THE SEASONAL ABUNDANCE OF *AEDES (STEGOMYIA) ALBOPICTUS* (SKUSE) (DIPTERA:CULICIDAE) IN UNIVERSITI SAINS MALAYSIA CAMPUS, PENANG.

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by

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KAJIAN KELIMPAHAN BERMUSIM NYAMUK *AEDES* (STEGOMYIA) *ALBOPICTUS* (SKUSE) (DIPTERA:CULICIDAE) DI KAMPUS UNIVERSITI SAINS MALAYSIA, PULAU PINANG

ABSTRAK

Kajian kelimpahan bermusim nyamuk *Aedes* (*Stegomyia*) *albopictus* (Skuse) dijalankan di Universiti Sains Malaysia, Pulau Pinang. Kajian ini dijalankan pada setiap minggu selama 13 bulan bermula November 2003 sehingga November 2004. 80 ovitrap telah digunakan sepanjang kajian di tiga kawasan utama iaitu di kawasan asrama pelajar, kawasan terbuka dan kawasan berhutan. Kajian ini juga dibuat untuk menentukan kesan faktor lain seperti taburan hujan, suhu dan kelembapan relatif ke atas spesies ini. Peta GIS dilakar untuk menentukan taburan *Ae. albopictus* secara geografi.

Secara amnya, peratus bagi jumlah kutipan telur *Ae. albopictus* ialah 34% bagi kawasan asrama pelajar dan kawasan berhutan manakala 32% bagi kawasan terbuka. Tiada perbezaan signifikan bagi jumlah kutipan purata telur di antara ketiga-tiga kawasan tersebut (ANOVA). Ini mungkin berlaku kerana kawasan kajian terletak berdekatan antara satu sama lain dan nyamuk *Ae. albopictus* betina menggunakan mekanisme kemandirian spesies yang mana nyamuk betina akan bertelur di beberapa kawasan yang berbeza dan tidak tertumpu pada satu kawasan sahaja.

Kajian juga mendapati hanya di kawasan berhutan mempunyai perkaitan yang kuat dengan taburan hujan lag-sebulan dan ini menunjukkan limpahan hujan

yang tinggi akan meningkatkan lagi bilangan telur di kawasan itu. Bagi faktor suhu, pertalian faktor ini adalah berkadar songsang dengan kelimpahan bermusim telur spesies ini di beberapa sub-kawasan terlibat. Analisis untuk mengkaji trend kelimpahan bermusim dan kelembapan relatif juga menunjukkan kelembapan relatif yang tinggi akan menghasilkan bilangan telur yang sedikit di kawasan kajian.

Peta GIS yang dilakar menggunakan ovitrap sebagai titik kawasan kajian untuk dianalisis secara spatial. Melalui peta ini didapati beberapa kawasan yang mempunyai kelimpahan bermusim *Ae. albopictus* yang tinggi ialah di kawasan yang mempunyai aktiviti seharian warga kampus berbanding kawasan hutan. Peta GIS juga digunakan untuk membuat anggaran dan andaian bilangan telur *Ae. albopictus* pada masa hadapan serta kawasan-kawasan yang berpotensi tinggi untuk pembiakan nyamuk.

THE SEASONAL ABUNDANCE OF *AEDES (STEGOMYIA) ALBOPICTUS* (SKUSE) (DIPTERA:CULICIDAE) AT UNIVERSITI SAINS MALAYSIA CAMPUS, PENANG

ABSTRACT

A survey was carried out to determine the population density of dengue vector, *Aedes albopictus* in Universiti Sains Malaysia, Penang, from November 2003 to November 2004. Eighty ovitraps were placed in the study areas and were collected weekly for 13 months. This surveillance was also carried out to verify the influence of temperature, rainfall and relative humidity on the amount of eggs collected in the ovitraps. GIS-based maps were used to plot ovitrap areas and predict the possible high-risk areas with a high density of this mosquito species.

The findings showed that total collection of *Ae. albopictus* eggs in hostel and wooded areas achieved the same percentage of 34% each while 32% obtained in open-space. There was no significant difference on the amount of eggs collected in the three areas (ANOVA). Hence, it explained that mean numbers of *Ae. albopictus* eggs collected in each study area did not differ from others because the study sites are located near and adjacent to each other and there was a probability that the female mosquito tend to lay eggs at numerous ovitrap stations, known as species survival mechanism.

This study also shows that only in wooded area has a strong relationship with lagged one month rainfall. Thus, the result shows that a higher amount of rainfall will increase the total eggs collected in wooded area. The study also indicated that a high mean of temperature and high relative humidity in the study area will decrease the amount of monthly egg collection.

GIS based maps were generated to produce an ovitrap map and were able to indicate hotspot areas that have high density of the *Ae. albopictus* eggs. As expected, the areas with most the human-based activities indicate a higher-risk area compared to wooded area. Forecast and buffer maps are then created to predict the abundances and other potential breeding areas of *Ae. albopictus* in future.

CHAPTER ONE

INTRODUCTION

1.0 Introduction

Universiti Sains Malaysia (USM) is a public university with a main campus in Penang, Malaysia. There are two other campuses, one in Penang as well, and the other on the East Coast of Peninsular Malaysia, in Kelantan. With around 35,000 students in 2005, USM is the biggest university in terms of enrolled students in Malaysia. USM was established as the second university in Malaysia in 1969 and it was first known as Universiti Pulau Pinang. At that time, it was operated in Bukit Gelugor, Penang. In 1971, USM's campus moved to its present 239.4-hectare site, which was the former site of military barracks.

From the outset, Universiti Sains Malaysia was given the mandate to provide, promote and develop higher education in the fields of pure sciences, applied sciences, pharmaceutical sciences, building sciences and technology, social sciences, humanities and education as well as to provide research, advancement and dissemination of such knowledge. The university was ranked 111th in the World University Ranking 2004 published by the Times Higher Education Supplement. However, it has dropped out of the list of top 200 universities in the world in 2005. USM now has 35,000 students including 28,000 undergraduates and 1,800 lecturers.

Reported dengue cases are on the rise in Penang. When the dengue season is approaching, precautionary techniques has been exercised nationwide. The Malaysia government is pulling out all stops to prevent an epidemic rather than rushing to control it when it hits. The Penang town municipal have directed the health department to keep updated and identify the hotspots of dengue outbreak. Dengue viruses has been transmitted from *Aedes* sp. Two known *Aedes* species in Malaysia are *Aedes albopictus* and *Aedes aeypti*. Although no dengue cases reported from USM so far, a surveillance on abundance of *Aedes* sp. should be carried out since neighbouring area were already affected with dengue viruses.

Aedes albopictus (Skuse) is known as the Asian Tiger mosquito (Robertson and Hu, 1988). Historically, this species had primarily an Asian distribution, being found from India in the west, China in the east and Indonesia in the south. It was not known to have spread further until in the 1980s when it began its current rapid global spread with infestation in USA and Europe. The global spread of *Ae. albopictus* during the past 20 years has caused alarm among some scientists and public health officers over the possibility that the introduction of this species will increase the risk of epidemic dengue fever, yellow fever and other arboviruses in countries where it has become established (Gubler, 2003).

In recent years, much effort has been directed towards understanding the invasive properties of *Ae. albopictus* from forested areas, where it originates as well as from indigenous to non-indigenous countries (Reiter, 1998). Like *Ae. aegypti*, *Ae. albopictus* is a woodland species that has successfully adapted itself to the urban habitat. There is evidence that *Ae. aegypti* may be competitively dominant in domestic urban premises, *Ae. albopictus* has the advantage in outdoor surroundings (Reiter and Darsie, 1984). *Aedes aegypti* has received considerable attention because of its importance as a vector of yellow fever and dengue fever meanwhile *Ae. albopictus* is important in maintaining the slyvatic cycle of the dengue fever viruses (Gubler and Bhattacharya, 1972).

The only human disease documented to be transmitted in nature in epidemic form by *Ae. albopictus* is dengue fever. The etiological agents of dengue and dengue hemorrhagic fever are four antigenically related, but distinct viruses, DEN 1, DEN 2, DEN 3, and DEN 4 that are classified in the genus *Flavivirus*, family *Flaviviridae*. In addition, there are multiple genotypes. All four serotypes can cause dengue and dengue hemorrhagic fever, diseases characterized by sudden onset of fever and headache often accompanied by myalgia, anorexia, arthralgia, and in the case of dengue hemorrhagic fever, increased vascular permeability (Halstead, 1997).

Daytime human biting collection has been the main method for sampling female mosquitoes. Biting collections are costly and laborious and put collectors at increased risk of contracting disease (Edman *et al.*, 1997). Thus, the ovitrap is the most common surveillance and sampling method for detecting and measuring mosquito abundance through their egg-laying activities (Service, 1992). The ovitrap is a sensitive method to detect the presence of *Aedes* sp. and are characterized by low operating cost (Bellini *et al.*, 1998).

Differences in abiotic factors such as temperature, precipitation and humidity could have a major influence on the distribution of mosquito species (Teng and Apperson, 2000). Mosquito abundance is often positively related to precipitation (Ho *et al.*, 1971) and this condition can provide fertile grounds for mosquito breeding (Ang and Satwant, 2001). GIS and spatial statistics are also valuable tools in the analysis of the long-term effects of radioactive exposure on the affected populations.

In the past two decades, there have been dramatic increases in the development of infrastructure, accommodations and various services to upgrade USM facilities. It was believed that this development has an impact on the abundance of *Aedes* mosquitoes by providing more habitats for these mosquitoes and thus leading to an increase in the abundance of dengue vectors.

1.1 Objectives of study

The objectives of this seasonal abundance surveillance of *Ae. albopictus* are as follows :

1. To determine the population density of the dengue vector, *Aedes albopictus* by ovitraps at Universiti Sains Malaysia campus, Penang.
2. To evaluate which outdoor breeding habitats are preferred by *Ae. albopictus*.
3. To elucidate the factors (climatological data and study areas) that may influence the *Ae. albopictus* density.
4. To study if there was a quantitative association between current and/or lag values of rainfall and the seasonal abundance of *Ae. albopictus* eggs.
5. To introduce the usage of digital mapping to forecast population density.

CHAPTER TWO
LITERATURE REVIEW

2.0 Dengue

2.0.1 General considerations

Dengue fever is the most important viral vector-borne disease in the world. The disease affects hundreds of millions of people every year, and it is transmitted predominantly by *Aedes* species which has adapted to living near human habitation (Hales *et al.* 2002). During the last decade, dengue infection with its complications has increased globally. It has been thought of as a primary urban disease. In recent years, the frequency of dengue epidemics in tropical and subtropical areas has increased. Since the emergence of DHF in the mid-1950s in Southeast Asia, the Americas and the Pacific region and its incidence is increasing rapidly worldwide (Gubler 2002).

Dengue virus consists of an antigenic subgroup of four closely related but antigenically distinct, viruses designated as DEN-1, DEN-2, DEN-3 and DEN-4 which belongs to genus *Flavivirus*, Family Flavivaridae (Gubler 1997). Epidemics of dengue annually affect millions of people in tropical areas of the world. Dengue haemorrhagic fever / dengue shock syndrome (DHF/DSS) is a severe form seen primarily in children although it can affect adults as well (Estrada-Franco and Craig 1995). Most DHF cases are diagnosed by clinical and haematological observations based on WHO criteria (WHO 1986).

Dengue fever is an acutely infectious mosquito borne viral disease characterized by episode of high fever, severe pain in postorbital region and in the muscles, joints and bones, accompanied by initial erythema and a terminal rash of varying morphology. A commercial kit for example Panbio-Australia usually was used to test blood samples from patients with acute febrile illness for dengue virus antibodies (Placheril 2004).

Aedes aegypti (L.) is the primary vector of dengue viruses in Southeast Asia with *Aedes albopictus* (Skuse) serving as secondary vector (Harinasuta 1984). Nonetheless, *Ae. albopictus* can transmit virus that causes Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) (Jumali *et al.* 1979). The decline and local disappearance of *Ae. aegypti* that followed the spread of *Ae. albopictus* in the southeastern states of North America has been well documented (O'Meara *et al.* 1995). *Aedes albopictus* being an invasive species, is an important arbovirus vector of dengue fever in Southeast Asia (Shroyer 1986).

The urban cycle of dengue, involving man as the vertebrate host and *Aedes* species as the vector, is the only recognized cycle in nature in most areas. This disease is transmitted by female *Aedes* mosquitoes. Two main mosquito vector species, incriminated in the transmission of dengue fever in Malaysia are *Ae. aegypti* and *Ae. albopictus* (Rudnick 1965). The two species are frequently found in and around human habitations. They breed in artificial and natural containers and receptacles that hold clean and clear water.

Its transmission cycle is the result of a complex system based on several main constituents: the presence of one or more serotypes of the dengue vectors, the density of the susceptible hosts and environmental conditions (Yoksan and Gonzalez 2002). Dengue is an endemic disease occurring throughout the country with a maximum number of cases reported. The reasons for this increase are due to the period of rapid urbanization and population growth (both local and migration to cities), a different lifestyle (throwing non-biodegradable containers), rapid transportation and poor living conditions (poor water supply and poor scavenging services at squatter areas). All these gave rise to increased breeding areas for the *Aedes* mosquitoes and easy spread of the virus (Satwant 2001).

Efficient virus transmission at low vector densities has been attributed to this mosquito propensity to imbibe blood meals almost exclusively from humans and to do so frequently (0.6-0.8 meals per day), something that increase their contact with human hosts and as a result enhances their opportunities for contracting or transmitting a virus infection. Dengue virus is one of those few pathogens that depend almost entirely on human for its survival. The four serotypes of dengue virus generally circulate either separately or in combination in a transmission cycle between *Ae. aegypti* mosquitoes and humans. This broad generalization has many exceptions, for example the virus is sometimes transmitted by *Ae. albopictus* in other locations for example in Singapore (Chan *et al.* 1971). Another exception is the occurrence of sylvatic transmission, and has been documented in limited areas where monkeys are important host (Traore-Lamizana *et al.* 1994).

However, the current pandemic of dengue that began in the late 1970s and that has involved all continents except Europe and Antarctica is a transmission cycle between *Ae. aegypti* and humans (Gubler 1997). As a result, dengue usually represents the simplest end of the spectrum of vector borne disease systems (Strickman and Kittapayong 2002). Generally, it is believed that dengue outbreaks occur during rainy seasons and an association was claimed between dengue outbreaks and increased dengue vector densities in many parts of the world (Nathan *et al.* 1998). Whereas epidemics coincide with the rainy season, the magnitude of the epidemics appears not to be related to rainfall (Chareonsook *et al.* 1999).

One area that has received particular attention is the association between climate variation and vector-borne diseases. Hales *et al.* (2002) mentioned that mosquito-borne disease transmission is climate sensitive for several reasons: mosquitoes require standing water to breed and warm ambient temperature is critical to adult feeding behavior, the rate of larval development and speed of virus replication. If the climate is too cold, viral development is too slow and mosquitoes are unlikely to survive long enough to become infectious. Although a suitable climate is necessary for disease transmission, other factors are needed for an epidemic to take place including source of infection, vector populations and a susceptible human population. Climate is one of the fundamental forces behind epidemics, and its effects become evident if adaptive measures falter or cannot be extended to all population risk (Hales *et al*. 2002).

In the vector mosquito, the virus, taken in during the bloodmeal from an infected patient, multiplies in the midgut of the mosquito. The viral particles were then released and either affect other cells and/or infect the salivary gland. In the salivary gland, the mosquito will transmit the virus to the host during feeding. The incubation period in the mosquito lasts about 7-10 days. A mosquito once infected with dengue virus is infected for life (Lee 2000).

2.0.2 Dengue fever in Malaysia

Among the vector-borne disease, dengue virus infections are emerging as the major ones in Southeast Asia (WHO,1986). Dengue fever (DF), DHF and DSS are now endemic in Southeast Asia. During the last decade, dengue infection with its complications has increased globally (Gubler 1997). Dengue like the mosquito that carries it, is found through tropical regions of the world. It was reported from over 100 countries with approximately 2,500 million people at risk.

DF and DHF have been serious *Aedes*-borne viral diseases in Southeast Asia since their first description in Penang in 1902 by Skae. Dengue is maintained by the indigenous *Ae. albopictus* and naturalized by *Ae. aegypti* (Ramalingam 1984). In studying the ecology of dengue in Malaysia, Rudnick (1978) showed clearly that dengue was a zoonotic disease of monkeys maintained by *Aedes pseudoniveus/subniveus* at canopy level. Transmission of dengue was thought to include silent jungle cycle, a rural endemic mild form maintained by *Ae. albopictus*, an urban cycle involving *Ae. aegypti*.

In recent years, the frequency of dengue epidemics in tropical and subtropical areas has increased. Among the vector-borne diseases, dengue virus infections are emerging as the major ones in Malaysia. In these epidemics, it was reported that all age groups were affected; the occurrence was high among children aged <6 years also presented symptoms of DHF (Ramalingam, 1984).

However, until today, transmission of dengue virus to human by *Aedes aegypti* and *Ae. albopictus* continues to be a serious problem in Malaysia. The number of reported cases of dengue haemorrhagic fever, which accounts for only a small proportion of total infections provides an index of total infections on a regional basis. Although reported cases fluctuated widely from year to year, the trend over the last decade has been upward; 27.5 cases/100,000 population in 1990 and the cases increased to 123.4 cases/population in 1998 during the global pandemic (Ang and Satwant 2001). In the year 2000, based on notification of clinically-diagnosed cases, 16.3 cases/100,000 population was reported. But unfortunately, dengue continues to be a public health problem in Malaysia when the incidences of dengue increases again from 36.4 cases/100,000 population in 2001, 63.6 cases/100,000 population in 2002 (Kementerian Kesihatan Malaysia (KKM), 2002).

The first report of dengue fever with haemorrhagic manifestations was made only on 1962 in Penang Island. Since then, the disease has become endemic throughout the country. In 1973 there was a major outbreak of DHF in Malaysia. Subsequently, in 1974, a plan of action for the prevention and control of DF and DHF in Malaysia was put into immediate effect and the disease was made notifiable (Rudnick 1965). A check by *The Star* showed that there were more than 3,000 reported dengue cases in 2005. Out of these, only 875 were confirmed cases. And seven people died from the disease. (The Star, 2006a). 58 dengue cases were reported for the first two months in 2006 and most of the cases were came from Sungai Dua and Taman Lip Sin (The Star, 2006b). Sungai Dua and Taman Lip Sin are neighbouring areas with USM.

Mosquito surveys in Penang Island revealed the presence of *Ae. aegypti* mosquitoes in relatively high abundance in the urban areas. *Ae. albopictus* were present in high abundance throughout the island in urban, rural and forest area. The ability of *Ae. albopictus* to transmit dengue virus was first shown in studies involving human volunteers as early as 1926 (Rudnick *et al*. 1965).

More recently, *Ae. albopictus* was shown to be able to transmit all four DEN serotypes. Various studies have shown *Ae. albopictus* to be associated with epidemics of dengue fever. However, because *Ae. albopictus* often overlaps in distribution with *Ae. aegypti*, another known vector of DEN viruses, it is often difficult to determine the relative contribution of the two species to disease transmission. Thus, *Ae. albopictus* may serve as an important maintenance vector of DEN viruses in endemic areas, and new endemic areas may be initiated by importation of vertically infected eggs (Gubler 2002).

During the 1960-1961 mosquito survey in Singapore, it was also noted that *Ae. albopictus* was common in both urban and rural areas. *Aedes albopictus* has also been considered as an important vector of endemic dengue in Southeast Asia. DHF is a disease of the urban human population with concentrations of cases in areas having a high density of human population. These also coincided with areas where both *Ae. aegypti* and *Ae. albopictus* have been widely found to be widely distributed and abundant (Chan *et al*. 1971).

2.1 Vector surveillance and control program

Since mosquitoes capable of transmitting disease such as dengue, it has so far not been possible to eliminate or eradicate even single species of mosquito from its native habitat and this is not for want of trying. An important way to control vector-borne disease is to control or limit the density of the vector such an extent that transmission of the pathogen or parasite is drastically reduced or even stopped. To achieve this we have to have detailed knowledge of all aspects of the vector such as correct identification of all stages, breeding habitat of the immature stages and detail adult biology (mating, resting, feeding habits, blood meal preference, egg laying habits, longevity and dispersal). Armed with this knowledge, the vector species can then be attacked at its weakest link (Ramalingam 1984). Reduction of the vector for dengue transmission, the *Aedes* mosquitoes remains the method of choice for controlling dengue (Ooi *et al*. 2001).

Dengue Fever and DHF are mosquito-transmitted arboviral diseases and their control depending on managing populations of *Ae. aegypti* (Gubler 1989). No effective vaccine or drug treatment for dengue fever is yet available, so management of disease has relied on vector control measures, such as reduction of breeding sites and use of insecticides. Such measures have succeeded in eradicating mosquitoes in some regions, but have proved difficult to maintain in the long term (Hales *et al*. 2002).

Dengue virus infections are significant and cause morbidity and mortality in many parts of the world. Dengue infection has the potential of rapid spread leading to an acute public health problem, thus special attention is required for its surveillance, prevention and control. Because vector control is the only option for dengue prevention, an assessment of breeding sources of *Aedes* is important for devising suitable control programs. Hence, preliminary entomological and serological surveys were carried out in rural areas in Malaysia to determine the prevalence of dengue vectors and their breeding habitats as well as the presence of dengue virus (Arunachalam *et al*. 2004).

An improved understanding of the relationships among various measures of entomological risk, human infection and disease prevalence and incidence would be important contributions for strengthening predictions for the effect of population replacement on dengue transmission. *Aedes aegypti* survive and efficiently transmit dengue virus even when their population densities are remarkably low (Scott *et al*. 2000).

Generally, the vectors that are important in the transmission of the diseases to human are those that are relatively host-specific in their attraction to human (anthropophilic), those that are common and widely distributed and those that live long enough to permit the disease organisms to develop to the infective stage (Miyagi and Toma 2000).

A successful population-replacement strategy that reduces mosquito density below threshold levels but fall short of *Ae. aegypti* eradication will reduce human herd community and accordingly increase the risk of virus transmission. Nevertheless, without information on the relationship between vector density and disease risk dengue programs will lack specific targets for vector densities. The new goal of dengue prevention and control programs became “cost-effective utilization of limited sources to reduce vector populations to level at which they are no longer of significant public-health importance” predicting and validating entomological threshold is one of the most important contributions that could be made to our understanding of dengue epidemiology and the application of population replacements strategies for disease prevention (Folks *et al*. 1995).

Assumptions about the relationship between mosquito density and dengue risk were not necessary for eradication programs. They are, however essential for vector control strategy. Nature of the relationship between density and risk, will vary temporally and spatially depending on factors like human herd immunity, density of human hosts, characteristics of mosquito-human interaction, virus introductions into the system, and weather: for example temperature and relative humidity, that affect mosquito biology and mosquito-virus interactions (Muttitanon *et al*. 2004).

While *Ae. aegypti* remain biting mainly indoor, *Ae. albopictus* bites both indoor and outdoor. As there are no specific treatment for dengue at the moment, vector control against the mosquitoes is given emphasis in the dengue control programs. The objective of vector control on the other hand are to reduce the density of vectors and to reduce man-vector contact. During outbreaks, the role of vector control is to prevent the spread of the disease by killing the infected and infective vectors (Tham 2001).

Entomological survey is an important and integral part of dengue prevention and control. The effect of the intervention by the community can directly affect the ecology of the vectors that is the *Aedes* mosquitoes. The behavior of these vectors and their close association with human habitation is an important consideration in choosing certain parameters to be measured.

2.2 Biology of *Aedes albopictus* (Skuse)

Mosquito population dynamics largely depend on biological and environmental conditions (Tsuda *et al*. 1991). Bates (1949) was the first mosquito biologist to categorize mosquito life cycles on the basis of shared life cycles strategies. Bates also recognized that tropical mosquitoes breed continuously unless their development is interrupted for the lack of water, and he grouped his life cycle types by habitat to indirectly reflect wet versus dry season abundance (Reinert 2001).

Mosquitoes are small, two-winged insects belonging to the family Culicidae of the order Diptera. They are among the best-known groups of insects because of their importance as pests and as vectors of diseases. Mosquitoes are easily distinguished by the combination of the following characters: a long proboscis projecting from the head; the presence of scales on the wing veins, a fringe of scales along the posterior margin of the wing, and the characteristic wing venation, the second, fourth and fifth longitudinal veins being branched (Miyagi and Toma 2000).

Because most female mosquitoes feed on blood, their mouthparts are highly specialized for piercing host skin and sucking blood (Wahid *et al*. 2002). Male mosquitoes do not take blood. Their food sources are mainly floral and extrafloral nectaries, honeydew or even plant tissue (Schlein and Muller 1995).

Both *Aedes* species are generally day-time biters and active during the day. During the day, both mosquitoes have peaks of landing and biting activity. The first peak of *Ae. albopictus* biting occurred about one hour after sunrise and reaches another peak before sunset (Abu Hassan *et al.* 1996). But mosquito biting rates change depending on mosquito age and time of the day. Host attack rates were highest in the morning, at 0800h, and in the evening between 1400 and 2000h and were lowest between 0200h and 0600h (Xue and Barnard 1996).

Important biological differences between widely distributed *Ae. albopictus* and these locally confined *Stegomyia* spp. are related to their dispersal and colonizing abilities. Comparative studies of behavior, ecology and physiology of *Ae. albopictus* and its associated *Stegomyia* spp. in Asia elucidate divergent habitat adaptations among *Stegomyia* that may be important in understanding the evolution of *Stegomyia* mosquitoes (Sota *et al*. 1992).

In Asia, where the species was introduced at the end of the 19th century, *Ae. aegypti* is a domestic antrophophilic mosquito, breeding around houses in artificial containers (Strickman and Kittapayong 1993), has a short flight range (Tsuda *et al*. 2001) and gets multiple bloodmeal before oviposition (Scott *et al.* 1997). Thavara *et al.* (1989) demonstrated that *Ae. albopictus* prefers to lay eggs in the field in containers with conditioned water that was left outside for a long period and with a stable flora together with the immature stages of this species.

Aedes albopictus exhibits greater flexibility in various traits than *Ae. aegypti*, such as choice of oviposition sites. However, the preferred breeding sites of the species are different and only slight overlap has been noted (Gould *et al.* 1970). The types of major breeding containers used by *Ae. albopictus* have been studied intensively considering few aspects such as container types, container covers and containers locations (Kittapayong and Strickman 1993). *Aedes albopictus* has a reputation to breed in a great variety of container habitats. *Aedes albopictus* breeds in both artificial and natural container. *Aedes albopictus* larvae develop in discarded tires, cemetery vases, water-filled tree holes and other containers (Hawley 1988). However, breeding containers of *Aedes* are associated with the environments and living habits of local residents which can vary by city and country (Teng *et al*. 1999). Juliano (1998) indicated that *Ae. albopictus* maintains a greater population rate of increase under conditions of crowding and resources stress, potentially giving it a comperative advantage over *Ae. aegypti*.

2.3 Life-cycle of *Aedes albopictus*

The mosquito gonotrophic cycle includes the search for host and the ingestion of a bloodmeal, the digestion of the meal and the maturation of ovaries, and the laying of mature eggs after a search for an oviposition site (Klowden and Briegel 1994). The oviposition behaviors of female mosquitoes were separated into two distinct behavioral categories: preoviposition and oviposition. Female mosquitoes choose oviposition sites during preoviposition stage. A female mosquito chooses oviposition sites by a combination of visual and chemical cues. Ovipositing mosquitoes “taste” the water associated with a potential ovipostion site to detect chemical cues (Bentley and Day 1989). *Aedes albopictus* is a multi-voltine species and should have a seasonal distribution and if in this case, larvae and egg collections should be evident in suitable breeding habitat throughout the year.

The complete life cycle from egg to adult at ambient temperature was 9-10 days. Studies at the Institute of Medical Research, Malaysia, have indicated that the females of *Aedes albopictus* are ready to oviposit 4-5 days after copulation. Females of *Ae. albopictus* lay an average of 79 eggs per female. The eggs hatched within 1-48 hour at ambient temperature (Lee 2000). The numbers of egg laid by *Ae. albopictus* depends on the physiological age of the mosquito, the body weight after emergence, and particularly the size of the blood meal (Estrada-Franco and Craig 1995). In the laboratory, egg mortality through 30 days for *Ae. albopictus* was strongly temperature and humidity dependent, with low humidity and high temperature producing greatest mortality (Juliano *et al.* 2002). Female adults are generalist blood feeders that lay desiccation-resistant eggs above the water line. These eggs hatch when flooded, and the larvae feed on microorganism and detritus (Daugherty *et al*. 2000).

The duration of larvae period is 6-8 days. Clearly, every larval mosquito habitat is selected by the gravid female. Whatever its nature, her act of oviposition usually takes place upon the chosen water. Sometimes too it occurs where water is not yet present but where it is destined to be: for example, of the latter practice is widespread among seasonally prevalent Culicidae in temperate regions and at high latitudes or altitudes where diapause is broken at the spring thaw, and also in tropical ones where hatching is triggered by heavy rainfall (Laird 1988). Mosquito larvae typically are filter-feeders, subsisting on microorganisms and particulate organic debris, supposedly with little ingestion of liquid (Dadd 1968). Water quality has been found to be an important limitng factor in breeding, where *Aedes* larvae has been found to require clear but not necessarily clean water (Lee 1991).

Under ideal conditions, *Aedes albopictus* remains in the pupal stage for about two days and as other *Aedes* species, *Ae. albopictus* males emerge before female (Estrada-Franco and Craig 1995). The mortality of eggs and larvae (combined) is about 9%, pupal mortality, 4% for this species. The life span of *Ae. albopictus* is 10-22 days for male and 12-40 days for females (mean 16 and 26 days respectively). The female:male ratio was about 1.4:1.0 for *Ae. albopictus* (Lee 2000). Besides that, the general belief has been known that *Aedes* female mosquitoes have a maximum flight range of only 50-100m during their entire lifetime (Liew and Curtis 2004).

2.4 Seasonal prevalence as a vector control program

In the past decades, there has been dramatic increase in the development of infrastructures. It is believed that these developments have had an impact on the abundance of *Aedes* mosquitoes by providing more habitats for these mosquitoes, thus, leading to an increase in the abundance of dengue vectors

(Thavara *et al.* 2001). The principle mosquito vector is *Aedes aegypti*, a highly domesticated species that has adapted in urban environment by using artificial containers that collect rainwater or those used for domestic water storage as a larval habitat. Other mosquito species such as *Aedes albopictus*, *Aedes polynesiensis*, and *Aedes medioviitarus* may be involved in suburban or rural maintenance cycles but less frequently responsible for transmitting epidemics of DF and DHF (Gubler and Clark 1996). Much effort has been directed towards understanding the invasive properties of *Aedes albopictus* from forested areas, where it originates as well as from indigenous to non-indigenous countries (Reiter 1998).

The global spread of *Ae. albopictus* during the past few years has caused alarm among some scientists and public health officials over the possibility that the introduction of this species will increase the risk of epidemic dengue fever, yellow fever, Japanese encephalitis, West Nile, Chikungunya and other arbovirus in the countries where it has become established (Gubler 2003).

This alarming situation of dengue has been caused by increased population growth and overcrowding, uncontrolled and underserviced urbanization (which severely affects the poor) and deterioration of public health infrastructure and mosquito control efforts (Kay and Nam 2005).

To plan vector control, it is necessary to identify the DHF vector and understand its basic biology and breeding habitats. Given the potential for *Ae. albopictus* to become a public health problem, it is important to acquire a thorough understanding of the biology of this species as a prerequisite for any control program. The purpose of *Aedes* surveillance is to obtain information on *Aedes* larval densities in terms of time and space. The main approach for *Aedes* surveillance is by regular larval survey. The purpose of larval survey is to find out the distribution, type and density of *Aedes* larvae in a given locality/facility (Tham 2000).

The lack of effective vaccines and treatment, the rapid urbanization, the emergence of insecticide-resistant mosquitoes and the increasing spread of viruses and vectors throughout the world require the development of alternative methods to control dengue transmission (Paupy *et al*. 2003).

An indirect measure of adult female presence or absence is the oviposition trap or ovitrap. Detection and measuring mosquito abundance through their egg-laying activities using ovitraps is the most common surveillance or sampling methods for this and some other *Aedes* mosquitoes, especially *Ae. aegypti* (Service 1992). Tham (2000) stated that ovitraps provide a sensitive and economical method for detecting the presence of *Ae. albopictus* in situations where the density is low and general larval surveys produce unsatisfactory results (e.g when the Breteau index is <5). Ovitraps can also be used to assess *Aedes* population fluctuation over a long-term period (Tham 2000). Tham (2000) also stated that ovitrap do not provide estimates of *Aedes* population densities but can give insights into relative changes in the adult female populations.

It was reported that under laboratory condition, females *Ae. albopictus* preferred to oviposit in habitats with a rough gray surface with low reflectivity rather than on smooth black surface with high reflectivity (Ho *et al*. 1971). It was also observed that ovitraps coated with red and black were preferred over ovitraps of other colours (Yap 1975). From previous experiments as stated above, it can be concluded that a darker surface of ovitraps enchance the oviposition of the *Aedes* mosquitoes.

Service (1993) mentioned that artificial containers can be placed in ecological niches, at different heights and in different mosquito-related habitats. Artificial habitats are sometimes used to monitor changes in seasonal abundance of mosquito breeding in natural sites in an area, but there maybe severe limitations in their ability to reflect true population changes. When water is maintained in artificial habitats when natural ones are drying out, because of their availability, they may attract abnormally large numbers of ovipositing females, and consequently not affect the decrease in population size that is occurring within the area. It is difficult to compare population size in different areas by using artificial habitats. For example, the absolute mosquito populations may be the same in two areas but if in one area there are twice as many breeding places as in the other, then artificial habitats will indicate that the population is about half that of the other. A correct interpretation would only be possible if the total number of available habitats in each area was known. Usually, the size, the attractiveness of the natural larval habitat varies and the problem becomes increasingly difficult. (Service 1993).

However, information on oviposition attractants for *Ae. albopictus* is rather limited at the present time (Thavara *et al*. 2004). There are several obvious advantages in using small artificial containers. Both of the total larval and egg population and predators and other associated fauna can frequently be counted with the minimum of effort, returned and recounted on successive sampling occasions. It was also a crucial effort to find out the importance of oviposition site preferences in planning vector programs against *Aedes* mosquitoes (Yap *et al*. 1995).

An ovitrap based transmission model was successfully developed and to determine the threshold of transmission. The year-to-year variations in the threshold of transmission of a particular locality may reflect the actual efficiency of the vector control operations; if the threshold increased, it would imply that higher vector populations density is required to initiate an outbreak, as the original population is now less efficient in transmission due to the effective vector control operations (Lee and Chang 1997). Thus, this model is not only able to determine the transmission threshold but also can be used as an epidemiological tool to evaluate the effectiveness of the dengue vector control operations in the field.

2.5 Environmental factors associated with *Aedes albopictus*

In general, insects are exceedingly sensitive to temperature and rainfall regimes, tropical and temperate species frequently show great variations in seasonal abundance (Samways 1995). Research on the distribution of *Ae. albopictus* has focused on the effects of the abiotic factors, such as temperature, humidity, conductivity, pH, dissolved oxygen and biotic factors that have a major influence on the distribution (Teng and Apperson 2000).

Mosquitoes are remarkably selective in their choice of breeding habitats, and it would appear that female species with diverse breeding habitats must respond differently to visual, chemical and tactile stimuli in selecting exactly the right kind of water in which larvae are best suited to develop (Gubler 1971). The mosquito is able to survive in a wide range of temperatures and climatic conditions as long as there are several sources of stagnant water such as open containers, which gather rainwater (Akram and Lee 2004). *Aedes albopictus* is adapted to both tropical and temperate climates and is capable of using a wide range of suitable containers (Hawley 1988). Changes in both temperature and precipitation affect the population of *Ae. albopictus* by disturbing the reproductive and mortality rates (Akram and Lee 2004). Higher temperatures decrease larval development times (Teng and Apperson 2000).

All insects can be considered to be poikilotherms, that is, their body temperature varies with that of the surroundings. Their basic metabolism is a function of the temperature of their surroundings, such that within a certain range the higher the temperature, the faster metabolic reactions are able to proceed. This means that any processes such as growth, development or activity are all dependent on temperature (Speight *et al*. 1999).

Because temperature and precipitation cover regionally, experiments manipulating both of these factors are needed to determine accurately how these abiotic factors influence *Ae. albopictus*. In addition, distribution of *Ae. albopictus* may be affected by anthropogenic changes in regional temperatures and precipitation regimes. Interactions of effects of temperature and precipitation also