conducted in Palestine, a wind speed of 2.5 km/hour aided the dispersal of *Ae. aegypti* up to 2.5 km. In addition, wind direction affects the dispersal of mosquitoes. For example, eggs were found in jars within 45° of the wind direction [140].

2.4.2 Non-climatic factors

Most of the non-climatic factors that trigger dengue transmission are related to population demographics and behavior. For example, a tendency of people to spend more time outdoor during the summer was shown to increase their exposure to mosquito bites and risks of arboviral infections [30,141]. In a previous study, investigators found a difference in dengue incidence between two towns along Mexico-U.S. borders, despite similar habitats of mosquitoes. This difference is attributed to air conditioner usage in the U.S., which reduced the outdoor exposure of the affluent population to mosquitoes [56].

Human movement. Human movements and introduction of new genotypes of dengue strains were shown to result in dengue epidemics in endemic areas. For example, importations of DEN-2 and DEN-3 in the 1970s had led to significant outbreaks in the Pacific and Indonesia, respectively. In addition, introductions of genotypes of DEN-2 (from Southeast Asia) and DEN-3 (from the Indian subcontinents) had caused major outbreaks of these strains in Latin America [22,142,143]. Southeast Asia is among the most congested regions having a population density of 132 per Km^2 (estimate of 2013)[144]. Interconnection of biological, social, ecological and technological processes at the regional level as well as at country and local levels furnish a suitable environment for the resurgence of dengue and other arboviral diseases [21,95]. The genetic diversity of dengue viruses besides the population movements between the region's countries and from outside is expected to increase the risk of dengue epidemics in this regions in the future [145].

2.5 Methods to study the ecology of dengue

2.5.1 Observational studies and experimentation

In general, observational studies are an indispensable tool to study disease ecology. Usually, specific sampling techniques (i.e. random, systematic or stratified sampling) are employed to verify the distribution or frequency of a variable over time or space (e.g. mosquito density per week or dengue cases per epidemiological week). However, this approach is challenged by possible biases that could be either random or systematic. Notably, systematic errors could be driven either by inappropriate selection criteria for the samples, insufficient sample size; non-standardized sampling procedure or instrument; or unknown factors or complex relationships between the factors [146].

On the other hand, experimentation, whether laboratory-based or a randomized controlled trial, provides a controlled foundation to investigate the driver(s) of spatial and temporal patterns. In a laboratory setting, specific climatic factor(s) can be manipulated, and the response of the mosquito and virus population(s) can be followed. Alternatively, semi-field work is useful if the objective is to investigate how different manipulated groups of the same element –human, mosquito or virus- respond to real fluctuations in ecological factors. The main limitation to experimentation is that we generalize a holistic picture for the disease transmission from a specific and necessarily limited situation.

2.5.2 Statistical methods

As discussed earlier, temporal patterns (e.g. abundance of *Aedes* or increase in DF cases) are considered seasonal only if the observation repeats between years. Seasonal peaks and dips can statistically be described by duration, onset time, median and mean dates [126]. Although, an increase in interannual variability in the above characteristics of seasonal patterns can be attributed to presence of confounding factors, however, descriptive statistics may not be appropriate in deciphering the reasons.

Another approach is to categorize the observations into contingency tables according to the season or multiyear period. After that, use of statistical tests for the differences between these groups (e.g. by Pearson's Chi-square) is the standard way to analyze seasonality. However, this approach oversimplifies the situation by assuming that all the patterns are seasonal or multiyear[126]. Moreover, annual grouped data impedes recognition of more than one intra-annual cycle.

A more comprehensive approach to study seasonality and other temporal patterns is the time series analysis. This method is useful in temporally fitting observational data. Both autocorrelation and spectral density functions are employed in plotting seasonality coefficients in an auto-correlogram and periodogram, respectively. Autocorrelation measures how serial observations correlate with each other at a time lag. Conversely, in spectral analysis, a cosine regression model is fitted to plot a spectrum of varying oscillatory frequency for the time-series data. An example of spectral analysis tools used in dengue studies is Morlet wavelet. The Morlet wavelet and other wavelet functions allow tracking the temporal evolution of periodic events and testing the association between two non-stationary time series [147]. In both methods (autocorrelation and spectral density), seasonality can be refined further by sorting the time series data into smooth and rough components [126].

Alternatively, empirical statistical modelling can be useful in predicting the amplitude of the temporal pattern. For example, seasonal patterns (such as that of dengue) can be described by cosine or sine functions in a Poisson regression. Such an approach can help in controlling confounding factors and makes it easier to study interannual epidemics observed in disease incidence. In addition, multiyear seasonal patterns can be investigated by using appropriate statistical models such quasi-cycles and Uniform Phase Chaotic Amplitude Dynamics (UPCA) [11,148].

A challenge in statistical models is that it may be difficult to control confounding factors by such external oscillators if the spatial pooling of observations is not adequately grained, thus leading to ecological fallacy in inferences [10]. In addition, group comparisons or data fitting by time series analysis does not answer the fundamental question of how intrinsic and extrinsic factors drive seasonality.

2.5.3 Mathematical modeling

A mathematical model is a numerical technique to represent the essential aspects of a system [149]. Mathematical modelling of infectious diseases is a process-oriented approach (i.e. mechanistic) that aims not only to simulate previous and forthcoming phenomena such as seasonality but also to explain how it is generated. Hence, input variables change their states with time according to the governing processes. Often, ordinary differential equations are used to describe changes over time in the state of these variables [11]. Also, mathematical modelling is useful in identifying which drivers are most important in the genesis of spatiotemporal patterns. This approach is accomplished by sensitivity tests where specific parameters are changed to see how the system behaves.

Mathematical models can be divided into two types: compartment-based and agent-based models. A compartment-based model is stochastic and top-down. It describes the interaction of the bulk population with the system and how this implicitly changes the state of the individuals. An example for compartment-based model is the Susceptible-Exposed-Infected-Recovered (SEIR) model [11]. In this example, the population is divided into four compartments. The change in the state of each group at each time step (t) follows a mass-balance of the total population in the study area (N):

$$N_t = S_t + E_t + I_t + R_t$$

Conversely, agent-based models are deterministic. They are bottom-up and explicitly describing changes in the individual state that affects the entire system state. An example in this category is HYDREMATS (Hydrology, Entomology and Malaria Transmission Simulator), developed by the Eltahir Group - MIT [150]. HYDREMATS couples the hydro-persistence of a breeding pool with the lifecycle and dynamics of each *Anopheles* individual. Interestingly, advancement of each mosquito individual in HYDREMATS is based on empirical enzyme kinetic model for *Ae. aegypti* [125,151,152]. Hence, the transmission rate is quantified at each time step while *Plasmodium* infections are shuttling between individual humans and mosquitoes represented in this mechanistic model.

A primary challenge in using mechanistic modelling is that they require comprehensive understanding of the processes that govern the system. For example, identifying rainfall as a driver of seasonality of dengue requires identifying each process in disease transmission that is influenced by wet/dry conditions. Another challenge is that a better simulation of the real world (i.e. data fitting) requires better parameterization. Thus, field and experimental observations are needed to estimate values of the parameters. Statistical approaches described earlier can also be employed in parameterization [11,153].

Development of mathematical models to study spatial and temporal patterns of dengue transmission has largely increased over the last two decades [154,155]. This approach has allowed mapping the potential risk of the disease and vector [12,15,60,156], forecast epidemics and temporal changes particularly in endemic areas [132,157–159] and under climate change scenarios [96,99,160,161], and pre-evaluate the outcomes of vector control strategies[161–164] and vaccination programs [154,155,165].

2.6 Dengue in Singapore

In 1966, a vector control unit was established within the Ministry of Health of Singapore after intense epidemics of DHF -- mainly affecting children -- struck the country [50]. Entomological surveys carried out between 1966 and 1968 had revealed the role of *Ae. aegypti* in dengue ecology in Singapore [166–168]. In 1972, the unit was moved to the Ministry of Environment and dengue was recognized as a notifiable disease (for the reporting system) in 1977. Responsibilities of dengue control program are currently divided between two governmental agencies in Singapore: 1) Ministry of health, which is responsible for clinical management of cases and disease surveillance; 2) National Environmental Agency (NEA), which is tasked with vector control [51].

Five key strategies are adopted by the dengue program of Singapore: early detection/case management, vector surveillance/source reduction, community involvement, health education, law enforcement, and operational research. NEA conducts epidemiological and entomological investigations, carries out vector control operations during outbreaks and supervises pesticides companies (which are contracted by townships to perform conventional vector control) [50,169].

The control program aims to prevent dengue transmission through anti-vector measures that reduce breeding of *Ae. aegypti* and *Ae. albopictus*. These interventions include house-to-house inspections, surveillance and monitoring of high-risk areas, scaling-up community awareness to prevent breeding in domestic containers, imposing legal procedures towards practices resulting in breeding of *Aedes*, and conduction of operational research.

Nevertheless, community involvement is an integral part of the control strategy. The pilot project in 1966-68 had shown that residential areas cleared by vector control teams get quickly infested, and efforts could not succeed unless the community is involved in source reduction [50]. Every year, NEA launches an outreach community campaign at the beginning of the dengue season (at the mid of the year) called “Do the Mozzie Wipeout”. Households are encouraged through the media to take five preventive actions: 1) change of water in plants bowls/vases every other day, 2) turnover of water storage containers, 3) drain water in plates of flower pots every alternate day, 4) cover unused holders of bamboo pole, and 5) remove blockage in roof gutters and place Bti monthly [51,52].

The key indoor breeding habitats of *Ae. aegypti* and *Ae. albopictus* found in the survey of 1966-1968 were ant traps. Earthenware jars and tin cans were the key outdoor habitats for *Ae. aegypti* and *Ae. albopictus*, respectively. Three types of housing were present in Singapore at that time: slum houses, shop houses and flats. The survey showed more storage containers in slum and shop houses, while, flowers and plants containers were common in apartments [166].

During the last two decades, construction sites have constituted a concern for dengue control program. Prevention of dengue transmission in construction sites was scaled up by NEA in 2015. Hence, NEA requires construction sites to recruit full-time Environmental control officers (ECO) to deal with issues related to public health and environment. A Code of practice was published by NEA to guide ECO on dengue control and other environmental issues encourage thermal fogging using malathion or pirimiphos-methyl at least one week when a dengue cluster is near, and Larvicide application in stagnant water that cannot be drained at least once a week. The ECO also help in identifying early dengue infections in the site using a thermometer for suspected individuals. In addition, stop-work orders are publicly announced to discourage companies from harboring breeding of *Aedes* in construction sites. Infected workers are required to sleep under bed nets or in air-conditioned sick bays [169].

Disease clusters are opened when two or more cases within a diameter of 150 meters -- of residence, work or school -- occur within two weeks [52]. A Dengue Community Alert System was established in 2013 to warn the local communities living near dengue clusters. Hence, three color codes are used in banners to inform the public: 1) red when there is a cluster > 10 cases; 2) yellow when there is < 10 cases, and 3) green when there no cases and the cluster is closed. The community outreach program in the epidemic periods and in clusters areas include health educational sessions such as seminars and roadshows and using the media [51]

Vector surveillance. Dengue control program uses House Index (HI) and the newly introduced gravitrap index as indicators for the risk of dengue transmission. A gravitrap is a small black container developed by NEA to monitor the adult population of the dengue vector in Singapore passively. Sticky films lining the inner wall of gravitraps trap gravid females when they seek stagnant water to lay their eggs. A hay infusion water is filled up to a level where is a mesh cover to prevent escapees of emerging adults. The gravitrap index is the percent of positive traps for *Aedes*. In a field survey, more adult mosquitoes were caught by gravitraps at lower floors (i.e. 2-6 floors) compared to higher ones (i.e. 7-13 floors), 64.9% compared to 35.1%, respectively [170]. By 2015, a weekly sentinel surveillance program was employed in Singapore using ~ 3,000 gravitraps implemented at 34 selected sites. The annual average of gravitrap index has shown an increase in 2015 compared to 2014, 15.3% and 7.6%, respectively [145].

Infectivity of mosquitoes. In addition, subsamples of collected adults are usually subject to viral investigations using RT-PCR. In a pilot work in 1995-1996, 20.9% and 20.1% of pools of females caught from dengue sensitive areas were positive for dengue virus for *Ae. aegypti* and *Ae. albopictus*, respectively. DENV-1 was detected in 78.3% and 77.55% of the positive pools, *Ae. aegypti* and *Ae. albopictus*, respectively. Minimum infection rates (MIR) of *Ae. aegypti* and *Ae. albopictus* were similar, 57.6 and 50 per 1,000. Interestingly, the earliest positive samples of *Ae. aegypti* preceded dengue outbreak in 1995-1996 six weeks while infected specimens of *Ae. albopictus* trailed peak time of the outbreak [46].

Legislations. Singapore issued three Acts to prevent and control dengue transmission: The Infectious Diseases Act (IDA); the Control of Vectors and Pesticides Act (CVPA); and the Environmental Public Health Act (EPHA). The IDA is dedicated to reporting, diagnosis, and treatment of dengue cases, the CVPA is tailored to the needs of vector control, and the EPHA is purposed to sanitation and other matters of public health. The IDA requires doctors and medical entities to notify the Ministry of health and NEA on suspected and confirmed dengue and DHF cases within 24 hours. Suspected individuals are also obliged to seek medical examination and to be quarantined in case of confirmation [55].

In 1968 Singapore had enacted a "Destruction of Disease Bearing Insects Act" to prohibit the community from inappropriate activities that increase production of *Aedes* mosquitoes. In 1998, this Act was repealed by the "Control of Vectors and Pesticides Act" (revised in 2002). The new Act strengthens NEA as the central authority to oversee and enforce the vector control and clarify limitations and power. Public health officers are entitled to access private properties for inspection and impose order on the owners to eliminate the conditions that led to the breeding of the vector. A penalty was instigated in 2005 to charge properties owners for *Aedes* breeding in their premises. In 2016, the fine amount was raised to 200.0S$ regardless of where positive premises lie (i.e. in dengue cluster or non-cluster areas). However, impact of enforcing the law compared to other measures that seek community involvement is questionable [55].

Insecticides. The sand granular form of the larviciding Temephos (Abate) is frequently applied in drains, roof gutters and other -- difficult to access -- breeding habitats. In addition, space spraying or fogging of pyrethroids is conducted by companies of vector control in residential yards and common places particularly in dengue clusters areas. Nevertheless, the continuous use of pyrethroids in dengue control since 1970s have resulted in insecticide resistance in *Ae. aegypti*. The knockdown resistance (kdr) mutation is involved in this resistance lowering a potential use of Insecticide treated nets in Singapore [171]. In addition, cross-resistance was detected in this species to DDT. Moreover, the use of vector control alternatives has been sought by the NEA, such as *Bacillus thurigensis* (Bti) – introduced in 2004-- and insect growth regulators (e.g. Pyroxene). In contrast, Temephos appeared to be effective in killing aquatic stages [169,172]. On the other hand, *Ae. albopictus* was previously shown susceptible to permethrin and pirimiphos-methyl and permethrin compared to *Ae. aegypti* in Singapore [173].

DENV serotypes. Singapore lies in a hyperendemic region where the four strains of dengue virus are simultaneously present. **Figure 2.5** shows the distribution of dengue serotypes in Singapore between 1993 and 2015. Accordingly, DENV-1 and DENV-2 constitute about 85% of the circulating virus strains in Singapore. Switches in predominance between these two serotypes were observed in 1995 from DENV-2 to DENV-1, 2001 from DENV-2 to DENV-1, 2007 from DENV-1 to DENV-2, and 2013 from DENV-2 to DENV-1. Three outbreaks were associated with these switches: DENV-1 in 1995-1997, 2004-2006, 2013-2015; and DENV-2 in 2007. Furthermore, importation of new virus genotypes beside evolution of local strains is among the factors that suggested to contribute to dengue outbreaks during the last two decades in Singapore. A switch of the genotypes of DENV could also increase fatality rates during epidemics [174]. A main difference between populations of DEN-2 and DEN-1 in Singapore is that the latter is composed of multiple genetic strains instead of clade lb (the dominant genotype of DEN-2 that also was responsible for dengue outbreak in Johor and Malacca in 2013-2014) [145]. The high viral diversity of dengue strains in Singapore – which is attributed to multiple introductions--could imply "better fitness" for the virus to be transmitted by *Ae. aegypti* in Singapore [169,175].

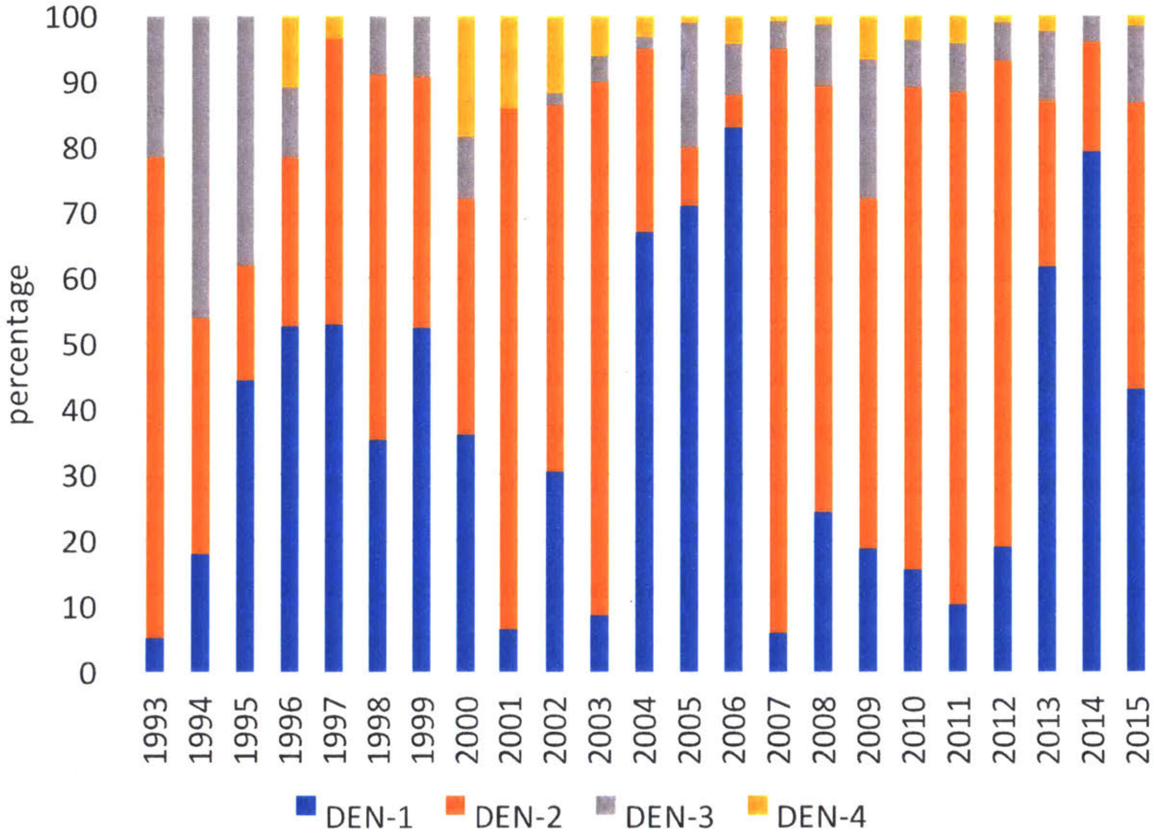


Figure 2.5 Distribution of dengue serotypes in Singapore (1993-2015). Data Source: Ministry of Health, Singapore.

Herd immunity. IgM/IgG diagnostic tests are used to detect antibodies to dengue serotypes in the blood. While a recent exposure to a dengue infection -- occurring within three to nine months of testing -- can be verified by IgM test, lifelong persistent antibodies of old infections could still be detected using IgG test. Estimation of dengue incidence and prevalence in endemic areas is limited by underreporting due to subclinical or asymptomatic cases. A cross-sectional survey during the outbreak of 2007 has shown that asymptomatic cases constitute 78% of the cases in seven endemic neighborhoods [54]. In another survey in 2010 to estimate seroprevalence of old infections in Singapore, the overall IgG seroprevalence was 44.4% for the population aged 1-79 years. The study showed an increase in the seroprevalence with age, from 11% in children aging 1-7 compared to 92.4% in senior adults above 60 years. The survey also showed that seroprevalence is 70.9% in homemakers and housewives compared to 18.9% in students and enrollees of national service. Malay people showed a significant lower seroprevalence compared to Chinese and Indian ethnic groups, 50.2% compared to 57% and 62%, respectively. No significant difference between resident and permanent resident was obtained, 57.4% and 53.4%, respectively. Although households living in landed houses showed lower seroprevalence (60.2%) compared to those living in private and public apartments, the difference was not significant. In addition, no difference was shown between floors levels of public and private apartments [45].

Effects of the climate. Previous studies have shown an association between dengue and precipitation and meant temperature in Singapore [176]. Chan and others (1967) showed a seasonal pattern for dengue, populations of dengue vectors (*Ae. aegypti* and *Ae. albopictus*) and dry seasons. Accordingly, population of *Ae. aegypti* appeared first during the dry February. The peak of dengue incidence in June had followed the peak densities of the two vectors shown in March. The dryness of February and April had resulted in two mosquito density peaks during March and June [168]. In a recent work by Hii and others (2012), an apparent peak of dengue cases is shown between July-October [132]. A rise in weekly temperature before 8-20 weeks was shown to precede an increase in dengue incidence in Singapore [177]. This lag time was shown to be 18 weeks during the major outbreak of 2005 [52].

Hii and others (2009) investigated how climate variability influenced dengue incidence from 2000-2007. They used mean temperature of 82.07°F and precipitation of 1.165 inches as median breakpoints to measure relative risk of dengue between 2004 -2007 [178]. Accordingly, dengue incidence increases by 80% when concomitantly mean temperature increases above 82.07°F and precipitation decreases below 1.165 inches in 1-20 weeks ahead. The first quarter of 2005 was drier in Singapore compared to the second and third quarters when dengue incidence increased. In another study, Hii and others (2012) utilized weekly mean temperature and cumulative rainfall to identify a16-weeks period as an optimum to forecast dengue outbreaks in Singapore. Their time series Poisson multivariate regression model was 96% and 98% sensitive to hindcast and forecast outbreaks during 2004-2010 and 2011, respectively [132].

In Singapore, ENSO was recorded in May-2002-March 2003, June 2004- Feb2005, Aug 2006-Jan 2007, and January 2015- September 2016. During El Niño years, Singapore experienced dry and warm weather particularly in the period of June-October which is the time of the Southwest Monsoon. In contrast, La Niña years were featured with wet weather during the same season. However, the very wet conditions between December and March had attenuated the impact of both El Niño and La Niña [179]. Consequently, higher mean temperatures and dry periods in 2004-2007 had increased relative risk of dengue outbreaks during this period [178]. Further, the strongest El Niño event during the last decade happened in 2015. Correlation between anomalies of Niño 3.4 SST and dengue incidence was compared for three periods of dengue outbreak, 1997-1998 and 2009-2010 and nine months in 2015. Interestingly, lagged correlations between dengue incidence and these anomalies showed a similarity between the incidents of 1997-1998 and 2015 [145].

Dengue and age. A difference in dengue incidence between age groups has been well documented in Singapore [45,180–182]. Since 1982, dengue incidence has steadily declined in all ages. Between 2004 and 2010, a survey for seroprevalence of past infection in adults 18-79 years old had shown a significant reduction, from 63% to 54% [180]. However, due to effective surveillance and vector control, children have remained very vulnerable in Singapore (< 16%) [45].

Figure 2.6 shows dengue cases by age group in Singapore between 2011 and 2015. It is clear people in the age range 15-44 years old has constituted 60% of the infected individuals. However, this finding does not necessarily imply that young adults are more vulnerable to mosquito bites. Instead, other intrinsic factors related to DEN-1 and DEN-2 could result in this observation. In the outbreak of 2007, dengue incidence in old age group > 55 years was noticed to correlate with DENV-2 [145]. In addition, a cross-sectional survey in 2009-2010 has shown that young people (16-40 years) are more susceptible to DENV-2 than DENV-2 [183].

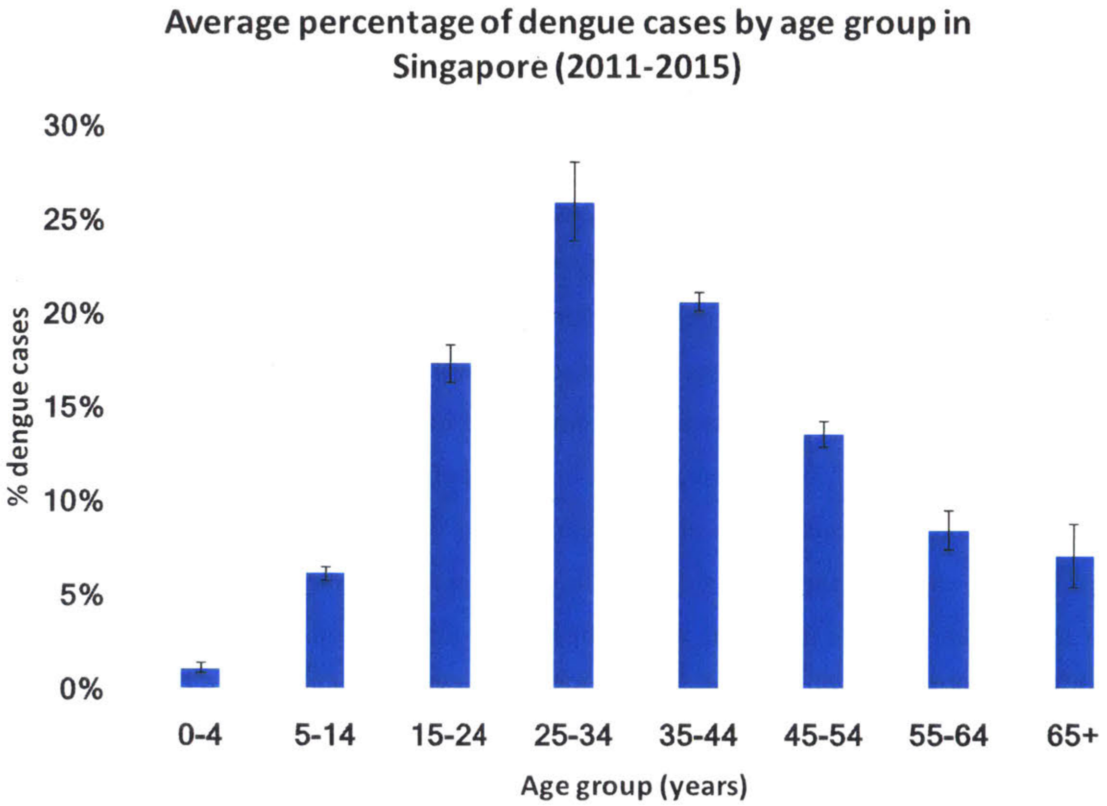


Figure 2.6 Average percentage of dengue cases in Singapore by age group (2011-2015). The error bars show standard deviation within each age group. Data Source: Ministry of Health, Singapore.

Chapter 3: Dengue Seasonality and Flushing Hypothesis

In dengue-endemic areas, transmission shows both a seasonal and interannual variability. To investigate how rainfall impacts dengue seasonality in Singapore, we carried out a longitudinal survey in the Geylang neighborhood from August 2014 to August 2015. The survey comprised of twice-weekly random inspections to outdoor breeding habitats and continuous monitoring for positive ones. In addition, observations of rainstorms were collected.

Out of 6824 inspected habitats, 67 contained *Aedes aegypti*, 11 contained *Aedes albopictus* and 24 contained *Culex spp*. The main outdoors habitat of *Aedes aegypti* was storm drains (54/67). We found that 80% of breeding sites in drains (43/54) were lost after intense rainstorms related to the wet phase of the Northeast monsoon (NE) between November 2014 and early January 2015. Subsequently, 95% (41/43) of these flushed drains had dried out during the dry phase of the NE in late January-February 2015. A return in the outdoor breeding of *Aedes aegypti* was observed after the onset of Southwest monsoon (SW) between May and August 2015. There was also a reduction in productivity of breeding habitats for larvae and pupae after the onset of the NE.

In wet equatorial regions like Singapore, rainfall varies with the monsoons. A monsoon-driven sequence of flushing and drying shapes the outdoor seasonal abundance of *Aedes aegypti*. This finding can be used to optimize vector control strategies and better understand dengue in the context of climate change.

3.1 Introduction

Dengue is an increasing public health problem in the world [15]. In endemic countries, dengue transmission shows both a seasonal and interannual variability [13,94]. Although there are various climatic and non-climatic factors that underlie temporal variability, seasonal patterns of dengue coincide with changes in monsoon systems in the tropics [132,184,185]. For example, in Mexico and Thailand, dengue incidence increases during their main rainy seasons between June and November [186,187]. Similarly, endemic countries in the southern hemisphere, like Brazil and Indonesia, witness dengue peaks in their rainy seasons between January and May [87,188]. Vector control is shown to be effective against dengue transmission when applied early in the season [13]. In addition, there is growing evidence for vector adaptation to outdoor breeding that can increase impact of the climate change on dengue [189,190]. Understanding the climatic drivers of seasonality can improve not only disease surveillance and control in endemic areas, but also global health efforts since tourists visit endemic countries on seasonal holidays [191].

Several studies on dengue and climate have revealed the pivotal role of temperature on both spread and seasonality of dengue [192–194]. Temperature affects larval behavior and development [127,152,195], survival and biting rate of the adult mosquito [30,122,127], and extrinsic incubation of the virus in the mosquito [119,120]. Moreover, daily temperature range (DTR) can influence infective probability of dengue virus (DENV) in *Aedes* females [196].

In wet tropical areas, there is little difference in temperature between the seasons, while rainfall occurs throughout the year and only differs in magnitude between the seasons. Rainfall mainly impacts dengue by generating physical conditions for the breeding of the vector. Rainwater can stagnate into a natural breeding habitat or feed an artificial one where mosquitoes can lay eggs [197,198]. On the other hand, rainfall intensity may have negative effects on larvae by pushing them down the water column or washing them out farther from the breeding site or shortening the survival of adults [129,199].

Singapore is a dengue-endemic country where the four serotypes of the virus simultaneously circulate in the city (DENV-1, -2, -3, and -4) [52,53,178,200]. While both *Aedes aegypti* and *Aedes albopictus* coexist in Singapore, the latter species is the main disease vector [177]. The city has been struck by repetitive outbreaks during the last two decades. This interannual variability of dengue is attributed to switches in dominant DENV strains and introductions of new virus genotypes [175]. In addition, the disease shows a seasonal peak around July - September and a relatively low incidence in February – April (see **Figure 3.1**).

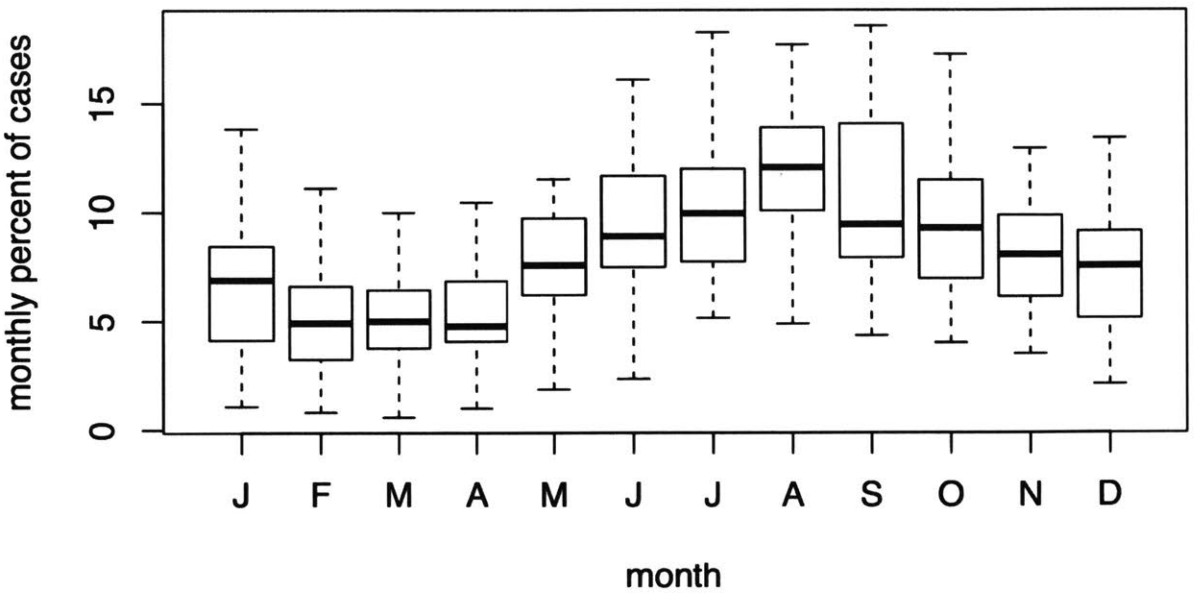


Figure 3.1 Seasonality of dengue in Singapore: boxplot of monthly percent of cases (1983-2015).

Singapore is also subject to two monsoons: a Northeast monsoon (NE) that results in heavy rainfall between November and March and a relatively drier Southwest monsoon (SW) between June and October [201]. Interestingly, the seasonal trough of dengue cases follows the NE. Daily rainfall intensities are higher by a magnitude of 12-25 mm during the wet phase of the NE (i.e. November-January) compared to other months (see **Figure 3.2A**). In addition, the dry phase of the NE places February as the driest month, where dry periods extend to four days and a total of only eight rainy days (see **Figure 3.2B** and **3.2C**, respectively). **Figure 3.3** shows the duration of storms in a rainy hour in Singapore between 1983-2011. Usually rainfall occurs afternoon in response to the diurnal heating of the land by the sun. A rainstorm in November - January extends on average 31 minutes compared to 19 minutes in February and 23 minutes in June-October.

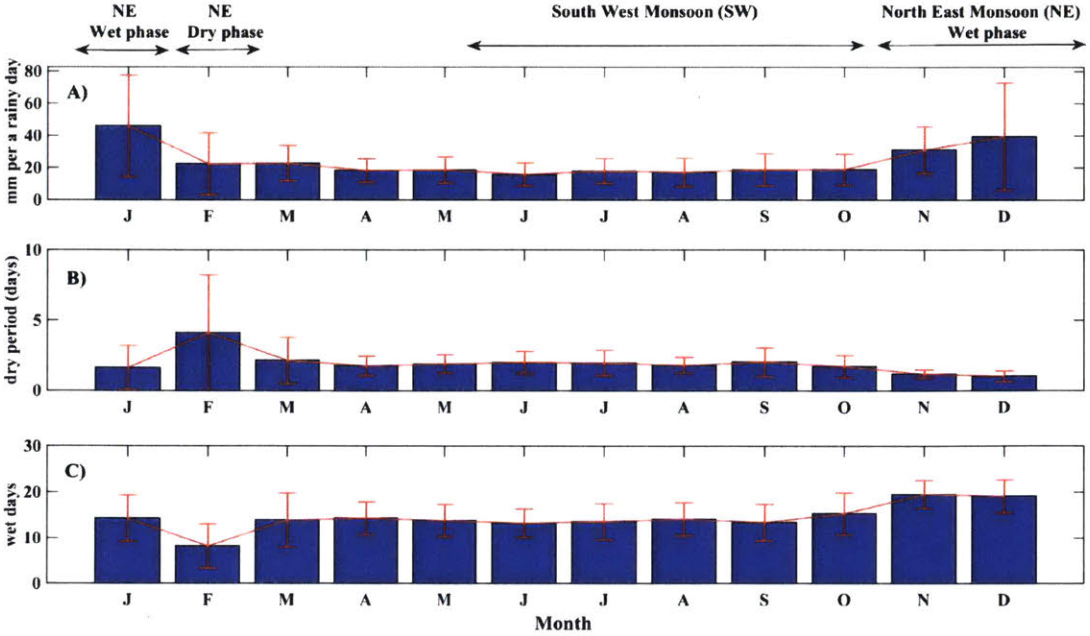


Figure 3.2 A) Mean daily intensity of rainfall in Singapore per a rainy day (1983-2011). The intensity is calculated by dividing amount of rainstorms by rainy days of a month. B) Mean duration of a dry period (i.e. sum of hourly stretches without rainfall) without a rainstorm. C) Monthly average numbers of rainy days; notice February is the driest month with only eight rainy days. Arrows indicate the Northeast (i.e. the wet and dry phases, NE1 and NE2, respectively) and Southwest (SW) monsoons. Data source: Changi station – National Environmental Agency (NEA).

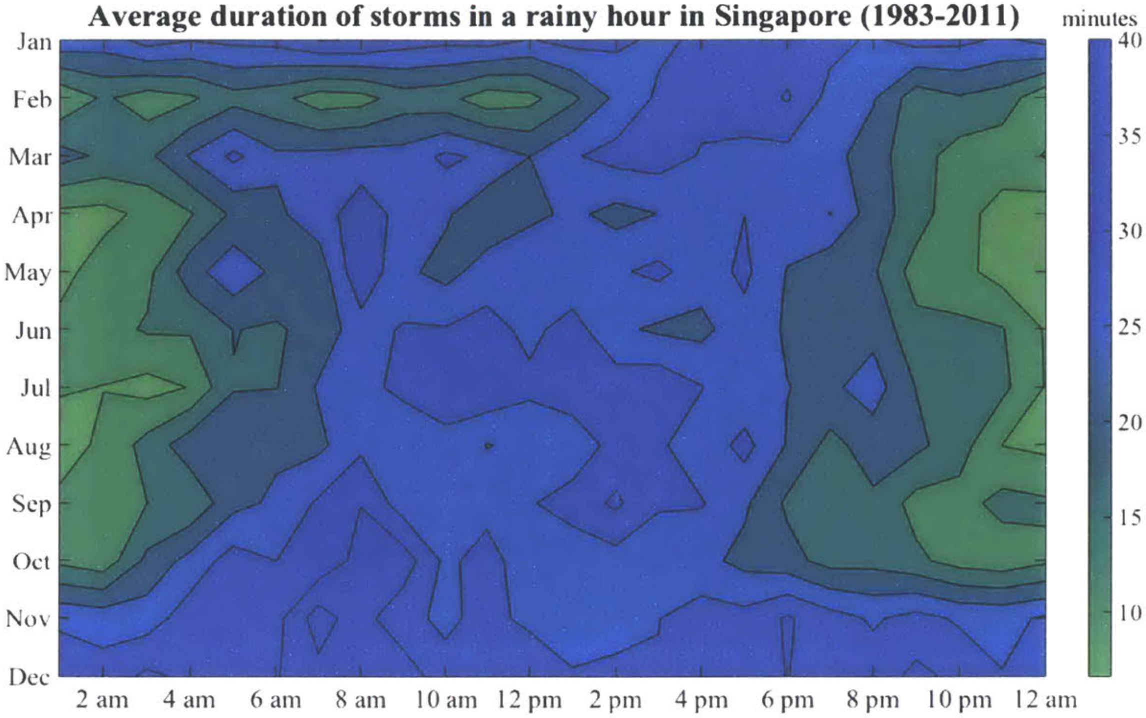


Figure 3.3 Duration of storms in a rainy hour in Singapore (1983-2011). Data source: Changi station – National Environmental Agency (NEA).

On the other hand, monthly temperature in Singapore ranges 26-28 °C. The seasonal difference in hourly temperature in Singapore between 1983 and 2011 did not exceed 2 °C (see **Figure 3.4**). The mean daily temperature of the hottest and coolest months, May and December, are 28.4 °C and 26.5 °C, respectively. Although, the daily temperature range (DTR) is 4-6 °C, the seasonal difference of DTR is small. The probability distribution of DTR during this period is shown in **Figure 3.5**.

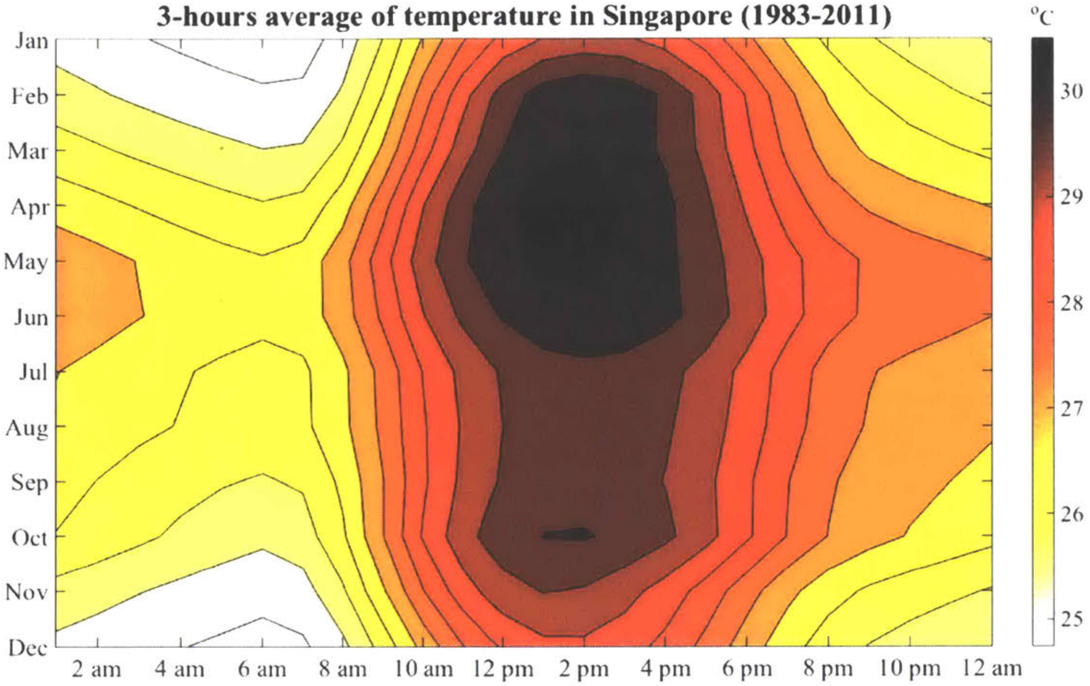


Figure 3.4 Variation of the 3-hours average of temperature in Singapore (1983-2011). Data source: Changi station– National Environment Agency of Singapore (NEA).

Past studies have shown statistical relationships between dengue and climate in Singapore. Heng and others showed a rise in weekly temperature 8-20 weeks in advance preceded an increase in dengue incidence in Singapore. This lag time was found to be 18 weeks during the major outbreak of 2005 [177]. Researchers also used weekly mean temperature and cumulative rainfall to identify a 16-week period as the optimum to forecast dengue outbreaks in Singapore [132]. In a recent work, absolute humidity showed strong predictive value for dengue incidence [133].

Here, we provide a mechanistic basis to explain the connection between dengue and rainfall in Singapore. We show that the NE is likely involved in a strong seasonal reduction of outdoor breeding of the dengue mosquito through a sequence of flushing and drying events.

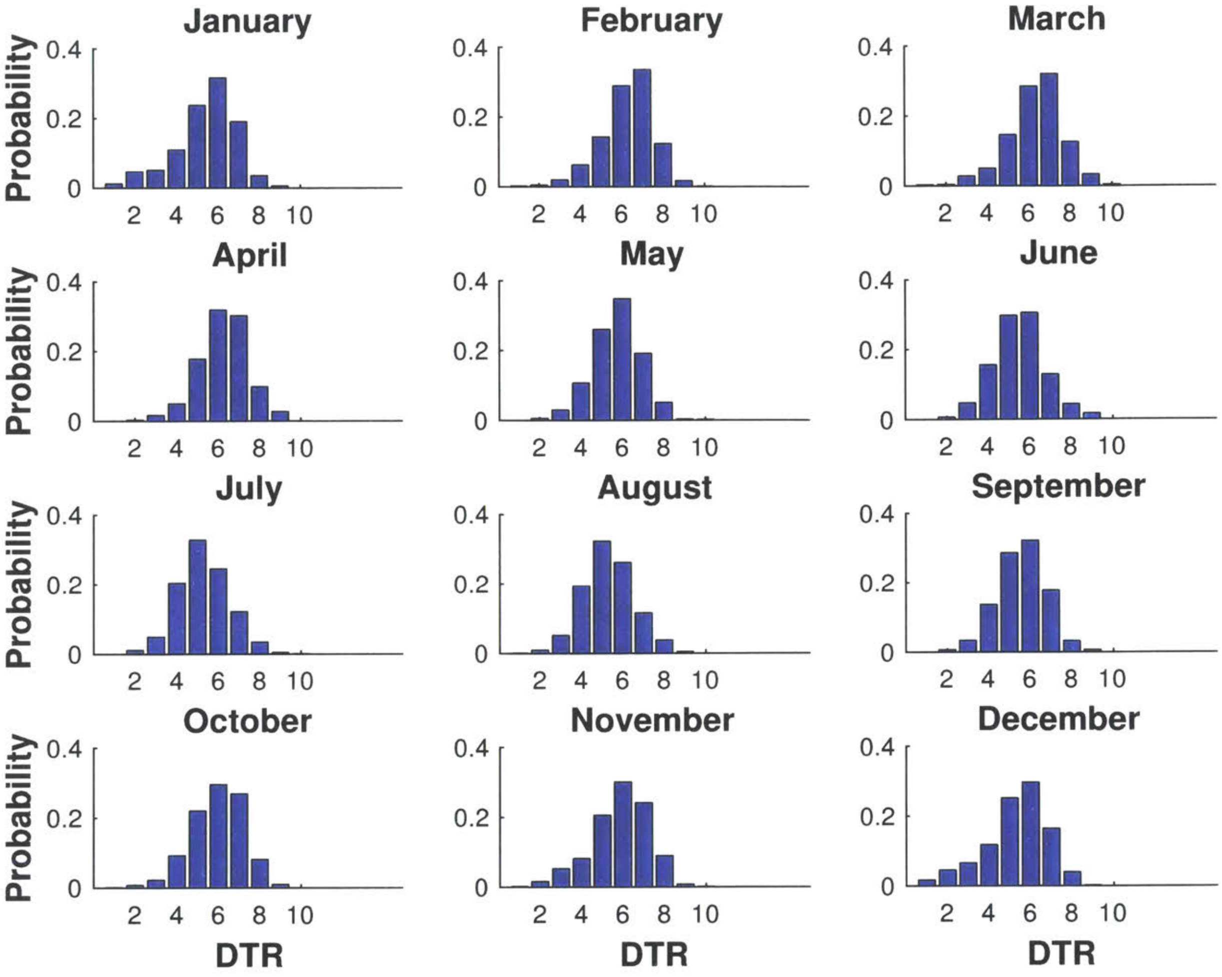


Figure 3.5 Probability distribution of the diurnal temperature range (DTR) in Singapore (1983-2011). Data source: Changi station– National Environment Agency of Singapore (NEA).

3.2 Methods

3.2.1 Hypothesis

A preliminary entomological survey was carried out in July 2013 in Singapore. Three neighborhoods were inspected for outdoor breeding of the dengue vector: Geylang (1.320° N, 103.891° E), Lorong Limau (1.323° N, 103.855° E) and Caldecott (1.337° N, 103.839°E). Accordingly, we found that roadside drains in back alleys are the main outdoor breeding habitats of *Ae. aegypti*. Breeding was also encountered in discarded receptacles indoors. While *Ae. albopictus* was identified in various outdoor discarded receptacles (but in association with the canopy), *Culex spp.* was mainly found in large drains on the main lanes and roads.

Based on the main observation of the preliminary survey (i.e., that dengue vector *Ae. aegypti* breeds in drains) combined with the above epidemiological and meteorological findings (see **Figure 3.1** and **Figure 3.2**), we developed a hypothesis to explain the connection between rainfall and outdoor breeding of the dengue vector in Singapore. During dry periods, only desiccation-resistant eggs can survive in drains and similar outdoor breeding habitats. We hypothesize that while a monsoon results in breeding of *Ae. aegypti* in drains, a monsoon with intense rainstorms can cause flushing of aquatic stages (see **Figure 3.6**). In order to test this hypothesis, we selected Geylang as study area.

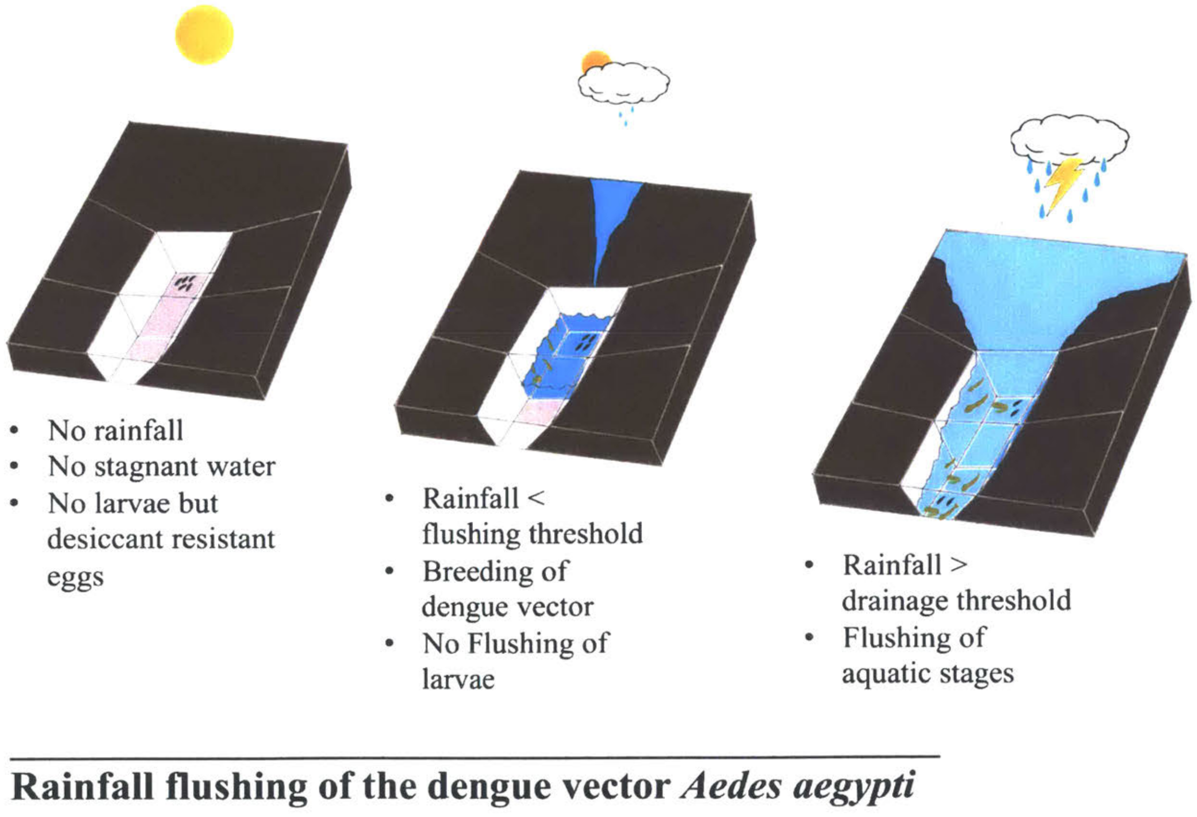
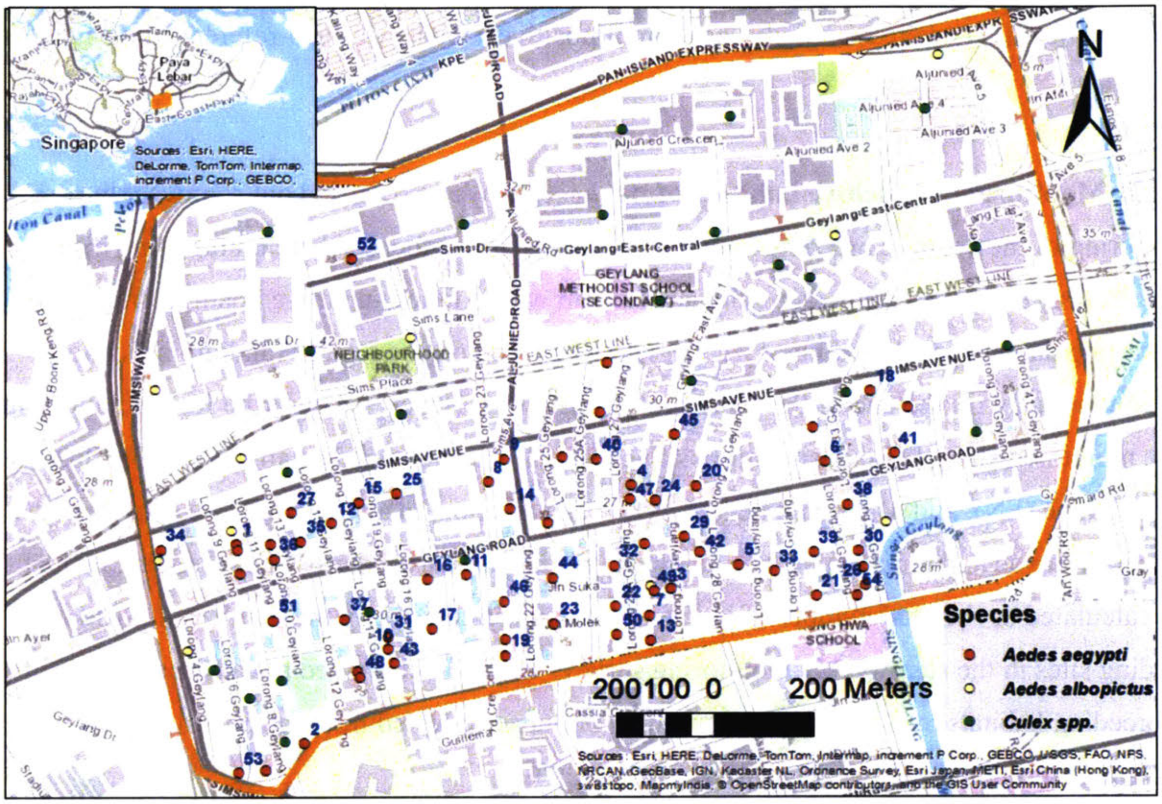


Figure 3.6 A descriptive sketch for the rainfall flushing mechanism shows how intense rainstorms during the monsoon results in washing breeding of dengue vector from stagnant drains.

3.2.2 Study area. Geylang neighborhood, east of the Singapore River, is a highly-urbanized neighborhood that has an area of about 3 km^2. Although Geylang has an estimate of 32,000 resident population, non-residents is believed to be larger because of the cheap housing that attracts foreign laborers. National Environmental Agency (NEA) recognizes Geylang as a hyperendemic area where a continuous reporting of dengue cases and disease transmission happens (see **Figure 3.7**).



Sources: Esri, HERE, DeLorme, TomTom, Intermap, increment P Corp., GEBCO.

Sources: Esri, HERE, DeLorme, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, Mapmyindia, © OpenStreetMap contributors, and the GIS User Community

Figure 3.7 The study area: Geylang neighborhood, Singapore. The figure shows locations of positive breeding sites for *Aedes aegypti, Ae albopictus* and *Culex spp*. Breeding drains of *Ae. aegypti* are given serial numbers that also used in Figure 3.9.

3.2.3 Entomological surveys

Entomological surveys were continuous between August 2014 and August 2015 except for two weeks between February 21st and March 10th. The surveys included two tasks of inspections:

3.2.3.1 **Semiweekly-random inspections.** We carried out a random aquatic survey twice a week. The inspector was equipped with torchlight, sieves, large-mouth pipettes, a white enamel pan and small shell vials. In each survey, the inspector examined all outdoor natural/artificial habitats in the selected blocks for aquatic stages. Samples of pupae and larvae were pipetted in labeled vials with 70% ethanol, and transferred to the laboratory for taxonomy. In addition, a subsample of aquatic specimens was held alive in a netted cup until adult emergence to confirm identification. Taxonomic keys [202–204] were used to identify the preserved larvae and emerged adults. For a positive breeding habitat of mosquitoes, type of habitat and presence of other aquatic insects were recorded. Location of positive habitats was geo-referenced using GPS tools.

3.2.3.2 **Monitoring of positive habitats of *Ae. aegypti*.** We also carried out semiweekly monitoring of the positive breeding habitats. In particular, we focused here on breeding history of *Aedes aegypti* in the drains. In particular, the aim was to follow-up these positive drains since the starting date when a breeding of *Ae. aegypti* was found (in the regular random inspections) and continuously till the end of the survey in August 2015. Hence, we describe four situations in these monitored sites: 1) Stagnant and Positive (SP), 2) Stagnant and Negative (SN), 3) Flushed and Negative (FN), and 4) dry and negative (DN). In addition, in a case of SP, we estimated the number of larvae and pupae in the site using larval dippers. Larval density per breeding habitat was calculated as the total number of larvae of *Ae. aegypti* divided by the number of positive breeding sites in the semiweekly monitoring survey. We also determined pupal-productivity of the breeding habitats by summation of numbers of pupae collected from the positive drains and non-drains during the semiweekly survey.

3.2.4 Microclimatic data on rainfall and flushing

A set of weather HOBO loggers was placed in Geylang between August 2014 and August 2015 to record hourly microclimatic conditions. These included: a rain gauge to record amounts of rainstorms, and water level logger. The siphon rain gauge tipping bucket (TR-525S) was calibrated in the laboratory according to the manufacturer. Next, the rain gauge was placed on the roof of a 7-storey building to prevent obstruction.

To characterize flushing events, we placed HOBO U20L logger in a back alley drain in Geylang. The water level logger records water temperature and absolute pressure every 10 minutes. Software uses absolute pressure, reference water level and density to calculate the water level.

The accuracy of the device is 0.1%. In addition, daily rainfall was obtained from the closest NEA weather station for the same period.

Logging of the rain gauge was intermittent because of periodic sensor errors. However, readings from nine months (between 9/15-9/25/2014, 12/07/2014-3/26/2015 and 5/28-8/26/2015) are retrieved. In order to overcome this discrepancy, we compensated missing data by their corresponding rainfall from the closest station Tanjong Katong. A regression analysis has showed that data of the weather station could strongly predict data of the rain gauge (R^2= 0.94).

3.3 Results

3.3.1 Main outdoors breeding habitats

Out of 6824 inspected sites, 3624 (53%) were wet habitats. Most of outdoor breeding habitats were open and closed drains, 45% and 40%, respectively. The remained of inspections (15%) were non-drains including canvas sheets, pails, plastic bags, and flowerpots. Interestingly, positive habitats contained *Ae. aegypti* (n=67), followed by *Culex spp.* (n=24) and *Ae. albopictus* (n=11)- **Table 3.1**.

Table 3.1 Total number of inspected and positive habitats in Geylang August 2014 - August 2015.

	Inspected habitats		**Positive habitats**		
Type of habitat	**Dry**	**Wet**	***Ae. aegypti***	***Ae. albopictus***	***Culex spp.***
Open drains	1267	1470	22	2	13
	(46.3%)	(53.7%)	(1.4%)	(0.14%)	(0.88%)
Closed drains	1463	1618	31	0	8
	(47.5%)	(52.5%)	(1.9%)		(0.49%)
Non-drains	470	536	14	9	3
	(46.7%)	(53.3%)	(2.6%)	(1.68%)	(0.56%)
Total	3200	3624	67	11	24
	(46.9%)	(53.1%)	(1.8%)	(0.3%)	(0.6%)

Note: A percentage in a breeding habitat is in reference to the corresponding number of wet habitats.

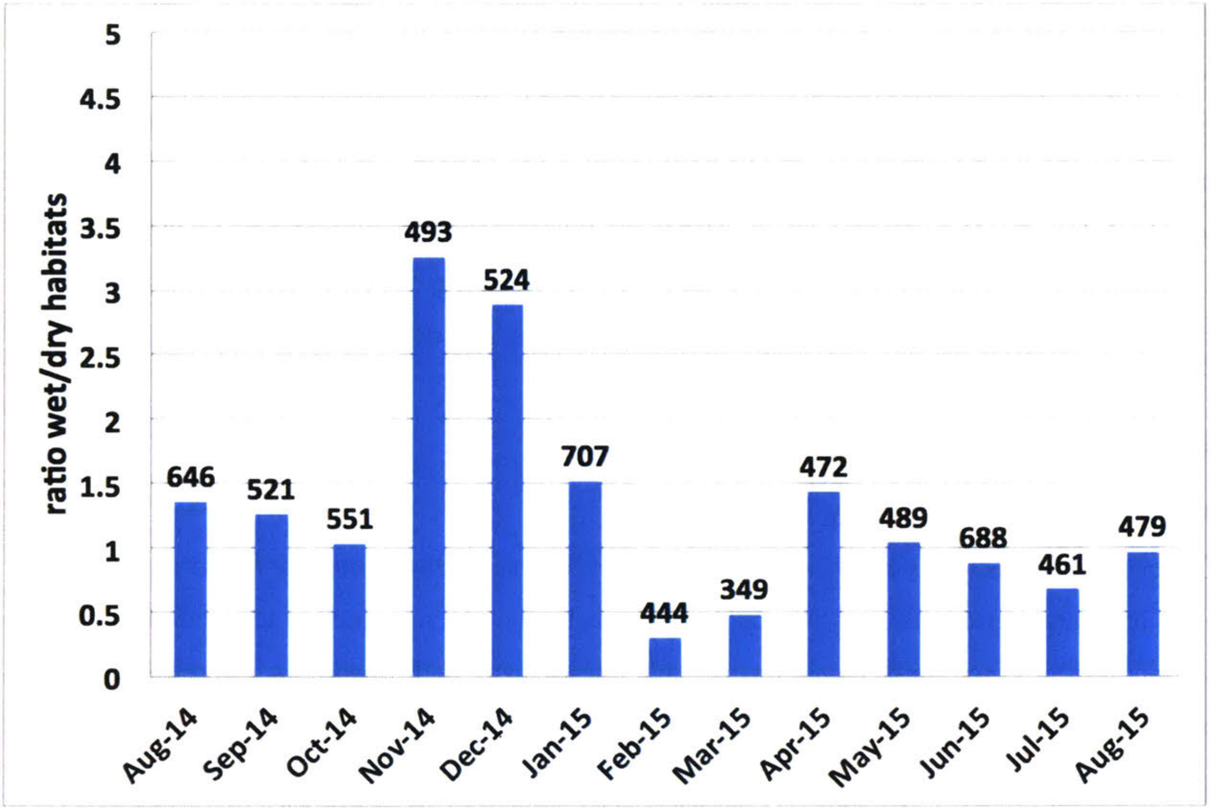


Figure 3.8 Monthly ratio of wet/dry habitats in Geylang (August 2014 - August 2015). Total numbers of inspected habitats are shown above bars. Arrows indicate periods of the wet and dry phases of the Northeast monsoon (NE1 and NE2, respectively) and Southwest monsoon (SW). Note: no inspections were carried out between 2/21/15 and 3/10/15.

Breeding of *Ae. aegypti* was mainly in storm drains (53/67). In addition, we found aquatic stages of non-mosquito species belonging to the families: *Chironomidae, Psychodidae* and *Viviparidae*. We also encountered rodents in the drains (Family: *Muridae*).

The monthly ratio of wet to dry habitats inspected in Geylang is shown in **Figure 3.8**. Accordingly, the number of wet habitats found during the wet phase of the NE is more than twice of the dry ones. On the other hand, wet habitats during the dry phase of the NE are less than half of the dry ones.

3.3.2 Flushing, drying and return of outdoor breeding

Figure 3.9 shows that breeding drains were mainly flushed in the wet phase of the NE. Moreover, the following dry phase of the NE had resulted in drying of 95.3% (41/43) of the flushed drains. Monitoring resumed in March showed that most of the previously positive drains were still dry 82.9% (34/41) while the wet ones (7/41) were negative. A return in outdoor breeding was shown after the onset of the SW. Hence, we found 11 positive drains for *Ae. aegypti* in June-August 2015. All these drains except one were within 200 meters from the previously positive ones prior to the NE period.

Locations of the breeding drains are in **Figure 3.7**. There is a clustering for breeding drains of *Ae. aegypti* in the southern part of Geylang.

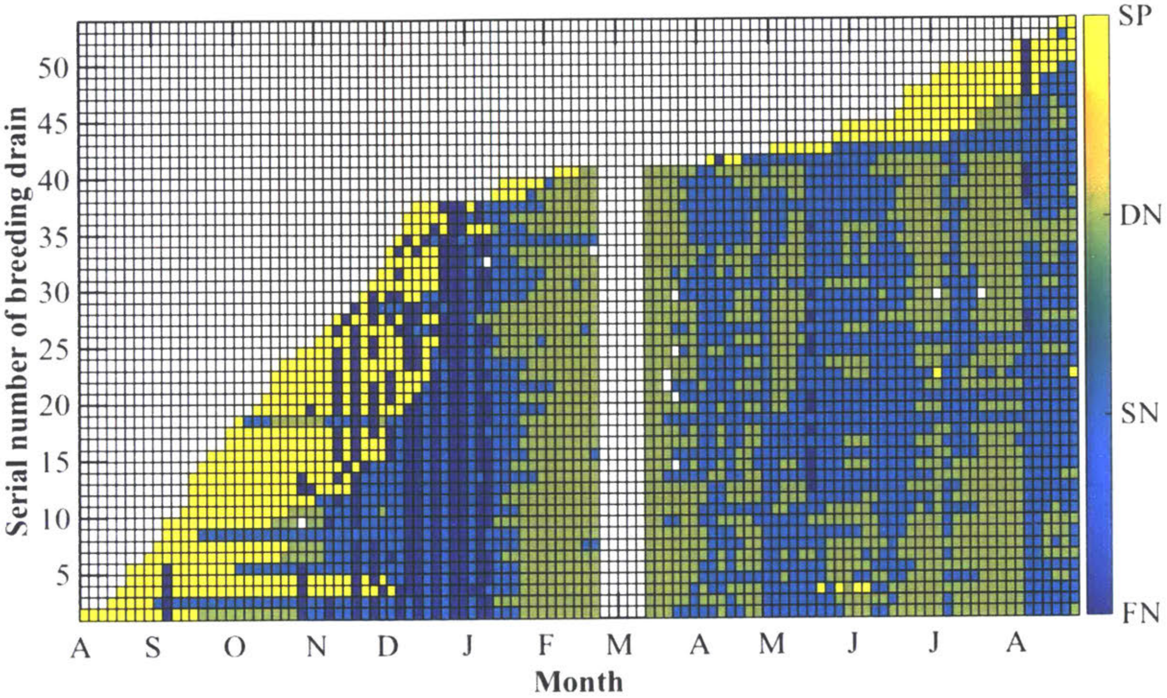


Figure 3.9 Timeline of the breeding drains of *Aedes aegypti* in Geylang, Singapore: August 2014- August 2015. (SP: stagnant and positive, DN: Dry and Negative, SN: Stagnant and Negative, FN: Flushed and Negative). Grids along the x-axis represent the twice-weekly follow-ups. White grids indicate no inspections were carried out in these drains (two weeks between 2/21/2015 and 3/10/2015). Locations of the breeding drains are shown in Figure 3.7.

3.3.3 Effects of rainfall on aquatic stages of *Ae. aegypti*

Figure 3.10A shows that 31.2% (19/61) of intense rainstorms (i.e. >10 mm) had occurred during the wet phase of the NE (i.e. November – December 2015). In addition, **Figure 3.10B** shows a similar pattern of increases in water level of the monitored drain.

The monsoonal pattern has also influenced larval density per breeding habitat as shown in **Figure 3.10C**. These larval densities were higher during the SW periods (averages were 38.1 and

27.7 in 2014 and 2015, respectively) compared to the NE period (average= 16.7). Similarly, the wetter monsoon had affected pupal-productivity of breeding drains for *Ae. aegypti* (see **Figure 3.10D**). Pupae had decreased after the onset of the NE from a total of 901 to 284 (i.e. 68.5% reduction). Although few breeding drains were encountered after the onset of the SW in 2015, the total of pupae was double that of the NE period (N=505).

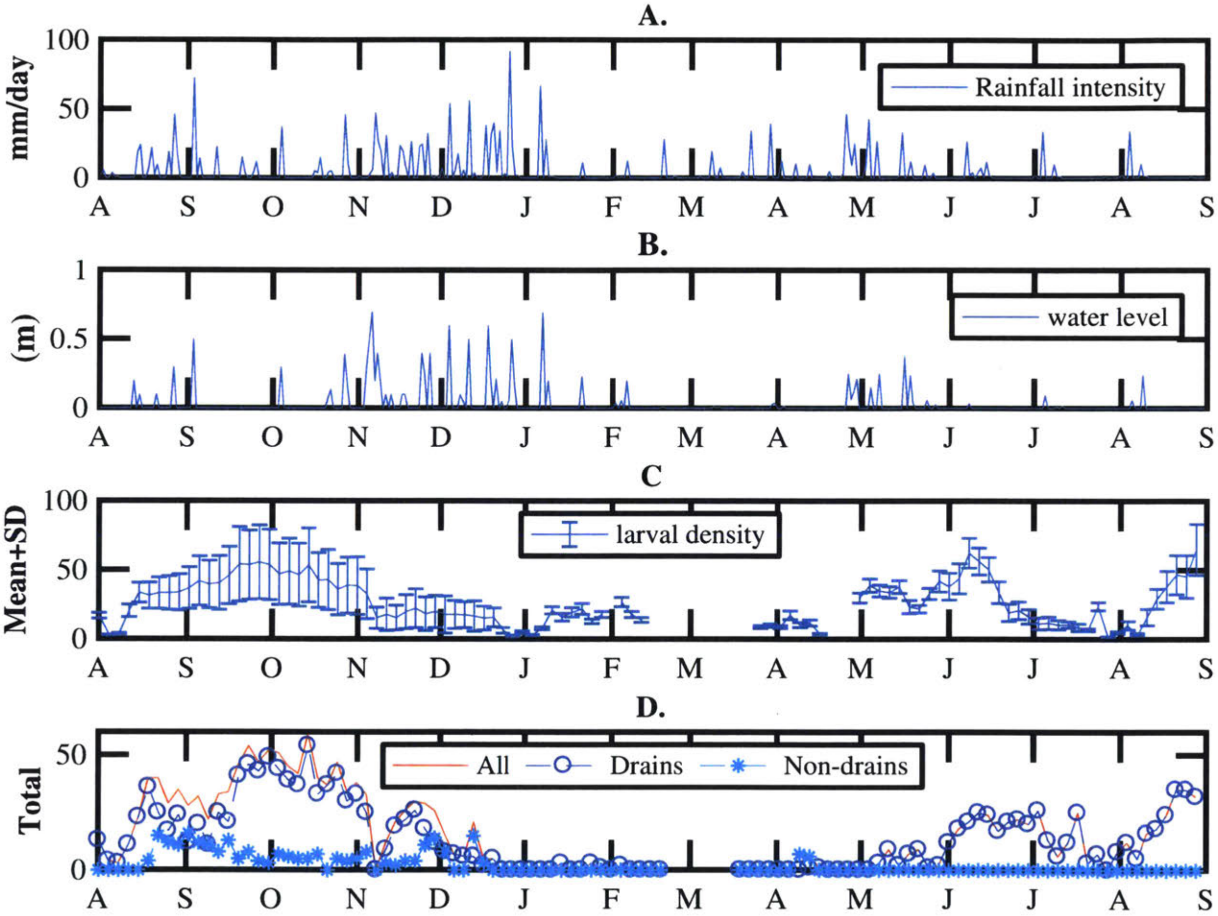


Figure 3.10 A. Intensity of daily rainstorms in Geylang (August 2014 - August 2015). B. Water level in a drain in Geylang (August 2014 - August 2015). C. larval density of *Aedes aegypti* per an outdoor breeding habitat in Geylang per semiweekly survey. D. Pupal-productivity of drains and non-drains for *Ae. aegypti* per semiweekly survey. Note: no monitoring was carried out between 2/21/2015 and 3/10/2015.

3.4 Discussion

In Singapore, dengue cases peak during the third quarter of the year while they dip in the first one. For this study, we show rainfall may influence dengue via a sequence of two processes acting on the outdoor population of vector mosquito: 1) intense rainstorms that flush out breeding drains of the main vector, and 2) acute drying that follows and impedes returning of *Aedes aegypti* breeding. While flushing happens when Singapore is under the wet phase of the NE, drying occurs when the monsoon which passes Singapore converges into Inter-tropical convergence zone (ITCZ) over Java [201].

Fluctuations of DTR around monthly mean temperatures 26.5- 28.4 °C are small in Singapore (i.e., < 1.1 °C). Lambrechts and others suggested that small DTR around a mean temperature 26 °C could induce the high season of DENV [196]. Hence, we argue that DTR effect on dengue seasonality in Singapore is modest.

We showed that the ratio of wet to dry habitats discovered during our random survey is larger in the wet phase of the NE than in the late dry one. However, wetness is not a sufficient condition for a mosquito to lay its eggs at a specific location. In fact, mosquitoes lay their eggs in specific breeding habitats that minimize mortality risk (e.g., predation or competition) and maximize nutritional benefits for their offspring [205]. One possible explanation for finding new breeding habitats during the wet phase of the NE is that they were flushed from indoor breeding sources. In addition, they can result from hatching of dormant eggs in the drains.

Likewise, we showed the effect of the NE on pupal-productivity and larvae of *Ae aegypti*. The rainfall and water level loggers verified these effects. However, the effect of intense rainstorms could be substantial on larval food; hence, on size of pupae and emerged adults. On the other hand, sampling of pupae from outdoor breeding habitat can be utilized in dengue surveillance in Singapore. Because sampling adults of *Ae. aegypti* is difficult, several studies have shown that pupal indices are useful in dengue surveillance [206–208].

Several factors may explain why few breeding sites found between June and August 2015. First, an intensive larviciding program for the drains is recently introduced in Geylang (observed by the investigators). Second, there was unusual drying in the drains particularly in June-July 2015 that resulted from El Niño. Indeed, there is ongoing strong El Niño in 2015 [209]. The impact of El Niño episodes on dengue in Singapore was previously recorded in Singapore in May-2002-March 2003, June 2004- Feb2005 and Aug 2006-Jan 2007 by Hii and others [178].

We have no information whether breeding in drains had resulted from oviposition at these sites or flushing of indoor or upstream sites. In fact, there were no inspections for indoors habitats due to ethical and legal considerations. For example, rain gutters, which are considered by National Environmental Agency of Singapore (NEA) as a key-breeding habitat for *Ae. aegypti*, could be the source that inoculated the breeding in drains. There is a need to assess the relative productivity of storm drains – in terms of *Ae. aegypti* pupae -- to that of other indoor containers. A further work is also needed to determine a flushing threshold that could result in reduction of breeding in drains. This threshold could be an attribute to the drainage network in a neighborhood.

To optimize dengue vector control in Singapore and similar wet tropical areas, we suggest seasonal strategies - as in Table 3.2. Targets and measures of vector control should consider the difference in outdoor abundance of the vector between pre-seasonal and seasonal periods of the year. A pre-seasonal control strategy should focus on elimination of indoor breeding habitats particularly during the monsoonal dry period. We recommend treatment of breeding drains and roof gutters by long lasting persistence larvicide before the rain arrives and the mosquito flourishes in outdoor habitats. This pre-seasonal strategy can be effective to reduce the disease risk before onset of the high season. Removal of discarded receptacles should be continued around the year. We also propose a focal space spraying -- using an adult insecticide -- when an outdoor breeding habitat encountered to minimize the dispersal of emerged adults within the flight range of *Ae. aegypti*.

There is a growing interest in the health consequences of climate change. While projections of the climate change show an increasing trend in temperature under "the business-as-usual" scenario, the effects are less understood on rainfall distribution and patterns. In equatorial regions, a non-stationary increase in rainfall is expected to follow the seasonal displacement of ITCZ [210]. In general, if climate change enhances the wet conditions around December or enhance the dry conditions around February, then that may impact the seasonality of Dengue in this region. The flushing-drying mechanism may play a role in shaping the impact of climate change on dengue and other related arboviral diseases.

3.5 Summary and Conclusion

Increasing concerns about the arboviral diseases transmitted by *Aedes aegypti* mosquito -- which include dengue, Chikungunya, yellow fever and Zika virus -- demand a better understanding for the breeding ecology of this mosquito. A common observation on mosquito-borne diseases in endemic countries is that they peak following the onset of the rainy season and increase in breeding habitats, while they trough in the dry season. On the contrary, in Singapore, which has no pronounced dry season, dengue cases decrease after a very wet monsoon. Here, we show that this monsoon is likely involved in a strong seasonal reduction of outdoor breeding of the dengue mosquito through a sequence of flushing and drying events. A flushing-drying mechanism may affect the seasonal abundance of the vector in similar eco-epidemiological settings.

In conclusion, rainfall has a mechanistic role in shaping seasonal abundance of the dengue vector *Ae. aegypti* in Singapore. This effect happens through a monsoonal-driven sequence of flushing and drying in outdoor breeding habitats. In light of global urbanization, urban drainage systems are expanding in well-structured urban setting like Singapore. Hence, vector control interventions can be very effective before the dengue season in such eco-epidemiological settings.

Table 3.2 Optimization of dengue vector control strategies according to the monsoon period in Singapore.

Strategy	**Pre-seasonal (or Low dengue season)**		**Seasonal (high dengue season)**
Targets (measures)	• Eliminate Indoor breeding (SR/LO/GT) • Adult control where indoor breeding found (IRS/SS). • Monitor outdoor habitats	• Eliminate Indoor breeding (SR/LO/GT) • A preseason treatment of dry drains/roof gutters (CL/BC) • Outdoor discarded receptacles (SR)	• Eliminate Outdoor breeding (SR/CL/BC) • Eliminate Indoor breeding (SR/LO/GT) • Adult control where outdoor breeding found (SS). • Monitor indoor/outdoor habitats
Hydrologic conditions	Flushing conditions (heavy rainstorms)	Drying conditions	Stagnation conditions
Monsoon	Early Northeast (NE) monsoon	Late NE monsoon	Southwest (SW) monsoon

SR= Source Reduction; LO= Lethal Ovitrap; GT= GraviTrap; IRS= Indoor Residual Spraying; SS= Space Spraying; CL= Chemical Larviciding; BC= Biological Control

Chapter 4: Urban Housing and Dengue Distribution in Singapore

Dengue is the fastest emerging human arboviral disease in the world. Vector control surveys in Singapore show a difference of indoor breeding between accommodation types. In this study, we classified residential areas into low-rise and high-rise housing and investigated the influence of the two patterns of urban housing on the distribution of dengue incidence and outdoor breeding at neighborhood and country scales.

In Geylang area, between August 2014 - August 2015, dengue incidence was higher in a subarea of low-rise housing compared to high-rise one, averaging 26.7 (Standard Error, SE = 4.83) versus 2.43 (SE = 0.67) per 1000. We found that outdoor breeding drains of *Aedes aegypti* have clustered in the low-rise housing subarea within the Geylang neighborhood. The pupal density per population was higher in the low-rise blocks versus high-rise ones, 246 (SE = 69.08) and 35.4 (SE = 25.49) per 1000, respectively. The density of urban drainage network in the low-rise blocks is double that in the high-rise ones, averaging 0.05 (SE = 0.0032) versus 0.025 (SE = 0.00245) per meter.

Further, a holistic analysis at a country-scale has confirmed the role of urban housing pattern in shaping dengue distribution in Singapore. We found that dengue incidence (2013-2015) is proportional to the fractions of the area (or population) of low-rise housing. The drainage density in low-rise housing neighborhoods is four times the corresponding estimate in high-rise building zones, 2.59 and 0.68 per meter, respectively.

Our findings suggest that the strategic plan of affording public housing in agglomerations of high-rise buildings would rather have a positive impact on dengue control if this urban housing develops at the expense of the built-up area in low-rise building. Moreover, the risk of dengue in low-rise housing is a result of conducive factors for indoor and outdoor breeding of the vector at this type of accommodation. We suggest that a "man-breeding" contact (rather than "man-vector") is responsible for the difference in the disease between urban housing types. The control strategy of dengue in Singapore should focus on disrupting this contact mechanism in low-rise housing.

4.1 Introduction

Dengue is the fastest reemerging human arboviral disease in the world. The disease incidence has increased 30-fold during the last 50 years, placing half of the global population at risk, mainly in its endemic territories in Asia, Latin America and some foci in Africa [15,34]. The economic burden of dengue is enormous. About 1300 disability-adjusted life years (DALYs) per a million people is attributed to dengue in endemic regions [19]. Despite high expectations for dengue vaccine trials [38], or field releases of a genetically modified mosquito [41,211], dengue epidemics are evolving at a higher rate. Indeed, various climatic (e.g., temperature and rainfall which affect the vector competence) and non-climatic factors (e.g., herd immunity, dengue serotype, and genotype) interact on regional and local scales and seem to be responsible for this increase in epidemics [30].

Clustering of Dengue --in space and time-- is a common observation in endemic areas [103,106,212], evident in the aggregation of dengue cases within a close distance ranging from 100 to 800 meters [49]. Some of this clustering is attributed to the short flight dispersal (i.e. usually < 100 meters) of the primary vector *Ae. aegypti* [139], affecting the man-vector contact.

In under-planned urban centers, inadequate water supply, poor sanitation, and disuse of non-biodegradable containers lead to *Aedes* breeding in storage containers inside houses [49,213]. On the other hand, in well-planned urban areas, *Aedes aegypti* breeds in various indoor habitats such as plant pots, bamboo pole holders and tanks of air-conditioners [30,198,214]. Further, there is growing evidence for vector adaptation to outdoor breeding habitats [189,190] or seasonal shifts between rain-fed and man-fed containers in urban areas [215]. The outdoor breeding habitats in urban settings include roof gutters, discarded receptacles, sewage, and storm drainage networks [190,216–218]. The outdoor breeding of *Ae. aegypti* can occur not only near housing, but also in public venues such as schools, restaurants, and workplaces [81,219]. The importance of the outdoor breeding habitats in busy urban epicenters is that they could work as outside transmission pockets affecting both residential and visiting population.

Dengue is an endemic disease in Singapore. Despite intensive control efforts to keep the population at a low risk of dengue, incidence has steadily increased since the 1990s. Three major dengue outbreaks struck Singapore in 2004-2005, 2007 and recently in 2013-2014 [52,53,178]. Both switches in dominant DENV strains and introductions of new virus genotypes have been considered as triggers for these outbreaks [175].

Dengue mosquito species *Ae. aegypti* and *Ae. albopictus* are sympatric in Singapore. However, *Ae. aegypti*, which is the primary vector, predominates in urban areas and shows a better vectorial competence for dengue transmission in the city [167,220]. Also, dengue virus was more detected in specimens of *Ae. aegypti* than *Ae. albopictus* in Singapore [221]. One of the key control strategies is to scale-up community participation in removing/destroying indoor breeding habitats of *Ae. aegypti*. This policy has resulted in a very low (< 1%) House Index (HI), which is the percent of domiciles with indoor breeding containers for *Aedes* [222].

The relationship between urban housing and dengue in Singapore was first documented in the 1960s [223]. At that time, slum houses showed the highest HI for *Ae. aegypti* while flats at multistory buildings had the lowest one. During the last 50 years, a progressive urban planning has changed the face of Singapore and slum houses had vanished. The strategic plan of Urban Redevelopment Authority (URA) of Singapore focuses on affording public housing in high-rise buildings as a solution for the growing population and scarcity of the land. Annual reports of the Ministry of Health (MoH) show a difference in dengue cases and incidence between three types of accommodations: HDB flats, compound houses and condominiums [169]. However, a further research is needed to investigate the impact of the urban planning and urban housing on magnitude and distribution of dengue in the country.

In this study, we classified the urban housing in Singapore by the height of buildings into low-rise (i.e., <= 3 floors) and high-rise housing. The high-rise buildings consist mainly of public housing owned by Housing and Development Board (HDB) and private condominiums (condos) for middle and upper classes. In contrast, the low-rise housing includes various types of landed private properties such as bungalows, shophouses, and terrace houses. **Figure 4.1** shows a difference in HI between low-rise and high-rise housing during the period 2000-2015. While low-rise housing accommodates only 6% of households in Singapore, the odds of an indoor breeding of *Aedes* in this type of accommodation is 3.1 times that in a high-rise one (95% C.I.=

2.9-3.3). Also, these vector control surveys --carried out by the National Environmental Agency (NEA)-- show that domestic containers top the indoor breeding habitats for *Ae. aegypti* (NEA, unpublished report, 2015).

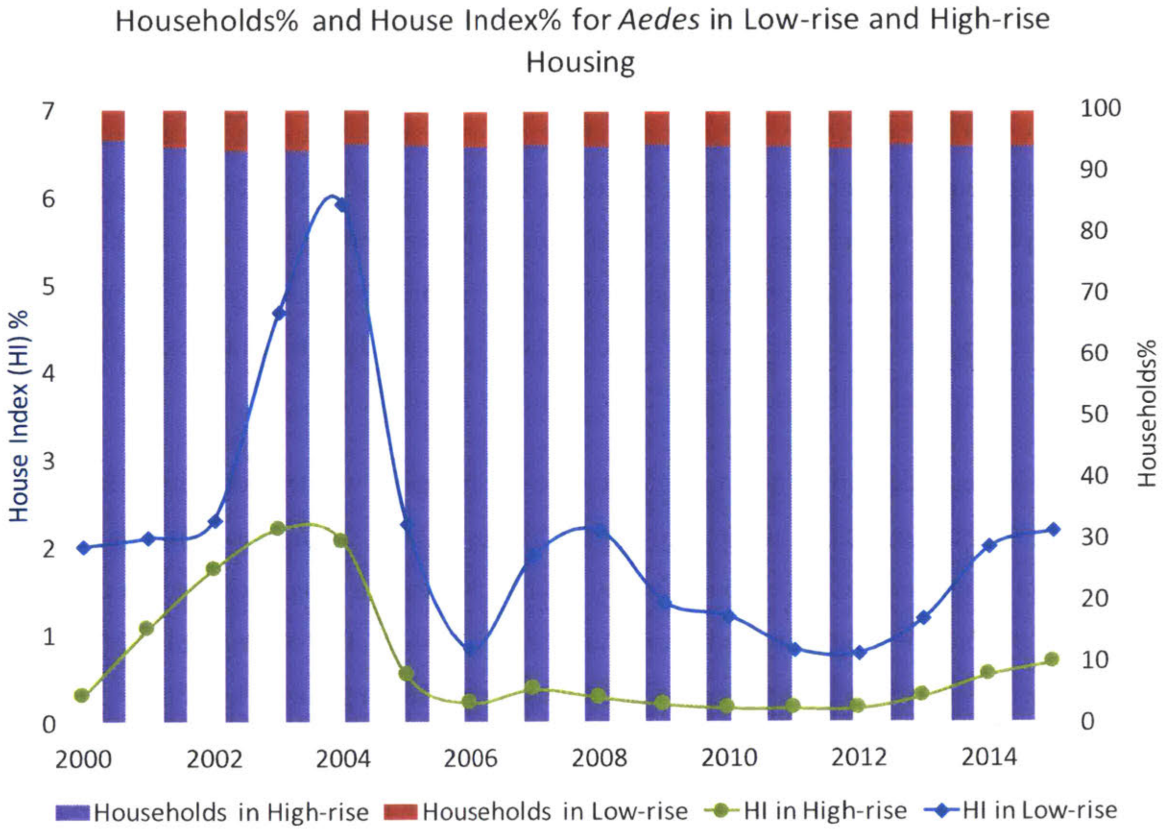


Figure 4.1 Households% and House Index% (HI) of *Aedes* breeding in Low-rise and High-rise housing, Singapore (2000-2015). [Data Source: MOH, NEA and DOS (Singapore)].

This study explores the influence of the pattern of urban housing on dengue distribution at a neighborhood and country scales. In a recent survey in Geylang between August 2014 and August 2015, we found that *Aedes aegypti* breeds mainly in clogged drains close to the housing [14]. These outdoor breeding habitats are clustered in the low-rise housing part of the Geylang. We document here a clustering of dengue cases in the same low-rise subarea. The low-rise portion has a denser urban drainage network. The outdoor abundance of *Ae. aegypti* --regarding pupal density and trapped adults-- shows a similar spatial difference. A further spatial analysis for the whole country revealed that dengue incidence is proportional to the fraction of low-rise housing. Likewise, the drainage network is denser in low-rise residential areas than high-rise ones. The strategic urban plan of Singapore to afford public housing in urban agglomerations of high-rise buildings does not increase the risk of dengue in Singapore. We also suggest that a man-breeding contact (rather than man-vector contact) is responsible for the difference in dengue incidence.

4.2 Methods

4.2.1 Neighborhood.

Geylang [1.3206° N, 103.8869° E] is one of the historic areas of the city. It is a hyperendemic area with a continuous circulation of the four serotypes [223,224]. For this study, we selected an area that extends from Pan Island Express (PIE) to Guillemard Road (from North to South), and from Paya Lebar Road to Sims Way (from East to West). It has a roughly rectangular shape of 1.9x1.1 km^2. Due to observed differences in the landscape of the built-up area, the study area is divided into two sub-neighborhoods. The East-West Mass Rapid Transit line (EW MRT) roughly separates landed low-rise private houses (mainly shop houses) to the south from high-rise housing to the north (see **Figure 4.2**).

4.2.2 Urban Housing data.

We obtained spatial housing data from Singapore Land Authority (SLA). This dataset includes built-up area by type of utility (i.e. commercial, industry, housing by type of buildings). Also, we used OpenStreetMap data to retrieve locations and length of roads by category (e.g., highways, residential, etc.) in Singapore.

Two fishnets were created using ArcGIS 10.2: 1). A neighborhood fishnet of 10x7 regular grids having 200 square meters each block, and 2) country fishnet of 14 x11 blocks of 1 square kilometer resolution. The fishnet was geographically rotated to fit the study domain. For the country-scale analysis, we calculated the fraction of low-rise housing as the built-up area of low-rise houses divided by the total residential area in that block.

Figure 4.2 A sketch map shows urban drainage network and other urban features in Geylang neighborhood.

4.2.3 Mapping the population distribution

Population census of Singapore in 2014 was obtained online from the Department of Statistics (DOS) of Singapore. This dataset includes population by subzone and type of housing. We projected the population distribution in each grid of the country and neighborhood fishnets. We calculated the fraction of the population living in the low-rise housing by dividing people in this housing type by estimated people in each block.

Anecdotal evidence suggests more non-residents exist in the low-rise housing. For example, because of low rental rates, foreign workers lodge in the shop houses. To test the effect of this assumption on dengue, we projected two population scenarios in the Geylang neighborhood: 1) a fixed population of only residents, and 2) dynamic population including non-residents and visitors.

The population distribution of Geylang is retrieved from the census data for 2014 published online by Department of Statistics -Singapore (DOS): http://www.singstat.gov.sg.

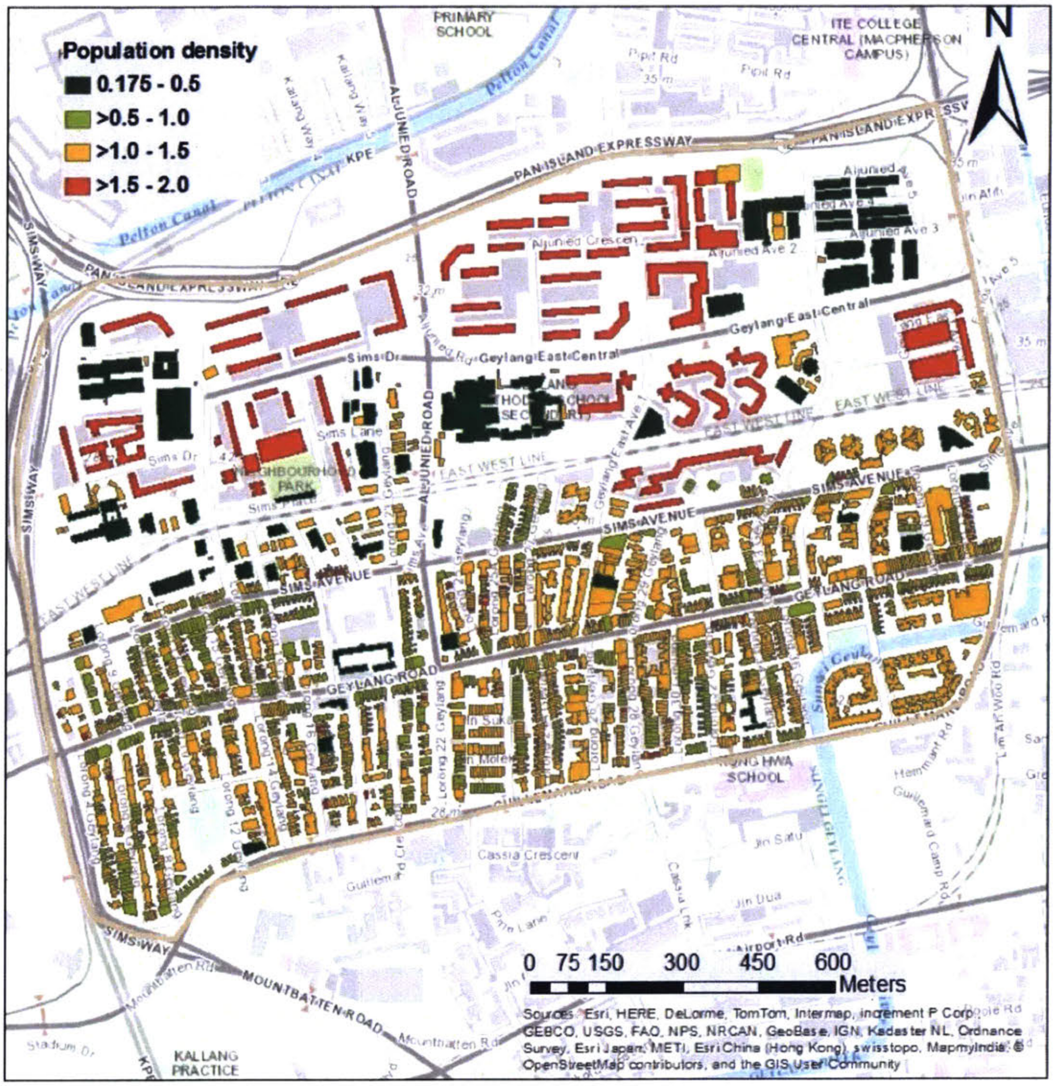
The dataset is organized by planning area, subzone, and type of dwelling (HDBs, condominiums, landed properties and others). However, the census does not go down to the exact resolution of the study area (i.e., pooled within the population of Aljunied subzone). Hence, the Aljunied subzone encompasses also the Dakota HDBs subarea.

We used ArcGIS 10.2 to delineate the footprints of buildings using a cadastral map of Geylang area provided by Singapore Land Authority (SLA). We verified residential ones and number of stories in each building by SLA data 2012, field observations and Google Earth Pro. We assumed that the population density per square meter of living area is uniform in all HDBs, condominiums or landed houses in Aljunied subzone. Finally, we projected estimated population density in each residential building while excluding the subpopulation in Dakota HDBs subarea.

On the other hand, the above dataset shows only the resident population. However, estimated population in Singapore is 5.47 million among which 3.87 million are residents while 1.6 million is non-resident. Unfortunately, similar census data on non-resident population for the same period is not available in Singapore. To estimate the non-resident population in the study area, we assumed the distribution of non-resident population is uniform between all dwelling units in Singapore.

4.2.3.1 Projection of a fixed population of only-residents

Using 2014 population data of DOS, SLA maps, Google Earth and ArcGIS delineation tools, we estimated the resident population of the study area as 32607. However, the population distribution differs between the high-rise and low-rise subareas (18,448 versus 14,159, respectively). In addition, in terms of inhabitants' density per 100 meters of living area (i.e., a living area is the building area multiplied by number of floors), the high-rise subarea is much crowded by an average 55% than in low-rise one (average 1.66 per 100 square meters of living area in high-rise versus 1.07 per 100 square meters in low-rise, respectively). The distribution of population density in Geylang, is shown **Figure 4.3**.



Sources: Esri, HERE, DeLorme, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

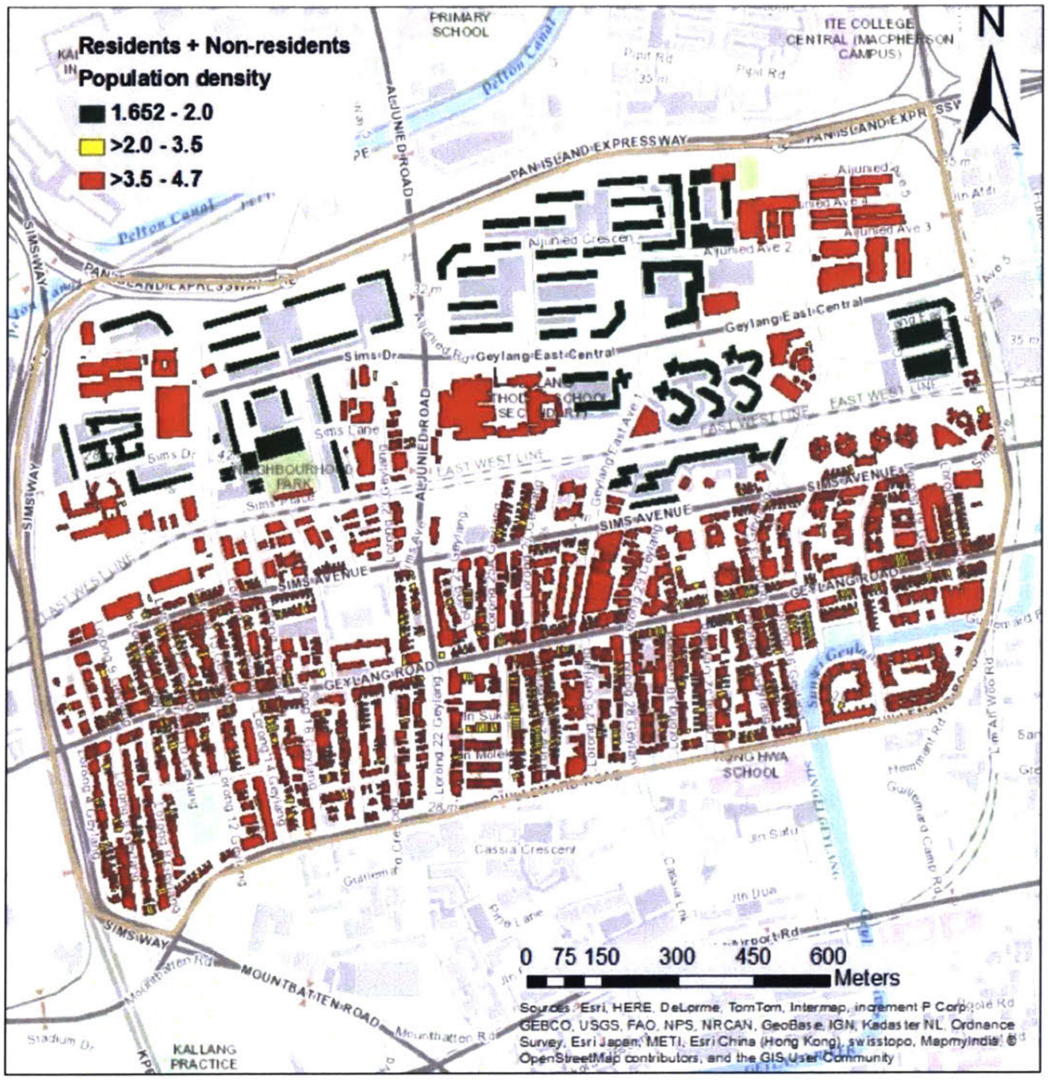
Figure 4.3 Density of the fixed population in Geylang. This includes residents only projected using 2014 statistics (density is per 100 square meters of living area).

4.2.3.2 Projection of a dynamic population including non-residents

Anecdotal evidence suggests more non-residents exist in the low-rise subarea. For example, because of low rental rates, foreign workers lodge in the shop houses. Most of the low-rise buildings have a shop on the ground floor and a residence on the top. In addition, some middle-class expats prefer condominium housing because of its flexible subleasing terms. We also need to consider visitors to public building such as hotels, restaurants and Chinese temples.

Hence, in a sensitivity analysis, we assumed that the total population in landed houses and condominiums is double to the one in HDBs because of the non-resident population. Thus, we projected the total density of resident and non-resident population per 100 square meters of living area (see **Figure 4.4**). We projected a total population of 64,629 which 38,698 and 25,940 people, lives in low-rise and high-rise subareas, respectively.

Accordingly, the dynamic population density in low-rise subarea is more crowded by an average 60% than in the high-rise ones (average 3.7 versus 1.66 per 100 square meters of living area in low and high-rise ones, respectively).



Sources: Esri, HERE, DeLorme, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

Figure 4.4 Density of the dynamic population in Geylang. This includes non-residents (density is per 100 square meters of living area).

4.2.4 Mapping the dengue incidence.

Two Epidemiological datasets were obtained for this study: 1) dengue cases in Geylang neighborhood (2010-2015), received from the Ministry of Health (MOH) – Singapore. This dataset includes the number of reported cases by location (i.e., street address) and epidemiological week for the period; 2) dengue cases in Singapore (2013-2015), retrieved mainly from a recent report by Hapuarachchi and others [51]. This article provides the spatial distribution of dengue in 2013-2014 using four quantiles of cases densities: 2 cases/km2, 16-25 cases/km^2, 56-61 cases/km^2 and >217 cases/km^2 [51]. We used ArcGIS 10.3 to digitize these maps and extract the datasets for 2013 and 204. All datasets were gridded in ArcGIS. The weighted average for quantiles of dengue density in a block was calculated before that was transformed into the number of cases. Also, dengue cases in 2015 --obtained from MoH-- were georeferenced and aggregated by their corresponding 1km^2 blocks.

We calculated dengue incidence as the number of cases in the grids divided by sum of the projected population in that grid. Incidence rates were laid over 3D maps for the study area. To generate a 3-D map for dengue incidence in the Geylang, a triangulated irregular network (TIN) was created in ArcScene 10.4, and the height of each building was calculated by multiplying the number of floors by 2.6 meters ceiling height (i.e. the minimum clearance height standard by HDB). Then, the disease incidence was overlaid on the horizontal surface of the gridded map.

4.2.5 Entomological data.

Entomological data were collected during a recent survey carried out between August 2014 and August 2015 in the Geylang. Outdoor breeding habitats were inspected twice-weekly, and active outdoor habitats of *Ae. aegypti* and *Ae. albopictus* were geo-referenced. Further details on this entomological survey can be found in [14]. In this study, we calculated pupal density per 1000 population by dividing estimates of *Aedes* pupae found in a grid cell by the total human population in that grid.

Besides, a total of 16 gravitraps were randomly placed outdoors in the two subareas of Geylang. The traps were inspected weekly for the presence of adults of dengue vector. Trapped adults on the inner film of the adhesive board were sorted out and identified using taxonomic keys [204].

4.2.6 The density of urban drainage network.

We delineated the urban drainage network (the system of conduits) at the neighborhood and country scale. Observations from the field confirmed that the roads could surrogate the drainage system in Geylang. Among categories of roads in OpenStreetMap data, we found that Residential and Service roads match the drainage network in the urban housing areas of Singapore. Further, Google Earth was used to check and correct the drainage data. The length of perimeters of the conduits of the network in each block was summed.

The following equation calculates the density of urban drainage network in each block:

$$\text{Drainage Density } (m^{-1}) = \frac{\text{Conduits length (m)}}{\text{Basin area } (m^2)}$$

4.2.7 Hot Spot analysis.

This technique was previously employed in several epidemiological studies of dengue [225,226]. In this work, we used Optimized Hot Spot Analysis (Getis-Ord Gi*) tool of *ArcGIS* 10.2 to identify significant hot spot grids of dengue occurred between 2010-2014, and whether these hot spots showed a difference between the low-rise and high-rise subareas.

The following equation gives the statistic:

$$G_i^* = \frac{\sum_{j=1}^{n} w_{i,j}x_{i,j} - \bar{X}\sum_{j=1}^{n} w_{i,j}}{S\sqrt{\frac{n\sum_{j=1}^{n} w_{i,j}^2 - \left(\sum_{j=1}^{n} w_{i,j}\right)^2}{n-1}}}$$

Where: x_i is dengue cases in block j, $w_{i,j}$ is the spatial weight between two blocks, n is the total number of the blocks. The following equations determine the average ($\bar{X}$) and variance (S):

$$\bar{X} = \frac{\sum_{j=1}^{n} x_i}{n}$$

$$S = \sqrt{\frac{\sum_{j=1}^{n} x_i^2}{n} - (\bar{X})^2}$$

The analysis compares the local sum of dengue cases in a location (and its surrounding) to the expected sum of cases in that location. The expected sum of cases was calculated using an epidemiological window. We selected two weeks and a diameter of 200 meters for the epidemiological window based on an autocorrelation ArcGIS tool. The Global Moran's I index was used to measure autocorrelation between the locations of dengue cases. The Moran's I index uses a range [-1,0, 1] to verify tendency of the distribution towards dispersal, random and clustering patterns, respectively. The peak of clustering intensity (i.e., cold and hot spots) was determined by the maximum value of standard deviation, Z-score. A null hypothesis for a Complete Spatial Randomness (CSR) was tested under $p < 0.05$ for the distribution of dengue cases within the Geylang area. The test was adjusted for spatial dependence using False Discovery Rate (FDR) correction method [227].

Accordingly, a grid was identified as a hot spot if it has a larger Z-score ($Z>1.96$) and its p-value <0.05. On the other hand, blocks of cold spots were identified if tested grids have small Z-score

($Z<-1.96$) but they are also significant ($p<0.05$). Further details on Optimized Hot Spot Analysis can be obtained online (ESRI. ArcGIS Resources: Optimized Hot Spot Analysis (Spatial Statistics); 2016) and in [228].

4.3 Results

4.3.1 Influence of urban housing on dengue clustering at a neighborhood scale

4.3.1.1 *Dengue incidence (August 2014 - August 2015).* A total of 353 cases were reported in the Geylang between August 2014 and August 2015. More dengue cases occurred in the subarea of low-rise houses than in the high-rise one. Using the fixed and dynamic population projections, average of dengue incidence per 1000 population in low-rise versus high-rise blocks, were 26.7 (Standard Error, SE = 4.83) versus 2.43 (SE = 0.67), and 7.93 (SE = 1.4) versus 1.74 (SE = 0.36), respectively (see **Figure 4.5**). Further, Chi-square test shows a significant difference ($p<0.001$) between the reported and expected dengue cases in the low-rise and high-rise blocks, using the fixed and dynamic population projections (**Table 4.1**).

Table 4.1 Observed and expected Dengue cases using estimates for fixed and dynamic populations in Geylang, Singapore (August 2014 –August 2015).

Category	Subarea	Projected Population	Dengue Cases		Chi-square test
			Observed	Expected	
Fixed population	**Low-rise**	14,159	310	153.28	χ^2 =154.54,
	High-rise	18,448	43	199.72	Df=1,
	Total	32,607	353	353	$p<0.001$
Dynamic population	**Low-rise**	38,698	310	211.34	χ^2 =71.59,
	High-rise	25,940	43	141.66	Df=1,
	Total	64629	353	353	$p<0.001$

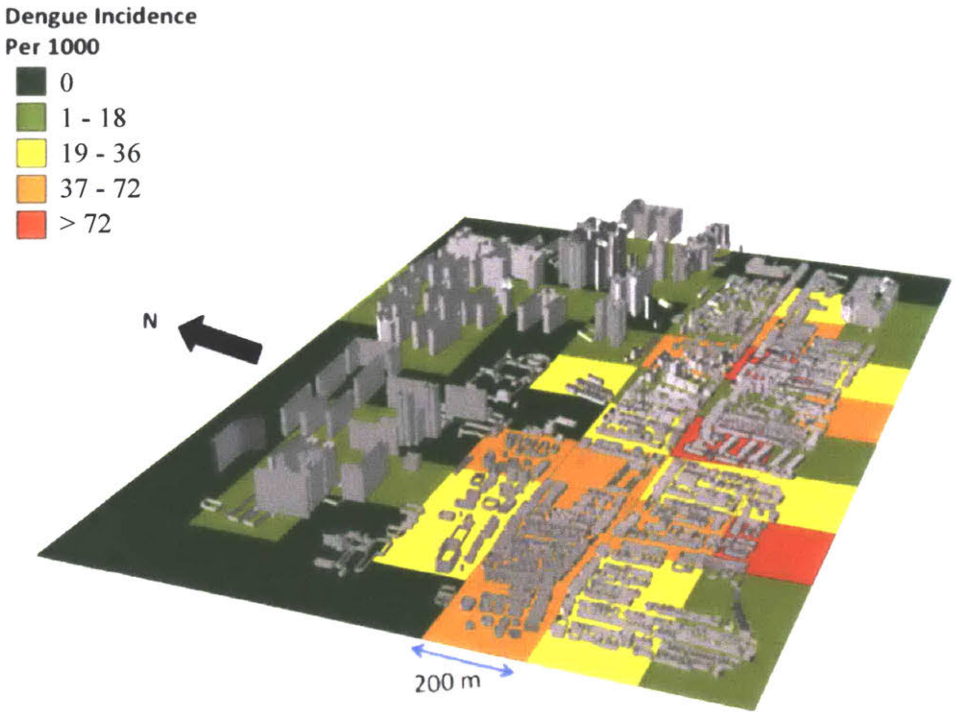


Figure 4.5 Dengue incidence per 1000 in Geylang Aug 2014 - Aug 2015. [Disease data source: Ministry of Health, Singapore].

4.3.1.2 Dengue entomology

4.3.1.2.1 Pupal density per population.

By gridding locations of breeding habitats, 27 blocks were positive for *Ae. aegypti* compared to 9 blocks for *Ae. albopictus*. Besides, 77.8% (n=28) of the positive blocks appear in the low-rise subarea. The average pupal density of *Aedes* in Geylang was 171.5 pupae per1000 population, considering a fixed population. Interestingly, the pupal density of *Ae. aegypti* shows a similar difference between low-rise and high-rise subareas of Geylang. These are 246 (SE = 69.08) and 35.4 (SE = 25.49) per 1000 population, in the low-rise and high-rise blocks, respectively (see Figure 4.6). There is a weak positive correlation between dengue cases and the total of pupae per block (Pearson's r=0.13, 95% C.I.= -0.13 to 0.37).

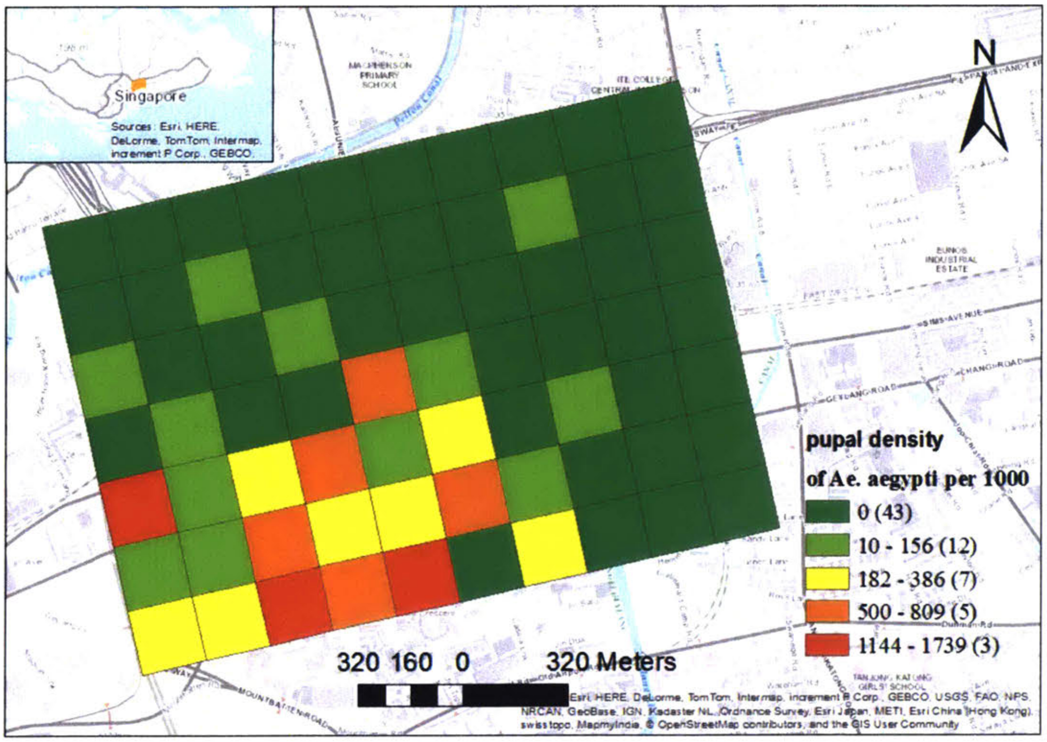


Figure 4.6 Annual pupal density in Geylang. Annual pupal density per 1000 population in 200 square meters blocks (August 2014 – August 2015).

4.3.1.2.2 Outdoor adult mosquitoes.

A total of 83 adult mosquitoes were trapped in Geylang between October and December 2015. *Culex* and *Ae. albopictus* dominated the collection, 33 and 32, respectively. However, there were 7/10 of the traps in the low-rise subarea were found positive for *Ae. aegypti* or *Ae. albopictus* compared to 3/6 of the traps placed in the high-rise one. Also, 88.8% (16/18) of caught females of *Ae. aegypti* were found in the low-rise subarea. In contrast, 87.5% (28/32) of trapped females of *Ae. albopictus* were in the high-rise section where a lush greenery surrounds the HDBs. Collections of the gravitraps are in Table 4.2.

Table 4.2 Number of outdoor gravitraps and total of caught mosquitoes in Geylang 24/October-31/December 2014

Subarea	**Gravitraps**		**Number of trapped mosquitoes**			
	Total	Positive[1]	*Ae. aegypti*	*Ae. albopictus*	*Culex spp.*	Total
Low-rise	10	7	16	4	21	41
	(62.5%)	(70%)	(88.9%)	(1.3%)	(63.6%)	(49.4%)
High-rise	6	3	2	28	12	42
	(37.5%)	(30%)	(11.1%)	(87.5%)	(36.4%)	(50.6%)
Total	16	10	18	32	33	83

[1] Positive gravitraps means for *Ae. aegypti.*

[2] Three gravitraps were lost within 2-4 weeks after installation.

4.3.1.2.3 Density of the drainage network and other urban features in Geylang

The density of the urban drainage network is higher in the blocks of low-rise subarea than in high-rise one, averaging 0.05 (SE = 0.0032) versus 0.025 (SE = 0.00245) per meter. There is a moderate positive correlation between the number of breeding habitats of *Ae. aegypti* and density of the urban drainage network in Geylang (Pearson's r= 0.57, 95% C.I.= 0.38 to 0.72, p<0.001), (see **Figure 4.7** for the boxplot). The correlation is positive but weak for the total of pupae (Pearson's r= 0.34, 95% C.I.= 0.1 - 0.55, p<0.001). Further, the correlation between the urban drainage network and dengue cases is also positive (Pearson's r=0.5, 95% C.I.= 0.29-0.67, p<0.001), (see **Figure 4.8**).

The high-rise subarea is also characterized by parking lots (n=17 vs. only two in the low-rise subarea, total area= 103,914 m^2) and bounded by greenery surroundings in the northern and western sides. Further, we observed that public buildings such as hotels, restaurants (including coffee shops), schools and houses of worship mainly aggregate in the low-rise area. (see **Figure 4.2**).

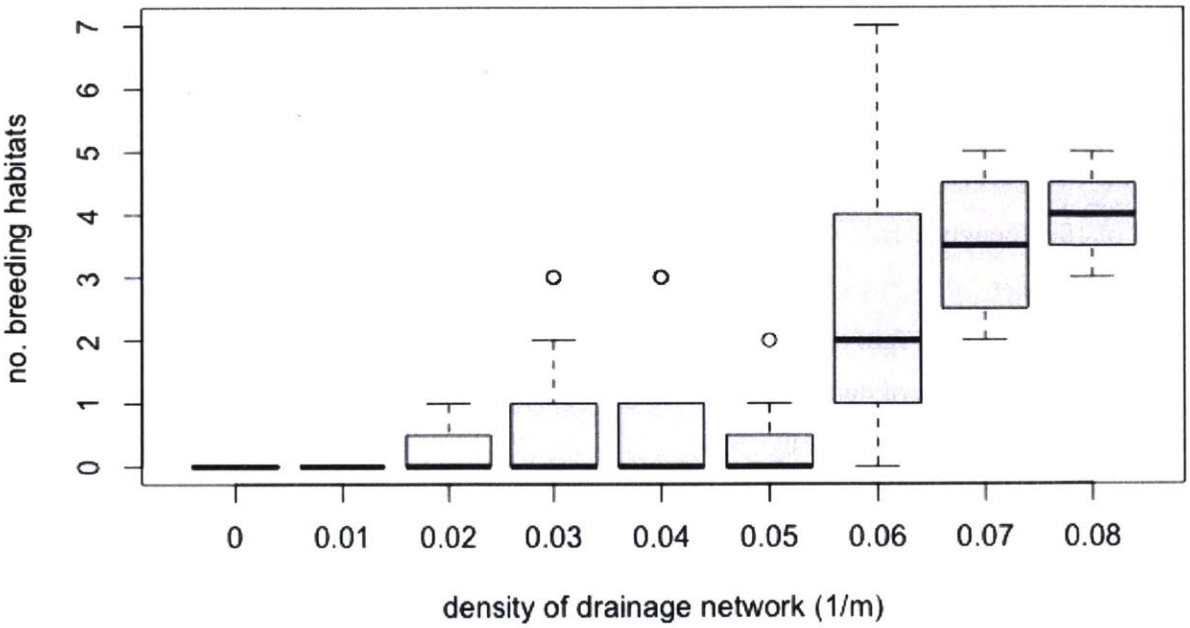


Figure 4.7 Density of the drainage network in a block versus number of outdoor breeding sites of *Aedes aegypti*, Geylang-Singapore (August 2014 –August 2015).

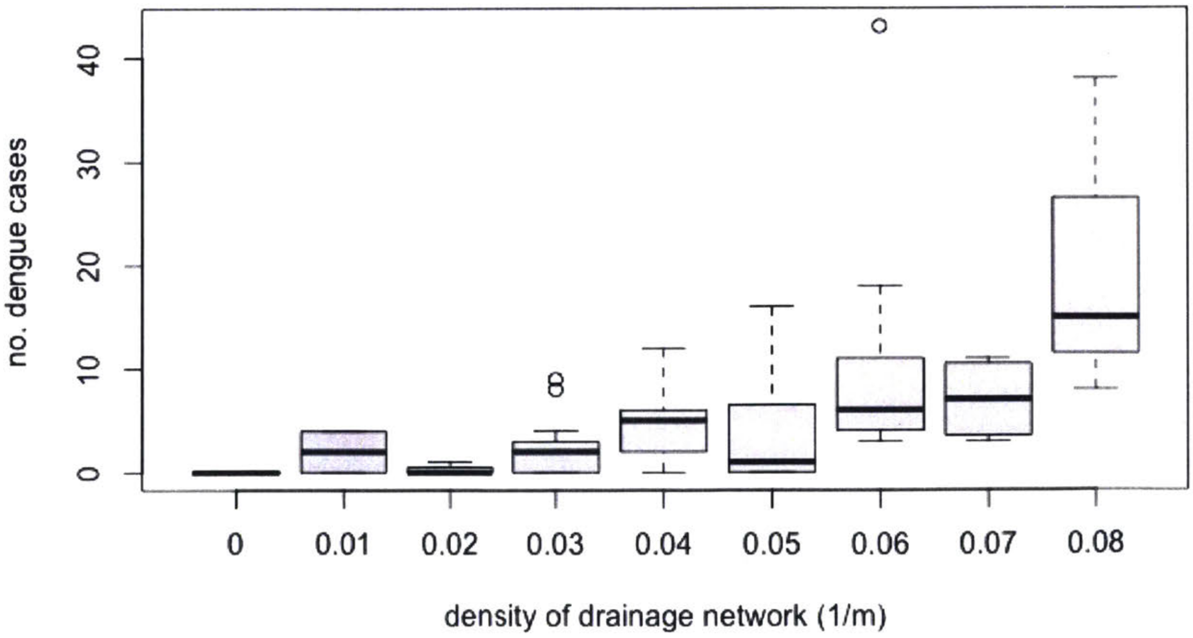


Figure 4.8 Density of the urban drainage network in a block versus dengue cases, Geylang-Singapore (August 2014 –August 2015).

4.3.1.2.4 Persistence of dengue difference in Geylang (2010 -2013).

A total of 1068 dengue cases were reported in Geylang between 2010 -2013. Cumulative incidence between 2010-2013 was more in the low-rise blocks than the high-rise subarea (average 98.6 versus 10.1)- (see **Figure 4.9**). Moreover, blocks of the former subarea had experienced more notification weeks of dengue cases during the four years period (average 30.3 versus 4.5 notified weeks) – (see **Figure 4.10**).

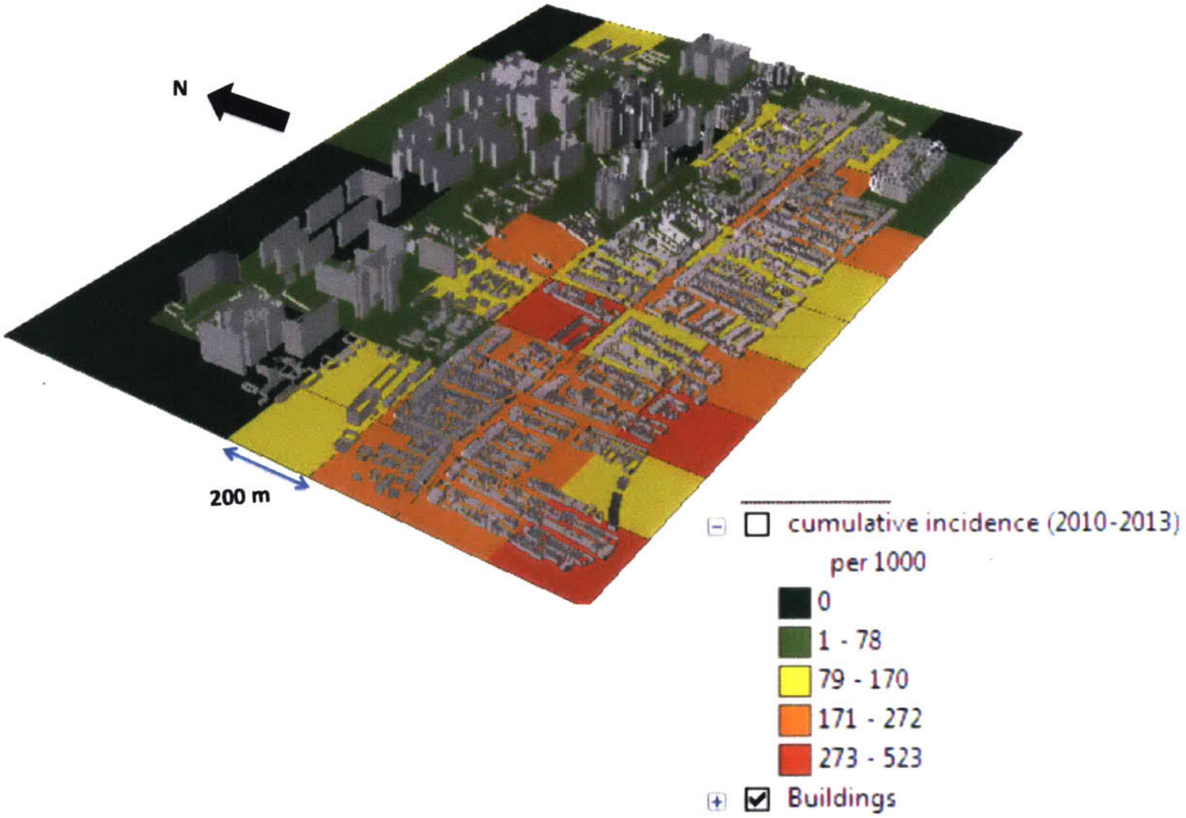


Figure 4.9 Dengue cumulative incidence per 1000 in Geylang 2010-2013. [Disease data source: Ministry of Health, Singapore].

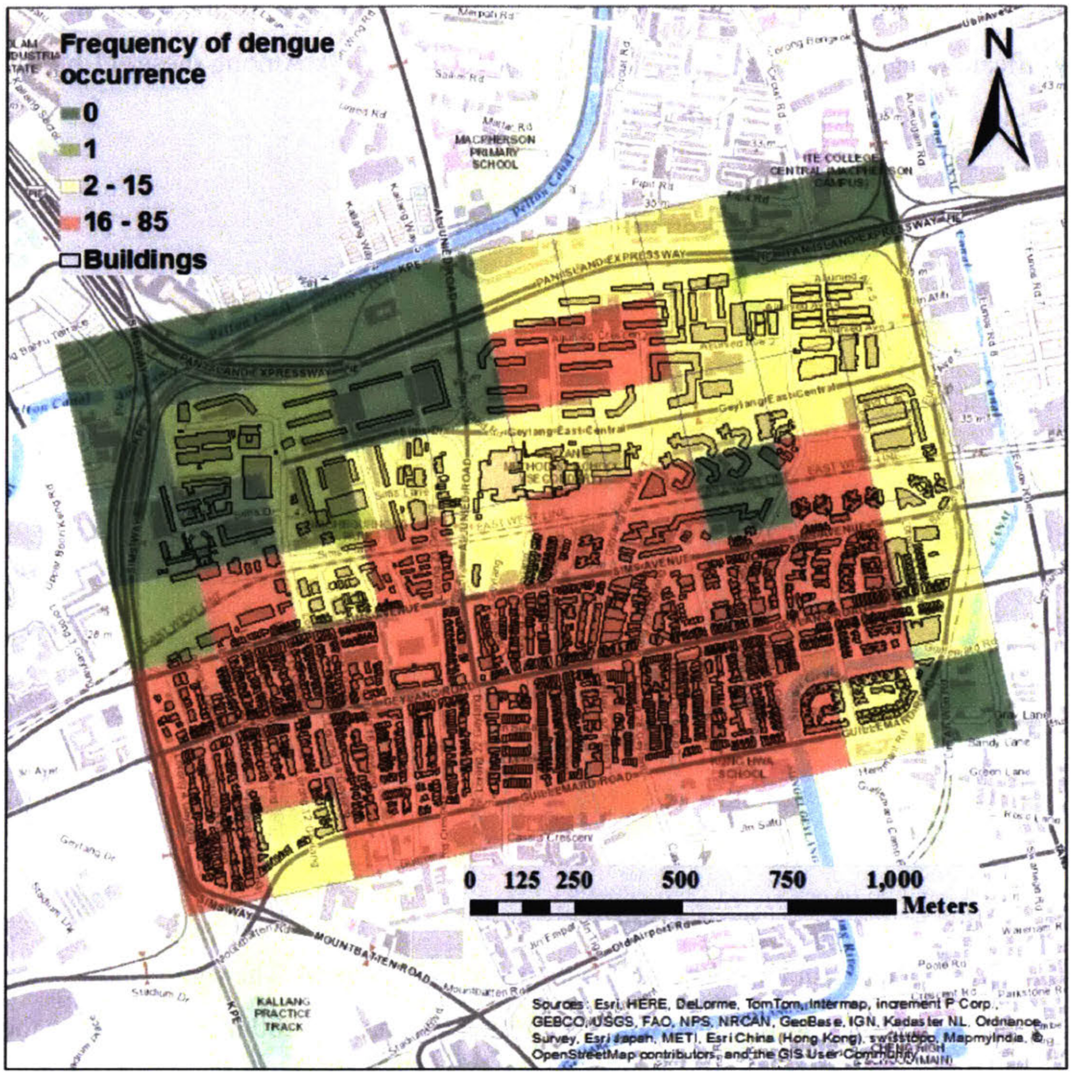


Figure 4.10 The frequency of dengue occurrence between 2010-2013 in Geylang. The occurrence is verified as the number of weeks with notification of dengue cases in a 5-years period in each grid of the study domain. [Disease data source: Ministry of Health, Singapore].

4.3.1.2.5 Cold and hot spots in Geylang.

Dengue cases in Geylang are statistically clustered using Moran's I index (I=0.26). Moran's I index showed the highest significant magnitude when the epidemiological window is 200 meter within two epidemiological weeks (Z-score=12.7; $p<0.001$).

Neglecting the temporal aspect, analysis of incremental spatial autocorrelation shows that the highest Z-score of clustering is obtained when the average distance between these cases is 200 meters (**Figure 4.11**). For the fixed population scenario, a total of 14 blocks is identified to represent significant dengue hot spots (with $\geq$ 90% confidence interval). Among these, 11 blocks clustered in the low-rise subarea (see **Figure 4.12**).

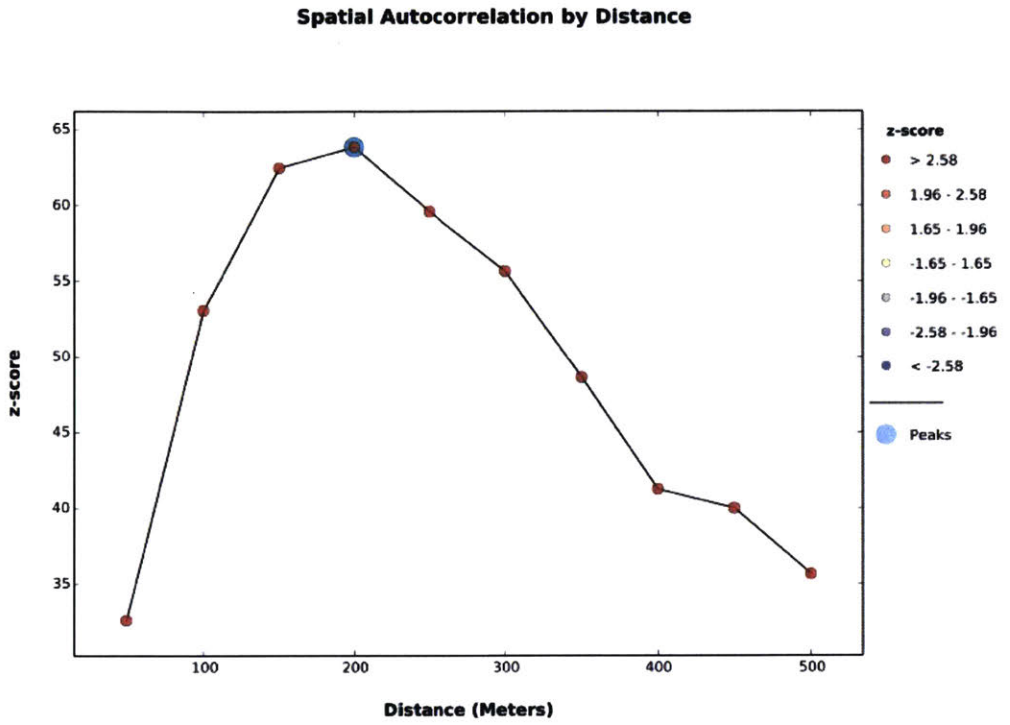
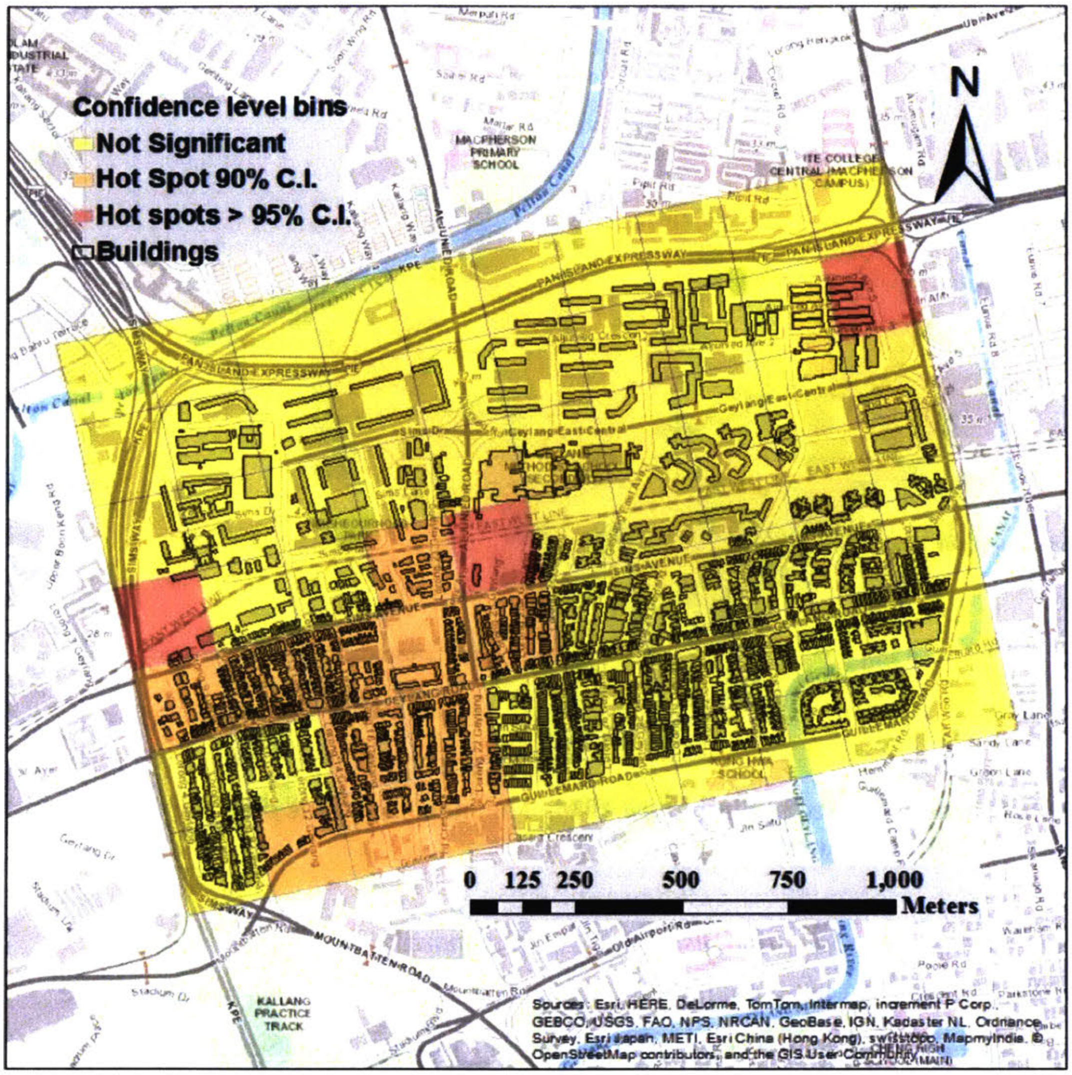


Figure 4.11 Spatial autocorrelation by distance (increments of 50 meters). The figure shows the highest Z-score for clustering was obtained when the average distance is 200 meters between the locations of dengue cases in Geylang, Singapore.



Sources: Esri, HERE, DeLorme, TomTom, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community

Figure 4.12 Hot spots grids of dengue incidence in Geylang (2010-2014). Considering a fixed population, and using a spatial-temporal window of 200 meters and 14 days.

4.3.2 Influence of urban housing on dengue distribution at a country scale.

The Singapore resident population of 3.87 million (as of 2014) is living in a total built-up area of 25.98 km^2. Out of 349 residential grids of 1 square kilometers, 52 and 175 are purely composed of people living in high and low-rise buildings, respectively. Population density per 1 km^2 in Singapore is shown in Figure **4.13**.

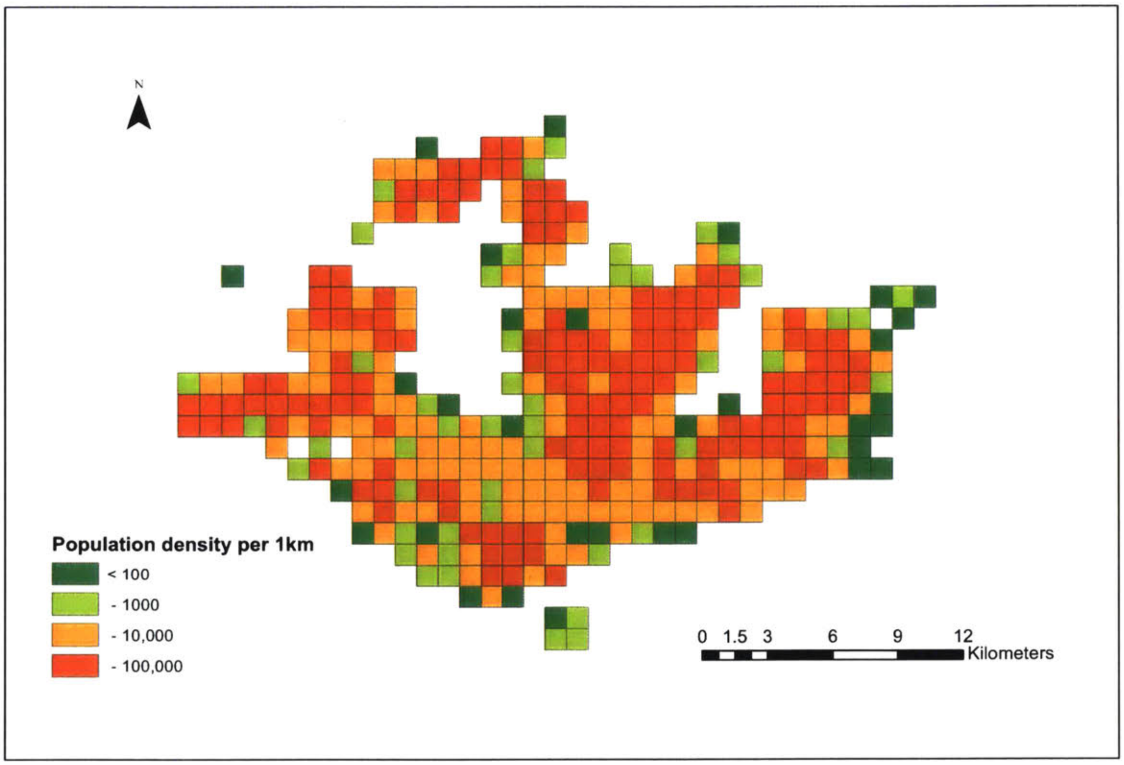


Figure 4.13 Population density in Singapore 2014. The map shows the distribution of the population density in Singapore per 1 square kilometer.

4.3.2.1 *Urban housing and population distribution in Singapore.*

Figure 4.14 and **Figure 4.15** show fractions of area and people living in low-rise grids. The figure confirms that the population is concentrated in high-rise housing in Singapore. The high-rise neighborhoods are in the northeastern part of the island.

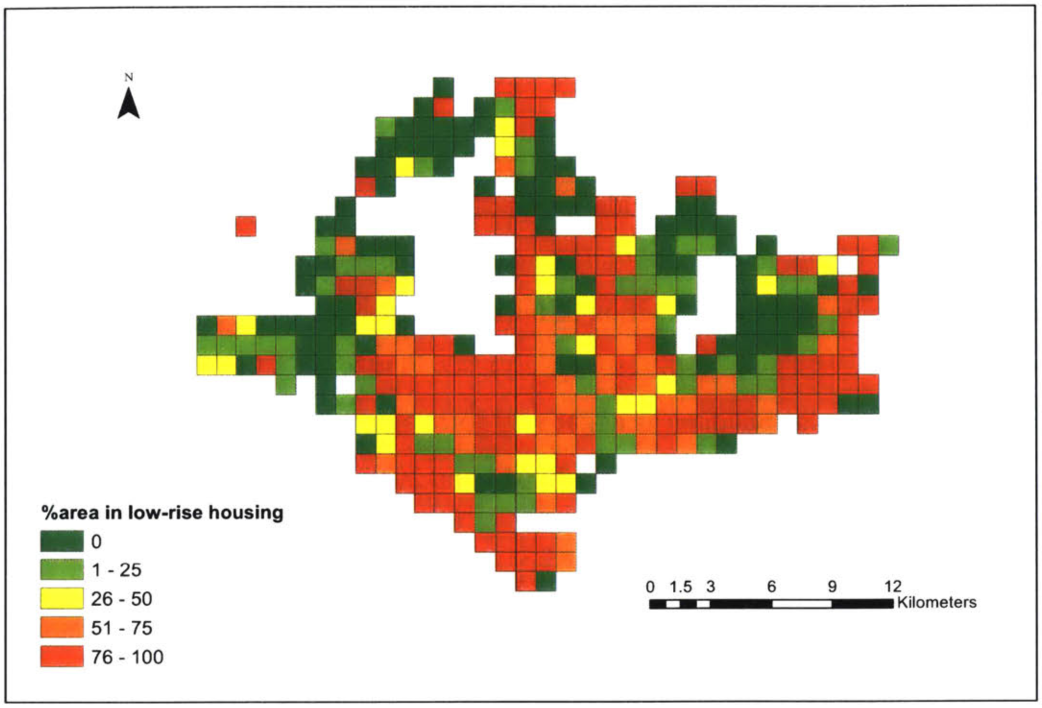


Figure 4.14 percent of area of low-rise housing per 1 square kilometer blocks in Singapore.

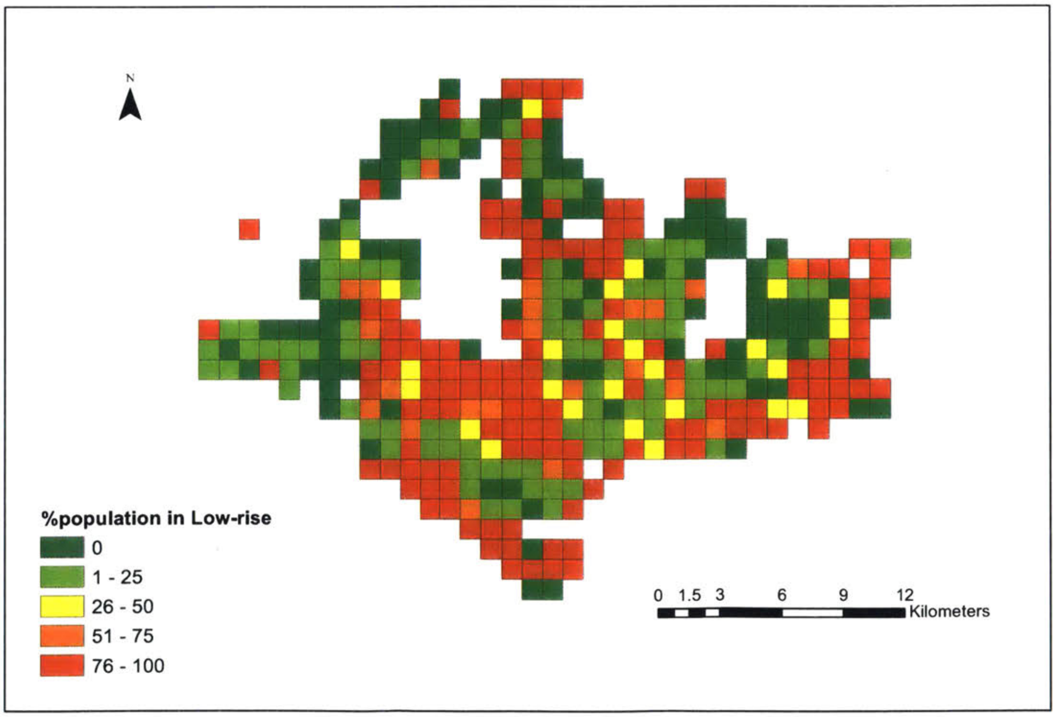


Figure 4.15 percent of resident population living in low-rise housing per 1 square kilometer blocks in Singapore.

4.3.2.2 *Distribution of Dengue incidence and urban housing.*

Figure 4.16 shows average dengue incidence for 2013-2015 in Singapore. As expected, the disease incidence is higher in low-rise blocks mainly in the central region of the city. Also, dengue incidence increased as the fraction of the area of low-rise housing increased (R^2=0.31, 0.38 and 0.3 in 2013,2014 and 2015, respectively), see **Figure 4.17**. Conversely, the fraction of the population in high-rise buildings is negatively correlated with dengue incidence during the same period (R^2=0.46, 0.54 and 0.48 in 2013,2014 and 2015, respectively), see **Figure 4.18**.

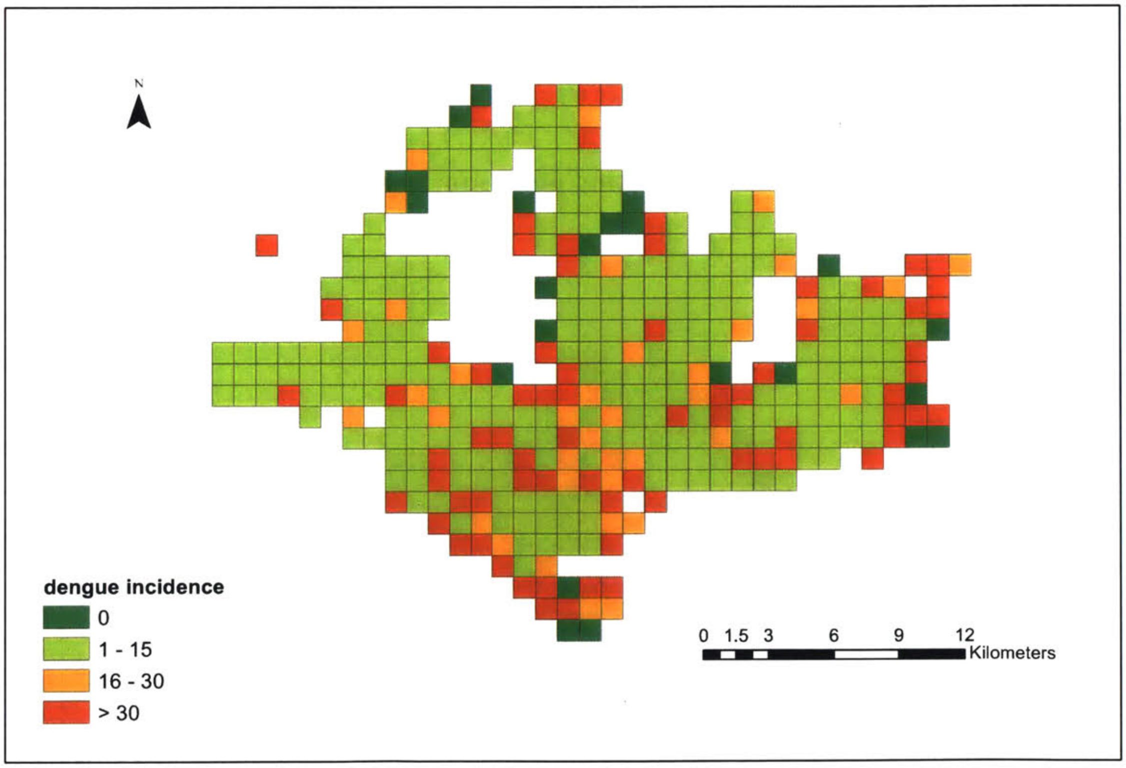


Figure 4.16 Average of dengue incidence per 1000 population in Singapore (2013-2015).

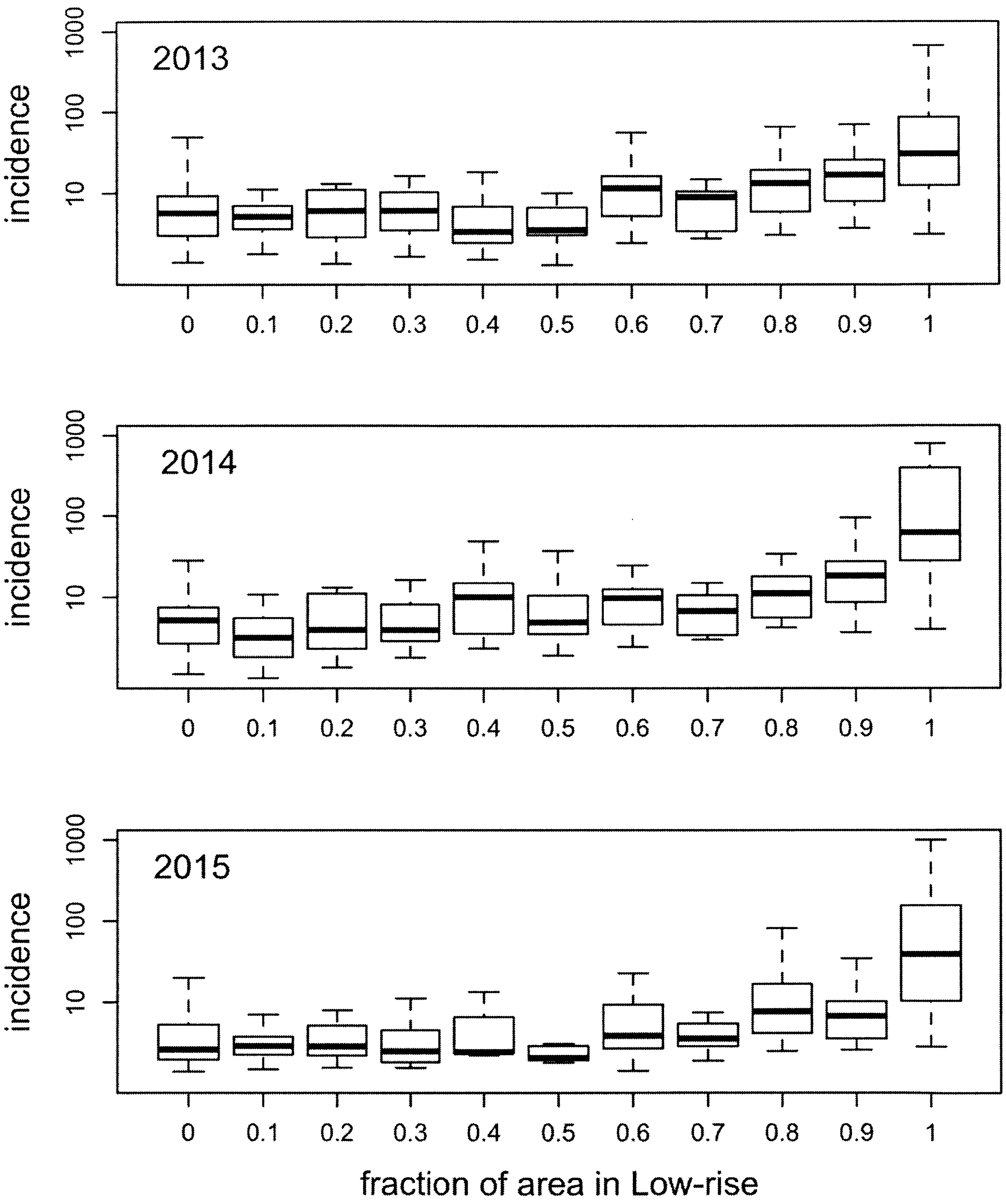


Figure 4.17 Boxplots for the fraction of area of low-rise housing versus dengue incidence in Singapore (2013-2015).

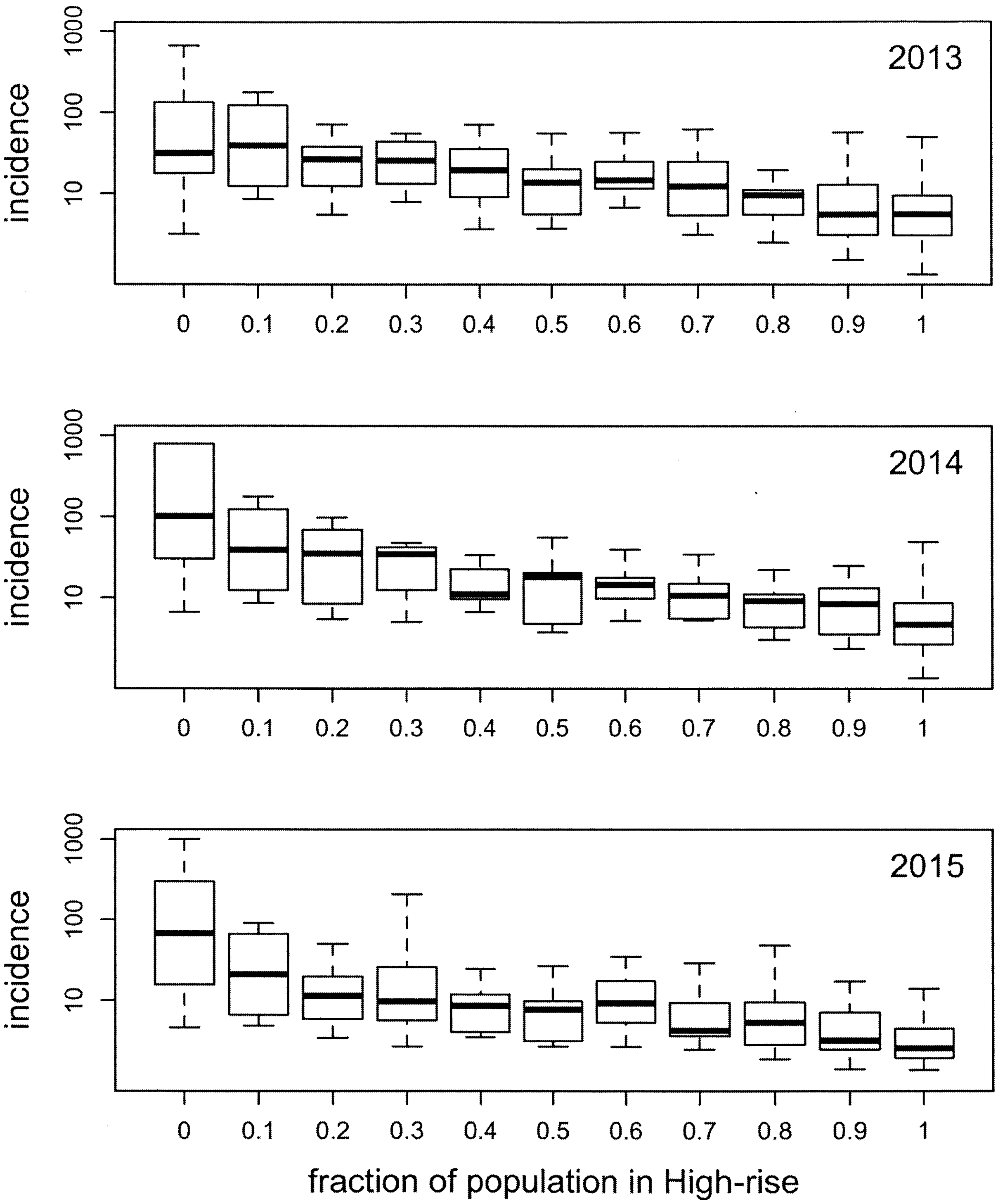


Figure 4.18 Boxplots for the fraction of population living in high-rise housing versus dengue incidence in Singapore (2013-2015).

4.3.2.3 Urban drainage and urban housing. **Figure 4.19** shows that average of drainage density in low-rise housing is four times that density in high-rise building areas, 2.59 and 0.68 per meter, respectively. This difference in means is significant using T-test (95% C.I.= 0.65 - 3.16, p=0.0029).

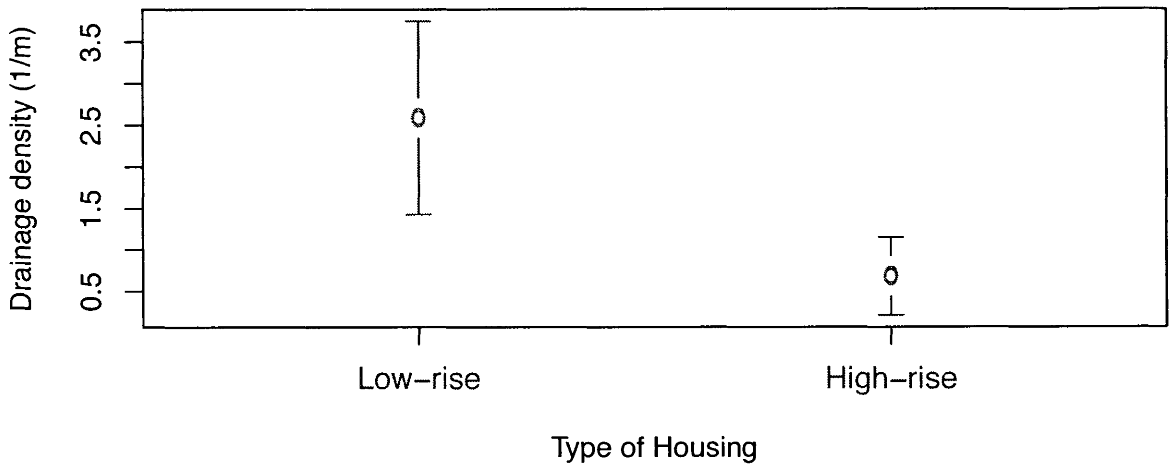


Figure 4.19 Mean and 95% Confidence Interval for average drainage density in low-rise (n=309) and high-rise(n=293) residential areas.

4.4 Discussion

This study quantifies the impact of patterns of urban housing on dengue incidence and distribution in Singapore at a country and neighborhood levels. Our findings suggest that the strategic housing plan of URA does not increase the risk of dengue transmission in the country. The expansion of high-rise public housing would rather have a positive impact on dengue control if this urban planning comes at the expense of the built-up area or population living in low-rise housing.

In the 1960s, researchers had reported a greater spatial spread for breeding of *Ae. albopictus* over *Ae. aegypti* in Geylang area [166]. They attributed this relative dispersal to Kallang basin serving at that time as refusal dump and large breeding swamp for this species. In contrast, *Ae. aegypti* had mainly infested indoors and primarily in low-rise neighborhoods composed of shop houses. The shop houses are still featured in conserved areas like Geylang and Little India; they are preserved because of their historical/architectural value to the country. Moreover, the growing affluent population of Singapore may prefer other types of landed houses instead of apartments in high-rise buildings.

The relationship between the incidence and area (or population) fraction of accommodation type implies clustering of dengue between the housing types has more influence on dengue distribution than other factors underlying clustering within specific housing types. Hence, either community practices— conducive for indoor breeding— are being different between low-rise and high-rise neighborhoods, or that conductivity for indoor and outdoor breeding habitats and their interactions are pertinent to the urban structure of the low-rise areas more than the community practices. Indeed, several socioeconomic factors could influence the community practices towards dengue. A previous study had revealed a difference in education and employment at households between hotspots and non-hotspot of dengue in Singapore [229]. However, landed houses were the non-hotspots in that study, having higher education and employment rates. Further research is needed to answer whether there is a more knowledge-practice gap on dengue control by the community in low-rise housing, or that this type of accommodation intrinsically favors breeding of the vector indoor and outdoor.

We also showed that density of the urban drainage system has a positive correlation with the number of dengue cases and breeding habitats at the neighborhood scale. The low-rise subarea of Geylang has a denser drainage network compared to the high-rise one. This difference in the drainage system between the two categories is shown to be significant in other areas of Singapore. While an extensive drainage network reduces the risks of flooding, this hydraulic system could increase dengue breeding habitats. In Singapore, the drainage system of rainwater is separate from the sewer system of wastewater. Thus, urban catchments and sub-catchments have extensive drainage network in neighborhoods. This system includes more than 7,000 kilometers of drains in areas that convey the rainwater to 32 rivers. The storm water is then directed to 17 reservoirs before being treated and pumped back into the water supply network [230]. Any defection or clogging along this extensive network of drains could result in water stagnation, which can turn into a breeding habitat for mosquitoes. Infestation of perimeter drains from domestic containers, and vice versa can maintain the vector population against the vector control. This interaction between indoor and outdoor breeding could be one of the reasons for the higher house index in the low-rise households.

A limitation of this study is a lack of spatially explicit data on the human population structure in Geylang. We tried to overcome this limitation by conducting a sensitivity analysis assuming that the non-resident population in the low-rise is twice that in high-rise areas. If this is true, then more cases are reported in the low-rise houses because herd immunity is lower in this section compared to the high-rise one. EXPLAIN BETTER According to a recent study, people living in old residential areas have higher seroprevalence (76.5% vs. 63.5) compared to new residential areas in Singapore [54].

While adults of *Ae. albopictus* were captured more than *Ae. aegypti* in Geylang neighborhood, specimens of *Ae. aegypti* were trapped mainly in the low-rise subarea. In endemic areas, *Ae. albopictus* shows a less vectorial competence to transmit dengue, especially when *Ae. aegypti* coexists [59]. Nevertheless, results of outdoor gravitraps should be considered as qualitative because of the small number of installed traps. A previous mark-release-recapture study had shown that height of the housing does not affect the dispersal ability of *Ae. aegypti* in Singapore [224]. However, availability of humans and breeding sites could have an impact on the mosquito dispersal. Here, we suggest that the man-vector contact happens close to the breeding habitats whether indoor or outdoor. For example, dengue cases cluster in the low-rise area of Geylang because they are close to breeding drains and mosquito does not need to fly far to get a blood meal.

Previous work has shown that feeding of *Ae. albopictus* on humans is less in rural areas and likely depends on human density in Singapore [220]. In contrast, the study showed that *Ae. aegypti* is a highly anthropogenic-blood feeder in urban and peri-urban areas. A lower population density in landed houses may widen the difference in human feeding behavior between *Ae. aegypti and Ae. albopictus*, hence, increase the relative importance of *Ae. aegypti* at these areas. In contrast, the large human population of HDBs and condos could limit the disease transmission considering the low seroprevalence rate (i.e. mosquitoes feed more on non-infectious individuals). Further work is required to investigate the frequency of man-breeding contact in low-rise housing in Singapore and how reducing this contact affects the disease transmission. Anti-vector measures in low-rise housing should focus on interrupting people contact with breeding habitats. These interventions may include the use of spatial repellents [231] and personal protection measures, limit people visits to positive areas during outbreaks and enhance a non-conducive environment for outdoor breeding (e.g., periodic cleaning of drains) or barriers for the interaction of indoor-outdoor habitats.

4.5 Summary and Conclusions

Dengue is among the increasing public health problems to many countries especially in large urbanized areas. Various climatic and non-climatic factors interact and shape the disease distribution in endemic cities. In Singapore, types of urban housing influence indoor breeding and dengue cases. The strategic plan of Urban Redevelopment Authority (URA) of Singapore focuses on affording public housing in high-rise buildings as a solution for the growing population. In this study, we revisit this observation at a neighborhood and country levels categorizing urban housing of Singapore into low-rise and high-rise housing. A low-rise housing subarea at the neighborhood shows more outdoor breeding in drains and denser drainage network compared to high-rise subarea. At the country scale, risk of dengue is related to the fractions of area or population living in low-rise housing. The distribution map of dengue incidence shows a similar profile to the distribution maps of low-rise housing. This finding suggests that the government strategic plan to afford public apartments in urban agglomerations of high-rise buildings does not increase the risk of dengue transmission. On the other hand, the higher risk in low-rise housing is likely related to more conducive factors for indoor-outdoor breeding habitats.

In conclusion, the differences of dengue and its primary vector between low-rise and high-rise housing suggest that man-breeding contact rather (than man-vector contact) influences dengue transmission in Singapore. The control strategy of dengue in Singapore should focus on disrupting this contact in low-rise housing.

Chapter 5: . Development of HYDRology, Entomology and DEngue Transmission Simulator (HYDREDETS)

Drains are one of the cryptic breeding habitats for mosquitoes that received little attention in public health. In this chapter, we describe a new mechanistic model that can be used to investigate the role of urban drainage networks in the outdoor breeding of *Aedes* and transmission of dengue virus and other arboviral diseases.

The HYDRology, Entomology and DEngue Transmission Simulator (HYDREDETS) consists of three coupled dynamic models, with an explicit spatial domain. Coupling of the urban hydrology model SWMM into HYDREDETS has enabled simulation of *Aedes* breeding in drains. The model was calibrated using observations obtained during the entomological survey 2014-2015, Geylang neighborhood, Singapore. Hence, the model allows studying rainfall-effects such as seasonal flushing/drying of larvae and pupae and re-inoculation of the drains by dormant eggs the following stagnation conditions.

HYDREDETS fills a gap in epidemiological modeling tools available for studying vector-borne diseases related to urban hydrology. This direction will open the door to incorporation of vector control measures within the guidelines and operating systems of urban drainage networks.

5.1 Introduction

By 2050, two-thirds of the world population will reside in cities and other urban areas as compared to 54% of the current population [232]. Challenges of this rapid urbanization to public health implicate the control and prevention of arboviral diseases. Dengue fever is among these vector-borne diseases, that has a significant burden on urban communities, with an annual estimate of 350 million cases. Half of the world population --mainly in the tropics—are at risk [12,15]. The disease is caused by four serotypes belonging to Flavivirus (DEN-1, DEN-2, DEN-3 and DEN-4) [233]. Several alpha cities around the world are hyperendemic for dengue (i.e. the four serotypes co-circulate) and exhibit transmission seasons, such as Bangkok, New Delhi, Sao Paulo, and Singapore [53,234–236]. While two mosquitoes *Aedes aegypti* and *Aedes albopictus* can transmit the disease, the former species is the primary vector in endemic areas.

The dynamics of dengue transmission in endemic cities involves climatic and non-climatic factors [192–194] and is influenced by the degree of urbanization[36]. In under-developed areas, inadequate water supply and the use of non-biodegradable containers can result in an indoor breeding of *Ae. aegypti* in storage containers and discarded receptacles [49,213]. In contrast, indoor breeding in well-developed cities is related to the lifestyle such as the use of ornamental and plant pots, bamboo pole holders and tanks of air-conditioners [30,198,214]. Besides, outdoors breeding occurs in rain-fed habitats such as roof gutters, sewage, and storm drainage networks [190,216–218]. There is a growing evidence for vector adaptation to outdoor breeding habitats that can increase impact of climate change on the *Aedes*-transmitted diseases [189,190].

Little attention is given to the cryptic role of the drainage network on dengue transmission in urban settings despite a cumulative evidence for breeding in catch basins [218,237–240]. Urban mosquitoes can efficiently breed in drains such as Culex mosquitoes (which are vectors for Filariasis, WNV and WEEE), *Ae. albopictus* and *Ae. aegypti* [190,240–243]. Organic matter accumulating in drains is used by gestating larvae and gravid females as breeding habitats to lay their eggs [205]. Also, physical properties of the drain water could influence the oviposition choice by females [244]. This outdoor breeding habitat could have a strong effect on spatiotemporal patterns of dengue transmission in endemic cities because they are more vulnerable to seasonal changes in temperature and rainfall. In a recent work, we showed that the main outdoor breeding habitats of *Ae. aegypti* in Singapore are storm drains in back alleys [14]. Clogging by litter in these ditches results in stagnation of rainwater and formation of breeding sites (see **Figure 5.1**). We also developed and tested a new hypothesis about the influence of rainfall on dengue seasonality in wet tropics. Field observations have revealed that a monsoon-related sequence of flushing and drying of breeding drains impacts seasonal outdoor abundance of the dengue vector in Singapore [14].

Figure 5.1. Outdoor breeding of the dengue vector *Aedes* aegypti occurs in clogged storm drain in Geylang, Singapore.

Previous works have shown that mathematical modeling is useful in studying the dynamics of dengue transmission [69,124,154,155,163,164,245–247]. Mathematical modeling is a process-oriented approach (i.e. mechanistic) that aims not only to simulate observed phenomena -- such as seasonality-- but also to explain how they are generated. Ordinary differential equations are used to describe changes over time in the state variables [11]. In general, mathematical models are divided into two types: compartment-based and agent-based models. A compartment-based model is stochastic, top-down, and describes the interaction of the bulk population with the system and how this implicitly changes the state of the individuals. Conversely, agent-based models are deterministic. They are bottom-up and explicitly track changes in the state of individuals that eventually affects the entire system. A primary challenge in using mechanistic modeling is that they require a comprehensive understanding of the processes that govern the system. For example, identifying rainfall as a driver of the seasonality of dengue requires identifying each process in disease transmission that is influenced by wet/dry conditions.

Another challenge is that simulation of the real world requires accurate parameterizations of processes. Thus, field and experimental observations are needed to estimate values of the parameters.

Since 1992, more than fifty models to simulate dengue transmission have been developed and published. While most of the published models are comprehensive including descriptions of the human and *Aedes* populations, several models have omitted the representation of the vector [155,248]. In addition, many models have simulated two or more serotypes of dengue viruses. These models also give attention to the antibody-dependent enhancement (ADE) and its implications in dengue transmission (i.e., "susceptibility, transmission and/ or mortality enhancement") [155,249–257]. Nevertheless, very few of these models are individual-based accounting for heterogeneities of human and mosquito populations [108,155]. Several of these compartmental stochastic models contain explicit spatial domains for human mobility [258–266] or dispersal of mosquitoes [163,164,267].

The modeling framework developed by Focks and others (1993 and 1995) has received great attention during the last two decades [123,152,164,268,269]. The model has two coupled dynamic models: Container-Inhibiting Mosquito Simulation Model (CIMSiM) and Dengue Simulation Model (DENSiM). CIMSIM is a compartmental model that simulates both the aquatic and adult stages of *Ae. aegypti* using daily weather files (e.g., air temperature, RH, and rainfall) and entomological parameters obtained from experimental and field studies [123,152]. The user needs to define the characteristics of breeding containers (i.e. surface area, depth, shading) and whether these containers are rain-fed or manually-fed including withdrawal rates for storage containers. On the other hand, DENSIM is agent-based model that simulates circulation of the four serotypes in the human population using feedbacks from CIMSIM for the mosquito population. The user needs to set death and birth rates of the human population, age structure of the population, serotype (s) and specific viremia parameters. DENSIM feeds-back to CIMSIM to simulate infections among the mosquito population[124]. A further study has shown that the CIMSIM-DENSIM model is sensitive to mosquito stage-survival, biting rate and IIP in humans [247].

In this study, we present a mathematical model to study how the hydro-persistence of breeding drains shapes the vector abundance and how this in turn influences dengue transmission in well-developed cities. Our group previously developed coupled models of hydrology and disease transmission to study malaria at the village scale and forecast the impact of climate change on malaria in Africa [270–272]. Here, we use the same approach to model dengue transmission at the neighborhood scale in Singapore. We called this model HYDRology, Entomology, and Dengue Transmission Simulator (HYDREDETS). The model consists of 3-coupled modules: urban hydrology, entomology, and epidemiology (Figure 5.2). We use the EPA Storm Water Management Model (SWMM) as the hydrological module of HYDREDETS. The entomology module features spatially- and temporarily-explicit representation of the aquatic habitat of *Ae. aegypti*, and description of behaviors of adult mosquitoes. The model also tracks transmission of each of the four serotypes of dengue in both the human and mosquito populations in Geylang neighborhood. The age structure of the population is also considered in HYDREDETS. We calibrated the model using entomological and hydrological observations of a field survey carried out in Singapore 2014-2015. A further evaluation of HYDREDETS including sensitivity analyses was conducted for dengue seasonality in Singapore, presented in **Chapter 6.**

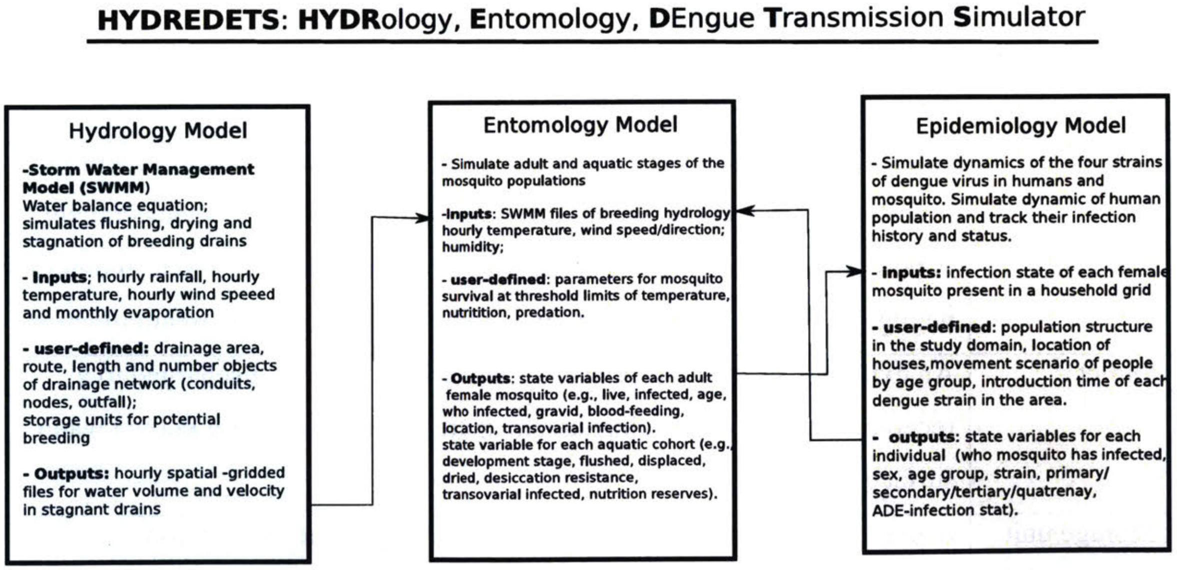


Figure 5.2. Overview of HYDREDETS model. The model consists of three coupled dynamic models: Hydrology model to simulate the water cycle of breeding drains, Entomology model to imitate the lifecycle and behavior of Aedes mosquito populations, and

Epidemiology model to track dengue infections in the human population. Outputs of the hydrology model: water volume and velocity are used by the entomology model in describing mosquito-breeding processes. Through infected mosquitoes and humans, the epidemiology and entomology models informs each other.

5.2 Materials and methods

5.2.1 Hydrology submodel

5.2.1.1 *Overview.* We used Storm Water Management Model (SWMM) as the hydrological module of HYDREDETS. SWMM is a compartmental model developed by Environmental Protection Agency (EPA) to simulate rainfall-runoff of urban drainage systems [273].
The first version of SWMM was released in 1971; and since that time the model has been gradually improved up to the recent version SWMM 5.1.012 (released in 2017). The model is widely used in scientific decision-making for the management of urban drainage systems by hydrologists, civil engineers and other specialists of water resources. Definitions of objects used in the model are presented in **Table 5.1**.

Table 5.1. Definitions of objects used in SWMM model. Modified after SWMM Manual [273]

Object	Definition
Rain gage	Source of rainfall to one or more subcatchments.
subcatchment	A land lot that receives rainfall associated with a rain gage and generates runoff that flows into a drainage system node or to another subcatchment.
Junction	A point in the conveyance system where conduits connect to one another with negligible storage volume (e.g., manholes, pipe fittings, or stream junctions).
Outlet	An exit point of the conveyance system where water is discharged to a receptor (such as a receiving stream or treatment plant) with known water surface elevation.
Storage unit	A pond, lake, impoundment, or chamber that provides water storage.
Conduit	A channel or pipe that conveys water from one conveyance system node to another.

Hence, we confined HYDREDETS to only two compartments: Land surface and conveyance network. The land surface covers the total area of subcatchments that collects rainfall of the spatial domain. Because of the built-up area, rainfall is subject to an initial abstract that depends on land use and interception characteristics of the surface (e.g., materials of rooftops). Also, part of the precipitation is lost from the system through evaporation (to the atmosphere) and infiltration (to the sub-surface). In residential neighborhoods, evaporation commonly occurs when the rainwater is intercepted in the roofs, lawn grass, paved areas and open fields [274]. The remainder of the storm water routes into the conveyance compartment as a surface runoff. The following mass-balance equation describes the change in depth of water excess after a rainstorm over a subcatchment:

$$\frac{\partial y}{\partial t} = p - e - i - q \ (5.1)$$

where: *y*=depth of ponding water atop the catchment surface (m); *t*=time (hour); *p*= precipitation rate (m/h); *e* = evaporation rate (m/h); *i* =infiltration rate (m/h); *q*=runoff rate per unit area (m/h). One of the assumptions of SWMM is that the flow rate in rectangular drains is uniform. The Manning equation gives the relationship between the volumetric flow rate and slope of the hydraulic grade line:

$$Q = \frac{1.49}{n} S^{1/2} R_x^{2/3} A_x \ (5.2)$$

where: n = a surface roughness coefficient for friction loss, S = average slope of subcatchment (m/m), A_x= area across the width of the subcatchment through which the runoff flows (m^2), R_x= The hydraulic radius associated with this area (m). The value of the Manning roughness coefficient depends on the surface cover. Input parameters required by the SWMM model are defined in Table 5.2.

Table 5.2. Input parameters used in SWMM model. Modified after SWMM Manual [273].

Variable/parameter	unit	type	Processes
Area (subcatchment)	hectare	Distributed	Precipitation area
Rainfall intensity	mm/hour	lumped	Water balance in drains
Evaporation	mm/hour	lumped	Water balance in drains (Flushing, stagnation, drying)

Temperature	℃	lumped	Water balance in drains (Flushing, stagnation, drying)
Wind speed	m/s	lumped	Water balance in drains (Flushing, stagnation, drying)
Elevation	m	-	Drainage slope
Shape of drain	-	Distributed	User-defined
Depth of nodes/conduits	m	Distributed	Stagnation
Flow Rate	cubic meters/sec (cms)	Distributed	Runoff/flushing
Infiltration Rate	mm/hour	Distributed	Water loss/drying of drain
Storage Volume	m3	Distributed	Stagnation
Velocity	m/s	Distributed	Displacement /flushing out
Area ponded	m2	Distributed	stagnation

The conveyance network is composed of links (i.e. channels and pipes), and nodes (i.e., connecters, storage units, and outfalls). While the entry point for the surface runoff into a drainage network is the inlet node, outlets are the exit point where the rain storms are diverted out of the system. In addition, two types of nodes were included in the model: junctions which are the connectors of the conveyance pipes, and storage units which are usually used as retention ponds in drainage systems.

The flow routing in the drainage network is governed by St. Venant equations for conservation of mass and momentum of shallow water:

$$\frac{\partial A}{\partial t} + \frac{\partial Q}{\partial x} - q = 0 \qquad (5.3)$$

$$\frac{1}{A}\frac{\partial Q}{\partial t} + \frac{1}{A}\frac{\partial (Q^2/A)}{\partial x} + gA\left(\frac{\partial y}{\partial x}\right) - g(S_0 - S_f) = 0$$

Where: Q= discharge through the conduit; A= cross-sectional area of the flow; y=Depth of the flow; S_0= Channel bottom slope; S_f = friction slope.

These equations are solved for a dynamic wave routing to track stagnation of water in breeding drains. Assumptions underlying St. Venant equations simplify the modeled flow as a one-dimensional, incompressible fluid prevailed by a hydrostatic pressure (compared to negligible vertical accelerations). The equations also assume that bottom slopes of both the channel and streamline curvature are small; and that channel's width is fixed [275].

The primary processes and physical objects used in the hydrology submodel are depicted in **Figure 5.3**. State variables include depth of runoff at the surface of subcatchments, water depth in nodes, and rate/velocity/area of flow at links. Changes in values of these discrete time variables depend on their previous states, meteorological conditions, and parameters (e.g., inverted elevation of nodes, shape and depth of links, imperviousness of subcatchments).

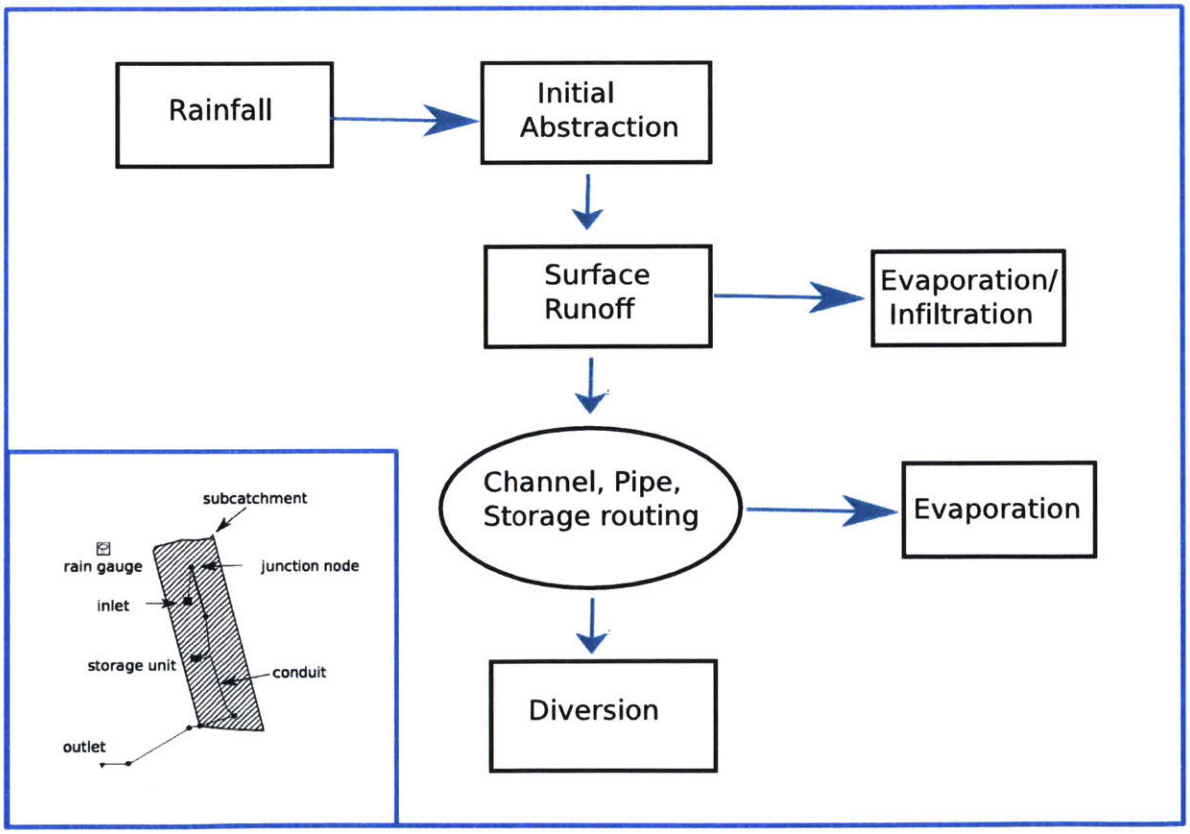


Figure 5.3. Main processes in the urban hydrology model. The inset shows the structural objects of SWMM used in HYDREDETS. (Modified after Rossman and Huber, 2016 [273]).

5.2.1.2 *Development and parameterization.* We developed urban drainage network for the Geylang in SWMM using ArcGIS tools, field observations for flow directions in Geylang and geospatial data. ArcGIS and ArcGIS-based software GeoSWMM [Streams Tech, Inc.] were employed in the delineation process to discretize the study area (see Figure 5.4). The catchment area of Geylang was discretized into 59 subcatchments. This procedure considers the urban design of the survey area, where high-rise and more open space exist in the northern part while low-rise and more alleys occur in the southern part of Geylang.

To calculate the slope of a subcatchment, SRTM DEM raster data (30 meters resolution) was downscaled and corrected by field observations for heights of buildings in Geylang. The Slope Calculation tool of GeoSWMM was used in this process.

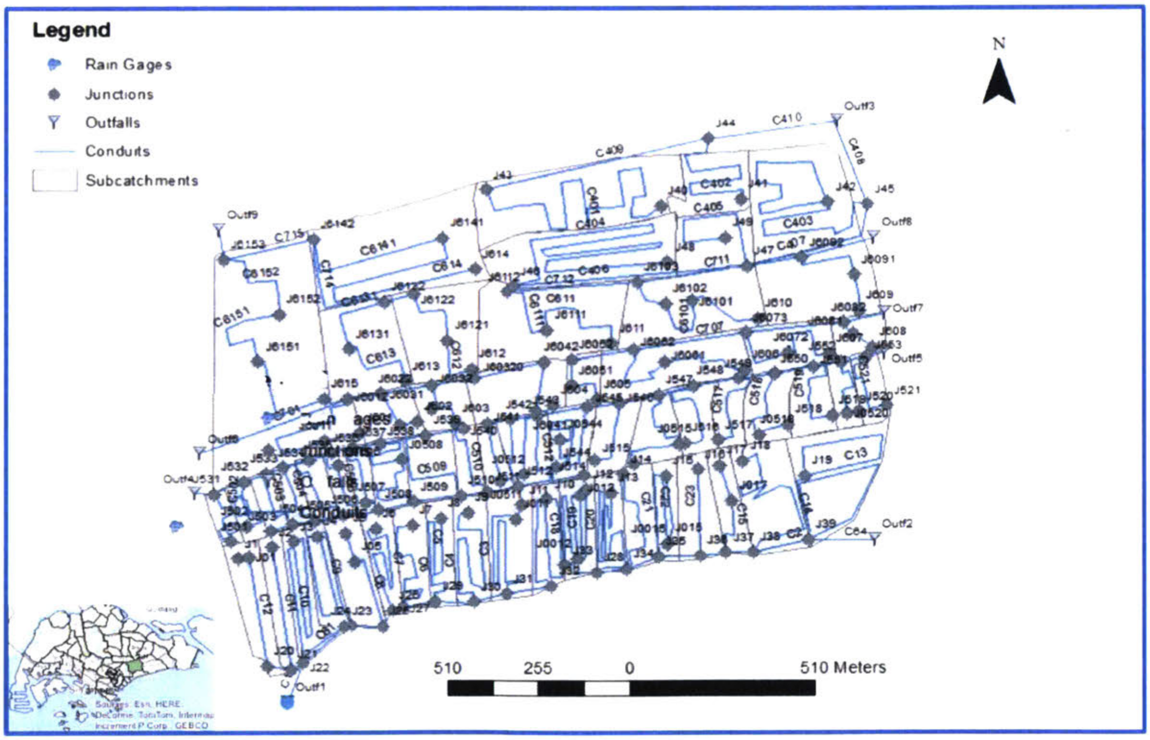


Figure 5.4. The urban drainage network of the study area, Geylang, Singapore. The drainage basin of Geylang is divided into 59 sub-catchments that collect storm water. The conveyance system consists of 168 junctions (nodes) and 192 conduits (links). Nine outfalls work as exit points of the drainage system. The inset map shows location of the Geylang neighborhood in Singapore.

Percent of impervious area in each subcatchment was parameterized using spatial data obtained from Singapore Land Authority (SLA). The SLA data contains shape and area of buildings' footprints and open spaces (e.g., parks) by their categories. We assigned built-up and non-built-up areas in each subcatchment before we calculated the area-weighted-average of imperviousness. Parameters of imperviousness zones according to the land use are given in [273]. Infiltration from the pervious area to the unsaturated zone was modeled in SWMM using Horton's equation. Maximum and minimum infiltration capacity(mm/hr), coefficients of infiltration capacity decay and recovery (1/hr) and maximum infiltration volume are parameterized using characteristics of storms and soil type. Properties of the soil of Geylang was reported by Urban Redevelopment Authority (URA) of Singapore. The top layer is silty sand with gravels and silts; the thickness range is 6.0-7.9m [276]. The average of depression storage in the catch basin was assigned using recommended values for impervious and pervious areas described in ACSE manual of practice [274,277]. One of the critical parameters of the SWMM model is the characteristic width (W) of surface runoff path. This parameter value affects the properties of runoff hydrograph. We parameterized W using an appropriate tool in GeoSWMM that employs the following equation:

$$W = \frac{A}{L} \qquad (5.4)$$

where: W= characteristic width of the subcatchment (m); A=Area of subcatchment (m^2); L= length of channels in the subcatchment (m).

Intersection tool of ArcGIS was used to calculate the sum of the length of channels in specific subcatchment.

Code of practice on surface water drainage issued by Public Utility Board (PUB) of Singapore was used to assign the size, geometry and the bed gradient of the drainage network [278]. Hence, a set of open and closed U-shaped and trapezoidal drains were employed in the model. Identified surface cover in each subcatchment and reference values for the Manning coefficient described in PUB manual [278] were used to give an estimate for this parameter in each subcatchment.

A wet time step of 15-minutes was used in the simulations because areas of the subcatchments are small to account for fast response time to rain events. Conversely, a dry time step of 1-day was employed in the runs since Singapore has a wet climate.

5.2.1.3. Formation of potential breeding drains. Stagnation in selected storage units was designed by increasing inverted elevation of upstream conduits (i.e. analogous effect when littering dams the outlet opening of the drains), see **Figure 5.5**.

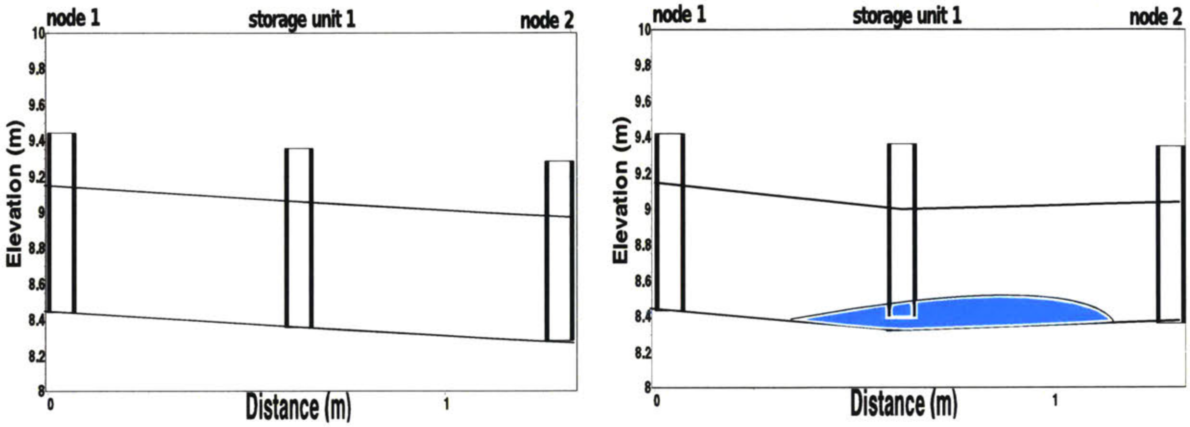


Figure 5.5. A water elevation sketch shows how clogged drains are modeled in HYDREDETS, a) no stagnation condition in a storage unit, b) stagnation condition in storage unit 1 designed by increasing inverted elevation of a downstream node 2.

Storage units are hydraulic nodes that allow ponding of excess flow atop the node and re-enter to the drainage system. The changes in stagnation volume (storage water) are governed by the following equations:

$$dS = A \times dh$$
$$\frac{dh}{dt} = \frac{Q_{in} - Q_{out}}{A} \qquad (5.5)$$

where: S= storage volume (m^3); Q_{in}= inflow (m^3); Q_{out}= outflow (m^3); h= the node head (inverted elevation+ water depth); A= surface area of the storage unit (m^2).

Storage units were originally introduced in SWMM to allow representation of detention ponds, simulations of riser inlets or elevated outlets (through weirs, orifices or other similar types of pipes). A depth-area relationship is required to parametrize fluctuation of the storage volume with the depth using either a tabular dataset or functional equation. Since the area-depth varies, we characterized this relationship using field observations (i.e., depth and area of clogged drains). Maximum depth of te storage units was also parameterized using field observations from

Geylang, Singapore. Allocation of potential habitats in the hydrology domain is based on the distribution of positive breeding drains from the field survey in 2014-2015 [14].

5.2.2 Entomology submodel

5.2.2.1 *Overview.* The structure of the entomology model is based on HYDREDEMATS model [150], while the parameters are derived from the accumulative research on CIMSiM model and related models [152,163,247]. Two objects are tracked in this model: individual adults and cohorts of aquatic stages (i.e., eggs, larvae, and pupae). The entomology module is connected to the hydrology component when gravid females seek breeding drains to oviposit eggs. While the aquatic cohorts advance towards emergence, they will be subject to nutritional and hydrological conditions of the breeding drains (i.e., flushing, drying, and stagnation). The entomology and epidemiology modules are coupled when adult mosquitoes stochastically get or give a dengue infection while seeking a blood meal from a human. Dispersal of adult mosquito population in the spatially explicit domain is influenced by mosquito behavior (blood-seeking and breeding seeking), wind speed and wind direction. Cycles of blood-feeding, resting and oviposition processes continue until the death of adults. The death of adult mosquitoes is also a stochastic process dependent on temperature and age. See Figure 5.6.

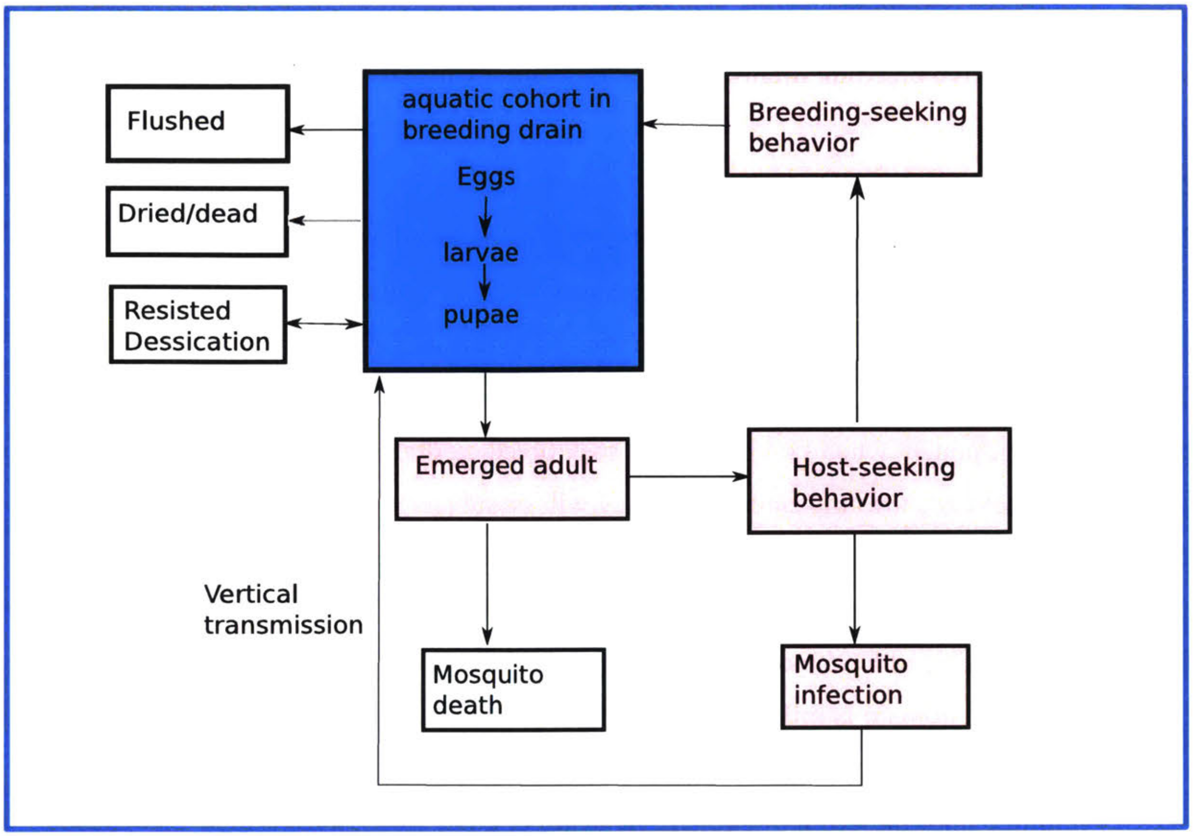


Figure 5.6. Structure of the entomology model of HYDREDETS. This mosquito population model simulates adult mosquitoes at individual level and aquatic stages as cohorts. Mechanistic effects of the hydrological conditions on breeding are incorporated in the model: flushing and drying of aquatic stages, and desiccation resistance of egg. Incorporation of vertical transmission in HYDREDETS is discussed in Chapter 6.

5.2.2.2 *development and parameterization*

Development of aquatic stages. Among the factors influencing the development of aquatic cohorts is water temperature. For this parameter, we used an empirical enzyme kinetic model for *Ae. aegypti* [125,152]. Progression of aquatic cohorts (i.e., from eggs, larvae, and pupae to adult emergence) is stochastically related to temperature-development rate by the following equations:

$$CD_t = \sum_{t=1}^{n} r_t\ (T) \qquad (5.6)$$

$$f(x) = \begin{cases} 0 & CD_t < a \\ \frac{CD_t - a}{b - a} & a \le CD_t \le b \\ 1 & CD_t > b \end{cases} \qquad (5.7)$$

where: CD= cumulative development rate, a and b are parameters for lower and upper thresholds for CD, f(x) is the probability of transition to the next aquatic stage.

We followed a previous approach to allow variability in advancing cohorts to the next stage using a uniform distribution conditioned to the range: $0.95 \leq CD \leq 1.05$ [247]. Also, air temperature influences adult survival, duration of the gonotrophic cycle, blood-meal frequency, extrinsic incubation period (EIP) of the virus, the probability of mosquito infection. We used temperature-dependent equations and parameters reported in previous works since they were tested and found to be reasonable in the tropics [123,247].

Flushing and displacement of aquatic stages. Flushing of the breeding habitats can affect the aquatic stages of *Ae. aegypti* in two ways:

1) displacement of aquatic stages from an upstream drain to a downstream drain using the following equations:

$$DD = v \times t$$

$$i = \begin{cases} i & DD \leq DD_{min} \\ 0 & DD > DD_{max} \\ i + DD & DD < DD_{max} \end{cases} \tag{5.8}$$

where: i= spatial index of a grid cell location; DD= Distance displaced (m); v=flow velocity (m/h); t= time (h); DD_{min} = minimum threshold distance(m); DD_{max} = maximum threshold distance(m). The zero value of the index i means that the cohort is flushed out of the spatial domain.

2) washing of the aquatic stages out of the system, which is incorporated in HYDREDETS by the following equations:

$$n_{(t+1)} \times V_{(t+1)} = n_{(t)} \times V_{(t)} \tag{5.9}$$

$$N_{(t+1)} = \frac{n_t \times V_t}{V_{(t+1)}}$$

where: n= mosquito density (No. mosquito/m^3); V = water volume in the drain (m^3); R= Rainfall intensity (mm/hour); A= Area of the drainage section (m^2).

Drying and desiccation resistance of eggs. We assumed that drying of a breeding drain does not immediately result in killing of larvae and pupae. Instead, these aquatic stages can tolerate dryness and survive for some period. Hence, we investigated a range of intervals between one hour and 72 hours for this assumption. Further, eggs of *Ae. aegypti* can survive desiccation conditions for several months [24]. However, the span of this desiccation time varies and is specific to mosquito populations. We incorporated desiccation resistance of eggs using the following equation:

$$E_t = \begin{cases} E_{t-c}, & V_t = 0 \wedge t < c \\ 0, & V_t = 0 \wedge t \geq c \end{cases} \qquad (5.10)$$

where: E= eggs cohort at time t, V= water volume in the drain (m^3), c= threshold time for surviving the desiccation period.

Oviposition. The relationship between oviposition behavior and physical attributes of breeding habitats is complex, and is influenced by larval food. Modeling of mosquito oviposition is revised to reproduce an inverse relationship between water depth and eggs laying by gravid mosquitoes. We neglected the effect of presence of conspecific/congeners in breeding habitats because inconsistent results had been reported for the effect of this factor on the oviposition behavior of *Ae. aegypti* [279,280]. Instead, we assumed that probability of a gravid female to lay eggs into drains is a function of water depth as described in [150,164]. Hence, a conditional probability function is used to allow oviposition:

$$f(x) = \begin{cases} 0 & d_t < a \\ k & a \leq d_t \leq b \\ k - \left(\frac{d_t - b}{c - b}\right) & b \leq d_t \leq c \\ 0 & d_t > c \end{cases} \qquad (5.11)$$

where: d= water depth in a drain (m); a, b and c are parameters for the minimum, upper and maximum limits of water depth to allow the laying of eggs in drains, k= constant probability.

Carrying capacity. Since inflows increase the nutritional content of drains but with a delay (that allows decomposition of organic material), we assumed that the maximum carrying capacity of breeding drains is a function of flushing in a previous interval. To simplify, constant functions for two different hydrological conditions are employed:

$$K_t = \begin{cases} C_1, & V_t < V_{t-n} \\ C_2, & V_t \geq V_{t-n} \end{cases} \qquad (5.12)$$

where: K= Maximum carrying capacity of a drain (g/m^3); n= delay interval, C_1 and C_2 are user-defined parameters (g/m^3) and $C_1 > C_2$.

Effect of larvae density on development. For the effect of larvae density on development, we followed the same approach in [150,151] to modify the growth rate –described in equation 5.6 --

as a function of the ratio of the overall biomass of larvae (i.e. 1st-4th instars of *Ae. aegypti*) to the carrying capacity of the drain:

$$B_t = \sum_{i=1}^{4} l_i w_i \qquad (5.13)$$

$$r = r\times\left(\frac{K_t - B_t}{K_t}\right)$$

Where: r_t = development rate (1/hour); B=the overall larval biomass in a drain (g/m^3); i= the four larval stages (I, II, III, and IV); L= total of larvae stage in the container; w= specific weight of larval stage.

We also assumed that the development rate r= 0.001 in breeding drains that have an overall biomass equal or greater carrying capacity (i.e. development does not entirely stop).

Effect of larvae density on mortality. Parameters for a minimum non-density mortality (m_{min}) for eggs, larvae and pupae are obtained from previous studies [164,247] – see Table 5.3. Also, we added a density-induced equation to account for both intra- and interspecific mortality:

$$m_i = m_{min} + \left(\frac{B_t}{K_t}\right) \qquad (5.14)$$

Where: m_i= mortality of larval cohort i.; m_{min}= minimum mortality rate (/hour).

Dispersal of adult mosquitoes. The modeling approach described in [270] for mosquito dispersal was modified with parameters that consider the diurnal activity and flight velocity of *Ae. aegypti* [24,128]. Dispersal of adult mosquitoes is a function of random flight (i.e. to find a blood meal source or breeding habitat) and influenced by wind speed and direction. Data from a mark-release-recapture study conducted previously in Singapore was used to parameterize the random flight of mosquitoes [224].

5.2.3 Epidemiology submodel

5.2.3.1 *Overview.* We used the classic modeling approach of Susceptible-Exposed-Infected-Recovered (SEIR) used for dengue transmission to build an agent-based model [11,164,165,281]. Hence, human individuals are explicit in the model domain with attributes of age, gender, and house location. The model tracks epidemiological changes of the population at an individual level. Hence, once a person is infected, the model continues to monitor for primary, secondary, tertiary and quaternary infections. While a permanent immunity is conferred when a human gets infected with specific serotype, a temporary cross-immunity to heterologous serotype is simulated after the recovery [155,254,281–283]. A schematic representation of the states and transitions of an individual is shown in **Figure 5.7**.

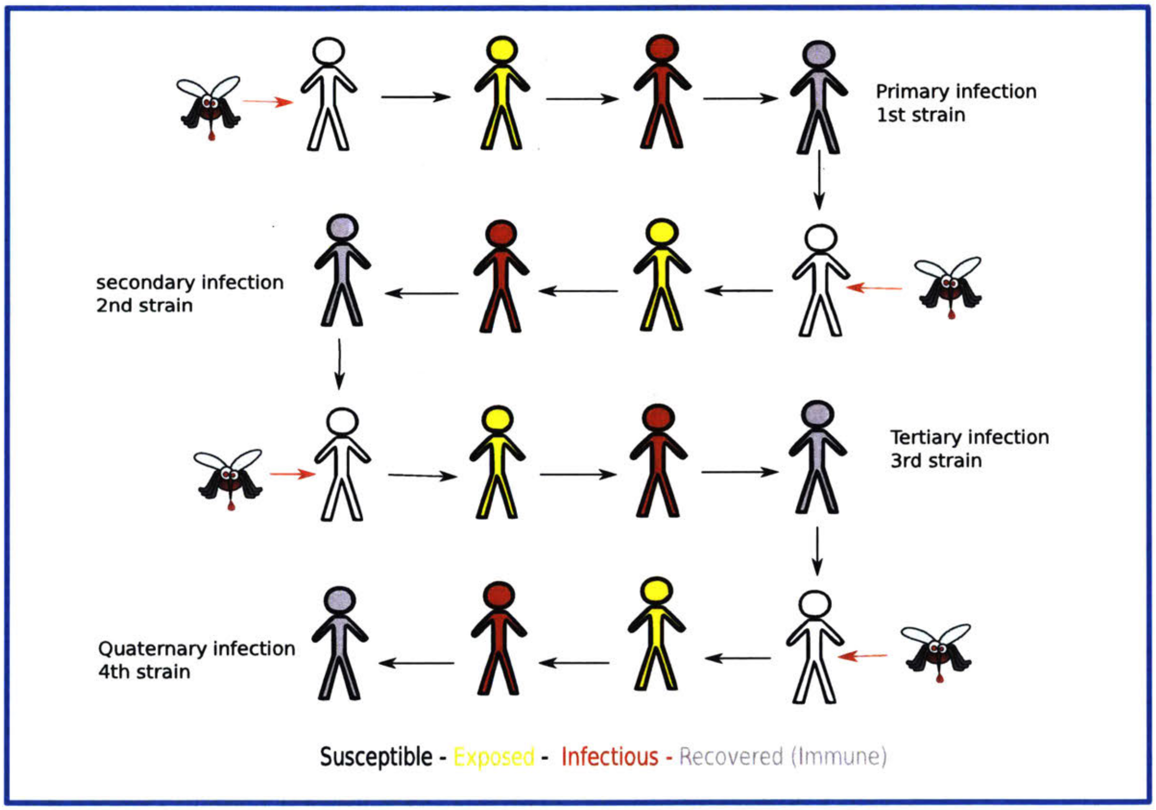


Figure 5.7. Structure of the epidemiology model of HYDREDETS. The model is based on the Susceptible-Exposed-Infected-Recovered (SEIR) approach. Susceptibility of an individual to the four dengue virus strains is considered in the model.

5.2.3.2 *Development and parameterization.* Parameters of virus incubation period in humans, the length of the infectious period, and duration of cross-immunity for the four strains were obtained mainly from the work on DENSiM [123,124,247], see **Table 5.4.**

Both human and mosquito infections are a stochastic individual-to-individual process. Among the human factors that influence dengue transmission are strain-specific-viremia, duration of human infection, and immunity [284]. Here, we consider these factors in the model. The probability of human infections stochastically depends on the number of infectious mosquitoes in a grid.

Similarly, probability of a mosquito to get a dengue virus while feeding on an infectious person depends on the number of infected persons in the block and temperature [196]. Once a mosquito becomes infected, it remains infected for the rest of the life. The extrinsic incubation period (EIP) of the dengue virus in the mosquitoes is a temperature-dependent process. For this, we used a thermo-kinetic model described in [247] to generate a development rate for the virus in mosquitoes. We also assumed that infection does not reduce life span of the individual mosquitoes. Moreover, a parameter of vector competence that depends on temperature and daily temperature range (DTR) was added to the model [196].

Modeling the population immunity. The immune individuals can become susceptible to another strain after the cross-immunity period. We considered a modification to an immunology model described in [285]. Hence, two conditions occur to human infections: 1) immunity increases when infected persons get a sequence of infectious bites; 2) immunity decreases when an infected person does not get an infectious bite. In addition, we set the maximum duration of cross-immunity to 24 months following the findings of a recent work that estimated a heterologous infection confer an immunological protection for a range of 12-36 months [234].

5.2.4 Model domain

HYDREDETS is a discrete event simulation model in both space and time. The spatial domain of the model is gridded in two-dimensional planar surface cells. Size and number of grids are flexible to adjust to the shape of the study area. The domain contains user-defined grids of outdoor breeding habitats, houses and non-residential (e.g., parks, public or commercial). The number of humans in a block depends on population distribution of the study area. The temporal domain allows coupling of processes of daily-parameters with sub-daily (e.g., hourly) ones through calls of time-variant-functions.

5.2.5 Model Calibration

5.2.5.1 *Hydrology submodel.* The water level of a particular drain in Geylang was recorded between August 2014 and August 2015 using a HOBO data logger [14]. This drain receives a continuous base flow. We calibrated a storage unit mapped in the hydrology submodel using physical dimensions (i.e. depth of the drain, depth of base flow during dry period, Manning roughness value for the drain material) and time series of water level in the drain. Other time series used in calibration process and for the same period include rainfall and water temperature, and averages of wind speed. In addition, monthly average of evaporation rate (/day) obtained from [286] was used to calibrate this parameter in SWMM , see **Figure 5.8**.

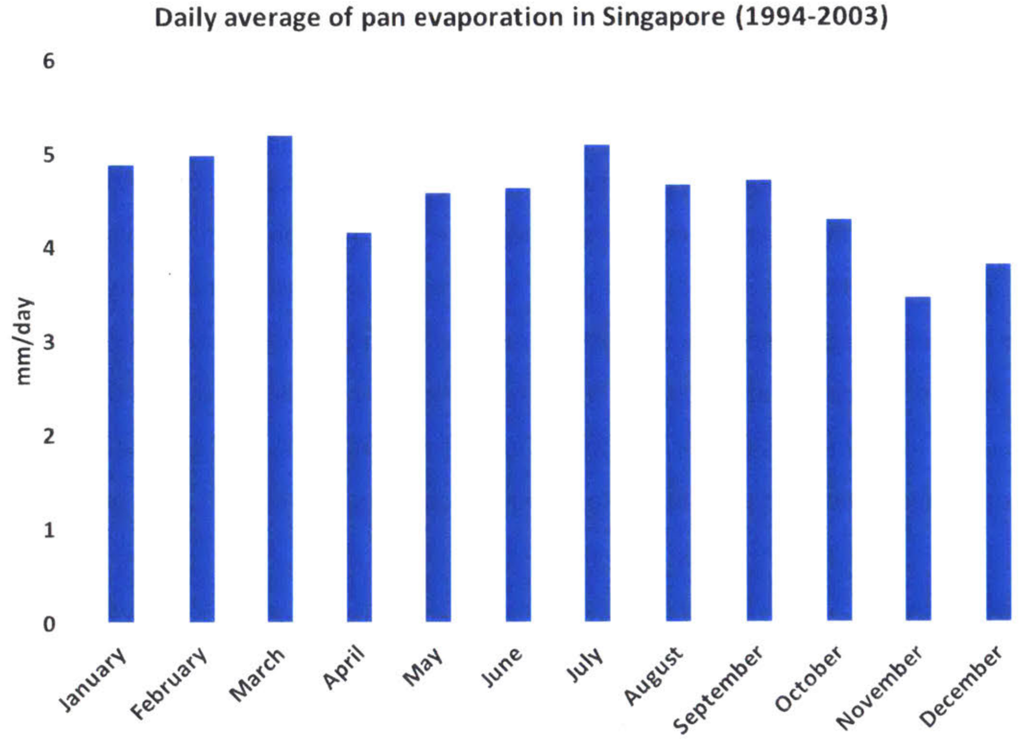


Figure 5.8. monthly average of evaporation rate for an evaporation pan, Changi airport, Singapore (Modified after [286]).

To find the appropriate time step for the simulations, we followed the method described in [287]. Hence, we tested different time steps for a single event till finding that one gives a minimum continuity error. The following time steps were used in SWMM: reporting step= 15 minutes; wet step = 15 minutes; dry time step= 24 hours.

5.2.5.2 *Entomology submodel.* We calibrated the entomology model using data from an entomological survey conducted in Geylang August 2014- August 2015. In this survey, semiweekly densities of larvae and pupae of *Ae. aegypti* in breeding drains were measured; further details are in [14]. Other datasets used in calibrations included rainfall, air temperature, relative humidity, wind speed and wind direction, obtained from the close meteorological station Tanjong Katong, for the same period. We compared the seasonal abundance patterns of the vector between the observations and simulations.

5.3 Results

5.3.1 Study area. Geylang neighborhood (1.32° N, 103.89° E) was selected for this study (see **Figure 5.4**). The area is hyperendemic with a continuous transmission of dengue. The total population of Geylang—as of 2015—is approx. 32,000. Geylang is a part of the Kallang Formation which consists of the Layer of Marine Clay and Old Alluvium [288]. The rectangular area of the spatial domain is 1.2 km^2 with a grid cell dimension of 10×10 m^2, and the total is 12000 grid cells. In addition to outdoor breeding data, we used SLA cadastral data to assign grid cells into potential breeding, housing, public/commercial, and open space ones. Census data of the Geylang in 2006 was obtained from Department of Statistics (DOS) of Singapore. Birth rate and death rate were retrieved from this DOS report were used in the simulations. Also, age-structure of the population of Singapore was used to parameterize the age structure of the human population of the epidemiology submodel. We allocated resident population of the Geylang to the housing grid cells (**Figure 5.9**).

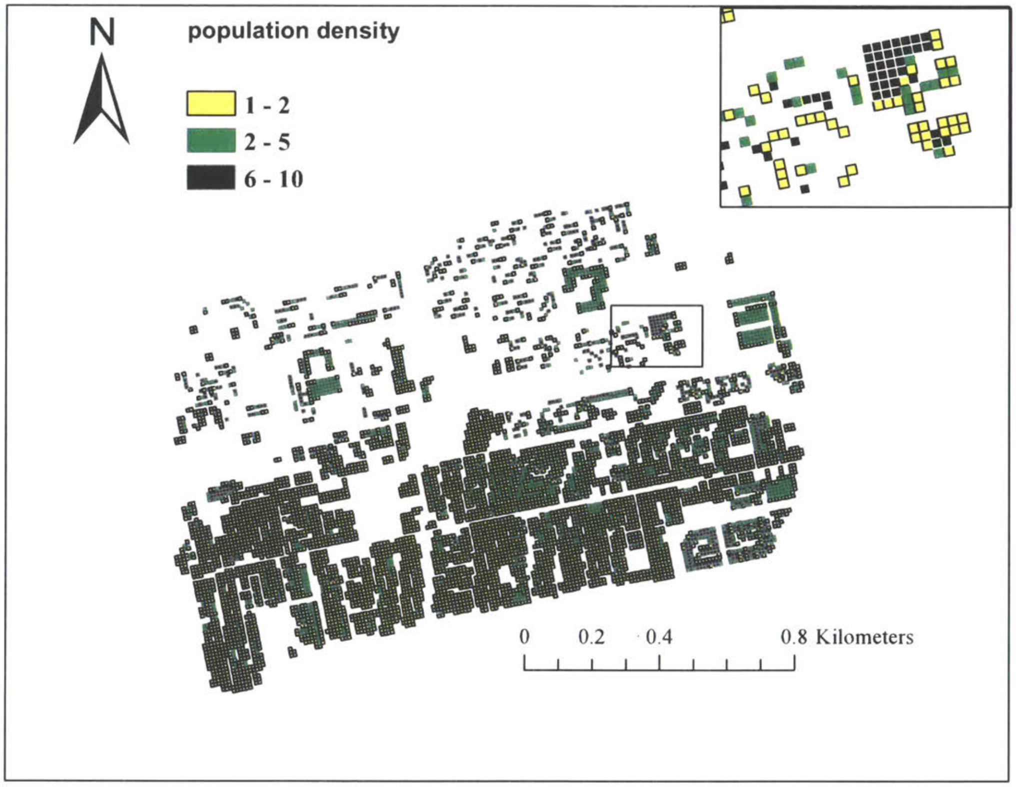


Figure 5.9. Population distribution of Geylang gridded to the spatial domain of HYDREDETS (grid cells of 10x10 m^2).

5.3.2 Hydrology

Results of the calibration of the hydrology submodel using the water level dataset are presented in **Figure 5.10** and **Figure 5.11**. A direct correlation between recorded rainfall and simulated water volume in the stagnant drain (see **Figure 5.10)**. **Figure 5.11** shows a good agreement between the observed and simulated water level of the storage node (R^2=0.73). The magnitude of water level is slightly higher for the observations compared to simulated ones.

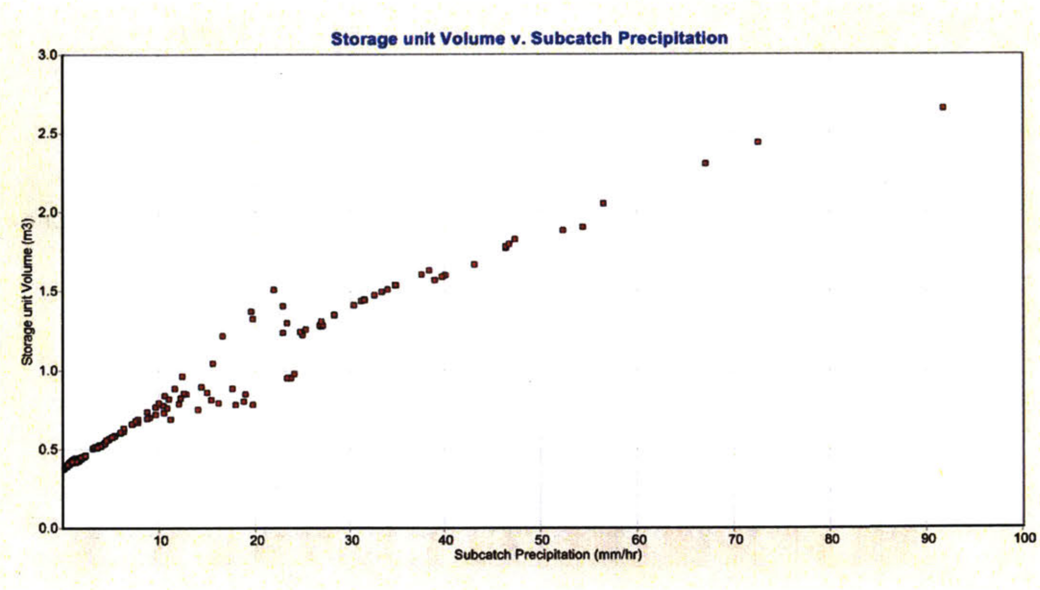


Figure 5.10. Simulation of water level of stagnant drain versus recorded rainfall in Geylang neighborhood (August 2014- August 2015).

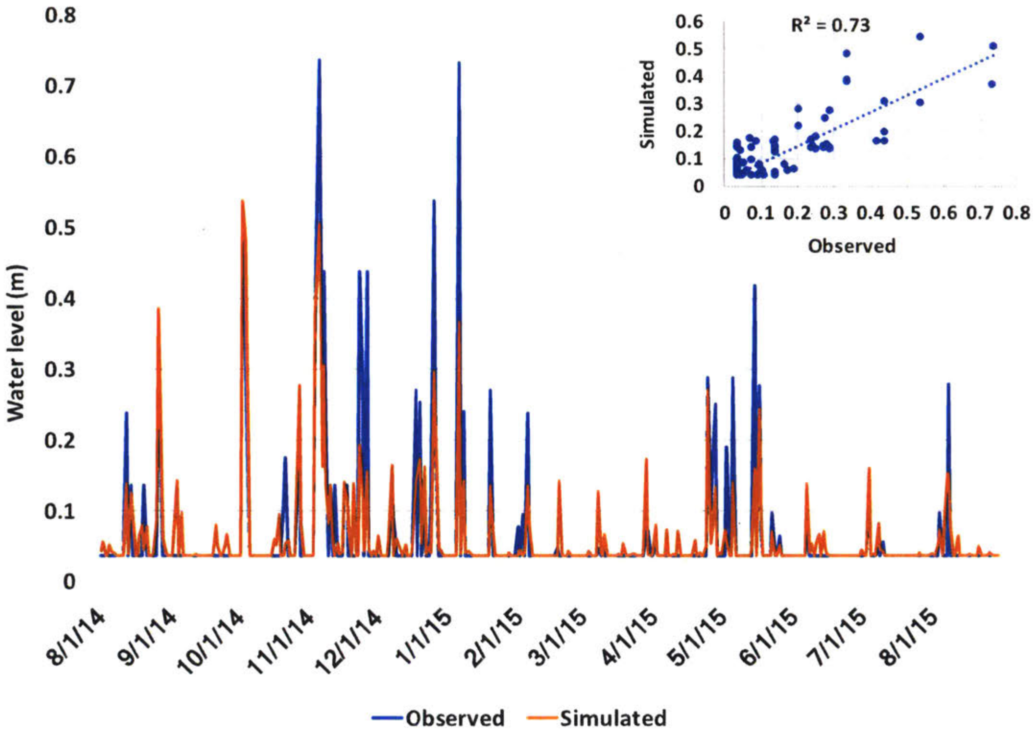


Figure 5.11. Observed and simulated water level of a stagnant drain in Geylang neighborhood (August 2014- August 2015).

5.3.3 Entomology

The entomology model was calibrated using the pupae and larvae datasets of Geylang. Pupae and larvae of *Ae. aegypti* show a good visual correspondence between the observations and simulations in their seasonal abundance (see **Figure 5.12**). The decline in abundance of the aquatic stages between November 2014 and January 2015 was reproduced by the model. HYDREDETS also has successfully simulated the impact of the seasonal drying conditions between February and May 2015 that led to the cessation of outdoor breeding of *Ae. aegypti*. However, since the field dataset was obtained using sampling techniques, the magnitude of the total numbers of pupae and larvae simulated was higher compared to the observations.

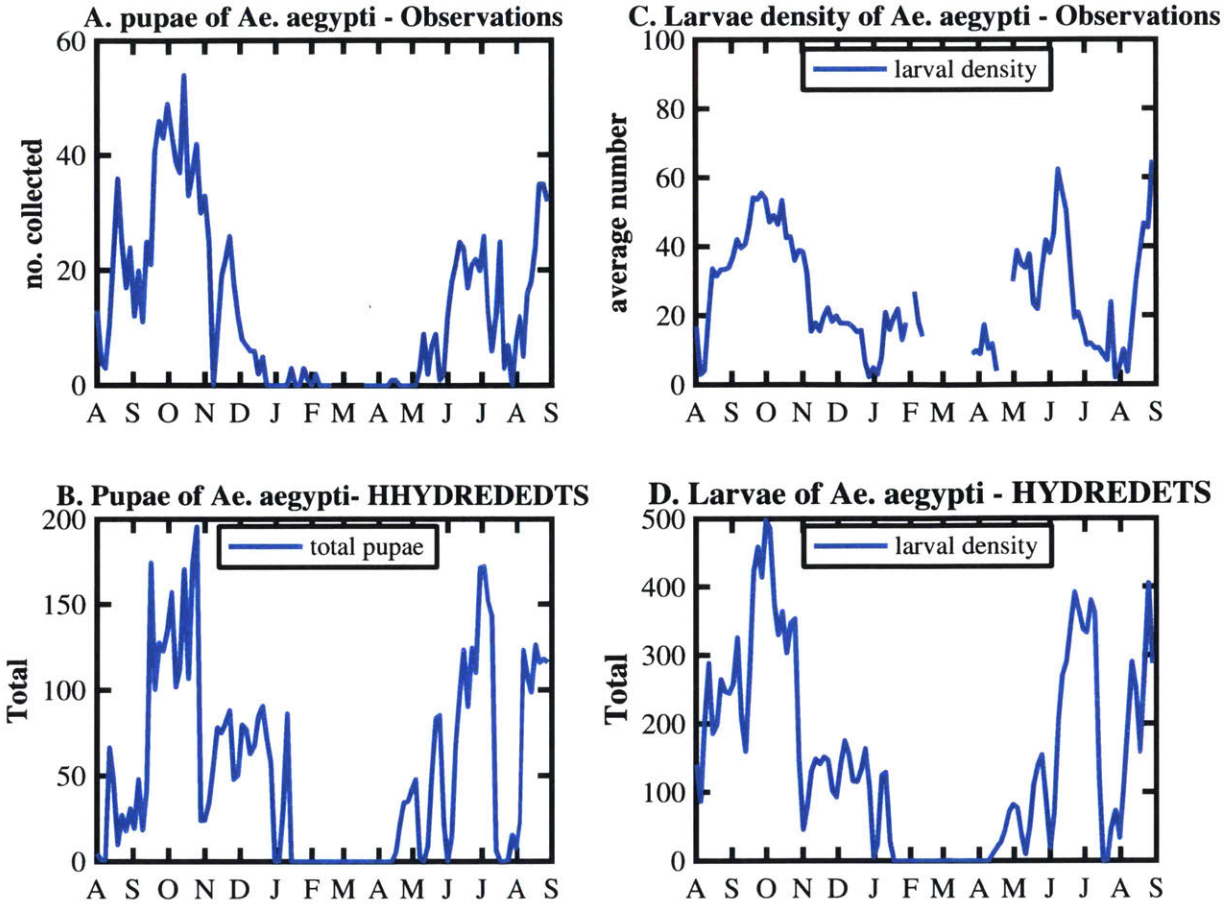


Figure 5.12. Calibrations of the entomology submodel of HYDREDETS (August 2014 – August 2015). A. Pupae of *Ae. aegypti* sampled from drains; B. total of simulated pupae; C. larval density of *Ae. aegypti* in drains, and D. total of larvae simulated. [Observation data after Seidahmed and Eltahir (2016)].

Flushing of aquatic stages. **Figure 5.13** shows rainstorm events had led to the flushing of the aquatic stages particularly between November 2014-January 2015 (i.e. the early phase of the Northeast monsoon). This seasonal process is verified by: the daily flushed proportion and total numbers, in the top and bottom subplots, respectively. While the upper panel of the figure shows that the frequency of the proportions flushed in November-January was higher compared to August-October, flushed numbers -- shown in the lower panel -- were higher in the latter period. This finding reflect that more vector is abundant during August- October.

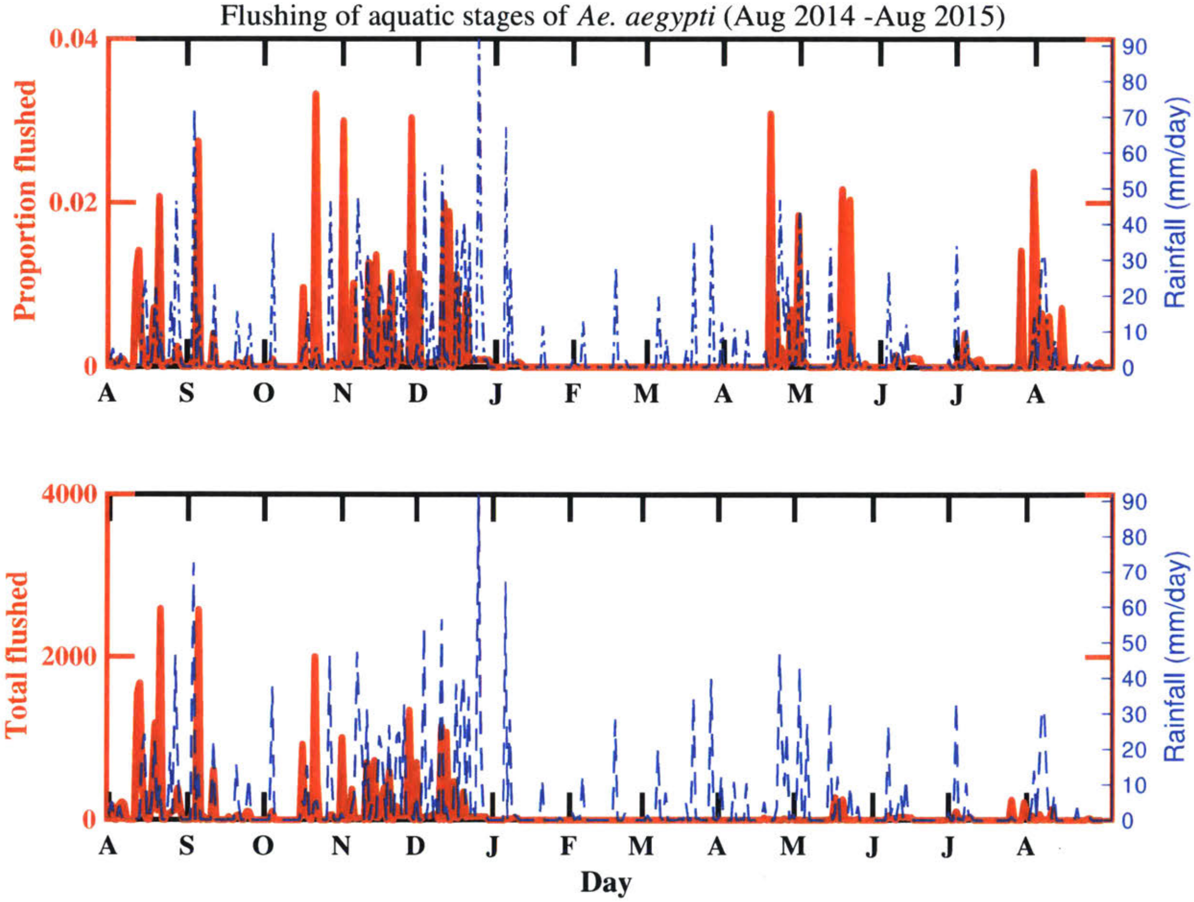


Figure 5.13. Daily flushing of aquatic stages of *Ae. aegypti* in Geylang neighborhood (August 2014 – August 2015) simulated using HYDREDETS. More flushing events had occurred between November-January (i.e. the early phase of the Northeast monsoon).

Displacement of aquatic stages. Similarly, displacement of aquatic stages during flushing events was farther during the wettest period: November-December 2014 compared to the summer season: July-October. The displacement was verified by the total distance displaced by all flushed cohorts (see **Figure 5.14**). The figure also shows the water velocity in a link (conduit) using the hydrology submodel.

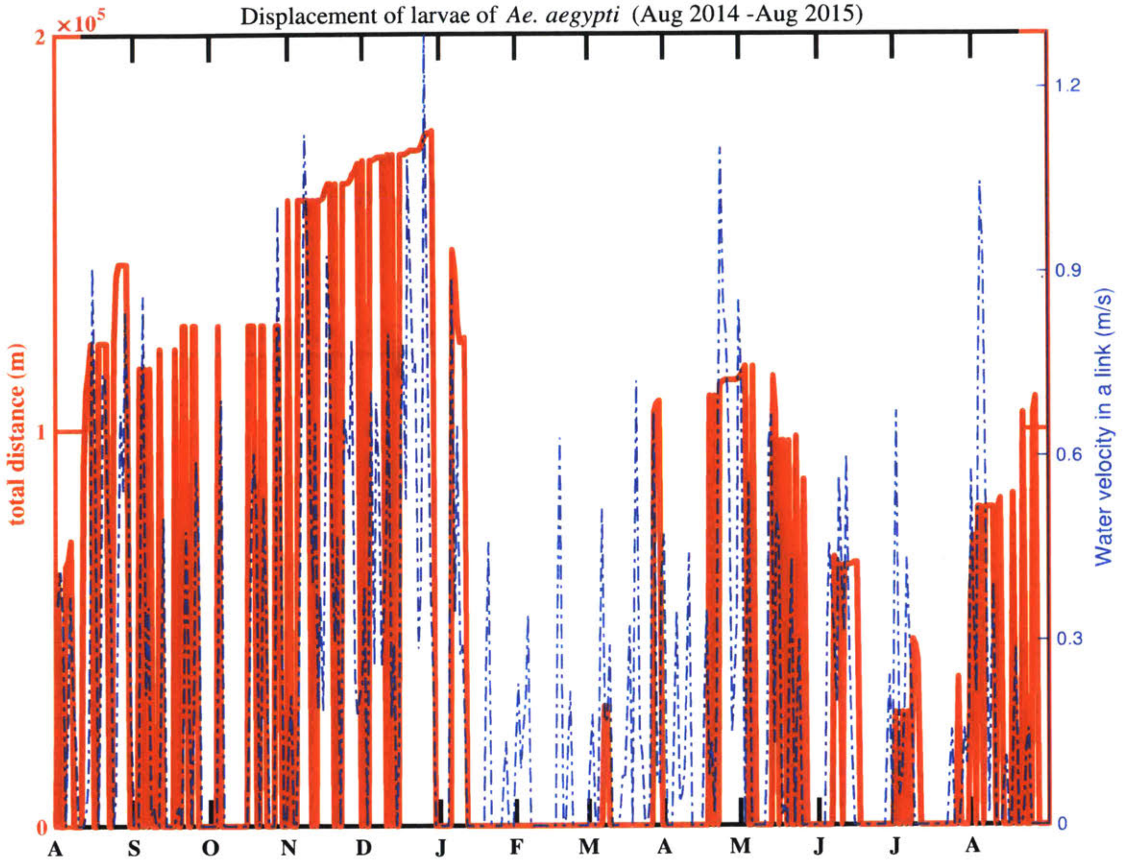


Figure 5.14. Daily displacement of aquatic stages of *Ae. aegypti* in Geylang neighborhood (August 2014 – August 2015) simulated using HYDREDETS. Total distance of displacements (meters) was larger between November-January (i.e. the early phase of the Northeast monsoon).

Desiccation resistance of eggs. **Figure 5.15** shows the daily number of desiccation-resistance eggs of *Ae. aegypti* (i.e. eggs that survived a dryness period) during the study period August 2014 – August 2015. Accordingly, the main drying conditions in breeding drains occurred during the late phase of the northeastern monsoon in 2015 between late January – early April. The largest number of eggs rescuing mortality had happened during this period. In addition, the El Nino episode mid of July 2015 had also resulted in a spike of desiccation-resistant eggs.

Dryness -mortality of larvae. **Daily average number of dried larvae of *Ae. aegypti* in Geylang neighborhood, simulated using HYDREDETS, is shown in Figure 5.16. The maximum**

mortality of larvae -- due to dryness -- had happened in January when drying conditions prevailed due to the late phase of the Northeast monsoon.

***Adults abundance*. Moreover, abundance of adult population of Ae. aegypti between August 2014 and August 2015 is shown in Figure 5.17. Accordingly, the decrease in mosquito adult abundance occurred between January and June when drying conditions prevailed due to the late phase of the Northeast monsoon.**

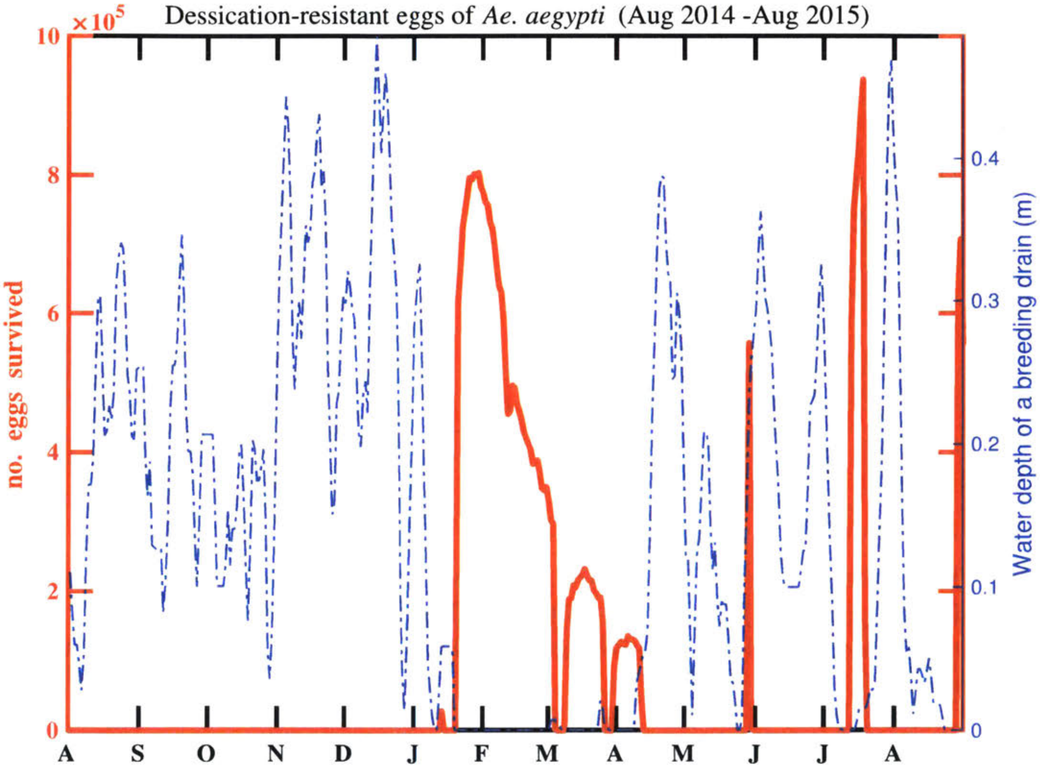


Figure 5.15. Daily number of desiccation-resistant eggs of *Ae. aegypti* in Geylang neighborhood (August 2014 – August 2015) simulated using HYDREDETS. Eggs survived the driest period between February-April (i.e. the late phase of the Northeast monsoon).

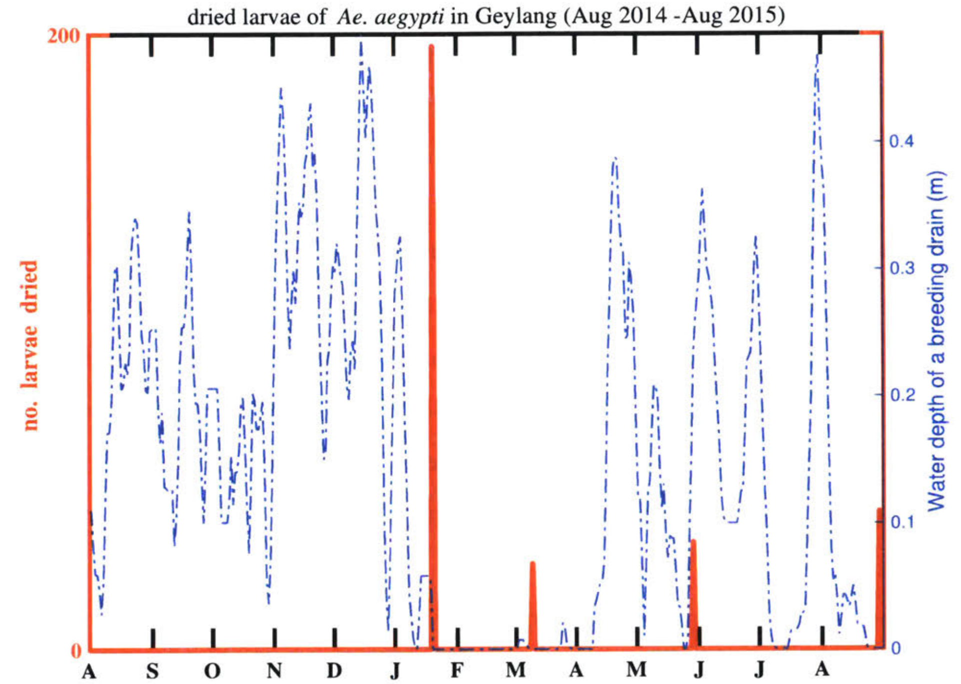


Figure 5.16. Daily average number of dried larvae of *Ae. aegypti* in Geylang neighborhood (August 2014 – August 2015) simulated using HYDREDETS. The maximum mortality of larvae -- due to dryness -- happened in January when drying conditions prevailed due to the late phase of the Northeast monsoon.

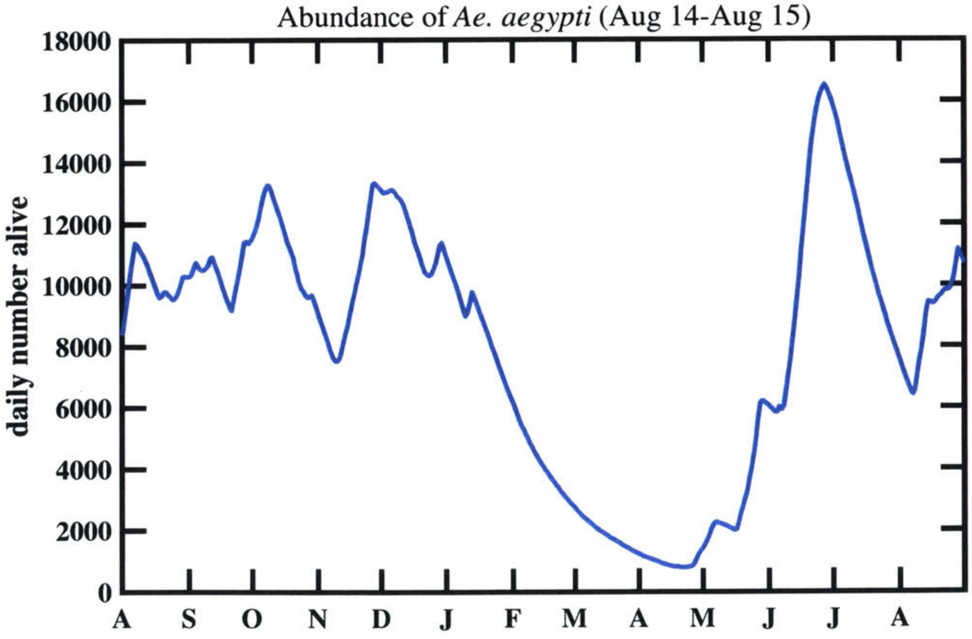


Figure 5.17. Daily average number of adults of *Ae. aegypti* in Geylang neighborhood (August 2014 – August 2015) simulated using HYDREDETS.

5.3.4 Epidemiology

The median duration of dengue transmission is calculated as the time between reported dengue cases in identified clusters. According to NEA (Singapore) this period varied between the years 1990-2015 with a range 4-20 days and overall median of 7 days (see **Figure 5.18**). We used this parameter to calibrate the epidemiology submodel (see **Figure 5.19**). Overall, the simulated median duration is 7 days, however, the infectious period fluctuates between the months. This calibrated parameter is also within the range of dengue viremia in symptomatic cases reported in previous works [30].

In addition, simulated dengue cases during the study period showed a seasonal pattern with a decline in cases between November and June (see **Figure 5.20)**. We compared this pattern to reported dengue cases in Geylang neighborhood and Singapore (see **Figure 5.21** and **Figure 5.22).** Interestingly, reported cases at the country scale shows a seasonal profile similar to that one simulated by HYDREDETS (**Figure 5.22)**. In contrast, HYDREDETS failed to capture the reported cases at the neighborhood scale which showed a continues decline after the onset of the Northeast monsoon (**Figure 5.21**). The disease surveillance of dengue in Singapore is a real-time notification system for new cases. However, the system is challenged by underreporting of asymptomatic cases. This limitation affects the verifiability of epidemiological measures at a neighborhood scale more than the country level. Further, persistence of the virus at a neighborhood scale is subject to several epidemiological and entomological factors. For example, presence of immediate susceptible or infectious individuals that could better drive the disease at the country level. The impact of the spatial scale on disease spatiotemporal patterns needs further work at the future. On the other hand, if the seasonal profile of vector abundance happens at the country scale, it is likely that HYDREDETS is capable to simulate dengue seasonality in Singapore.

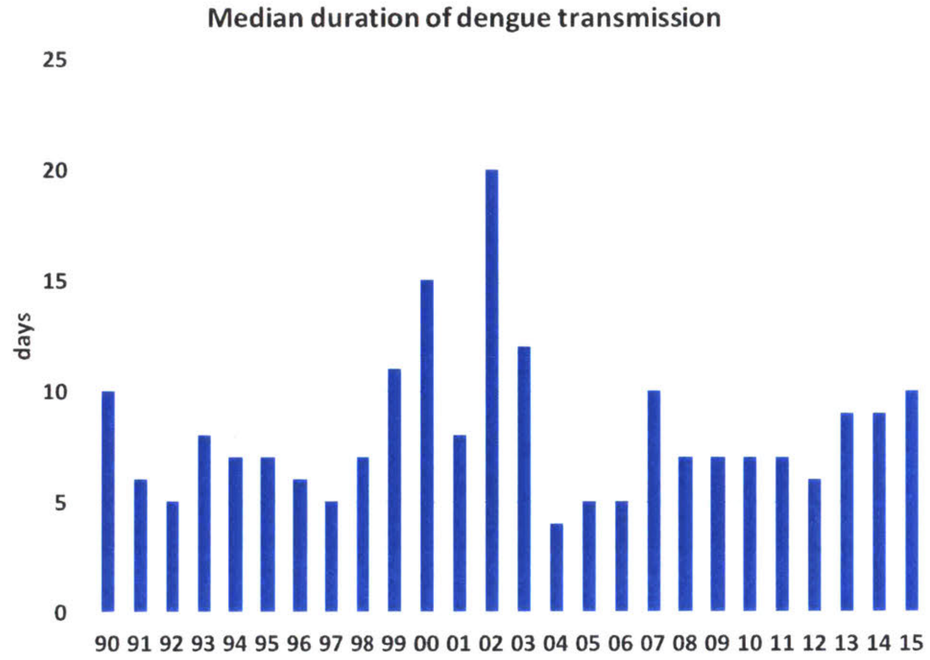


Figure 5.18. Median duration of dengue transmission in Singapore (1990-2015). Data source: Ministry of Health, Singapore.

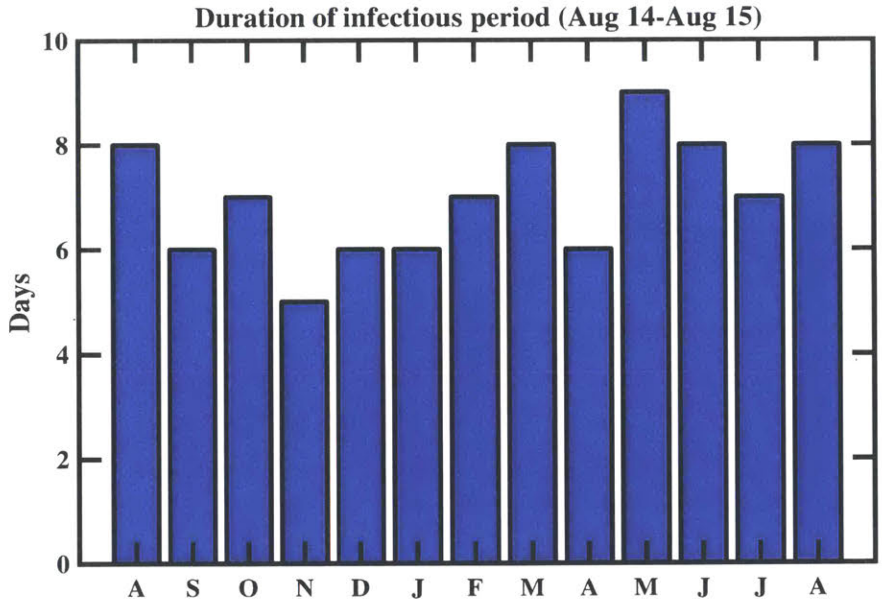


Figure 5.19. Median duration of dengue transmission simulated by HYDREDETS (August 2014 – August 2015).

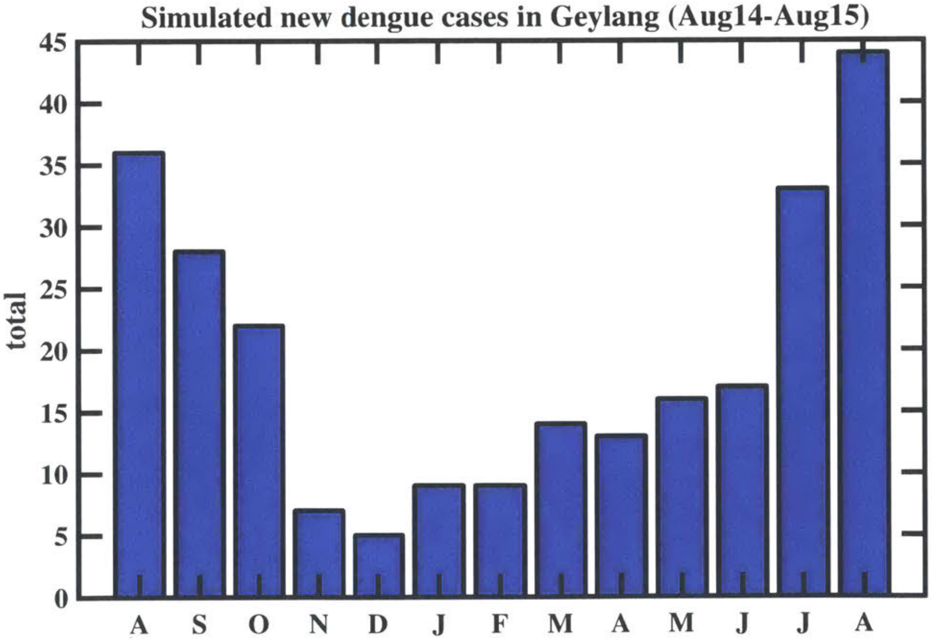


Figure 5.20 Simulation of new dengue cases in Geylang neighborhood (August 2014 – August 2015).

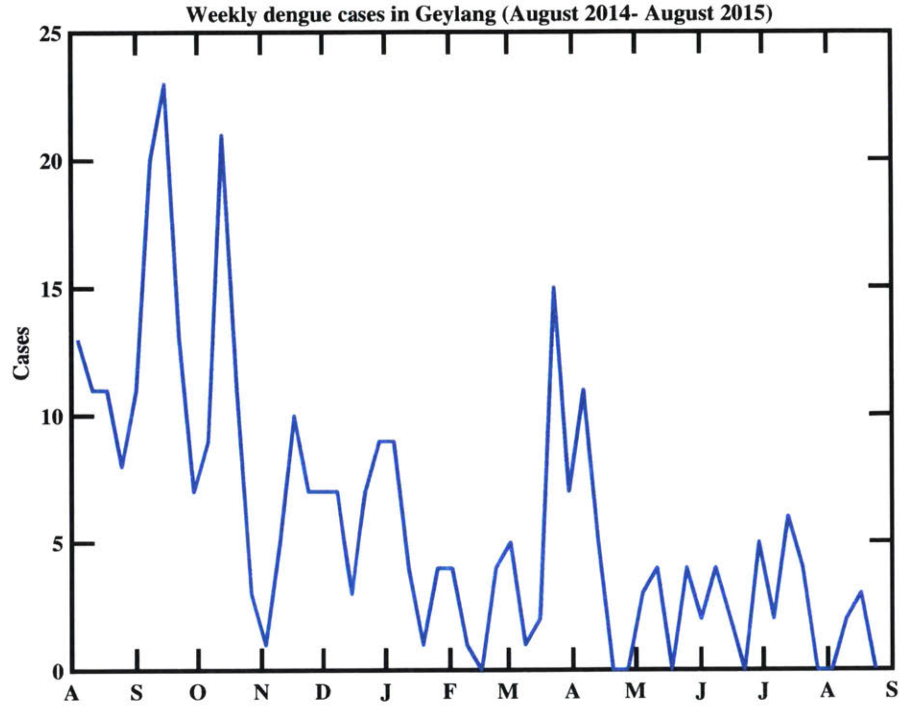


Figure 5.21 Weekly cases of dengue in Geylang neighborhood (August 2014 – August 2015). Data source: Ministry of Health, Singapore.

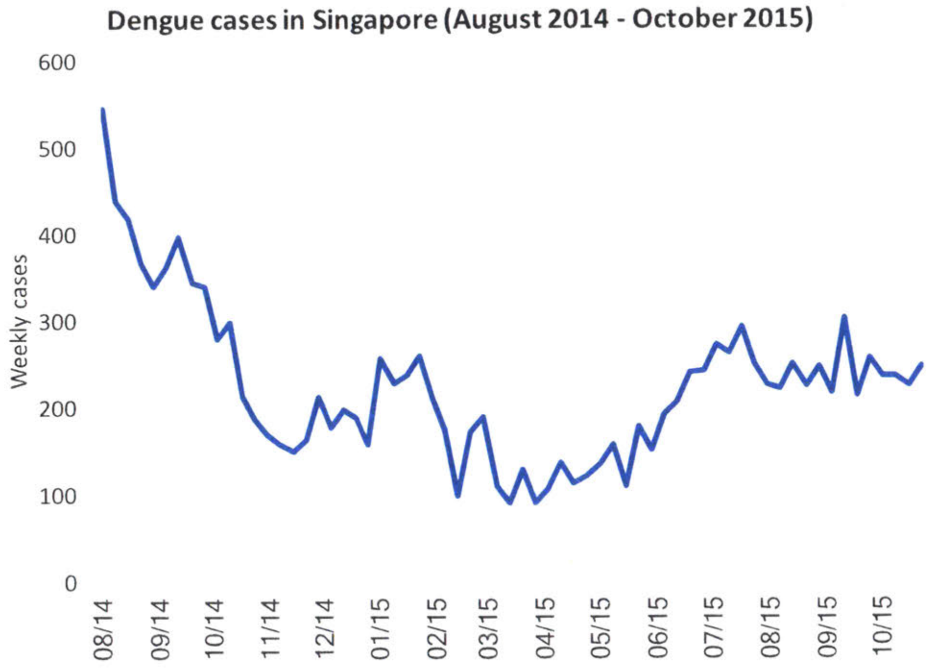


Figure 5.22 Weekly reported cases of dengue in Singapore (August 2014 – October 2015). Data source: Ministry of Health, Singapore.

5.4 Discussion

In this study, we developed a modeling framework to: 1) simulate breeding of dengue mosquito in urban drains using SWMM model for urban hydrology, 2) simulate human population in a spatial domain, 3) couple humans, adult mosquitoes and dengue virus strains to simulate the disease transmission. Few dengue models mechanistically had simulated breeding habitats of the vector such as CIMSiM/DENSiM [123,124], Skeeter Buster[163], and a new model by Karl and others[164]. However, these models contain discrete water containers (e.g., barrels and buckets) either indoor or outdoor. Up to our knowledge, this is the first modeling framework that investigate the role of urban hydrology and drainage systems in the transmission of dengue or other mosquito-borne diseases.

Several studies from Latin America, Australia, and Southeast Asia have demonstrated that subterranean structures of storm water are significantly contributing to the abundance of the dengue vector in urban areas [14,190,216]. In a vector survey conducted in a Mérida City (México), investigators reported a massive breeding of *Ae. aegypti* in the sewer system. This breeding was mainly attributed to improper connections in the conveyance network as well as littering [289]. However, the importance of this type of breeding habitats in dengue transmission is poorly addressed in epidemiological studies as well as in public health and vector control studies.

The neglected role of storm drains in dengue transmission is mainly due to the tendency of vector control and surveillance programs to focus on inspection of man-made indoor breeding containers. For example, a classic response to a dengue outbreak is to identify houses of infected people and deploy vector control teams to inspect and eliminate indoor breeding containers and discarded receptacles outdoor. However, entomological monitoring of storm drains is difficult, and the teams usually address by random sampling. Hence, HYDREDETS can strengthen the vector surveillance of municipalities and urban areas.

In contrast, HYDREDETS distinctively gives attention to the urban hydrology and the role of the drainage systems in dengue transmission. The model accounts for the impact of this breeding habitat on dengue transmission in urban areas. We showed here that HYDREDETS allows studying storm-effects such as seasonal flushing/drying of larvae and pupae and re-inoculation of the drains by dormant eggs in the following stagnation conditions. Modeling these hydrological-dependent processes was possible using SWMM model.

During the last forty years, the EPA SWMM model has been extensively used in design and operation of combined or separate drainage systems in the urban areas. The model has been reported to be sensitive to inverted elevation of the storage unit, Manning roughness value, and depth of water in upstream conduits [273]. In urban areas, hydraulic slope of the drainage systems usually is less steep compared to that one for undeveloped landscape. This is partly attributed to the presence of buildings which enforce sometimes the flow direction opposite to the natural flow, as well as loss of energy associated with surface roughness (i.e. Manning value). A sensitivity analysis for the hydrological and hydraulic parameters of SWMM, applied on catchment area in Istanbul city, had shown that the model is more sensitive to characteristics of the storms, catchment area, roughness of the conveyance structures compared to imperviousness and slope [290]. One of the limitation of this study is the lack of field measurements of these parameters specific to the study area. However, we used the best available information about these parameters recommended by SWMM, PUB and ASCE manuals.

In conclusion, HYDREDETS fills a gap in the epidemiological modeling of vector-borne diseases related to management of storm water. We showed that SWMM and other urban hydrology models can be useful in studying vector-borne diseases and public health. This direction will open the door to consider vector control within the guidelines and operating protocols of the urban drainage networks. In a previous work, we proposed to optimize vector control in Singapore by selection of interventions according to the monsoon time (see **Table 3.2**). Further, we suggest including the operation protocols of the drainage systems as environmental interventions. For example, rerouting the outflow path in neighborhood during the summer season into shallow or clogged drains could reduce breeding of *Ae. aegypti* in these subterranean habitats.

Table 5.3. Parameters used in the entomology submodel of HYDREDETS

Stage	parameter	Distribution	unit	Default (min, max)	certainty	Reference
Eggs	Development rate of eggs	uniform	1/hr	Temperature-dependent equation (0.017, 0.027)	High	[123,152]
Eggs	Minimum hatch temperature	Uniform	℃	22 (13.5, 26.4)	High	[123,152,247]
Eggs	Nominal survival	Uniform	/day	0.99 (0.792, 1)	High	[123,152,247]
Eggs	High-temperature threshold	Uniform	℃	30 (20.6, 38.5)	High	[123,152,247]
Eggs	Survival at or above high lethal temperature threshold	Uniform	/day	0.05 (0.04, 0.06)	High	[123,152,247]
Eggs	duration for desiccation resistance	uniform	month	6 (1,12)	Low	[24]
Eggs	High lethal temperature threshold	Uniform	℃	47 (38.5, 56.4)	High	[123,152,247]
Larvae	Development rate of larvae	uniform	1/hr	Temperature-dependent equation (0.005, 0.013)	High	[123,152,247]
Larvae	Dry container survival	Uniform	/day	0.05 (0.04, 0.06)	High	[123,152]
Larvae	Minimum weight for survival	Uniform	mg	0.0009 (0.00072, 0.00108)	High	[123,152,247]

Larvae	Nominal survival	Uniform	/day	0.99 (0.792, 1)	High	[123,152,247]
Larvae	Minimum weight for pupation	Uniform	mg	0.1 (0.08, 0.19)	High	[123,152,247]
Larvae	Pupation survival	Uniform	/day	0.95 (0.76, 1)	High	[123,152,247]
Larvae	High-temperature threshold	Uniform	℃	39 (30.2, 41.5)	High	[123,152,247]
Larvae	Survival at or above high lethal temperature threshold	Uniform	/day	0.05 (0.04, 0.06)	High	[123,152,247]
Larvae	High lethal temperature threshold	Uniform	℃	44 (41.5, 52.8)	High	[123,152,247]
larvae	maximum weights for larval stages	uniform	mg	L1= 0.0875, L2= 0.313, L3 = 1.71, L4 = 4.11	High	[24,123]
Pupae	Development rate of pupae	uniform	1/hr	Temperature dependent equation (0.016, 0.027)	High	
Pupae	High lethal temperature threshold	Uniform	℃	44 (41.5, 52.8)	High	[123,152,247]
Pupae	Emergence survival	Uniform	/day	0.83 (0.664,0.996)	High	[123,152,247]
Pupae	Female emergence ratio	Uniform	/day	0.5 (0.4,0.6)	High	[123,152,247]

Pupae	Nominal survival	Uniform	/day	0.99 (0.792-1)	High	[123,152,247]
Pupae	High-temperature threshold	Uniform	℃	41 (30.2 -41.5)	High	[123,152,247]
Pupae	Survival at or above high lethal temperature threshold	Uniform	/day	0.05 (0.04 - 0.06)	High	[123,152,247]
Pupae	Emergence survival	Uniform	/day	0.83 (0.664, 0.996)	High	[123,152,247]
Adults	Rate of gonotrophic cycle (duration to oviposition)	uniform	1/hr	Temperature-dependent equation (0.001, 0.002)	High	[123,152,247]
Adult	Mortality rate within the bio-range	Uniform	1/day	Temperature-dependent equation (0.026, 0.33)	High	[120]
Adults	High-temperature threshold	Uniform	℃	40 (30,45)	High	[123,152,247]
Adults	Survival at or above high lethal temperature threshold	Uniform	1/day	0.05 (0.04,0.06)	High	[123,152,247]
Adults	High lethal temperature threshold	Uniform	℃	50 (45,60)	High	[123,152,247]
Adults	Minimum oviposition temperature	Uniform	℃	18 (14.4, 21.6)	High	[123,152,247]
Adults	Frequency of blood meal	uniform	/cycle	Temperature-dependent equation (0.33,1)	High	[62,291]

Adults	Interrupted feeds	uniform	1/meal	3 (0,5)	High	[123,152,247]
Adults	Taking blood meal probability	uniform	1/day	Temperature-dependent equation (0.1, 0.9)	High	
Adults	Proportion of mosquitoes feeding on humans- aegypti	Uniform	-	0.9 (0.72, 1)	High	[124,247]
Adults	Proportion of mosquitoes feeding on humans- albopictus	Uniform		0.6 (0.5, 1)	High	[292]
adults	Clutch size of eggs laid by female per gonotrophic cycle	uniform	# eggs	63	High	[267,293]
Adults	Effective Flight velocity	uniform	m/ hr	4.3	High	[128]

Table 5.4. Parameters used in the epidemiology submodel of HYDREDETS

parameter	**distribution**	**unit**	**Value (max, min)**	**Certainty**	**Reference**
Vector competence (probability that an acquired dengue virus infects the mosquito)	Uniform	-	Temperature-dependent equation (0.1,0.9)	High	[196]
Extrinsic Incubation period the virus in the mosquito (EIP)	Uniform	hour	Temperature-dependent equation (0.006, 0.001)	High	[124,247]
probability for transmission of dengue virus from human to mosquito	Uniform	-	Temperature-dependent equation (0.1,0.9)	High	[196]

DEN-1 viremia duration (days)	Uniform	day	5 (3, 8)	High	[124,247]
DEN-2 viremia (MID-50)	Uniform	dose/ml	1,000,000	High	[124,247]
Duration of heterologous immunity	Uniform	days	730 (365, 1095)	High	[234]
DEN-1 viremia (MID-50)	Uniform	dose/ml	1,000,000 (4, 8)	High	[124,247]
DEN-1 Latent period in humans	Uniform	days	4 (2, 8)	High	[294]
DEN-1 viremia period in humans	Uniform	days	5 (3, 8)	High	[124,247]
DEN-2 viremia (MID-50)	Uniform	doses/ml	1,000,000 (10^4 , 10^8)	High	[124,247]
DEN-2 Latent period in humans	Uniform	days	4 (2, 8)	High	[294]
DEN-2 viremia period in humans	Uniform	days	5 (3, 8)	High	[124,247]
DEN-3 viremia (MID-50)	Uniform	dose/ml	1,000,000 (4, 8)	High	[124,247]
DEN-3 Latent period in humans	Uniform	days	4 (2, 8)	High	[294]
DEN-3 viremia period in humans	Uniform	days	5 (3, 8)	High	[124,247]
DEN-4 viremia (MID-50)	Uniform	dose/ml	1,000,000 (4, 8)	High	[124,247]
DEN-4 Latent period in humans	Uniform	days	4 (2, 8)	High	[294]
DEN-4 viremia period in humans	Uniform	days	5 (3, 8)	High	[124,247]
Probability of infection in biters at or below low titer set point (4 logs)	Uniform	-	0.1 (0, 0.3)	High	[124,247]
Probability of infection in biters at or above high titer set point (8 logs)	Uniform	-	1 (0.8, 1)	High	[124,247]
Nominal probability of mosquito to human infection	Uniform	-	0.9 (0.72, 1)	High	[124,247]

EIP factor at or below low set point (3 logs)	Uniform	-	1.25 (1.1, 1.4)	High	[124,247]
EIP factor at or above high set point (8 logs)	Uniform	-	0.75 (0.6, 0.9)	High	[124,247]
Probability of infection in biters at or below low titer set point (4 logs)	Uniform		0.1 (0, 0.3)	High	[124,247]

Chapter 6: Simulation of Dengue Seasonality in Singapore using HYDREDETS

Aedes aegypti and *Aedes albopictus* are the main vectors of dengue, Chikungunya, and other related arboviruses. The two species coexist and transmit dengue in several endemic areas. In this chapter, we extend HYDREDETS model -- described in **Chapter 5** -- to simulate the coexistence of *Ae. aegypti* and *Ae. albopictus*, and retrospectively investigate the role of these two species in shaping dengue seasonality in Singapore between 2000-2011. Hourly meteorological datasets of temperature, rainfall, relative humidity, wind speed and wind direction were obtained for the period 2000-2011. Beside the previously modeled processes (i.e., breeding in drains and effects of flushing/drying conditions on the abundance of the vector population), we model in this work vertical transmission and investigate its epidemiological significance in Singapore. We also investigate the role of human movements in the spatial domain which could overcome the short flight range of *Aedes aegypti* and *Aedes albopictus* and impact outdoor transmission. Further, sensitivity analyses for the newly incorporated processes are conducted.

6.1 Introduction

In dengue-endemic countries, vector surveillance is an indispensable tool to both forecast the disease transmission patterns and optimize vector control. Because no vaccine is available yet to stop the disease transmission especially in endemic countries, vector control remains the sole intervention for dengue particularly at the present crisis of emergence of arboviruses. Nevertheless, dengue entomological indices (e.g., house index and pupal demographic index) are logistically difficult to monitor in highly populated areas. In contrast, modelling tools can be useful not only in monitoring the disease but also evaluation of vector control interventions and insecticide resistance [162,295–297]. Mathematical modeling also allows investigation of drivers of spatial and temporal patterns of dengue transmission which in turn could result in prevention of dengue outbreak and better vector control.

Although dengue seasonality has been documented in endemic areas and largely attributed to the influence of monsoon changes on vector populations, few models have mechanistically incorporated enforcing of the climate on *Aedes* mosquito (instead of using sine functions) [124,154,281,298]. Nonetheless, this reduction approach limits capability of dynamic models to investigate drivers of intra- and interannual variability of the disease transmission [248]. Further, climatic drivers of intra-annual patterns are not necessarily similar between endemic areas. For example, types of breeding habitats of *Aedes* mosquitoes differs between rural and urban areas and they are influenced by economic, cultural and social factors [213,299,300].

In **Chapter 5** we developed, a new mechanistic model, HYDREDETS (Hydrology, Entomology, Dengue, Transmission Simulator), to investigate the role of urban hydrology in vector abundance and disease transmission. HYDREDETS couples the EPA Storm Water Management Model (SWMM), with a mosquito population and epidemiology dynamic models. We previously calibrated HYDREDETS using field observations of the Geylang area in Singapore between August 2014 and August 2015. The model has allowed investigating the effect of climate-enforcing process such as rainfall-flushing and drying of breeding habitats on seasonality of the vector and disease. In this chapter, we retrospectively investigate dengue seasonality in Singapore using weather datasets and dengue reported cases for the period 2000-2011.

To better model dengue ecology in Singapore, we updated HYDREDETS to address the coexistence of *Ae. aegypti* and *Ae. albopictus* which is documented in Singapore [46,60,223]. The tiger mosquito *Ae. albopictus* is the native species in Singapore, while *Ae. aegypti* invaded the island from Africa after European colonization of Southeast Asia in the 19th centaury [301]. Though the earlier species was incriminated in outbreaks of Chikungunya in the city, the role of *Ae. albopictus* in dengue transmission is insufficiently investigated [220,302,303]. Few modelling frameworks were developed for *Ae. albopictus* particularly in temperate areas to study Chikungunya [246,304–306]. However, modeling the sympatric presence of *Ae. aegypti* and *Ae. albopictus* is needed in endemic areas where the two species shape the spatiotemporal patterns of dengue.

Both *Ae. aegypti* and *Ae. albopictus* fly for short distances (in order of tens to hundreds of meters) and stay close to the blood source (e.g., humans or other warm-blooded hosts) and oviposition. This behavior allows mosquitoes cohorts to cluster in the intra and inter-house environment, and avoid negative impact of wind speed [25,139,307]. In contrast, persistence of dengue viruses in endemic areas necessitates a continuous contact between susceptible and infectious people (via the mosquito vector) which in this situation only possible through human movements. Indeed, recent works have demonstrated that routine and social commutes of individuals affect the spatiotemporal patterns of dengue in endemic areas and can result in regional epidemics [110,111]. Incorporation of individual mobility in dengue models was accomplished using different modeling approaches of cellular automation [261–263,265,266], complex networks [258,264,308], deterministic reaction-diffusion [155,309,310], and mechanistic [164]. In Singapore, intertwining of the outdoor breeding of *Aedes* and human movements suggests that the risk of outdoor transmission is complex. To study the impact of human mobility on outdoor transmission of dengue, we added an age-structured mobility scenario to HYDREDETS.

We also incorporated vertical transmission in HYDREDETS. Vertical transmission (VT) is the ability of insect vectors to acquire a viral or bacterial pathogen horizontally from humans or other vertebrate hosts and pass this infection to their offspring generations. Another term used interchangeably to describe this phenomenon -- but not the same -- is transovarial transmission (ToT), which implies a vertical transmission via infection of ovaries (versus trans-eggs infection). Previous studies have shown an inconsistent but low rates of VT for dengue. The ranges of vertical transmission rate (VTR) and filial infection rate (FIR) were 0-41.2% and <5%, respectively [73]. Hence, a further examination is needed to investigate the importance of VT in dengue transmission particularly in endemic areas. A summary of infection rates reported in the literature is shown in **Table 6.1**.

Table 6.1. Summary of infection rates of dengue vertical transmission by vector species, serotype, and location reported in the literature. (Summarized after [73])

Species	**Serotype**	**Measure(1)**	**Range of infection rate**	**Location/Origin of DENV**	**Reference**
Ae. albopictus	DENV-1	MIR	0.38-28/1,000	Brazil, Bolivia, Fiji, Puerto Rico	[311–317]

Ae. albopictus	DENV-1	VTR	11 - 41.2%	Jamaica, Florida (USA)	[318,319]
Ae. albopictus	DENV-1	FIR	0.4-17.4%	Jamaica, USA	[311,318]
Ae. albopictus	DENV-1	VTR	0.4 - 100%	Fiji	[312,320,321]
Ae. albopictus	DENV-2	VIR	4/7 individuals, 4/11 individuals	Brazil	[315,322]
Ae. albopictus	DENV-2	MIR	0.91-98/1,000	Not specified	[311,312,315]
Ae. albopictus	DENV-3	MIR	0.23- 9.4/1,000	Brazil	[318,323]
Ae. albopictus	DENV-4	MIR	0.22-5.2/1,000	Brazil, Puerto Rico	[323]
Ae. aegypti	DENV-1	FIR	13.0%	Jamaica	[318]
Ae. aegypti	DENV-2	FIR	1.4 -3.7%	Thailand	[312]
Ae. aegypti	DENV-1	MIR	0.65-15/1,000	Many virus strains, Not specified	[324]
Ae. aegypti	DENV-2	MIR	0.26-51/1,000	Myanmar, India, Indonesia, Brazil, New Guinea, Malaysia	[325,326]
Ae. aegypti	DENV-3	MIR	0.27- 6.5/1,000	Mexico, India, Bolivia	[327]
Ae. aegypti	DENV-4	MIR	0.091-15/1000	Trinidad, Mexico	[327–329]
Ae. aegypti	Unknown	MIR	0.28/1,000	India, Tamil Nadu,	[330]
Ae. aegypti	DENV-3	VIR	2.7-67.5%	Thailand	[331,332]

(1) Measure of infection rate: FIR= Filial Infection Rate; MIR= Minimum Infection Rate; VIR= Vertical Infection Rate; VTR= Vertical Transmission Rate. For calculation of these measurements see **Chapter 2**.

6.2 Materials and Methods

6.2.1 Data sets

Hourly datasets of precipitation, temperature, humidity, wind speed and wind direction were obtained for Changi Airport station. Figure 6.1 shows the seasonal profiles of rainfall, air temperature, relative humidity and wind speed in Singapore between 2000 -2001. The figure shows a remarkable seasonal pattern for rainfall (i.e. twice amount of total precipitation in November-January compared to June-September). The seasonal difference of mean temperature does not exceed 1.5 °C between the seasons. In addition, seasonal variations in relative humidity and wind speed are small.

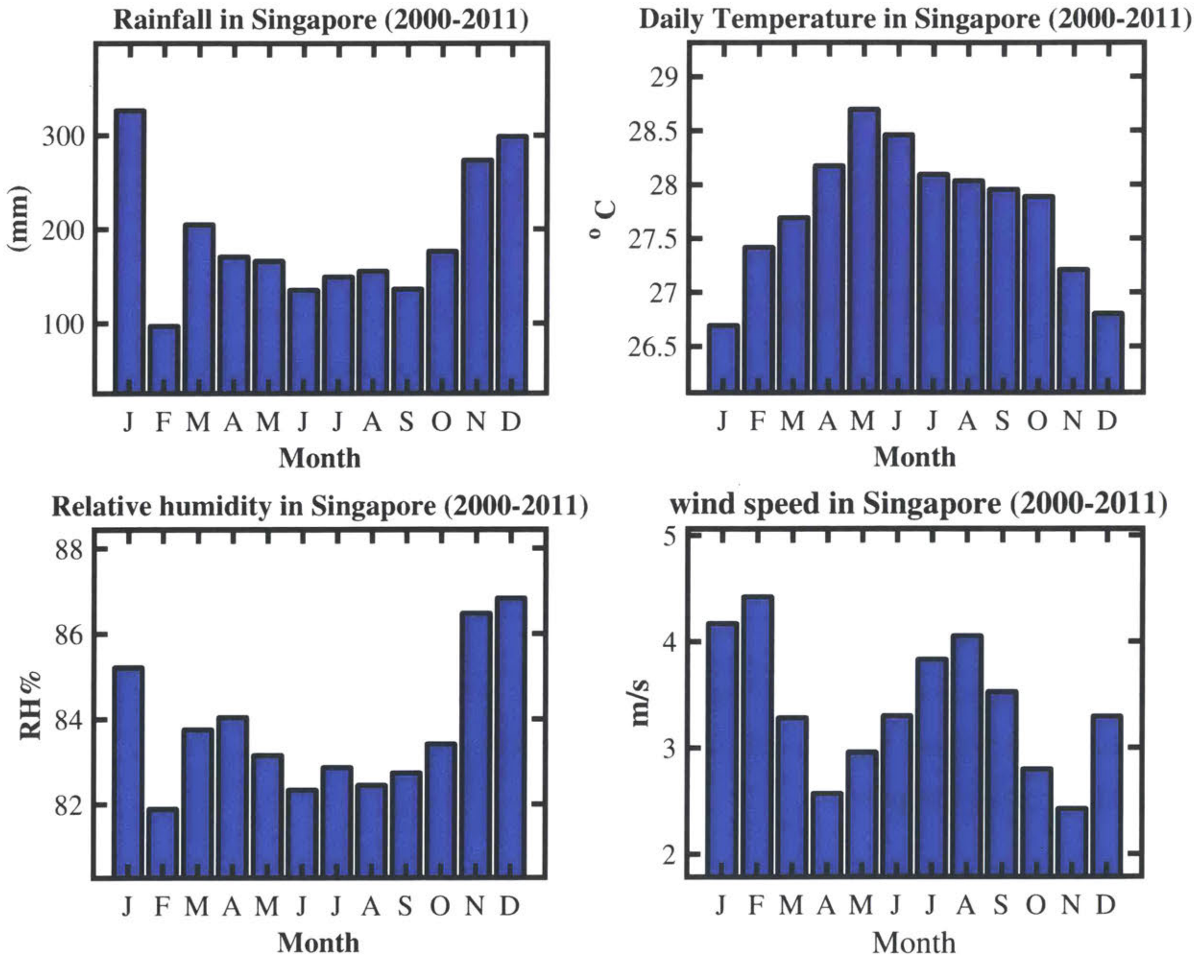


Figure 6.1. Monthly averages of total rainfall, daily temperature, relative humidity and wind speed recorded in Changi airport, Singapore (2000-2011). Data source: NEA, Singapore

6.2.2 Modeling the coexistence of *Ae. aegypti* and *Ae. albopictus*

We extended the modeling framework of HYDREDETS by including adults and aquatic cohorts of *Ae. albopictus*. Parameters of development and survival rates for this species under coexistence and separate breeding were obtained from a previous work [305,333,334], summarized in **Table 6.2**. In addition, these studies demonstrate that three processes are unique for this species: the effects of interspecific competition in co-breeding habitats, preference for blood feeding and diapause. We neglected the latter process in Singapore because this mechanism enables temperate strains of *Ae. albopictus* to evade the winter conditions [65,246,304,306].

Interspecific competition. The effect of interspecific competition between the two *Aedes* populations in case of a co-breeding event is simulated following Lotka-Volterra model [335,336]. Past studies have demonstrated that coexistence of these two species affect the survival of aquatic stages in breeding habitats. While larvae of *A. albopictus* has a better competitive advantage in breeding containers of low food quality, eggs of *Ae. aegypti* can better resist dry conditions [68,336]. Hence, we modified the density-induced mortality equation described in **Chapter 4** to account for interspecific effects:

$$m_i = m_{min} + \left(\frac{\alpha_{nm} B_t}{K_t}\right) \qquad (6.1)$$

Where: m_i= mortality of larval cohort i., α_{nm}= parameters for the effect of interspecific competition on density-dependent mortality (nm= the effect of *Ae. aegypti* on *Ae. albopictus* and vice versa, n≠m).

However, since the coexistence of these two species in Singapore is stable (i.e. no entire replacement for the native population *Ae. albopictus* by the invasive species *Ae. aegypti*), we argued that competition parameters should be within a range of a similar magnitude. For this purpose, we used parameters for the interspecific competition described in a previous study [337]. In addition, two thresholds are used to trigger competition effects: 1) onset of drying conditions (affecting mortality of desiccation-resistant eggs of *Ae. albopictus*), and 2) onset of flushing conditions (affecting survival of larvae of *Ae. aegypti*).

Blood feeding. *Ae. albopictus* in Singapore has remained highly anthropophilic despite urbanization and landscape changes over the last 40 years [220,338]. In contrast to *Ae. aegypti* which solely feed on humans, *Ae. albopictus* is an opportunistic feeder (i.e. the species has no preference for specific hosts and can feed on other available blood sources) [51,339]. Hence, we assumed feeding probability of *Ae. albopictus* on humans is slightly lower than *Ae. aegypti*, See **Table 6.2**.

Table 6.2. Summary of parameters of *Ae. albopictus* and *Ae. aegypti* under separate and coexistence scenarios used in HYDREDETS

parameter	unit	Inter-competition		Intra-competition		references
		Ae. albopictus	*Ae aegypti*	*Ae. albopictus*	*Ae aegypti*	
Adult longevity	days	1.21	1.00	1.45	1.00	[334]
ratio blood meals offered taken	(0-1)	1.13	1.00	1.11	1.00	[334]
No of blood meals	-	0.75	1.00	0.76	1.00	[334]
Ratio successful reproductive opportunities	(0-1)	1.19	1.00	1.08	1.00	[334]
mean lifetime fecundity	eggs /female	0.83	1.00	0.65	1.00	[334]
development time	days	0.84	1.00	0.79	1	[334]
Biting rate	(1/day)	B2 = 0.5 *B1	B1 (1)	B2 = 0.5 *B1	B1(1)	[62,333,334, 340,341]
Probability feeding on humans	(0-1)	0.5 (0.24- 1)	1[0.88-1]	0.5 (0.24— 1)	1[0.88-1]	[15,63,155,292,334,341–346]
Transmission probability from mosquitoes to humans	(0-1)	0.5[0.1-0.75]	0.5[0.1-0.75]	0.5[0.1-0.75]	0.5[0.1-0.75]	[155,334]
Transmission probability from humans to mosquitoes	(0-1)	0.033	0.10	0.033	0.10	[334]
Mortality rates for larvae	(0-1) /day	$\mu 2$(3)	$1.1 * \mu 1$(2)	$\mu 2$	$\mu 1$	[15,334]
desiccation-resistance survival rate for eggs	/day	0.9*e1	e1(4)	0.9*e1	e1	[68]
EIP development rate	/day	EIP2(6)	EIP1(5)	EIP2(6)	EIP1(5)	[346]

(1) $B1=0.0043*T + 0.0943$.

(2) $\mu 1 = 1/(1.22 + \exp(-3.05 + 0.72T)) + 0.196$; if $T < 22$ °C; $\mu 1 = 1/(1.14 + \exp(51.4-1.3T)) + 0.192$; if $T \geq 22$ °C.

(3) $\mu 2 = 1/(1.1 + \exp(-4.04 + 0.576T)) + 0.12$; if $T < 15$ °C; $\mu 2 = 0.000339T2 - 0.0189T + 0.336$; if 15 °C $\leq T < 26.3$ °C; $\mu 2 = 1/(1.065 + \exp(32.2-0.92T)) + 0.0747$; if $T \geq 26.3$ °C.

(4) $e1=0.99$ (0.792, 1).(5) $EIP1 =1.3 (4 + \exp(5.15-0.123T))$; (6) $EIP2 =4 + \exp(5.15-0.123T)$.

6.2.3 Modeling human movements.

Human movements can impact dengue transmission as shown in previous studies [110,111]. Hence, we allowed diffusion of people between the housing grid cells (described previously in **Chapter 5**, **Figure 5.9**) and other grids containing shops and schools (described in **Chapter 4**, **Figure 4.2**), with the following assumptions:

1- Human movements during the working week depends on age. Hence, we assumed: children < 5 years and elders > 70 to remain most of the time in the house-grids, other active age groups to have a schedule for the day hours 17:00-6:00 and 7:00-16:00, indoors and outdoors, respectively.

2- Movements of the active-age groups happens to specific grids all the weekdays except on weekends. The assumption here is that people usually commute between two specific locations (e.g., home to school) during the weekdays. We included grids of commercial and public buildings previously identified in the study area (**Chapter 4**) in this scenario.

3- Weekends (i.e. Saturdays and Sundays) are marked with shopping and recreational movements to random grids between the day hours 11:00-19:00. We used grids of parks, hawkers, restaurants, and shops identified in the study area map for this purpose.

6.2.4 Modeling vertical transmission.

Previous experimental studies suggest that vertical infection occurs mechanistically during oviposition when eggs are fertilized and after the development of eggs ([316], cited in [73]). Hence, vertical transmission is a true transovarial infection (i.e. transmission happens inside the ovaries). Hence, probability of dissemination of dengue infection from infected gravid females to their offspring is incorporated in HYDREDETS using infection rates reported in previous studies. In Singapore, a previous study showed 1.33% and 2.15% positivity for dengue viruses in wild males of *Ae. aegypti* and *Ae. albopictus*, respectively [339]. Hence, we assigned probabilities of 2% and 3% for infected females to pass infections to their laid eggs, *Ae. aegypti* and *Ae. albopictus*, respectively. Likewise, we followed the assumption in [347] that EIP of dengue virus takes place during the aquatic development before the emergence (i.e. emerged mosquitoes are immediately infectious). In addition, HYDREDETS tracks infected cohorts while they are developing in breeding drains.

6.2.5 Model validation

To validate HYDREDETS, a time series of reported dengue cases in Singapore between 2000-2011 was referenced as the observational point (see **Figure 6.2**). The figure shows seasonal and interannual patterns of dengue during this period. We used HYDREDETS in this study to investigate the intra-annual pattern. The model was first run for 12 years using climatic datasets of the year 2000 to eliminate the transient effect of the initial conditions that strongly influence the long-term dynamics of the virus populations (i.e. we assumed a steady state for dengue in Singapore because the city is an endemic area). Then, HYDREDETS was run for the twelve years on an hourly basis (i.e., a total of 105,120 time steps).

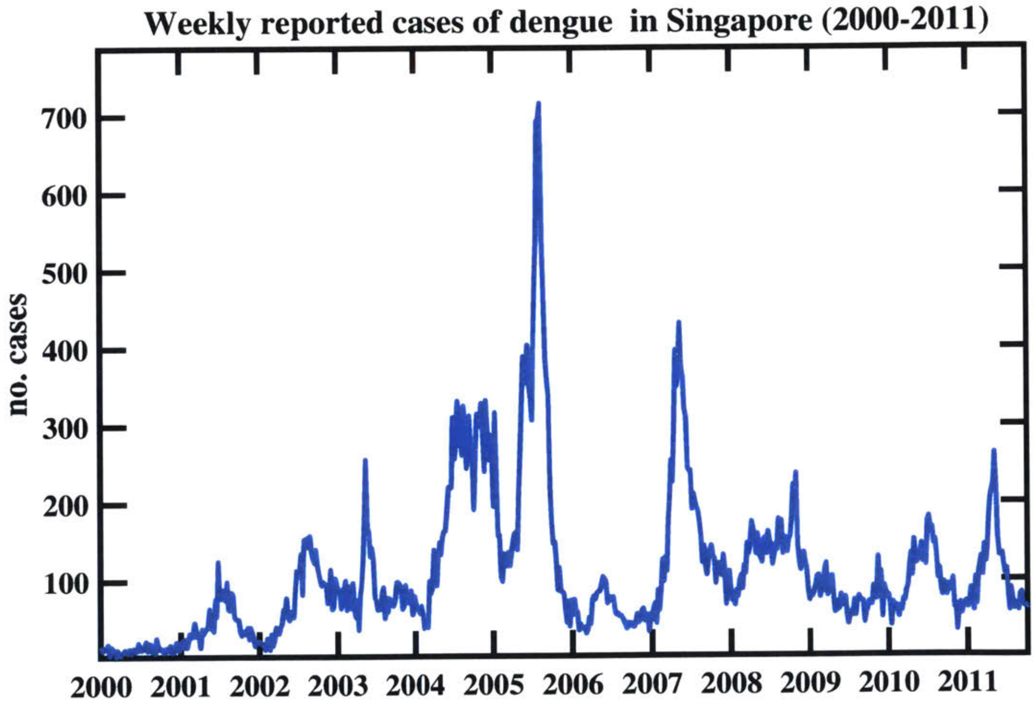


Figure 6.2. Weekly reported cases of dengue in Singapore, 2000 – 2011. (Data source: Ministry of Health, Singapore).

6.2.6 Sensitivity analysis

Sensitivity analyses were conducted for uncertain or unknown parameters. The tested parameters are the newly incorporated processes, which are: 1) flushing/displacement of aquatic stages; 2) desiccation resistance of eggs; 3) transovarial transmission of dengue virus; 4) movement of the human population. We used two approaches in these analyses: enforcing of these parameters (i.e., triggering on/off), and changing the magnitude of the parameters (i.e. +50% and -50%). Total numbers of simulated dengue cases in March and August (the lowest and highest months for the intra-annual cycle) were averaged for the period (2000-2011). For this variable, sensitivity experiments were compared to the simulations of default parameters.

6.3 Results

6.3.1 Hydrology.

Characteristics of runoff and flow routing in Geylang simulated by the SWMM submodel of HYDREDETS are summarized in **Table 6.3** and **Table 6.4**, respectively. Seasonal profile of water in storm drains in Geylang (2000-2011) is presented in **Figure 6.3** and **Figure 6.4**. The simulated outflow (i.e. drained water from a clogged drain) shows a seasonal pattern with a twice volume in December –January compared to the summer July-September. This seasonal flushing corresponds with the annual profile of rainfall recorded in Singapore (**Figure 6.3**).

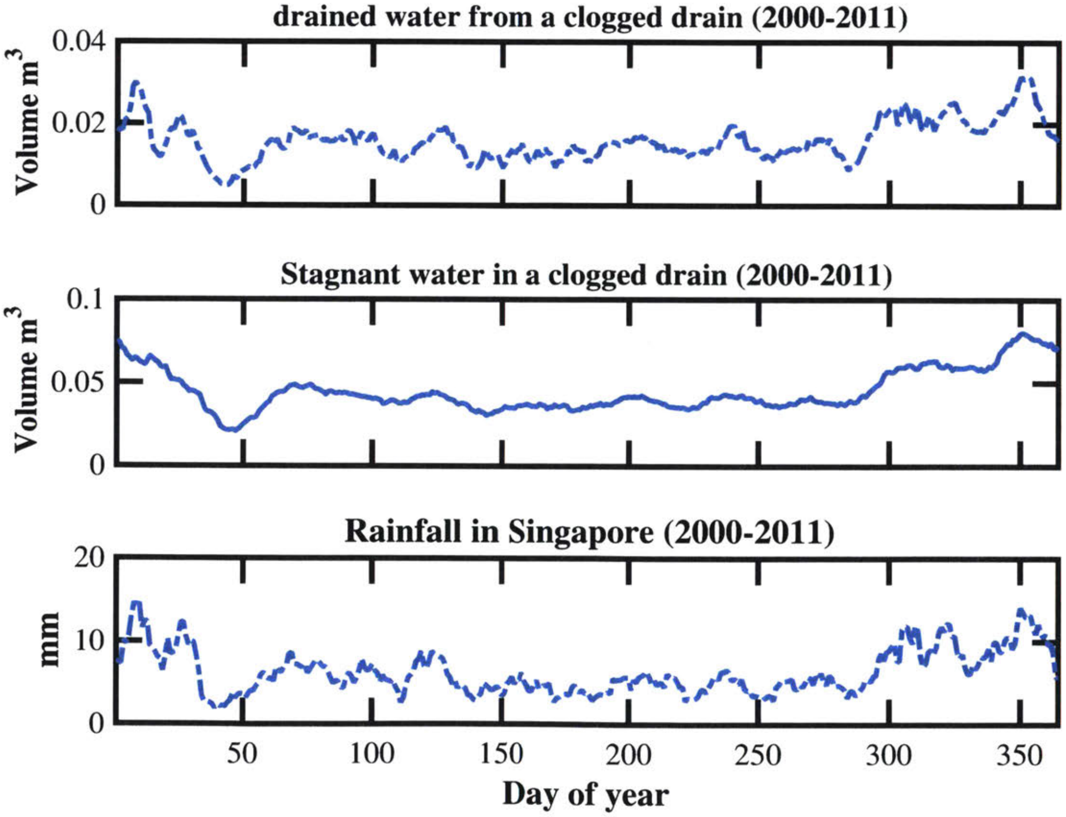


Figure 6.3. Seasonal profiles of a) outflow (drained water), b) stagnant water in a clogged drained simulated by HYDREDETS, and c) rainfall (2000-2011).

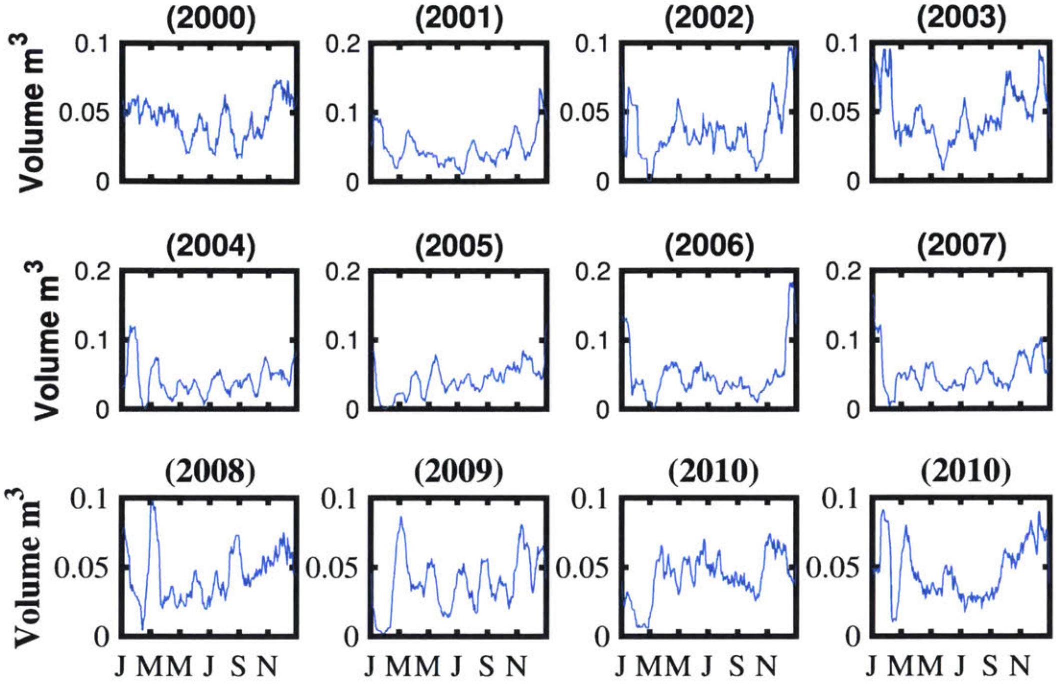


Figure 6.4. stagnant water in a clogged drained simulated by HYDREDETS (2000-2011).

Table 6.3 Characteristics of runoff in Geylang neighborhood – Singapore, simulated by HYDREDETS (2000-2011)

Runoff Quantity Continuity	**Volume (hectare-m)**	**Depth (mm)**
Total Precipitation	546.21	2524.2
Evaporation Loss	19.64	90.78
Infiltration Loss	107.81	498.22
Surface Runoff	421.69	1948.73
Final Surface Storage	0	0
Continuity Error (%)	-0.54	

Table 6.4 Characteristics of flow routing in Geylang neighborhood – Singapore, simulated by HYDREDETS (2000-2011)

Flow Routing continuity	**Volume (hectare-m)**	**Volume (10^6 liter)**

Wet Weather Inflow	421.7	4217.02
External Outflow	304.427	3044.297
Internal Outflow	111.851	1118.523
Storage Losses	0	0
Initial Stored Volume	0.006	0.061
Final Stored Volume	0.164	1.641
Continuity Error (%)	1.248	

6.3.2 Entomology.

Effects of temperature on development. The simulated development time for pupae, larvae, eggs and gonotrophic cycle range was: (2.3-2.6), (3.6-5.9), (1.5-2.0) and (2.5-1.7) days, respectively (see **Figure 6.5**). The difference in EIP duration of dengue between the hottest and coolest months (May and June) is less than two days, 10.5 and 8.8, respectively (see **Figure 6.6**). These results reveal modest effects of temperature variability in Singapore on vector abundance and EIP.

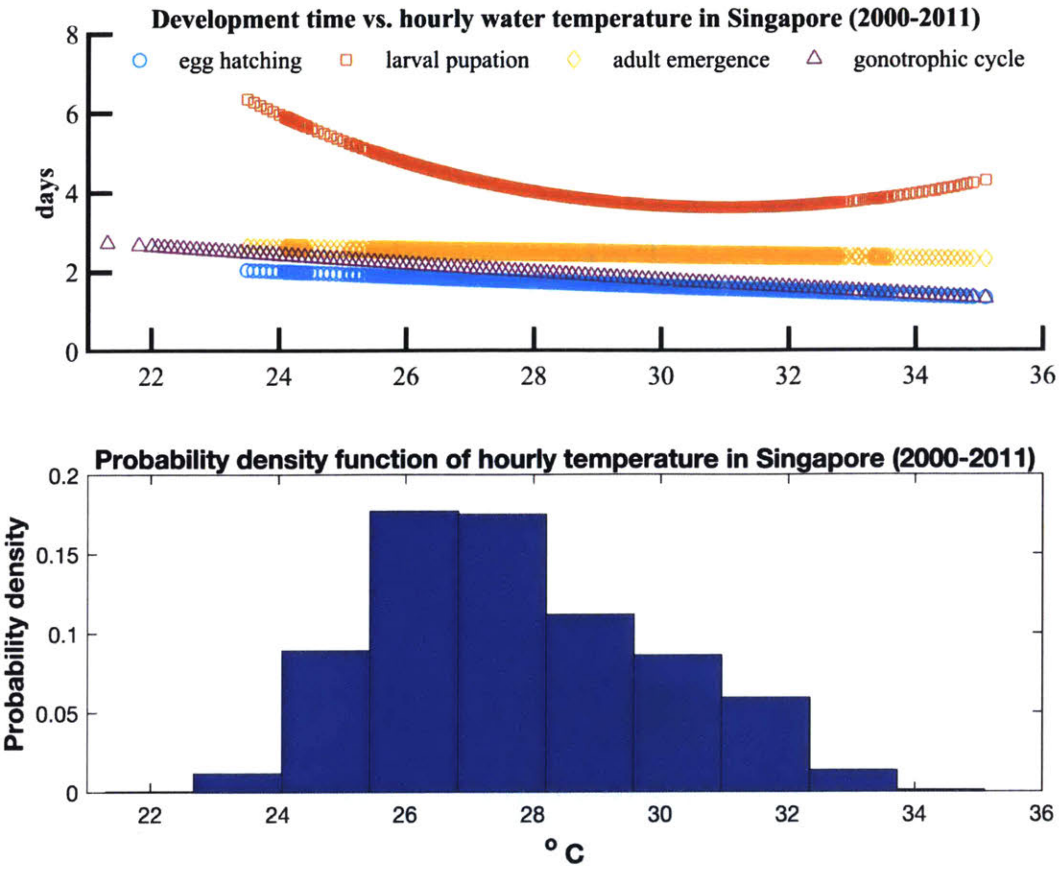


Figure 6.5. Profiles of temperature development processes using temperature dataset for Singapore (2000-2011). Data source: NEA, Singapore.

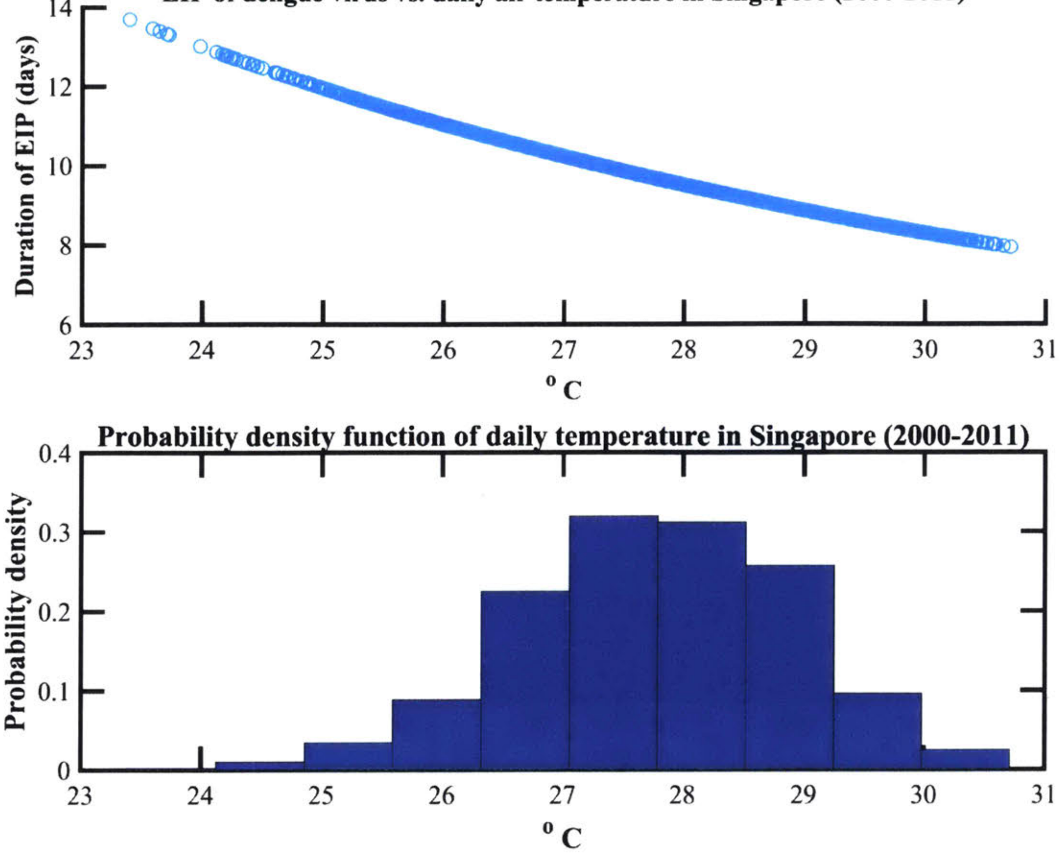


Figure 6.6. Extrinsic Incubation period (EIP) of dengue virus versus daily air temperature in Singapore (2000-2011). Data source: NEA, Singapore.

Vector abundance. The daily densities of females of *Ae. aegypti* and *Ae. albopictus* exhibit a seasonal cycle during the study period with a peak in June-October and a dip in February-March, **Figure 6.7** and **Figure 6.8**, respectively. The average abundance of *Ae. albopictus* is slightly higher compared to *Ae. aegypti* (6,636 and 6,353, respectively).

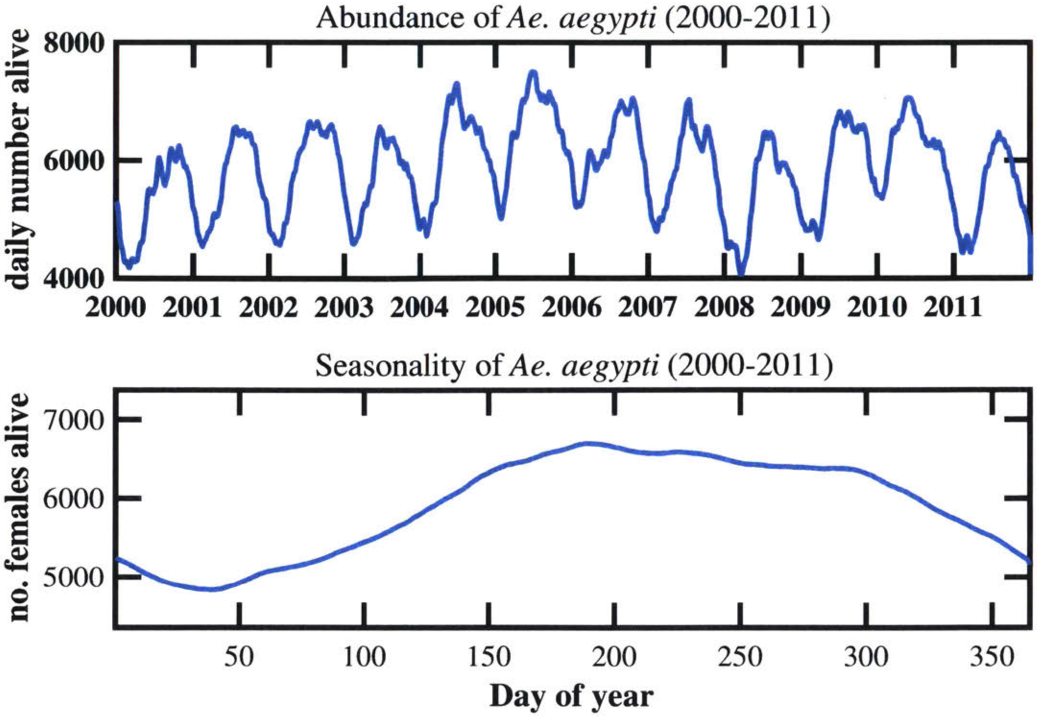


Figure 6.7. Seasonal abundance of *Ae. aegypti* in Singapore simulated by HYDREDETS.

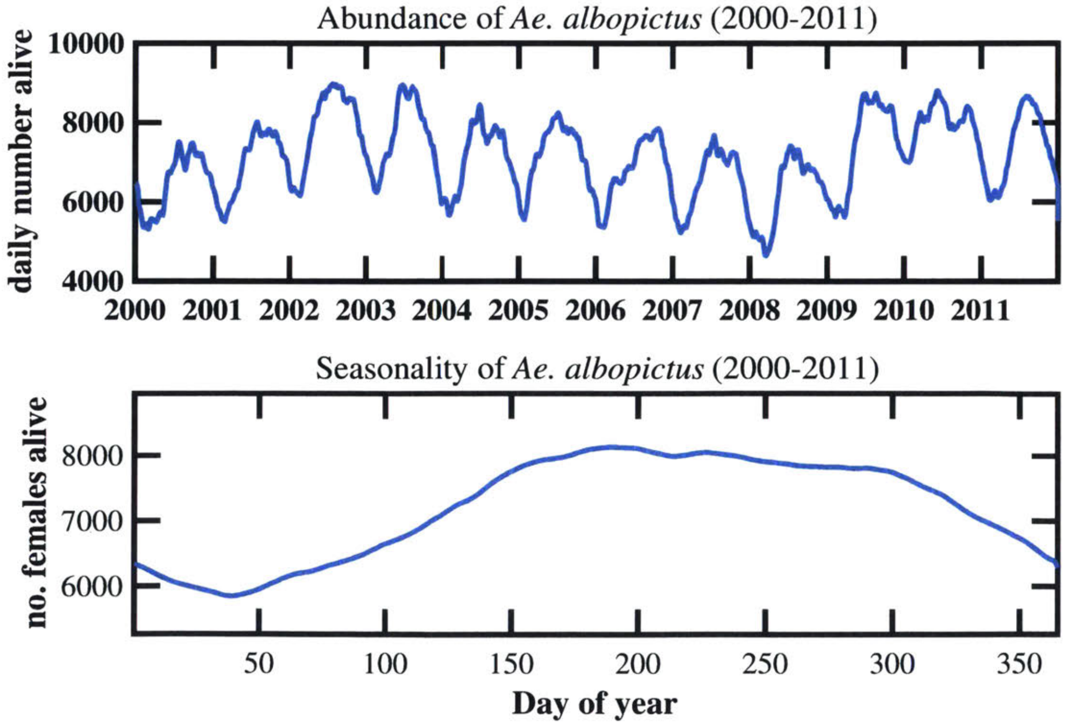
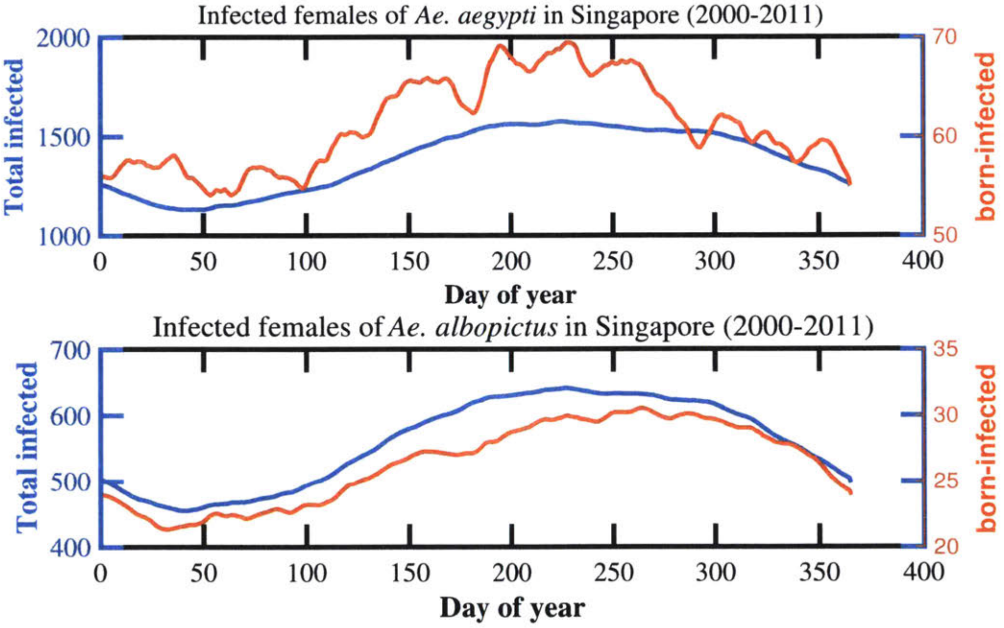


Figure 6.8. Seasonal abundance of *Ae. albopictus* in Singapore simulated by HYDREDETS (2000-2011).

Infection rates of mosquitoes. Total numbers of infected females and infected-borne ones for *Ae. aegypti* and *Ae. albopictus* are shown in **Figure 6.9**. Beside the observed seasonal pattern for dengue infections in the mosquito population, we calculated the infection rate of dengue in the two mosquito populations (number of infected females / total number of females), **Figure 6.7** and **Figure 6.8.** Overall, ranges of infectivity rates are (21.7% - 28.5%) and (7.1%-9.9%), for *Ae. aegypti* and *Ae. albopictus*, respectively. In addition, infected-borne females constitute 4.6% and 4.7% of the infected populations of *Ae. aegypti* and *Ae. albopictus*.


Infected females of *Ae. aegypti* in Singapore (2000-2011)

Infected females of *Ae. albopictus* in Singapore (2000-2011)

Figure 6.9. Seasonal pattern of dengue infected females of Ae. aegypti and *Ae. albopictus*. The figure shows average numbers of infected females and borne-infected ones in Singapore (2000 -2011) simulated by HYDREDETS.

Rainfall-Flushing effect. The seasonal impact of rainfall on flushing of the larvae is presented in **Figure 6.10**. The figure shows a correspondence between the outflow of breeding drains (see **Figure 6.3**) and daily ratio of flushed aquatic stages, with a seasonal peak observed between November-January.

Effects of drying conditions. Effects of drying of drains on mortality of larvae and desiccation resistance of eggs are shown in **Figure 6.11**. Interestingly, eggs of *Ae. aegypti* benefits from a physiological ability to resist the drying of February month. 52% of laid eggs by *Ae. aegypti* in this month survived drying conditions compared to other months which showed a range of (0.5%-13%). In contrast, percentage of larvae killed by the drying conditions was inconsistent between the months. The maximum and minimum mortality of larvae -- due to dryness -- has ranged between 18% and 0.5%, February and November, respectively.

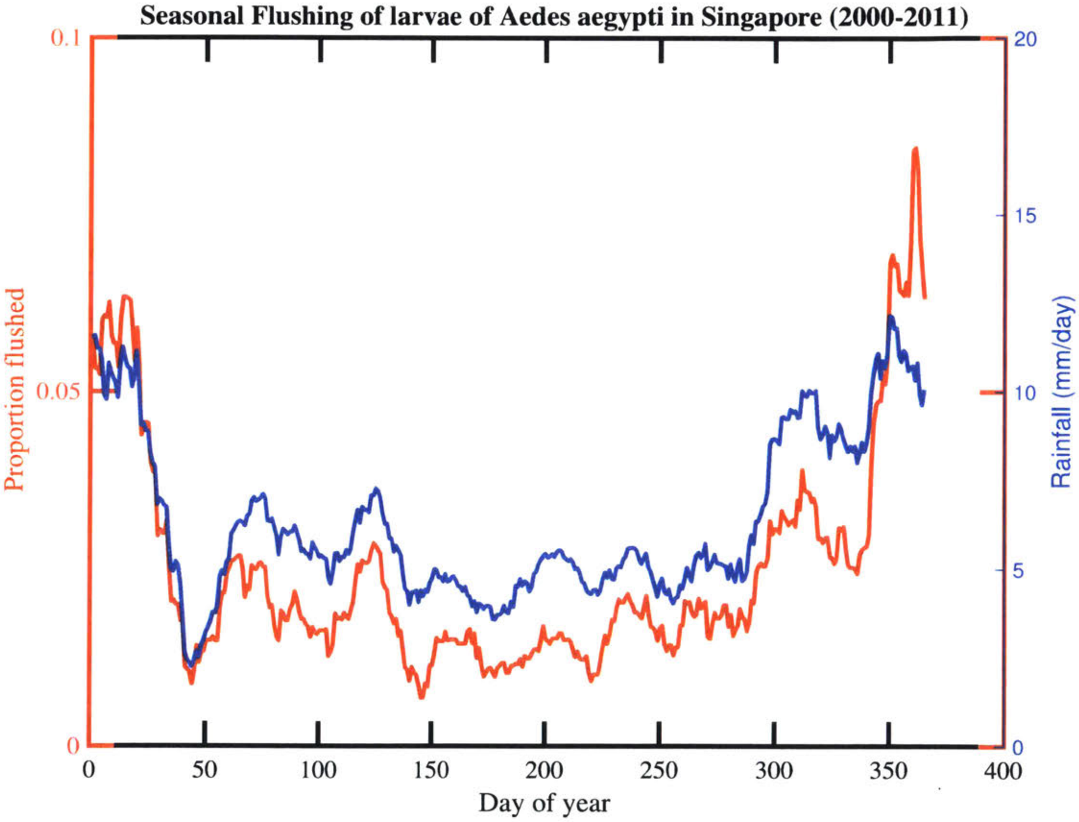


Figure 6.10. Seasonal flushing of larvae of *Aedes aegypti* using HYDREDETS, Singapore (2000-2011). The figure shows a correspondence between the observed seasonal profile of rainfall and the daily proportion of flushed aquatic stages.

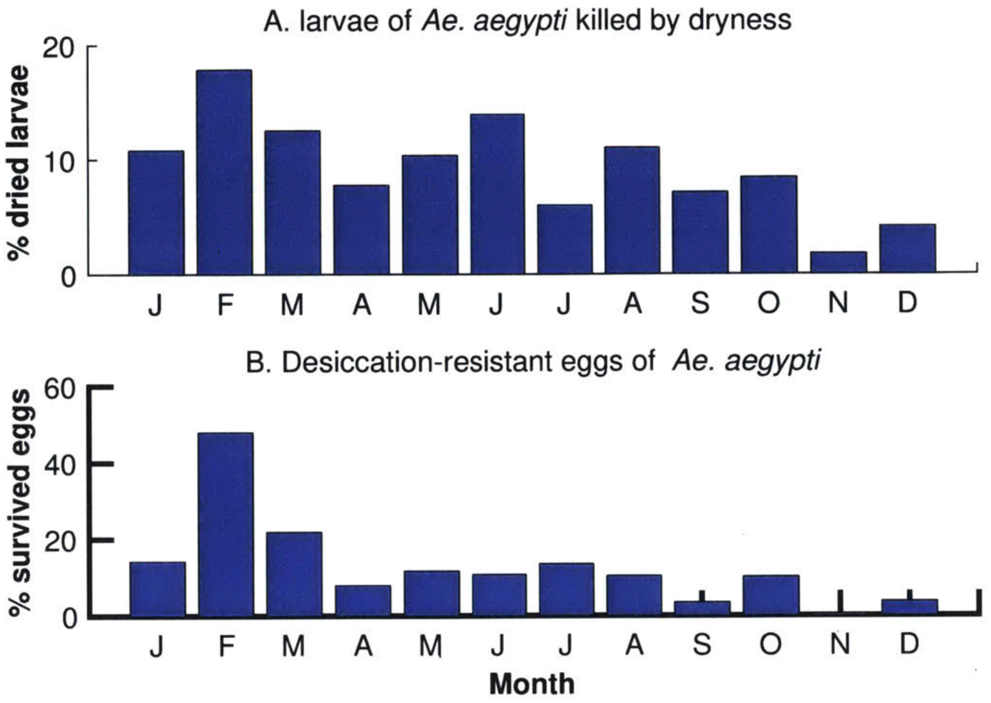


Figure 6.11. The effect of seasonal drying of breeding drains on: A) mortality of the larvae, and B) desiccation-resistance eggs of *Aedes aegypti* in Singapore (2000-2011). While the seasonal drying conditions in February caused mortality of larvae and pupae, proportion of survived eggs were higher in this month.

6.3.3 Epidemiology. Seasonal cycle and time series of dengue new and active cases simulated by the model versus reported cases in Singapore (2000-2011) are shown in **Figure 6.12** and **Figure 6.13**. The seasonal peak of dengue cases occurred in June-September compared to the low dengue season February – May. Although HYDREDETS has simulated the seasonal pattern of dengue in Singapore, the magnitude of the seasonal difference is lower compared to the observations, 30% times versus 60%, respectively.

Simulated dengue cases by virus strain and type of infection are presented in **Table 6.5**. Primary and secondary infections constitute 63% and 36.9% of the total cases, respectively. However, these infection types are dominated by two virus strains: DENV-1 (51.7%) and DENV-2 (47.9%).

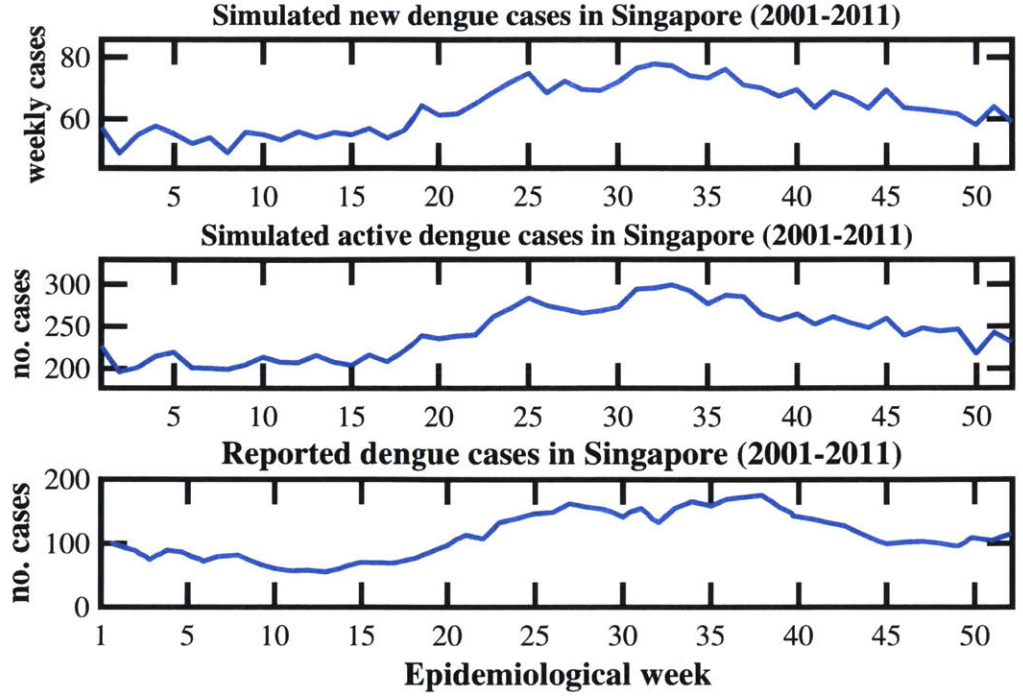


Figure 6.12. Seasonality of dengue cases simulated by HYDREDETS (2001-2011). The figure shows a correspondence between new and active simulated cases, and reported ones. Incubation and infectious periods (of the SEIR model) are used to track active cases.

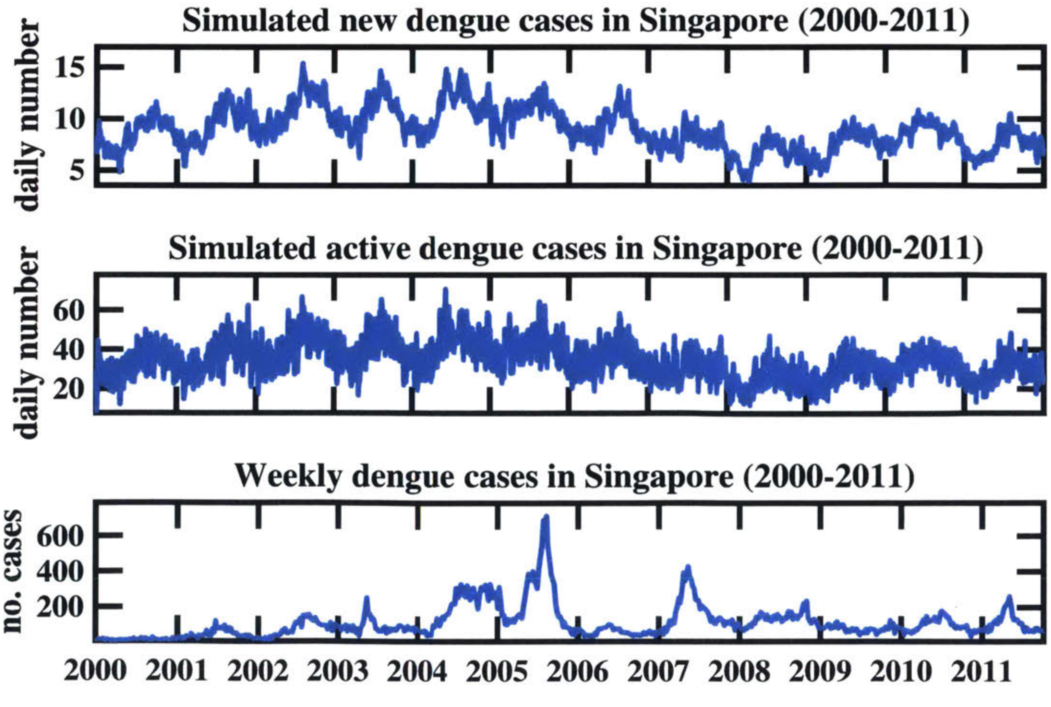


Figure 6.13. Time series of simulated and reported dengue cases in Singapore (2001-2011).

Table 6.5 Percent of dengue cases by strain and type of infection using HYDREDETS (2000-2011).

	Type of infection				
Strain	**Primary**	**Secondary**	**Tertiary**	**Quaternary**	**Total**
DENV-1	29.4%	22.3%	0.1%	0.0%	51.8%
DENV-2	33.4%	14.5%	0.1%	0.0%	48.0%
DENV-3	0.1%	0.0%	0.0%	0.0%	0.1%
DENV-4	0.1%	0.0%	0.0%	0.0%	0.1%
Total	63.0%	36.9%	0.2%	0.0%	100.0%

6.3.4 Vertical transmission. In **Figure 6.14**, filial infection rate FIR (i.e. proportion of infected cohorts emanating from an infected parent, see section 2.2.3 for measure calculations) exhibits a seasonal pattern between (2001 -2011). **Figure 6.15** shows number of human dengue cases that are attributed to bites of infected-born females of *Aedes*. On average, 4% of FIR has resulted in 10% of dengue cases in Singapore, being contracted through bites of born-infected mosquitoes.

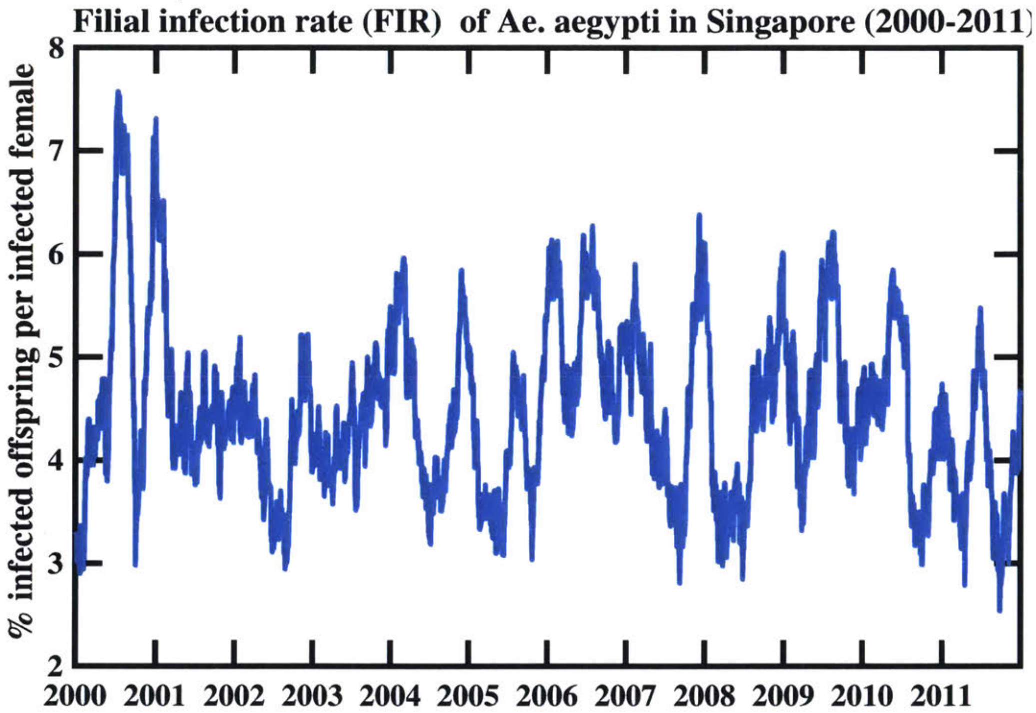


Figure 6.14. Time series of filial infection rate (FIR) of dengue virus in *Ae. aegypti*, Singapore (2000- 2011).

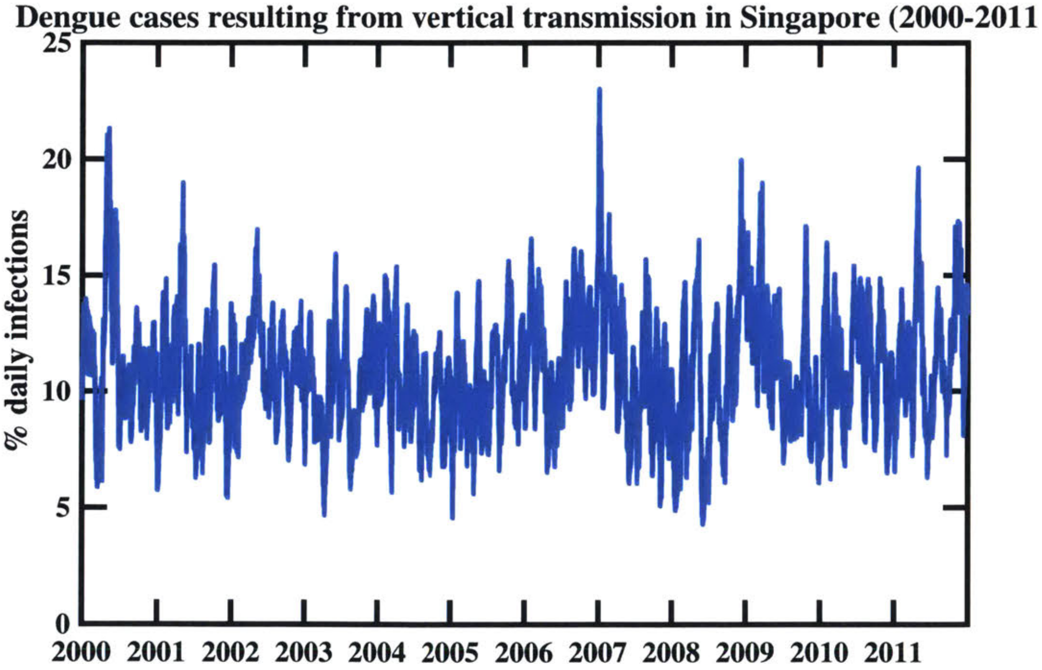


Figure 6.15. Time series of dengue cases attributed to bites of infected-borne mosquitoes simulated by HYDREDETS (2001-2011). Vertical transmission is responsible for 10% of dengue cases simulated by the model.

6.3.5 Human movements. Figure 6.16 shows the impact of human movements on dengue between 2000 and 2011. An average of 62% of simulated dengue cases occurred outdoor.

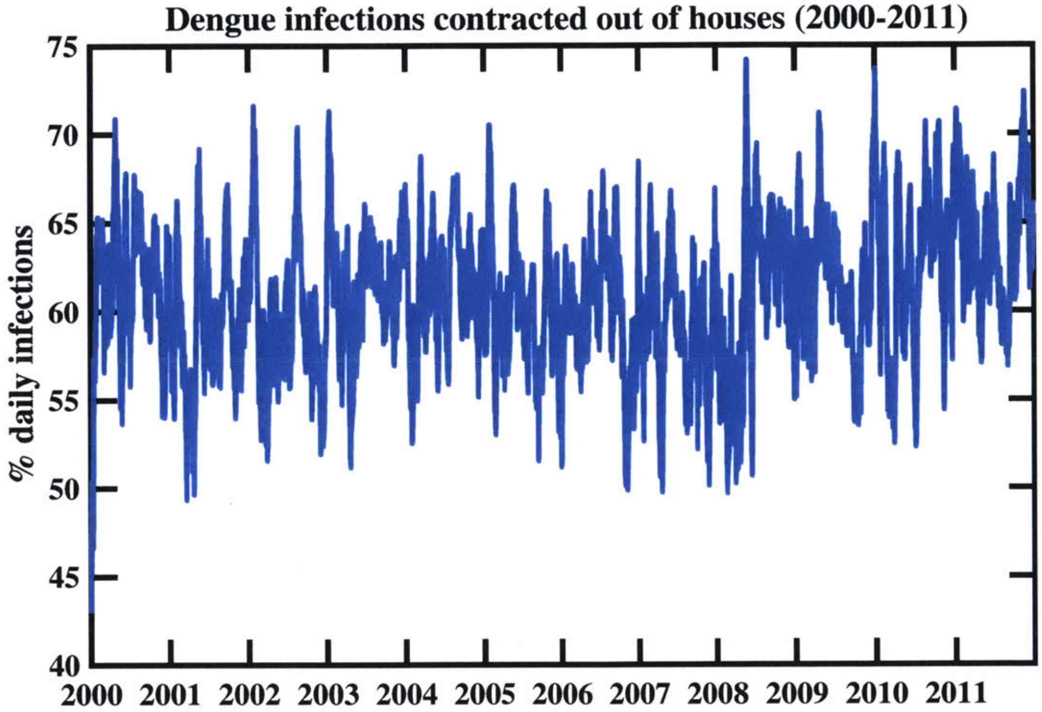


Figure 6.16. Time series of dengue infections contracted outdoors simulated by HYDREDETS (2001-2011). The figure shows 60% of dengue cases are contracted outdoors.

6.3.6 Spatial distribution. One important feature of HYDREDETS is that simulated processes are resolved at high spatial resolution. This modeling approach consider the scale of interaction of these processes that lead to the transmission of dengue. The distribution of breeding habitats simulated in HYDREDETS is shown in **Figure 6.17**. Accordingly, most of the breeding habitats occurred in the southern part of Geylang area where the low-rise housing and more stagnant drains were previously observed. Similarly, abundance of simulated adults and infected females of *Ae. aegypti* are more in the low-rise area of Geylang, see **Figure 6.18**. **Figure 6.19** shows more dengue cases are simulated in the southern part of Geylang area (where low-rise housing and more breeding drains) compared to the northern part (the high-rise area).

Figure 6.17. The distribution of breeding habitats of *Aedes aegypti* and *Ae. albopictus* simulated in Geylang area using HYDREDETS. The figure shows a random monthly snapshot for the study period (2000-2011).

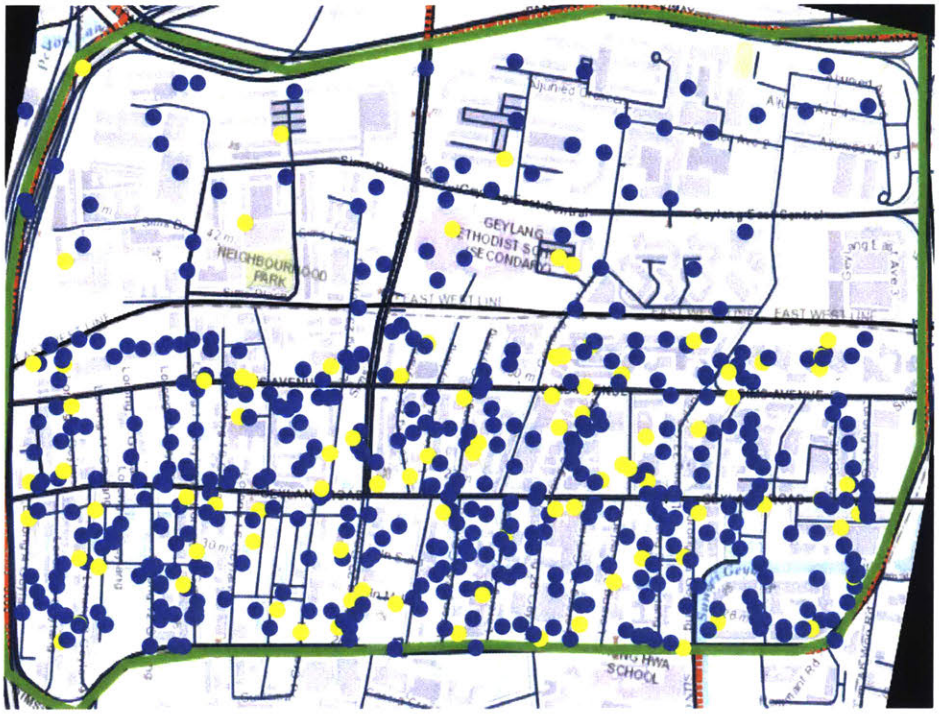


Figure 6.18. The distribution of simulated adults of *Ae. aegypti* (blue) and infected females (yellow) in Geylang area using HYDREDETS. The figure shows a random monthly snapshot for the study period (2000-2011).

Figure 6.19. The distribution of simulated dengue cases in Geylang area using HYDREDETS. The figure shows a random monthly snapshot for the study period (2000-2011).

6.3.7 Sensitivity analyses. In general, results of sensitivity analysis show that HYDREDETS is robust in simulating dengue seasonality in Singapore. However, the model is sensitive to human movements, flushing-displacement of the aquatic stages and vertical transmission of dengue virus, **Table 6.6**. If "no human movements" is assumed in HYDREDETS, only 40% of dengue cases could occur. Also, the seasonal difference between August and March will decrease in the "no human movement" scenario. In a comparison to the defaults, a "no displacement" scenario has decreased monthly cases by 40%, and seasonal difference (93 vs. 57 cases, for the displacement and no displacement scenarios, respectively). Also, a "no vertical transmission scenario" decreased monthly cases by 25% compared to the default scenario.

In addition, sensitivity tests for ±50% changes in magnitude are presented in **Table 6.7**. Increasing the desiccation period by 50% has slightly decreased the seasonal difference in monthly cases between August and March, compared to decreasing this period by 50%, 74 and 98, respectively.

Table 6.6 Results of sensitivity tests conducted for selected processes of HYDREDETS. The tested outcome is the monthly average of dengue cases of the default simulation compared to the sensitive simulations for March and August, ± SE.

Process	**Yes (default)**		**No**	
	March	**August**	**March**	**August**
Flushing displace of aquatic stages	239 ±16	332 ±18	138±12	195±10
Desiccation resistance of eggs	239 ±16	332 ±18	233 ±14	331 ±18
Vertical transmission of dengue virus	239 ±16	332 ±18	184 ±13	249 ±17
Movement of the human population	239 ±16	332 ±18	105 ±15	140 ±20

Table 6.7 Results of sensitivity tests conducted for selected parameters of HYDREDETS. The tested outcome is the monthly average of dengue cases of the default simulation compared to the sensitive simulations for March and August, ± SE.

Parameter/change in value	**-50%**		**+50%**	
	March	**August**	**March**	**August**

Duration of desiccation period	223 ±11	321 ±13	214 ±14	288 ±17
Tolerance time of drying conditions	288 ±14	339 ±16	268 ±13	368 ±13
Flushing ratio of aquatic stages	251 ±19	339 ±18	240 ±13	343 ±11
Displacement of aquatic stages	232 ±15	320 ±19	233 ±20	324 ±22

6.4 Discussion

We used HYDREDETS to investigate drivers of dengue seasonality in Singapore between 2001-2011. Although the simulations showed a relatively low seasonal difference compared to observations, the model successfully captured the temporal pattern. Hence, mechanistic assumptions underlying HYDREDETS could well explain the drivers of seasonality of the disease in the city. In **Chapter 5**, we demonstrated how rainstorms -- associated with the Northeastern Monsoon- between November and January cause flushing of aquatic stages, and how drying conditions in February killed larvae and turned desiccation-resistance eggs *of Ae. aegypti* into a dormancy state. In this study, the long term seasonal abundance of the vector has coincided with the simulated dengue cases. This finding supports the hypothesis that dengue seasonality in Singapore could be a result of flushing-drying sequence in outdoor breeding habitats [14].

HYDREDETS also shows a moderate to slight sensitivity to the newly incorporated processes: human movements; flushing displacement of aquatic stages; vertical dissemination of the dengue virus from infected mosquitoes to offspring, and tolerance of the aquatic stages to drying conditions. The movement of the human population could have a greater effect on dengue seasonal patterns because more seasonally abundant mosquitoes does not fly longer and the disease need a contact between infected and susceptible populations (i.e. humans and mosquitos). A limitation to this study is that we conducted qualitative sensitivity analyses only for the new parameters. In addition, no statistical analyses were performed for the results. A further statistical and modeling work is needed to thoroughly explore the impact of these parameters on the model results, and to test the model in other similar urban settings. Sensitivity analyses could help to maximize parsimony of the model and exclude unnecessary variables [155]. For instance, the effect of temperature on development rates from eggs to adults has a narrow range in Singapore and equatorial regions and this could allow simplification of the thermokinetic equations used in development rates and save computational time of model runs.

Among the limitations of this study is the assumption that dengue seasonality in Singapore is related only to outdoor habitats, particularly storm drains. In contrast, indoor breeding of *Ae. aegypti* is well documented [166,348]. However, little is known about the seasonal abundance of indoor habitats. Also, *Ae. albopictus* coexists in Singapore but a little is known about its role in dengue transmission. Previous modeling framework for *Ae. albopictus* also assumed that advancement and survival of life stages are temperature-dependent. Interestingly, diapause mechanism is incorporated in some of these models to allow simulations of the vector in temperate areas [246,304]. Thresholds of air temperature and durations of day hours are used to trigger the start/end of diapause season. In addition, first events of rainfall illicit the hatching of dormant eggs during the non-diapause season [246,304]. However, parameters of these models particularly for vectorial capacity are mostly dependent on previous studies of *Ae. aegypti*. Further field studies that address indoor breeding habitats *Ae. aegypti* and population dynamics of *Ae. albopictus* are needed.

Another shortcoming to this work is a lack of representation of vector control interventions in HYDREDETS. A recent modeling work conducted in Cairns (Australia) has shown that both type and time of vector control operations could significantly impact dengue outbreaks [164]. In Singapore, stringent anti-vector measures are usually deployed particularly during the outbreaks years [50,52,200]. Applications of larviciding or other interventions could affect the interannual cycle of the disease (result in dampening the season peak or shift the season). Nevertheless, the simulations of this study may imply that vector control efforts during the period 2001-2011 did not result in changing the seasonal profile of dengue in Singapore.

In this study, we showed that horizontal transmission could result in an infection rate of 19.5% and 4.3% in *Ae. aegypti* and *Ae. albopictus*, respectively. In a previous survey in Singapore samples of females of *Aedes* mosquitoes collected within the surveillance program of NEA (1997 – 2000) were tested for dengue viruses using RT-PCR. Accordingly, dengue viruses were detected in 6.9% and 2.9% of the tested females of *Ae. aegypti and Ae. albopictus*, respectively [221]. Also, a longitudinal survey has demonstrated that 1.33% and 2.15% of the caught male adults of *Ae. aegypti* and *Ae. albopictus* were positive for dengue viruses [339]. In this work, vertically infected females were only 1.1% and 8.5% of the populations of *Ae. aegypti* and *Ae. albopictus*.

Theoretical studies argue that evolutionary forces of vertically transmitted pathogens selectively attenuate virulence of infections, and do not reduce fecundity and fertility of their vectors [349]. Several studies have failed to detect VT in mosquito field collections because of various factors including DENV strains, mosquito populations, sampling, and screening techniques. Although low infection rates for VT of dengue have been reported in previous studies, still a horizontal transmission (HT) mode does not well explain sustainability of infection in endemic areas particularly during unfavorable conditions (e.g., winter and dry seasons in temperate and arid areas, respectively) or after insecticide control campaigns for outbreaks. Mathematical models show that persistence of DENV in long-term simulations requires high infection rates of VT and this could be important through infected diapause eggs [347,350]. Several hypotheses such as the reintroduction of new virus strains by movement of people and longer viremia duration in asymptomatic cases have been proposed to support the role of HT in the virus persistence [73]. However, population genetics studies of DENV serotypes show that same strains are conserved in their endemic areas [351]. Also, increase of herd immunity after epidemics remains for approximately 1-3 years before the cross-immunity wanes in past infected people [234].

In conclusion, HYDREDETS is capable to capture and explain the seasonal patterns of dengue in Singapore (2000-2011). The model is sensitive to human movements, flushing displacement of aquatic stages, vertical dissemination of the dengue virus from infected mosquitoes to offspring, and tolerance of the aquatic stages to drying conditions.

Chapter 7: Conclusions

7.1 Summary of Results

Dengue is an increasing global public health challenge. The primary goal of this thesis was to investigate the role of *Aedes aegypti and Ae. albopictus* in shaping the spatiotemporal patterns of dengue transmission in Singapore. To address this goal, we carried out entomological surveys to observe effects of wet/dry seasons in the outdoor breeding of the disease vectors; conducted spatial analyses to explore the impact of urban ecology on dengue distribution, and developed mechanistic models to simulate the seasonality of dengue transmission by *Ae. aegypti* and *Ae. albopictus* in Singapore.

Chapter 2 reviews the relevant literature on the disease ecology. Four related Flavivirus strains are involved in the domestic transmission cycle of dengue. The global distribution of *Ae. aegypti* and *Ae. albopictus* demonstrates the dominance of the earlier species in endemic areas in the tropics while the latter species is expanding in the temperate areas. Two modes of transmission characterize dengue epidemiology: vertical route (from parent mosquitoes to their offspring), and horizontal path (from mosquitoes to humans and *vice versa*). Spatiotemporal patterns of dengue reveal that the disease is sensitive to the climate and socioeconomic factors surrounding local communities. Despite the continuous efforts to curb the disease in Singapore, there is an increase in frequency and magnitude of dengue epidemics during the last two decades. While a switch in serotypes and immunity factors are suggested to explain this upsurge, seasonal patterns indicate that the vector control is undermined by less understanding about the breeding ecology of the vectors.

Chapter 3 describes and tests a new hypothesis to explain dengue seasonality in Singapore. Seasonal peak of cases during the summer coincides with the Southwest monsoon, while the low season between February and May follows the wettest and driest phases of the Northeast monsoon. We hypothesize and test that a seasonal sequence of flushing-drying conditions in breeding drains shapes dengue seasonality in Singapore. Further, field observations between August 2014 and August 2015 in Geylang neighborhood has confirmed this hypothesis. Stagnation in positive breeding drains during August-October 2014 had ended by the onset of flushing and drying conditions related to the Northeast monsoon. The semi-weekly surveys also demonstrate a return in breeding conditions between June and August 2015.

Chapter 4 explores the interaction between the outdoor breeding of the vector in the drainage system and landscape. Two patterns of urban housing are documented in Singapore: High-rise and low-rise. Observational and spatial analyses show correlations between low-rise housing areas and clustering of dengue cases and vector abundance. The drainage network is denser at this type compared to high-rise housing areas. Further, the distribution of dengue incidence at a country-scale shows a similar pattern to the distribution of the attributes of low-rise housing. The increases of built-up area and the population living in low-rise housing were shown to correlate with dengue incidence.

Chapter 5 presents a new mechanistic model for dengue transmission. The HYDRology, Entomology and DEngue Transmission Simulator (HYDREDETS) couples three dynamic sub-models. The EPA Storm Water Management Model (SWMM) is employed to simulate hydrological conditions of breeding drains. *Aedes aegypti* population and epidemiology models are developed to simulate vector abundance and disease transmission patterns. HYDREDETS is calibrated using parameters from previous works and datasets for Geylang neighborhood. The model reproduces the observations of seasonal flushing/drying of larvae and pupae and re-inoculation of the drains by dormant eggs in the following stagnation conditions. HYDREDETS is the first model to consider urban hydrology of the drainage networks in studying arboviral and vector-borne disease.

Chapter 6 investigates dengue seasonality in Singapore using HYDREDETS. The model is further modified to include the sympatric populations of *Ae. albopictus*, vertical mode of transmission, movements of an age-structured human population in Singapore. Simulations using datasets for the period 2001-2011 shows that HYDREDETS (and underlying assumptions of incorporated processes) captures and explains the seasonal patterns of dengue in the city. HYDREDETS is sensitive to human movements, flushing displacement of aquatic stages, vertical dissemination of the dengue virus from infected mosquitoes to offspring, and tolerance of the aquatic stages to drying conditions.

7.2 Major contributions

The primary objective of this work is to understand better ecological factors that underlie the spatiotemporal patterns of dengue transmission in Singapore. The dissertation contributes to the knowledge and control of dengue -- and vector-borne diseases-- in five major areas:

1. The study describes a new hypothesis to explain the role of monsoons in shaping the spatiotemporal patterns of dengue in wet tropics. Contrary to conditions in arid regions, monsoons with intense precipitation in the wet tropics do not necessarily result in the formation of more breeding habitats for the mosquito vector. Instead, stagnation conditions are annihilated by extreme rainstorms that lead to the washing of the aquatic stages and reduce the seasonal abundance of the vector. It is important to emphasize that this hypothesis does not exclude the role of other climatic factors (mainly temperature) is shaping the spatiotemporal patterns of the disease and vector.

2. The thesis highlights the role of urban drainage and subterranean structures in dengue ecology. Identification of cryptic breeding habitats for *Aedes* mosquitoes is among the challenges to dengue control programs in endemic countries. Here, we propose guidelines to optimize vector control according to the monsoon time. Indoor breeding should intensively be targeted when climatic conditions do not allow outdoor breeding whether these are flushing, drying or even high temperature. Besides, intervention measures should be carefully selected to avoid ineffective outcome and waste of resources. For example, oiling of drains during the onset of wetting conditions could leave only modest impact on reducing vector abundance.

3. The research results emphasize the importance of housing and urban planning on dengue ecology. In Singapore, a housing policy to provide affordable public apartments in congregations of high-rise buildings does not increase the risk of dengue transmission. Urban infrastructures in the rapid urbanization trend in the world should be well designed and maintained with an objective towards improving public health.

4. In contrast to previous studies that neglected the role of outdoor transmission in dengue epidemiology, this work highlights the need to reconsider assumptions about the man-vector contact in dengue epidemiology. Since adults of *Ae. aegypti* and *Ae. albopictus* remain close to their breeding habitats, a "man-breeding" contact prevails and requires access and movements of humans. This notion could help to explain why dengue is a very localized disease and could have further implications for dengue control.

5. The study adds a new mechanistic tool to the repertoire of epidemiological models available in public health. The model allows studying dengue and other vector-borne diseases such as Chikungunya and Zika virus. The theoretical framework described in this study is comprehensive, coupling urban hydrology, dengue entomology, and epidemiology. Incorporation of flushing effects of rainfall on washing and displacement of aquatic stages of *Aedes* was shown necessary in dengue seasonality in Singapore. Also, the framework is unique in modeling the coexistence *Ae. aegypti* and *Ae. albopictus*.

7.3 Policy Implications

In absence of antiviral medicines or vaccine, anti-vector measures remain the only method to prevent and control dengue. Findings of this study have several important implications on the policy of dengue control program of Singapore.

First, the current policy of the control program focuses on surveillance and control of indoor breeding of *Aedes* and transmission of dengue. Law enforcement and community involvement are focused on the prevention of indoor breeding of *Ae. aegypti* and *Ae. albopictus*. Although this policy was successful to reduce the burden of dengue in 1970s and 1980s, the current upsurge of dengue in the city --since 1990s-- necessitates a revision of this strategy. The goal of the vector control programs is to suppress the population(s) of *Aedes* mosquito or their contact with humans below levels that could result in dengue epidemics. This revision requires further field, experimental and modeling studies that not only investigate the bionomic role of *Aedes* mosquitoes in dengue transmission but also test the effectiveness of anti-vector measures. Longitudinal studies for indoor and outdoor habitats that can span over years will be important to reframe the control policy of the program towards effective outcomes.

Second, there is a need to improve the surveillance system of dengue and *Aedes* in Singapore. Although, Singapore has one of the best integrated framework to collect entomological and epidemiological data, it is not clear how this information is useful in prevention of dengue, Chikungunya and other *Aedes*-transmitted diseases. For example, there is no thresholds for pupal density or house index that are robust to categorize the epidemiological situation during the year or between the regions of the city. We agree that a part of this problem is due to the absence of sensitive sampling tools for *Aedes* mosquitoes, however, use of better statistical and modeling tools could help identify not only which collected parameters are useful but also needed ones.

7.4 Future work

This dissertation provides opportunities for further research and investigations in three main areas:

- Dengue vector control and urban hydrology. The purpose of construction of storm drainage systems in cities is to minimize the risk of flooding. Based on this study, there is an opportunity to optimize the guidelines and operation protocols of the combined and separate drainage systems. For example, rerouting the outflow during stagnation time to increase washing of breeding sites in areas that are prone to dengue transmission. Also, structural changes in the conveyance system can be made to result in the fast drying of drains such as increasing the design slope. Indeed, the material and shape of pipes can be improved to prevent mosquitoes breeding.

- Dengue and climate variability. Previous studies had documented a connection between dengue outbreaks and El Niño events. However, none of these studies had addressed the impact of this phenomenon on dengue seasonality. In general, El Niño events results in dry and warm conditions in Southeast Asia. However, if the El Niño onset happens during the wettest monsoon between November-January, this interference could lead to more stagnation conditions and increase dengue risk. In contrast, the onset of El Niño during summer between June and September could decrease the risk of dengue if this event results in drier conditions than expected. The flushing hypothesis and modeling framework developed in this dissertation can be useful in studying the impact of climate variability on dengue seasonality the in wet tropics.

- Dengue and climate change. The climate of Singapore is governed by two monsoons which are cool and wet but differ in patterns of precipitation. While global circulation models strongly agree that temperature will increase in Southeast Asia by the end of this century (~ 0.8 - 3.2°C), there is some uncertainty about the change in rainfall (~ 1-8% increase). Hence, modeling work is needed to answer which one of the climatic factors will be crucial to dengue transmission under climate change. Forcing HYDREDETS or other dengue model using projections of emission scenarios should give a way to anticipate the impacts and plan to mitigate this effect on dengue. However, catchments in urban areas are usually small presenting a challenge for calibration of statistical and modeling techniques used in any downscaling exercise.

7.5 Recommendations

In order to improve the vector control strategy in Singapore and similar endemic countries, we recommend to:

1. Reorient the control strategy to give more attention to the surveillance and control of cryptic outdoor breeding habitats near low-rise housing areas. For example, periodic inspections for the drainage network in low-rise areas during the summer time in Singapore could be more effective in reducing the risk of *Aedes*-transmitted diseases.

2. Strengthen inter-sectoral coordination between the dengue control program and authorities responsible for the management of storm water and drainage systems. For instance, Public Utility board is the water authority of Singapore that manage the drainage systems in the city. Cleansing of blockage at the drainage system can be useful to prevent formation of breeding drains. However, a sustainable approach to this problem should seek better design of hydraulic structures of conveyance networks that prevent stagnation conditions.

3. Consider effects of housing types and drainage systems on dengue in urban planning. Although the strategic housing plan of Singapore concentrates on affording inexpensive apartments in high-rise buildings, this plan should also set directions for the control of dengue and other vector-borne diseases in human settlements. The density of the drainage

network in these housing agglomerations should be the least required to prevent both flooding and breeding of urban mosquitoes. Also, redevelopment of low-rise housing areas is needed to improve both indoor and outdoor environments that are conducive for dengue transmission.

4. Use mechanistic models as tools for planning, monitoring and evaluation of interventions against dengue and other *Aedes*-transmitted diseases. However, developing these modeling frameworks will require coordinated efforts between the governmental agencies, universities and modeling experts. In addition, web-based platforms of these models are needed to timely inform the community about the risk of dengue. The feedback from the community can be useful to improve the model outputs.

5. Strengthen regional collaboration between Singapore and other endemic countries in South East Asia. In particular, population movements between Singapore, Malaysia and Indonesia could be a source of introduction of new genotypes of dengue virus. The risk of an imported infectious person visiting or temporary lodging at a hotel in low-rise housing areas can be higher. These countries should establish a platform that allow exchange of information for new genotypes and incidence of the disease, so that the control program should be able to effectively monitor and contain epidemics.

6. Develop better surveillance tools to monitor both vector abundance and virus prevalence at low-rise housing areas. These tools should be less expensive and powerful in sampling under-infested sites (for example, cryptic outdoor breeding habitats for adults and aquatic stages of *Ae. aegypti* and *Ae. albopictus*). In addition, periodic serological surveys are needed to monitor the serotypes of dengue virus in the resident population.

7. Develop effective vector control products and delivery systems that target breeding habitats of *Aedes* mosquitoes. In particular, there is a need for formulations of larvicides that are more resistible to washing or dilution effects during rainstorm events. The application tools of these products should also be effective in reaching inaccessible sites for treatment.

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