THE ECOLOGY AND BIOLOGY OF *Aedes aegypti* (L.) AND *Aedes albopictus* (Skuse) (DIPTERA: CULICIDAE) AND THE RESISTANCE STATUS OF *Aedes albopictus* (FIELD STRAIN) AGAINST ORGANOPHOSPHATES IN PENANG, MALAYSIA

by

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EKOLOGI DAN BIOLOGI *Aedes aegypti* (L.) DAN *Aedes albopictus* (Skuse) (DIPTERA: CULICIDAE) DAN STATUS KERINTANGAN *Aedes albopictus* (STRAIN LAPANGAN) TERHADAP ORGANOFOSFAT DI PULAU PINANG, MALAYSIA

oleh

MANORENJITHA MALAR A/P SIVANATHAN

Tesis yang diserahkan untuk memenuhi keperluan bagi Ijazah Sarjana Sains

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LIST OF ABBREVIATIONS AND SYMBOLS

cm	centimeter
mm	millimeter
mg	milligram
mg/L	milligram per liter
rH	relative humidity
N	number of samples
p	significant
S.E	Standard error
temp.	temperature
DF	Dengue fever
DHF	Dengue haemorrhagic fever
°C	degree Celsius
%	percentage
±	plus minus
LC	Lethal concentration

Ekologi dan biologi *Aedes aegypti* (L.) dan *Aedes albopictus* (Skuse) (Diptera: Culicidae) dan status kerintangan *Aedes albopictus* (strain lapangan) terhadap organofosfat di Pulau Pinang, Malaysia

ABSTRAK

Penyampelan populasi telur (luar kediaman) dijalankan di dua lokasi pinggir kota di Pulau Pinang iaitu di Paya Terubong (Air Itam) dan Bagan Dalam (Butterworth). Terdapat korelasi kuat antara curahan hujan dan populasi telur di Paya Terubong manakala korelasi yang sederhana di Bagan Dalam. Hampir 99% telur yang dikutip di Paya Terubong dan 95% telur yand dikutip dari Bagan Dalam menetas menjadi *Aedes albopictus* dalam makmal. Bilangan *Aedes aegypti* yang menetas di makmal adalah sangat sedikit di kedua-dua lokasi. *Aedes albopictus* merupakan spesis yang dominan di kedua-dua lokasi kajian.

Penetasan telur paling tinggi direkodkan untuk *Aedes aegypti* (BD-strain) bagi telurnya yang berusia sebulan. Bagi telur *Aedes albopictus* (PT-strain) pula, penetasan tertinggi di catatkan bagi telur berusia 3 minggu. Tempoh perkembangan dari larva instar pertama hingga kemunculan nyamuk dewasa bagi *Aedes aegypti* (VCRU-strain), *Aedes aegypti* (BD-strain),*Aedes albopictus* (VCRU-strain) dan *Aedes albopictus* (PT-strain) adalah lebih kurang 6.25 ± 0.26 hari, 6.84 ± 0.30 hari, 8.36 ± 0.18 hari dan 7.73 ± 0.24 hari masing-masing. *Aedes albopictus* (VCRU-strain) betina yang hanya diberikan makanan sukrosa 10% merekodkan purata jangka hayat yang tertinggi iaitu 55.5 ± 1.25 hari manakala *Aedes albopictus* (PT-strain) jantan yang hanya diberikan makanan sukrosa 10% pula

menunjukkan purata jangka hayat terendah iaitu 37.7 ± 0.74 hari. Kitar gonotropik *Aedes aegypti* (BD-strain) dan *Aedes albopictus* (PT-strain) adalah masing-masing 3.00 ± 0.83 hari dan 2.73 ± 0.18 hari. Larva dari kumpulan yang diberi makanan optima menunjukkan kemunculan pupa pertama dan terakhir yang singkat dan kadar mortaliti yang rendah berbanding dengan larva dari kumpulan yang diberi makanan suboptima. Kumpulan larva yang diberikan makanan optima menghasilkan paling banyak telur iaitu 86.2 ± 2.20 telur bagi *Aedes aegypti* (BD-strain) dan 67.5 ± 4.37 telur bagi *Aedes albopictus* (PT-strain). Lebih banyak telur dilihat pada substrat berbentuk kon berbanding dengan substrat rata. Bilangan telur yang direkodkan pada substrat berbentuk kon adalah 122.7 ± 1.83 telur dan 96.5 ± 4.08 telur masing-masing bagi *Aedes aegypti* (BD-strain) dan *Aedes albopictus* (PT-strain).

Larva *Aedes albopictus* dari lapangan masih belum rintang terhadap temephos walaupun sudah lama digunakan di Pulau Pinang. Malah larva *Aedes albopictus* dari lapangan tidak menunjukan kerintangan apabila diuji dengan larvisid lain dari kumpulan organofosfat. Chlorpyrifos menunjukan aktiviti insektisid yang tertinggi berbanding dengan larvisid lain.

The ecology and biology of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) and the resistance status of *Aedes albopictus* (field strain) against organophosphates in Penang, Malaysia

ABSTRACT

Outdoor Ovitrap surveys were carried out in two suburban locations in Penang; Paya Terubong (Air Itam) and Bagan Dalam (Butterworth). Statistical analyses showed a strong correlation between rainfall and egg population in Paya Terubong while Bagan Dalam showed a moderate correlation. Almost 99 % eggs collected in Paya Terubong and 95 % of eggs collected in Bagan Dalam emerged as *Aedes albopictus* in the laboratory. Only a small percentage of *Aedes aegypti* emerged from the paddles collected in both locations. *Aedes albopictus* was the most predominant outdoor species in both sampling area.

Aedes aegypti's eggs and *Aedes albopictus*'s eggs which were submerged after 1 month and 3 weeks, respectively, produced the highest hatching rate. The development from the first instar larva to adult stage for *Aedes aegypti* (VCRU-strain), *Aedes aegypti* (BD-strain),*Aedes albopictus* (VCRU-strain) dan *Aedes albopictus* (PT-strain) was about 6.25 ± 0.26 days, 6.84 ± 0.30 days, 8.36 ± 0.18 days dan 7.73 ± 0.24 days, respectively. Female *Aedes albopictus* (VCRU-strain) fed with 10% sucrose only showed the highest mean survival which was 55.5 ± 1.25 days while male *Aedes albopictus* (PT-strain) fed with 10% sucrose recorded 37.7 ± 0.74 days which was the shortest mean survival. Gonotrophic cycle for *Aedes aegypti* (BD-strain) and *Aedes albopictus* (PT-strain) were 3.00 ± 0.83 days

and 2.73 ± 0.18 days, respectively. Larvae reared under optimal condition showed a very short pupation period and low mortality rate compared to larvae reared under suboptimal condition. Larvae reared under optimal condition produce more eggs. The number of eggs recorded for *Aedes aegypti* (BD-strain) was 86.2 ± 2.20 eggs and for *Aedes albopictus* (PT-strain) was 67.5 ± 4.37 eggs. Both *Aedes sp.* showed preference to cone shaped substrate than flat substrate. Number of eggs recorded on cone shaped substrates for *Aedes aegypti* (BD-strain) and *Aedes albopictus* (PT-strain) was 122.7 ± 1.83 eggs and 96.5 ± 4.08 eggs respectively.

Field collected *Aedes albopictus* larvae are still susceptible to temephos although temephos has been used in Penang for a long period. No resistance was shown by *Aedes albopictus* (field strain) when tested against other organophosphate larvicides. Chlorpyrifos showed the highest insecticidal activity compared to other larvicides.

CHAPTER ONE
General introduction

1.1 Introduction

During the 19th century, dengue was considered a benign sporadic disease that caused epidemics at long intervals. However, in the past five decades, the incidence was reported to have increased 30-folds (Kindhauser, 2003). An estimated 100 million cases of dengue fever, 500 000 cases of dengue haemorrhagic fever and 25 000 deaths have been reported annually (Gubler *et al*., 1998).

In humans, dengue infection ranged from simple fever to much more severe and sometimes fatal dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Halstead, 1980; Hawley, 1988; WHO, 1995). In Southeast Asia, dengue haemorrhagic fever (DHF) is a major cause of mortality among children (Halstead, 1980; WHO, 1997c) while dengue fever (DF) occurs epidemically (WHO, 1997c) and affecting older children and adults (WHO, 1997a). The outbreaks are often described as explosive (Halstead, 1997; WHO, 1997c).

There are four antigenically related, but distinct, dengue virus serotypes; DEN-1, DEN-2, DEN-3 and DEN-4 (Gubler, 1997; Miyagi and Toma, 2000), all of which can cause dengue fever and dengue haemorrhagic fever. The dengue virus belongs to genus Flavivirus (Westaway, *et al*., 1985; Miyagi and Toma, 2000). Human are the primary vertebrate host of all four serotypes and *Aedes* mosquitoes of the subgenus *Stegomyia* was the primary mosquito vectors (Gubler, 1997; Miyagi and Toma, 2000).

The transmission of dengue fever in Malaysia is caused by two *Aedes* mosquito; *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) (Smith, 1956). *Aedes albopictus* is a semidomestic mosquito while *Aedes aegypti* is a domestic mosquito in urban area (Chan *et al*., 1971c). These species are effective vectors of dengue because of their ability to breed in artificial containers in and around the house, close to human being (Cheong, 1967).

Since 1999, dengue cases in Malaysia have been reported on the increase. Number of cases reported in 1999, 2000, 2001, 2002, 2003 and 2004 are 10146 cases, 6692 cases, 15446 cases, 30807 cases, 30221 cases and 32422 cases respectively. The increase in mortality rate was also observed. Number of deaths reported in 2004 was 102 cases compared to 37 cases only in 1999 (WHO DengueNet, 2006).

Major global demographic changes (unplanned urbanization and concurrent population growth), increased travel by airplane and non effective mosquito control programme (Singh, 1996; Kindhauser, 2003), insufficient and non-dependable water supply and inadequate solid waste management (CTD, 1995), increasing resistance of vectors and pathogens, decreasing number of new insecticides and drugs and finally expanding of habitats because of global warming (Yap *et al*., 2003) had attributed to the re-emergence of dengue fever.

Before any attempt is made to employ either chemical or biological controls, the ecology of vector mosquito and the mechanism of disease transmission must be understood. It is important to recognize the vulnerable time in the life cycle of a vector and correct forecast of population increase under known environmental condition. This information provides a sound basis for the planning of an effective control programme (WHO, 1972).

1.2 The objective of these studies:

The purpose of this study is to determine the role of the vector mosquitoes in the dynamics of transmission by studying their bionomics and seasonal prevalence. The outcome of this study is essential for mosquito control. For that reason the following objectives have been drawn up for this study:

- to study the seasonal abundance of *Aedes* eggs in an outdoor environment in Bagan Dalam, Butteworth and Paya Terubong, Air Itam over a period of 14 months
- to study the population densities of *Aedes aegypti* and *Aedes albopictus* in both study areas
- to study the differences between laboratory reared and field collected *Aedes aegypti* and *Aedes albopictus*; duration of the immature stages and survival and longevity
- to study the hatching rates of *Aedes aegypti* and *Aedes albopictus*'s eggs air dried between one week to six months

- to study the effect of overcrowding and starvation on pupation period and mortality rate of field strain *Aedes aegypti* and *Aedes albopictus*
- to study the effect of larval diet on the fecundity of field collected *Aedes aegypti* and *Aedes albopictus*
- to study the duration of oviposition and gonotrophic cycle of field collected *Aedes aegypti* and *Aedes albopictus*
- to study the substrate preferences of field collected *Aedes aegypti* and *Aedes albopictus*
- to study the resistance status of field collected *Aedes albopictus* larvae against organophosphate insecticides

CHAPTER TWO

Literature Review

2.1 History of *Aedes* in relation to dengue in Malaysia

In 1956, Smith reviewed two reports, first by Skae in 1902, who described an outbreak in Penang which was one of a series of dengue epidemics affecting most of the port cities of the Far East in 1900-1901. The second report reviewed was by More in 1904, who reported an epidemic occurring in Singapore at the same time and also several incidence of the disease in Penang Port, Province Wellesley and parts of the Federated Malay State.

Smith (1956) correlated these outbreaks with the then distribution of *Aedes aegypti* mosquitoes which occurs only in the seaports and along parts of the seacoast at the time. No distribution of *Aedes aegypti* was recorded in inland. He assumed that the dengue fever is endemic since sporadic and localised outbreaks occurred frequently,

Serological evidence produced by Smith (1958) showed that dengue virus infection was widespread in the human population. In rural areas where *Aedes aegypti* was absent, *Aedes albopictus* was assumed to be the vector since dengue antibody was detected in residents of those particular areas.

In follow up studies, Smith (1958) found that only the monkeys, of the tree-dwelling mammals could be confirmed serologically as dengue positive. Not only he assumed that a forest-canopy mosquito species was probably the vector of dengue virus among monkeys in the forest, but he also suggested that *Aedes albopictus* may be the transmitting link between monkeys in the forest and man in rural areas.

Rudnick (1978; 1986), has concluded that dengue in Malaysia is a zoonosis where monkeys are the host in the canopy while the vectors are *Aedes niveus*, (the tree hole breeding and canopy biting group of mosquitoes). He also summarised that the dengue in Malaysia as silent enzootic jungle cycle, a rural endemic form involving *Aedes albopictus* which produces a mild illness, an urban endemic form with epidemics which involves both *Aedes albopictus* and *Aedes aegpyti*, and a severe form associated with *Aedes aegypti*.

2.2 Mosquito

2.2.1 Classification of mosquitoes

Mosquitoes are among the best known groups of insects, because of their importance to man as pests and vectors of some of the most distressing human diseases. They are small, two winged insects belonging to the family Culicidae of the order Diptera (two winged flies) (Muesebeck, 1952; Goma, 1966). Nearly three quarters of all mosquito species was found living in the humid tropics and subtropics (Miyagi and Toma, 2000).

As of 1997, Abu Hassan and Yap (1997), stated that 3100 species of mosquitoes from 34 genera has been identified. Three subfamilies are recognised among the Culicidae: the Toxorhynchitinae, Anophelinae and Culicinae (Kettle, 1984; Abu Hassan and Yap, 1997).

Subfamily Toxorhynchitinae comprises a single genus, *Toxorhynchites*. There are about 76 species in this single genus. *Toxorhynchites* are not medically important. Unlike Anophelinae and Culicinae, both sexes of Toxorhynchitinae posses a proboscis which is curved backwards, thus making them incapable of piercing skin and transmitting disease (Service, 1996).

There are 3 genera in subfamily Anophelinae, however; only *Anopheles* is of any medical importance (Service, 1996). There about 60 species of *Anopheles* mosquitoes known to be vectors of malaria (WHO, 1997c). Some *Anopheles* species are also transmitting filariasis and arboviruses (Service, 1996; Abu Hassan and Yap, 1997; WHO, 1997c).

Culicinae are the major vectors of arboviruses and filarias, and important vectors of human disease (Kettle, 1984). Medically most important genera in subfamily Culicinae are *Culex*, *Aedes*, *Mansonia*, *Haemagogus* and *Sabethes* (Service, 1996; WHO, 1997c). According to Kettle (1984), there are more than 2500 species of Culicinae of which the main genera are *Aedes* with over 900 species. *Aedes* is best known vectors of yellow fever and dengue fever. Some *Aedes* species are also vectors of some filariasis and viral disease (WHO, 1997c).

2.2.2 Classification of *Aedes* mosquito

Classification of *Aedes aegypti* and *Aedes albopictus* (Knight and Stone, 1977)

Aedes aegypti (Linnaeus, 1762)	***Aedes albopictus*** (Skuse, 1894)
Kingdom: Animalia	Kingdom: Animalia
Phylum: Arthropoda	Phylum: Arthropoda
Class: Insecta	Class: Insecta
Order: Diptera	Order: Diptera
Family: Culicidae	Family: Culicidae
Subfamily: Culicinae	Subfamily: Culicinae
Genus: *Aedes*	Genus: *Aedes*
Species: *aegypti*	Species: *albopictus*

2.2.3 Identification of adult *Aedes aegypti* and *Aedes albopictus*

Adults *Aedes aegypti* and *Aedes albopictus* can easily be differentiated by the patterns of white scales on the dorsal side of the thorax (Figure 2.1). For *Aedes aegypti*, the pattern consists of two straight lines surrounded by curved lyre-shaped lines on the side (Harwood and James, 1979; Cheong, 1986; Goddard, 1993; WHO, 1995; Abu Hassan and Yap, 1997). In contrast, *Aedes albopictus* has only a single broad line of white scales situated in the middle of the thorax (Cheong, 1986; Goddard; 1993; WHO, 1995).

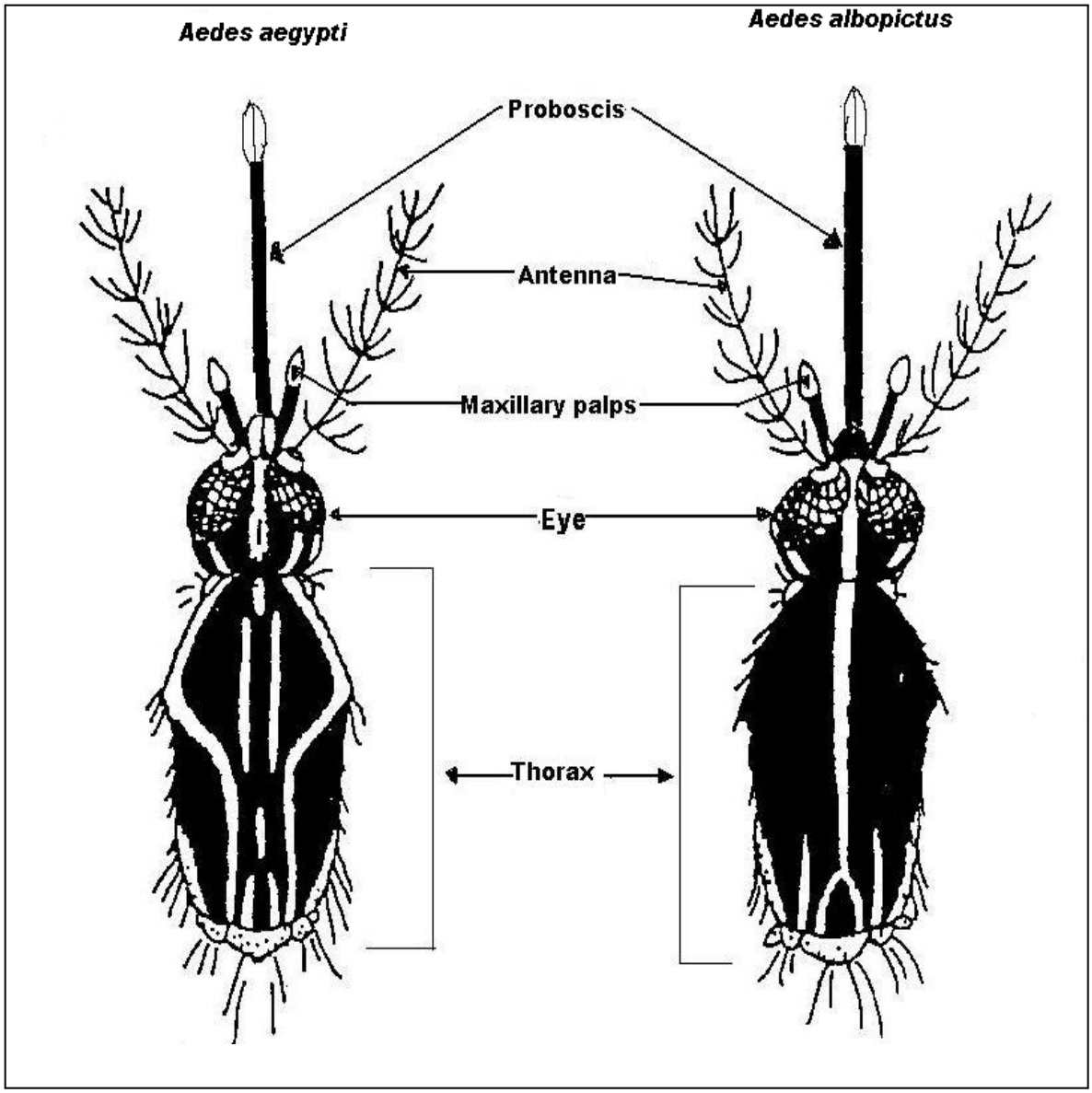

Figure 2.1: Thorax of adult female *Aedes* mosquito (modified from WHO, 1995)

2.2.4 Identification at the larval stage

At the larval stages, the two *Aedes* species can be differentiated by the shape of the comb scales on the eighth segment of the abdomen and the shape of the pecten teeth on the siphon (Figure 2.2). In *Aedes aegypti* larvae, the comb teeth have well developed lateral denticles but the pecten teeth have less defined denticles. Whereas in *Aedes albopictus* larvae, the comb teeth have no lateral denticles but the pecten teeth have three well defined pointed denticles (Cheong, 1986; WHO, 1995).

2.2.5 *Aedes aegypti* (Linnaeus)

Aedes aegypti is a tropical mosquito (Plat 2.1 and Plat 2.2). It is believed that *Aedes aegypti* originated from Central Africa, where it is found in greatest abundance (Smith, 1956; Cheong, 1986). Being a domestic breeder, it found breeding places on sailing ships on those days, where it has been distributed to all parts of the world (Chandler, 1945; Smith, 1956; Gubler, 1997).

Aedes aegypti is one of the most efficient mosquito vectors for arboviruses, because it is highly antropophilic and thrives in close proximity to humans (WHO, 1997a) preferring to live indoors (WHO, 1997a; WHO, 1997c). It is commonly in urban areas especially in the most densely populated districts (Rudnick, 1966). This species breeds in domestic and predomestic water containers (Service, 1992) that contain clean water (WHO, 1997c).

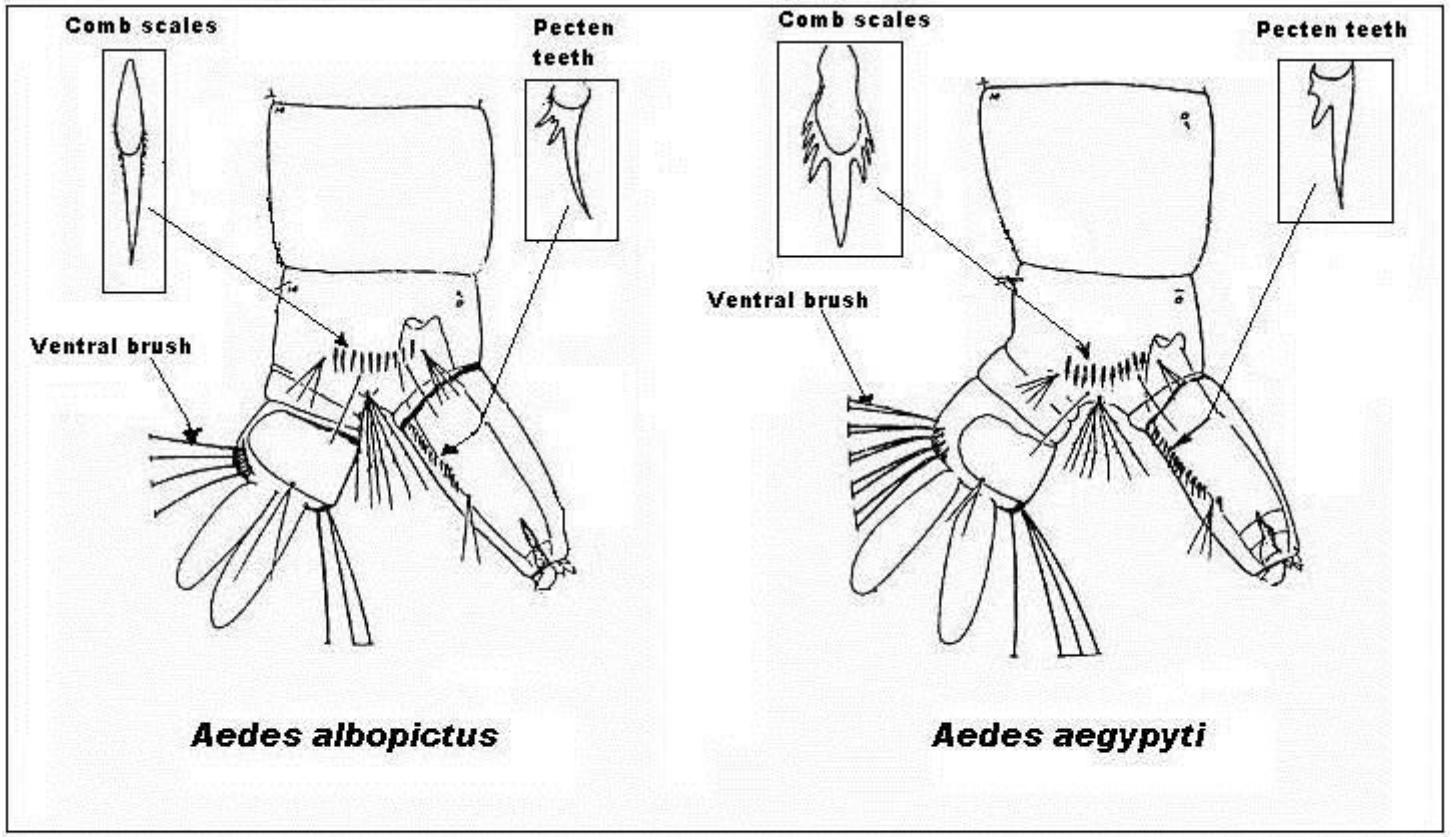

Figure 2.2: Terminal segments of larvae (modified from WHO, 1995)

Plate 2.1: *Aedes aegypti*

Plate 2.2: Thorax of *Aedes aegypti*

Aedes aegypti is usually found between latitudes 35°N and 35°S (WHO, 1997a). During the warm season, *Aedes aegypti* were found expanding its geographic distribution to more northern and southern latitudes (WHO, 1997a; Gubler, 1997).

2.2.6 *Aedes albopictus* (Skuse)

Aedes albopictus (Plat 2.3 and 2.4) is believed to have originated in the tropical forest of Southeast Asia (Smith, 1956; Hawley, 1988) where many closely related species are known to exist (Smith, 1956). *Aedes albopictus* occurs throughout the geographical region consisting of the countries of South East Asia and it has been found in all types of country, urban, suburban, rural (Hawley, 1988), farmland or deep forest (Rao, 1967). Hawley (1988), in his conclusion stated that *Aedes albopictus* is a highly adaptable species because it seems to be able to recolonize tree holes in forests after transported to a new region, thus making it hard to control.

A photoperiod-induced egg diapause of *Aedes albopictus* allows them to colonize temperate and northern latitudes. In addition, egg cold hardiness of temperate strains *Aedes albopictus* enables them to survive the suboptimal winter temperatures in the northern latitudes (Hawley, 1988).

Plate 2.3: *Aedes albopictus*

Plate 2.4: Thorax of *Aedes albopictus*

2.3 Ovitrap

"Ovitrap" or "oviposition trap" (Pratt and Jacob, 1967; WHO, 1995; Tham, 2000) is a simple devise used to attract the female *Aedes* to oviposit. The presence of eggs on the paddle is proof that the species is present in that particular area (Pratt and Jacob, 1967). The development of ovitrap has created a new approach in monitoring *Aedes* population (Fay and Perry, 1965).

The following guidelines should be observed before placing an ovitrap (Pratt and Jacob, 1967; Evan and Bevier, 1969):

i. Place the ovitrap at ground level, where it will not be disturbed by children or pets
ii. Place the trap away from home lawn sprinklers or excess rainwater.
iii. Place it close to shrubbery or accumulations of junk and trash or any typical adult mosquito resting sites
iv. Place the trap in partial or total shade to avoid direct sunlight
v. Place it at the back of a house where there are more shelter and breeding places for mosquito
vi. Place the ovitrap where the mosquito can detect or see the trap
vii. Place the trap far from piles of tires because *Aedes* mosquito especially *Aedes albopictus* prefer tires over other containers

The females are apparently influenced by dark colours, water and the presence of a rough surface (Pratt and Jacob, 1967). Traps are generally serviced every 7 days to remove eggs from previous trapping and to replace water for the next sampling (Jakob and Bavier, 1969).

A weekly trapping period provides for continuous trapping which reduces bias due to bad weather (Fay and Eliason, 1966) and increases the percentage of positive ovitraps (Frank and Lynn, 1982). It seems unlikely that a longer trapping period would produces adults in warm weather (Ritchie, 1984).

A longer trapping period (seven days) is economical, efficient and more sensitive (Fay and Eliason, 1966; Tanner, 1969; Ritchie, 1984; WHO, 1995; Tham, 2000) and increases the statistical reliability of the data (Ritchie, 1984).

There are a few types of ovipositional traps invented to survey egg population in the field. A lot of modifications were made to simplify the technique. Oviposition jar is made of flint glass and coated with glossy black paint and pressed wood paddles (Pratt and Jacob, 1967). Yap (1975) replaced the jar with drinking glass and used the hardboard as substrates. Chan (1972) also found that condensed milk cans painted black is a good oviposition trap. Toma *et al*. (2003) employed black plastic flower pots as ovitraps and strips of Mansonite® were used as substrates to collect eggs.

Instead of paddles, Fay and Eliason (1966) glued a panel of brown blotting paper onto a wooden tongue depressor while Steinly *et al*., (1991) used strips of seed germination paper to monitor the egg population. This method however, has been modified to be used in not only in artificial container (inside black cans) but

also in natural conditions (tree holes and tires). Recently, sticky ovitraps has been invented as new surveying methods (Ordonez-Gonzales *et al*., 2001). However, this method does not provide egg population instead it captures gravid females to determine dispersal distances of mosquito.

2.4 The biology of *Aedes aegypti* and *Aedes albopictus*

Mosquitoes have four distinct stages in their life cycle, namely, egg, larva, pupa and adult (Goma, 1966; WHO, 1972). The first three stages are passed in water but the adult is an active, flying insect that feeds on blood or plant juices (WHO, 1972; Burgess and Cowan, 1993).

2.4.1 Egg biology

The eggs of *Aedes* mosquitoes do not have a frill or floats but elongate-oval in shape. The outer shells of the eggs are patterned with small reticulations. (Goma, 1966). The eggs of both species are superficially similar, shiny jet black (Linley, 1989) (Plat 2.5 and 2.6).

The *Aedes* eggs are white in colour and soft when they were newly laid; but later the eggs turn black and become quite hard (Schlaeger and Fuchs, 1974; Christopher, 1960). Before the eggs mature, the newly laid eggs also undergo some increase in size (Christopher, 1960).

Plat 2.5: *Aedes* eggs on the filter paper.

Plat 2.6: *Aedes* eggs (upclose)

Eggs of *Aedes aegypti* and *Aedes albopictus* are laid in a batch singly or individually by the female (Kettle, 1984; Rhodain and Rosen, 1997) above the water surface on the damp substrates. The eggs are normally laid above the water line of breeding places. It can remain dry for months but still remain viable and hatch when they become flooded with water (Goma, 1966; Harwood and James, 1979; Service, 1996). Eggs of *Aedes* mosquito hatch in instalments and may require repeated immersions in water followed by short periods of desiccation (Gillett, 1951).

2.4.2 Larval biology

Mosquito larvae (Plat 2.7 and 2.8) undergo four larval stages that require five to 10 days for completion (Hawley, 1988). The variation of duration depends on temperature (WHO, 1972) or larval diets (Hawley, 1988).

Mosquito larvae are never found in turbulent waters because the larva unable to withstand wave action (Bates, 1970). Mosquito larvae are found with all types of aquatic habitats and water. The larvae commonly are found in waters containing microflora and fauna and debris of plant and animal origin (Clements, 1963).

Mosquito larvae move about mainly in two ways; by jerks of the body and by propulsion with the mouth brushes (WHO, 1972). Mosquito larvae normally dive to the bottom when the water surface is suddenly disturbed or if a shadow passes over them (Goma, 1966; WHO, 1972).

Plate 2.7: Clumps of *Aedes aegypti* larvae

Plate 2.8: *Aedes aegypti* larva (upclose)

According to Christopher (1960), in *Aedes aegypti* and *Aedes albopictus* and other mosquito species, there are four larval instars, each instar terminating with a moult or ecdysis. One of the first sign that ecdysis is about to take place is the appearance of dark bands across the thorax due to the circularly wrapped lateral hairs of the next instar shining through the cuticle

Newly hatched first instar is about 1 mm in length, but grows during the instar to nearly twice the size. The first instar is recognized by the presence of an egg-breaker on the dorsum of the head and consists of a strongly sclerotised, very sharp, pointed, curved and flattened cone set in an oval area of soft membrane. It is used to cut through the egg shell and allow the larva to escape by simply forcing off a circular cup from the anterior end of the egg.

After ecdysis, the second instar larva, is much the same in length as the fully grown first instar, but is bulkier and the swollen head is enormous. The tracheal trunks are now enlarged and lined with taenidia and the terminal portions in the siphon have ballooned. As in the first instar, the head darkens and the body becomes long and cylindrical. During this instar the larva grows in length from 2 to 3 mm.

The third instar larva has certain characters of its own and to a large extent the head measurement is more variable than in any other stage, since there are large and small headed forms. It is very apt to have the ecdysis lines on the head capsule open for some time before ecdysis is definitely in progress. The pre-ecdysis stage is rather long and thin and is larger than the previous instar. It shows this character more cons piously. The tail comb-spine is a prominent structure in this instar.

The fourth instar at a corresponding size is much stouter due to the development of the thoracic imaginal buds and an accumulation of fat body. The fourth instar shows the rudiments of the pupal respiratory trumpets. The most prominent structure in this instar is the tail comb-spine.

2.4.3 Pupal Biology

As ecdysis approaches the final or pupal ecdysis, larva becomes plump and increasingly turgid (Goma, 1966). The larva tends to cease feeding and to remain at rest at the surface. When first emerged, the pupa (Plat 2.9 and 2.10) is white, but in a short time shows pigment changes (Christopher, 1960). They are comma shaped (Goddard, 1993) and also called “tumblers”. The pupal stage is quite short and usually last 1 to 2 days (Lee, 2000). The mosquito pupa is active, unlike pupa of the most insects (Goma, 1966; Bates, 1970; Harwood and James, 1979).

Plate 2.9: Clumps of *Aedes* pupae

Plate 2.10: An uplcose view of *Aedes* pupa

2.4.4 Adult biology

2.4.4.1 Resting

In searching for resting places adult mosquitoes frequent a wide range of places. Mosquitoes are generally found in areas where the air is relatively static and the humidity high (Goma, 1966).

During day time most mosquito species prefer to rest in dark places and avoid light (Goma, 1966). *Aedes aegypti* adults prefer to rest inside the house where it is dark (WHO, 1995; Rodhain and Rosen, 1997). Macdonald *et al*. (1965), found most of them resting In temporary objects (on clothing and mosquito nets) while a few percentage was found resting on furniture and other semi-permanent articles In Malaysia, *Aedes albopictus* adults have been found resting outdoors in clearing and rubber plantations (Estrada-Franco and Craig, 1995).

2.4.4.2 Survival and longevity

In tropical regions, the life span of adult mosquitoes ranges from a few days to several weeks and it is frequently longer in temperate regions. The life span of female for species that overwinter as adults may approach one year (Clements, 1992a). The longevity of *Aedes albopictus* under natural environment is not fully known but it is expected to be shorter than under laboratory conditions (Ho *et al*., 1972). Laboratory studies showed that male and female *Aedes* mosquito survive an average of 20 to 30 days respectively (WHO, 1995).