LIFE TABLE, DESSICATION TOLERANCE, BREEDING HABITATS AND MIDGUT *Lactobacillus* IDENTIFICATION IN DENGUE VECTORS IN PENANG ISLAND, MALAYSIA.

by

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*In memory of my beloved elder brother Hassan Maikime, my elder sister Falmata Maikime and my son Aliyu who all passed away during the period of this study. May their souls rest in perfect peace.

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LIST OF ABBREVIATIONS

BI	Breteau index
CAT	Catalase
CI	Container index
E	East
GLU	Glucose utilization
GM/Mor	Gram stain and morphology
HI	House index
LAC	Lactose utilization
MEL	Melibiose utilization
NA	Not applicable
N	North
n	Number of samples
NH4-arg	Ammonia from arginine
OXI	Oxidaze
O/F	Oxidative/fermentative
PC	Positive container
RAF	Raffinose utilization
Sg. Ara	Sungai Ara
Sg. Nibong	Sungai Nibong
WHO	World Health Organization
sp	Species

JADUAL HIDUP, TOLERANSI KEKERINGAN, HABITAT PEMBIAKAN DAN PENGECAMAN *Lactobacillus* DALAM USUS TENGAH VEKTOR DENGGI DI PULAU PINANG, MALAYSIA.

ABSTRAK

Virus denggi menjangkiti lebih 100 juta orang setiap tahun dan kemungkinan untuk mengurangkan insiden denggi dikaji di seluruh dunia. Kawalan vektor adalah salah satu pilihan, kerana gangguan penyebaran parasit denggi adala strategi kawalan penyakit yang paling berkesan. Atas sebab ini, adalah penting untuk mengkaji bionomiks vektor. Kajian ini dijalankan dalam empat fasa, iaitu; jadual hayat, toleransi kekeringan, habitat pembiakan dan pengenalan *Lactobacillus* di usus tengah dalam vektor denggi. Sifat-sifat pembentukan jadual hayat strain liar *Aedes albopictus* dan *Aedes aegypti* telah dianalisis berdasarkan kepada peringkat-umur, dan jadual hidup dua seks. Hasil kajian termasuk tempoh praoviposisi dewasa, jumlah tempoh praoviposisi, min kadar peningkatan intrinsik (r), min kadar peningkatan finit (ʎ), kadar pembiakan bersih (R_0), dan min masa generasi (T). Jumlah masa pembangunan pra-dewasa adalah 9.47 hari bagi *Ae. albopictus* dan 8.76 hari bagi *Ae. aegypti*. Jangka hayat adalah 19.01 hari untuk *Ae. albopictus* dan 19.94 hari untuk *Ae. aegypti*. Kematian berlaku terutamanya semasa peringkat dewasa. Min masa pembangunan untuk setiap peringkat berkait lemah dengan suhu kedua-dua *Ae. albopictus* ($r = 0.21$, $P < 0.05$) dan *Ae. aegypti* ($r = 0.31$, $P > 0.05$). Pada bahagian kedua kajian, satu penilaian toleransi kekeringan pupa *Ae. albopictus* dan *Ae. aegypti* berumur 1 hari dan 2 hari telah dijalankan di bawah suhu dan kelembapan ambien. ANOVA satu hala dan korelasi Pearson telah digunakan untuk menganalisa data. Pupa yang berumur satu

hari menunjukkan mortaliti 100% untuk kedua-dua spesies. Kematian yang rendah dicatatkan bagi pupa berumur 2 hari *Ae. albopictus* (33%) dan *Ae. aegypti* (28%). Bagi kedua-dua spesies, pupa berumur 2 hari tahan kekeringan manakala pupa berumur 1 hari tidak tahan kekeringan. Walau bagaimanapun, kedua-dua spesies menunjukkan sedikit perbezaan dalam toleransi kekeringan. Bahagian ketiga kajian ini telah dijalankan untuk menentukan kelimpahan, taburan dan kepelbagaian potensi habitat bekas pembiakan kedua-dua vektor denggi di tempat-tempat awam di Pulau Pinang, Malaysia. Premis di restoran-restoran, sekolah, taman dan masjid telah ditinjau secara serentak. Statistik deskriptif digunakan untuk mendapatkan bilangan bekas dalam lokasi. Kelimpahan (min ± SE) bekas pembiakan antara tapak telah dibandingkan menggunakan ANOVA satu hala. Ujian-t digunakan untuk membandingkan jumlah bilangan *Ae. albopictus* dan *Ae. aegypti* yang dikaji. Sebanyak 3,741 bekas pembiakan dan 19, 537 tidak matang dicatatkan semasa musim basah (Min ± SE = 3520. 500 ± 891,51, df = 3, $p < 0.05$) dan musim kering (Min ± SE = 636,250 ± 187,43, df = 3, $p < 0.05$). Bahagian terakhir kajian ini dijalankan untuk mengasingkan dan mengenal pasti *Lactobacilli* dalam usus larva dan dewasa liar *Ae. aegypti*. Pengasingan dan pengenalpastian simbion bakteria telah dilakukan dengan menggunakan ujian gram pewarnaan, ujian motililiti, ujian catalase dan ujian biokimia. Morfologi disahkan menggunakan skim standard yang memudahkan pengenalpastian bakteria asid laktik. Sejumlah 174 spesies bakteria telah diasingkan dan pasti dikenal, 135 strain dari dalam usus larva dan 39 jenis dari dalam usus dewasa (Min ± SE: 4.00 ± 0.72, $P < 0.05$). Spesies yang terpencil adalah: *L. fermentum, L. casei, L. acidophilus, L. viridescens, L. brevis dan L. gasseri*. Boleh disimpulkan bahawa *Ae. aegypti* mempunyai potensi untuk menjadi perumah simbion bakteria dan boleh digunakan untuk program paratransgenesis.

LIFE TABLE, DESSICATION TOLERANCE, BREEDING HABITATS AND MIDGUT *Lactobacillus* IDENTIFICATION IN DENGUE VECTORS IN PENANG ISLAND, MALAYSIA.

ABSTRACT

Dengue viruses infect over 100 million people every year and possibilities to reduce dengue incidence are studied world-wide. Vector control is one of the options, as interruption of transmission of dengue parasites is clearly the most effective disease control strategy. For this reason it is important to study the bionomics of the vectors. This study was conducted in four phases, namely; life table, desiccation tolerance, breeding habitats and identification of midgut *Lactobacillus* in dengue vectors. Life table developmental attributes of wild strains *Aedes albopictus* and *Aedes aegypti* were analyzed based on the age-stage, two-sex life table. Parameters measured are adult preoviposition period, total preoviposition period, mean intrinsic rate of increase (r), mean finite rate of increase (l), net reproductive rates (R_0), and mean generation time (T). The total pre-adult development time was 9.47 days for *Ae. albopictus* and 8.76 days for *Ae. aegypti*. The life expectancy was 19.01 days for *Ae. albopictus* and 19.94 days for *Ae. aegypti*. Mortality occurred mostly during the adult stage. The mean development time for each stage weakly correlated with temperature both for *Ae. albopictus* ($r = 0.21$, $P > 0.05$) and *Ae. aegypti* ($r = 0.31$, $P > 0.05$). In the second part of the study an assessment of desiccation tolerance of 1 day old and 2 day old *Ae*. *albopictus* and *Ae*. *aegypti* pupae was conducted under ambient temperature and humidity. One way ANOVA and Pearson‘s correlation were used to analyze the data. One day old pupae expressed hundred percent

mortality for both species. Low mortality was recorded for 2-day old *Ae. albopictus* (33%) and *Ae. aegypti* (28%) pupae. For both species, 2 day old pupae .resisted desiccation while 1 day old pupae did not. However, the two species shows slight variation in their desiccation tolerance. The third part of the study was carried out to determine abundance, distribution and diversity of potential breeding container habitats of the two dengue vectors in public places in Pulau Pinang, Malaysia. Premises at Restaurants, schools, parks and mosques were surveyed simultaneously. Descriptive statistics were used to obtain mean number of container within locations. Abundance (mean ± SE) of breeding containers between sites was compared using One Way ANOVA. T-test was used to compare total number of *Ae. albopictus* and *Ae. aegypti* surveyed. A total of 3, 741 breeding containers and 19, 537 immature was recorded during wet season (Mean ± SE = 3520. 500 ± 891.51, $df = 3$, $p < 0.05$) and dry season (Mean ± SE = 636.250 ± 187.43, $df = 3$, $p < 0.05$). The last part of the study was carried out to isolate and identify *Lactobacilli* in larval and adult midguts of wild *Ae. aegypti*. The isolation and identification of the bacterial symbionts was done using gram staining test, motility test, catalase test and biochemical tests. Morphological features were confirmed using standard scheme that simplify the identification of lactic acid bacteria. Total of 174 bacterial species were isolated and identified, 135 strains from larval midguts and 39 strains from adult midguts (Mean ± SE: 4.00 ± 0.72, $P < 0.05$). The isolated species were: *L. fermentum*, *L. casei*, *L. acidophilus*, *L. viridescens*, *L. brevis* and *L. gasseri*. It can be concluded that *Ae. aegypti* has the potential to harbor bacterial symbionts, hence they can be used for paratransgenesis program.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Introduction

Dengue fever infection is one of the most important arboviral diseases in humans and it is endemic in Africa, the Americas, Eastern Mediterranean, Southeast Asia and the Western Pacific (Opena et al. 2013). It is estimated that 50 – 100 million dengue infections occur each year (Sabchareon et al. 2012). An estimated 3.45-3.61 billion people (53% - 55% of the world population live in areas at risk of dengue from 124 countries (Eisen et al. 2008). Outbreaks exert a huge burden on populations, health systems, and economies in most tropical countries of the world (Sabchareon et al. 2012). In Malaysia, dengue was first documented in 1902, while the first outbreak occurred in Penang in 1962 (Lee and Inder Singh 1993). Though endemic in the sixties, dengue haemorrhagic fever (DHF) emerged as a major public health problem in Malaysia from 1973 onward (Shekhar and Huat 1992). Efforts have been mainly made to control dengue through chemical insecticides, but with limited success (Dieng et al. 2010a). In an epidemic situation, the local health authority each time carryout thermal fogging and Ultra-Low Volume (ULV) space spray (Yeap et al. 2011). Unfortunately the dengue vectors have developed resistance to most insecticide groups at the time when specific therapeutics and vaccines to prevent the infection currently do not exist (Raharimalala et al. 2012). The use of certain insecticides have been found to increase pest problems (Elliott et al. 1978).

Control of the disease depends largely on the control of the vector. Thorough understanding of the ecology of pest has been considered crucial for an ecologically sound integrated pest management program (Huang and Chi 2012).

Therefore, mosquito surveillance plays an important role in formulating a good control program (Silver 2007). Simultaneous control of adult and larval stages is essential in dengue vector control to prevent transovarial transmission of dengue virus (Pinheiro et al. 2005). Life tables are tables of data on survivorship and fecundity of individuals within a population. A standard method is to collect data on a cohort, or group of individuals all born in the same time period. Understanding these demographic processes and how they affect populations is a central concern of population and conservation biologists (Ehrlich and Hanski 2004).

Desiccation tolerance is the capacity of a creature to endure or withstand life-threatening dryness. Pupae of some mosquito species, however, are able to resist desiccation to some extent, and when removed from water may remain alive and active for some hours; while mosquito larvae, on the contrary, unless kept moist, quickly begin to dry and die (Christopher 1960). Pupae of mosquito that were "dried" for 24 hr, still emerged from pupal case (Young 1922). It has also been demonstrated that adults and eggs of *Ae. albopictus* and *Ae. aegypti* species differ in desiccation tolerances (Sota et al. 1992). *Aedes albopictus* eggs are much more sensitive to desiccation than are *Ae. aegypti* eggs (Juliano et al. 2002).

Despite possibilities to generate modified vectors (Catteruccia et al. 2000), a technique for replacing wild population with a vector-incompetent population is lacking (Riehle and Jacobs-Lorena 2005). Symbiotic Lactic Acid Bacteria (LABs) can be exploited in strategies towards paratransgensis-based dengue vector management. Since dengue vectors can only develop when they find a suitable breeding site, feed and reproduce only when bacteria are present, knowing the bacterial communities in their breeding sites and midgut is thus a priority in the search for effective vector intervention strategies (Riehle and Jacobs-Lorena 2005). The LABs constitute an important part of mosquito midgut micro-biota. In humans, LAB breakdown food, enhance nutrient bioavailability and immune system, regulate cholesterol and fight off unfriendly organisms that might cause diseases.

To date, dengue lack effective treatment or licensed vaccine (Durbin and Whitehead 2012), despite the availability of several candidates (Thomas and Endy 2011). Although a vaccine has been introduced, but with limited scope of usage. Taking these factors into consideration, with the harmful impacts of insecticide use and concerns about climate change, bio-rational approaches have gained popularity. The only way is managing the vectors.

1.2 Objectives of the Study

1. To assess monthly abundance, distributions and diversity of container habitats in public sites in Penang Island by determining type, category (material) and movability of encountered container habitats.

2. To compare larval development of wild strains of *Aedes* mosquitoes (*Aedes albopictus* and *Ae. aegypti*); studying their differences in population characteristics by constructing age-stage, two-sex life table.
3. To assess the effects of age, humidity and temperature on desiccation tolerance of pupae of *Ae. albopictus* and *Ae. aegypti*.
4. To isolate and identify microbial communities in midguts of most prevalent larval and adult *Aedes* mosquitoes from milk source containers with a focus on the species of *Lactobacilli.*

CHAPTER TWO

LITERATURE REVIEW

2.1 *Aedes* aegypti and *Ae. albopictus* mosquitoes

Aedes aegypti and *Ae*. *albopictus* have been incriminated as vectors of dengue fever and dengue haemorrhagic fever viruses in numerous studies (Chan et al. 1971; Nogueira et al. 1999). The capacity of *Ae. albopictus* and *Ae. aegypti* mosquitoes to transmit several deadly arboviruses like dengue and related diseases is well documented (Morens and Fauci 2008). Dengue ranks as the most important mosquito borne viral disease in the worl, infecting over 100 million people every year with 30-fold increased in incidence during the past five decades (Kyle and Harris 2008). Some 50 – 100 million new infections are estimated to occur annually in more than 100 endemic countries (Sabchareon et al. 2012), with a documented further spread to previously unaffected areas (La Ruche et al. 2010). Dengue viruses infect over 100 million people every year (Hahn et al. 2001), and often affecting very poor populations (Shepard et al. 2013). The true numbers are probably far worse since severe underreporting and misclassification of dengue cases have been documented (Shepard et al. 2013).

Aedes albopictus which breeds in both man-made containers such as cans, tires, and water jars; as well as in natural containers such as bamboo, bromeliads coconut shells is more cosmopolitan in its breeding habitats and rests both inside and outside homes, making control difficult. Niche segregation among container-inhabiting mosquitoes based on container type selection has been reported (Sunahara et al. 2002). *Aedes albopictus* can also inhabit rural areas without any artificial containers, posing additional health problem (Morens and Fauci 2008) with high preference for gardening utensil (Saleeza et al. 2011). While thriving in peri-domestic environments and depositing eggs in natural and artificial containers, *Ae. albopictus* was found to be an important container-inhabiting mosquito that transmit disease agents, outcompete native species, and continue to expand its range in the United States (Kraemer et al. 2015). Dengue viruses are the causative agents of dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) in humans (Idrees and Ashfaq 2012). The contemporary worldwide distribution of the risk of dengue virus infection and its public health burden are poorly known(Bhatt et al. 2013)

(Bhatt et al. 2013) estimated that there to be 390 million dengue infections per year, an infection total more than three times the dengue burden estimate of the World Health Organization (Organization 2009). Dengue claims an estimated 20,000 victims per year. Over 40% of the world's population is now at risk and 75% of the population at risk lives in the Asia Pacific region, and dengue fever cases are in alarming state in Malaysia. Today, dengue ranks as the most important mosquito-borne viral disease in the world and has been described as world's fastest growing vector-borne disease, making human life and economic costs staggering (Sabchareon et al. 2012). There are over 2.5 billion people living in high-risk areas with 390 million infections per year (Bhatt et al. 2013). The resultant diseases extract an immense toll in terms of loss of life, human suffering, incapacitation and economic losses. Control measures of the spread of dengue and related diseases depend largely on the management of the vector. These new risk maps and infection estimates provide novel insights into the global, regional and national public health burden imposed by dengue. As more information were collated on the ratio of dengue haemorrhagic fever to dengue fever cases, and the ratio of deaths to dengue haemorrhagic fever cases, the global figures were revised to 50–100 million infections

(Tsuda et al. 2006), although larger estimates of 100–200 million have also been made (Bhatt et al. 2013). These estimates were intended solely as approximations but, in the absence of better evidence, the resulting figure of 50–100 million infections per year is widely cited and currently used by the World Health Organization (Sabchareon et al. 2012).

2.2 Identification of *Ae. albopictus* and *Ae. aegypti*

Aedes albopictus is characterized by a black and white body, thorax with a single and broad line with black and white stripped legs. Its body is about 2-10 mm long and the body size in adult depends on larval population density and food supply within the breeding water (Walker et al. 2011). Though morphologically very similar, the males are smaller than the females and the antennae of the males are bushier and contain auditory receptors. In a study on ten images of both male and female *Ae. albopictus*, (Belkin 1962), found that, the average length of the abdomen was 2.63 mm, the wings 2.7 mm with the proboscis 1.88 mm. Difference between *Ae. aegypti* and *Ae. albopictus* is shown in Table 2.1, Figures 2.1 and 2.2.

Table 2.1. Characteristic difference between *Aedes eagypti* and *Aedes albopictus*

Stage	*Aedes aegypti* Linnaeus, 1762	*Aedes albopictus* (Skuse, 1894)
Adult	Thorax with silvery straight and curved lines.	Thorax with a single broad line.
	Prefer indoor breeding sites.	Prefered outdoor breeding sites.
Larva	Spines at the base of pleural hairs on thorax are large and ending in a single point.	Spines at the base of pleural hairs on thorax are ending in several points.
	The lateral hairs on the saddle are single.	The lateral hairs on the saddle are double.
	Comb hair at 8^{th} abdominal segment with well-developed lateral dentricles.	Comb hair at 8^{th} abdominal segment without lateral dentricles.

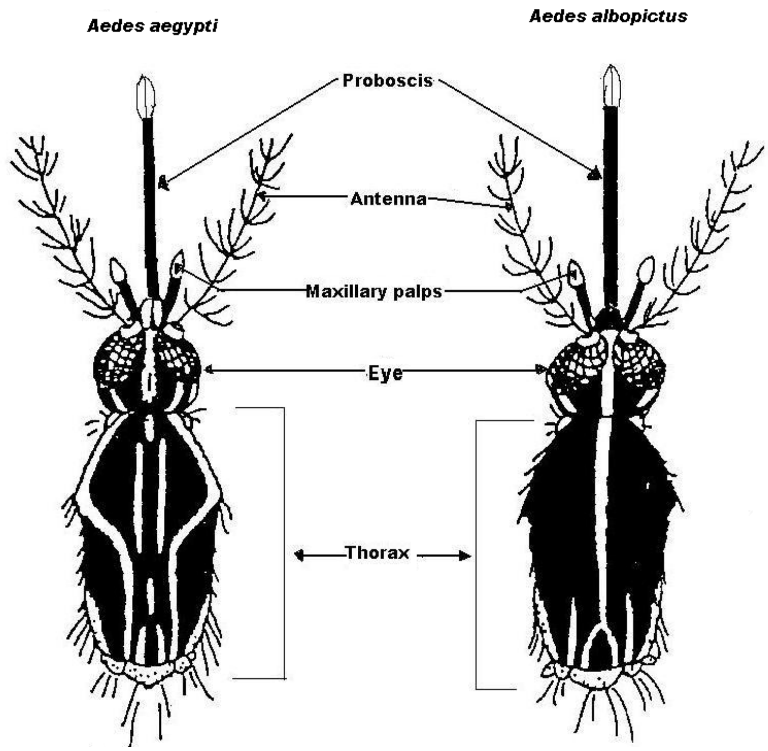

Figure 2.1. Morphological difference between *Ae. aegypti* and *Ae. albopictus* adults. Source: Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) Magnolia PressAuckland, New Zealand.
http://medent.usyd.edu.au/arbovirus/mosquit/photos/mosquitophotos.htm#aegypti

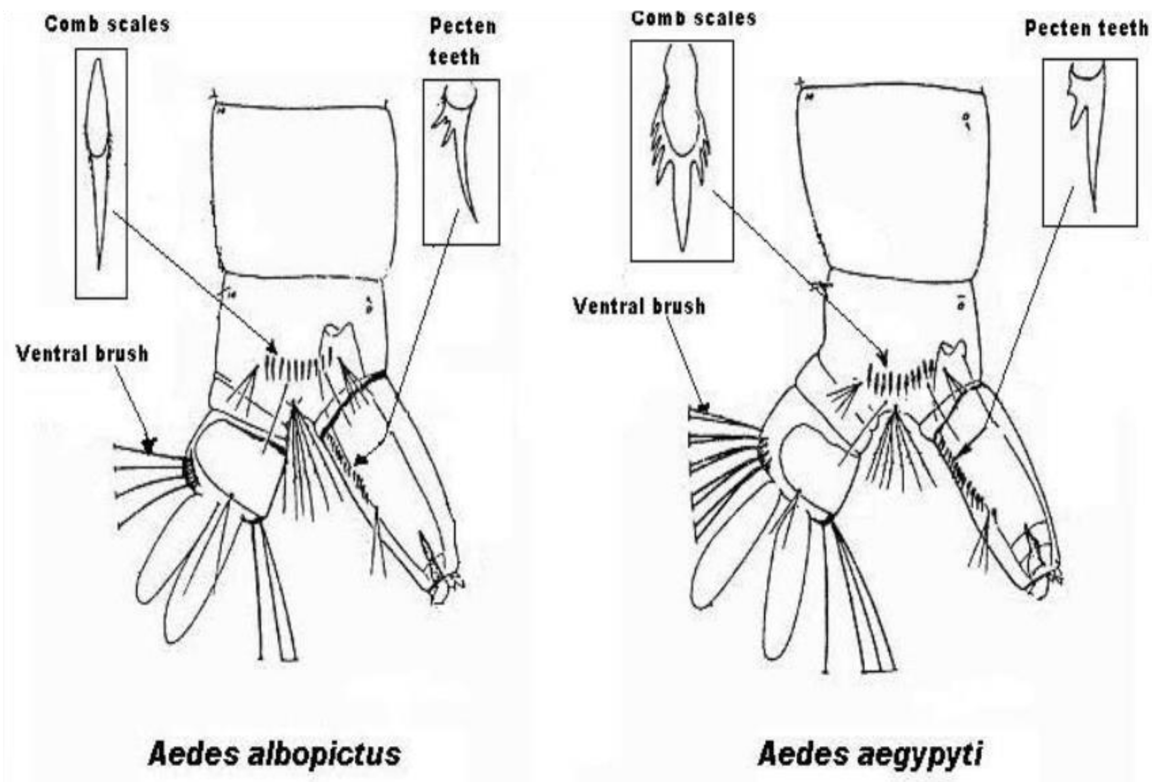

Figure 2.2. Morphological differences between adults of *Ae. aegypti* and *Ae. albopictus* at larval stage. Source: Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) Magnolia PressAuckland, New Zealand http://medent.usyd.edu.au/arbovirus/mosquit/photos/mosquitophotos.htm#aegypti

2.3 *Aedes* Mosquitoes Behaviour

Dengue vectors have well adapted to metropolitan environments. Most of the dengue cases reported were from urban areas with high population density and rapid development activities, a factor considered favouring dengue transmission. However, behaviourally the vectors exercise dynamicity in selection of habitats. While inhabiting forest fringes or secondary forest habitats, *Ae. albopictus* can also be found in green areas in town (Abu

Hassan 1994). Mixed breeding of *Ae. albopictus* with *Ae. aegypti* in ant-traps and earthenware jars outside the house has been reported in Malaysia (Dhang 2009).

(Thavara et al. 2001) showed two peaks of biting and landing activities of *Aedes* mosquitoes. One peak was just after sunrise and the other just before sunset. The biting activities dropped significantly during the hot period of the day at noon and is more pronounced in non-forested than forested areas (Reiter 2001). The search for host is demonstrated in two phases. First, the mosquito presents non-specific behavioural approach until the perception of host stimulants, while the second phase is the targeted approach. *Aedes albopictus* is generally a diurnal (day-time) biter and is more active during the day (Nichols and Vogt 2008). During the day (after sunrise and before sunset) there are peaks of biting and landing activities. (Ehrlich and Hanski 2004) described the flight range of the adult as quite-short, being a scrub-habitat mosquito. The flight range of the adult Asian tiger mosquito (*Ae. aegypti*) was limited to a distance of approximately 91-274 meterss and they have not been observed to fly in strong winds (Fukuda et al. 1997).

2.4 Life history of *Aedes mosquitoes*

Aedes mosquitoes like all other mosquitoes have distinct life stages consisting of egg, larva, pupa and adult. The first three stages are aquatic while the adult is the free flying insect feeding on humans, animals and plant juices (Reiter 2001).

2.4.1 Eggs

Eggs of *Aedes* mosquitoes are black in colour, rugby ball-shaped and singly laid. Engorgement to oviposition takes 2-5 days (Bentley and Day 1989). The eggs are laid singly on the surface of the water and usually hatch when flooded. (Sulaiman et al. 1996) reported that, eggs of *Aedes* mosquitoes are laid just at or below the water-line in the containers and will hatch in approximately 72 hours. *Aedes* eggs also hatch better in deoxygenated water. Freshly laid eggs of *Aedes* mosquitoes are capable of remaining dormant until flooding and ecological factors trigger the pharate larvae (Opena et al. 2013). Under unrestrained temperature and humidity, *Ae. albopictus* exhibited augmented egg hatch, a short but improved immature survival and fecundity (Aida et al. 2011). A single female lays about 60-70 eggs in initial oviposition.

The gonotrophic cycle of a female *Aedes* mosquito is defined as the time elapsed from blood feeding to oviposition. The duration of the cycle is influenced by temperature and blood-meal type and frequency under natural conditions. It is reported that, the duration of gonotrophic cycle of field collected *Ae. aegypti* is as short as 3 d during the hot season (Pagès et al. 2009). Several other factors, for example, the size of females and hence the volume of blood meal also impacted the number of eggs produced by a female.

2.4.2 Larvae

Aedes larvae breed in clear and unpolluted but not necessarily in clean water(Dieng et al. 2010a). The larvae use a snorkel-like air tube called siphons to breathe air at the posterior end of the body. *Aedes* larvae have only a single pair of hair tufts on the breathing tube, and the tube is relatively short and stout (Holt *et al*., 1994). The larvae, which are also wrigglers, occur in variety of aquatic situation; in ponds, artificial containers, in tree holes and in other situations, but each species usually occurs only in a particular type of aquatic habitats.

Larvae usually go through larval moults before changing into pupae. In 2 - 3 days of hatching, the first instar larvae moult to 2^{nd} instar and may continue to moult the next day. Transformation to 3^{rd} instars occur in 3 - 4 days after hatching and in the 4^{th} to 5^{th} day, the larvae will moult to the 4^{th} instar. At this stage they are much larger in size and feeds both at the bottom of the pan and near the surface of the water. Temperature, food supply, crowding and sex are among the factors influencing larval size and development (Couret et al., 2014). In many insects, mortality rate differs according to the developmental phase (Southwood, 1978). Understanding the immature stages and factors affecting each stage may help in larval control (Aida et al. 2011) reported that the period of larval and pupal stages is 8 -14 days in the tropical climate and longer in the temperate climate. (Sowilem et al. 2013) reported that, *Ae. aegypti* males strain emerged at 12.8 days faster than the females (15.3 days). The emergence time for both the sexes is slower than that reported by (De Pauw et al. 2009) for Argentinean *Ae. aegypti* colonies (males: 7.2-8.1 days; females: 8.13-9.3 days). (Sowilem et al. 2013)

2.4.3 Pupae

The pupae are the stage just before transforming into adults. The mosquito pupae do not feed (Ibarra et al. 2003) and lack mechanism for the intake of nourishment (Marston et al. 1993). The pupal stage is quite short in some mosquitoes and usually last 1 to 2 days and adult mosquito emerges from the pupa in some species after 3-4 days (Ibarra et al. 2003).

Under ideal conditions, it takes about two days for *Ae. albopictus* pupa to develop (Aida et al. 2011). However, unlike the other stages, pupal stage in *culex* mosquitoes shows no adaptation for prolonged period under unfavourable condition(Marston et al. 1993) (Marston, 1970). The mosquito pupa is active, unlike pupa of most insects (Goma 1966). However, they tend to remain still in one place throughout their life (Sivanathan 2006). The pupae are comma-shaped and if disturbed, they swim up and down in the water in a jerky fashion (Focks and Chadee 1997).

Mosquito pupae live in water but show no resistance to prolonged submergence in water as respiration is wholly aerial and unlikely to make use of dissolved oxygen owing to their thick and impenetrable cuticle (Goma 1966). However, unlike the other stages, pupal stage shows no adaptation for prolonged period under unfavourable condition (Goma 1966) and the length of the pupal stage is very likely a function of temperature (Christopher 1960). At temperature of 27-37°, mosquito pupae complete development in one to three days; between 15° and 22°, the stage requires three to nine days; no growth occurs at temperature less than 10° while 40° proved lethal for 50% of pupae (Manrique-Saide et al. 2008). A period of 32-36 h pupal development was recorded for *Ae. albopictus* males and 49-52 h for females (Focks and Chadee 1997). Pupae of some mosquito species, however, are able to resist desiccation to some extent, and when removed from water may remain alive and active for some hours; while mosquito larvae, on the contrary, unless kept moist, quickly begin to dry and die (Christopher 1960).

Table 2.2. Duration of instar's period. (Adapted from Claudia 2012).

Duration (hr)	Instar				Pupa	Timing of stage
	1st	**2nd**	**3rd**	**4th**		
20	100100	---	---	---	---	All 1st stage up to 20 hours
22	89	+	---	---	---	--------
23	67	11	---	---	---	--------
24	56	33	---	---	---	--------
25	44	44	---	---	--- }	50 per cent ecdysis I-II 25-26 hours
26	31	56	---	---	---	
27	3	69	---	---	---	--------
28	---	97	---	---	---	--------
30		100	---	---	--- }	--------
	---					All second instars 30 - 42 hours
42	---	100	---	---	---	--------
45	---	74	26	---	---	--------
46	---	37	63	---	---	50 per cent ecdysis II - III 46 hours
47	---	17	83	---	---	--------
48	---	27	73	---	---	--------
49	---	+	100	---	---	--------
50	---	15	85	---	---	--------
52	---	+	100	---	---	--------
54		2	98	---	--- }	--------
	---					All third instar 54 – 68 hours
68	---	---	100		---	--------
69	---	---	73	27	---	--------
70	---	---	60	40	---	--------
71	---	---	58	42	--- }	50 per cent ecdysis III – IV 71 – 72 hours
72	---	---	32	68	---	--------
73	---	---	24	76	---	--------
76	---	---	17	83	---	
78	---	---	---	100	---	--------
92	---	---	9	91	---	--------
94	---	---	---	100	---	--------
96	---	---	6	94	---	--------
98	---	---	---	100	---	--------
102	---	---	---	100	+	First male pupa 102 hours
117	---	---	9	91	---	--------
122	---	---	---	50	50	Bulk of male pupated 122 hours
139	---	---	---	+	100	--------
141	---	---	---	12	88	--------
144		---	---	+	100	Bulk of female pupated 144 hours

2.4.4 Adults

After 48 hours of emergence, the adult female *Aedes* takes its first blood meal and can also take multiple blood meals between different gonotropic cycles (Bentley and Day 1989). The newly emerged adult mosquito rests on the surface of the water until it is strong enough to fly (Christopher 1960). Female mosquitoes require a blood meal to lay eggs. Male mosquitoes do not feed on blood. Diseases are transmitted when female mosquitoes feed on an infected host and then feed on an uninfected host (Delatte et al. 2010). The tip of the abdomen of the female is usually pointed, with the cerci protruding, and the thorax often has silvery or white markings (Silver 2007). The entire body of the adult is divided into three distinct regions; head, thorax and abdomen (Carpenter and LaCasse 1974). The male and female adult *Aedes* mosquitoes can survive an average of 20 and 30 days respectively (Rueda et al. 1990).

2.5 Abundance and distribution of *Aedes* mosquitoes breeding sites

Urbanization can lead to an increase in the amount of artificial containers, including other useful materials like water-storage tanks and many urban trash such as discarded tires and bottles. As a result of human activities, artificial containers are common in urban settlements throughout the world despite their utility. In 2002, (Sunahara et al. 2002) described urban environments as heterogeneous set of dwellings for residential and other land-use types, providing number of habitats that can be also used by arthropods.

In 2007, (Ahmed et al. 2007) attributed the abundance of *Aedes* breeding sites to the lack of knowledge by the city dwellers as a large number of them did not know the exact types of *Aedes* breeding containers. Strong breeding sites seasonality has been reported by (Dieng et al. 2010a). Both species of *Ae. aegypti* and *Ae. albopictus* are adapting to urban and suburban areas (Chen et al. 2006). *Aedes aegypti* is highly domesticated and adapted to the urban environment, whereas *Ae. albopictus* is less domesticated and abundant in the rural environment (Chan et al. 1971). (Cheong et al. 1986) suggested that, *Ae. aegypti* decreases in number away from centres of human population, and is absent in forest and most rural areas. The high incidence of dengue is closely associated with the abundance of the vectors. Therefore, both species play an important role in the transmission of the dengue virus.

Aedes albopictus occurs over a wide geographic range and encounters a wide range of ambient temperatures. The expansion of *Ae. albopictus* range in North America is likely to continue, and regional differences in temperature may affect its population dynamics, as is the case in other mosquitoes (Rueda et al. 1990). In tropical and sub-tropical climates, *Ae. albopictus* is abundant all year round, however, in temperate climates such as the Midwestern United States and Japan, the active season for larval stages is limited to late spring through early fall, with larval abundance greatest in July- August (Toma et al. 1982).

2.6 Breeding habitats of *Aedes* mosquitoes

Aedes aegypti and *Ae. albopictus* are the main and secondary dengue vectors, respectively that have well adapted to metropolitan environments (Nene et al. 2007). Though, *Ae.*

aegypti was formerly a forest species while *Ae albopictus* was a woodland species (Lourenço-de-Oliveira et al. 2004). However, the two species are practically cohabiting in many areas and regions and partaking the same breeding locations (Dieng et al. 2012). Formerly thought to lack the obvious home life of *Ae. aegypti*, *Ae. albopictus*, has just been found to have adjusted to enclosed environments on Penang Island (Dieng et al. 2010a). An overlapping pattern of distribution of *Ae. aegypti* and *Ae. albopictus* has been reported in Malaysia where both species were found adapting to urban and suburban areas (Sulaiman et al. 1996).

Although *Ae. albopictus* is found in endemic Asian regions; they are successfully adapting themselves to cooler regions (Lourenço-de-Oliveira et al. 2004), and is one of the 100 world's worst invasive species (Sanders et al. 2010). In the warm and humid tropical regions, the mosquitoes are active the entire year long, however, in temperate regions they hibernate over winter. While inhabiting forest fringes or secondary forest habitats, *Ae. albopictus* can also be found in green areas in town (Abu Hassan 1994). In a study in Putrajaya and Kuala Selangor in Malaysia, *Ae. albopictus* was found as the most prime species (Saleeza et al. 2011). Mixed breeding of the two species has been reported in outdoor containers in a house to house survey (Chen et al. 2006) (Chang & Jute, 1994). *Ae. aegypti* and *Ae. albopictus* were found to have extensively shared 55.40% of the total positive ovitraps in Georgetown, Penang (Rozilawati et al. 2015).

2.7 Feeding behaviour and oviposition of *Aedes* Mosquitoes

The females require blood meal for developing their eggs and rarely suck nectar and other sweet plants juices for energy just as the males do (Merritt et al. 1992). The size of mosquito determines the size of the blood meal taken, but it is usually around two micro litres (Hawley et al. 1989). This blood meal is often interupted short prior to ingesting enough for egg development, making it necessary for the mosquito to bite multiple hosts for complete development of their eggs, thereby making them effective disease transmitters. Carbon dioxide and organic substances produced from the host, humidity, and optical recognition are important in host location (Eisen et al. 2008).

In mosquito survival, selection of oviposition site has been found as the most important behavioural component (Bentley and Day 1989). *Aedes albopictus* oviposit in both natural and artificial containers while *Ae. aegypti* prefer man-made and some natural containers (Vezzani and Carbajo 2008) and lower temperature (Le Goff et al. 2012). In 2012 both *Ae. aegypti* and *Ae. albopictus* appeared to prefer darker coloured backgrounds for oviposition, with special preference for black over lighter colours (Merritt et al. 1992). Daily environmental condition found to have influence of peak oviposition (Tsuda et al. 2006). Natural and artificial container habitats are heterogeneous with factors such as the environmental, temporal and spatial fluctuations in temperature, water volume, and nutrient conditions. All these factors may have an effect on oviposition selection, survival, and fitness of progeny of *Ae. albopictus* (Sunahara et al. 2002). In rural areas of Malaysia, *Ae. albopictus* showed colour preference in oviposition by selecting red and black ovitraps to yellow; green, blue, plain and white (Yap 1975). But no trends based on container colour were observed (Harrington et al. 2001).

2.8 Life table characteristics of *Aedes* Mosquitoes

The mosquito life history parameters include female gonotrophic cycle and fecundity, egg hatching, birth and death rates, stage-specific survivorships, longevity and adult emergence (Grieco et al. 2003). The life cycle characteristics of *Ae. aegypti* mosquitoes vary considerably with ecobiological particularities of each location (Juliano et al. 2004). These include biotic factors such as population structure and dynamics, the presence of controphic species in larval habitats, the type and frequency of adult blood-meal and predators. The prevailing ecological factors include temperature, rainfall, type and number of larval water habitats, insecticidal applications, distance from human dwellings etc (Juliano et al. 2002). (Tabashnik and Croft 1985) reported that, insecticide resistance in insects was associated with annual generation turnover in their population: the shorter the mean generation time, the higher the likelihood of developing insecticide resistance by these species (Werren 1997). A considerable variation exist between the life cycle characteristics of *Ae. aegypti* with ecological attributes such as population structure and dynamics, the presence of con-species in larval habitats, the type and frequency of adult blood-meal and predators of each location. The life table method has been used to study survivorship and reproductive strategies of *Culex* and *Aedes* mosquitoes (Christopher 1960).

Numerous life table studies were carried out on important vectors such as *Ae. aegypti* in order to gain information on population biology and dynamics, potential for colonization in the laboratory, vectorial capacity and impact on the risk of disease outbreaks and finally to determine the effect of habitat modification like deforestation or the effectiveness of various control measures on a specific species (Focks and Chadee 1997; Grieco et al. 2003).

Understanding the immature stages and factors, like, mortality which affects each stage of development of these agents of transmission may assist in control measures (Harcourt 1969). (Grieco et al. 2003) have described mosquito life history parameters to include egg hatching, birth and death rates female gonotrophic cycle and fecundity; stage-specific survivorships; longevity and adult emergence. Stage and age-specific horizontal life tables have been used in summarizing the life history characteristics of a given species under different natural and controlled laboratory conditions (Rajesh et al. 2013). The two-sex life table analysis provides a complete account of the stage differentiation of *C. megacephala* (Gabre et al. 2005).

In order to obtain information on certain parameters such as population biology and dynamics, potential for colonization in the laboratory; vectorial capacity and risk of disease outbreaks as well as to determine effect of habitat modifications (e.g. deforestation) or the effectiveness of various attempted control measures on a specific species, construction of numerous life table studies on important vectors such as *Ae. aegypti* have existed(Afrane et al. 2007). Based on the ecological (population structure) and biological (dynamics), characteristics of the location such as presence of controphic species, type and frequency of adult blood-meal and predators, the life cycle characteristics of *Ae. aegypti* mosquitoes varies considerably. Julio, (2009) reported that temperature, rainfall, type and number of larval water habitats, insecticidal applications, distance from human dwellings as the prevailing ecological factors (Ahmed et al. 2007). (Juliano et al. 2004) reported presence of genetic differences among subpopulations of *Ae. aegypti* using molecular DNA markers. Variable vectorial capacities for dengue viruses exist among