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"STUDIES OF BREEDING HABITATS AND SEASONAL OCCURRENCE OF MOSQUITOES IN PUTRAJAYA AND KUALA SELANGOR, WITH LABORATORY EXPERIMENTS OF GUPPIES AND DRAGONFLY NYMPHS AS POTENTIAL BIOCONTROL PREDATORS AGAINST MOSQUITO LARVAE"

Field of Study:

ENVIRONMENTAL ENTOMOLOGY

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ABSTRACT

Mosquito control is essential for the control of vector borne diseases. Many synthetic insecticides are widely used for controlling adult and larval mosquito populations. However, there are multirole effects: e.g. the harmful effects of chemicals on non-target organisms, the development of resistance to these chemicals in mosquitoes and the recent resurgence of different mosquito-borne diseases. The objectives of this study are to determine the potential breeding habitats of the mosquitoes, mosquito indices, mosquito species, density of mosquito larvae, perceptions of respondents on bio control and to conduct captivity studies on predator–prey relationships. Entomological surveillance was carried out in six localities in the urban and suburban areas from January until December 2010 to identify potential breeding sites for mosquitoes and mosquito species populations. A total of 442 representative households in six localities were selected. Breeding habitats were sampled outdoors in the surroundings of the housing areas. There was a significant difference in the number of mosquito larvae collected, where the urban areas had a higher density in contrast to suburban areas. The study indicated that the most predominant species found in both areas was *Aedes albopictus* with gardening utensils as a preferred breeding habitat for urban area and artificial containers for suburban area. Entomological indices were calculated to predict future outbreaks in the localities. Ovitrap surveillance was carried out in one year to study the relationship between ovitrap surveillance and environmental parameters, which revealed no significant difference in the population numbers for both areas and no correlation to the environmental factors. Questionnaires on the perceptions of chemical in mosquito control and the potential use of bio control were distributed to staffs in health office and also public in both study areas. In general the public had high uncertainties (scoring on 'not sure' for all the 4 questions given ranging from 47.9% to 27%. This is due to the public being unfamiliar to bio control as indicated in question 1 (56%) in contrast to staff very aware on bio control (75%). Fatigue was the

most frequently reported symptom by staff and breathing difficulty reported by public. Natural bio control agent surveillance was conducted in both study areas. *Poecilicia reticulata* and Odonata nymph species was the most natural predator collected at study areas. Three species of Odonata nymphs consumed more Aedes species than Culex species but there was no significant difference in the predator feeding efficiency. In terms of prey preferences of guppy, both male and female consumed more Aedes species than Culex species. The behaviour of mosquito larvae species and predator (guppy and Odonata nymph) species showed direct influence on the predatory activities. All predators exhibited diurnal activities; they were day-time stalkers and actively consumed more mosquito larvae during the day time. The efficiency of predatory activities depends on several factors such as water volume, number of predator, and number of prey density. These results concluded that both common biocontrol agent (guppies) and potential biocontrol agent (Odonata nymphs) are efficient predators in laboratory experiment and thus likely candidates to be utilized as an environmental friendly mosquito management strategy.

ABSTRAK

Kawalan nyamuk adalah penting bagi pengawalan penyakit bawaan vektor. Banyak racun serangga sintetik digunakan secara meluas bagi mengawal populasi nyamuk dewasa dan larva. Walau bagaimanapun, terdapat kesan-kesan pelbagai peranan: contohnya, kesan bahan kimia yang memudaratkan kepada penduduk bukan sasaran, pembinaan ketahanan terhadap bahan-bahan kimia ini oleh nyamuk dan kemunculan semula penyakit bawaan nyamuk yang berbeza. Objektif kajian ini ialah untuk menentukan potensi pembiakan habitat nyamuk, indeks nyamuk, spesies nyamuk, kepadatan larva nyamuk, persepsi responden mengenai kawalan biologi dan menjalankan kajian kurungan terhadap hubungan mangsa-pemangsa. Penelitian entomologi dijalankan di enam lokasi di kawasan bandar dan pinggir bandar dari Januari hingga Disember 2010 untuk mengenal pasti potensi tempat pembiakan nyamuk dan populasi spesies nyamuk. Sejumlah 442 wakil isi rumah di enam kawasan telah dipilih. Habitat pembiakan telah disampel di kawasan luar persekitaran kawasan-kawasan perumahan. Terdapat perbezaan yang signifikan dalam bilangan larva nyamuk yang dikumpulkan, iaitu kawasan bandar mempunyai kepadatan yang lebih tinggi, berbeza dengan kawasan-kawasan pinggir bandar. Kajian ini menunjukkan bahawa spesies yang paling pradominan dijumpai di kedua-dua kawasan adalah *Aedes albopictus* dengan peralatan berkebun sebagai habitat pembiakan pilihan bagi kawasan bandar dan bekas-bekas buatan bagi kawasan pinggir bandar. Indeks entomologi telah dikira untuk meramal wabak pada masa depan di kawasan-kawasan tersebut. Pengawasan ovitrap telah dijalankan selama satu tahun untuk mengkaji hubungan antara pengawasan ovitrap dan parameter alam sekitar, dan ia menunjukkan bahawa tiada perbezaan yang signifikan dalam bilangan populasi bagi kedua-dua kawasan dan tiada korelasi dengan faktor persekitaran. Borang

soal selidik mengenai persepsi terhadap bahan kimia dalam kawalan nyamuk dan potensi penggunaan alat kawalan biologi telah diedarkan kepada kakitangan pejabat kesihatan dan juga orang awam di kedua-dua kawasan kajian. Umumnya, orang ramai mempunyai ketidaktentuan yang tinggi (pemarkahan 'tidak pasti' bagi semua 4 soalan yang diberikan dalam julat antara 47.9% hingga 27%). Ini kerana orang awam tidak mengetahui kawalan biologi sebagai yang dinyatakan dalam soalan 1 (56%) berbanding dengan kakitangan pejabat kesihatan yang sangat menyedari mengenai kawalan biologi (75%). Keletihan adalah simptom yang paling kerap dilaporkan oleh kakitangan pejabat kesihatan dan kesukaran bernafas dilaporkan oleh orang ramai. Pengawasan agen kawalan biologi asli telah dijalankan di kedua-dua kawasan kajian. Spesies *Poecilicia reticulata* dan nimfa *Odonata* adalah pemangsa paling semula jadi yang dikumpulkan di kawasan-kawasan kajian. Tiga spesies nimfa *Odonata* memakan lebih banyak spesies *Aedes* daripada spesies *Culex* tetapi tidak terdapat perbezaan yang signifikan dalam kecekapan makan pemangsa. Dari segi keutamaan mangsa ikan gapi, kedua-dua ikan jantan dan betina memakan lebih banyak spesies *Aedes* daripada spesies *Culex*. Tingkah laku spesies larva nyamuk dan spesies pemangsa (ikan gapi dan nimfa *Odonata*) menunjukkan pengaruh langsung terhadap aktiviti-aktiviti pemangsa. Semua pemangsa mempamerkan aktiviti-aktiviti diurnal; mereka adalah pemburu di waktu siang dan memakan lebih banyak larva nyamuk secara aktif pada waktu siang. Keberkesanan aktiviti-aktiviti pemangsa bergantung kepada beberapa faktor seperti isi padu air, bilangan pemangsa, dan bilangan kepadatan mangsa. Kesimpulan daripada keputusan ini ialah kedua-dua agen kawalan bio biasa (gapi) dan agen kawalan biologi berpotensi (nimfa *Odonata*) merupakan pemangsa yang cekap dalam uji kaji didalam makmal dan dengan itu merupakan pilihan-pilihan yang mungkin boleh digunakan sebagai strategi pengurusan nyamuk yang mesra alam sekitar.

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&	and
$^{\circ}C$	degree centigrade
$=$	equal
$>$	greater than
$\geq$	greater than or equal to
$<$	less than
$\leq$	less than or equal to
%	percent
$+$	plus
$\times$	times
L	litre
1st	first
2nd	second
3rd	third
4th	fourth
AI	*Aedes* index
Ae.	*Aedes*
An.	*Anopheles*
ANOVA	analysis of variance
Ar.	*Armigeres*
AR	Augumentative release
BI	Breteau index
Biocontrol	Biological control

Bti	*Bacillus thuringiensis israelensis*
ChE	cholinesterase
COMBI	Communication for Behavioural Impact
CI	Container index
cm	centimetre
Cx.	*Culex*
DDT	Dichlorodiphenyltrichloroethane
DF	Dengue fever
DHF	Dengue Haemorrhagic fever
DO	Dissolve oxygen
g	gram
G. affinis	*Gambusia affinis*
h	hour
IMR	Institute for Medical Research
km	kilometre
L	litre
m	meter
mg	milligram
mm	millimetre
MOH	Ministry of health
N. flactuans	*Neurothemis flactuans*
P. reticulata	*Poecilia reticulata*
O. chrysis	*Orthetrum chrysis*
O.sabina	*Orthetrum sabina*

RBC	Red blood cell
RH	Relative humidity
S.E	Standard error
sp.	species
Tx.	*Toxorhynchites*
ULV	Ultra low volume
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 Background

Mosquitoes have an almost worldwide distribution, being found throughout the tropics and temperate regions. They can thrive in a variety of habitats whether fresh, brackish clear, turbid or even polluted water. Although there are about 3,500 known species and subspecies, there are probably more than 1,000 species that have yet to be found and described. The biodiversity of mosquitoes is evident, with many genera having a worldwide distribution and some genera with limited or endemic distribution (Rueda, 2008). Mosquitoes can be harmful by acting as vectors that can spread diseases such as Dengue, Malaria, Filariasis, Yellow fever, and Japanese encephalitis.

Putrajaya is the new Administrative Center of the Government and it is set to be a model garden city with sophisticated information network based on multimedia technologies. About 70% of Putrajaya is still preserved as natural habitats (Perbadanan Putrajaya, 2004). There is a lot of vegetation in the area which provide suitable resting places for *Aedes* mosquitoes. Urbanization is one factor that increases the number of suitable habitats for *Aedes* mosquitoes especially for *Aedes aegypti* (WHO, 2008). In urban areas where vegetation is abundant, both *Ae. aegypti* and *Ae. albopictus* can found together. In general, *Ae. aegypti* is the dominant species in urban areas but depending on the availability and types of larval habitat (WHO, 2006). Design and planning are powerful tools that can either support or undermine the quality of development and conditions for sustainability in all communities (McClure, 2007).

Public areas, particularly residential developments, have been located in close proximity to major mosquito or biting midges major breeding sites, some of which are construction sites. The presence of vegetation corridors between community areas and these breeding sites provide dispersal routes for biting insects to populate community areas. Trees and shrubs with dense foliage, planted near dwellings, will provide harbourage sites for mosquitoes and biting midges (Scott, 2002).

Certain pesticides and chemicals can significantly and effectively control the population of mosquitoes. However, the chemicals can pollute the entire water in the breeding areas, causing additional environmental problems. These harmful chemicals can no doubt destroy the mosquitoes but at the same time directly or indirectly will accumulate within the different members of the food chain and get magnified which may cause serious health problems to the predators at higher tropic levels (Aditya & Mahapatra, 2003).

Many synthetic chemicals are widely used for controlling adult and larval mosquito populations. However, the harmful effects of chemicals on non-target populations and the development of resistance to these chemicals in mosquitoes along with the recent resurgence of different mosquito-borne diseases have prompted thus research in order to explore alternatives in terms of simple, sustainable methods in mosquito control as supported by Milam *et al*. (2000). The eradication of adult mosquitoes using adulticides is not a wise strategy, as the adult stage occurs alongside human habitation, and they can easily escape from control measures (Service 1983 & 1992).

Chemical compounds have been used in public health control program especially in mosquito population control including organochlorine, organophosphates, carbamate and pythroids. The insecticides that are normally used in mosquito control are DDT, temephos, fenitrothion, malathion, propoxur and permethrin. DDT was used to control Malaria cases and Temephos (ABATE®) is regularly used in containers for control *Aedes* mosquito larvae (Chareonviriyaphap *et al*. 1999). WHO (1975) defined resistance as "the developed ability in a strain of insects to tolerate doses of insecticides which prove lethal to the majority of individuals in a normal population of similar species. Many researchers have reported the chemical resistance in mosquito vectors (Andrade & Mondolo 1999; Chareonviriyaphap *et al*. 1999; Hidayati *et al*. 2005; Prapanthadara *et al*. 2002).

Ever since the usage of chemicals in the control mosquito populations become more effective and have been used for long time most of researches reported the resistance of chemical to mosquito are well documented (Chareonviriyaphap *et al*. 1999; Kasap *et al*. 2000; Seccacini *et al*. 2008). In Thailand (Somboon, *et al*. 2003) *Ae. aegypti* and *Ae. albopictus* were highly resistant to DDT and in Malaysia (Chen *et al*. 2005; Hidayati *et al*. 2011) *Ae. aegypti* and *Ae. albopictus* have developed some degree of resistance to temephos and highly resistant to Malathion. Hidayati *et al*. (2005) showed that *Cx. quinquefasciatus* larvae developed higher resistant to Malathion and permithrin compared to *Ae. aegypti* and *Ae. albopictus.* The study of chemical resistant in *Cx. quinquefasciatus* mosquito has also been done as this mosquito is known to be harmful to human health.

Nazni *et al*. (2005) have carried out the insecticide test to adult and larvae of *Cx. quinquefasciatus* both of which were reported to be highly resistant to malathion and DDT. In terms of insecticide resistant, DDT is the least effective of insecticide. Other insecticides used to test the insecticide resistant such as Malathion, fenitrothion, propoxur, permethrin, lamdacyhslothrin and cyfluthrin. Selvi *et al*. (2005) also reported the chemical resisitance are *Cx. quinquefasciatus* mosquito.

Biological control of mosquito larvae with predators would be a more-effective and eco-friendly method, avoiding the use of synthetic insecticide and pollution to the environment. The selection of biocontrol agents should be based on its self-replicating capacity, preference for the target pest population in the presence of alternate natural prey, adaptability to the introduced environment, and overall interaction with indigenous organisms (Kumar & Hwang, 2005). One example of potential biocontrol for dragonfly nymph *Brachythemis contaminata* (Family: Libellulidae) against the larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* was investigated by Singh *et al*. (2003) and found that they had good predatory potentials and can be used as a biological control agent for the control of mosquito breeding.

1.2 Problem Statement

Mosquitoes are very important from the standpoint of human welfare because the females are bloodsucking, many species bite people, and they serve as vector in transmission of several important and dangerous human disease (Triplehorn & Johanson, 2005). The role of blood-sucking arthropods as agents of human and animal diseases was established in the last quarter of the 19th century (Clements, 1992), where it was known that *Ae. aegypti* and *Ae. albopictus* acted as reservoir for dengue virus. The dengue virus was transmitted to humans by the bites of infected female *Ae. aegypti* and *Ae. albopictus* (Heymann, 2004).

Insecticides dominated vector control approaches after their introduction, but damage to the environment, vector resistance to insecticides, and community resistance to their use have resulted in a new focus on biological control measures (WHO, 2003a).

As environmental effects of chemical pesticides became better understood, there is increasing pressure to replace the more toxic materials. In some cases biological controls can help reduce or sometimes replace the use of toxic chemicals (William, 2003). The use of synthetic chemical is known to contaminate drinking water supplies. Additionally, there are many available investigations which reported mosquitoes that are resistants to insecticides frequently used and making it even more difficult to control adult mosquitoes. Basically, larval mosquito populations should be the first target of all control measures (Service, 1992; Briegel, 2003). According to Kumar and Hwang (2005) the use of chemical in control of mosquitoes can an effect non-target populations as well as the environment. Mosquitoes can become resistance to insecticide and thus, make their control to be more difficult in the future. Chua *et al*. (2005) reported dead animals such as ants and spiders (which are non target insects) within 48 hours after chemical fogging in their studies.

As mentioned by Chareonviriyaphap *et al*. (1999) the long-term intensive use of chemical pesticides to control insect pests and disease vectors is often cited as the reason behind the development of insecticides resistance in insect population. For instance in Thailand mosquito became resistant to DDT that was used in the control of mosquito populations. Beside that the use of chemical control also brought issues of costing as the relatively high costs were needed to buy the insecticide, operation cost for the distribution of ABATE to houses, and labour cost for the worker sparying insecticides (Gratz, 1967). One of the possible ways of avoiding development of insecticide resistance in field is using non chemical control method for example biocontrol agent (larvivorous fish) (Raghavendra & Subbarao, 2002). Biological control measures were commonly used before the introduction of insecticides in the 1940s (WHO, 2003a).

As seen in Figure 1.1 Dengue is now the most important viral disease transmitted by mosquitoes, having been recorded from more than 100 countries, and the number of cases world-wide is increasing (Service, 2000). Malaysia is one of the 30 most highly endemic Dengue cases reported by World Health Organization (Figure 1.2). Other common diseases in Malaysia as reported by Ministry of Health were Malaria and Filariasis (Table 1.1). The crisis of dengue outbreaks occurred in Kuala Lumpur and Selangor state. AFP claimed that in 2009, it was worst outbreak ever but this is not just a Malaysian problem, but a global problem. In 2008, a total of 49,335 cases of dengue fever were reported, amounting to an increase of 489 cases or 1% as compared to the 48,846 cases reported in 2007(MOH, 2009). Data on dengue fever in Putrajaya and Kuala Selangor were collected from Ministiry of Health between 2000 until 2012 (Figure 1.3, Figure 1.4).

As the effective vaccine for dengue is not yet available, vector control against Aedes mosquitoes is emphasized in the dengue control programme (Lam, 1993; Koenraadt, 2006). Dengue is a significant public health issue in urban and suburban areas (Liaqat *et al.* 2013). The common vector-borne diseases in Malaysia are tabulated as below.

Table 1.1 The common diseases in Malaysia as reported by Ministry of Health 2008, such as Dengue, Malaria, and Filariasis

Types of Disease	Peak of transmission season	Endemicity	Risk Population
Dengue	June- August		Congested urban areas
Malaria	Peak transmission season	Endemic in certain parts of East Malaysian States of Sabah & Sarawak and interior areas of Penisular Malaysia.	2.5 million
Filariasis	Peak transmission season	Microfilaremia rate : 0.14%	1,018,000 populations in endemic areas (3.7%)

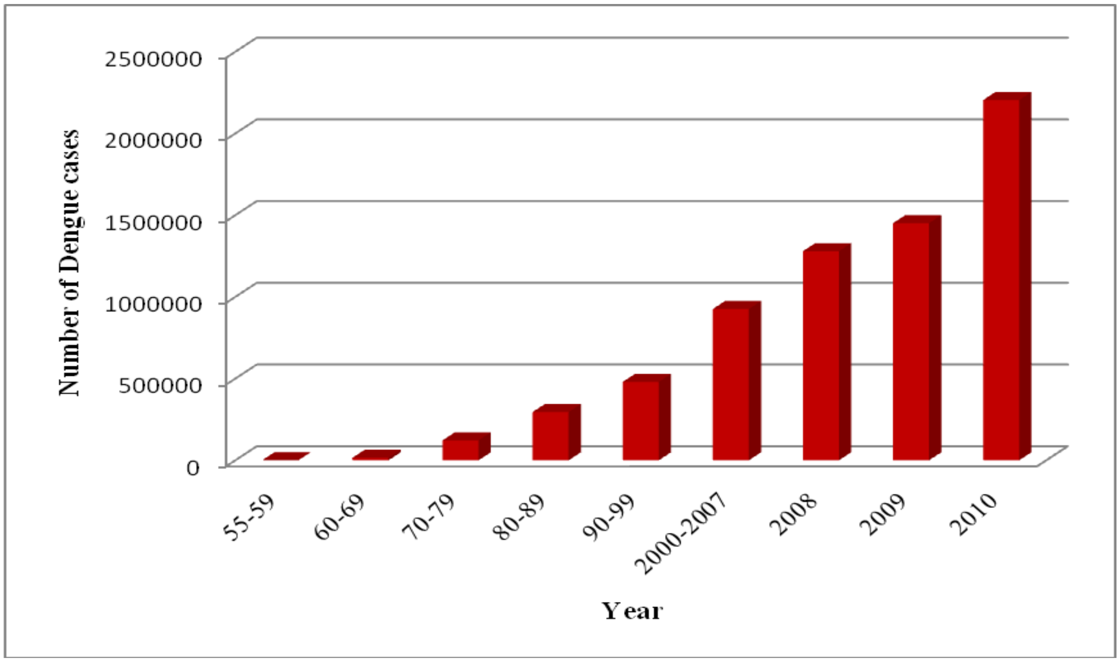


Figure 1.1 Average numbers of dengue and severe dengue cases reported by WHO annually from 1955–2007 and the number of cases reported in recent years, 2008–2010 (WHO, 2012)

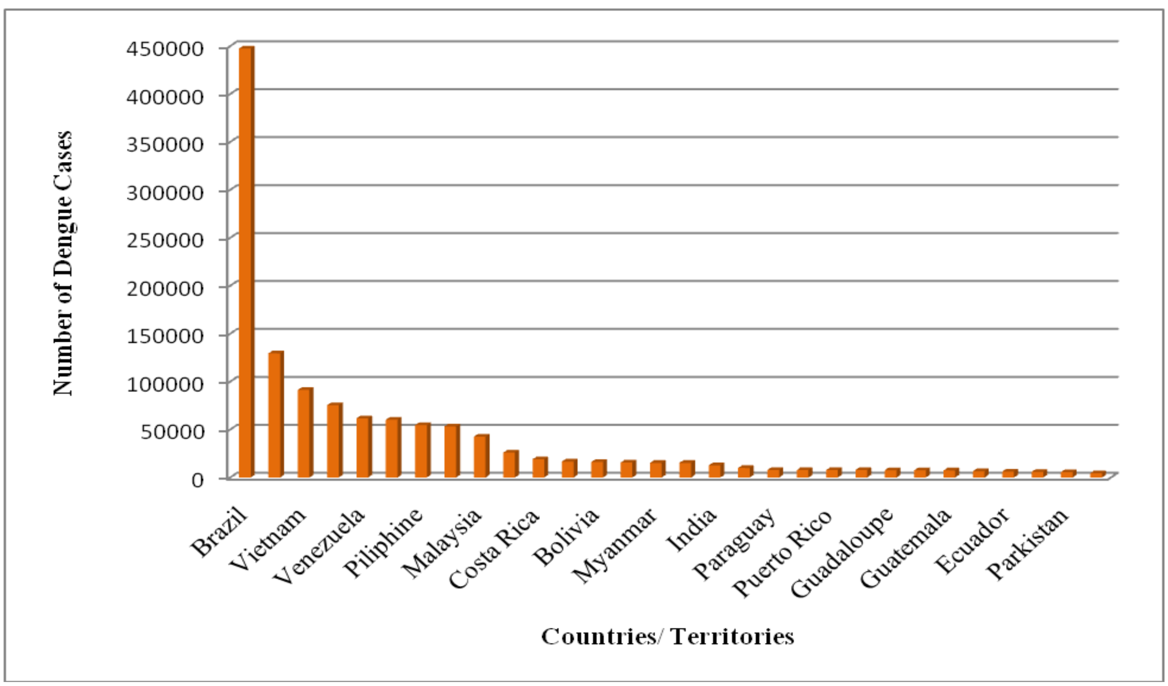


Figure 1.2 Average number of dengue cases in 30 most highly endemic countries as reported by WHO 2004–2010 (WHO, 2012)

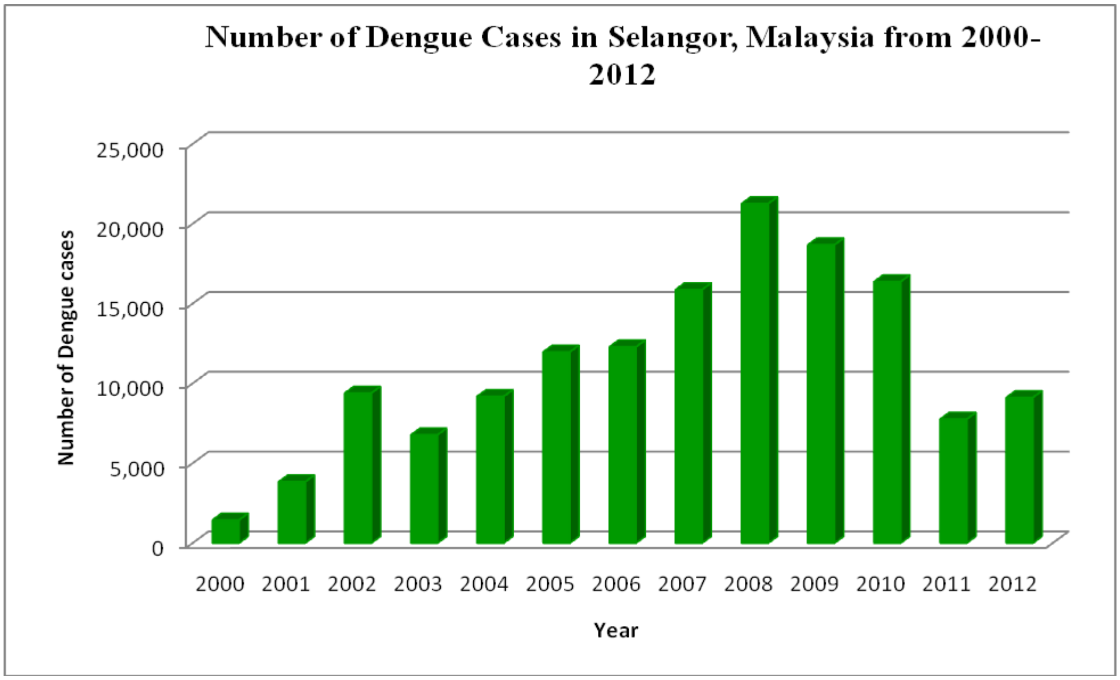


Figure 1.3 **Number of Dengue Cases in Selangor from 2000 until 2012 as reported Jabatan Kesihatan Negeri Selangor (JKNS 2013)**

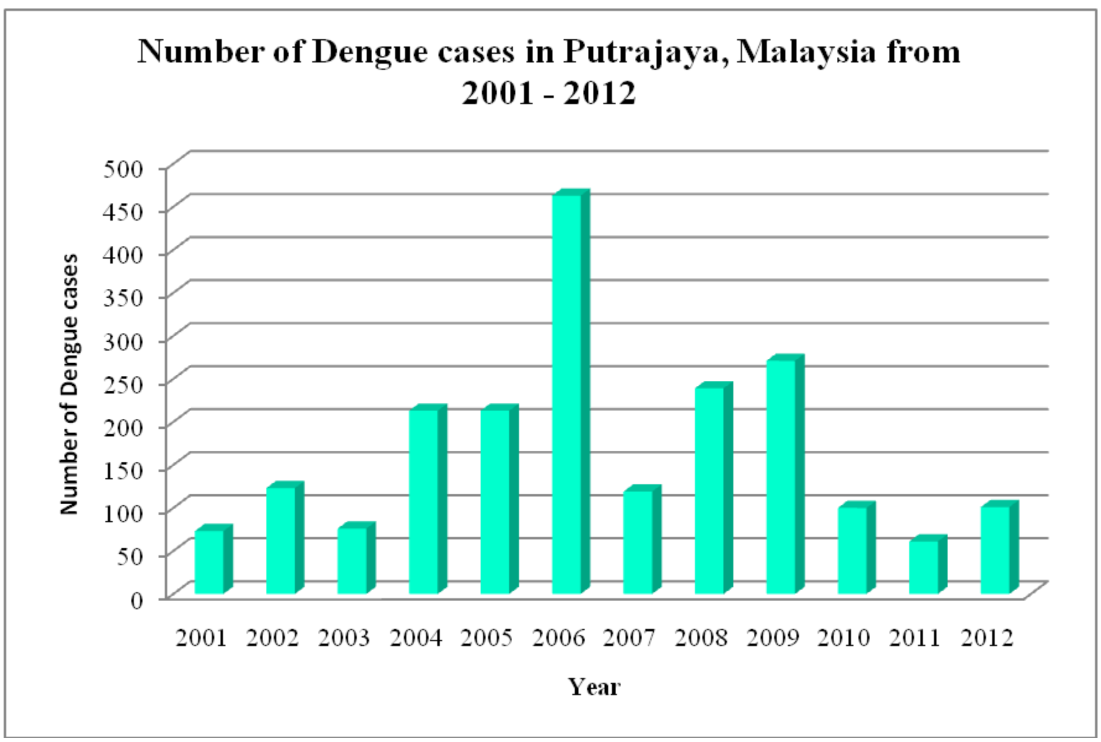


Figure 1.4 **Number of Dengue Cases in Putrajaya from 2001 until 2012 (Putrajaya Health Office, 2013)**

1.3 Research Objectives

1.3.1 General Objective

The control of mosquitoes is a very important effort because these insects are the primary vectors in the transmission of several important and dangerous human diseases. Since the excessive use of insecticide can also be harmful to human health thus it is important to evaluate the effectiveness of biological control as one of the beneficial ways in vector control. Hence, the specific objectives of the present study are:

1.3.2 Specific objective

i. To determine the mosquito larvae species, their larvae density and their breeding places in the areas of Putrajaya and Kuala Selangor.

ii. To calculate the entomological indices from the data obtained in the residential areas in Putrajaya and Kuala Selangor.

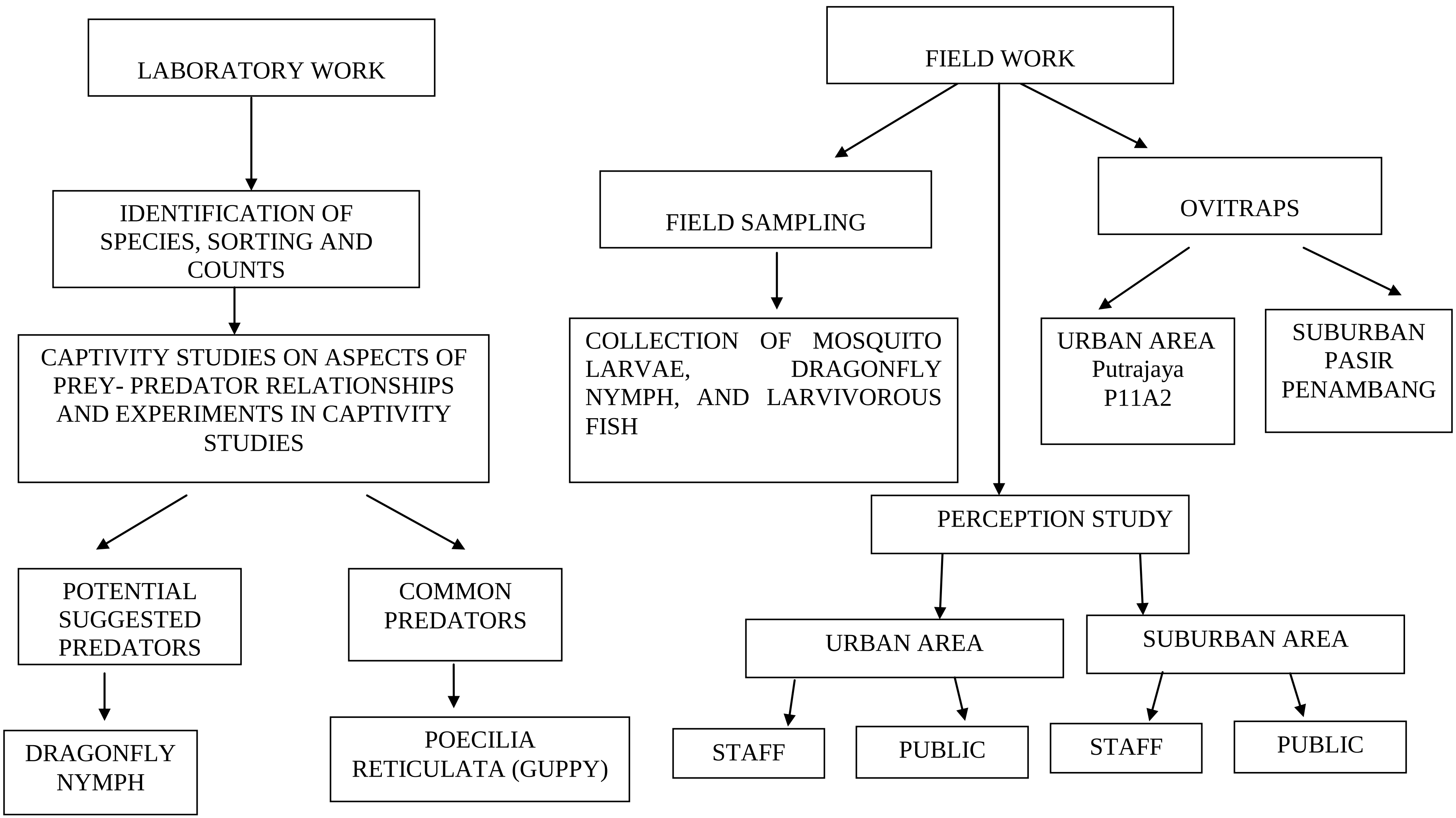
iii. To study the relationships between ovitraps survey and environmental parameters.

iv. To obtain the perceptions of chemical in mosquito control and the potential use of biocontrol for two target involved groups.

v. To survey for natural predators within study sites to enable identification of potential biocontrol agents.

vi. To conduct captivity studies on predator–prey relationships in order to assess the efficiency of selected predators also to evaluate factors influencing predation activities such as density and physical variables.

Figure 1.5 **A schematic flowchart to show the components of the research work**



CHAPTER 2

LITERATURE REVIEW

2.1 Mosquitoes in General

There are about 3200 species and subspecies of mosquitoes belonging to 37 genera, all contained in the family Culicidae. This family is divided into three subfamilies: Toxorhynchitinae, Anophelinea (anophelines) and Culicinae (culicines). Mosquitoes have a world-wide distribution; they occur throughout the tropical and temperate regions and extend their range northwards into the Artic Circle. The only areas from which they are absent are Antarctica, and a few islands. They are found at elevations of 5500 m and down mines at depths of 1250 m below sea level. The most important pest and vector species belong to the genera *Anopheles*, *Culex*, *Aedes*, *Psorophora*, *Haemagogus and Sabethes* (Service, 2000).

In Malaysia, there are 434 species representing 20 genera of mosquito fauna (Abu Hassan & Yap, 1999). *Ae. albopictus* and *Ae. aegypti* mosquitoes were vector that transmitted dengue fever and dengue haemorrhagic fever (Lee, 2000). *Culex* mosquitoes are commonly referred to as Japanese encephalitis (JE) vectors. However, it is important to know that not all *Culex* mosquitoes are JE vectors. Only two species *Cx. tritaeniorhynchus* and *Cx. gelidus* are suspected as the principal JE vectors. *Cx. quinquefasciatus* mosquitoes one of species that are found commonly in Malaysia is a vector of urban filariasis (Yap, *et al*. 2000). Nine species of *Anopheles* mosquitoes have been shown to be capable of being vectors of diseases: *An. maculatus*, *An. balabacensis*, *An. dirus*, *An. letifer*, *An. campestris*, *An. sundaicus*, *An. donaldi*, *An. leucosphyrus* group and *An. flavirostris* (Rahman *et al.* 1997).

2.2 Breeding Places of Mosquitoes

Design of construction sites, such as the building of roads, drainage and canal developments, may create potential breeding sites for mosquitoes because of environmental modifications (Scott, 2002). Rooftop gutters have been banned in new developments Building Plan approval process because it can pose a high potential breeding habitat of mosquito (Benjamin, 2008). Breeding sites of mosquito can be divided into two main categories: breeding sites with clean waters and breeding sites with polluted water. Normally *Aedes* species prefer breeding sites with clean waters and on the other hand *Culex* species prefer breeding sites with polluted waters (WHO, 1986).

Although some *Aedes* species breed in natural habitats such as marshes and ground pools, including snow-melt pools in the artic and subartic areas, many others especially those that live in the tropical areas would exploit artificial, man-made container- habitats besides natural phytothelmata for example trees-holes, bamboo stumps, leaf axils, rock-pools, village pots, tin cans and tyres. *Ae. aegypti* breeds in village pots and water storage jars placed either inside or outside houses. Larvae occur mainly in those with clean water intended for drinking. In some areas, *Ae. aegypti* also breeds in rock-pools and tree-holes. *Ae. albopictus*, which is a vector of dengue in South-East Asia, breeds in natural and man-made container-habitats such as tree-holes, water pots and vehicle tyres. This species was introduced into the USA in 1985 as dry, but viable eggs which had been oviposited in tyres in Asia and then exported (Service, 2000).

Cx. quinquefasciatus, the vector of urban filariasis for some areas, normally breeds in on-site sanitation systems such as wet pit latrines and septic tanks that contain polluted water rich with organic matters. Other breeding sites are pools and disused wells used for dumping garbage (WHO, 1986).

The larvae and pupae of *Mansonia* species attach themselves to aquatic plants for them to be able to breathe. Therefore to control this species, the aquatic plant or vegetation have to be destroyed or removed The aquatic plants and vegetation provide suitable hiding places for mosquito larvae to escape from larvivous fish. In large water bodies such as pond and lakes, vegetation would be removed by using herbicides or release fish to eradicate the mosquito population. The mosquito species *An. stephensi*, a vector of malaria in some urban areas in south Asia, it normally found to breed in wells, ponds, cisterns and water storage container (WHO, 1986).

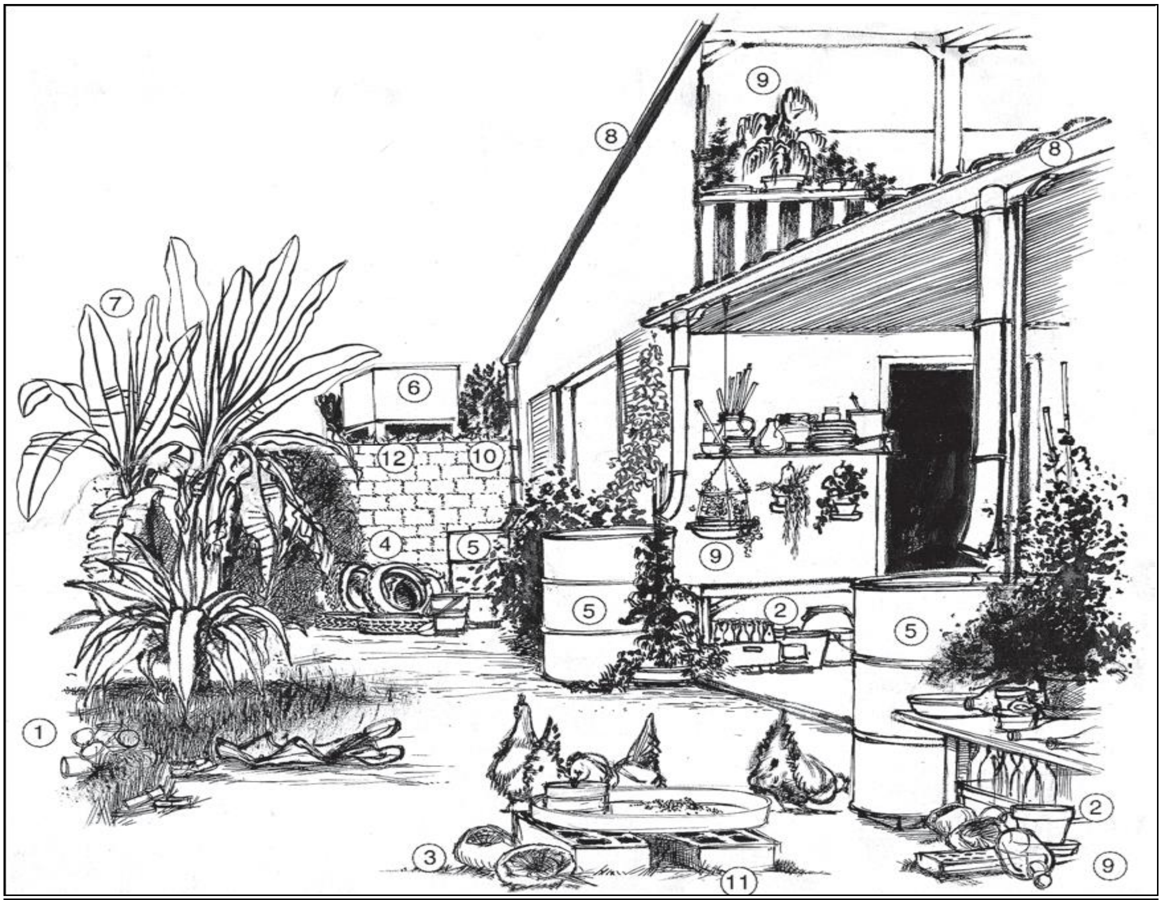


Figure 2.1 **Some examples of outdoor breeding places of *Aedes* mosquitoes. Breeding occurs in (1) discarded cans and plastic containers, (2) bottles, (3) coconut husks, (4) old tyres, (5) drums and barrels, (6) water storage tanks, (7) bromeliads and axils of banana trees, (8) obstructed roof gutters, (9) plant pot saucers, (10) broken bottles fixed on walls as a precaution against burglars, (11) holes in unused construction blocks, and (12) the upper edge of block walls (Rozendaal, 1997).**

2.3 Taxonomy and Life Cycle of Mosquitoes

The mosquito or Culicidae, is a family of about three and a half thousand species within the order Diptera, the two winged flies (Clements, 1992). Only female mosquitoes bite animals or humans for a blood meal to nourish their eggs. Males differ from females by having feathery antennae and mouthparts not suitable for piercing skin. Nectar is the principal food source for males (Dykstra, 2008).

Mosquitoes have a relatively short life and a complete metamorphosis from eggs, larvae, pupa and adults. There are four stage of larvae such as 1st instar, 2nd instar, 3rd instar and 4th instar (Figure 2.2). In larvae stage they are aquatic and depend on water for development until adults emerge. A gravid adult female mosquito will find suitable places to lay eggs or search for the oviposition sites. These sites will be the water surface of open water or water holding containers like tins, flower pots and tyres (Webb & Russell, 2007).

Mosquito larvae are legless, but they retain a well-formed head and so do not appear maggot-like. The preferred larval habitats are small or shallow bodies of water with little or no water movements for example shallow pools, sheltered stream edges, marshes, water-filled tree holes, leaf axils or man-made containers. Most species live in fresh water but a few are adapted for a life in brackish or saline water in salt marshes, rock pools or inland saline pools. The young mosquito larva is fully adapted for living in water, and two features which determine its manner of life are (1) use of atmospheric oxygen for respiration and (2) use of water–borne particles as food. The food resource of mosquito larvae includes particulate matter and others such as aquatic microorganisms, algae and particles of detritus that are largely derived from decayed plant tissues. The growing mosquito larva moults four times. On the first three occasions the larvae leave their cast cuticles and have similar physical appearance to larvae. During the period of the fourth moult the imaginal disks develop rapidly, changing the form of the insect crudely to that of an adult, and at the stage they are known as pupa (Clements, 1992). Every species of mosquito larvae have their own resting position (Figure 2.3). There are four common positions of mosquito larvae such as surface, bottom, wall and middle. Surface means spiracular siphon of the larvae in contact with water-air interface. Bottom refers to larvae within 1mm of the bottom, wall position is the postion where the larvae within 1 mm of the walls and middle is referring larvae more than 1mm from any surface and not in contact with the water – air interface (Kesavaraju, *et al*. 2007).

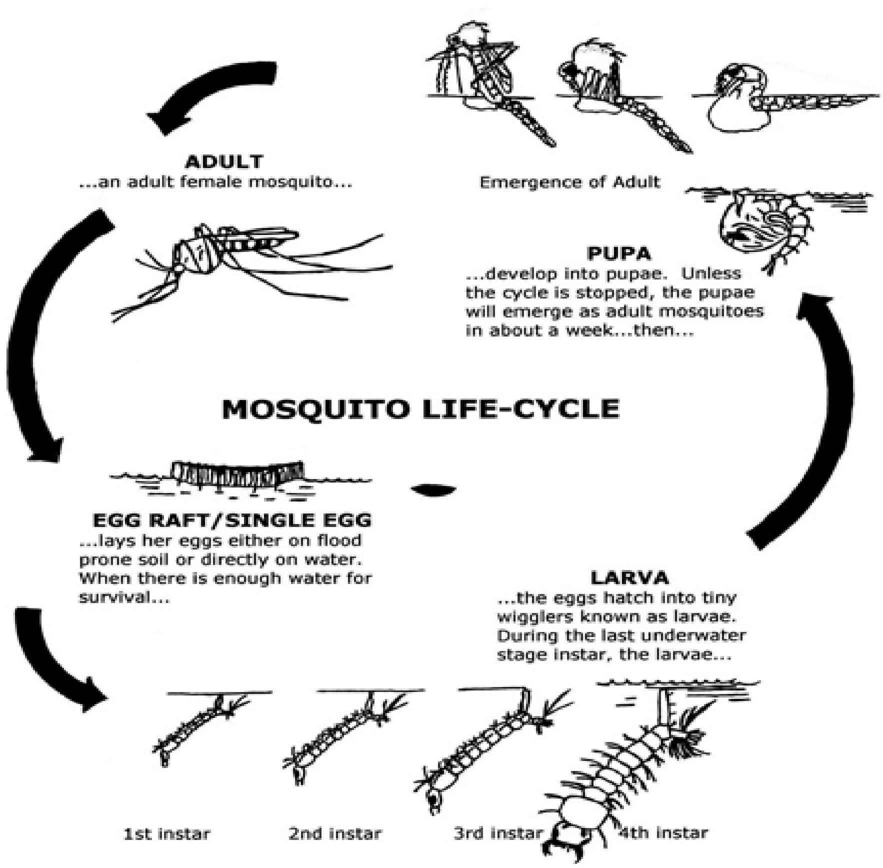


Figure 2.2 Mosquito Life Cycle

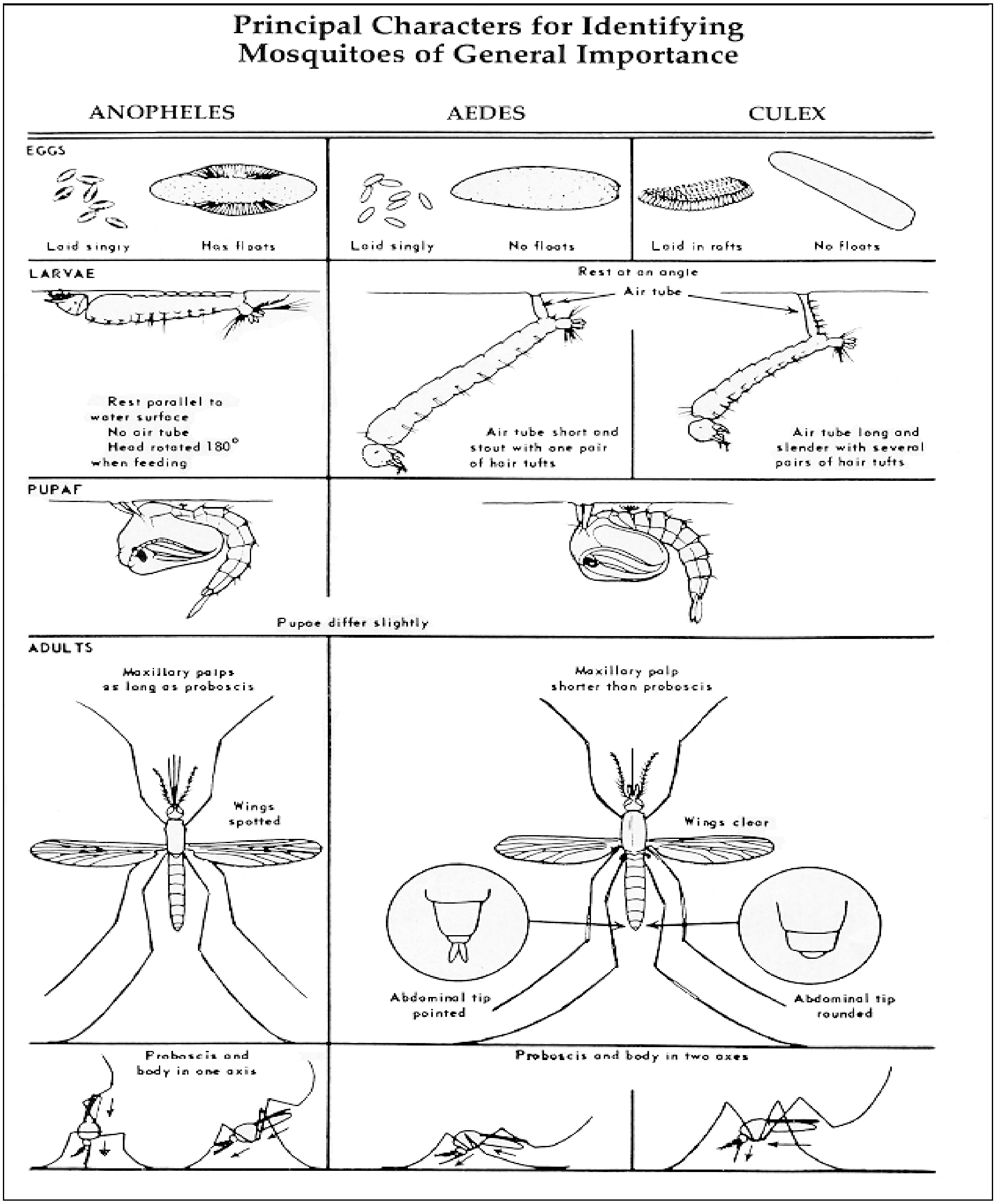


Figure 2.3 **Some of the main characteristics for differentiating *Anopheles*, *Aedes* and *Culex* mosquitoes (Rozendaal, 1997)**

2.4 Biology of *Aedes* Mosquitoes

The distribution of *Aedes* mosquitoes are world-wide, the range of *Aedes* mosquitoes extends well into northen and Artic areas, where they can be vicious and serious pests to people and animals. Eggs are usually black, more or less ovoid in shape and are always laid singly. Eggs are laid on damp substrates just beyond the water line, such as on damp mud and leaf litter of pools, on the damp walls of clay pots, rock-pools and tree holes. *Aedes* eggs can withstand desiccation, the intensity and duration of which varies, but in many species they can remain dry, but viable, for many months. When flooded, some eggs may hatch within a few minutes, while others of the same batch may require prolonged immersion in water; thus hatching may be spread over several days or weeks. Many *Aedes* species breed in small container–habitats such as tree-holes, and plant axils which are susceptible to drying out; thus the ability of eggs to withstand desiccation is clearly advantageous. The life cycle of *Aedes* mosquitoes from eggs to adults can be rapid, taking as little as about 7 days, but it more usually takes 10-12 days; in temperate species the life cycle may last several weeks to many months, and some species overwinter as eggs or larvae. The adult mosquitoes of *Aedes* normally bite during the day or early evening. Most biting occurs out of doors and adults usually rest out of doors before and after feeding (Service, 2000).

2.5 Mosquito Related Disease

Table 2.1 Summary of types of vector borne diseases by the mosquito species indicating their habitat and ecology (MOH, 2008)

Type of vector borne diseases	**Primary and Secondary Vectors**	**Information on vector species**		
		Feeding Behaviour	**Resting behaviour**	**Adult larval Ecology**
Dengue	*Ae. aegypti* *Ae. albopictus*	Peak bitting: dawn and dusk	Rest indoor and outdoor (vegetation foliage)	Clean and clear stagnant water in natural & artificial receptacles.
Malaria	*An. maculatus*	Zoophilic Exophagic	Exophilic	Slow flowing clean and clear water exposed to sunlight
	An. balabacencies	Zoophilic Exophagic	Exophilic	Small pools of muddy water in the forest and periphery
	An. latens	Simio-anthrophagic	Exophilic	Small pools of muddy water in the forest and periphery
	An. sundaicus	Zoophilic Exophagic	Exophilic	Coastal/ Brackish water
	An. letifer	Zoophilic Exophagic	Exophilic	stagnant, somewhat acidic water, usually in shade
	An. donaldi	Zoophilic Exophagic	Exophilic	Stagnant pools, edge of forest
	An. campestris	Anthropophagic Endophagic	Endophilic	Still fresh water rice fields, marshes, drains.
Filariasis	*Mansonia uniformis*	Exophagic & Zoophilic. Biting starts immediately after dust	Exophilic	Open ponds and swamps with floating and emergent vegetation
	Mansonia bonneae *Mansonia dives*	Zoophilic Exophagic	Exophilic	Swamp forest breeders

Dengue fever and dengue haemorrhagic fever, caused by dengue viruses, are increasing importance. The vectors are four man-biting species of *Ae. aegypti*, *Ae. albopictus, Ae. scutellaris* and *Ae. polynesiensis* which breed efficiently in urban environment (Clements, 1992). Dengue is widely distributed in the tropics, occurring through-out most of South-East Asia, the Pacific, the Indian subcontinent, Africa, the USA down to northern parts of South America, and in the Caribbean. A more severe form, dengue haemorrhagic fever, causes infant mortality and has appeared in many parts of South-East Asia and also India. Both dengue and haemorrhagic dengue are transmitted by *Ae. aegypti* and in South-East Asia to lesser extent also by *Ae. albopictus.* Japanese encephalitis (JE) is present in Malaysia, Japan, China, Korea and other areas of South-East Asia and India. Transmission to birds, humans, and pigs is mainly by *Culex tritaeniorhynchus,* which is a common rice field breeding mosquitoes (Service, 2000). In Thailand, *Ae. aegypti* has been documented as the principal of vector Dengue transmission Paeporn, *et al*. (2003). Bancroftian filarisis is an infection with the nematode *Wuchereria bancrofti,* which normally resides in the lymphatics in infected people. *W. bancrofti* is transmitted by many species, the most important being *Cx. quinquefasciatus, An. gambiae*, *An. funestus, Ae. polynesiensis, Ae. scapularis* and *Ae. pseudoscutellaris* (Heymann, 2004).

2.6 Mosquito Control in Malaysia

Mosquitoes such as *Aedes, Culex, Anopheles* and *Mansonia* are anthropophilic which are responsible for many diseases. Mosquitoes larvae are controlled mechanically, biologically, chemically or environmental management (Herman & Michael, 2002; McCall & Kittayapong, 2007). In Malaysia, vector control methods which include source reduction, environmental management, and larviciding with use of chemicals insecticide. In controlling of adult mosquitoes, the common methods include personal protection measures (household insecticide products and repellent) for long term control and space spray (both thermal fogging and ultra low volume sprays) as short term epidemics measures (Yap *et al*. 1994). Several initiatives have been taken to strengthen dengue control. Some of the alternatives include repriortizing *Aedes* surveillance aimed at new breeding sites, strengthening information system for effective disease surveillance and response, legislative changes for heavier penalties, strengthening community participation and intersectoral collaboration, changing insecticide fogging formulation, mass abating and reducing case fatality (Teng & Singh, 2001).

According to Lam (1993) the strategies used in the prevention and control of dengue are directed to both larval and adult stages. For larval control, the activities carried out are source reduction measures, use of temephos larvicide, regular house inspection and enforcement of the Destruction of Disease-bearing Insects Act (DDBIA, 1975). Control measures include fogging activities when a case is notified and conducting case investigations and contact tracing. Health education activities are carried out routinely as an integrated approach for the prevention and control of dengue. Communication for Behavioural Impact (COMBI) is a planning tool for communication and social mobilization activities in support of program goals and objectives. COMBI also was implemented in certain location in Malaysia.

To control an outbreak of disease, fogging should be initiated immediately over a minimum area of 200 m radius around the affected places (Lee, 2000). The activities carried out by the Ministry of Health and the Ministry of Housing and Local Goverment are house inspection, fogging, larviciding and enforcement of Destruction of Diseases Bearing Insect Act, 1995. House and premises inspection for *Aedes* and 'search and destroy' activities to reduce breeding sites in all premises are carried out regularly by the health personnel. Enforcement of law on those found breeding *Aedes* mosquitoes within their premises is usually taken as last resort, on uncooperative members of the public in the gazetted areas, after all efforts in health education on the need to destroy all potential breeding places of *Aedes*, have failed (Singh, 2000). The most extensive effort to control *Ae. albopictus* and *Ae. aegypti* in Singapore include environmental management, health education. Legal measures and community participation and chemical control are reserved solely for outbreaks of dengue hemorrhagic fever (WHO, 1986b).

2.6.1 Chemical Control of Mosquito

In order to control and reduce the mosquito population, chemical applications are the main control agents in several countries. This method was used to prevent mosquito borne diseases. The major classes of insecticide used are pyrethroid, organophosphate, carbarnate and organochlorine (Nauen, 2007). All residents in affected area should be encouraged to apply temephos (ABATE ®) in all water- storing containers. For this purpose, sand granule formulation is recommended at a dosage of 10g/90 L water (about 1 mg/ L) (Lee, 2000). Larviciding for example with temephos to destroy larval stage of *Aedes* is also carried out by the health personnel (Singh, 2000). Dengue control in Malaysia is primarily based on case surveillance by notification of suspected dengue cases by doctors and vector control by space spraying of insecticides (Kumarasamy, 2006).

Chemical insecticides are dispered by ultra-low-volume or/ and thermal fogging. Operations should be initiated immediately when first case is reported. Fogging should be conducted within a minimum distance of 200 m radius (flight distance of *Aedes*) from affected house/houses. Two treatments should be conducted at 10-day intervals and the chemical used is preferably pyrethroids (Lee, 2000). Fogging is done in areas where a case is reported, in outbreak areas, and areas identified as high risk (high density of *Aedes* mosquito) (Singh, 2000).

Larviciding or "focal" control of *Ae. aegypti* is usually limited to domestic-use containers that cannot be destroyed, eliminated, or otherwise managed. It is difficult and expensive to apply chemical larvicides on a long-term basis. Therefore chemical larvicides are best used in situations where the disease and vector surveillance indicate the existence of certain periods of high risk and in localities where outbreaks might occur (WHO, 2002).

Malathion was used in the 1970s after the 1^{st} nation wide outbreak in 1974 (Vythilingam *et al*. 1992). It is a broad spectrum non-systemic organophosphate insecticide. It became the insecticide of choice in the control of vector-borne disease in several countries including Malaysia. This is because malathion possesses fast action and low acute toxicity to both humans and animals (Becker *et al*. 2010) as compared to other organosphosphates (Jamal *et al*. 2011). However, due to smell and oily residues left on floors and walls of residents' houses, malathion was later replaced by pyrethroid (water-based formulation) in 1996. Resigen and Aqua-resigen are the water-based pyrethroid fogging formulations suitable to be used in many residential sites, both indoor and outdoor.

(Teng and Singh, 2001). Pemethrin is a broad spectrum pyrethroid insecticide. It is currently insecticide used in Malaysia in order to control mosquito population (Wan-Norafikah *et al*. 2010).

2.7 Undesirable Effect of Insecticide Use in Mosquito Control

Although a few IGRs (Insect Growth Regulators) are effective against *Aedes* mosquitoes, their slow action is not favourably perceived by the consumers (Yap *et al*. 1994). The concept of space spraying of insecticides using the new ultra low volume technology was initiated in the early 1970s as the recommended method to control *Ae. aeqypti* (Lofqren, (1970); Pant, (1983); & Mount, (1985). Needless to say, this strategy, which has been recommended for over 40 years, has been a complete failure (Gubler, (1989); Newton & Reiter, (1992).

In Southeast Asia, which bears the brunt of the global disease burden, dengue is a leading cause of hospitalization and death among children in most countries (WHO, 2010). In fact, there have been only four major drivers of this dramatic increase in incidence and geographic expansion of dengue: 1) population growth in tropical developing countries, 2) unprecedented urban growth in those same countries, 3) lack of effective mosquito control in tropical urban centers, and 4) globalisation (Gubler, 2011a).

The use of chemical control has adverse effect to human (Jaga & Dharmani, 2003; Syamimi, *et al.* 2011), non target organism, chemical resistance (Chen, *et al*. 2005; Hidayati *et al*. 2011) and costing (Halasa, *et al.* 2012). Insecticides play a vital role in the fight against these mosquito borne diseases by controlling the vectors themselves in order to improve public health; however, resistance to commonly used insecticides is on the rise (Nauen, 2007).

Dengue represents a substantial burden in many tropical and sub-tropical regions of the world including Malaysia and also economic burden of dengue illness in Malaysia (Shepard, *et al.* 2012). Total costs included both direct costs from medical expenditures and prevention activities and indirect costs from lost productivity (Halasa, *et al.* 2012; Wettstein, *et al.* 2012). To reverse the trends of increased incidence and geographic expansion of epidemic dengue, we will need to use all of the tools that are available to use, both old and new. This includes integrated use of chemical, biological and genetic control tools for *Ae. aegypti*, combined with a top-down bottom-up strategy that includes the use of vaccines and drugs as they come online (Gubler, 2011b).

2.7.1 Insecticide Resistance

Insecticide resistance is viewed as an extremely serious threat to crop protection and vector control, and is considered by many parties, including industry, the WHO, regulatory bodies and the public, to be an issue that needs a proactive approach (Nauen, 2007). Many vector surveillance and control have been frequently carried out in Malaysia. Chemical control plays a major role in vector control but their effectiveness has been threatened by the development of resistance among vectors. There is a growing concern on the resistance towards insecticides which are commonly used during fogging in residential housing areas in Malaysia (Hidayati *et al*. 2011).

The countries of the South-East Asia Region rely on pesticides for the control of vector borne diseases. For example many countries achieved significant success in malaria control in the early period of DDT use in the 1950s and 1960s. However, the development of vector resistance in subsequent years contributed to the failure to achieve effective control and alternative insecticides such as malathion, fenitrothion and bendiocarb, were introduced in the Region (WHO, 1992). Insecticide resistance is generally considered to undermine control of vector-transmitted diseases because it increases the number of vectors that survive the insecticide treatment (Rivero, *et al.* 2010). The widespread use of insecticide has led to insecticide resistance in mosquitoes, which will be a problem for the control of disease (Robert & Andre, 1994). The uses of Temephos in controlling immature stages of mosquito have been shown to be effective. However, after more than 30 years of usage ABATE (temephos) has been shown to have decreased its effectiveness. It is due to resistance being developed by mosquitoes (Lee, 1984).

Paeporn *et al*. (2003), from the results of their study, suggested that temephos resistance could be developed in *Ae. aegypti* under selection pressure and that the main mechanism is based only on esterase detoxification. In India, in the present situation of insecticide resistance status in malaria vectors, the fate of vector control mainly relies on the strategies for the management of insecticide resistance in malaria vector. The approaches have been the replacement of insecticide by an effective and preferably by a new group of insecticides. This situation has led to the development of multi resistant malaria vectors (Raghavendra & Subbarao, 2002).

Malathion and pemethrin are the common adulticides used in the vector-control in Malaysia (Chan *et al*. 2011). However, repeated usage of the same type of insecticides in fogging activities has caused rising of resistance among mosquito population (Loke *et al*. 2012). Therefore, it is necessary for constant monitoring to ensure that these insecticides are still effective against the mosquitoes as fogging with insecticides the major controlling method of vector-borne disease used in Malaysia.

Mosquitoes became resistant to chemicals or insecticides that have been used in the control of the population in larvae and adults stages because of several reasons. For example, *Ae. aegypti* more resistant than *Ae. albopictus* to temephos. Thia is due to *Ae.*

aegypti species prefer to rest indoor and likely to the exposed to household insecticides that are normally used in indoor areas (Chen *et al*. 2005). Furthermore temephos, Malathion, and permethrin have been always used for vector control especially during outbreak in Malaysia (Chen *et al*. (2005); Nazni, *et al*. (2005). Somboon *et al*. (2003) suggested the ineffectiveness in use of permethrin and deltamethrin because these chemicals were currently used for controlling mosquito populations.

WHO (2011) reported *An. gambiae*, a malaria vector is resistant to all insecticide classes and resistance is extremely prevalent; more than two-thirds of mosquitoes survive the diagnostic dose for 4 of the 5 insecticides tested (permethrin, deltamethrin, DDT, fenitrothion and bendiocarb). *Cx. quinquefasciatus*, one of the vectors of filariasis, is found mainly in urban areas and has developed resistance to many types of organochlorines, organophosphorus compound and carbamates (WHO, 1992). Kumar *et al*. (2011) reported that Cx. *quinquefasciatus* is highly resistant to DDT, malathion and incipient resistance pyrethroids (deltametrin, cyfluthrin, permethrin, and lambdacyhalothrin). Nineteen species of *Aedes* are now recorded as resistant. Seventeen of them show resistance to DDT and 12 to one or more organophosphorous compounds. *Aedes aegypti* has shown resistance to carbamates and phrethroids in certain areas as well as to DDT and organophosphorus compounds (WHO, 1986).

2.7.2 Health Effect

All pesticides are associated with some risk of harm to human health and the environment. Organophosphate pesticides are a group of chemicals that are mainly used in agriculture. Organophosphate exposure is a major public health issue in terms of health, morbidity, health care and general safety from toxicity (Fenske, *et al.* 2002). Exposure to pesticides in public places is an unexpected, unintentional, nonoccupational form of exposure among general public (Maddy & Edmiston, 1988). Organophosphate exposure can produce acute toxicity, resulting in high morbidity and even death. The toxicity of an organophosphate is determined by the exposure level of the organophosphate in the environment, the dose absorbed, and the level of ChE depression in an individual. The pesticide-related illness that people suffer from chronic exposure to low to moderate doses of organophosphates is a public health concern (Jaga & Dharmani, 2003). Biological monitoring of organophosphate pesticides includes a method of surveillance for assessing exposure by measuring ChE activity in Red Blood Cells serum. This is applied mainly to the workers exposed to organophosphates. However, ChE activity is measured to assess acute organophosphate toxicity from any exposure, including the nonoccupational situations, since ChE depression is diagnostic of organophosphate toxicity. The cholinesterase (ChE) levels in relations to exposure and symptoms of organophosphate toxicity are show in (Table 2.2).

Table 2.2 Guidelines for cholinesterase (ChE) levels in relations to exposure and symptoms of organophosphate toxicity

ChE level (activity)	Feature
75% to 100% of baseline	Normal, asymptomatic
50% of baseline	Symptoms present
20% to 50% of baseline	Mild exposure, minimal symptoms
10% to 20% of baseline	Moderate exposure, muscle fasciculations, myosis
0% to 10% of baseline	Severe poisoning, life – threatening symptoms, acute cholinergic crisis

(Sullivan & Blose 1992; Schenker, *et al.* 1992)

2.7.3 Cost

Most of the vectors have developed resistance to one or more commonly used insecticides. The use of alternative insecticides may be less cost effective and thus cause financial and operational difficulties. In malaria control programmes for example, the replacement of DDT by malathion increased the cost and replacement by other organophosphorus compounds, carbamate or synthetic pyrethroids may cost even more (WHO, 1986). To avoid the development of insecticide resistance the subsequent replacement of insecticide to a new one is needed. It may be mentioned that subsequent change of insecticides has burdened the programme with increase costs (Raghavendra & Subbarao, 2002). It also involves direct and indirect costs of hospitalization and control of vector by using chemical control (Halasa, *et al.* 2012).

The impact of dengue can be enormous and can place a significant burden on families, communities, and nations. The impact on the family can includes loss of life, unplanned expenditures for medical care and hospitalization of sick family members, school and work absenteeism, and a loss of income if the patient is the family's source of income. The impact on a community and nation can include a productivity loss in the workforce due either to illness in economically active age groups or to the need to take care of ill family members; health-care services that are greatly strained or that collapse outright because of sudden, high demand caused by thousands of cases entering the health system during an epidemic; unplanned expenditures for large-scale emergency control actions; and a loss of revenue from tourism as a result of negative publicity (WHO, 2013). There are two main components that should be considered in a dengue cost study: (i) healthcare costs (hospital-related costs, outpatient-related costs) and (ii) program costs (prevention and control, including vector control, costs, education and community mobilization costs, and surveillance costs) (Armien, *et al.* 2012). Application of *P*. *reticulata* was less costly than that of temephos (chemical control). The cost of fish application can be further reduced if the community is involved in the application (Kusumawathie, *et al.* 2009).

2.8 Biological Control of Mosquitoes

Biocontrol or biological control is the method to control populations of pest by using other living organisms (Becker, 2006). The biological control of mosquitoes and other pests involved introducing into the natural environment, the identified natural enemies, such as parasites, disease organisms and predatory animals. The effective use of these agents required a good understanding of the biology and behaviour of the target pests to be controlled as well as the local environmental conditions. Such methods could be most effective when used in combination with others, such as environmental manipulation or the application of larvicides that would not harm the biological control agents. Several organisms had proved effective against mosquito larvae such as larvivorous fish, mosquito of the genus *Toxorhynchites,* dragonflies, damselflies, cyclopoid copepods, nematode, *Bacillus thuringiensis* H-14 and *B. sphaericus* (WHO, 1986). Biological control of mosquitoes was very popular during the early part of this century, but with the development and availability of chemicals such as organochlorines and organophosphates it was replaced by insecticidal control. However, because of problems with insecticide resistance and greater awareness of environmental contamination there has been renewed interest in biological methods (Service, 2000).

There were two known approaches to biocontrol of pests: inoculation and inundation. Inoculation, also referred to as “classical biocontrol’, entailed introducing natural enemies (parasites, parasitoids, pathogens or predators) of a pest into an environment where they are not yet present. This approach, with requisite precautions has been observed, can be feasible in situations where a pest had been introduced into a new country without its complement of natural enemies. If the inoculation proved to be successful, the natural enemies multiply naturally until they reach a level such that they either eliminate the pest or keep the pest populations down to a level deemed acceptable to humans. Inoculation seemed rarely successfull, partly because damage thresholds recognised by humans are usually far lower than that natural enemies could achieved (after all, an oligophagous predator needed to have some prey to feed on), and partly because, if natural enemies attained high densities, either at the time of release or subsequently, they typically dispersed, thus reducing their effectiveness for local suppression (Corbet, 1999).

The other approach to biocontrol is known as inundation or augmentative release (AR). This entailed prior estimation of the numbers of natural enemies needed (within a given area and a given time) to achieve suppression to the required level, and then releasing sufficient numbers into a closed system, i.e. an environment from which they could not disperse. If the requisite conditions were satisfied, AR could be highly successful (Corbet, 1999).

2.8.1 Larvivorus Fish as Biocontrol Agent

The larvivorous fish are generally feeding mainly on insect larvae and pupae. The most potential larvivorous fish that were used in mosquito control belong to the fish families Poeciliidae, Cyprinidae, Cyprinodontidae and Chichlidae (WHO, 2003a). Mosquito control using fish has focused primarily on *Gambusia affinis* and *P. reticulata* (Table 2.3). The most widely and firstly used biocontrol agents of mosquito populations were the larvivorous fish of mosquito fish, *Gambusia affinis*, and *G. holbrooki*. These species are effect on native faunal composition and they become unable to control mosquito in small containers, tree holes and suitable breeding sites of medically important mosquitoes (Kumar & Hwang, 2006). Another commonly used fish is the South African guppy, *P. reticulata* which can tolerate organic polluted waters and is also more heat tolerant. Other types of fish that have been used to control mosquito larvae, are carps, *Cyprinus carpio* found in Chinese rice fields, edible catfishes, *Clarias fuscus* that lives in water storage tanks in Myanmar to control *Ae. aegypti* and a Tilapia species *Oreochromis*, found in Africa and *Aplocheilus* species which can be found in Europe and Asia (Service, 2000). Many of larvivorous fish were used in controlling mosquito all over the worlds (Table 2.4 and Table 2.5).

Guppies (*P. reticulata)* were used to control dengue vector of *Ae. aegypti* in domestic water storage containers in rural areas in Cambodia (Chang *et al*. 2008) and *P. reticulata* was tested in India to assess their predation on *Cx. quinquefasciatus*, tubificid worm and chironomid larvae (Manna *et al*. 2008). Besides guppy fish was cultured along with Indian carps and the money generated was used for village development in India (WHO, 2003a). During the 20th century, several fish species were introduced outside their natural habitats such as the mosquito fish that can tolerate a broad range of environmental conditions and can exist in high densities with no specific diet. Their high fecundity, viviparity and low fry mortality resulting in rapid population growth can be efficient predators for mosquito control (Moyle & Cech 1982).

According to Chatterjee and Chandra (1997) the efficiency of *G.affinis* under experimental studies in laboratory was good as they consume all species of *An. subpictus* larvae, *Cx. quinquefasciatus* larvae and *Ar. subalbatus* larvae.

In China the health authorities have also used fishes to eradicate mosquito larvae of *Ae. aegypti* species in water containers. Other fishes, such as *Claris fuscus*, *Tilapia nilotica*, and *Macropodus* sp. have been used in many regions of China to eliminate larvae in domestic water containers with considerable success it was found that the catfishes were particularly effective as predators (Neng, *et al*. 1987). According to Lowe, *et al*. (2000) *G. affinis* and *G. holbrooki* have been designated among 100 invasive species worldwide because of their ability to spread widely and their negative impact on aquatic communities. In Malaysia, the used of fish as biocontrol as early as 1915 for the control of malaria vectors (Strickland, 1915).

Table 2.3 Summary of the contrasting characteristic of 2 types of larvivorous fishes according to (Chandra, 2008)

Criteria	***Gambusia affinis* (Baird & Girard), 1853**	***Poecilia (Lebistes) reticulata* (Peters), 1859**
Common name	Top minnow	Guppy
Size	Male - 3.5 cm, Female - 6 cm.	Male - 2 cm; Female - 4 cm.
Distribution	A native of coastal waters of United States from New Jersey southwards, introduced into India about 40 years ago from Italy and Thailand.	It is originally from tropical America. The native distribution includes The Netherlands, West Indies and from Western Venezuela to Guyana. It was imported to India more than once, and restricted to south India and some other parts.
Ecology	Found in freshwater, brackish water and salt marshes with high salinity.	*Poecilia* cannot tolerate low temperature. A prolific breeder in tropical waters requiring a temperature between 22 and 24°C,
Food	Feed on aquatic and terrestrial insects. Terrestrial insects that fall in the water show preference to mosquito larvae	*Poecilia* lives on artificial food and prefers mosquito larvae. In contrast to Gambusia they have able tolerance to polluted waters.

Table 2.4 Summary of the larvivorous fish use in mosquito control by country (WHO, 2003a)

Country	Larvivorous fish
Afghanistan	*Gambusia affinis*
Bahrain	*Aphanius dispar*
Cyprus	*Gambusia affinis*
Djibouti	*Aphanius dispar*
Egypt	*Gambusia affinis*
Iran	*Gambusia affinis*
Iraq	*Gambusia affinis, Gambusia holbrooki*
Jordan	*Gambusia affinis*
Kuwait	*Aphanius dispar*
Lebanon	*Gambusia affinis*
Morocco	*Gambusia affinis*
Oman	*Aphanius dispar*
Parkistan	*P.reticulata*
Saudi Arabia	*Aphanius dispar*
Somalia	*Oreochromis spilurus spilurus*(Tilapia)
Sudan	*Gambusia affinis*
Syria	*Gambusia affinis*
Tunisia	*Gambusia affinis*
United Arab Emirates	*Oreochromis*(Tilapia) and *Aphanius dispar*
Yemen	*Aphanius dispar*

Table 2.5 Summary of reports on the use of fish as biocontrol agents for mosquito species

No.	Biocontrol agents	Prey	Country / Reference
1.	*P. reticulata*	*Anopheles alonitus*	Indonesia (Nalim *et al*. 1988)
2.	*P. reticulata*	*Ae. aegypti* larvae	Cambodia (Chang *et al*. 2008)
3.	*P. reticulata*	*Cx. quinquefasciatus* larvae, tubificid worm and chironomid	India (Manna *et al*. 2008)
4.	*P. reticulata*	*Chironomous* larvae, mosquito larvae and worm	Nigeria(Anogwih & Makanjuola, 2010)
5.	*P.reticulata* and *Gambusia affinis*	*Ae. aegypti* larvae	India (Ghosh *et al*. 2011)
6.	Guppy (*P.reticulata*) and Panchax Minnow (*Aplocheilus Panchax*)	*Cx. quinquefasciatus* larvae	India (Gupta & Banerjee 2013)
7.	Larvivorous fish (*Macropodus cupanus*)	*Culex* larvae	India (Jacob *et al*. 1983)
8.	Larvivorous fish (*Aphanius dispar*)	*Cx. quinquefasciatus*, *Ae. aegypti* and *Anopheles stephensi* larvae	India (Haq& Yadav, 2011)
9.	Larvivorous fish - *Ambassis* (=*Chanda) nama* - *Parrambassis* (=*Chanda*) *ranga* - *Colisa fasciatus* - *Esomus danricus* - *Aplocheilus panchax*	*Cx. quinquefasciatus* larvae	India (Aditya *et al*. 2012)
10.	*Aphyosemion gularis* fish	*Anopheles* larvae	Nigeria (Okorie & Abiodun, 2010).
11.	*Aphyosemion gularis* fish	*Anopheles* larvae, *Anopheles pupa*, *culex* larvae, *chironomid larvae and ostracods*	Nigeria (Okorie & Abiodun, 2011).
12.	*Pseudomugil signifier* Kner and *Gambusia holbrooki* (Girard)	*Cx. annulirostris* larvae	Australia (Willems *et al*. 2005)
13.	Exotic fish predators (*Cryprinus carpio Linnaeus,*	*Anopheles stephensi* larvae	India (Ghosh *et al*. 2005)

	Ctenopharyngodon idella, Oreochromis niloticus and Clarias gariepinus)		
14.	*C.decemmaculatus* and *J. multidentata* fish	*Cx. pipiens* larvae	Argentina (Marti *et al*. 2006)
15.	*Oreochromis spilurus spilurus* (Tilapia)	*Anopheles* larvae	Somalia (Alio *et al*. 1985)
16.	*Clarius fuscus* and *Tilapia nilotica*	*Ae. aegypti* larvae	China (Neng & Shu–sen, 1985)
17.	*Oreochromis niloticus niloticus* (Nile Tilapia)	Mosquito *larvae*	India (Ghosh 2006)
18.	*Gambusia affinis*	*Anopheles* larvae	Iran (Tabibzadeh *et al*. 1973)
19.	*Aplocheilus blockii* (Dwarf panchax),	*An. stephensi*	India (Kumar *et al*. 1998*)*
20.	*Oryzias melastigma* (Estuarine ricefish)	*Anopheles*	India (Sharma & Ghosh, 1989)
21.	*Macropodus cupanus* (Spike tailed paradise fish)	*Cx. fatigans*	India (Mathavan *et al*. 1980)
22.	*Carassius auratus* (Gold fish)	*An. subpictus, Cx. quinquefasciatus* and *Ar. subalbatus*	India (Chatterjee *et al.* 1997)
23.	*Xenentodon cancila* (Fresh water gar fish)	*An. subpictus, Cx. quinquefasciatus* and *Ar. subalbatus*	India (Chatterjee & Chandra, 1996)
24.	*Channa gachua*	Mosquito *larvae*	India (Phukon & Biswas 2011)
25.	*Channa gachua, Puntius sophore* and *Trichogaster fasciata*	Mosquito *larvae*	India (Phukon & Biswas 2013)
26.	*Aplocheilus panchax*	*Anopheles annularis* larvae	India (Pemola *et al*. 2010)
27.	*Aplocheilus panchax*	Anopheline mosquito larvae	India (Pemola & Jauhari, 2011)
28.	*Aplocheilus panchax*	*Cx. quinquefasciatus* larvae	India (Manna *et al*. 2011)
29.	*Oreochromis niloticus* L. (*Tilapia nilotica*)	*An. gambiae* and *An. funestus*	Kenya (Howard *et al.* 2007)
30.	*Nothobranchius guentheri*	*Culex spp.*	Africa (Reichard *et al.* 2010)
31.	Least chub (*Iotichthys phlegethontis*) and western mosquitofish (*Gambusia affinis*)	*Culex spp.*	Utah (Billman *et al.* 2007)

32.	*Poecilia reticulata*	*Cx. pipiens fatigans*	Thailand (Bay & Self, 1972)
33.	*Retropinna semoni (Retropinnidae); crimson-spotted rainbowfish, Melanotaenia duboulayi (Melanotaeniidae); empire gudgeon, Hypseleotris compressa (Eleotridae); estuary perchlet, Ambassis marianus (Ambassidae); firetail gudgeon, Hypseleotris galii (Eleotridae); fly-specked hardyhead, Craterocephalus stercusmuscarum (Atherinidae); and Pacific blue-eye, Pseudomugil signifer (Atherinidae)* – Australian Native fish species	*Cx. annulirostris*	Australia (Hurst *et al.* 2006)
34.	Aquarium fishes (*Betta splendens*, *Pseudotropheus tropheops*, *Osphronemus gorami* and *Ptrerophyllum scalare*)	*An. stephensi* larvae	India (Ghosh *et al.* 2004)
35.	*Anabas testudineus*, *Clarias batrachus* and *Heteropneustes fossilis*	*Cx. quinquefasciatus*	India (Bhattacharjee *et al.* 2009)
36.	*Tilapia guineensis* and *Epiplatys spilargyreius*	Mosquito larvae	Kenya (Louca *et al.* 2009)
37.	*Aphanius dispar* (Rüppell)	*An. stephensi*, *An. subpictus*, *Ae. aegypti* and *Ae. vittatus*	India (Haq & Srivastava, 2013).
38.	Ornamental fish (Blue Gourami, Goldfish, Black Molly, Angel Fish and Swordtail)	*Cx. quinquefasciatus*	India (Tilak *et al.* 2007)
39.	*Poecilia reticulata*	*Cx. quinquefasciatus*	Bangladesh (Elias *et al.* 1995)
40.	*Carrasius auratus* (goldfish), *Poecilia reticulata* and *Aplocheilus sp.*	*Culex sp*	India (Gupta & Banerjee, 2009)
41.	*Oreochromis spilurus spilurus*	Mosquito larvae	Somalia (Mohamed, 2002)

42.	Five indigenousornamental fish species (*Mystus bleekeri,Channa stewartii, Rasbora daniconius, Colisa fasciatusand* and *Danio aequipinnatus*)	Mosquito larvae	India (Das, 2012)
43.	*Aplocheilus dayi Steindachner, D. malabaricus Oreochromis mossambicus* Peters, *Oreochromis niloticus L.*, and *Poecilia reticulata* Peter	Anopheline mosquito larvae	Sri Lanka (Kusumawathie *et al.* 2006)

2.8.2 Guppies as Biocontrol Agent

a) Guppies species

Taxonomic name: *Poecilia reticulata* Peters, 1859

Synonyms: *Acanthophacelus guppii* (Günther, 1866), *Acanthophacelus reticulatus* (Peters, 1859), *Girardinus guppii* Günther, 1866, *Girardinus reticulatus* (Peters, 1859), *Haridichthys reticulatus* (Peters, 1859), *Heterandria guppyi* (Günther, 1866), *Lebistes poecilioides* De Filippi, 1861, *Lebistes poeciloides* De Filippi, 1861, *Lebistes reticulatus* (Peters, 1859), *Poecilia reticulatus* Peters, 1859, *Poecilioides reticulatus* (Peters, 1859)

Common names: guppie (Afrikaans), guppii (Japanese), guppy (English), hung dzoek ue (Cantonese), ikan seribu (Malay), lareza tripikaloshe (Albanian), lebistes (Portuguese), lepistes (Turkish), Mexicano (Portuguese), miljoenvis (Afrikaans), miljoonakala (Finnish), million fish (English), millionenfisch (German), millions (English), poisson million (French), queue de voile (French), rainbow fish (English), sarapintado (Portuguese), Sardinita (Spanish), Wilder RieChanguppy (German), zivorodka duhová (Czech) (ISSG, 2006).

Poecilia reticulata is a small benthopelagic fish native to Brazil, Guyana, Venezuela and the Caribbean Islands. It is a popular aquarium species and is also commonly used in genetics research. In the past *Poecilia reticulata* was widely introduced for mosquito control but there have been rare to non-existing measurable effects on mosquito populations. It can occupy a wide range of aquatic habitats and is a threat to native cyprinids and killifishes. It is a carrier of exotic parasites and is believed to play a role in the decline of several threatened and endangered species. *P. reticulata* males are smaller; reaching an average length of 3.5cm compared 5cm in females (ISSG, 2006).

The poeciliid fishes include a number of species which have been introduced by human agency well beyond their natural geographic range. Two species, viz., Lebistes reticulatus (Peters) and Mollienisia sphenops (Valen-ciennes), occur in Singapore and both are well established (Alfred, 1966).

The guppy fish grow to about 6 centimeters in length and the females can produce 40–50 offspring after a 1-month gestation period. Guppy fish are extremely efficient at eating larvae; in Cambodia, guppies reportedly eat an average of 102 larvae a day. Guppy fish can be mass-produced easily as they breed year round and can be bred in ponds cleared of other larvivorous fish and weeds, in hatcheries built for the purpose, or in large water jars as in Cambodia (WHO, 2003a).

b) Habitats

Fish of the Poecilidae family inhabit fresh and brackish waters (Nelson, 1994) and have been introduced widely and indiscriminately in many parts of the world as mosquito control agents. The common guppy is a small poecilid fish that lives in freshwater ponds and streams. Guppy are found in a range of fresh and brackish warm water habitats and also in slow flowing water typically associated with well-vegetated margins of ponds/streams.

The guppy is a native species to the Caribbean Islands (Netherlands Antilles, Trinidad and Tobago, Barbados, Windward and Leeward Islands), Venezuela and coastal islands, Guyana and northern Brazil. It has been introduced to about 50 countries in Asia, including Malaysia, Australasia-Pacific, Europe, North America, and South America (Figure 2.4) (Webb, *et al.* 2007).

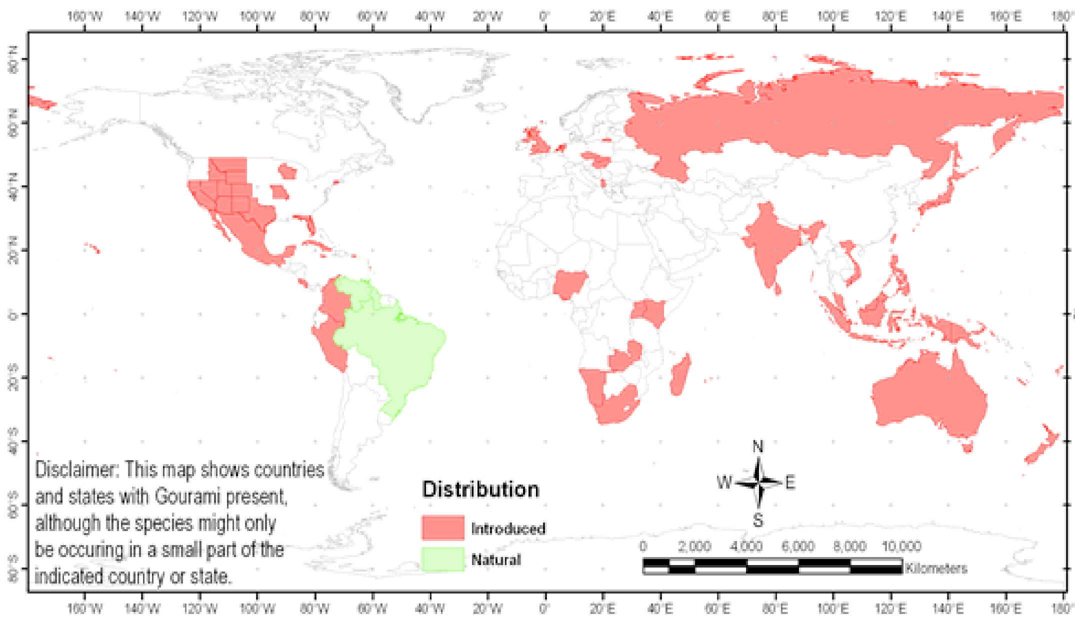


Figure 2.4 Worldwide distribution of guppy

c) Behaviour

The behaviour of guppies includes social, schooling, diurnal, and polygynous. Extensive research is still to be done on the social organization of guppy populations. Shoals are small, 2-20 individuals allowing direct interaction between members, and come into contact every 14 seconds. Shoals composition can be entirely males, females or mixed sex; each moving in uniformity. At night guppies disperse into smaller shoals; reassembling each morning (Croft *et al.* 2003). Females in wild populations develop familiarity with shoal through social learning, learning behaviours and characteristics of members, which help in finding shoals, known as stable partner association.

Little published information on the feeding behaviour of guppies (Houde, 1997). Feeding accounts for 15-30% time budget in males, 45-73% in females (Dussault & Kramer, 1981). When grazing on benthic algae *P. reticulata* pecks rapidly using teeth to loosen algae in scraping motion. Body moving as a whole, the guppy approaches food in forward, downward movement with mouth closed, pecks with jaw maximally protracted, closes mouth, retracts jaw leaving the food vertically (Magurran, 2005). Dussault and Kramer (1981) discovered pecking occurs at intervals of 0.55 seconds, jaw movement at 0.17 seconds and substrate contact at 0.03 seconds ingesting algae of as much as 25% of their body daily when feeding continuously. Guppies nip at insects, detritus and other fish. In single-sex shoals, females feed to bottom of water spending less time than males finding feeding sites, usually relying on previously used sites, males move between previous and new feeding sites (Dussault & Kramer, 1981).

d) Applied research (guppy as a biocontrol agent)

The diminutive but extremely prolific guppy was originally introduced for mosquito control (probably sometime in the early 1900s), and has since colonised many of Singapore's disturbed freshwater bodies. It is a very successful little fish, being able to survive in conditions which few other fish can tolerate, e.g., polluted canals and even sewage tanks (Lim & Ng, 1999).

For dengue control, guppy have been used successfully as biological control agents in water jars and other large containers in many countries, including Thailand and Cambodia (Chang *et al.* 2008). The researchers reported that *P. reticulata* have been used in all over the world and in variety of breeding habitats. For example a laboratory experiment was carried out to assess the efficiency P. *reticulata* against *An. subpictus* larvae. As a results *P. reticulata* can consume 32 and 18 4th stage larvae of *An. subpictus* in 24 (Chatterjee & Chandra, 1997).

Field trials had been conducted by Nalim and Tribuwono (1987), they found *P. reticulata* was effectively controlled *An. aconitus* in rice field with the community participation. They also noticed a sharp decline in the number of malarial cases after introduction of effective biocontrol procedures with larvivorous fish.

Several studies also were conducted in man-made habitat e.g. Sabatinelli *et al*. (1991) reported that the indigenous fish, *P. reticulata*, effectively suppressed larval and adult population of *An.gambiae* in washbasins, and cisterns by 85 per cent in a single year using 3-5 fish in a water surface of 1 m^2. Gupta *et al*. (1992) reported that in India, *P. reticulata* effectively reduced the breeding of *An. stephensi* and *An. subpictus* population breeding in containers. In India, Saha *et al*. (1986) studied the use of guppy (*P. reticulata*) as a powerful biocontrol agent in mosquito control. They found density of *Cx. quinquefasciatus* was reduced in the presence of *P. reticulata* compared to drain sithout *P. reticulate.* The role of *P. reticulata* in the control of mosquito breeding in the wells was also investigated in several district in India (Sharm & Ghosh, 1989; Ghosh, *et al.* 2005).

2.8.3 Dragonfly as Biocontrol Agent

a) Classification and morphology

Dragonfly nymphs are distinguished by a squat and stocky body. The gills are encased within the abdomen and are aerated by a pump that can also provide locomotion. Damselfly nymphs, on the other hand, are elongate and thin, have external gills on the tip of their abdomen, and move with a sinuous fish-like motion. Both groups have a labium (a set of extendable jaws), which they can fire out to catch passing prey (Blakesley, 2005). Dragonflies and damselflies undergo incomplete metamorphosis from egg to nymph to adult, but others insects such as butterflies undergo complete metamorphosis from egg to larvae to pupa and emerge as adults (Venable, 2005). The youngest larvae may be only a couple millimetres (1/16 inch) long, whereas mature nymphs of some species attain a length of more than 3.5 centimetres (about 1-1/2 inch) (Keller *et al.* 2007).

Odonata spend most of their life cycle in an aquatic nymph stage. The adult stage is spent as an aerial organism, and the eggs are then laid back in the aquatic environment. Because two life stages are based in the water, Odonata are good indicators of wetland health. Most of a dragonfly's life is spent in the larval stage and it is among larvae that the greatest range of form is found. Some species have variable numbers of larval moults depending on food supply, temperature and other factors. Development commonly takes 1-2 years but it can last for as long as 6 years in petalurids and 5 years in some gomphids. Its duration depends partly on altitude and latitude. Rates of larval development depend partly on inherited mechanisms and partly on environmental factors such as temperature and food abundance. Factors which affect the distribution of larvae may include the pH of water, the amount and type of aquatic vegetation and whether the water is stationary or running (Miller, 1987).

Every dragonfly's life begins as a larva in water. The larvae look so different that most people would not even recognise them as dragonflies. The tip of the abdomen of damselflies bears leaf like external anal gills, whereas dragonflies carry pointy spines, the so-called "caudal pyramid". Even the dragonfly larvae are something special: They are the only insects equipped with a "pre mentum". This structure lies below the larvae's mouth and has sharp hooks designed to hold onto a prey. It can be hurled forward almost like a harpoon. The larvae of some species lurk hidden in the sediment, others rest among water plants, preying on gnat larvae, worms, small crustacean, and other small water animals (Rademacher, 2011).

Dragonflies belong to the Order Odonata. Based on morphology, the order Odonata are divided into three groups, *viz*. damselflies (Zygoptera), Anisozygoptera and dragonflies (Anisoptera). The adults of damselflies and dragonflies are different based on wings where the Zygoptera (damselflies), with fore and hind wing similar, and Anisoptera (true dragonflies) with wings of different shape (Orr, 2005; Subramaniam, 2005). There are 10 families under Zygoptera which are Chlorocyphidae, Euphaeidae, Calopterygidae, Synlestidae, Amphipterygidae (including: Philogangidae), Platystictidae, Protoneuridae, Platycnemididae, and Coenagrionidae (Synonym: Agrionidae). In Anisoptera, includes Gomphidae, Lindeniinae, Aeshnidae, Cordulegastridae, Macromiidae, Corduliidae, and Libellulidae. Anisozygoptera has only one family, Epiophlebiidae (Nasemman, *et al.* 2011).

In dragonflies, mature males and females often look very different, the males regularly being more conspicuous and brightly coloured. However, freshly emerged and young males often resemble paler females in colouration. Wing venation and often patterns on the thorax is not sex dependant. Since males are more common near water, the majority of individuals observed are likely to be males (Bedjanič *et al.* 2007).

b) Habitat

The odonata species are widely distributed and are particularly prominent around aquatic ecosystems in tropical countries. The adults odonata mate near water bodies, and the females lay eggs in water soon thereafter. Dragonflies are hemimetabolous (they do not have a pupal stage), and most have an aquatic larval stage. There are a few truly marine species, several that live in brackish water, and many that survive in arid regions where the larvae can develop quickly in the warm waters of temporary ponds before they dry up. Others live in flowing water, some even in waterfalls, where the larvae cling to moss on the rocky surface (Miller, 1987).

According to Orr, (2005) in Peninsular Malaysia and Singapore there are more than 230 dragonfly species and most of them encountered near their freshwater. Many habitats are suitable such as suburban drains, garden ponds, open lakes, dams, marshy wayside places, swamp forest, streams, seepages in mixed dipterocarp forests and montane forests. Greatest diversity occurs around swift, clear streams in lowland dipterocarp forest, and certain swamp forest habitats. Andrew, *et al.* (2008) reported the life history of odonates is closely linked with water bodies. They use a wide range of flowing and stagnant water bodies. Odonata species also can the found in the higher latitudes (Norma-Rashid, 2010; Oppel, 2005). In Malaysia many researchers collected numerous Odonata species in different habitats such as in Forest Reserve (Norma-Rashid, 2009), wetland areas at East Malaysia (Dow & Unggang, 2010) several island in the Strait of Malacca (Norma-Rashid *et al*. 2008), fresh water swamp lake (Norma-Rashid *et al*. 2001) Sekayu recreational forest, Terengganu (Wahizatul *et al*. 2006) and Sungai Bebar, Pahang (Dow *et al.* 2006). Factor influencing the distribution of dragonfly diversity can be divided into histrorical (geological) and ecological factors (Kalkman *et al*. 2008). According to Sharma *et al*.

(2007) the wide diversity of odonate in the environment might be playing a potential role in keeping the insect pest population under control.

c) Behavior

Dragonfly larvae are generalized, obligate carnivores, which feed on almost any kind of animals which they can perceive and which are of an appropriate size (Corbet, 1962). Nymphs are categorised into three groups, according to their behaviour: climbers, sprawlers, or burrowers. Nymphs of darners are climbers and climb in and out of submerged weed beds. Sprawlers usually have flattened bodies and lie flat on the mud with legs outstretched. Burrowers live shallowly buried in the silt and sand with the upturned tip of the abdomen reaching up to the water for respiration. The burrowers have nearly cylindrical bodies and legs with stout modifications for burrowing. Burrowers include the nymphs of dragonflies such as club-tails. Only the crawlers and burrowers occur in rapidly flowing waters. Some burrowers use the crevices of stones for shelter (Venable, 2005).

Dragonfly larvae possess a highly specialised mouthpart, the labial mask, which can be shot out rapidly, grasping small prey animals with the hooks at the tip (Pritchard, 1965, cited in Miller, 1987). Dragonfly larvae detect prey by sight, by touch, or by both means. Larvae which live on the bottom of ponds, such as those from the family Libellulidae, have small eyes, long antennae and long legs covered in fine hairs (setae) covering the often flattened body. The long legs and flat body help prevent them from sinking into the mud. The setae act to clothe the insect in debris, helping to conceal it (Miller, 1987). The dragonfly and damselflies nymphs predate on mosquito larvae as a food and the adults dragonfly were attack adults mosquitoes efficiently (Kumar & Hwang, 2005).

When dragonflies are in the nymphal stage, they eat tiny water creatures such as microorganism as the nymphs grow, they eat water fleas, mosquito and mayfly larvae that live in the same habitat. As the nymph grows it will eat small fishes, tadpoles, water beetles and large worms. Dragonflies are definitely not harmful to humans. They do not bite or sting. They are very beneficial because of their feeding habit including exploiting the mosquitoes, flying ants, swarming termites, flies, gnats, and anything small enough for them to catch (Venable, 2005; Subramaniam, 2005).

Dragonfly larvae differ greatly from the adults. They do not share the bright coloring of their adult counterparts; instead, their drab colors camouflage them from predators. The larvae of most species are exclusively aquatic. The larvae of some species actively stalk their prey, whereas others lay in wait for the arrival of their next meal (Keller *et al.* 2007).

Prey is always detected at a short distance, not exceeding the length of the larva itself. The progressive increase in importance of the eyes might be expected to have affected the diurnal rhythm of feeding activity. Thus it appears to have done by determining the kind of feeding behavior which takes place during daylight, rather than by restricting the activity to that time (Corbet, 1962). To feed, dragonfly larvae use a modification of the lower lip (the labium). The labium has a pair of spines at the tip and it is hinged at the base so it can be withdrawn under the head. When the larva is within range of prey it is shot out at high speed and the prey is impaled on the spines. The labium is then retracted to below the mouth and the prey can be devoure (Miller, 1987).

During the daytime a larva usually remains immobile until it perceives a moving organism. After this, its feeding behaviour may be said to consist of three phases (Koehler, 1924 cited in Corbet, 1962). First, it orientates itself correctly to the organism, sometimes by walking slowly towards it; second, it ejects the labium and grasps the prey; and third, it uses the mandibles to masticate and ingest the prey. It is consistent with their habit of remaining still and awaiting the arrival of their prey, that larvae should be able to withstand long periods without food, and it has been noted that two species of Australian Anisoptera were able to survive starvation for at least three and eight months, respectively (Tillyard, 1910 cited in Corbet, 1962).

d) Applied research (Dragonfly as biocontrol agent)

Dragonfly nymph was used as biocontrol agents to control of many species mosquito larvae (Figure 2.6). In any ecosystem the dragonflies are one of the dominant invertebrate predators. Both adults and larval stages are predators to other preys and they play a significant role in the food chain of ecosystem (Vashishth *et al*. 2002) also they act as bioindicator for the quality of biotope (Subramaniam, 2005). In review papers of aquatic predator Kumar and Hwang (2006) indicated that the nymphs of dragonfly and damselflies are predators of mosquito larvae. The use of dragonflies as potential biological control against malaria and other insect borne diseases has rarely been studied (Chandra, 2007).

The successful story about dragonfly as biocontrol agent was reported by Sebatian *et al*. (1990) in Myanmar. They use augmentative release (AR), an approach which is entails prior estimation of the number of natural enemies needed (within given area and a given time) to achieve suppression to the required level and then releasing sufficient numbers into closed environment. In the experiment in Myanmar the larvae of *C. servilia* were used as predator against *Ae. aegypti* larvae in water containers. This field experiment, after 6 weeks the density of prey was reduced at lower level. The releases of dragonfly nymph were carried out during the monsoon season which is the time when the Dengue fever was transmitted. Dragonfly nymphs of *Brachytron pratense* proved to be an effective predator against larvae different mosquito species under laboratory conditions and fields (Chandra, *et al*. 2006). In another study done by Mandal *et al*. (2008) it is indicated that the different Odonate species consume different number of larvae of *Cx. quinquefasciatus* under laboratory conditions. Odonata nymphs as biocontrol agents use for control of mosquito species (Table 2.6).

Dragonflies are sometimes called “mosquito hawks” because they catch and eat high number of mosquitoes. In contrast studies done by Breene *et al*. (1990) it wasfound that there were no mosquito larvae in the gut of the damselfly larvae (*Enallagma civile*). Their analysis revealed that the larvae preyed upon chironomid larvae, and they also found corixids, cladocerans, ostracods, and aquatic mites. No remains of mosquito larvae were detected in any of the specimens, even though mosquito larvae (*Aedes*, *Culex*, *Culiseta*, *Mansonia*, and *Psorophora*) were observed in the pond where the damselfly larvae were collected.

Despite the preference of several species for diffuse light or shade, Odonata are essentially lovers of sunshine. Odonata, being cold-blooded creatures, mostly only appear when the sun is shining. Warm sunny days will bring forth many species over almost any kind of water and there will be plenty to observe as they couple, mate and oviposit. Generally speaking Odonata are late riser and early retire but there are a number of crepuscular species, for example all members of *Gynacantha* and their closest relatives fly well after dusk and again before sun rise. Some species which take to the wing only after dark or at dusk live entirely on mosquitoes: proving a real boon to those living in malaria areas (Silsby, 2001).

Table 2.6 Summary of reports on the use of Odonata nymphs as biocontrol agents for mosquito species

No.	Biocontrol agents	Prey	Country / References
1.	*Mesogomphus lineatus*	*Cx. fatigans* larvae	India (Mathavan, 1976)
2.	*Mesogomphus lineatus*	*Cx. fatigans* larvae	India (Pandian, *et al*. 1979)
3.	*Pantala flaviscens* and *Tramea abdominalis*	*Cx. quinquefasciatus*	Brazil (Santamarina & Mijares, 1986)
4.	*Sympetrum frequens*	*Anopheles sinsensis*	(Urabe *et al*. 1986)
5.	*Bradinopyga jaminata* and *Brachythemis contaminata*	Mosquito larvae	(Thomas *et al.* 1988)
6.	*Crocothemis servilia* (Drury)	*Aedes aegypti* larvae	Myanmar (Sebastian, 1990)
7.	*Pantala hymenaea*	*Cx. quinquefasciatus* larvae and midge *Chironomus plumosus* (L.)	Mexico (Quiroz-Martinez, *et al*. 2005)
8.	Odonate nymphs (*Brachytron pratense* nymphs)	*Anopheles subpictus* larvae	India (Chandra, *et al*. 2006)
9.	Odonate nymphs (Dragonfly/damselfly nymphs) 1 species of dragonflies nymph (*Aeshna flavitrons* and *Sympetrum durum*) 2 species of damselfly nymph (*Coenagrion kashmirum, Ischnura forcipata* and *Rhincocypha ignipennis*)	4th instars *Cx. quinquefasciatus* larvae	India (Mandal, *et al*. 2008)
10.	*Ceriagrion coromandelianum* and *Brachydiplax chalybea chalybea*	4th instars *Cx. quinquefasciatus* larvae	India (Saha, *et al*. 2012)
11.	*Pyrrhosoma sp.* (nymphal Damselfly)	*Ae. aegypti* larvae	India (Midhun, & Dhanakkodi, 2013).
12.	*Urothemis signata signata* (Rambur)	*Culex* larvae	India (Kumari & Nair, 1983)

2.9 Other Biocontrol Agents of Mosquitoes

2.9.1 *Toxorhynchites* Larvae

Mosquitoes in the genus *Toxorhynchites* (Theobald), commonly referred to as ‘‘Tox,’’ are predacious as larvae on mosquitoes and other aquatic organisms that inhabit natural and artificial containers, e.g., tree holes, leaf axils, discarded tires, drums, plastic buckets, cisterns and boat hulls. As adults, they feed on nectar rather than blood. Toxorhynchites have been investigated periodically since the late 1930s as a potential alterative control method for mosquitoes found in this habitat (Schreiber, 2007).

Toxorhynchites is the sole genus in the tribe *Toxorhynchitni*, and its distribution is almost entirely tropical or subtropical (Table 2.7). Without known exception, *Toxorhynchites* larvae are obligate predators. The adult females of all species feed only on nectar and other sugar-containing fluids and are autogenous. The third and fourth instar larvae of a number of *Toxorhynchites* species feed on *Toxorhynchites* eggs floating on the water surface (Clement, 1999). All the instars of *Toxorhynchites* spp. are predacious as larvae on mosquitoes and other aquatic organisms. They are found in both natural habitats and artificial containers. Feeding rates and total prey consumption during larval development depend on a number of abiotic (water temperature and light level) and biotic (prey size and prey type) factors (Schreiber, 2007).

The use of *Toxorhynchites splendens* as a biocontrol of mosquito is well documented (Aditya *et al*. 2006; Aditya *et al*. 2007). These have been introduced into container habitats in certain areas in Fiji, Samao and Hawaii to control larvae of other container-breeding mosquitoes but the results obtained have not been very encouraging (Service, 2000). According to Nyamah *et al*. (2011) *Tx. splendens* was observed to co-exist with larvae of *Ae. albopictus* and *Cx. fuscocephala* in the ovitraps. They suggested that the

Tx. splendens larvae is a good biocontrol agent in control of mosquito populations as *Tx. splendens* larvae are environmentally friendly and attack larval stages. In Singapore as reported by Chan, (1968) three prey species were found with *Tx. splendens* larvae such as *Ae. albopictus*, *Culex* spp. and chironomids. The normal prey for *Tx. splendens*is is *Ae. albopictus* larvae. *Tx. splendens* larvae are more effective in the control of *Ae. albopictus* in rural areas than *Ae. aegypti* which are found in urban settings. It is because *Tx. splendens* larvae are rarely found in populated areas which are in urban areas. They also depend on nectar of flowers thus areas with vegetation are their preferences habitat.

Table 2.7 Summary of reports on the use of *Toxorhynchites splendens* as biocontrol agents for mosquito species

No.	Biocontrol agents	Prey	Country / Reference
1.	*Toxorhynchites splendens*	*Armigeres subalbatus and Cx. quinquefasciatus* larvae	India (Aditya *et al*. 2007)
2.	*Rhantus sikkimensis* and larvae of *Toxorhynchites splendens*	4th instars *Cx. quinquefasciatus* larvae	India (Aditya *et al*. 2006)
3.	*Toxorhynchites splendens*	*Ae. albopictus* larvae	Malaysia (Nyamah *et al*. 2011)
4.	*Toxorhynchites rutilus*	Mosquito larvae	India (Sahib, 2011)
5.	*Toxorhynchites splendens*	*Ae. albopictus* and *Ae. aegypti* larvae	Singapore (Chan, 1968)
6.	*Toxorhynchites rutilus*	*Ae. aegypti* larvae	USA (Lounibos *et al*. 1998)
7.	*Toxorhynchites splendens*	*Ae. aegypti , Ar. subalbatus, An. stephensi* and *Cx. quinquefasciatus* larvae	India (Pramanik & Raut, 2003)
8.	*Toxorhynchites violaceus*	*Ae. aegypti* larvae	Brazil (Albeny *et al*. 2011)
9.	*Toxorhynchites amboinensis*	*Ae. aegypti* larvae	Indonesia (Annis *et al*. 1990)
10.	*Toxorhynchites amboinensis*	*Ae. polynesiensis* larvae	French Polynesia (Mercer *et al*. 2005)
11.	*Toxorhynchites brevipalpis*	*Ae. aegypti* larvae	Tanzania (Trpis *et al.* 1973)

2.9.2 *BTI*

Bti (*Bacillus thuringiensis israelensis*) was commoly used and applied in control of mosquito larvae and recently, *B. sphaericus* larvicide has been successfully applied in various mosquito control (Table 2.8). The used of Bti (*Bacillus thuringiensis israelensis*) against *Ae. aegypti* in earthen jar containing landscaping aquatic plant showed that container with aquatic plants for landscaping should be treated more frequently than container without aquatic plant. The mortality ranged from 77.34% -100% for jars with aquatic plants and 80.66%-100% for jars without aquatic plants (Chen *et al*. 2009).

A new variety- serotype H-14 is particularly active against mosquito and black fly larvae. It is most active against *Aedes*, *Culex*, and *Psorophora* spp., and slightly less so against *Anopheles*. *Bt H-14,* which is commercially available under a number of trade names, is a proven, environmentally-nonintrusive mosquito larvicide. It is entirely safe for humans when the larvicide is used in drinking water in normal dosages. *Bt. H-14* formulations tend to rapidly settle at the bottom of water containers, and frequent applications are therefore required. The toxin crystal is formed alongside the spore. Larval enzymes digest the crystal, releasing the toxin within seconds of ingestion, and larvae are killed within hours of ingesting a lethal dose (WHO, 1982). The mosquito indices of BI, CI and HI decreased gradually after application of Bti H-14 at rural areas in Thailand. It shows that the Bti is most effective in control of mosquito larval populations in water jars (water container) which is the main positive breeding site for mosquito larvae (Phan-Urai *et al*. 1995).

Table 2.8 Summary of reports on the use of *Bacillus thuringiensis israelensis* (Bti) as biocontrol agents for mosquito species

No.	Biocontrol agents	Prey	Country / Reference
1.	*Bacillus thuringiensis israelensis* (Bti)	*Ae. aegypti* larvae	Malaysia (Chen *et al*. 2009)
2.	*Bacillus thuringiensis israelensis* (Bti)	*Cx. saltanesis* larvae	Brazil (Zequi & Lopes, 2007)
3.	*Bacillus thuringiensis israelensis* (Bti) and *Mesocyclops thermocyclopoides*	*Ae. aegypti* larvae	Thailand (Kittayapong *et al*. 2006)
4.	*Bacillus thuringiensis israelensis* (Bti)	*Ae. aegypti* larvae	Thailand (Phan-Urai *et al*. 1995)
5.	*Bacillus sphaericus* strain 2362	*Cx. quinquefasciatus* larvae	Thailand (Mulla *et al.* 2001)
6.	*Bacillus sphaericus*	*Culex pipiens* larvae	Israel (Uspensky *et al.* 1998)

2.9.3 Copepoda

In Vietnam the Copepoda, *Mesocyclops* were successful in the control of larval *Ae. aegypti* where it reduced the number of mosquito population in containers (Nam *et al*. 1998) and larval *An. albimanus* and in term of costing the use of *Mesocyclops* as predator is inexpensive and easy to transport (Marten *et al*. 1989). Marten (1990) in his study introduced *Macrocyclops albidus* in tire piles that contained *Ae. albopictus* larvae, as a results it reduced the population *Ae. albopictus* larvae and *Mesocyclops longisetus* was also used to control *Ae. albopictus* larvae in tires (Luciana *et al*. 1996).

The field trial of application of *Mesocyclops* species has also been done in many habitats such as tires, temporary pools, marshes, rice fields, residential roadside ditches and domestic containers. From the results different species of Cyclopoid can eliminate or effective against different types of mosquito species which are in suitable habitat. For instance *Mesocyclops longisetus* can effecitively eliminate mosquito larvae of *Ae. aegypti* in cisterns, 55-gallon drums and domestic container. They also suggest that 2 species of

Mesocyclops longisetus and *Macrocyclops albidus* could be of use to control larvae *Anopheles* spp. and *Cx. quinquefasciatus* (Marten, *et al*. 1994b). Cyclopoid will survive well in two conditions (i) if they get enough food supply and (ii) need proper habitat which is near vegetation with no direct sunlight (Jorge, *et al*. 2004; Marten, *et al*. 1994b). Many species of Cyclopoid have been proven as one of the biocontrol agents of mosquito (Table 2.9).

Table 2.9 Summary of reports on the use of *Cyclopoid* as biocontrol agents for mosquito species

No.	Biocontrol agents	Prey	Country / References
1.	*Mesocyclops* (Copepoda:Cyclopoida)	*An. albimanus* larvae	Colombia (Marten *et al*. 1989)
2.	*Acanthocyclops vernalis*, *Diacyclops navus*, *Macrocyclops albidus*, *Mesocyclops edax*, *Mesocyclops longisetus*, and *Mesocyclops* sp. (Cyclops)	*Ae. albopictus* larvae	New Orleans (Marten *et al*. 1989)
3.	*Mesocyclops longisetus* and *Macrocyclops albidus*	*Anopheles* spp. and *Cx. quinquefasciatus* larvae	New Orleans (Marten *et al*. 1994a)
4.	*Mesocyclops longisetus*, *Mesocyclops thermocyclopoides*, *Mesocyclops venezolanus* and *Macrocyclops albidus*	*Ae. aegypti* larvae	New Orleans (Marten *et al*. 1994a)
5.	*Mesocyclops longisetus* and *Macrocyclops albidus*	*Cx. pipiens* larvae	Uruguay (Maite *et al*. 2008)
6.	*Mesocyclops* (Copepoda:Cyclopoida)	*Ae. aegypti* larvae	Vietnam (Vu *et al*. 1998)
7.	*Macrocyclops albibus* (Copepoda,Cyclopidae)	*Ae. albopictus* larvae	New Orleans (Marten 1990b)
8.	*Mesocyclops thermocyclopoides* (Copepoda:Cyclopoida)	*Cx. quinquefasciatus* and *An. stephensi* larvae Alternate prey – *Moina macrocopa* and *Ceriodaphnia cornuta*)	Delhi, India (Kumar & Rao, 2003)
9.	*Mesocyclops aspericornis*	*Ae. aegypti* larvae	India (Ramanibai & Kanniga, 2008)
10.	*Mesocyclops longisetus*	*Ae. albopictus* larvae	Brazil (Santos *et al*. 1996)
11.	*Macrocyclops albibus*	*Ae. albopictus* and *Ae. aegypti* larvae	USA (Rey *et al*. 2004)
12.	*Mesocyclops longisetus*	*Ae. albopictus*, *Ae. triseriatus* and *Cx. quinquefasciatus* larvae	USA (Soumare & Cilek , 2011)
13.	*Mesocyclops aspericornis*, *Mesocyclops thermocyclopoides* and *Mesocyclops woutersi*	*Ae. albopictus* and *Ae. aegypti* larvae	Vietnam (Kay *et al*. 2002)

14.	*Mesocyclops brevisetosus*	*Ae. aegypti, Cx. quinquefaciatus*, and *An. farauti*	Indonesia (Yoyo *et al*. 2006)
15.	*Mesocyclops longisetus* (Copepoda)	*Ae. albopictus* and *Cx. quinquefasciatus* larvae	USA (Soumare *et al.* 2004)
16.	*Acanthocyclops robustus, Diacyclops uruguayensis, Macrocyclops albidus andMesocyclops longisetus*	*Ae. aegypti* and *Cx. pipiens*	Argentina (Tranchida *et al.* 2009)

2.9.4 Backswimmer

Backswimmer is one of the predators that were used to control mosquito larvae (Figure 2.10). The backswimmers, *Notonecta undulata*; (Hemiptera: Notonectidae) had been used against the larvae of *Anopheles quadrimaculatus* and greatly reduced the survivorship of larvae and the number of mosquito larvae (Knight *et al*. 2004). A study on the predatory effect of backswimmer *Anisops sardea*, on oviposition habitat selection of mosquitoes and other dipterans have been carried out and the results showed that certain mosquito species try to avoid *Anisops* pools when ovipositing (Eitam, *et al*. 2002). In contrast study reported by Zuharah and Lester (2010) where mosquito larvae ignore the presence of *Anisops* in the same habitats. They concluded that the mosquito larvae had no ability to detect the presence of predators, or perhaps the cues from *Anisops* predators were not sufficiently strong enough to alarm these mosquitoes. Besides mosquitoes backswimmers also prefer other preys which are *Daphnia*, *Ceriodaphnia* cladocerans, copepods and rotifers (Gilbert, *et al*. 1983).

Table 2.10 Summary of reports on the use of backswimmer as biocontrol agents for mosquito species

No.	Biocontrol agents	Prey	Country / Reference
1.	Backswimmer (Notonecta undulate; Hemiptera:Notonectidae)	*An. quadrimaculatus* larvae	USA (Knight *et al*. 2004)
2.	*Anisops wakefieldi*	Cladocerans, copepods and rotifer	USA (Gilbert & Burns, 1999)
3.	*Anisops wakefieldi*	*Cx. pervigilans* larvae	New Zealand (Zuharah & Lester, 2010)
4.	*Notonecta hoffmani*	*Cx. pipiens* larvae	USA (Scott & Murdoch, 1983)

2.9.5 Frog

Other predators of mosquito larvae and pupae include tadpoles of frogs and toads and various aquatic insect larvae, but these have been rarely proved to be effective as control agents. New finding by Bowatte *et al*. (2013) reported that different species of tadpoles of four species of randomly selected genera *Bufo, Ramanella, Euphlyctis* and *Hoplobatrachus* predate on *Ae. aegypti* (vector mosquito of dengue virus) eggs.

2.9.6 Water Bugs & Beetles

Others predators that are used as potential biocontrol of mosquitoes are water bugs, beetle (Table 2.11), flatworm and planaria (Table 2.12). *Acilius sulcatus* (Coleoptera: Dytiscidae) was used to control *Cx. quinquefasciatus* larvae (Chandra *et al*. 2008). The used water bugs *Sphaerodema annulatum* predate on *Ar. subalbatus* (Aditya *et al*. 2005) and *Cx. quinquefasciatus* (Aditya *et al*. 2004) was also carried out under experiment condition. Ohba and Takagi (2010) assessed the predatory ability of adult Japanese diving beetles on 4^{th} instars of *Cx. tritaeniorhynchus* which is one of the principle vectors of Japanese encephalitis under laboratory conditions. Other biocontrol agents that were used to control mosquito larvae such as planaria (Legner, *et al*. 1975), flatworm (Tranchida, *et al*. 2009), turtle (Marten, 2007), wolf spider as predator against *Anopheles gambiae* (Futami *et al.* 2008), waterboatmen (*Micronecta grisea*) (Amrapala, *et al.* 2009) and aquatic insects of *Gyrinus natator*, *Nepa cinerea* and *Cybister tripunctatus* (Mohanraj *et al.* 2012).

Table 2.11 Summary of reports on the use of beetle and water bugs as biocontrol agents for mosquito species

No.	Biocontrol agents	Prey	Country / Reference
1.	*Diplonychus sp*. and *Anisops sp*.	*Cx. annulirostris* larvae	Australia (Shaalan *et al*. 2007)
2.	*Acilius sulcatus* (Coleoptera: Dytiscidae)	4th instars *Cx. quinquefasciatus* larvae	India (Chandra *et al*. 2008)
3.	Adult Japanese diving beetles	*Cx. tritaeniorhynchus* larvae	Japan (Ohba & Takagi, 2010)
4.	(Agabus; Coleoptera: Dytiscidae) - *Agabus punctatus* and *Agabus disintegrates*	Mosquito larvae, copepods and ostracods	USA (Culler & Lamp, 2009)
5.	Heteropteran water bug Diplonychus (*D. Annulatus, D. Rusticus* and *Anisops bouvieri*)	*Cx. quinquefasciatus* larvae	Kolkata, India (Saha *et al*. 2008)
6.	Heteropteran water bug Diplonychus(*D. Annulatus, D. Rusticus* and *Anisops bouvieri*)	a) 2nd instar and 4th instar *of Cx. quinquefasciatus* larvae b) 2nd instar and 4th instar of chironomid	Kolkata, India (Saha *et al*. 2010)
7.	Diplonychus indicus (Hemiptera: Belostomatidae)	*Ae. aegypti* larvae	India (Sivagnaname, 2009)
8.	Water bug *Sphaerodema annulatum*	*Cx. quinquefasciatus* larvae	India (Aditya *et al*. 2004)
9.	Water bug *Sphaerodema annulatum*	*Ar. subalbatus* larvae	India (Aditya *et al*. 2005)
10.	Water bug *Laccotrephes griseus*	*Cx. quinquefasciatus* larvae	India (Ghosh & Chandra, 2011)
11.	Hemiptera (*Gerridae Hydrometridae, Veliidae* and *Notonectidae*) and Coleoptera (*Dytiscidae*)	*An. gambiae s.l.* and *An. funestus*	Kenya (Muiruri *et al.* 2013)

Table 2.12 Summary of reports on the use of Flatworm/ Planaria as biocontrol agents for mosquito species

No.	Biocontrol agents	Prey	Country / Reference
1.	Flatworm species (Platyhelminthes:Turbellaria)	*Ae. aegypti* and *Cx. pipiens* larvae	Argentina (Tranchida *et al*. 2009)
2.	Planaria (*Dugesia bengalensis*)	*Anopheles and Cx.* larvae	India (Kar & Aditya 2003)
3.	Planaria (*Dugesia bengalensis*)	*Culex* larvae and chironomid midge	USA (Legner, *et al*. 1975)
4.	Planaria (*Dugesia Tigrina*)	*Ae. albopictus* and *Cx. quinquefasciatus*	Brazil (Melo & Andrade, 2001)

2.10 Factors Affecting Predation Activities

Study conducted by Saha *et al.* (2007) showed that the predation activities of bugs were depending upon the prey and predator densities. As results from their study, the number of prey consumed varied significantly between prey and predator densities indicating their capability to consume more prey at higher density. Ghosh *et al.* (2004) revealed that the significance of predatory efficacy with reference to prey density and water volume (search area). Okorie and Abiodun (2010) in their study on potential of larvivorous fish against mosquito larvae found that size of predator, prey densities and time (light on and light off) were affecting predation activities of predator. In India, Ghosh *et al.* (2005) reported that predatory efficacy was positively related with prey density and inversely related with water volume (search area).

Prey species is one of the factors that are affecting predation activities. Soumare and Cilek (2011) found that *Mesocyclops longisetus* appeared to preferably prey on *Aedes* larvae compared with *Culex.* This situation happens due to less contact between *Mesocyclops longisetus* and *Culex* larvae. As *Mesocyclops longisetus* spend much of its time at the bottom of the container where *Culex* spends time on water surface. Wijesinghe *et al.* (2009) reported the larvivorous fish consumed *Aedes* species greater than *Toxorhynchites* larvae. Besides prey species, predator species is one of the factors that affect predation activities (Cavalcanti *et al.* 2007). Kweka *et al.* (2011) stated that predator species had a significant impact on the predation rate in the 24 hour evaluations of fish towards mosquito larvae.

Size of the prey was also a factor affecting predation activities. Different predator species preferred different size of prey. For example *Anisops*, preferred small size prey. In contrast *Diplonyvhus annulatus* preferred large size of mosquito larvae (Saha *et al.* 2010). The work of Willems *et al.* (2005) points out that prey densities and vegetation densities were affecting predation activites however prey size (larval instars) was no significant difference in affecting predation as fish consumed all types of mosquito instars. In contrast study by Shaalan *et al.* (2007) and Marti *et al.* (2006) as the prey stages were affecting the predation activities.

Most of the researchers had reported many factors affecting the predation activities. The followings are the factors that affecting the predation activities:

1) body size predator/energy (ability to kill more preys), prey densities and number of predator (Aditya *et al.* 2006)

2) water volume (foraging area), aquatic vegetation and prey size (Shaalan *et al.* 2007)

3) predator ability, time, number of predator, prey densities and water volume(search area) (Mandal *et al*. 2008)

4) water volume(searching area), number of predator and prey densities (Chandra *et al*. 2006)

5) water volume(searching area), number of predator and prey densities (Chandra *et al*. 2008)

6) age of predator, prey densities and prey species (Aditya *et al*. 2007)

7) behaviour of prey species (Kar & Aditya 2003)

8) body size of predator and behaviour of predator how they captured the prey (Ohba & Takagi, 2010)

9) Sexes of predator (Chang *et al*. 2008)

10) Body size of predator fish, prey species and number of predator (Manna *et al*. 2008).

11) Prey stages (instars), and body size of predators (Tranchida *et al*. 2009).

12) Prey size or instars, predator species, vegetation, and prey densities (Willems *et al*. 2005).

13) Prey densities, water volume, and predator species (Ghosh *et al*. 2005).

14) Prey densities, predator species and behaviour (searching ability) (Marti *et al*. 2006).

15) Prey species and predator species (Culler & Lamp, 2009).

16) Prey species, number of predator and prey densities (Anogwih & Makanjuola, 2010).

17) Prey behaviour both activity and position of mosquito larvae (Juliano & Reminger, 1992; Juliano *et al.* 1993; Yee *et al.* 2004; Kesavaraju *et al.* 2007)

18) Behavioral responses to water-borne cues (Kesavaraju & Juliano, 2004; Kesavaraju & Juliano, 2008; Kesavaraju *et al.* 2008; Kesavaraju *et al.* 2011)

CHAPTER 3

MATERIALS AND METHODS

3.1 Background of Study Location

The study locations are the residential areas of Precinct 9, Precinct 11, and Precinct 16 Putrajaya which are categorised as the urban areas and Kuala Selangor as the suburban areas. The selection of this areas based on the high incidence of dengue cases as stated in Putrajaya Health Office annual report and Kuala Selangor Health Office annual report for the last 4 years beginning in 2006.

Precinct 9 is located on the western edge of Putrajaya at 2 56’ N, 101 40’ E and with a total site area of 466.4 acres, it is one of the bigger precinct in Putrajaya (Figure 3.1). The main character of the precinct is defined by the high- rise high density residential blocks. These blocks, up to 15 storeys high, are laid out to form a line of towers that defines the western edge of Putrajaya. Figure 3.1 shows the land use distribution in Precinct 9, Putrajaya. The total site area of this precinct is 466.4 acres. At 269.5 acres or 58 % of the total site area, residential areas form the single largest land use component within the precinct. This is followed by open space at 20% and public facilities at 8%. There is a small neighbourhood commercial component of 3 acres which amount to less than 1 % of the total site area. Utilities and infrastructure take up the rest (13%).

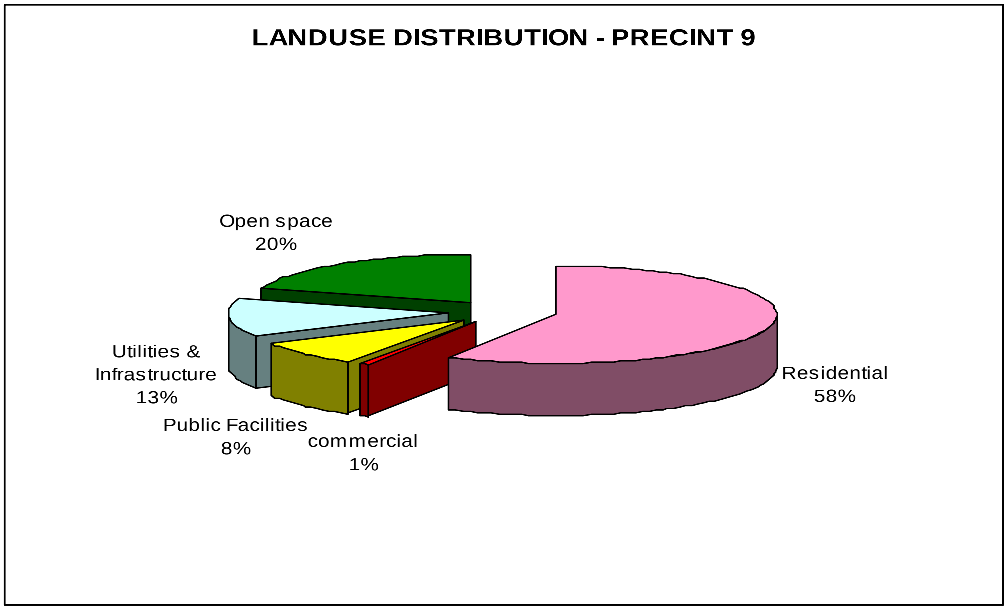


Figure 3.1 **Land use Distribution Precinct 9, Putrajaya, Perbadanan Putrajaya, (1997)**

Precinct 11 is located at the north-west corner of Putrajaya at 2 57’ N, 101 40’ 35.07” E and with a site area of 1049 acres, it is the largest precinct in the Periphery (Figure 3.2). It is planned as a wholly medium density residential precinct and is well serviced by roads and public facilities. Figure 3.2 shows the land use distribution in Precint 11, Putrajaya. The total site area is 1049 acres which, 44% or 463.4 acres are residential areas. Open space is the next largest component with 20.4% of the total site area. Public facilities take up 19.8%, while utilities and infrastructure take up 10%. A military camp of 53.7 acres and commercial areas totalling 3 acres, accounts for 5.1 % and less than 1% of the total site area respectively. Apart from the medium density residential developments of up to 6 storeys high, other major development components include a Health Centre, a School Complex, a Post Office, a Police Station, a Mosque, a Surau, another religious facility, a Branch Library, a Public Market, a Multi-Purpose Community Hall, a Putrajaya Service Centre, a Golf Course, an Area for Service Industries and a Bus Depot.

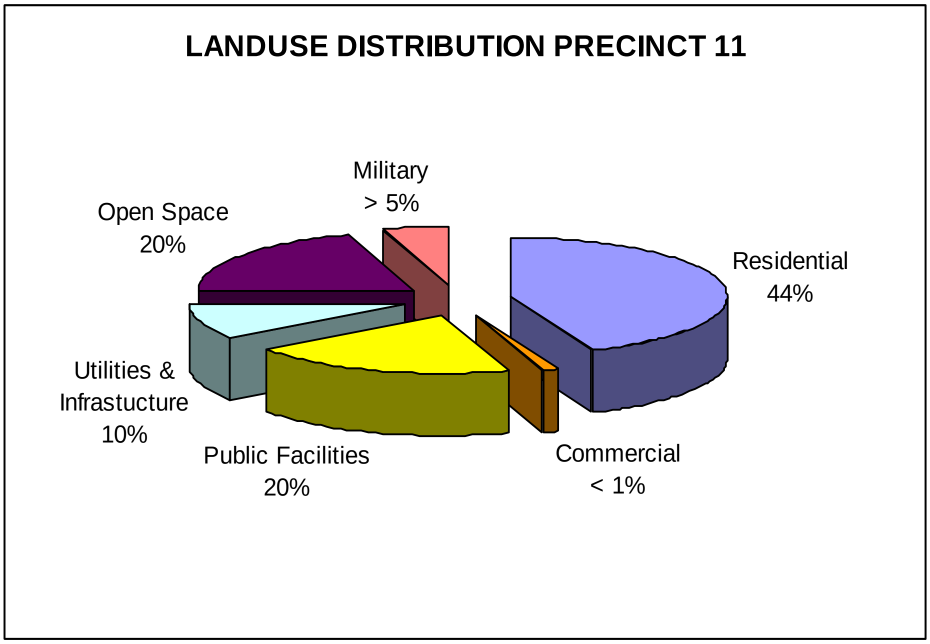


Figure 3.2 Land use Distribution Precinct 11, Putrajaya, Perbadanan Putrajaya, (1997)

Precinct 16 is located close to the northern Core Area precincts 2 55' N, 101 42'18.62" E and with a total site area of 384.1 acre, it is a medium-sized precinct in Putrajaya (Figure 3.3). Planned as a Special Precinct to accommodate the official residence of the Deputy Prime Minister, its major characteristic is its proximity to the Government Precinct and the rest of the Core Area. Consisting of medium and medium-high density housing, the character of the precinct is defined by these housing of up to 12 storeys high. Figure 3.3 shows the land use distribution in Precint 11, Putrajaya. The total site area within this Precinct is 384.1 acres. Of this, residential areas make up the largest land use component at 48% of the total site area, which includes 31.5 acres or 8% for the Official Residence of the Deputy Prime Minister. Open space makes up the second largest land use component at 27%. Public facilities account for 7.8% while the rest is taken up by utilities and infrastructure at 16%. There is a small neighbourhood commercial area of 4 acres, which is about 1% of the total site area. The main development components are the residential buildings. Of up to 12 storeys high, these medium density houses are complemented by a various public facilities. These include a School Complex, a Post Office, a Mosque, an Other Religious Facility, a Branch Library, a Public Market, a Community Hall and an Information Centre.

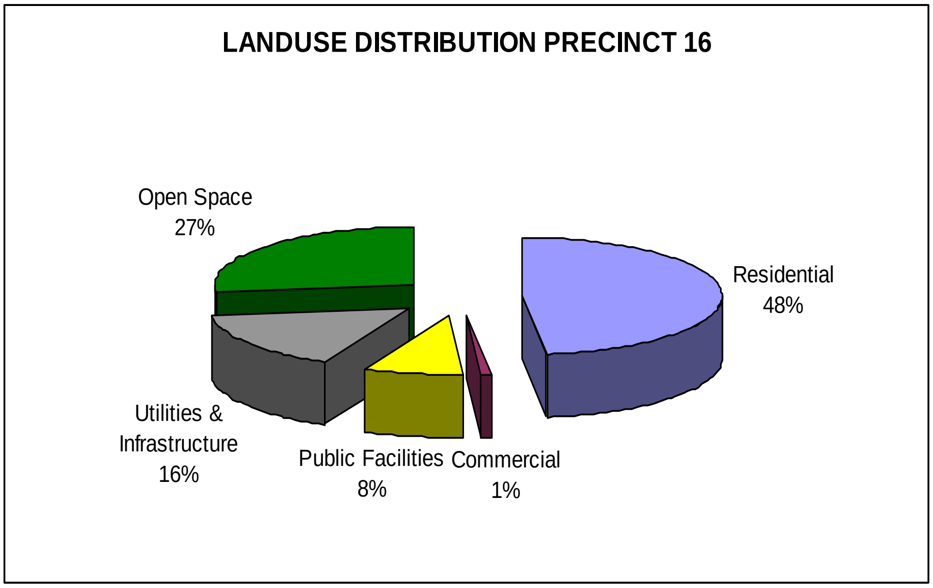


Figure 3.3 Land use Distribution Precinct 16, Putrajaya, Perbadanan Putrajaya, (1997)

Kuala Selangor is the second largest district in the State of Selangor and it is rich in historical relics. Economic resources in Kuala Selangor of the residents are in the fields of agriculture, rearing of livestock, service sector, manufacturing as well as tourism. Kuala Selangor is situated at 67 km southeast of Kuala Lumpur. The study areas are Seri Pagi (Saujana Utama), Kampung Bestari jaya (Mawar) and Kampung Bestari Jaya (Bunga Raya). Seri Pagi community was located in Bandar Saujana Utama (BSU) near Sungai Buloh. This area about 1000 acres was developed by Glomac Berhad a real estate company in Malaysia, since 1998. After more than ten years of development, estimated of populations Bandar Saujana Utama to more than 30,000 people.

Kampung Bestari Jaya (Mawar) and Kampung Bestari Jaya (Bunga Raya) are located in Batang Berjuntai, Kuala Selangor. In 2007, the name Batang Berjuntai was renamed “Bestari Jaya” by the government. This village is located 55 kilometres away from Kuala Lumpur, 30 kilometres away from Rawang, and 20 kilometres away from the town of Kuala Selangor. The population at Bestari Jaya is mainly made up of 3 races which are Malay, Indian and Chinese. Ninety percent of the residents are Malay, followed by Indians and Chinese. The residents of Bestari Jaya stay in houses along the roads, living on their own land or in housing estates. The types of houses that can be found are terrace, single-storey terrace, and shop houses.

3.2 Sampling

3.2.1 Sampling Population and Sampling Technique

Sampling population for mosquito larvae surveillance was mosquito density included larvae and also pupae. Sampling technique for mosquito larvae surveillance was systematic whereby every 3^{rd} house was inspected to detect mosquito breeding in the potential breeding sites. For sampling for potential biocontrol agents, natural predators such as dragonfly nymphs, damsefly and *P. reticulata* also known as guppy fish were searched in the potential breeding sites like concrete drains and small streams at residential areas in Putrajaya and Kuala Selangor. Disposable pipette was used for the sampling of mosquito larvae and other macro invertebrate and dip nets were use to sample natural predators. For ovitrap surveillance the sampling technique was systematic whereby every third house and an ovitrap was placed at outdoor areas at residential areas (Plate 3.1, Plate 3.2).

3.3 Relevant Data Collection

Data or document was collected from larval survey activities, and Annual report from Putrajaya Health Office and Kuala Selangor Health Office. One of the most important uses of documents is to corroborate evidence gathered from other sources. Data and documentary evidence act as a method to cross- validate information gathered from interview and observation given that sometimes what people say may be different from what people do. The environmental data such as temperature, relative humidity and rainfall were collected from Meteorology Department.

3.4 Field Survey

In this study sampling and surveys activity were carried out every month from January until December 2010 in the potential mosquito breeding sites at in six study location. The ovitrap surveillance was carried out from March 2010 until February 2011 at two selected residential areas in Putrajaya (Presinct 11A2) (urban area) and Kuala Selangor (Pasir Penambang) (suburban area).

3.4.1 Mosquito Larval Survey

Larval survey was carried out in the potential mosquito breeding sites in residential areas in Putrajaya and Kuala Selangor with the assistance from the Assistant Environmental Health Officer and the staffs of Vector control Unit from Putrajaya Health Office and Kuala Selangor Health Office. There were three persons involved in larval surveys which include two staffs from health office and myself. There are a total of 873 houses in Putrajaya and 600 houses in Kuala Selangor. The number of houses inspected during larval surveys was 262 houses 30% in Putrajaya [62 houses in Precinct 9, 108 houses in Precinct 11 and 92 houses in Precinct 16] and 180 houses were inspected 30% in Kuala Selangor from the total [90 houses in Seri Pagi, Saujana Utama, 45 houses in Kampung Bestari jaya (Mawar) and 45 houses in Kampung Bestari jaya (Bunga Raya)]. Every third house was inspected for mosquito larvae population.

All water-holding containers of a household were inspected for larvae or pupae. Detailed investigations with respect to larval densities and their preference towards breeding containers, in different environmental conditions were taken in all the study areas in Putrajaya and Kuala Selangor. Larval survey was carried out at outdoor areas only, not inspection in indoor area involved. During larval surveys the staff from health office were ask permission from the residents before searching and looking for the potential breeding habitats stated. Mosquito larvae were obtained and collected from the potential mosquito breeding sites and placed into the universal specimen bottles. These bottles were labelled with information of date, time, location of breeding habitats, and the collector name's.

3.4.2 Ovitrap Survey

The ovitrap consists of a 1 liter black plastic container filled with 300 ml of tap water. The paddle is made from wooden measuring 12cm x 2cm placed inside the container. The wooden paddle was wrapped with tissue paper. This method was used to identify eggs easily by letting the eggs hatched to larvae. "Ovitraps" are devices used to detect the presence of *Ae. aegypti* and *Ae. albopictus* where the population density is low and larval surveys are largely unproductive (e.g. when the Breteau index is less than 5), as well as under normal conditions. They are particularly useful for the early detection of new infestations in areas from which the mosquitoes have been previously eliminated.

The following guidelines should be observed before placing an ovitrap.

Adopted from Pratt & Jacob, 1967; Evan & Bevier, 1969):

i. Place an ovitrap at ground level, where it will not be disturbed by children or pets.
ii. Place a 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 place.
iv. Place a 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 an ovitrap where the mosquito can detect or see the trap
vii. Place a trap far from piles of tires because *Aedes* mosquito *Aedes albopictus* prefer tires over other containers

An ovitrap survey was conducted from March 2010 until February 2011 at the Presint 11A2 Putrajaya (urban area) and Pasir Penambang, Kuala Selangor (suburban area). The total number of ovitraps were placed in 30% of total number of houses in residential area which amounted to 50 ovitraps in Putrajaya and 70 ovitraps in (Pasir Penambang) Kuala Selangor. An ovitrap was placed outdoor in secure and cool shaded area (Plate 3.3). All the ovitraps were collected after 5 days and brought back to laboratory (Plate 3.4). The positive ovitraps with eggs were maintained and were allowed to hatch. All the larvae present in the positive ovitraps were identified and counted during the 3rd instars. The mosquito larvae were identified under microscope (model Leica 2000) in the laboratory using the guidelines set by Ministry of Health Malaysia (1986) and WHO, (2003b). After species identification, the specimens were preserved in 70% alcohol and kept in valve bottle. All specimens should have the ecological information associated with locality where it was found, collection date and the collector's name.

Plate 3.1 Ovitraps placed outdoor randomly **Plate 3.2 Ovitraps placed outdoor randomly**

Plate 3.3 Ovitraps placed outdoor **Plate 3.4 Ovitraps collected and placed in the lab**

3.4.3 Natural Predator Survey

The natural predator survey was carried out at potential breeding habitats near the residential areas. The potential biocontrol agents such as dragonfly nymphs, guppy fish, and other aquatic insects were caught using fine nets from the small streams, drainage locality, streams in oil palm plantations, marshes and concrete drains (Plate 3.5, Plate 3.6, Plate 3.7, Plate 3.8, and Plate 3.9).

The adults dragonflies were also collected in both study areas in suburban (Plate 3.10) and urban (Plate 3.11). For the adults sampling was done in open fields. The adult specimens were caught using aerial nets and were placed in triangular paper envelopes, preferably one specimen to an envelope. The locality data and collection date were written on the outside of envelope. The adults were keept in cool conditions to ensure they stay alive until time to preserve them. All the specimens of aquatic insects were brought back in the lab. Only guppy fish and dragonfly nymphs were used in behaviour study in the lab after the dominant species identified in both study areas. The dominant species of dragonfly nymphs was used in behaviour studies.

Plate 3.5 Sampling location in urban area (small stream)

Plate 3.6 Sampling location in urban area (drainage locality)

Plate 3.7 Sampling location in suburban area (Stream in oil palm plantation)

Plate 3.8 Sampling location in suburban (Marshes)

Plate 3.9 Sampling location in urban area (Concrete drain)

Plate 3.10 Sampling location in suburban area

Plate 3.11 Sampling activities in concrete drain urban area

3.5 Laboratory–Based Experiment

3.5.1 Identification of Larvae Mosquitoes

The identification of mosquito larvae was done with the help of compound microscope (model Leica 2000). In this study 1st and 2nd instars were calculated and discarded because immature at these stages could not be identified. Only 3rd and 4th instar of mosquito larvae were identified species. The key for identification purpose to species level was based on the guidelines produced by the Ministry of Health Malaysia (1986) entitled Guideline for Prevention and Control measure of Dengue Fever/ Dengue Haemorrahagic Fever and WHO, (2003b). The specimens were preserved in 70% alcohol stored in vials with information labels. The standard information contained were ecological information associated with locality where it was found, collection date and the collector's name. The taxonomic label includes species name, identification date and determiner's name. All collections will be deposited in the University of Malaya Zoological Museum (UMZM) and will be given the appropriate catalogue numbers.

3.5.2 Experimental Methods

Mosquito species of *Ae. albopictus, Ae. aegypti* and *Cx. quinquefasciatus* were collected from the laboratory colony at the Institute for Medical Research (IMR). The 4th instars of mosquito larvae were used in the experiments. Feeding efficacy of predator dragonfly nymphs of selected dominant species in the study areas, and guppies (*P. reticulata* both males and females) was carried out under laboratory conditions in 3 replicates for every predator chosen for the experimentation. The body lengths of guppies and dragonfly nymphs were measured by using digital calliper. The body weight of guppies and dragonfly nymphs were measured before and after the experiments by using electronic balance (MODEL BL-2200H).

The prey-predator relationships and feeding efficacy and other listed behavioural aspects that were scored are as follows:

- predator and prey escape strategies
- duration of first attack from the first introduction of predator
- predator preference

3.5.3 Prey – Predator Relationship by Using *Poecilia Reticulata* (Guppies)

The category for common predator that was used in this experiment was *P. reticulata* (guppies). These guppies were collected in the drainage systems of Putrajaya and Kuala Selangor. All fish were recorded for their wet weights and lengths before and after experiment. Before start of experiment all fish used were acclimatised to laboratory conditions and were placed in plastic aquarium L 22 cm x H 13 cm x W11 cm. Within one week prior to the actual date of experimentation, all fish were provided with blood worm and fish food as a diet. Guppies were starved for 24 hours before introduction to the experimental aquaria, as the hunger level of fish is 24 hours. The experimentation aquaria contained 1L of pond water for the feeding efficacy experimentation. For this experiment the daily feeding rate of guppies towards three species of mosquito larvae were recorded. The single fish of *Poecilia reticulata* was exposed to a total of 100 of 4^{th} instar larvae *Ae. albopictus, Ae. aegypti* and *Cx. quinquefasciatus.* Therefore, three aquaria were setup for every mosquito species and three replicates of experiments were done on separated days. The time of first attack of guppies against every mosquito larva was recorded and the daily feeding rate was recorded every 3 hour interval. The same mosquito larvae that were left uneaten at end of experiment and fish were not used in subsequent experiments. At every 3 hour interval, the water from experimentation aquaria was sieved and transferred to a white tray for counting the number of mosquito larvae not eaten to obtain the number of mosquitoes consumed by predator fish. After that the numbers of mosquito larvae consumed were replenished into the aquaria. The experiment was carried out within 24 hours from 05.00: 1700h for light on and 17:00-05:00h light off. From this setup the active periods of *P. reticulata* consuming mosquito larvae can also be determined, that is whether their active feeding times were during the day time or the night time.

The second experimental setup was to assess the relationship of feeding rate with the different water volumes contained in the aquaria, also with the number of predator and prey densities. In these experiments 4 aquaria were set up for every mosquito species. This experiment was also carried out with 3 replicates of experiment on separate dates. In one experiment 12 aquaria were setup were used, 4 aquarium for *Ae. albopictus* larvae, 4 aquaria for *Ae. aegypti* and another 4 for *Cx. quinquefasciatus*

1) Aquarium A Female fish (1× 1 ×100) – Single fish with 1L of water volume and 100 4^{th} instars of mosquito larvae
2) Aquarium B Female fish (1 × 2 ×100) - Single fish with 2L of water volume and 100 4^{th} instars of mosquito larvae
3) Aquarium C Female fish (2 × 1 × 100) – Two fishes with 1L of water volume and 100 4^{th} instars of mosquito larvae
4) Aquarium D Female fish (1 × 1 × 200) – Single fish with 1L of water volume and 200 4^{th} instars of mosquito larvae
5) Aquarium A Male fish (1 × 1 × 100) – Single fish with 1L of water volume and 100 4^{th} instars of mosquito larvae

6) Aquarium B Male fish ($1 \times 2 \times 100$) - Single fish with 2L of water volume and 100 4^{th} instars of mosquito larvae

7) Aquarium C Male fish ($2 \times 1 \times 100$) – Two fishes with 1L of water volume and 100 4^{th} instars of mosquito larvae

8) Aquarium D Male fish ($1 \times 1 \times 200$) – Single fish with 1L of water volume and 200 4^{th} instars of mosquito larvae

3.5.4 Prey – Predator Relationship by Using Dragonfly Nymph

The dragonfly nymphs species used in these experiments were *Orthetrum chrysis*, *Orthetrum sabina* and *Neurothemis fluctuans* which were the dominant species in both study areas. All the three species of dragonfly nymphs were measured for the body lengths of every single species used by using a digital calliper before and after experiments. The mosquito larvae and their predator dragonfly nymphs were being maintained in the laboratory separately. Three species of dragonfly nymphs were exposed with three species of mosquito larvae *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* in different aquaria. Before the experimentation the dragonfly nymphs were supplied with aquatic insect. Nine aquaria were used which contained pond water and were oxygenated using air pumps. Every aquarium was labelled with the name of predator and name of mosquito species. During the experiment three species of dragonfly nymphs *O.chrysis*, *O. sabina*, and *N. fluctuans* were allowed to feed on 100 4th instar mosquito larvae of *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus.*

The number of mosquito larvae consumed by the nymphs of dragonfly was counted every 3 hour interval for 24 hours. The duration of time taken (first attack) by each dragonfly nymph to attack or consumed mosquito larvae were recorded. The numbers of mosquito larvae ingested by the dragonfly nymphs were counted by pouring through a fine mesh sieve to collect all of the mosquito larvae and were transferred to a white pan for counting of the larvae not consumed. After each 3 hour interval, the aquaria were replenished with the number of larvae that were eaten, along with the same volume of water, to maintain the same prey density. This experiment was conducted three times on three separate days (n= 3) with the same number of nymph for accuracy. After 24 hours all remaining mosquito larvae and dragonfly were removed from the aquarium. These mosquito larvae and dragonfly nymphs were not used in subsequent experiment. The active period of dragonfly nymphs consuming mosquito larvae were assessed in this experiment by setup the time with 12 hour in day time and 12 hour in the night time. This experiment was conducted to see the prey-predation relationship. *Ae. albopictus, Ae. aegypti* and *Cx. quinquefasciatus* were used as prey for the dragonfly nymphs. This experiment conducted also provided data on the most preferred species by dragonfly nymphs, the active time for every predator and daily feeding rate.

In another experiment the aquaria were set up to assess the relationship of predation efficiency and other factors that influenced the predation activities. The 36 aquaria were set up with different predator and prey species. This experiment were repeated on 3 separate day

1) Aquarium A *Orthetrum chrysis*(1 × 1 × 100) – Single dragonfly nymph with 1L of water volume and 100 4th instars of mosquito larvae
2) Aquarium B *Orthetrum chrysis*(1 × 2 × 100) – Single dragonfly nymph with 2L of water volume and 100 4th instars of mosquito larvae
3) Aquarium C *Orthetrum chrysis*(2 × 1 × 100) – Two dragonfly nymph with 2L of water volume and 100 4th instars of mosquito larvae
4) Aquarium D *Orthetrum chrysis*(1 × 1 × 200) – Single dragonfly nymph with 2L of water volume and 200 4th instars of mosquito larvae
5) Aquarium E (1 × 1 × 100) *Orthetrum sabina*, – Single dragonfly nymph with 1L of water volume and 100 4th instars of mosquito larvae
6) Aquarium F (1 × 2 × 100) *Orthetrum sabina*, – Single dragonfly nymph with 2L of water volume and 100 4th instars of mosquito larvae

7) Aquarium G (2 × 1 × 100) *Orthetrum sabina,* – Two dragonfly nymph with 1L of water volume and 100 4th instars of mosquito larvae

8) Aquarium H (1 × 1 × 200) *Orthetrum sabina,* – Single dragonfly nymph with 1L of water volume and 200 4th instars of mosquito larvae

9) Aquarium I (1 × 1 × 100) *Neurothemis fluctuans* – Single dragonfly nymph with 1L of water volume and 100 4th instars of mosquito larvae

10) Aquarium J (1 × 2 × 100) *Neurothemis fluctuans* – Single dragonfly nymph with 2L of water volume and 100 4th instars of mosquito larvae

11) Aquarium K (2 × 1 × 100) *Neurothemis fluctuans* – Two dragonfly nymph with 1L of water volume and 100 4th instars of mosquito larvae

12) Aquarium L (1 × 1 × 200) *Neurothemis fluctuans* – Single dragonfly nymph with 1L of water volume and 200 4th instars of mosquito larvae

3.6 Secondary Data

Data for the number of mosquitoes borne diseases cases were obtained from Putrajaya Health Office and Kuala Selangor Health Office. The environmental data for environmental temperature, humidity and rainfall data were obtained from Malaysian Metrological Department (MMD) for the months of January 2010 until February 2011 in Putrajaya areas and from May 2010 to February 2011 in Kuala Selangor. This environmental parameter was used to determine relationship between ovitraps surveillance with the environment conditions. Temperature was measured in degrees Celsius and is defined as mean average of maximum and minimum temperature. Relative humidity is the average monthly humidity based on daily records and is expressed as the percentage. Rainfall, measured in millimeters, is the amount of rainfall in a month. The secondary data on medical examination for staff that handled with machine fogging and were involved in fogging activities also obtained from Ministry of Health (MOH). Data on chemical use in fogging activities were also obtained from MOH.

3.7 Questionnaire

Questionnaires were distributed among vector staff unit in Putrajaya Health Office and Kuala Selangor Health Office and also residents in both study locations (Appendix A). The questionnaire for staff was divided into four sections which included: 1) respondent profile 2) knowledge about prevention of Dengue Fever and insecticide use for mosquito control 3) knowledge about biological control and 4) knowledge about used of insecticide during fogging activities, sign and symptoms of insecticide exposure. Questionnaires for residents in urban and suburban areas were divided into three sections comprised of: 1) respondent profile 2) knowledge about prevention of Dengue Fever and insecticide use for mosquito control 3) knowledge about biological control. The questionnaires were prepared in both languages English and Bahasa Malaysia to ensure accuracy of understanding and comprehension among the respondents. The questionnaires were adapted from WHO (2009) field surveys of exposure to pesticides standard protocol with additions and modification to meet the objectives of this research project.

3.7.1 Pilot Test

The term 'pilot studies' refers to mini versions of a full-scale study (also called 'feasibility' studies), as well as the specific pre-testing of a particular research instrument such as a questionnaire or interview schedule (van Teijlingen, & Hundley, 2001). The pilot test was carried out in the same population but outside the areas of the study, in order to identify any problem in comprehension and obtained feedback on potential difficulties when answering the questions and filling the form. Thirty questionnaires were distributed in urban and suburban areas before the actual study was conducted. The participants were asked the same questions as the actual study participants. Baker found that a sample size of 10–20% of the sample size for the actual study is a reasonable number of participants to consider enrolling in a pilot study. Stoper (2012) also mentioned that the respondents for the pilot study should not less than 30.

3.7.2 Questionnaires Validification

Content validity of the questionnaire was ensured by issusing out to qualified persons and experts who are experienced in vector controls of mosquitoes from MOH. The questionnaire was amended according to the suggestions given.

3.7.3 Sampling Technique

The sampling technique in this study follows sampling design by Kothari, (2004). The sampling technique is as follow:

Step 1: Sampling population. The population of this study were staff from Health District office and residents or public in Putrajaya (urban) and Kuala Selangor (suburban).

Step 2: The sampling unit was district in Malaysia which is Putrajaya (urban) and Kuala Selangor (suburban).

Step 3: In this study the sampling frame refers to Health District Office and residents in Putrajaya and Kuala Selangor. This source list was obtained from MOH.

Step 4: Sample size. This sample size was selected based on Krejcie & Morgan, (1970) table.

Step 5: Parameter of interest. In determining the sample design, one must consider the question of the specific population parameters which are of interest. Parameter of interest in this study refers to perception of staff and public on biocontrol of mosquitoes.

Step 6: Cost considerations, from practical point of view, have a major impact upon decisions relating to not only the size of the sample but also to the type of sample. This fact can even lead to the use of a non-probability sample.

Step 7: Deciding sampling procedure and technique in selecting sample size.

3.7.4 Sample size

The sample size calculation for this study is derived from Krejcie & Morgan, (1970) (Appendix B). Based on the Krejcie and Morgan, (1970) sample size of residents in urban area was 269 and suburban were 234. Sample size for staffs in Putrajaya Health Office was 18 staffs and 20 staffs from Kuala Selangor Health Office.

3.8 Data Analysis

3.8.1 Entomological Analysis

For practical reasons, the most common survey methodologies employ larval sampling procedures rather than egg or adult collections. The basic sampling unit is the house or premise, which is systematically searched for water-holding containers (WHO, 1995).

To evaluate the distribution and density of the mosquito species in the study areas, the following parameters were calculated:

(a) Aedes index (AI): percentage of houses infested with larvae and/or pupae.

$$AI = \frac{\text{Number of houses found positive for } \textit{Aedes aegypti/Aedes albopictus}}{\text{Number of houses inspected}} \times 100$$

(b) Container index (CI): percentage of water-holding containers infested with larvae or pupae.

$$CI = \frac{\text{Number of positive containers}}{\text{Number of containers inspected}} \times 100$$

(c) Breteau index (BI): number of positive containers per 100 houses inspected.

$$BI = \frac{\text{Total number of containers positive for } \textit{Aedes aegypti/Aedes albopictus}}{\text{Number of houses inspected}} \times 100$$

Containers were examined for the presence of mosquito larvae and pupae. The collection of specimens for laboratory examination was necessary to confirm the presence of species. The commonly-used larval indices (AI, CI, and BI) are useful for determining general distribution, seasonal changes and principal larval habitats, as well as for evaluating environmental sanitation programmes (WHO, 1995).

3.8.2 Classification of Priority Areas for Vector Control

According to the Guideline for Prevention and Control of Dengue Fever and Dengue Hemorrhaguc Fever (1986) from Ministry of Health Malaysia, the priority areas for vector control are those having a concentration of cases and/ or high vector density whereby special attention should be focused on areas where people congregate. Priority areas are identified for regular *Aedes* mosquito surveillance and control activities. The priority areas are classified according to the following:

Priority I	Localities where an outbreak or case of dengue has occurred in the past.
Priority II	Localities (urban or suburban) with high *Aedes* Index (AI) $\geq$ 5% and Breteau Index (BI) $\geq$ 20.
Priority III	Localities (urban or suburban) with high *Aedes* Index (AI) $\leq$ 5% and Breteau Index (BI) $\leq$ 20.
Priority IV	Rural areas where there are no cases of dengue and low *Aedes* Index

3.8.3 Ovitrap Index (OI)

Ovitrap Index (OI), the percentage of positive ovitrap against the total number of ovitraps recovered for each ovitrap surveillance for each study site.

$$OI = \frac{\text{Numer of positive ovitrap}}{\text{Total number of ovitrap recovered}} \times 100$$

3.8.4 Statistical Analysis

Data on the number of mosquito larvae collected, types of breeding sites, mosquito indices and feeding experiment may desirably be presented as a graph prepared with Microsoft Excel. All the data were analyzed using SPSS version 17. To determine the difference in mosquito larvae species collected during larvae surveillance was analysed using one way ANOVA. Data were analyzed to find the relationship between mosquito densities in ovitraps collected and climatic factors using Pearson correlation and multiple regression techniques.

The difference in feeding consumption of mosquito larvae between dragonfly nymph species was assessing using one way ANOVA. Two - way ANOVA were used to determine the different in mosquito larvae species by Odonata species. The data of daily consumption rate of both male and female guppies toward mosquito species were analysed using two - way ANOVA.

Feeding consumption of male and female guppy and three mosquito larvae species were analysed by using Two-way ANOVA. Two – way ANOVA analysis also were used to analysed the feeding consumption of Odonata species and mosquito larvae species during light on and light off and feeding consumption of male and female guppy and mosquito larvae species during light on and light off. The relationships between feeding consumption and variation of water volume (1 liter and 2 liter), prey species (*Aedes albopictus*, *Aedes aegypti* and *Culex quinquefasciatus*), number of predator (1 and 2 predators), and prey densities (100 and 200), were analysed using multiple regression. All level of significance was determined at $p < 0.05$ which was considered significant whereas above that non significant (N.S).

CHAPTER 4

RESULTS: DIVERSITY AND POPULATION STUDIES

4.1 Diversity and Ecological Studies

4.1.1 Mosquitoes Diversity in Urban and Suburban Areas

A total of 227 of positive containers, 2257 mosquitoes were collected with 258 early instars (1st and 2nd instars), 1748 late instars (3rd and 4th instars) and 251 pupae in both study areas. Figure 4.1 shows that the number of late instar larvae collected in both study areas were higher than that of early instar and pupa. Only late instars were identified to species level.

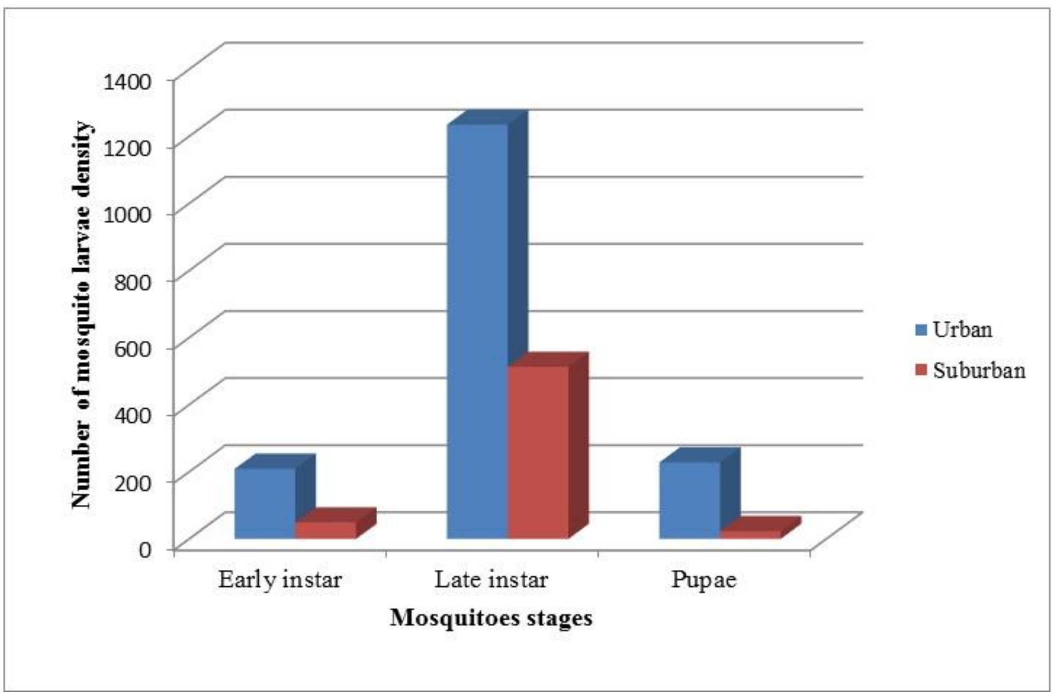


Figure 4.1 **The number of mosquito life-stages found in both urban and suburban areas during the larval surveys**

From the total number of late instars (3rd and 4th instar) mosquito larvae collected, 1596 were *Ae. albopictus* larvae, 126 larvae of *Ae. aegypti* and 32 of *Cx. quinquefasciatus* larvae. Figure 4.2 shows the number of mosquito species larvae collected from both study areas. There was a significant difference in the number of mosquito larvae species collected (one way ANOVA. $F(2, 69) = 15.04$) $p < 0.05$. Among the three common mosquito species present in both study locations, *Ae. albopictus* was the dominant species in both study locations.

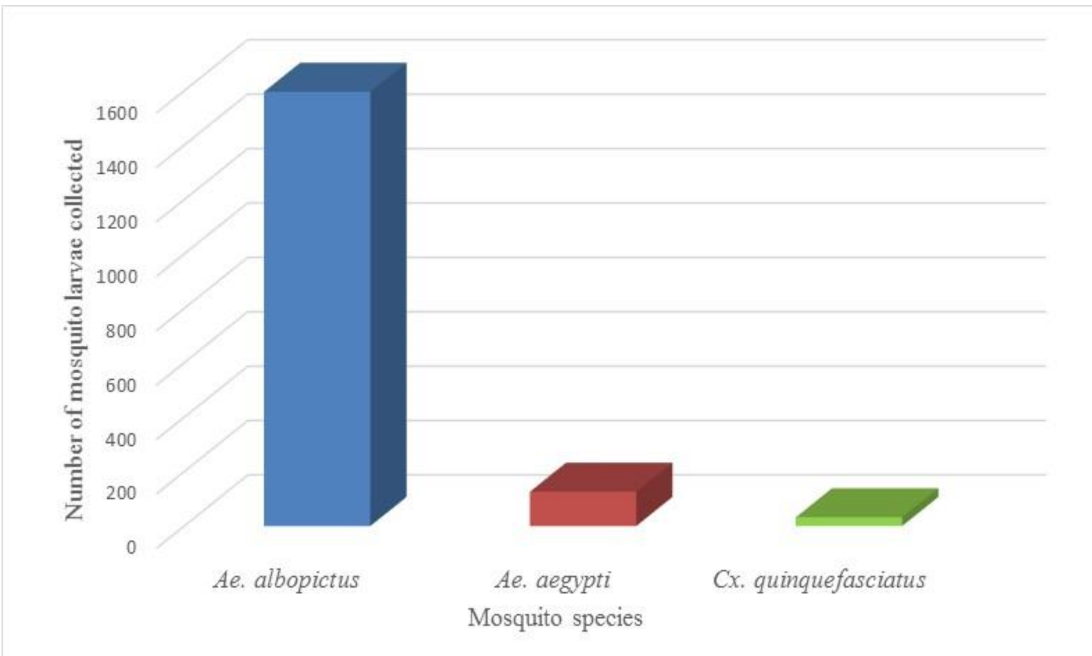


Figure 4.2 **The species of mosquitoes and number of their larvae collected in both study areas during the larval surveys**

During 1 year mosquito surveillance from January 2010 until December 2010 three species of mosquito larvae were collected in Putrajaya anad Kuala Selangor. The two Genus of mosquitoes that were collected was *Aedes* mosquito larvae (Plate 4.1) and *Culex* mosquito larvae (Plate 4.2).

Plate 4.1 *Aedes* larvae

Plate 4.2 *Culex quinquefasciatus* larvae

The results revealed that six localities in both study areas were found positive for mosquitoes breeding. A total of 262 houses in three localities in urban areas were search for mosquitoes breeding for montly basis. Breeding were detected in 37 houses in Precinct 9 (Table 4.1), 76 houses in Precinct 11 (Table 4.2) and 27 houses in Precinct 16 (Table 4.3). About 6379 water containers were search for mosquito breeding, out of which 200 were found positive for *Aedes* breeding in three localities in Putrajaya. In suburban areas 180 houses in three localities were search for mosquitoes breeding. The positive houses were detected in 16 houses in Seri Pagi, Saujana Utama (Table 4.4), 10 houses in Bestari Jaya (Mawar) (Table 4.5), and 7 houses in Bestari Jaya (Bunga Raya) (Table 4.6). During larval survey a total of 4457 containers were examined. Out of these, 60 containers were found to be positive for *Aedes* larval breeding.

Table 4.1: Prevalence indices of *Aedes* in Precinct 9, Putrajaya

Months	**House check**	**House positive**	**Container check**	**Container positive**	**AI (%)**	**CI (%)**	**BI (%)**
January	62	10	186	26	16	14	41.9
February	62	4	198	7	6.4	3.5	11.3
March	62	2	213	2	3.2	9.5	3.2
April	62	1	167	2	1.6	1.2	3.2
May	62	2	173	2	3.2	1.2	3.2
June	62	10	251	22	16	8.8	35
July	62	1	89	1	1.6	1.1	1.6
August	62	0	106	0	0	0	0
September	62	2	132	2	3.2	1.5	3.2
October	62	2	98	1	3.2	1	1.6
November	62	1	89	1	1.6	1	1.6
December	62	2	101	3	3.2	3	4.8

Table 4.2: Prevalence indices of *Aedes* in Precinct 11, Putrajaya

Months	House check	House positive	Container check	Container positive	AI (%)	CI (%)	BI (%)
January	108	16	250	28	14.8	11.2	25.9
February	108	3	216	4	2.8	1.9	3.7
March	108	1	135	1	1	1	1
April	108	3	143	3	2.8	2	2.8
May	108	3	119	3	2.8	2.5	2.8
June	108	6	175	7	5.6	4	6.5
July	108	4	169	4	3.7	2.4	3.7
August	108	0	157	0	0	0	0
September	108	4	207	5	3.7	2.4	4.6
October	108	3	211	3	2.8	1.4	2.8
November	108	3	176	3	2.8	1.7	2.8
December	108	3	217	10	2.8	4.5	9.2

Table 4.3: Prevalence indices of *Aedes* in Precinct 16, Putrajaya

Months	**House check**	**House positive**	**Container check**	**Container positive**	**AI (%)**	**CI (%)**	**BI (%)**
January	92	8	245	23	8.7	9.3	25
February	92	5	250	13	5.4	5.2	14
March	92	1	226	1	1	4.4	1.1
April	92	1	188	1	1	5.3	1.1
May	92	1	178	1	1	5.6	1.1
June	92	5	225	7	5.4	3.1	7.6
July	92	1	178	1	1	5.6	1.1
August	92	0	189	0	0	0	0
September	92	1	201	2	1	1	2.2
October	92	1	170	1	1	0.6	1.1
November	92	1	180	1	1	0.5	1.1
December	92	2	171	3	2.2	1.8	3.3

Table 4.4: **Prevalence indices of *Aedes* in Seri Pagi, Saujana Utama, Kuala Selangor**

Months	House check	House positive	Container check	Container positive	AI (%)	CI (%)	BI (%)
January	90	3	190	3	3.3	1.6	3.3
February	90	5	185	12	5.6	6.5	13
March	90	2	189	4	2.2	2.1	4.4
April	90	2	200	5	2.2	2.5	5.6
May	90	1	179	1	1.1	0.5	1.1
June	90	1	198	3	1.1	1.5	3.3
July	90	1	120	2	1.1	1.7	2.2
August	90	0	186	0	0	0	0
September	90	0	157	0	0	0	0
October	90	1	159	2	1.1	1.3	2.2
November	90	0	172	0	0	0	0
December	90	0	180	0	0	0	0

Table 4.5: Prevalence indices of *Aedes* in Kampung Bestari jaya (Mawar) Kuala Selangor

Months	House check	House positive	Container check	Container positive	AI (%)	CI (%)	BI (%)
January	45	1	120	1	2.2	0.8	2.2
February	45	3	95	6	6.7	6.3	13
March	45	1	90	2	2.2	2.2	4.4
April	45	1	115	2	2.2	1.7	4.4
May	45	2	121	2	4.4	1.7	4.4
June	45	1	96	3	2.2	3	6.6
July	45	0	80	0	0	0	0
August	45	1	78	2	2.2	2.6	4.4
September	45	0	101	0	0	0	0
October	45	0	92	0	0	0	0
November	45	0	87	0	0	0	0
December	45	0	95	0	0	0	0

Table 4.6: Prevalence indices of *Aedes* in Kampung Bestari jaya (Bunga Raya) Kuala Selangor

Months	House check	House positive	Container check	Container positive	AI (%)	CI (%)	BI (%)
January	45	1	89	1	2.2	1.1	2.2
February	45	1	96	2	2.2	2.1	4.4
March	45	2	90	1	4.4	1.1	2.2
April	45	1	101	2	2.2	2.0	4.4
May	45	1	112	1	2.2	0.8	2.2
June	45	1	105	3	2.2	2.9	6.6
July	45	0	121	0	0	0	0
August	45	0	104	0	0	0	0
September	45	0	78	0	0	0	0
October	45	0	94	0	0	0	0
November	45	0	90	0	0	0	0
December	45	0	92	0	0	0	0

In the annual mosquito survey for urban and suburban area it was found that the *Aedes* Index (AI) for urban areas in is above the standard value, from January until December 2010 except in August AI dropped to 0%. The higher *Aedes* Index (AI) was recorded in January in Precinct 9, Putrajaya which is 16% (Figure 4.3). *Aedes* Index (AI) in Kuala Selangor was higher in February (6.7 %) in Bestari Jaya (Mawar) and it was below the standard value from August until December in Seri Pagi and Bestari Jaya (Mawar) (Figure 4.4). In Bestari Jaya (Bunga Raya), the *Aedes* Index above standard from January to June and below standard from July to December 2010.

All the results of Container Index (CI) in both study locations were below the standard except in the urban area (Precinct 9 and Precinct 11) where the Container Index was 14% and 11.2% in January (Figure 4.5). The Breateau Index (BI) was above standard in January, February and June for Precinct 9 and Presinct 11 Putrajaya and in January, February, June and December for Precinct 16 Putrajaya (Figure 4.7). The higher BI recorded in January in Precinct 9, Putrajaya which is 41.9. In the Kuala Selangor areas the reading was 13 in February (above standard) recorded in Seri Pagi and Bestari Jaya (Mawar) (Figure 4.8).

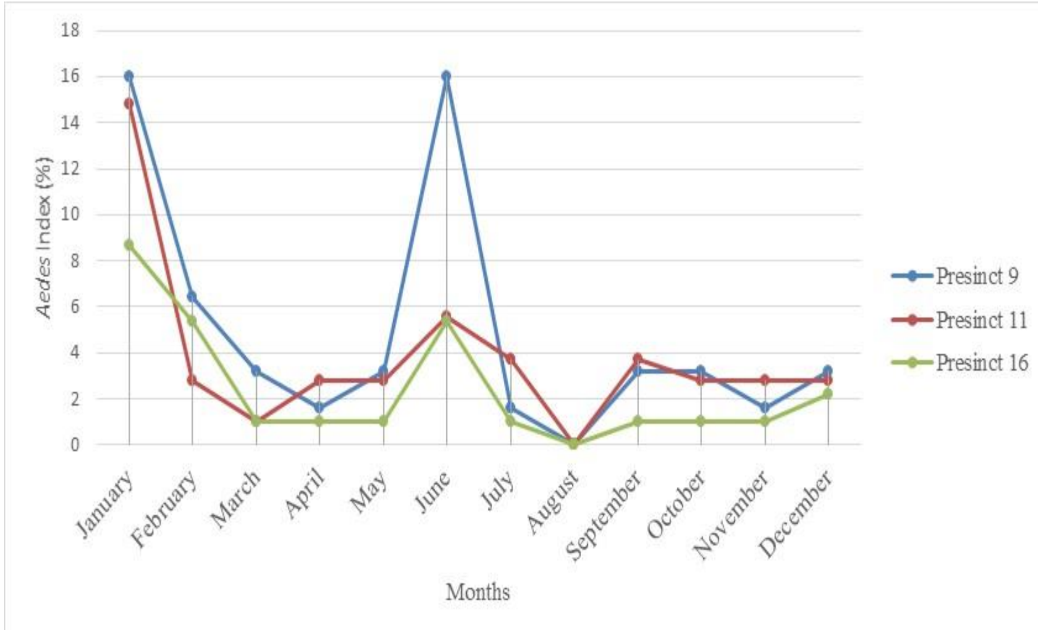


Figure 4.3 ***Aedes*** **Index (AI) calculated for urban areas**

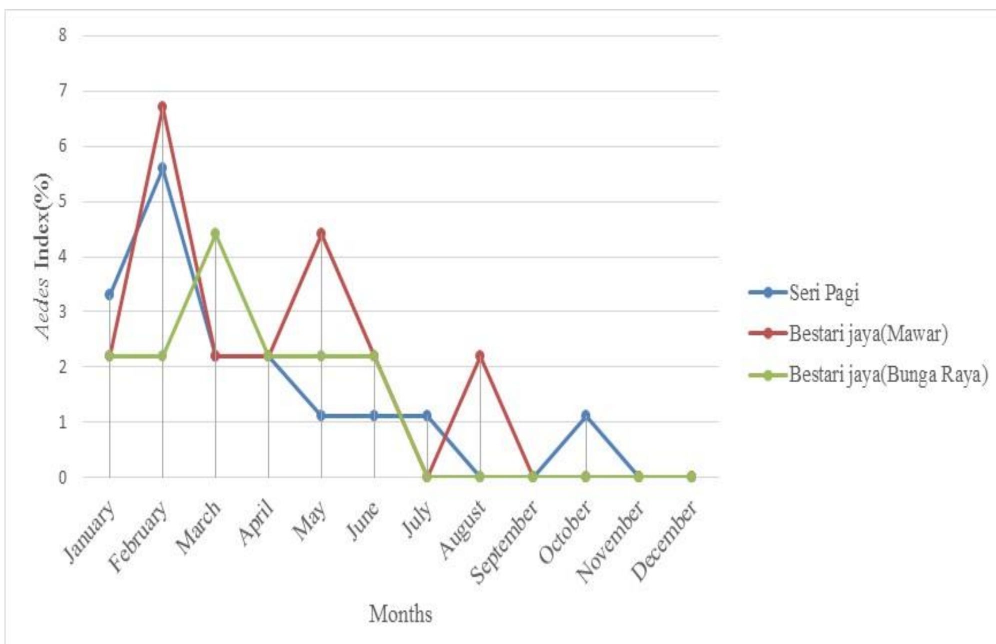


Figure 4.4 ***Aedes*** **Index (AI) calculated for suburban areas**

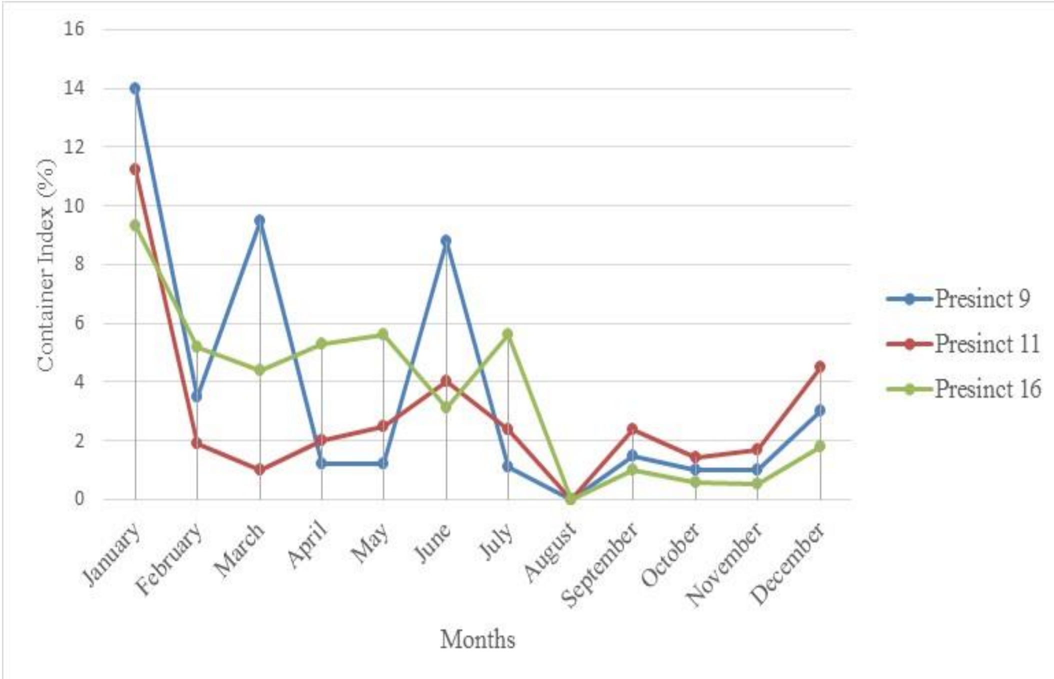


Figure 4.5 Container Index (CI) calculated for urban areas

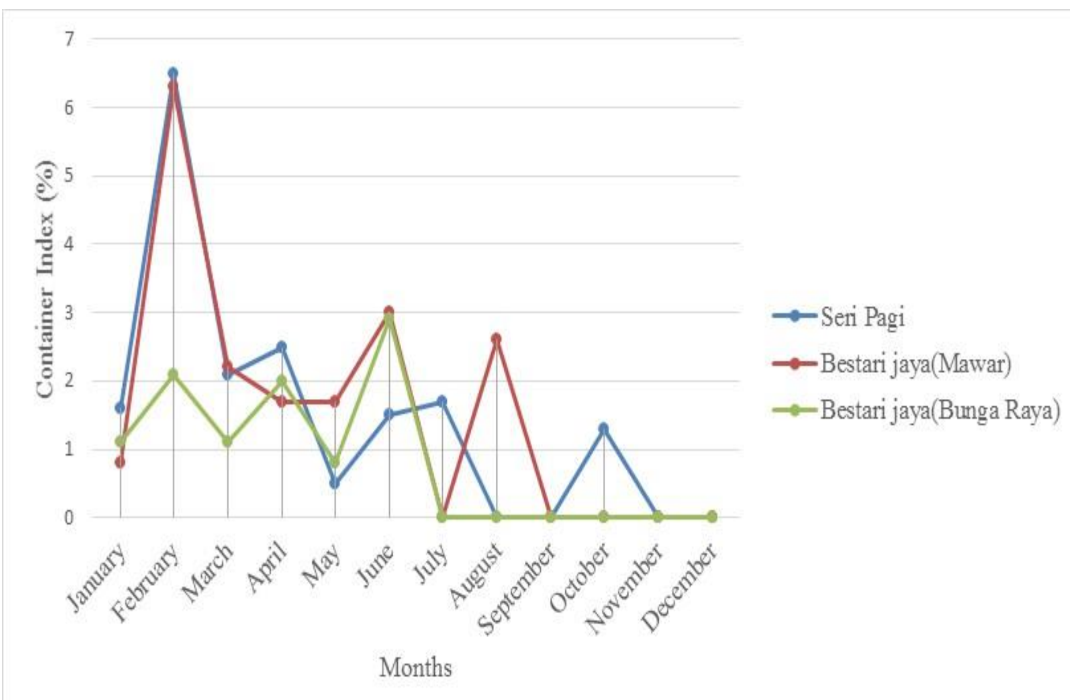


Figure 4.6 Container Index (CI) calculated for suburban areas

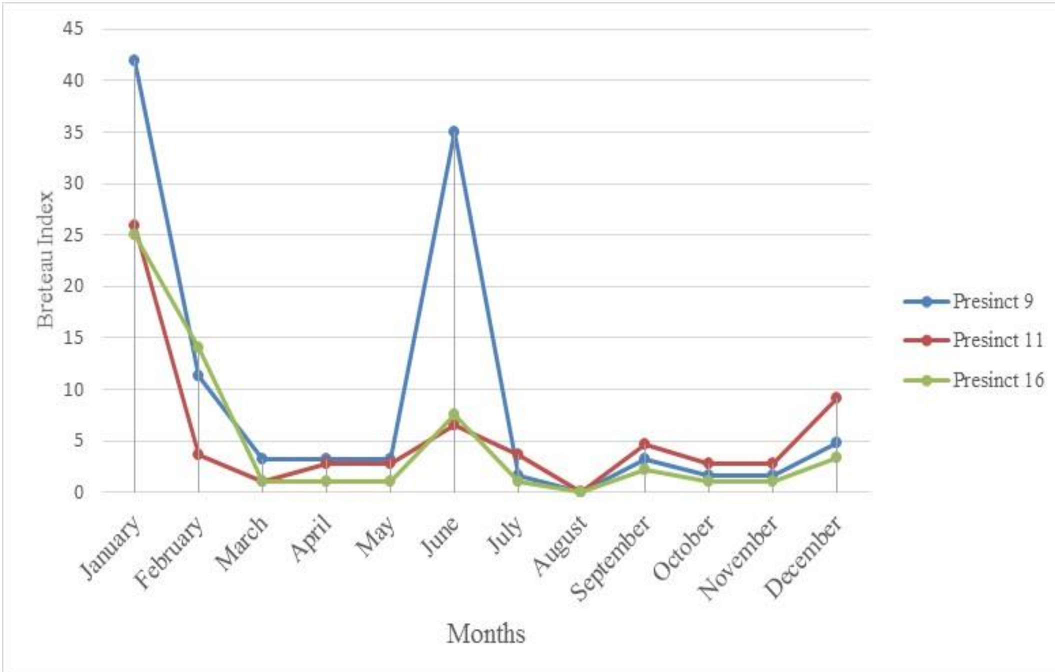


Figure 4.7 Breteau Index (BI) calculated for urban areas

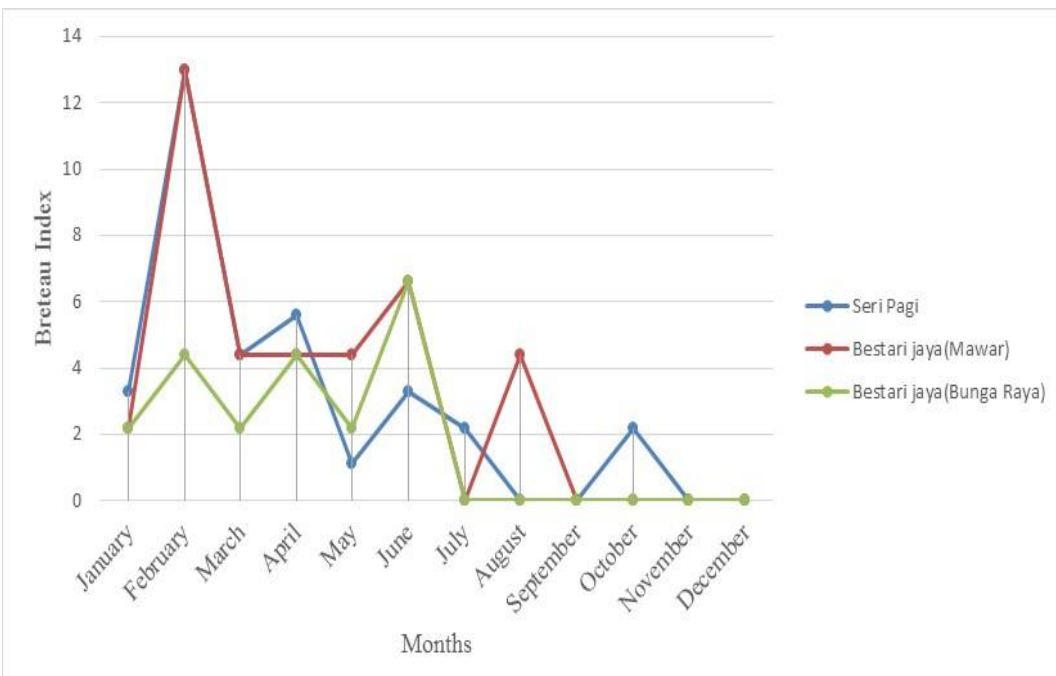


Figure 4.8 Breteau Index (BI) calculated for suburban areas

4.2.1 Ecological Studies

Table 4.7 shows number of different types of mosquito larvae collected ni Putrajaya and Kuala Selangor areas. There were eight types of different habitat found in Putrajaya and six types of habitat in Kuala Selangor areas. Figure 4.9 illustrates the types of breeding habitats identified during the larvae survey in urban areas of Putrajaya. The main breeding habitat for urban areas was found to be gardening utensils with a propotion of 44%(n = 88) Gardening utensils which include flower pots (Plate 4.3a), artificial pond (Plate 4.3b), flower pot plate (Plate 4.3c), watering can (Plate 4.3d), plastic flower pots (Plate 4.3e) and unused flower pots. Other breeding habitats for mosquitoes were artificial containers (23%)(n= 46) which included animal drinking dish (Plate 4.4) and toys, building designs (9%)(n = 18) which included floor trap (Plate 4.5), sand trap (Plate 4.6) and floor (Plate 4.7), discarded items (7%)(n = 14) such as unused containers, shoes and plastic bags. Rubbish bins (6%) (n=12), unused tyres (5%)(n= 10) (Plate 4.8), water storage containers (3%)(n=6) and natural habitat (3%)(n=6). Natural habitats composed of tree holes (Plate 4.9), and fallen leaf (Plate 4.10).

Figure 4.10 showed the types of breeding habitats identified during the larvae survey in Kuala Selangor. From the results obtained the preferred breeding habitat for suburban area was artificial containers (48%)(n=29) followed by gardening utensils (23%)(n=14). Other breeding habitats for mosquitoes were water storage containers (11%)(n=6), discarded items (8%)(n=5), unused tyres (8%)(n=5) and rubbish bins (2%)(n=1).

Table 4.7: **Number of mosquitoes larvae collected in different types of breeding habitats**

Types of breeding habitats	**Urban areas**	**Suburban area**
Gardening utensils	88	14
Artificial containers	46	29
Natural habitats	6	0
Tyres	10	5
Discarded items	14	5
Building designs	18	0
Water storage	6	6
Rubbish bins	12	1
Total	**200**	**60**

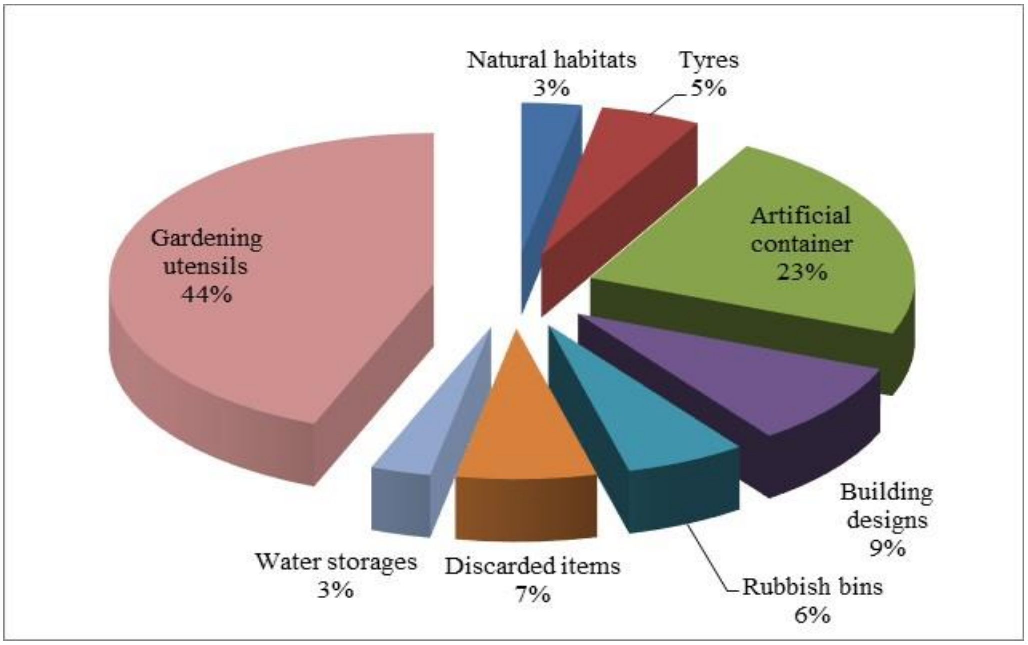


Figure 4.9 **Percentage of mosquitoes collected in different types of mosquitoes breeding habitats that were identified during the larval surveys in Putrajaya**

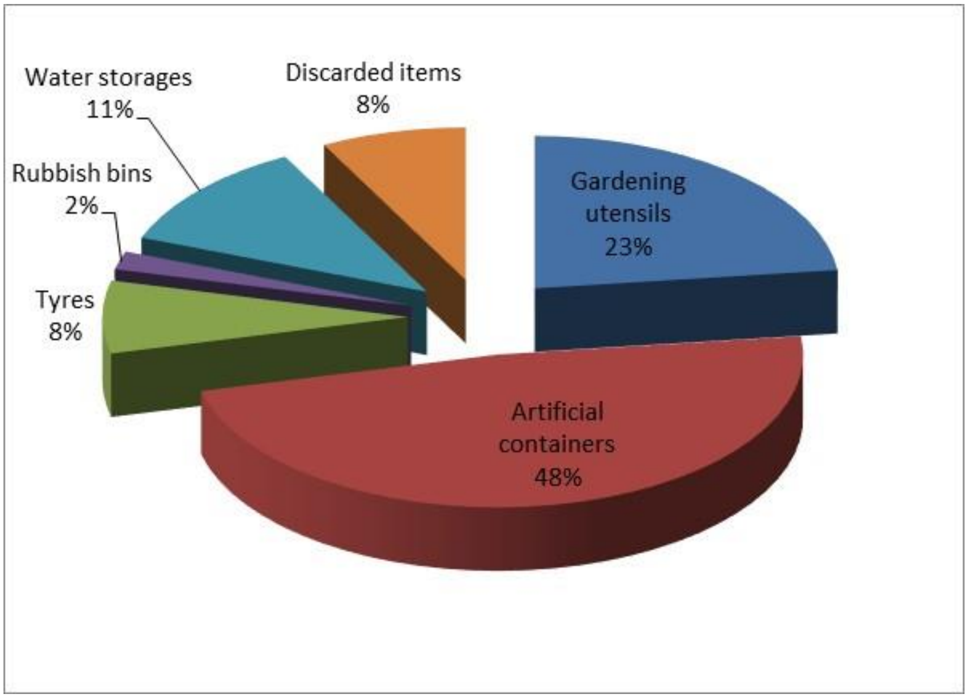


Figure 4.10 **Percentage of mosquitoes collected in different types of breeding habitats that were identified during the larval surveys in Kuala Selangor**

Plate 4.3a Flower pots

Plate 4.3b artificial pond

Plate 4.3c Flower pot plate

Plate 4.3d Watering can

Plate 4.3e Plastic flower pot

Plate 4.4 Animal drinking dish

Plate 4.5 Floor trap **Plate 4.6 Sand trap**

Plate 4.7 Floor **Plate 4.8 Unused Tyres**

Plate 4.9 Tree holes **Plate 4.10 Fallen leaf**

CHAPTER 5

RESULTS: OVITRAP SURVEY

5.1 Ovitrap Survey

Only one species was collected during the ovitrap survey. *Ae. albopictus* larvae was found in 290 ovitraps in urban and 359 ovitraps in suburban areas (Table 5.1). A total of 6481 *Ae. albopictus* larvae were collected the during one year survey in both study areas with 2953 larvae collected in urban areas and 3528 larvae collected in suburban areas. The number of larvae collected were higher in suburban areas than urban areas. In urban areas the number of mosquito larvae collected from the ovitrap was higher in December 2010 with 379 larvae and the lowest number of mosquito larvae collected was recorded in March 2010. In suburban areas where the highest number of mosquito larvae 546 collected in May 2010 and the lowest number (63) recorded during September 2010. The mean number of larvae per ovitrap of *Ae. albopictus* in urban and suburban areas ranged from 4.96 ± 1.043 - 19.22 ± 1.301 and 3.12 ± 0.78 -20.21 ± 1.27, respectively. The result shows significant difference between numbers of mosquito collected in both study areas were varied between months (Table 5.2).

Putrajaya, P11A2 (urban) was selected as locality for ovitrap surveillance and Pasir Penambang in Kuala Selangor (suburban). Total number of ovitraps collected were different every month from both study areas (Figure 5.1). From the results Ovitrap Index (OI) was higher in June 2010 in urban area with 72% and in April 2010 in suburban area with 80%. The lowest OI recorded in March 2010 with 12.5% for urban area and in

September 2010, October 2010, and January 2011 with same value of 27.14% for suburban areas.

The results of number of mosquito larvae collected in ovitrap in relation to environmental factors such as humidity, rainfall and temperature are presented in Figures 5.2-5.7 in both study areas. Environmental data were collected in both study locations from Malaysia Meteorological Department. The climatic variables include rainfall, temperature and humidity. The high temperature was recorded in June 2010 with 30.9 °C in urban areas (Figures 5.2) and 27.4 °C in suburban areas (Figures 5.5). The high humidity data were recorded in December 2010 with 79.1% in urban (Figures 5.3) and 85.4% in suburban areas (Figures 5.6). In urban area the heavy rain was recorded in September 2010 with 512.8 mm and the little rain was recorded in October 2010 with 99.6 mm (Figure 5.4). In suburban areas, the heavy rain was recorded in March 2010 with 375.0 mm and little rain was recorded in July 2010 with 81.7 mm (Figure 5.7).

The statistical analyses were performed between mosquito density and environmental factors. The mosquito density has a moderate positive correlation with rainfall in urban areas and negative correlation with rainfall in suburban areas. The results also reported that the negative correlation between mosquito density and temperature was very strong in suburban area and positive correlation in urban areas. The lowest positive correlation reported in both study locations (Table 5.2). However statistically there were no significant differences between mosquito density and climatis factors in this study.

Table 5.1 The Ovitrap Index (%) and comparison of number of larvae per ovitrap of *Ae. albopictus* in urban and suburban areas from March 2010 until February 2011

Month	Number of positive ovitraps		Ovitrap Index (OI)		Number of Mosquito larvae collected in ovitraps	
	Urban (out of 50)	Suburban (out of 70)	Urban	Suburban	Urban	Suburban
March 2010	6	50	12.5%	71.4%	36	156
April 2010	18	56	36%	80%	346	378
May 2010	21	35	42%	50%	305	546
June 2010	36	28	72%	66.7%	260	322
July 2010	29	27	64.4%	40%	233	205
August 2010	28	20	58%	28.6%	139	243
September 2010	28	19	56%	27.1%	228	63
October 2010	20	19	40%	27.1%	286	229
November 2010	30	33	60%	47.1%	342	481
December 2010	27	24	54%	34.3%	379	141
January 2011	28	19	56%	27.1%	217	384
February 2011	20	29	13%	41.4%	182	380

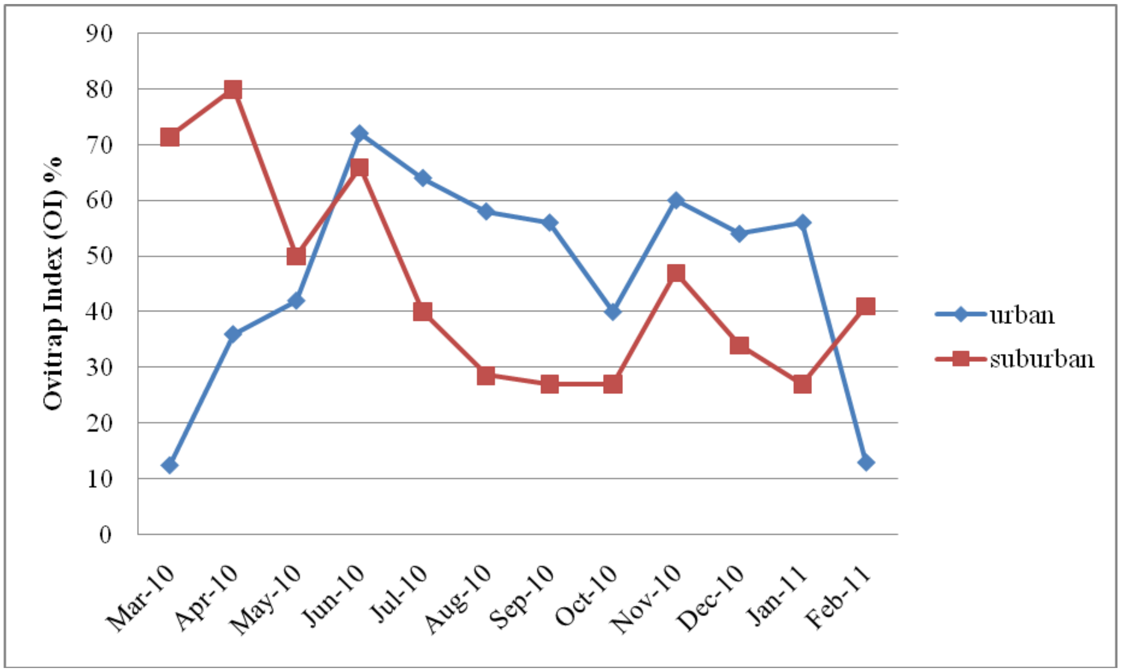


Figure 5.1 Ovitrap Index in both urban and suburban study areas

Table 5.2 Two-way ANOVA was used to analyze the mean number larvae between urban and suburban within one year survey.

Source	**df**	**F**	**P value**
Month(M)	11	14.88	$p<0.05$
Locality (L)	1	0.361	NS
M × L	11	18.023	$p<0.05$

Table 5.3 Correlation coefficient between mosquito density and climatic factors

Correlation	Urban	Suburban	P value
Mosquito density and rainfall	0.082	- 0.148	NS
Mosquito density and humidity	0.118	0.129	NS
Mosquito density and temperature	- 0.071	0.325	NS

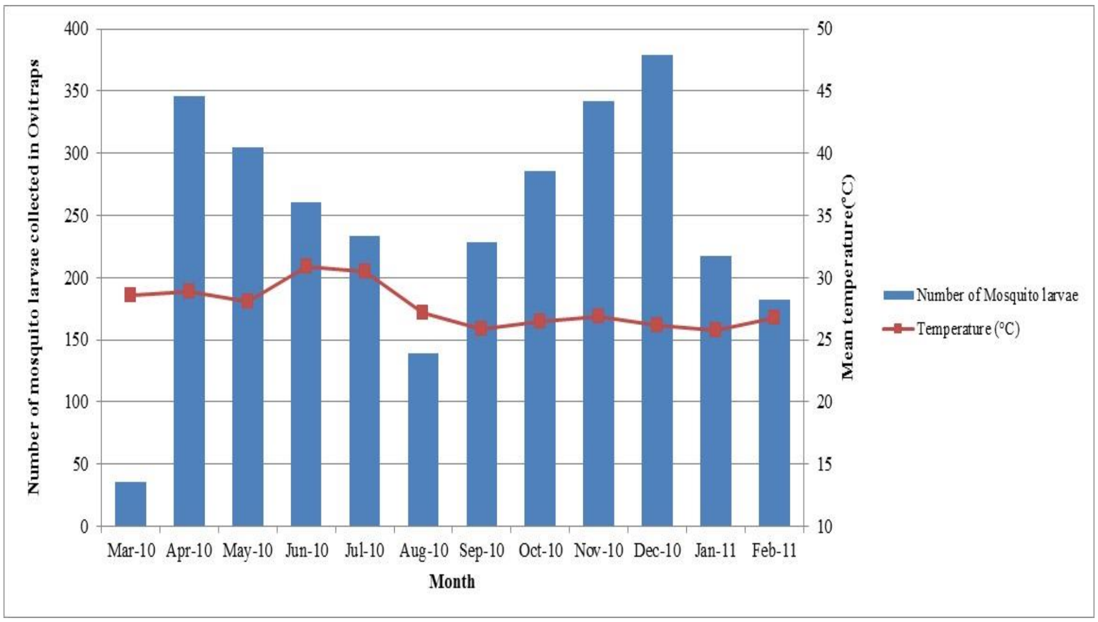


Figure 5.2 Monthly collections of mosquito larvae in ovitraps in relation to temperature in urban area

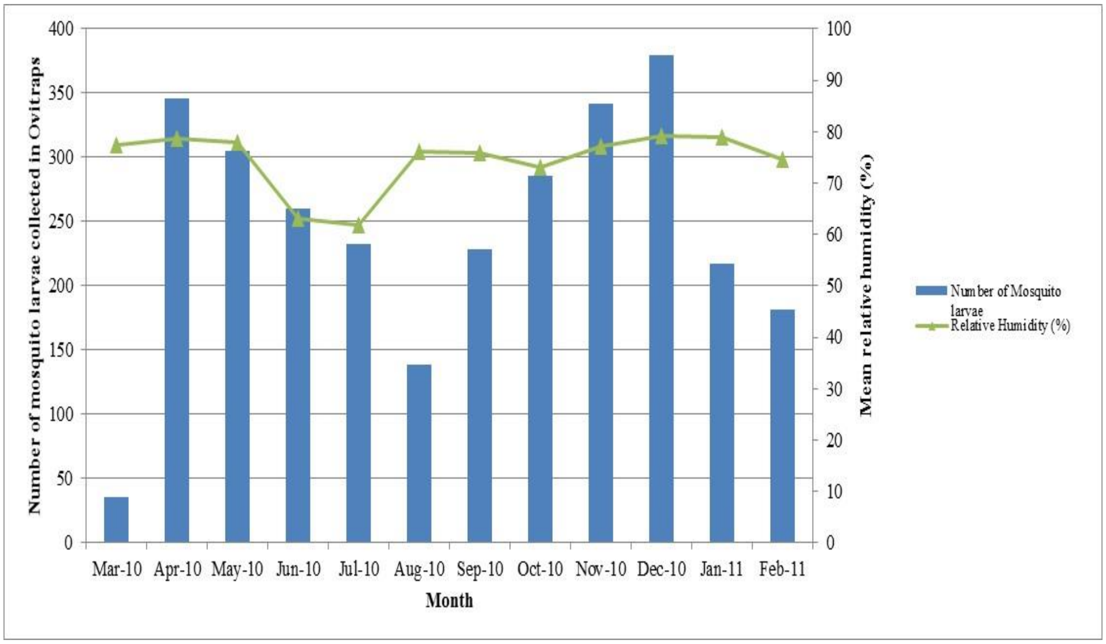


Figure 5.3 Monthly collections of mosquito larvae in ovitraps in relation to relative humidity in urban area

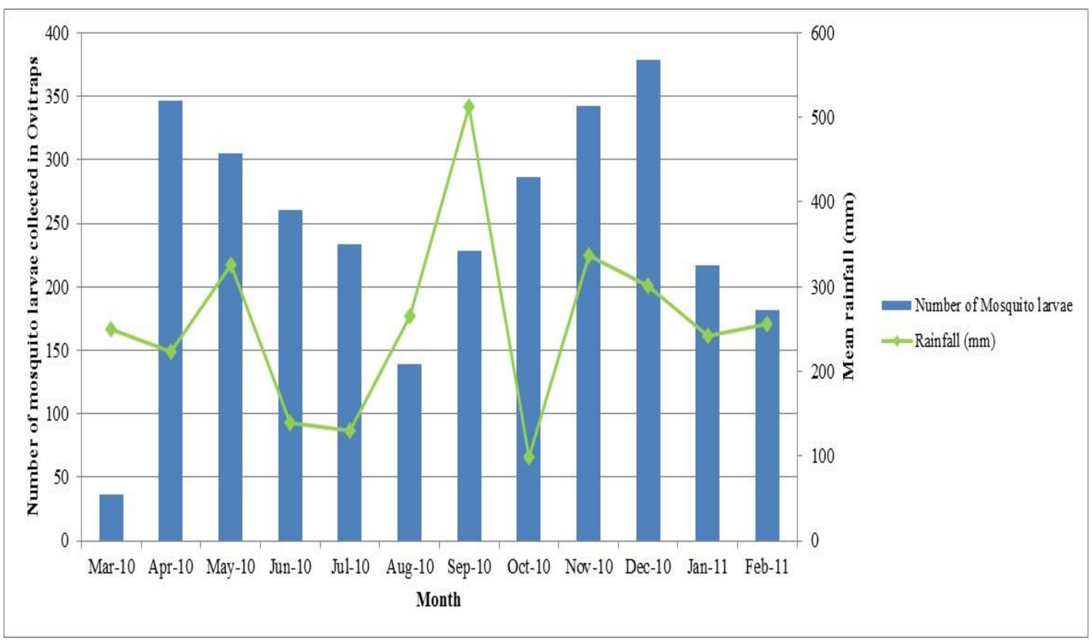


Figure 5.4 Monthly collections of mosquito larvae in ovitraps in relation to rainfall in urban area

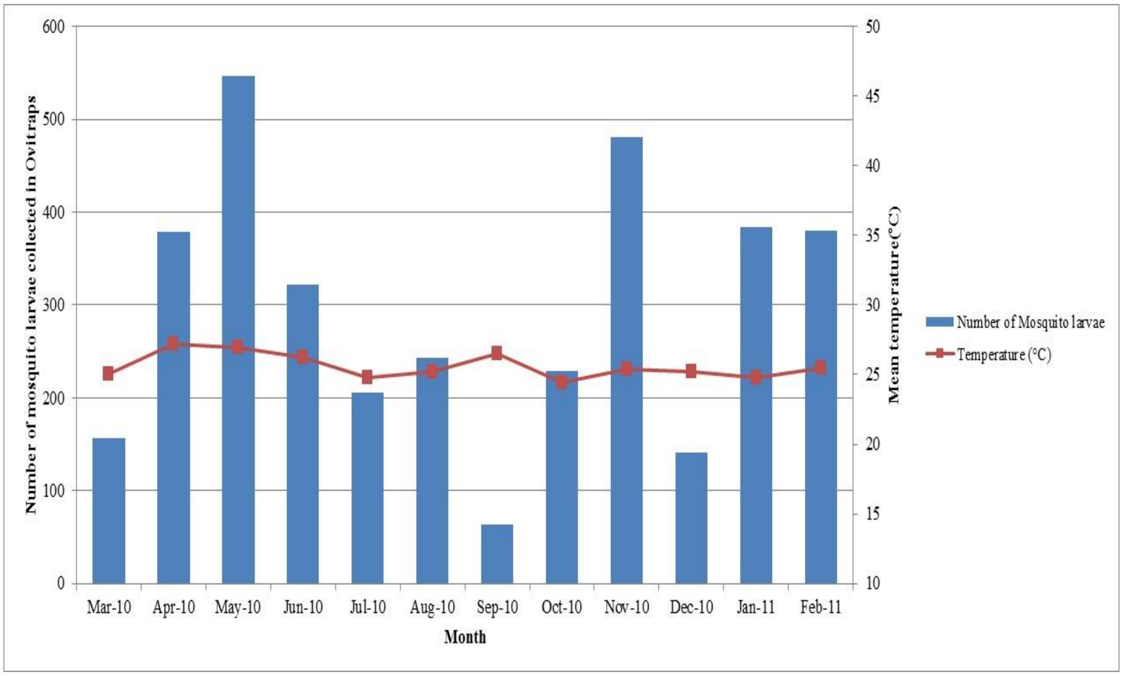


Figure 5.5 Monthly collections of mosquito larvae in ovitraps in relation to temperature in suburban area

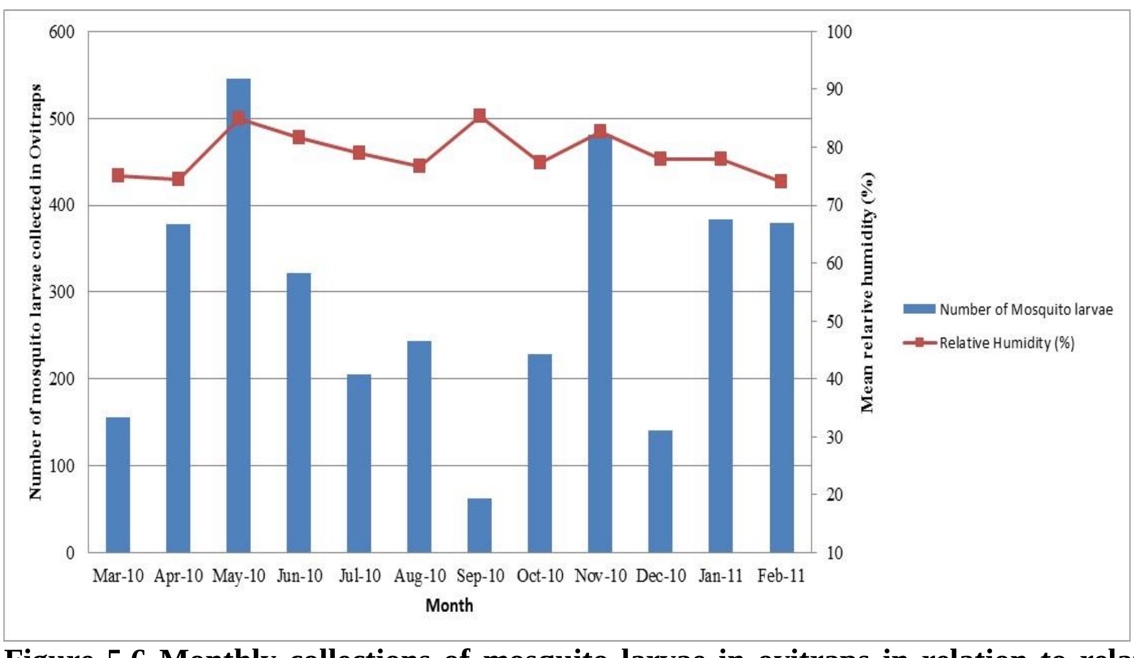


Figure 5.6 Monthly collections of mosquito larvae in ovitraps in relation to relative humidity in suburban area

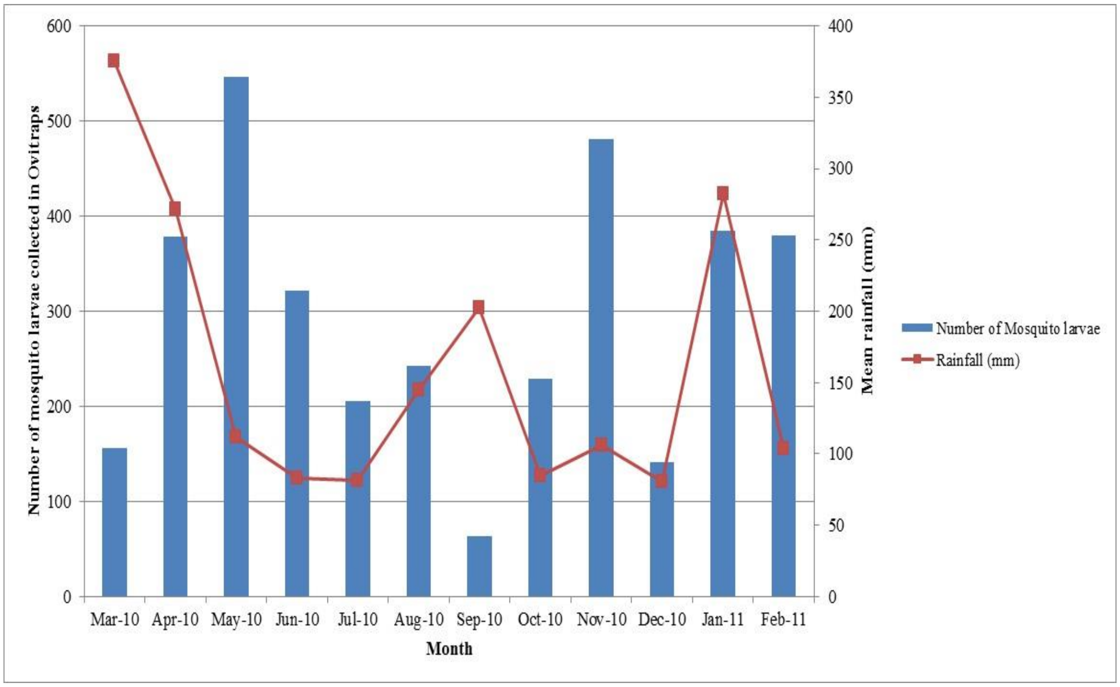


Figure 5.7 Monthly collections of mosquito larvae in ovitraps in relation to rainfall in suburban area

CHAPTER 6

RESULTS: PERCEPTION ON THE USE OF CHEMICALS IN MOSQUITO CONTROL AND UTILIZATION OF BIOCONTROL

6.1 Demographic Information

Demographic characteristics of staff Vector Unit and residents in both study areas was shown in Table 6.1. A total of 438 responded to questionnaire during the survey activities. Five hundred and three questionnaires were distributed to residents/public in both study areas. Four hundred and two questionnaires were returned. One hundred and one questionnaires were returned that were not considered useable. The unusable questionnaires were either blank or respondents would not be able to complete the questionnaires. With 402 returned questionnaires out of 503, response rate was (80%). The response rate for staff in both study areas was (94%), where 38 questionnaires were distributed among staff and 36 were returned and use able.

Table 6.1 summarised the social and demographic data of respondents. The staffs involved in vector control unit were males (94%) and 4% females. The propotion of age groups between 36 to 41 years old was 31%, aged between 18-23 and 24-29 were 19%, aged 48 and above 17%, and aged between 42-47 years old 14%. The staffs comprised only two races; Malay (94%) and Indian (4%). Some of them had completed secondary school (28%) and among them (33%) had certificate (Pembantu Kesihatan Awam) from Ministry of Health (MOH) and achieved higher education at Diploma (14%) and 6% at degree levels.

The residents/public involved in this study was 49% males and 51% females. Most of them 24% aged between 24-29 years old, aged between 30-35 years old were 21, aged between 36-41 years old were (16%), aged between 42-47 years old (12%) and aged between 48 and above were (9%). Among them were Malay (97%) and India (3%). All the public were completed secondary school and among them 28% had achieved higher education at diploma and 18% were degree holders.

Table 6.1 Social and demographic characteristics of respondents in both study areas

Variables	Staff (n=36)		Public (n=402)	
	Frequency(f)	Percentage (%)	Frequency(f)	Percentage (%)
Gender				
Male	34	94	196	49
Female	2	6	206	51
Age(years)				
18- 23	7	19	72	18
24-29	7	19	98	24
30-35	0	0	83	21
36-41	11	31	65	16
42-47	5	14	48	12
48 and above	6	17	36	9
Race				
Malay	34	94	390	97
Chinese	0	0	0	0
Indian	2	6	12	3
Others	0	0	0	0
Educational Status				
PMR	7	19	32	8
SPM	10	28	113	28
STPM	0	0	36	9
Certificate	12	33	35	9
Diploma	5	14	104	28
Degree	2	6	74	18

6.2 Perception on Control Measures of Mosquitoes

The staffs reported that cleaning up mosquito breeding areas (32%) was the most common strategy used while the public perceived fogging method (29.2%) being the most frequently methos used, as shown in Figure 6.1. Other control measures reported by both groups were the use of larvicides 25.2% of staff and 28.9% of public. The use of guppy fish was perceived 17.2% of staff and 15.5% of the public.

Figure 6.1 and Table 6.2 illustrate the perception from both and the public on chemical control of mosquito from both target groups. A total of 72.2% of staff and 83% of the public were concerned that fogging activities may affect their health. Subsequently, 66.7% of staff has responded that fogging activities did not affect the environment. Nonetheless, 56.7% of the public agreed that fogging activities may affect the environment.

In the questionnaires the respondents also have to answer question regarding the effect of the use of insecticide apart from causing health problems to humans (Figure 6.2). The most obvious effect of insecticide reported by both groups was negative effect to the environment from staff (34%) and public (25.7%), the use of insecticide will kill other non target organisms besides mosquitoes; staff (34%) and public (26.7%) and the use of insecticides is very costly; staff (20%) and public (26.9%). Other effects such as effect animal had smaller percentage of scores; staff (12%) and public (19.8%).

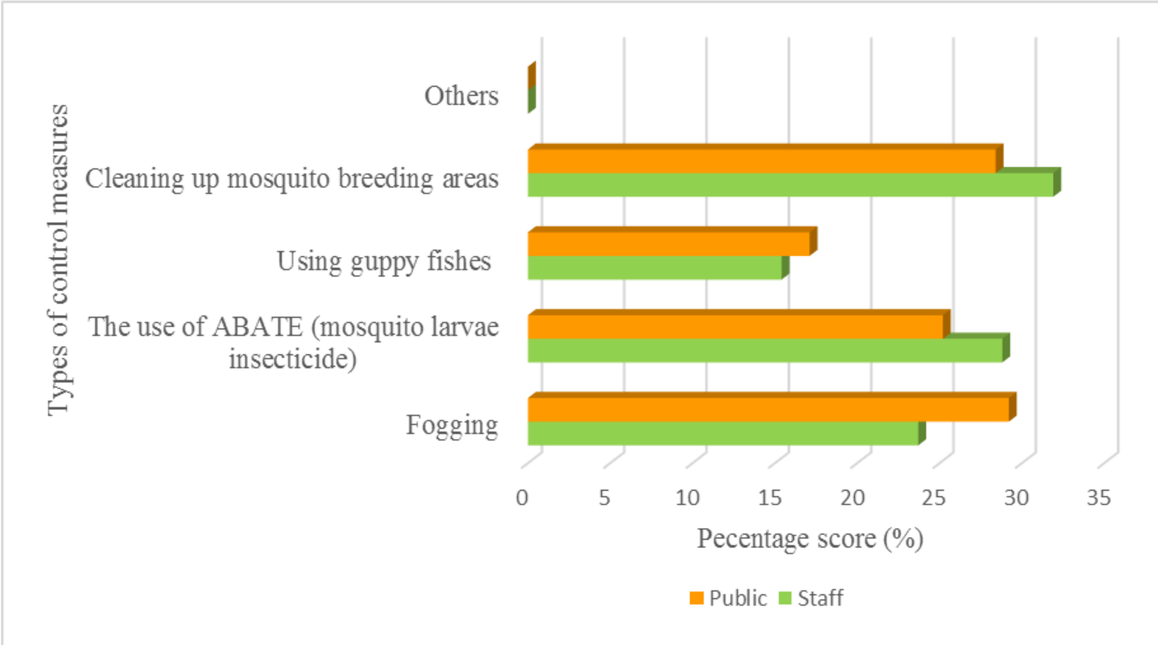


Figure 6.1 Perceptions on control measures of mosquitoes from both target groups

Table 6.2 Perceptions on control measures of mosquitoes from both target groups

Question	**Staff**		**Public/ Residents**	
	Yes (%)	**No (%)**	**Yes (%)**	**No (%)**
Do you worry about how fogging that is used to kill adult mosquitoes will affect you and your family's health?	29(72.2)	7(27.8)	334 (83)	68 (17)
In your opinion, does fogging negatively affect the environment?	12(33.3)	24(66.7)	227 (56.7)	175 (43.3)

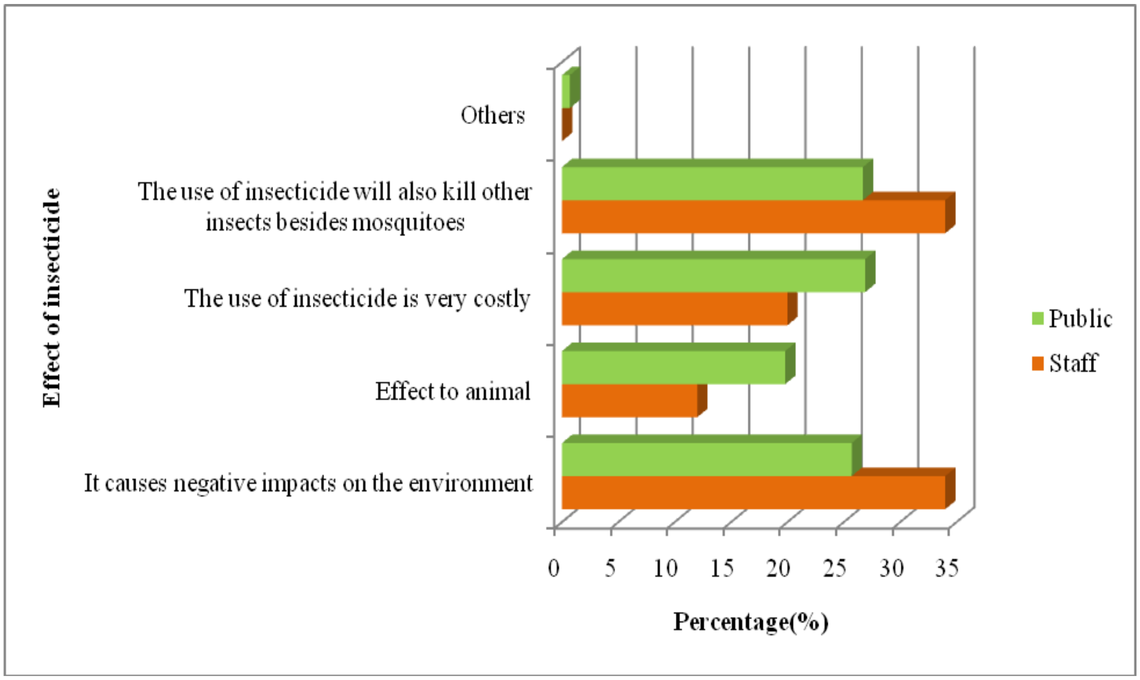


Figure 6.2 Perceptions on the effects of insecticide from both target groups

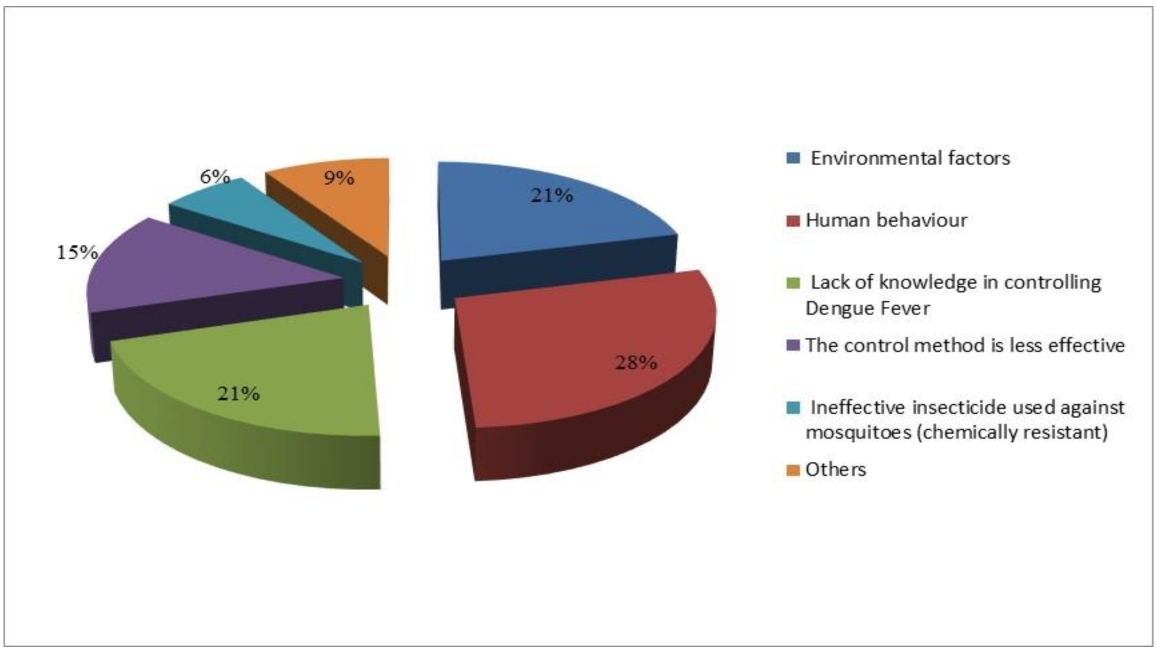


Figure 6.3 Perception of staff on factors contributing to the increase of dengue cases

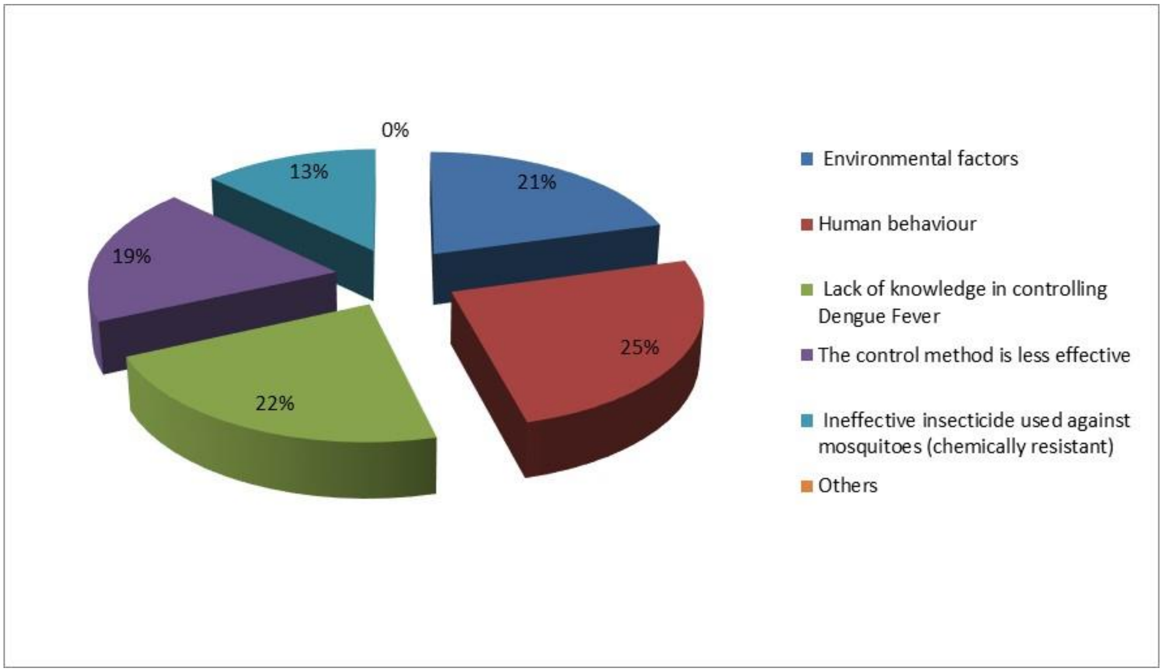


Figure 6.4 Perception of public on factors contributing to the increase of dengue cases

6.3 Factors Contributing to Increase of dengue Cases

In this survey both groups of staff and public were asked about factors that would contribute to the increase of dengue cases in Malaysia (Figure 6.3 and Figure 6.4). Both groups reported that human behaviour was the main factor contributing to dengue cases in Malaysia 28% from staffs and 25% from public perceptions followed by lack of knowledge in controlling dengue fever with 21% from the staff and 25% from the public. Other factors included 21% each both responses from the staff and public due to environmental factors. Ineffectiveness of control measure also was one of the main reasons that contribute to the increased of dengue cases which each responded 15% and 19% from the staff and public respectively. Finally, 6% of staff and 13% of public answered that mosquito had become more resistant to such insecticide.

6.4 Perception on Biocontrol Agent Uses to Control Mosquito Population

Part D of the questionnaire was about biocontrol agent used to control mosquito population. Figure 6.5 shows results of staff's and public's perception on biocontrol method and effect of biocontrol. Seventy five percent of staffs knew about biological control methods (biocontrol) in controlling mosquito population, while 17% do not know about biocontrol and 8% indicated unsure of biocontrol. From the public's perception more than half (56%) knew about biocontrol method, while 17% do not know and 27% not sure. Most of the staffs (80.5%) responded that biological method was effective in controlling mosquitoes and 47.9% of the public was not sure. Most of target groups responded that biological method not pollute the environment from staff (77.8%) and public (52.9%). While, 40.7% of public were not sure that wheater biological control can pollute the environment. In their opinion, 83.3% from staff and public (53.6%) responded that the biological method was safe for human health and (42.5%) public were not sure.

Figure 6.6 shows the types of biocontrol agent gathered from the questionnaires responded by both public and staff group. The majority of the group responded that guppy is the effective biocontrol agent (public, 39.3%) and staff (42.2%). The next method is by using toxo mosquito which generate 14.3% of the public and 15.3% of the staff. In addition, 4.4% of the public and 3.4% of the staff selected dragonfly nymph and only 1.6% of the public considered bettle as one of the available options of biocontrol agent.

The role of biocontrol was investigated from the perception of staff and general public. Overall and as expected the staffs had significantly higher positive scores by responding ‘Yes’ to all questions as shown in Figure 6.5. The range of percentage scores on ‘Yes’ for staffs are 83.3% to 75% in contrast to public with arange of 56% to 44.6%. In general the public had higher uncertainties (scoring on ‘not sure’) for all the 4 questions given ranging from 47.9% to 27%. This was due to public being unfamiliar with biocontrol as indicated in question 1 (56%) in contrast to staff very aware of biocontrol (75%). It can be highlighted here that staffs were convienced on the biocontrol effectiveness (80.5%) and safely on human health (83.3%). On the other hand the public had doubts on biocontrol effectiveness (44.6%) and safely on human health (53.6%).

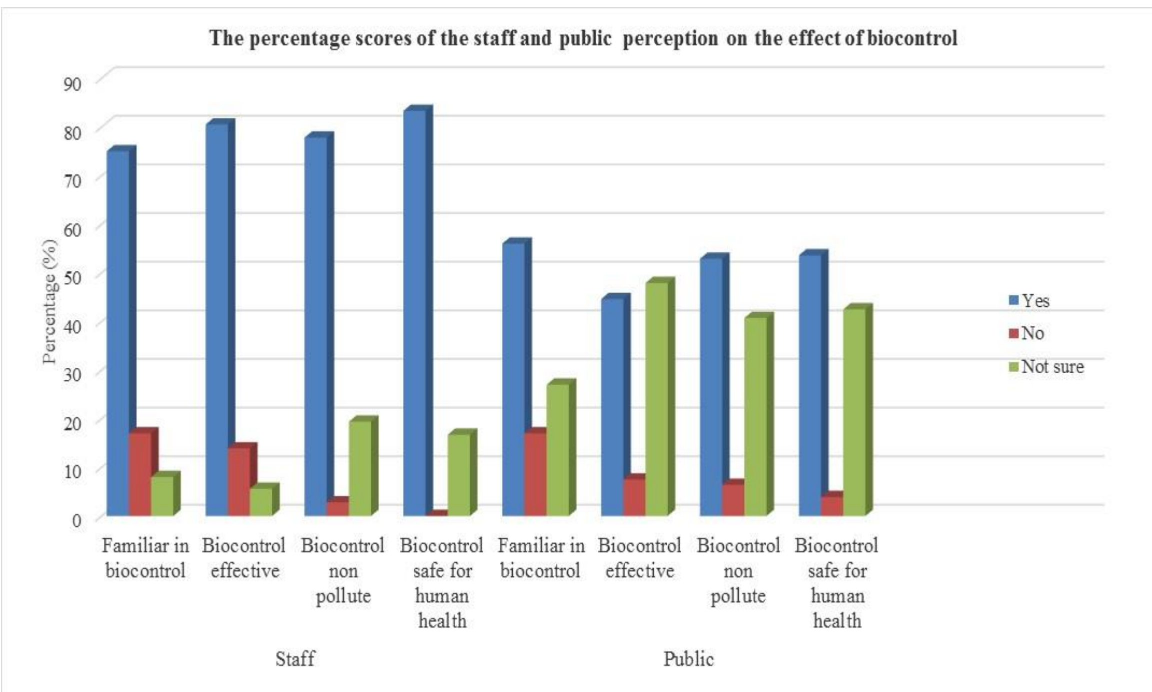


Figure 6.5 Perception on effect of biocontrol from both target groups

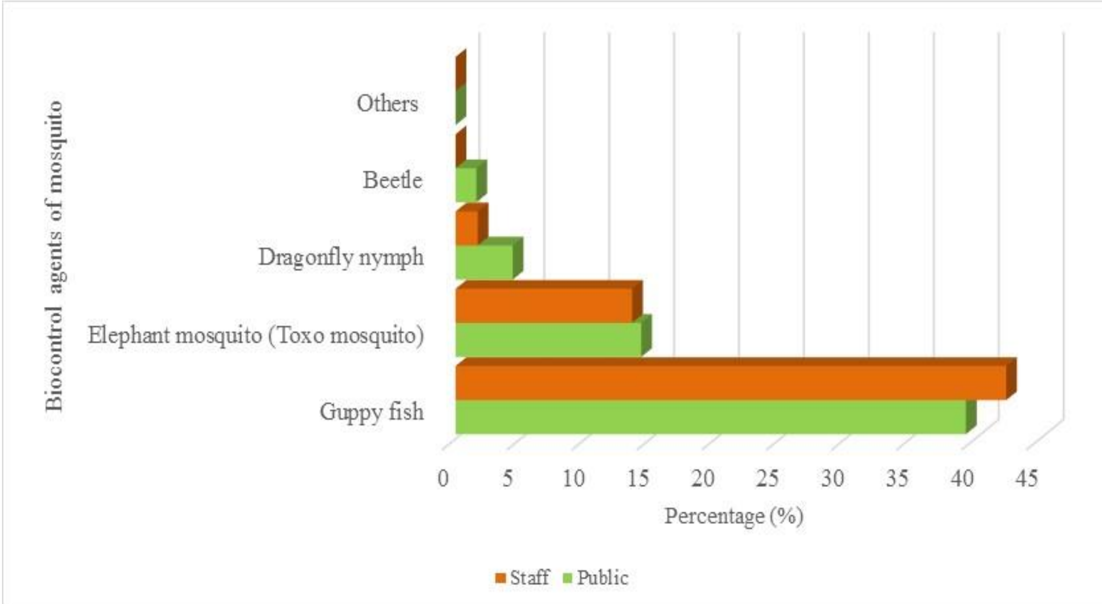


Figure 6.6 Perception on biocontrol agent used to control mosquito population from both target groups

6.5 Self-Reported Symptoms Experienced by Respondent in Both Study Areas

Figure 6.7 shows multiple health effect experienced by workers after undertaking the fogging activities. This includes 27.3% which relates to fatigue, followed by 15% of them responded with having dizziness. Subsequently 12% having blurred vision, 10.6% suffered breathing difficulty, 7.6% felt itching symptoms and 6% of them experienced chest tight. In addition, others symptoms included back pain (45%) and 3% each answered uncontrolled shivering and swollen knee joint. Lastly 1.5% responded both having abdominal pain and heat sensation while urinating. The workers also shared their experience of changing behaviour such as feeling anger as a result due to the exposure of insecticide more than 5 years.

Figure 6.8 showed that 80% of the public responded that they had health symptoms of insecticide during fogging activity, 26.9% having difficulty of breathing, 23.5% dizziness, 13% having nausea, 9.6% having chest tight, 7% having blurred vision, and 5% experienced vomiting. Others symptoms include 4.8% of iching, 3.8% of fatigue,1.7% having bleeding nose, 1.4% of shaking, both 1.2% each experienced of abdominal pain, and heat sensation.

Table 6.3 shows no correlation between age, education level, length of service and frequency of exposure of staff against health effect. While, table 6.4 also shows no correlations between age and education level of residents against health effect. Data of pesticide use for the control of *Aedes* mosquito adult and larva in Malaysia from 2009 – 2013 was obtained from MOH (Table 6.5 and Table 6.6). Insecticides used to control adult mosquitoes includes sumithion L40, gokilahts, aqua resigen, actellic 50EC, and malathion. For larvae control such as Bti 12 AS, Bti WG, Abate 500E, and Abate granule.

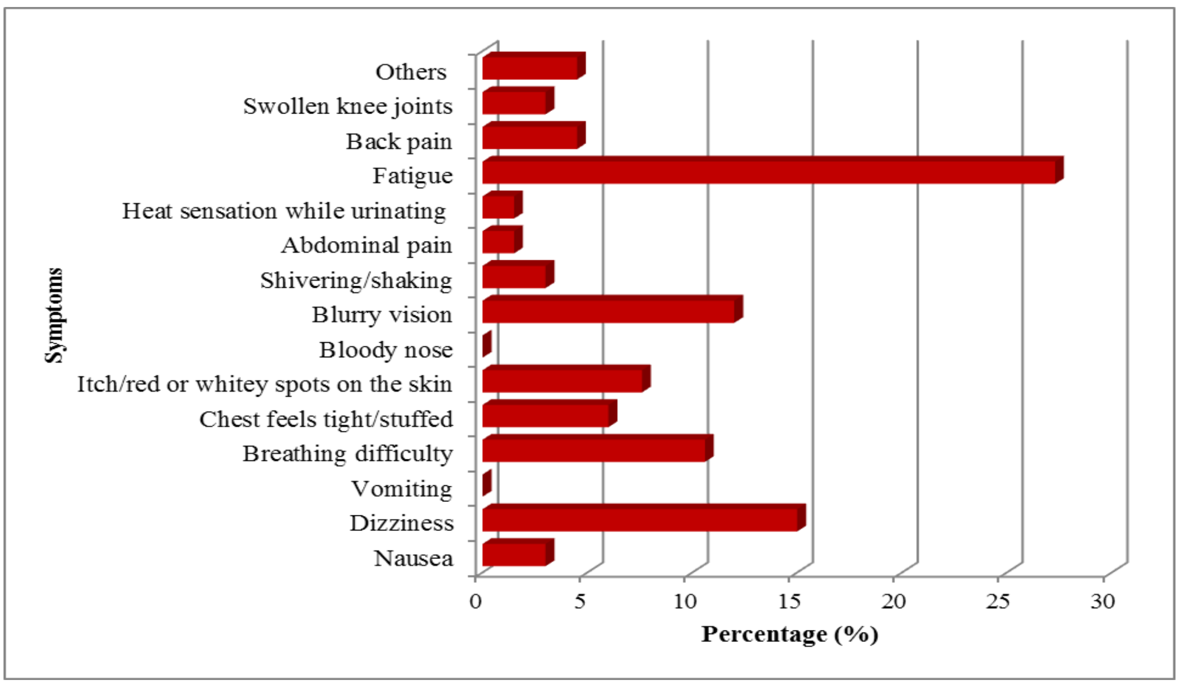


Figure 6.7 Self reported symptoms experienced by staff in both study areas

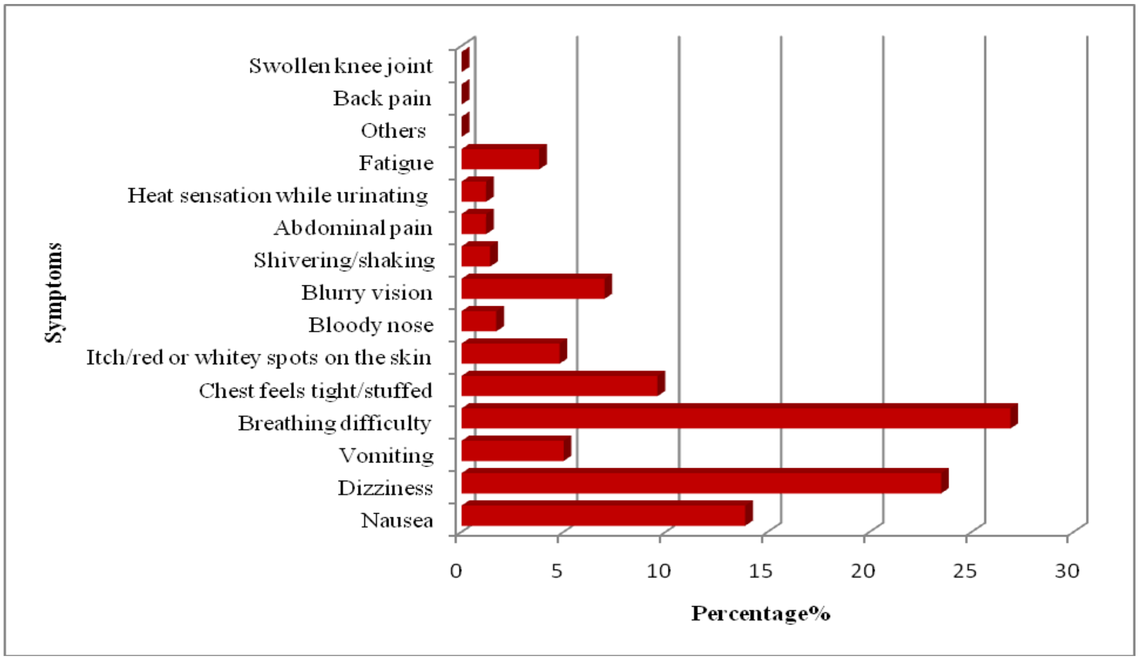


Figure 6.8 Self reported symptoms experienced by public in both study areas

Table 6.3 Association between age, education level, length of service and frequency of exposure of staff against health effect

Factor	P value
Age	0.13, $p > 0.05$
Education level	-0.13, $p > 0.05$
Length of service(working experience)	0.24, $p > 0.05$
Frequency of exposure	0.07, $p > 0.05$

Table 6.4 Association between age and education level of residents against health effect

Factor	P value
Age	-0.045, $p > 0.05$
Education level	-0.030, $p > 0.05$

Table 6.5 **List of pesticide used for the control of *Aedes* mosquitoes (adults and larvae) by Malaysia Ministry of Health from 2009 – 2013**

Insecticide used for control *Aedes* mosquitoes in Malaysia			
ADULT	**Active Ingredient**	**LARVAE**	**Active ingredient**
Sumithion™ L40	fenitrothim	VectoBac™ 12 AS	Bti
Gokilahts™	α – cyphenothim	VectoBac™ WG	Bti
Aqua resigen™	permethrin	Abate 500E	temephos
Actellic™ 50EC	pirimiphos	Abate granule	temephos
malathion	methyl malathion		

Table 6.6 List of pesticide used by Malaysia Ministry of Health from 2009 – 2013

Year	Insecticide
2009	Actellic™ 50EC
	Aqua resigen™
2010	Actellic™ 50EC
	Aqua resigen
2011	Actellic 50EC
	Aqua resigen
	Sumithion L40
2012	Sumithion L40
	Gokilahts
	Aqua resigen
2013	Sumithion L40
	Gokilahts
	Aqua resigen

For Local authority, malathion is commonly used along with other insecticides such as resigen and mospray for mosquito control.

CHAPTER 7

RESULTS: FIELD SURVEY OF NATURAL PREDATORS IN STUDY AREAS

7.1 Survey of the potential Natural Predator from Both Study Areas

A survey of potential natural biocontrol agents for mosquitoes was carried out at both study areas. Six types of potential natural predators were collected which composed of 48% *P. reticulata* (guppy), 30% of dragonfly nymph, 9% of damselfly nymph, 6% of tadpoles, 4% of water bugs and 3% of worm. The predominant species were sampled were subsequently used in the predation experiments. There were *Poecilia reticulata* (guppy) and dragonfly nymph; *Orthetrum sabina*, *Orthetrum chrysis* and *Neurothemis fluctuans.*

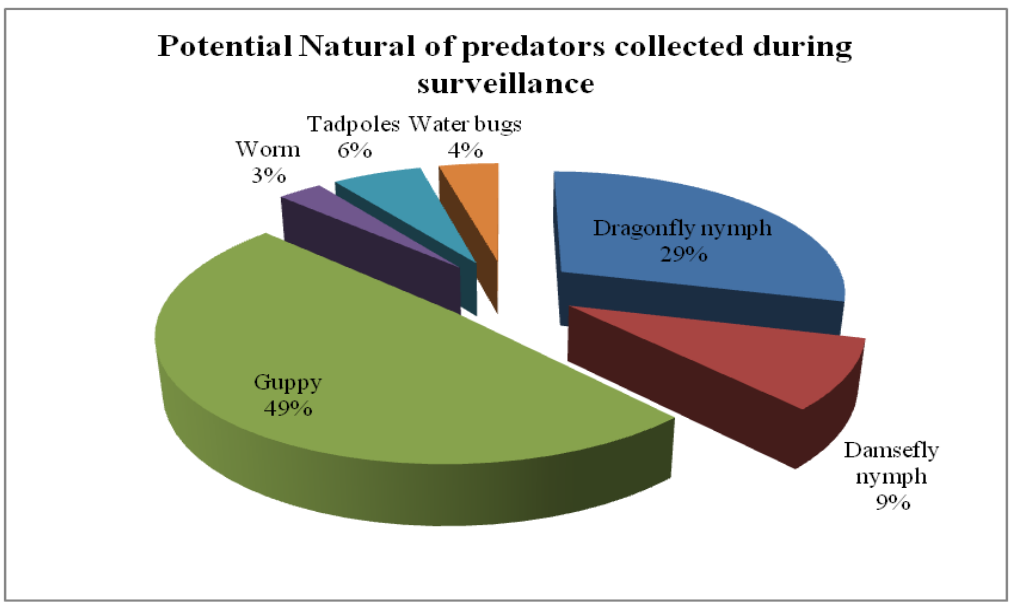


Figure 7.1 The percentages of potential natural predators that were collected in both study areas.

7.2 Survey of Odonata and guppy from the Study Areas

The general survey for potential biocontrol agents that were conducted in the study areas revealed high number for guppies and dragonflies (Figure 7.1). In contrast to the extensive biocontrol research on guppies the potential of dragonfly is unexploited. Thus a specific sampling was focused on the dragonfly. The total number of 427 dragonfly nymphs (Plate 7.1) were caught in both study areas which belonged to 6 common species (Figure 7.2). The dominant species in both study areas was *Neurothemis fluctuans* (Fabricius, 1793) commonly known as Coppertone velvetwing with a total of 112 individuals. Other species collected were *Orthetrum sabina* or commonly knowns as Sober skimmer, (Drurry, 1770) (105), *Orthetrum chrysis* (Selys, 1891) or Redfaced skimmer (92), *Trithemis aurora* (Burmeistar, 1839) (Down dropwing) (65), *Trithemis festiva* (Rambur, 1842) (Indigo dropwing) (26) and *Brachydilax chalybea* or Yellow patched lieutenant (27). A total of 712 guppies, *Poecilia reticulata*, were collected from small streams and drains in urban and suburban areas (Plate 7.2).

Eight species of adult dragonflies were collected in both study areas (Table 7.1). Only one species that was not found in urban areas is *Brachythemis contaminata* (Fabricius, 1793). Other species found were *Orthetrum chrysis* (Plate 7.3, Plate 7.4), *Orthetrum sabina* (Plate 7.5), *Neurothemis fluctuans* (Plate 7.6), *Rhyothemis phyilis* (Plate 7.7), *Trithemis festiva* (Plate 7.8), *Trithemis aurora* and *Brachydilax chalybea* The adult dragonfly species was not use in the experiment, only dragonfly nymphs were use as biocontrol agent in the feeding experiment.

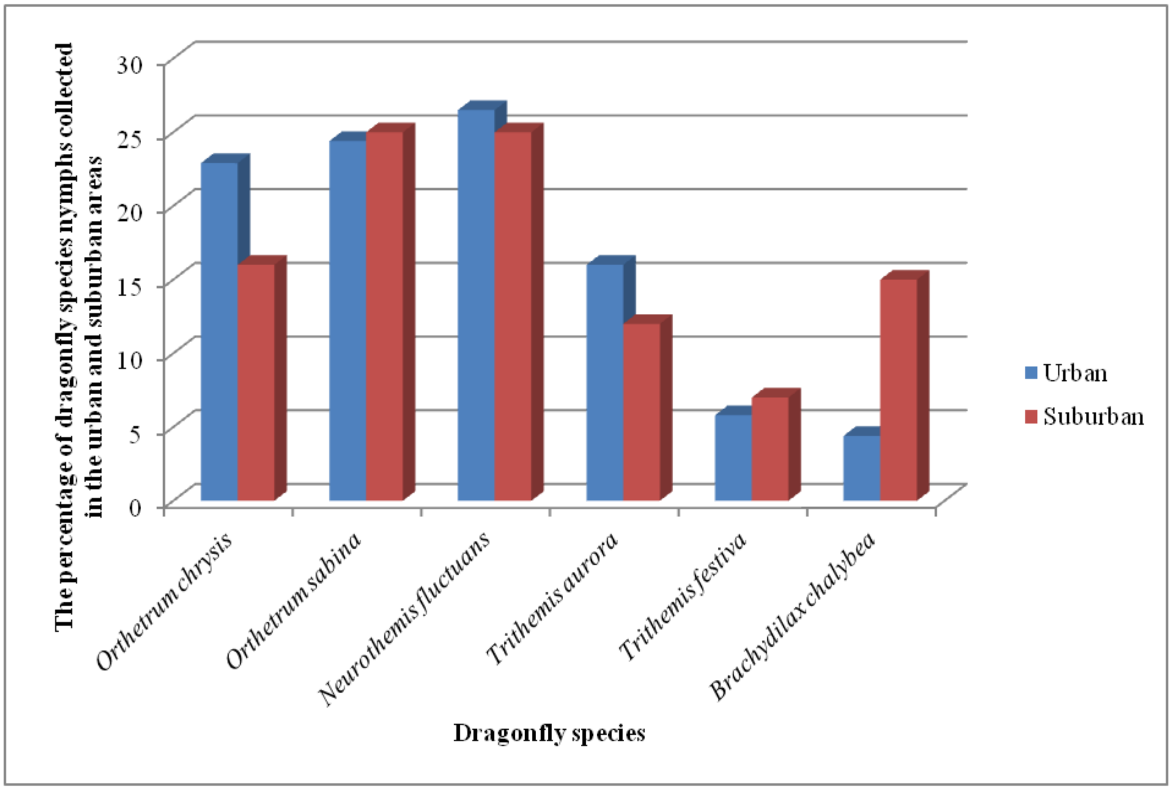


Figure 7.2 **The percentage of dragonfly species nymphs collected in the urban and suburban areas**

Table 7.1 **The percentage of adults Odonata species found in both study areas urban and suburban**.

No.	Odonate species	Urban	Suburban
1.	*Orthetrum chrysis*	19.2%	18.1%
2.	*Orthetrum sabina*	22.3%	23.4%
3.	*Neurothemis fluctuans*	26.4%	27.5%
4.	*Trithemis aurora*	10.4%	10.5%
5.	*Trithemis festiva*	5.2%	5.3%
6.	*Brachydilax chalybea*	8.8%	7.0%
7.	*Rhyothemis phyilis*	7.7%	4.7%
8.	*Brachythemis contaminata*	0	3.5%

Plate 7.1 Dragonfly nymph

Plate 7.2 Guppy*(P.reticulata)*

Plate 7.3 *O. chrysis*

Plate 7.4 *O. chrysis*

Plate 7.5 *O. sabina*

Plate 7.6 A male adults *N. fluctuans*

Plate 7.7 *R. phyllis*

Plate 7.8 An adults male *Trithemis festiva*

CHAPTER 8

RESULTS: CAPTIVITY STUDIES ON PREY-PREDATOR EXPERIMENT

8.1 Predators Behaviour

The predation activities of dragonfly nymphs and guppies on mosquito larvae species where the behaviour of both predators were recorded. The categories of predator behaviour recorded were searching, following, pursuing, attacking, capturing and motionless (Table 8.1).

Table 8.1 Comparison between *P. reticulata* (guppy) and Dragonfly nymph

Behaviour categories	**Ethogram**	***Poecilia reticulata* (guppy)**	**Dragonfly nymph**
Searching	moving but not orienting towards prey	Searching all the time until they can capture the prey	Ambush strategy by waiting for prey to approach closer
Following	moving and orienting towards prey	Yes and very active	No , just waiting for the prey
Pursuing	following prey at burst speed	Yes	No
Attacking	striking at prey	Yes and all the time	Attack and ambush when prey come closer to them
Capturing	engulfing and handling prey	Very fast	Very fast, capture prey when they come closer
Motionless	no locomotion but head and eyes may be observing prey	Very active, fast movement and always search and attack the mosquito larvae	Yes, they are motionless until when preys come close to them, they were ambush and attack the mosquito larvae.
Vertical stratification of activity	Level of water at which they are, lost active	Surface area	Bottom substrat

8.2 Prey Behaviour

The prey behaviour of mosquito larvae were also recorded during predation activities and the behaviour of prey were categorized as shown in the Table 8.2. Two categories of prey behaviour were recorded: movement and resting behaviours. Both *Ae. albopictus* and *Ae. aegypti* larvae had similar behaviour but their behaviour were different from *Cx. quinquefasciatus* larvae.

Table 8.2 Comparative behaviour of *Ae. albopictus, Ae. aegypti and Cx. quinquefasciatus*

Behaviour categories	***Ae. albopictus***	***Ae. aegypti***	***Cx. quinquefasciatus***
Movement behavior	*Aedes* larvae spent more of their activity time trashing below the water surfaces	*Aedes* larvae spent more of their activity time trashing below the water surfaces	*Culex* spent more time at the surfaces
Resting behavior	Larvae move freely in the water	Larvae moved freely in the water	Larvae of this species tend to hang to the surface of the aquarium

8.3 Feeding Experiments of Dragonfly Nymphs

The daily feeding rate was assessed by exposing 100 4th instar mosquito larvae of every species to a single predator species. The overall feeding rates of *O. sabina* were significantly higher than the overall feeding rates of *O. chrysis* and *N. flactuans* (Figure 8.1). Table 8.3 shows the results of two-way ANOVA for the feeding experiment (recorded in 3-hour intervals within 24 hours) of the three species of dragonfly nymph namely *N. fluctuans*, *O. sabina*, and *O. chrysis* on larvae of mosquito species namely *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus*. There was no significant difference [F (2, 27) = 3.42, NS] among the three species of dragonfly nymphs in terms of mosquito larvae consumption of the three species of mosquito larvae namely *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus*.

However, in terms of prey preference, there was a significant difference [F (2, 27) = 5.35, $p < .05$] in terms of the mosquito species most preferred by the dragonfly nymphs It was observed that the dragonfly nymphs consumed more *Ae. aegypti*. The Odonata predators showed specific prey preference; *N. fluctuans* and *O. sabina* consumed more *Ae. aegypti* larvae than *Ae. albopictus* larvae and *Cx. quinquefasciatus* larvae, while *O. chrysis* do not show any larvae preference as they consumed 3 species of mosquitoes larvae *Cx. quinquefasciatus* larvae, *Ae. aegypti* larvae and *Ae. albopictus* larvae.

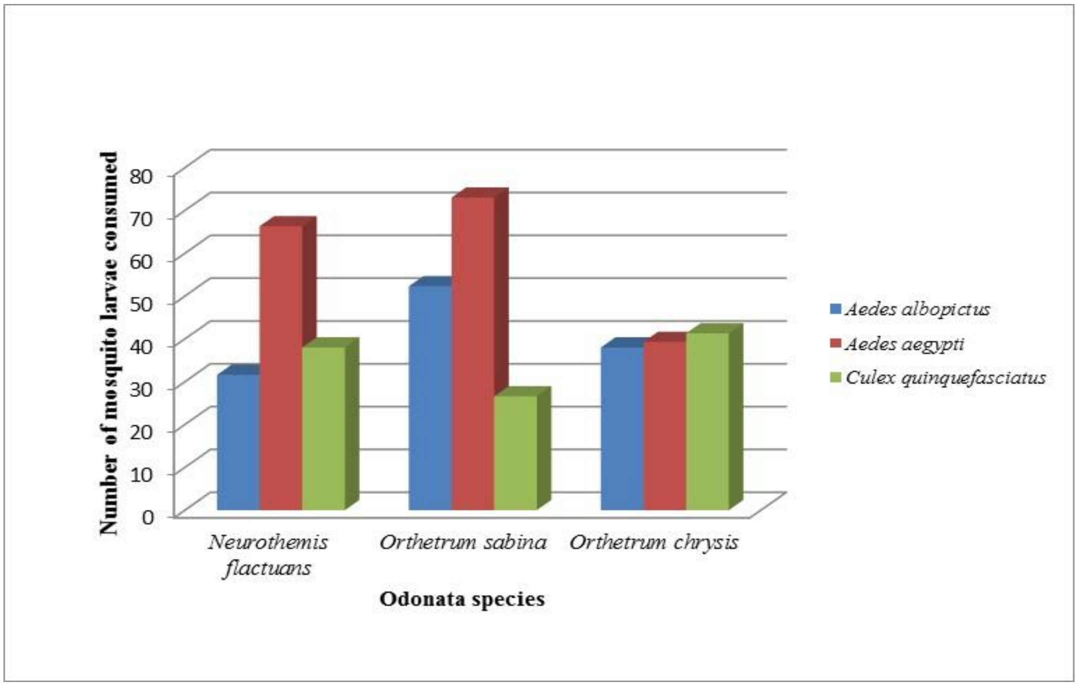


Figure 8.1 **Feeding rates of Odonata species on *Cx. quinquefasciatus, Ae. albopictus* and *Ae. aegypti* larvae**

Table 8.3 **Results of two-way ANOVA on feeding consumption of dragonfly nymph towards three species of mosquito larvae *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus***

Source	**df**	**F**	**P value**
Dragonfly nymphs(D)	2	3.42	NS
Mosquito larvae species (L)	2	5.35	$p<0.05$
D × L	4	2.31	NS
Error	18		
Total	27		

8.3.1 Feeding Experiment of between Light on and Light off

Figure 8.3 shows the results of the experiment that have been conducted in two different situations of light on and light off for 3 selected species of dragonfly predators; *Neurothemis fluctuans, Orthetrum sabina* and *Orthetrum chrysis* preying on 3 species of mosquitoes *Ae. aegypti* larvae, *Ae. albopictus* and *Cx. quinquefasciatus*. It was found that they were significantly more active in predation behaviour during light on across all species and there was difference between species [ANOVA, $F (2, 216) = 14.09$ $p< 0.05$] (Table 8.5).

The feeding rate between light on and light off also varied between the three Odonata species. In general all Odonata species were more active during the light on (5am – 5pm) rather than during the light off between 5pm until 5am (Figures 8.3). All Odonata species preferred or consumed more *Aedes* species than *Culex* species. The resulting prey preference in light on and light off were *Ae. aegypti* > *Cx. quinquefasciatus* > *Ae. albopictus* larvae. The patterns of different odonate nymph species with respect to the different times of a day were conducted under laboratory conditions towards mosquito larvae (Figures 8.4 – 8.6).

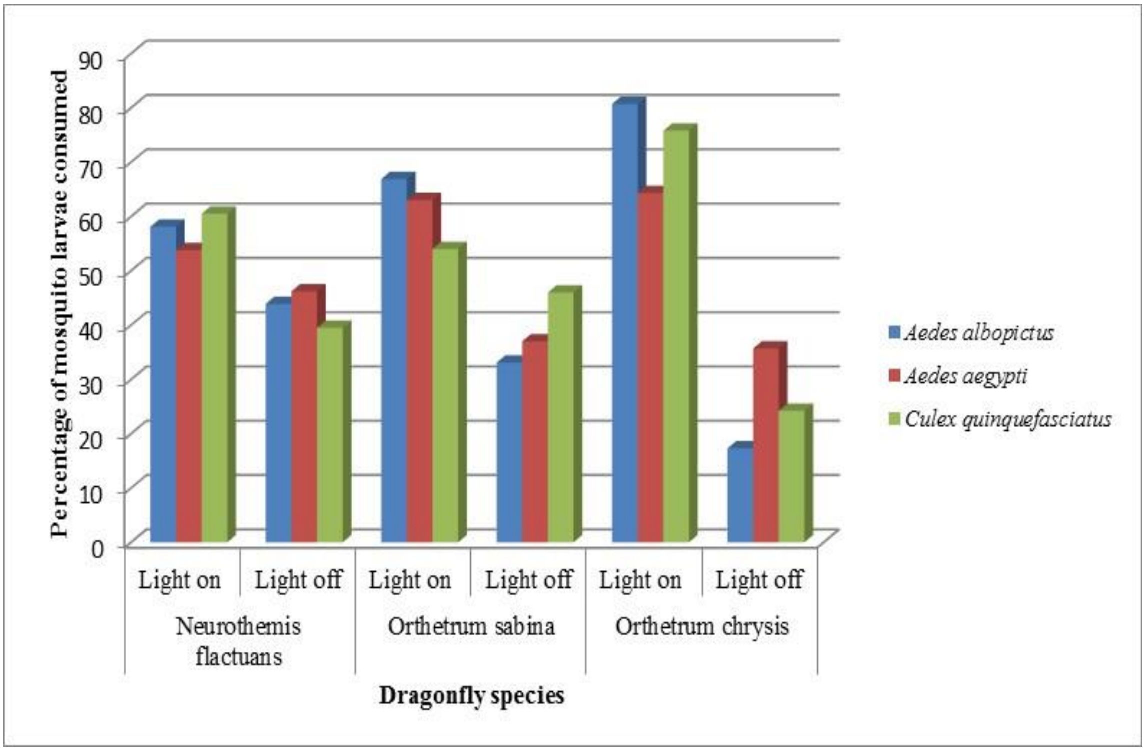


Figure 8.2 The percentage number of 3 mosquitoes prey species consumed by 3 species of dragonfly predators.

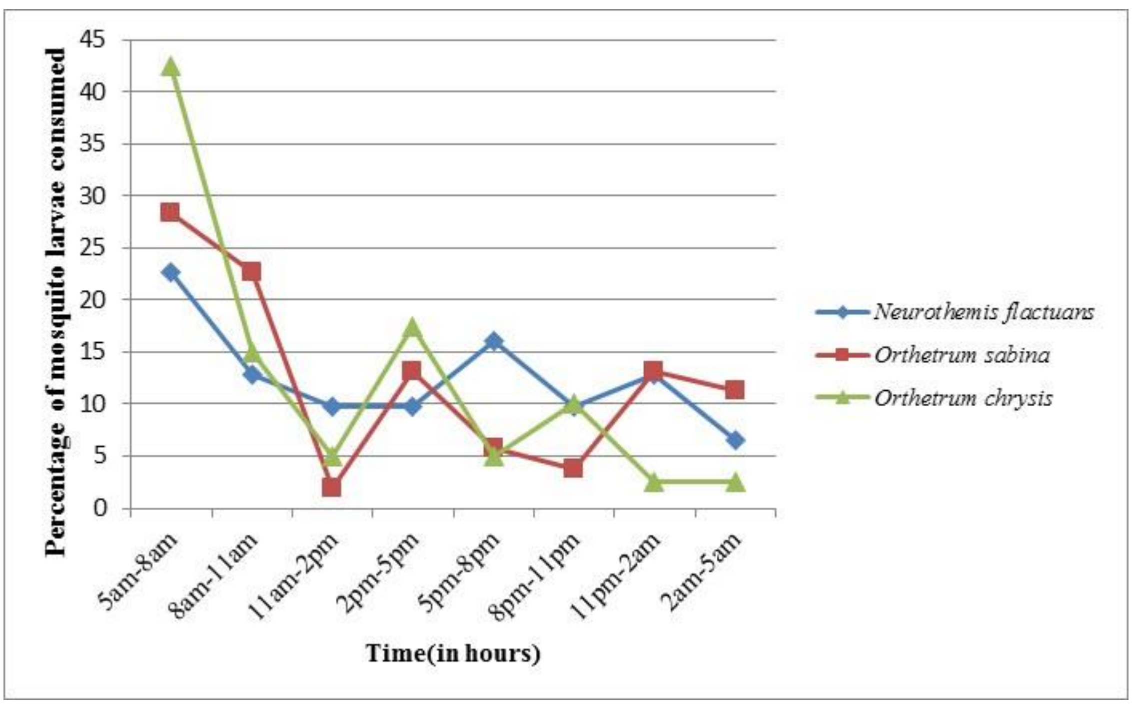


Figure 8.3 **Comparative consumption patterns of different odonate nymph species with respect to the different times of a day, under laboratory conditions towards *Ae. albopictus* larvae (*n* = average across 3 replicates)**

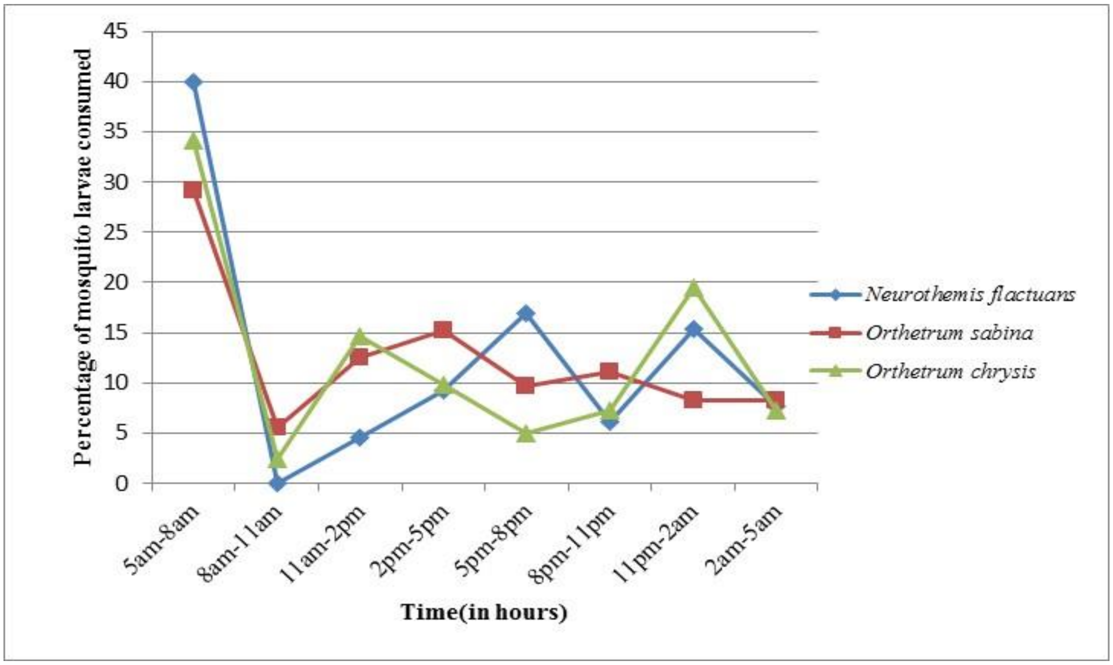


Figure 8.4 **Comparative consumption patterns of different odonate nymph species with respect to the different times of a day, under laboratory conditions towards *Ae. aegypti* larvae (*n* = average across 3 replicates)**

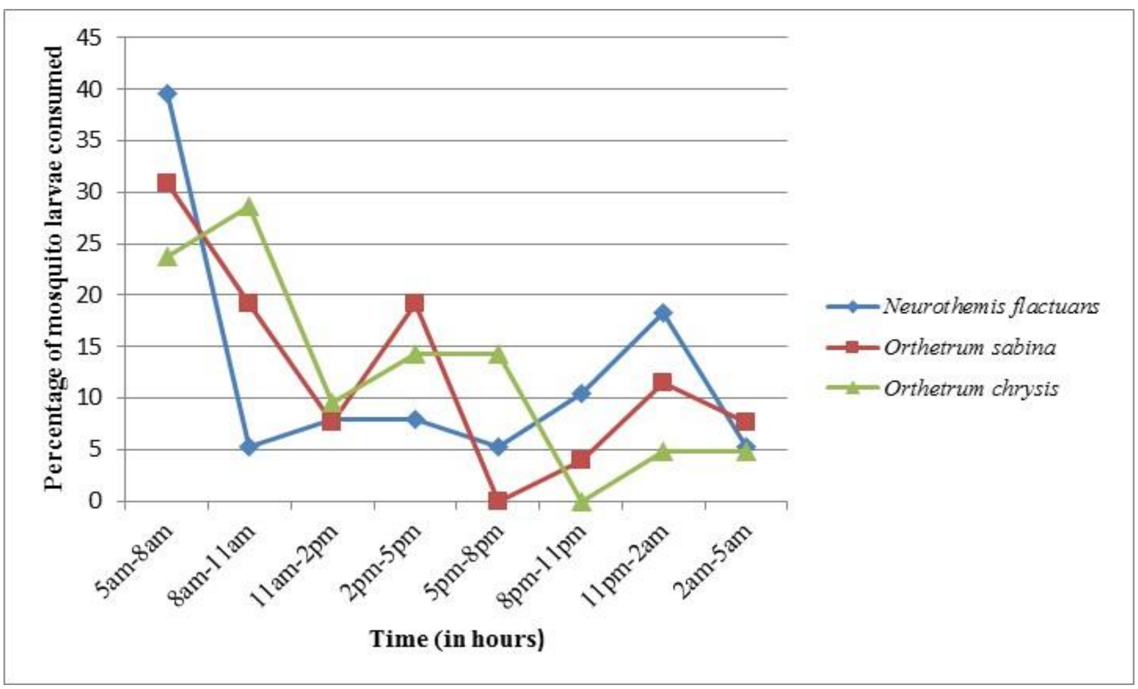


Figure 8.5 **Comparative consumption pattern of different odonate nymph species with respect to the different times of a day, under laboratory conditions towards *Cx. quinquefasciatus* larvae (*n* = average across 3 replicates)**

Table 8.4 **Results of two-way ANOVA on feeding consumption of Odonata species and mosquito larvae species during light on and light off.**

Source of variation	**SS**	**df**	**MF**	**F**	**P value**
Time	640.667	1	640.67	14.09	.00
Odonata species	69.481	2	34.74	.76	.47
Time x Odonata species	109.778	2	54.89	1.21	.30
Error	9549.333	210	45.47		
Total	17260.000	216			

8.4 Feeding Experiment of *Poecillia reticulata*

The daily feeding rate was assessed by exposing the 100 individuals of 4th instar mosquito larvae of every species to single predator species. The number of larvae left was recorded at every 3-hour intervals and the experiment was carried out for 24 hours. Overall there was a higher consumption of mosquito larvae by female in contrast to male guppies as shown in Figure 8.2 [ANOVA, $F(1, 144) = 4.127$ $p < 0.05$]. It was observed that the female guppies were more aggressive than male guppies as they consumed more mosquito larvae species. Both male and female guppies spent most of their time on surface water and were active in searching mosquito larvae, but the female guppies were more aggressive than male guppies. When the mosquito larvae were released in the aquaria, the first attack of guppy was very fast.

There was significant difference between male and female guppy with mosquito species [ANOVA, $F(2, 144) = 4.98$ $p < 0.05$]. Feeding rate of male and female guppy was different between mosquito species. Table 8.4 illustrates the lower consumption of *Cx. quinquefasciatus* larvae by the guppies in contrast to both species of *Aedes*. Female guppy also showed the similar result as they consume more on *Ae. aegypti* larvae, followed by *Ae. albopictus* and *Cx. quinquefasciatus*. This trend in common to both male and female guppies showing similar preferences for all 3 species of mosquito larvae [ANOVA, $F(2, 144) = 0.48$ NS].

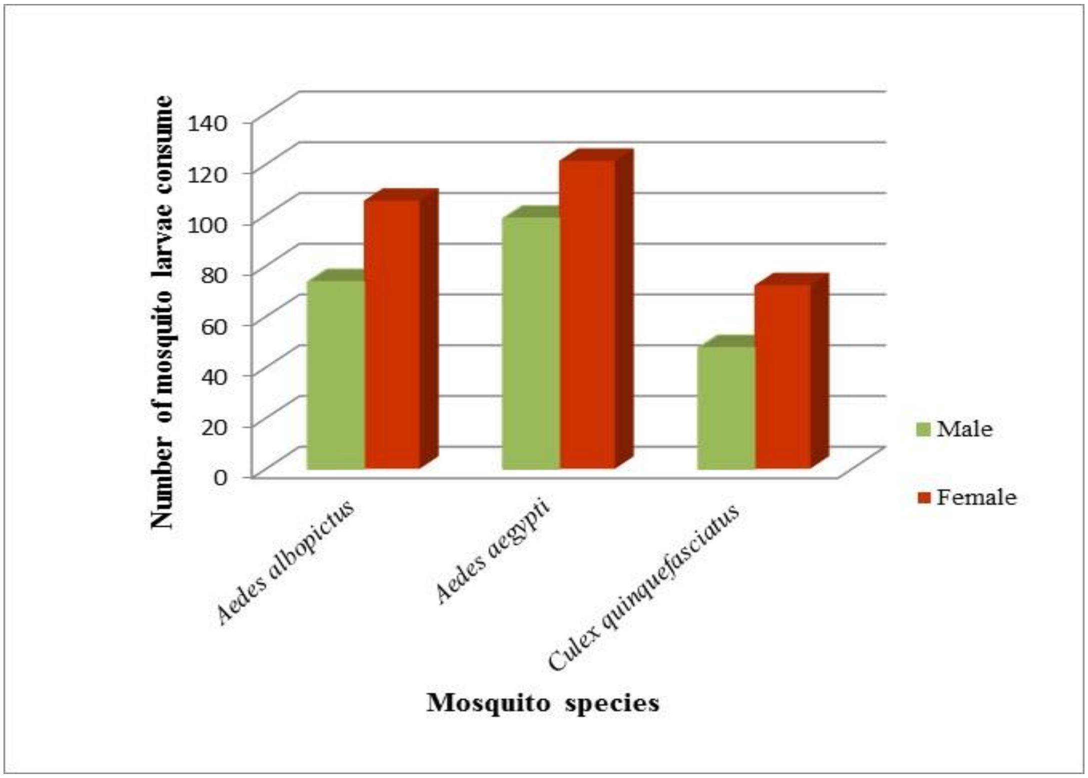


Figure 8.6 **Feeding rates of male and female guppies on *Cx. quinquefasciatus, Ae. albopictus* and *Ae. aegypti* larvae**

Table 8.5 **Results of two-way ANOVA on feeding consumption of male and female guppy and mosquito larvae species**

Source of variation	**SS**	**df**	**MF**	**F**	**P value**
Larvae species	948.39	2	474.19	4.975	.008
Guppy (male/female)	393.36	1	393.36	4.127	.044
Larvae species × guppy (male/female)	9.06	2	4.53	.048	.954
Error	13152.50	138	95.30		
Total	31360.00	144			

8.4.1 Feeding Experiment of between Light on and Light off

The feeding rate between light on and light off also varied between male and female guppies, but both were active during light on. As shown in Figure 8.7, both predators were active during light on as they consumed more mosquito larvae during this time. The statiscally shows F (1, 144) = 29.33 p< 0.05 by using ANOVA analysis (Table 8.6). The comparative consumption pattern of male and female guppy with respect to the different times of a day, under laboratory conditions towards *Ae. albopictus* larvae (Figure 8.8), *Ae. aegypti* larvae (Figure 8.9) and *Cx. quinquefasciatus* larvae(Figure 8.10). The duration between 5am-5pm (as light on) and 5pm-5am (as a light off) to see the different pattern of consumption. Boths guppy male and female were active during light on when exposed with all three larvae species.

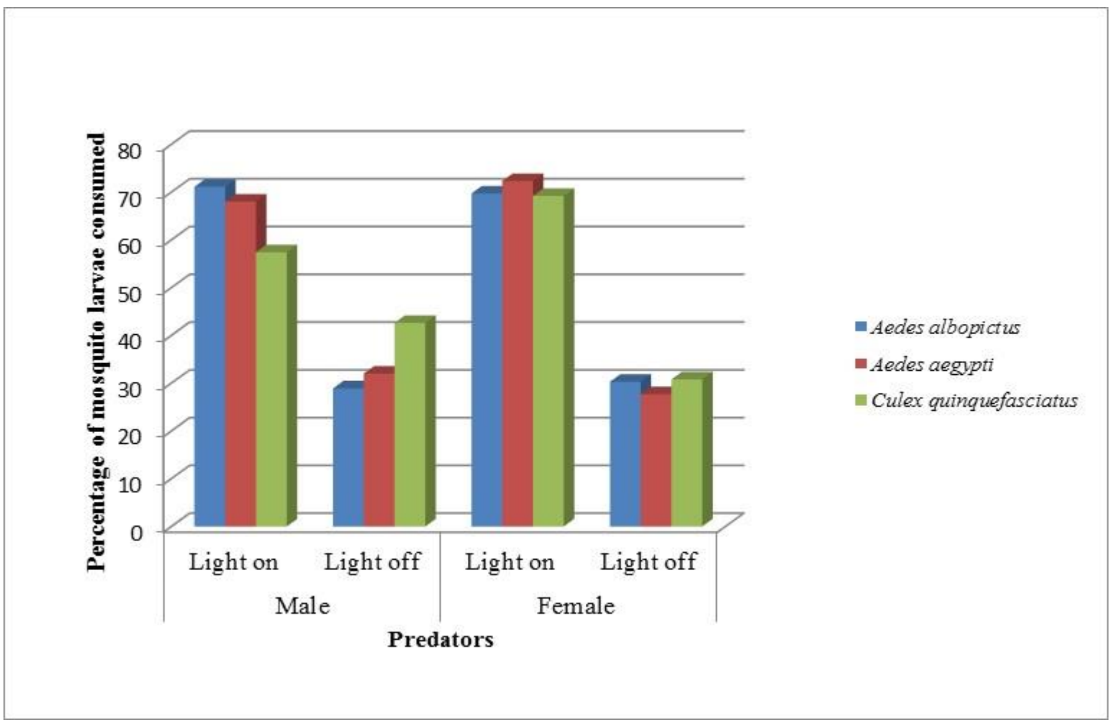


Figure 8.7 The percentage of 3 mosquitoes prey species consumed by guppies.

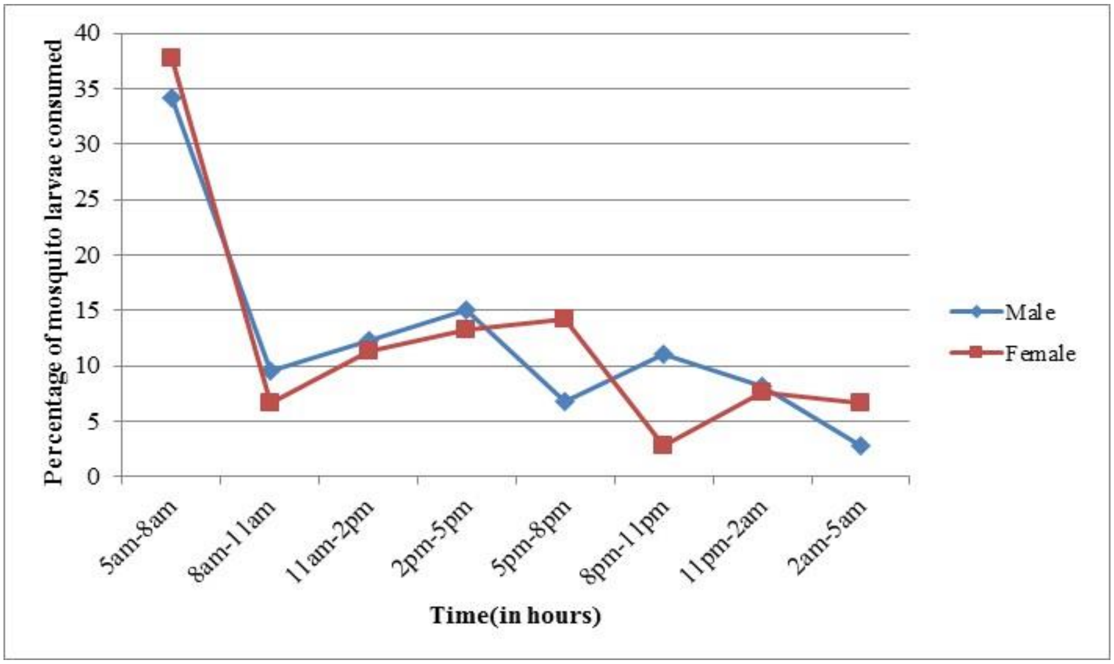


Figure 8.8 Comparative consumption patterns of male and female guppies with respect to the different times of a day, under laboratory conditions towards *Ae. albopictus* larvae (*n* = average across 3 replicates)

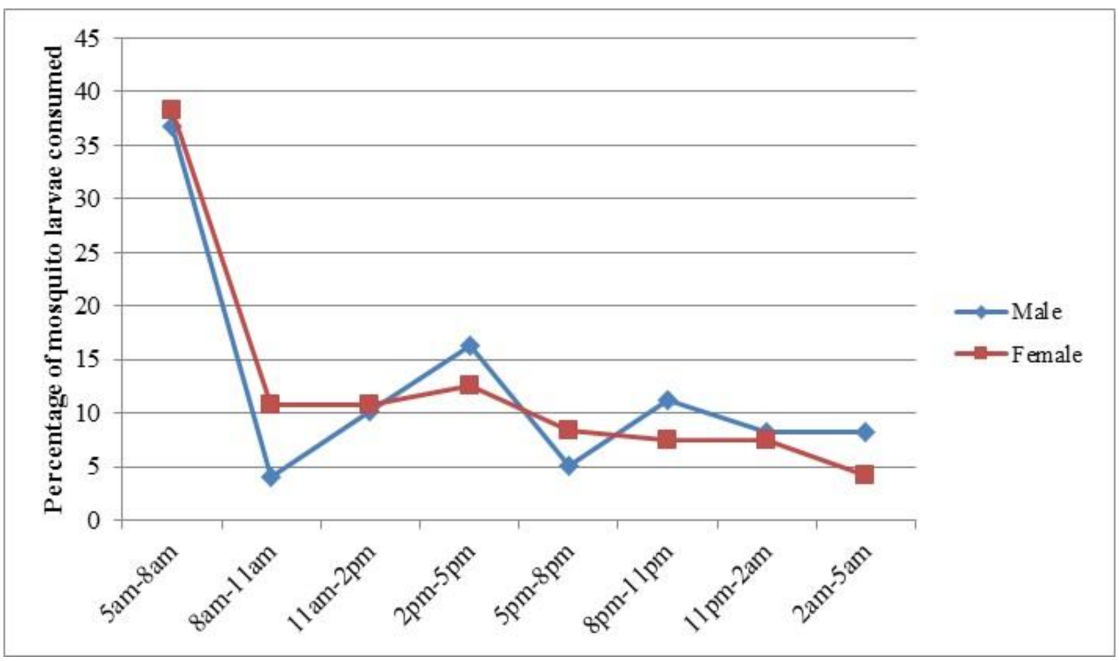


Figure 8.9 Comparative consumption patterns of male and female guppies with respect to the different times of a day, under laboratory conditions towards *Ae. aegypti* larvae (*n* = average across 3 replicates)

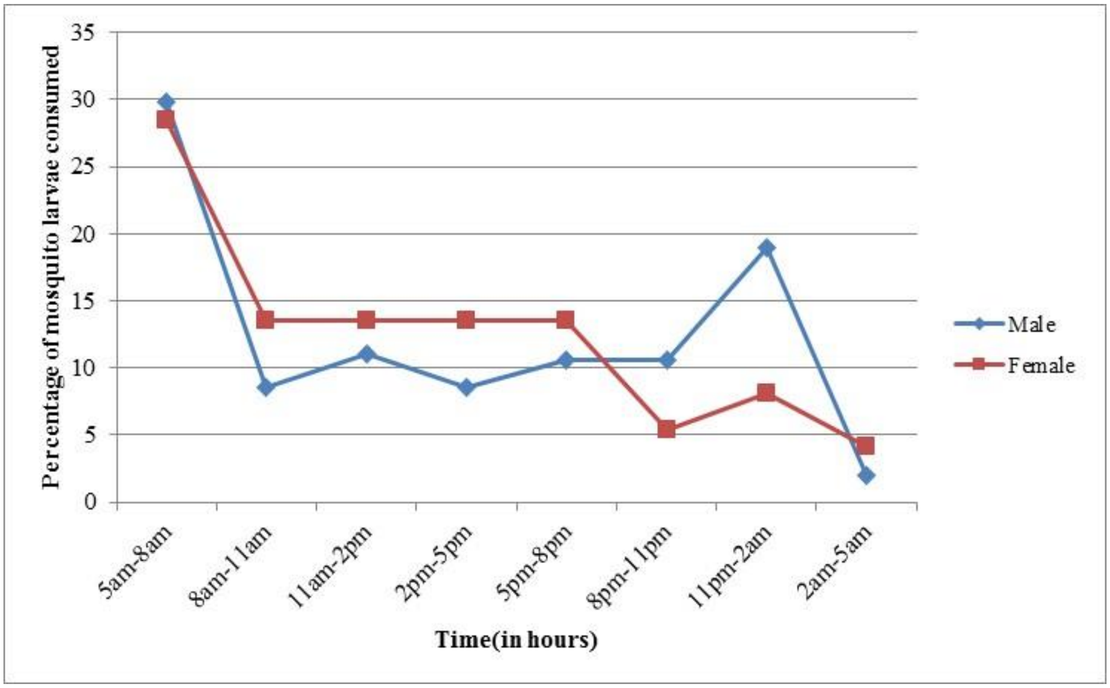


Figure 8.10 **Comparative consumption pattern of male and female guppies with respect to the different times of a day, under laboratory conditions towards *Cx. quinquefasciatus* larvae (*n* = average across 3 replicates)**

Table 8.6 **Results of two-way ANOVA on feeding consumption of male and female guppy and mosquito larvae species during light on and light off.**

Source of variation	SS	df	MF	F	P value
Time	2417.36	1	2417.36	29.33	.000
Guppy (male& female)	393.36	1	393.36	4.77	.000
Time x Guppy(male& female)	156.25	1	156.25	1.90	.031
Error	11536.33	140	82.40		.171
Total	31360.00	144			

8.5 Predation Experiment

8.5.1 Predation Experiment of Dragonfly Nymphs and *Poecilia reticulata*

The predation experiment of dragonfly nymphs towards mosquito larvae species with variation in number of predator, water volume and number of predators were presented in Figures 8.11 – 8.13. To investigate the efficiency of predatory of the selected 3 species of dragonfly nymph on 3 species of mosquito larvae, 3 types of variable were introduced: (i) the predator number was either 1 or 2, (ii) the water volume was either 1 or 2 liters and (iii) the prey density was either 100 or 200 in number of individuals. Three Odonata species were used in this experiment, they were *N. fluctuans, O. sabina* and *O. chrysis* as a predator and three mosquito larvae species as a prey: *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* larvae.

Overall, the 3 species of dragonfly nymphs were consumed higher number of mosquito larvae in 2 conditions which is in (2 predators× 1 liter of water ×100 mosquito density) and in (1 predator × 1 liter of water × 200 mosquito density). However, dragonfly nymphs were consumed small number of mosquito larvae when exposed in 2 liters of water volume.

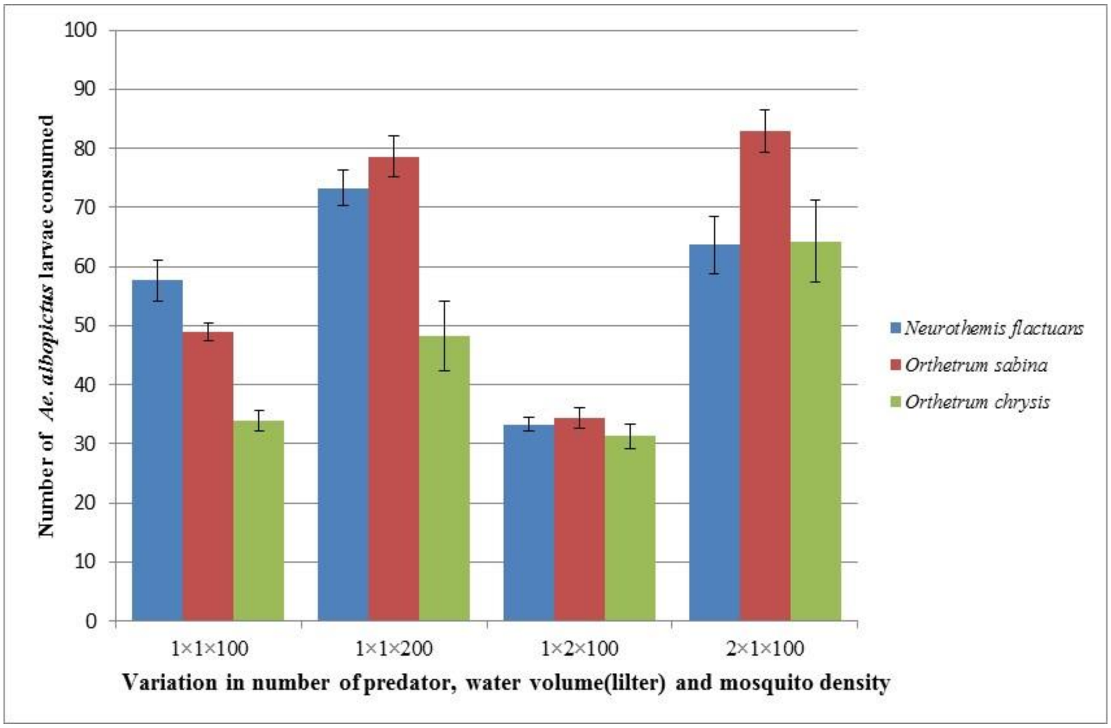


Figure 8.11 **Variations in daily feeding rate of three Odonata nymph species on the fourth-instar *Aedes albopictus* larvae with variation in prey density, water volume and number of predator**

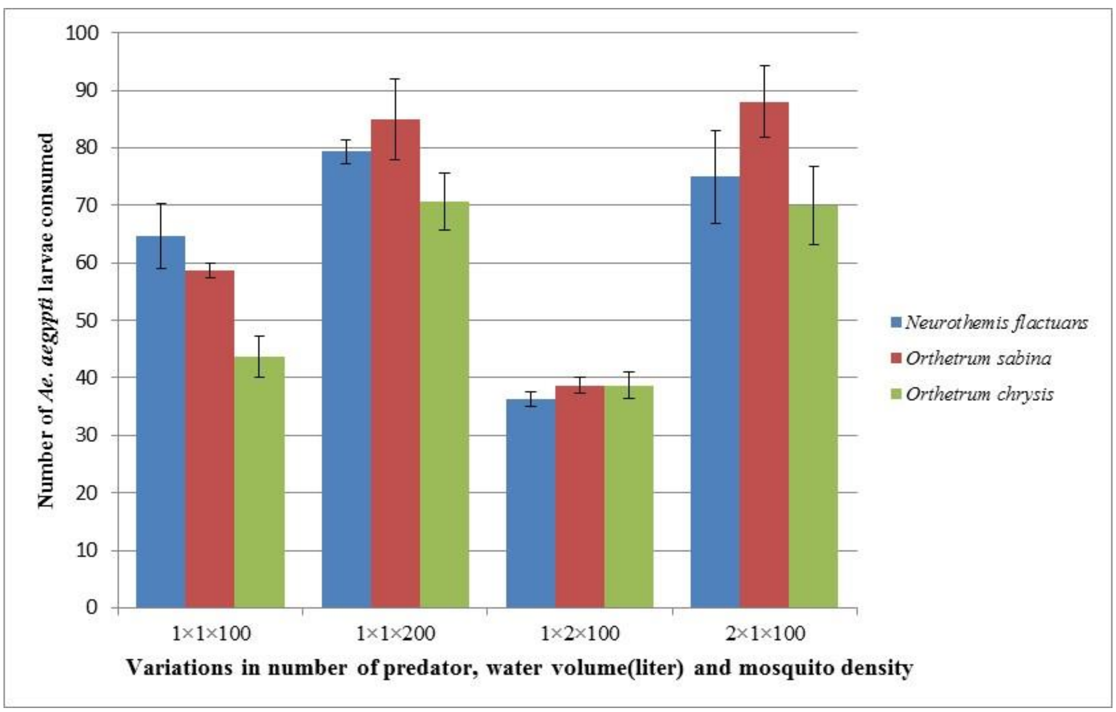


Figure 8.12 **Variations in daily feeding rate of three Odonate nymph species on the fourth-instar *Aedes aegypti* larvae with variation in prey density, water volume and number of predator**

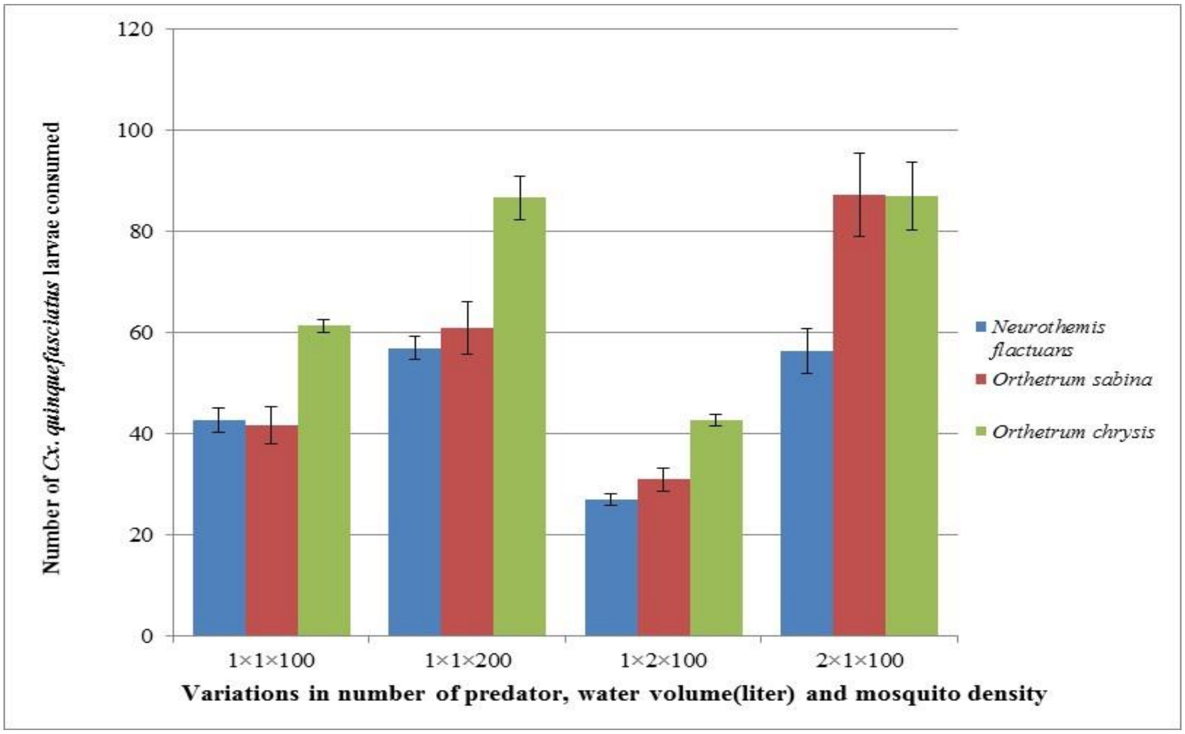


Figure 8.13 Variations in daily feeding rate of three Odonate nymph species on fourth-instar *Cx. quinquefasciatus* larvae with variation in prey density, water volume and number of predator

The predation experiment also observed in male and female guppy towards three species of mosquito larvae (Figures 8.14 – 8.16). In all three experiments it showed that female guppy consumed higher number of mosquito larvae than male guppy except in one condition when male guppy consumed more larvae of *Cx. quinquefasciatus* larvae in (2 predators × 1 liter of water × 200 mosquito density).

Female guppy consumed high number of mosquito larvae in (1 predator × 1 liter of water × 200 mosquito density) of *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* larvae (Figures 8.14 – 8.16).

Male guppy consumed high number of mosquito larvae in different condition for example they consumed more *Ae. albopictus* and *Cx. quinquefasciatus* larvae in (2 predators × 1 liter of water × 100 mosquito density) but they consumed more *Ae. aegypti* larvae (1 predator × 1 liter of water × 200 mosquito density). In all three experiments, both male and female guppies consumed small number of mosquito larvae when exposed with (1 predator × 2 liter of water × 100 mosquito density), where the water volume was increased.

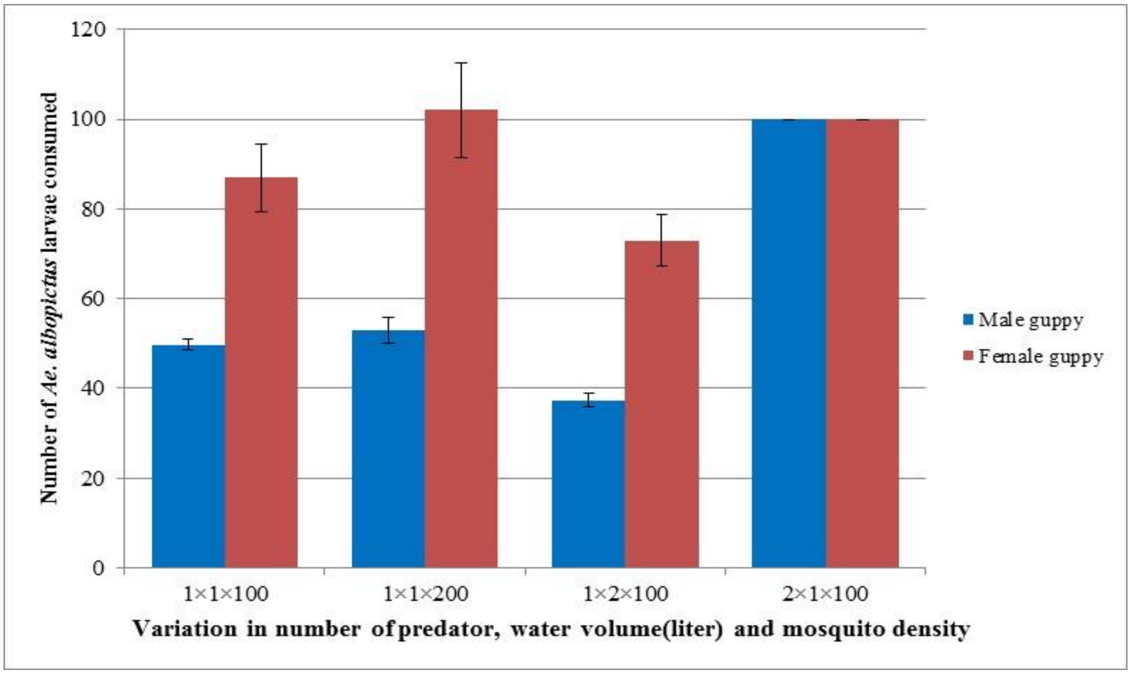


Figure 8.14 Variations in daily feeding rate of male and female guppies on fourth-instar *Aedes albopictus* larvae with variation in prey density, water volume and number of predator

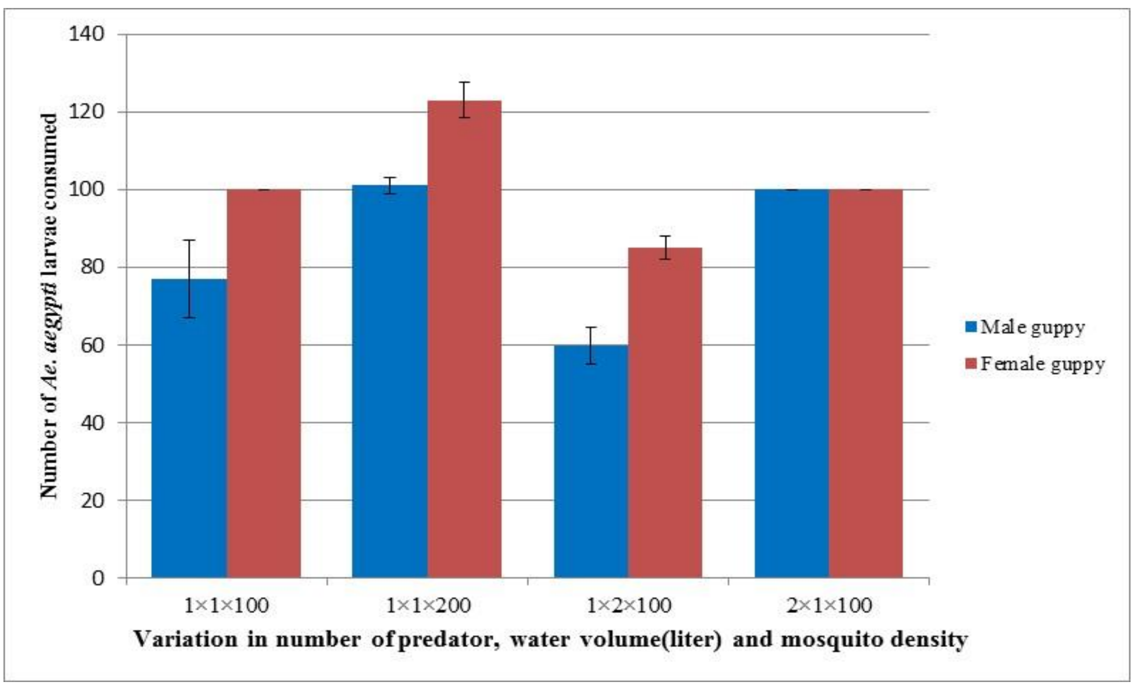


Figure 8.15 Variations in daily feeding rate of male and female guppies on fourth-instar *Aedes aegypti* larvae with variation in prey density, water volume and number of predator

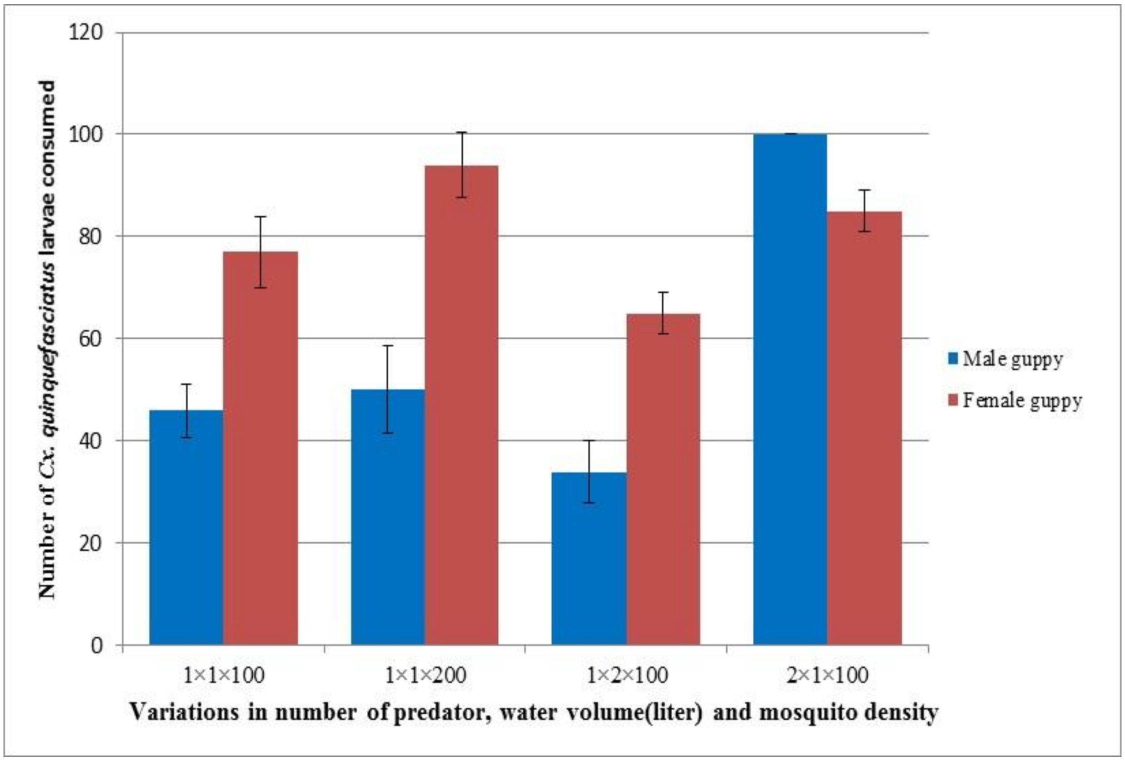


Figure 8.16 Variations in daily feeding rate of male and female guppies on fourth-instar *Cx. quinquefasciatus* larvae with variation in prey density, water volume and number of predator

Table 8.7 shows the regression equations provided by multiple regression analyses for predation on *Ae. albopictus* larvae by Odonata species. From the regression equation, it was observed that factors such as number of predator, water volume and prey density influenced the feeding consumption of Odonata species. For *N. fluctuans* prey density was significantly affected the predation activities. The feeding rate of *N. fluctuans* was higher when the prey density was low than when the prey density was high. The factor that influences predation activities for *O. sabina* was water volume. Its show negative relationship between feeding consumption and water volume; the feeding rate decreased with increased water volume. The feeding rate of *O. chrysis* increased when the number of predator increased.

Multiple regression analyses for predation of *Ae. aegypti* larvae by odonate species have been depicted in Table 8.8. Only water volume and prey density were influenced the predation activities. For *N. fluctuans* and *O. chrysis* the prey density was influenced the predation activity. The feeding rate was higher when the prey density was low than when the prey density was high. Water volume influenced the predation activities of *O. chrysis.* The *O. chrysis* eat more larvae during water volume decreased.

Three factors such as number of predator (X1), water volume (X2) and prey density (X3) were influenced the predation activities(Table 8.9). For *N. fluctuans,* water volume was influenced the predation activities. Two factors influenced predation activities between *O. sabina* and *Cx. quinquefasciatus* larvae. When the number of predator increased the feeding rate also increased and they eat less when number of prey increased. However, for *O. chrysis* only one factor influenced the predation activity which is prey density.

Table 8.7 **The regression equations of predation on *Aedes albopictus* larvae by different Odonate nymphs (Y) against the number of predator (X1), water volume (X2) and prey density (X3) as variables**

Predators (Odonata species)	Regression equations	R value
Neurothemis fluctuans	Y = 96.444 – 31.556 X3	0.88
Orthetrum sabina	Y = 106.111 – 35.889 X2	0.75
Orthetrum chrysis	Y =11.444 – 26.44 X1	0.78

Table 8.8 **The regression equations of predation on *Aedes aegypti* larvae by different Odonate nymphs (Y) against the number of predator (X1), water volume (X2) and prey density (X3) as variables**

Predators (Odonata species)	Regression equations	R value
Neurothemis fluctuans	Y = 109.667 – 36.667 X3	0.87
Orthetrum sabina	Y = 115.778 – 38.556 X2	0.78
Orthetrum chrysis	Y = 84.222 – 22.778 X3	0.61

Table 8.9 **The regression equations of predation on *Cx. quinquefasciatus* larvae by different Odonate nymphs (Y) against the number of predator (X1), water volume (X2) and prey density (X3) as variables**

Predators (Odonata species)	Regression equations	R value
Neurothemis fluctuans	Y = 77.000 – 25.000 X2	0.84
Orthetrum sabina	Y = 35.667+ 36.00 X1– 20.333 X3	0.89
Orthetrum chrysis	Y = 114.000 – 35.667 X3	0.79

Table 8.10 shows the regression equations provided by multiple regression analyses for predation on *Ae. albopictus* larvae by male and female guppies. From the regression equation, it was observed that factors such as number of predator, and water volume influenced the feeding consumption of male guppy. Whereas for the female guppy only water volume significantly affected the predation activities. It showed negative relationship between feeding consumption and water volume; the feeding rate decreased with increased water volume. The feeding consumption increased when the numbers of predator increased.

Multiple regression analyses for predation of *Ae. aegypti* larvae by guppies have been depicted in Table 8.11. Only water volume and prey density influenced the predation activities. The feeding rate was higher when the prey density increases and feeding rate increases when water volume decreases.

Table 8.12 showed the factors that influenced the predation of both male and female guppies towards *Cx. quinquefasciatus* larvae. When the number of predator increased the feeding rate also increased and feeding rate decreased when search area was increased (water volume increased).

Table 8.10 The regression equations of predation on *Aedes albopictus* larvae by male and female guppy (Y) against the number of predator (X1), water volume (X2) and prey density (X3) as variables

Predators	**Regression equations**	**R value**
Male guppy	Y = 16.67 + 48.67 X1 – 14.00 X2	0.99
Female guppy	Y = 119.67 – 23.33 X2	0.66

Table 8.11 The regression equations of predation on *Ae. aegypti* larvae by male and female guppy (Y) against the number of predator (X1), water volume (X2) and prey density (X3) as variables

Predators	**Regression equations**	**R value**
Male guppy	Y = 125.33 – 32.67 X2	0.75
Female guppy	Y = 92.00 – 15.00 X2 + 23.00 X3	0.96

Table 8.12 The regression equations of predation on *Cx. quinquefasciatus* larvae by male and female guppy (Y) against the number of predator (X1), water volume (X2) and prey density (X3) as variables

Predators	**Regression equations**	**R value**
Male guppy	Y = – 13.33 + 56.67 X1	0.92
Female guppy	Y = 105.67– 20.33 X2	0.67

CHAPTER 9

DISCUSSION

9.1 Mosquito Diversity in Urban and Suburban Areas

Mosquitoes are a significant vector and human health issue in the world. Mosquitoes can be found in a variety of habitats in urban and suburban areas. Vector ecology and disease epidemiology are strongly affected by environmental changes. The present study revealed that the six localities in both study areas showed different number of mosquito larvae density. As reported by many researchers, factors contributing risk factors that contribute to the abundance of mosquito larvae were higher density of human populations which also mean more opportunities for *Ae. albopictus* blood feeding, tropical urban environment, crowded human living populations, increasing human population mobility and habitat modifications by humans positively influenced the diversity of the mosquito species (El-Badry & Al- Ali, 2010; Gubler, 2011b; Thongsripong *et al.* 2013; Li *et al.* 2014).

Our results contradicted the previous finding of a study done in the Sisaket province in Thailand that the number of mosquitoes collected in urban did not differ from the rural areas because the degree of urbanization there was low (Hammon *et al.* 1960).

Many species of mosquitoes can be found worldwide and the mosquito species may be different in terms of habitats, seasonal factors and other factors. Chong and Wada (1988) mentioned that different number of species occurred due to several factors such as the collection techniques, geographical variations or general change in the population distributions of various mosquito species and the climatic variations. For instance, a survey which was carried out in Saudi Arabia, found five species consisting of *Ae. caspius* Pallas, *An. multicolour* Cambouliu, *Cx. perexiguus* Theobald, *Cx. pipiens* L. and *Cx. pusillius*

Macquart (Ahmed *et al*. 2011). This study found three common species which are *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* in both urban and suburban areas. A study reported by Yap (1975) in Malaysia found three common mosquito larvae species of *Cx. quinquefasciatus* (Say), *Ae. albopictus* (Skuse) and *Ae. aegypti* (Linnaeus) abundant in both urban and suburban areas. Abu Hasssan *et al.* (2005) reported three common mosquito larvae species of *Cx. quinquefasciatus, Ae. albopictus* and *Ae. aegypti* found in construction sites in East Malaysia. In contrast, a study conducted by Dev *et al.* (2014) has revealed that both *Ae. aegypti* and *Ae. albopictus* were widely abundant in city and suburban, breeding in a wide variety of resources. Thongsripong *et al.* (2013) conducted a study on diversity of mosquito species in six different habitats. They concluded that the relative abundance of vector varied by habitats with the lowest diversity and highest abundance of certain vectors occurring in urban environments, whereas other vectors were most abundant in different habitats depending on their biology.

From this study *Ae. albopictus* was found to be predominant in both study areas as larvae survey was carried out in the outdoor areas only where *Ae. albopictus* is known to be a container breeder and mostly found in outdoor areas. However, Dieng *et al*. (2010) observed *Ae. albopictus* larvae in most containers within homes in Northern Peninsular Malaysia and Ae. *albopictus* lives longer in the indoor environment.

In contrast, Vijayakumar *et al.* (2014) reported that *Ae. albopictus* larvae are the most common species distributed equally in urban and rural areas in India and this is due to the significant presence of vegetation in the study area. Their finding supports this study as both urban and suburban study areas have a lot of vegetation. Moreover, other researchers also stated that in the domestic environments, *Ae. albopictus* prefers vegetation and feeds and rests outdoor (Niebylski *et al.* 1990; Iliga *et al.* 2001).

Maimusa *et al*. (2012) reported *Cx. quinquefasciatus* larvae coexisted with *Ae. albopictus* larvae. Beside *Ae. albopictus* larvae, others species that was found during the larvae surveillance were *Ae. aegypti* and *Cx. quinquefasciatus* with smaller percentages in both urban and suburban areas. This study was supported by others researchers who found *Aedes* species with *Cx. quinquefasciatus* with smaller percentages (Vijayakumar *et al.* 2014; Philbert & Ijumba, 2013). *Culex* mosquito species breeds in a wide range of habitats. For instance they were found in tanks, puddles, tyres tracks, pools metal and plastic containers. *Cx. quinquefasciatus* is predominantly associated with urban areas but occurring also in rural. *Cx. quinquefasciatus* preferentially breeds in organically rich water (Mwangangi *et al*. 2009; Okiwelu & Noutcha, 2012). Genus *Culex* is mainly found in highly polluted urban habitats like drainages (Chaves *et al.* 2010). Asha and Anesh (2014) reported that they found *Culex* species as the most predominant genus among others genera of *Aedes*, *Anopheles*, *Mansonia* and *Armigeres*. Stoops *et al*. (2008) collected five *Culex* vector species in the rice fields of Indonesia which were *Cx. fuscocephala*, *Cx.gelidus*, *Cx.pseudovishnui*, *Cx. tritaeniorhyncus* and *Cx. vishnui.* In the East Malaysia the dominant species of *Cx. tritaeniorhynchus* was found in rice fields (Surtees, 1970). Mwangangi, *et al.* (2009) found *Anopheles* and *Culex* mosquito larvae species living together in puddles, tyre tracks and pools containing highly turbid water. This study confirms the mixed breeding pattern of *Cx. quinquefasciatus* and *Ae. aegypti* in urban areas and *Cx. quinquefasciatus* and *Ae. aegypti* in suburban areas.

9.2 Mosquito Breeding Habitat

Mosquitoes are known to breed successfully in many types of areas including natural habitats and artificial containers that contain stagnant water. In this study the main breeding habitats for mosquito were different between urban and suburban areas where in urban areas the major breeding habitats for mosquitoes were gardening utensils whereas in suburban areas the major breeding habitats were artificial containers. This finding is similar to Takagi *et al*. (1990) who also reported a variety and different density of potential containers in rural and urban areas. Wongkoon *et al*. (2013) also found different breeding sites in urban and rural areas in Thailand which comprised of natural and trash containers. This happens may be because the breeding sites identified in different areas reflect the change in ecology, cultural and social behaviour of human population and life style changes of human communities (George & Chattopadhyay 2001; Tyagi *et al.* 2003). According to Singh *et al.* (2013) the contribution of *Aedes* breeding was affected by different income group of communities in India. They found different localities contributing different breeding sites of mosquito larvae. Higa (2011) stated that since the lifestyle and customs of people vary among countries and regions, the environments for *Ae. aegypti* and *Ae. albopictus*, for instance the larval breeding sites which are usually artificial containers, housing structures, garden and others also vary.

The gardening utensils include potential places for mosquito breeding which comprised of flower pots, flower pot plates and watering cans. From direct observation in the residential areas it seemed that all residents have a mini garden outside their houses which naturally increase the potential places for mosquitoes. Nyamah *et al*. (2010) also found that the main breeding sites for *Aedes* spp. consisted of garden accoutrements such as flower pots, flower pot plates, vases and watering cans. Li *et al.* (2014) conducted a study on *Ae. albopictus* larval habitat and they found that mosquito habitats are flowerpots and plastic buckets in urban areas and plastic buckets and disposal containers in suburban areas which are similar in this study. It is proposed that the residents there should have proper waste management system to prevent them from throwing rubbish with unused containers outside their houses and eventually can encourage mosquitoes to breed. Discarded items found during larvae survey activity included tin, polystyrene and others. In contrast, Philbert and Ijuma (2013) concluded that the flower pots were the least preferred mosquito breeding sites in their mosquito surveillance study in Tanzania.

The unused flower pot that contains water was the suitable place for mosquito breeding. It was also found that the residents did not maintain their garden well and often discard unused containers in the garden. In tropical countries, anything that retained water would be potential breeding sites for *Aedes* mosquitoes within human dwellings (Isaacs, 2006). Containers that retain water for long time will make good or suitable breeding habitats of mosquitoes like the artificial containers in Putrajaya and Kuala Selangor. Besides garden utensils other breeding places in urban areas in decending propotions were artificial container (23%), building design (9%), discarded items (7%), rubbish bins (6%), tyres (5%), water storage (3%) and natural habitat (3%). In both study areas, the higher proportion of the breeding sites were artificial containers than in natural containers. This result supports a study by Wongkoon *et al.* (2007) who found that there were higher number of mosquito larvae in articifial containers than natural containers. This could be due to the availability of the artificial containers which were higher in both study areas than the natural habitats. Kristen *et al.* (2012) suggested that artificial containers such as tyres, buckets, planter dishes, traps and natural tree holes are the major breeding habitats of *Aedes* mosquitoes.

Other structures of building design which include sand trap, floor and floor traps of houses in Putrajaya also provide potential breeding places for mosquitoes. It was apparent that every house in Putrajaya was designed equipped with sand traps which increased the sites for mosquitoes to breed. According to Wongkoon *et al.* (2013) as water supply is readily available in the urban areas, residents do not need to store water inside and around the house. The possible larval habitat for Aedes mosquitoes in the urban areas is the concrete drainage systems. Construction techniques and design of the construction sites, such as the building of roads, drainage and canal developments, may create artificial breeding sites for mosquitoes and biting midges because of the environmental modifications (Scott, 2002). This is also supported by Gustave *et al.* (2012) in a study where they found roof gutters are becoming the most important *Ae. aegypti* breeding sites with consequences on dengue transmission and vector control.

In this study, discarded tyres were one of the breeding sites found in both study areas. In India, Tanzania and United State discarded tyres were found as the most efficient breeding places as recorded with the highest number of *Aedes* larvae species (Vijayakumar *et al.* 2014; Philbert & Ijuma, 2013; Bartlett-Healy *et al.* 2012). Discarded tyres were also found to be the positive breeding habitats for mosquito larvae especially *Ae. albopictus* which preferred to breed in tyres as supported by the work of Rao (2010) however in Philippines *Ae. aegypti* larvae was found in used tyres (Cruz, *et al*. 2008). In India both species *Ae. albopictus* and *Ae. aegypti* larvae were found in used tyres (Kusumawathie & Fernando, 2003). Kling *et al*. (2007) reported that the discarded tires were important larvae breeding sites for larvae of multiple species. In their study, they found the *Culex restuans* as dominant species in tyres at the unforested site and *Ochlerotatus triseriatus, Anopheles barberi* and

Orthopodomyia signifera were found primarily in the forested areas. The difference in the mosquito composition between the forested and an unforested location was due to the detritus type, amount and nutrient content found in the trapped water containers. Previous study by Qualls and Mullen (2006) reported that *Ae. albopictus* was the most common species collected from tyres in Alabama in the absence of *Ae. aegypti* found in the tyres during the survey that was conducted outdoors. It seemed that *Ae. albopictus* was displaced by *Ae. aegypti* as the tire breeder. Studies in some other countries like India (Kusumawathie & Fernando, 2003); Philippines (Cruz *et al*. 2008) and Trinidad (Hemme *et al*. 2009) have reported water storage containers as the main breeding habitats for *Aedes* mosquitoes.

The major breeding habitat in the suburban area was artificial containers comprised of 48% and other breeding habitats were gardening utensil (23%), water storage (11%), tires (8%), discarded items (8%) and rubbish bin (2%) in smaller proportions. In Brazil, they found non-useful or non-returnable containers such as metal can and plastic bottle as major breeding habitats that were positive for *Ae. aegypti* larvae (Mazine *et al*. 1996). In India wastes of four major categories, namely earthen, porcelain, plastic and coconut shells were positive with *Aedes* larvae and the number of waste containers varied significantly with respect to locations, types and months (Banerjee *et al*. 2012).

As mentioned by Li *et al.* (2014) five factors that influence the presence of *Ae. albopictus* larvae were urban habitats, preference to breed in water surface (water depth), clean water rather than polluted water, shaded areas, habitats or breeding sites with food sources such as leaves. Mosquito larvae breeding sites can be found in both natural and man-made habitats. Some mosquito species preferred natural habitats while others preferred man-made containers. In Sarawak, East Malaysia, the dominant species in urban areas were *Ae. albopictus* and *Ae. aegypti.* The breeding sites for *Ae. albopictus* include man-made containers and natural habitats like coconut husks, bamboo stumps and *Colocasia* axils but in contrast, *Ae. aegypti* was only found in man-made containers (Surtees, 1970). According to Rao (2010), *Ae. albopictus* is a container breeder which breeds in both natural and man-made habitats. *Ae. albopictus* is more likely to be found in natural containers or outdoor man-made habitats containing a greater amount of organic debris (Rattanarithikul & Panthusiri, 1994). Wongkoon *et al.* (2007), revealed that *Aedes* larvae preferred outdoor breeding sites in containers without lids. This is because the organic material and leaf litter falling into the water containers serve as the nutrient for mosquito larvae. In other study, the researchers found that the highest number of *Ae. aegypti* larvae and pupae were found in roof gutters containing water with sediment and water with vegetal detritus (Gustave *et al.* 2012).

Thavara *et al*. (2001) reported that *Ae. albopictus* most preferred outdoor breeding habitats in Thailand and from the results of their study almost 1000 outdoor natural breeding sites that were surveyed around the island had 45% of the 623 coconut husks and 10% of 360 coconut floral spathes infested with *Ae. albopictus* larvae. Studies conducted by Nyamah *et al*. (2010) in Malaysia found that all the containers containing *Ae. albopictus* were found outdoors, while three out of four containers positive for *Ae. aegypti* were also found outdoors. Whereas *Ae. aegypti* commonly breeds and feeds inside houses, *Ae. albopictus* is more common outside, in open spaces with shaded vegetation and suitable breeding sites such as car tyres and garbage dumps (WHO, 1986). However in Indonesia, *Ae. aegypti* larvae were found outdoor rather than indoor areas (Syarifah *et al*. 2008). This study also indicated that *Ae. albopictus* was the most dominant mosquito species found in both study areas together with other species that were found outdoors such as *Ae. aegypti* and *Cx. quinquefasciatus*. *Ae. aegypti* was found outdoors together with *Ae. albopictus* and *Cx. quinquefasciatus*. This result is supported by Rathor (1996) who discovered that *Ae. aegypti* was breeding in natural receptacles like tree holes, but always near human habitation. Other study indicated the same results with the results reported here by Chareonviriyaphap *et al*. (2003) who found that both species *Ae. albopictus* and Ae. *aegypti* breed outside the houses. In contrast with other researchers, it was found that the density of *Ae. aegypti* was high indoor, while that of *Ae. albopictus* was high outdoor (Hawley, 1988; Rodhain & Rosen, 1997).

In India, domestic containers such as cement tank and plastic container contribute to the major breeding habitats for *Aedes* mosquitoes (Balakrishnan *et al*. 2006). Preechaporn *et al*. (2006) reported that *Ae. albopictus* established well and in greater numbers than *Ae. aegypti* in both dry and wet seasons and in all three topographical areas of mangrove, rice paddy and mountainous areas. Most of the *Ae.albopictus* larvae were found in outdoor containers in mangrove and mountainous areas. The storage jars and cement water storage tanks (in bathroom) were the main breeding sites of *Aedes* larvae both indoor and outdoor in both wet and dry seasons. In Thailand, researchers reported that *Ae. albopictus* larvae were found in all water containers outdoor but *Ae. aegypti* was found both in indoor and outdoor containers. This indicated that *Ae. aegypti* and *Ae. albopictus* larvae have different preferred development site that slightly overlap (Wongkoon *et al.* 2007). Lee (1991) also reported that both *Ae. aegypti* and *Ae. albopictus* were breeding indoors and outdoors in a variety of containers. The dominant indoor breeder was still *Ae. aegypti* but both species were equally present in outdoor containers. This changing pattern in the breeding habitats of *Ae. aegypti* may be significant epidemiologically since it is a highly domesticated mosquito and dependent on humans for blood. Other study found only *Ae. aegypti* larvae in indoor areas in the defrostwater collection trays of refrigerators (Srinivasan *et al*. 2007).

The source reduction program which should be implemented as the main breeding habitats of mosquito is artificial containers in suburban and urban areas. Many researchers reported that the abundance of mosquito density depends on environmental factors such humidity, rainfall, temperature and precipitation (Ansari & Razdan, 1998; Chong & Wada 1988; Wada *et al*. 1993). However, besides these, other factors such as the life style of the people as well as the condition of sanitation should also be causative to the density and diversity of breeding containers (Takagi *et al*. 1990) and the availability of breeding sites (Yang *et al*. 2005). It is suggested that the Kuala Selangor (suburban) residents should have proper waste management system and not discard unused containers outside their houses which can become the habitats for mosquito breeding. The source reduction program should be implemented to solve the mosquito problems in these areas.

There are a number of control measures that can be applied for the mosquito breeding prevention. One example which should be promoted is the public participation and change of habits in minimizing the breeding sites by eliminating the unused containers within the vicinity of houses, drainage clearing and proper maintenance of the garden. The unused containers should be disposed properly. The authority should provide proper waste management system for all housing areas. The environmental sanitation such as regular garbage collection and piped water supply would be the most effective larval control measures (Takagi *et al*. 1990).

Health education would be one of the important ways to educate residents on the management of their waste. Residents should be alert and concerned about their housing areas especially when these can contribute to mosquito breeding. The authority should educate and advise the residents on the potential mosquito breeding habitats, the outbreak of diseases as a consequence of the presence of mosquito populations, the dangers of these diseases, how to control and awareness of the controlling measures at the same time promoting the idea that 'prevention is better than cure'. Hence, we can conclude that residents in Putrajaya, within the urban locality should maintain their gardens to ensure the prevention of mosquito breeding especially when using flower pots which contribute as the major breeding sites. In Kuala Selangor, suburban locality, it is suggested that the residents should have a proper waste management system for the housing area because the artificial containers outside their houses were the main breeding habitats for the mosquitoes.

9.3 Entomological indices in both study areas

Larvae survey or entomological survey is an important measure which contributes to calculation of important indices, mainly *Aedes* Index (AI), Breateau Index (BI) and Container Index (CI). These indices are useful in predicting areas with high density of mosquito larvae and proper control measures can be taken. Other useful information which can be obtained such as the mosquitoes density, mosquitoes species, breeding habitat of mosquitoes, (Rozilawati *et al*. (2011); Sharma *et al*. (2008); Singh *et al*. (2010) and can predict the outbreak from the indices for instance the Breateau Index threshold levels indicating risk for dengue (Sanchez *et al*. 2010). The larvae survey was not only done in residential areas or human dwelling (Basker & Ezhil 2012) but in India they also conduct this at the airport and sea port (Gill *et al*. 1996). The indexes are used as prediction or indicators where the control measures will take by the government to the area which is the indices were above the standards.

Three indices were calculated and the results revealed at certain months AI, BI and CI were above standard of MOH. According to Sekhon and Minhas (2014) the high values of three indices may cause the dengue outbreak in future. Katyal *et al.* (1997) and Singh *et al.* (2008) reported during outbreak in India, the three indices AI, BI and CI was recorded with high value of index reading. Singh *et al.* (2014) concluded that the hight entomological incides is due to most of the people may not be aware of the factors exacerbating mosquitoes breeding conditions. A similar observation was made by other researchers (Tandon and Roy, 2000; Singh *et al.* 2008; Singh *et al.* 2010; Singh *et al.* 2011).

As a results from this study both the authorities and communities should caution to the necessary control measures in order to avoid the possibility of future outbreaks of Dengue fever. Similarly, in Vietnam the researchers found that the incidence of dengue fever was significantly associated with the following factors such as higher household index, higher container index and higher Breteau index (Pham *et al.* 2011). Sanchez *et al.* (2006) found that larval indices are useful for identifying high-risk areas for dengue virus transmission.

This study revealed that the readings of the three indices of the *Aedes* Index (AI), Breateau Index (BI) and Container Index (CI) were influenced by the state of awareness of the residents. Other researcher found that the environmental factors such as rainfall, humidity and temperature which could contribute to the dynamic fluctuations of indexes Chong and Wada (1988). Pham *et al.* (2011) reported the risk of dengue was also associated with elevated temperature, humidity and rainfall and also the reading of indices. They suggested that indices of mosquito and climate factors are the main determinants of dengue fever in Vietnam. This finding suggested that the global climate change will likely increase the burden of dengue fever infection in Vietnam, and that intensified surveillance and control of mosquito during high temperature and rainfall seasons may be an important strategy for containing the burden of dengue fever.

Land-use change, including deforestation for agriculture and urbanisation, has coincided with increase in vector-borne diseases worldwide. Land-use change is likely to regulate immature (larvae and pupae) mosquito populations through changes in local temperatures owing to manifold changes to the physical environment (Leisnham *et al.* 2006). Barker *et al.* (2010) found that the seasonal factors such as temperature influenced the abundance of mosquitoes besides, the availability of larval habitats.

The environmental parameters that influence mosquito activities were temperature, relative humidity and rainfall. During this study the heaviest rainfall was in September 2010 which recorded 512.8 mm of the rain. There was little rain in October, July, and June. Chakravati and Kumaria (2005) indicated that analysis of three climatic factors such as rainfall, temperature and relative humidity was really important as these factors could affect the mosquito breeding activities. Moreover, the climatic factors also affect the dengue cases. As the rain increases, the cases of dengue also started rising and with declining rainfall, dengue cases also demonstrated a gradual decline (Karim *et al*. 2012).

Surendran *et al*., (2007) reported the density of *Ae. aegypti* and *Ae. albopictus* larvae were significantly depending on seasonal factor, for instance lower number of *Aedes* larvae recorded during dry season. Furthermore the rainfall showed a positive effect on the density of mosquito larvae. In seasonal country like Japan, Thailand and India the seasonal factor also influences the abundance of *Aedes* mosquitoes and the seasonal abundance of larvae was different in years but generally lower in summer and high in during post monsoon (Ansari & Razdan, 1998; Mogi *et al*. 1998; Rao, 1967; Wada *et al*. 1993).

As proven the abundance of mosquito larvae were high during monsoon and post monsoon season because of very favourable climatic conditions (Katyal *et al*. 2003). However, Srinivasan *et al*. (2007) reported the most abundance of pupae and larvae collected during larvae surveillance was during summer as compared with monsoon season in both towns. Barker *et al*. (2010) found that the seasonal pattern of mosquito may be driven by temperature and availability of larval habitat. In their work, they use a light trap to collect the adult mosquitoes in two different places. The results showed that the abundances of mosquitoes increased in the regions that were drier and warmer. This was due to the adult female mosquito seeking blood meals once temperatures increase.

Singh *et al.* (2014) concluded that the entomological survey should be undertaken effectively in the known endemic localities and the information should be utilized to forecast the possibility of future outbreaks. Malaysia which is a non-seasonal country the environmental factors had no significant influence on the density and abundance of mosquito larvae. Other obvious contributing factors are the availability of potential breeding sites and behaviour of residents.

9.4 Ovitrap Index in Urban and Suburban Areas

Ovitraps survey was conducted over a year for both study locations urban and suburban from March 2010 until February 2011. According to Focks (2003) the ovitrap is an excellent tool and most effective to detect the presence of mosquito larvae. This study observed monthly variations of mosquito populations in both study areas. This concurs the study done by Maimusa *et al*. (2012) but in contrast to the work done by Chen *et al*. (2005) where they observed weekly variations of mosquito populations. From these surveillance 2953 larvae of mosquito collected from ovitrap in Putrajaya and 3528 larvae collected in Kuala Selangor. This study indicated that only *Ae. albopictus* species was found in urban and suburban areas during ovitraps survey which is to be expected as all the ovitraps were placed in the outdoor areas only. Similar to results reported by Cheung and Fok (2009), *Ae. albopictus* was the only *Aedes* vector detected and its distribution was extensive in various areas during summer months in Hong Kong. *Ae. albopictus* preferred to breed outdoor areas as reported by Thavara *et al*. (2001), in their study. Similar results were also found by Rozilawati *et al*. (2007) *Ae. albopictus* was the dominant species in both study areas urban and suburban though larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were found in smaller percentages. Other study by Norzahira *et al*. (2011) also found *Ae. albopictus* was dominant species as they collected higher number of *Ae. albopictus* than *Ae. aegypti* in the ovitraps. In contrast as reported in (Chen *et al*. 2005; Lim *et al*. 2010; Malinda *et al*. 2012) where they are found *Ae. aegypti* was the dominant species in the study areas in urban areas and in India, *Ae. aegypti* was found to be the dominant species for both indoor and outdoor ovitraps for the residential areas (Surendran *et al*. 2007). In a recent study conducted by Wan-Norafikah *et al*. (2012) they also found both *Ae. albopictus* than *Ae. aegypti* in the ovitrap surveillance in Kelantan,Terengganu and Sabah. However, in their study conducted in Kuala Lumpur where the ovitrap were placed both outdoors and indoors only *Ae. albopictus* species was caught (Wan-Norafikah *et al*. 2009). The presence of *Aedes* mosquito larvae in the ovitraps that were placed in the high-rise apartments also reported the presence of mosquitoes not only on the ground level but also found on different higher floors of the apartment (Chadee 2004; Wan-Norafikah *et al*. 2010). According to Chan and Counsilman (1985), breeding places of *Aedes* mosquitoes were most prevalent in the slum areas. However, the ovitrap surveillance in this study locations showed that *Aedes* mosquitoes were associated with not only the slum areas, but also the general residential areas, as supported by similar findings by Chen *et al*. (2005) and Pemola *et al.* (2013).

In general the ovitrap index (OI) was higher in urban than in suburban areas in most of the month. In urban area the heavy rain was recorded in September 2010 with 512.8 mm and little rain was recorded in October 2010 with 99.6 mm but the number of mosquito collected in September was not highest as expected. Baruah and Dutta (2012) also reported the same situation which was suspected to have created problems in adult dispersal and mating. The highest abundance took place after heavy rainfall. In this study there was no correlation between Ovitrap Index and environmental parameters also same results with Sulaiman and Jeffrey 1986, study in Malaysia and in Japan (Mori & Wada, 1978).

This study found that temperature was not correlated to mosquito density. It is because the temperature recorded in urban areas for example was not the favourable temperature for mosquito growth. As mentioned by McMicheal *et al.* (1996) the suitable range of temperature for mosquito growth is between 25°C to 27°C. From the Meteorology data recorded in urban areas certain months exceed the favourable temperature for mosquito growth. The results reported by Vezzani, *et al*. (2004) found that Ovitrap Index was higher during summer than other season and the highest abundances of *Aedes aegypti* was with mean temperature above 20°C and accumulated rainfalls above 150 mm. According to Murty *et al.* (2010) other than temperature, rainfall was also found to correlate with the mosquito density. In their study, they noticed that temperatures between 22°C and 34°C with lower to medium humidity (42.7% to 69.6%) had facilitated the higher population density in both rural and urban areas in India. As reported by Karim *et al*. (2012) temperature was found to be closely related with rise of dengue infections. According to Rueda *et al*. (1990) the development and survival rates of adults and larvae of *Cx. quinquefasciatus* and *Ae. aegypti* depended on temperatures. Temperature affected the head capsule widths, body lengths and weight of both larvae species. The body size generally decreased as temperature increased. El-Badry and Al- Ali (2010) observed the density and distribution of *Ae. aegypti* depended on temperature and available moisture. The higher density and distribution of *Ae. aegypti* of mosquito was detected in low temperatures of the months.

Maimusa *et al*. (2012) reported that rainfall had significant correlation on the *Aedes* populations. According to Wiwanikit (2005; 2006) the rainfall influenced the dengue incidence in Thailand. The study found a high correlation between rainfall and the prevalence of clinical cases of dengue in Thailand. The study concluded that other confounding factors like ambient temperature and humidity which also determine the transmission of dengue should be looked into, before concluding that the increased prevalence is a result of rainfall alone. In the Philippines, the researcher also found that rainfall had significant correlation to dengue incidence (Sia Su, 2008). More over a study conducted by Ali Alshehri (2013) reported there is a strong relation between mosquito density and climatic factors for temperature and relative humidity. The results also showed high dengue cases in the city of Jeddah. Promprou *et al.* (2005) indicated that climatic factors play an important role in the cycles of Dengue Haemorrhagic fever. However, the relative importance of these climatic factors varied with geographical areas. This statement supports our study as the climatic factors were not correlated with the mosquito density in ovitrap. As mentioned by McMicheal *et al.* (1996) Lindsay and Mackenzie (1997), changes in climate may influence the abundance and distribution of vectors and intermediate hosts which is second host.

High humidity enhances mosquito and biting midge survival but reduces their flight activities. Normally, flight activity will cease when the relative humidity is above 90 per cent. In sub-tropical areas, most mosquitoes stop feeding when the temperature falls below 10°C. Prolonged extreme temperatures of 10°C and 35°C will greatly reduce the survival rate of most adult mosquitoes and biting midges. However, high temperatures will warm the water or substrate in breeding sites, resulting in shorter development periods for eggs, larvae and pupae. Hence, pest problems always occur during warmer times of the year. High rainfall helps to maintain permanent mosquito breeding sites, such as swamps and ponds, as well as creating extensive breeding sites in low lying grassy areas. Heavy rain can also flush mosquito larvae out of their breeding sites and drown pupae (Scott, 2002).

Ae. albopictus and *Ae. aegypti* prefer different environments and surroundings as the habitat. According to Rudnick *et al*. (1986) *Ae. albopictus* prefers outdoor areas, forest fringe habitats, disturbed and a lot of vegetation with tree surrounding the breeding site whereas *Ae. aegypti* prefers indoor. Basker and Ezhil (2012) also found the *Aedes* mosquito preference of shade area with vegetation. This situation happens in both study areas where urban area Putrajaya was disturbed with development, high population and have vegetation surrounding residential areas.

In suburban area of Pasir Penambang, a fisherman village with forested habitats, the resident keep water storage in containers for washing boat and other activities. Chan *et al*. (1971) reported that the domestic containers used as water storage is one of the breeding habitats of *Ae. aegypti* and *Ae. albopictus* in Singapore. The solid waste management systems were also poor and the resident disposes rubbish around their houses. The lifestyles of resident contribute to providing potential breeding sites for mosquitoes. Therefore the numbers of mosquito larvae collected in ovitraps were found to be higher in suburban than an urban area. A study conducted by Pemola *et al*. (2013) concluded a high density of dengue vectors in the residential area warrants the vector surveillance activities along with awareness programme.

9.5 Perception on the use of Chemicals in Mosquito Control and Utilization of Biocontrol

9.5.1 Perception of Control Measures of Mosquitoes

During questionnaires distribution, 80% of public and 94% of staff completed the questionnaires. Richardson (2005) revealed that 50% is regarded as an acceptable response rate. Other researchers stated that the response rate should be more than 50% as an acceptable response rate (Cook *et al.* 2000; Dommeyer *et al.* 2002; Watt *et al.* 2002; Ballantyne 2003; Nair *et al.*2005). This study has successfully obtained a good significant rate of response.

All staff and public involved in this study were directly exposed to fogging activities. Staffs selected in this study were involved either during insecticide solution preparation and/or its application in the field. The perception of control measures of mosquitoes is important for a successful community program or implementation of new control measures. WHO (1983) stated that in order to measure community program success, it is necessary to know the community's perceptions about mosquitoes, control measures and how best communities can participate in the control efforts.

The most effective control measure chosen by staffs was cleaning up the mosquito breeding site, whereas the public responded that the most effective measure used was fogging. This result was similar reported by Lennon (2004). Yohannes e*t al.* (2005) & Singh *et al.* (2006) revealed that the source reduction or cleaning up of mosquito breeding sites was one of the most effective ways to control mosquito population. A study reported by Kumar and Gururaj (2005) found that most of community are not aware of control measures of mosquitoes. Only 29.8% of urban and 12.5% of rural residents were aware that keeping surroundings clean was the direct control measures for mosquito control. The present study showed that although the respondents had a good knowledge on source reduction or cleaning up the mosquito breeding site as effective control measure of mosquitoes however, they were not practicing what they knew. Since the major breeding habitat found was higher in artificial container than natural habitat. Similar outcome was reported by Habibullah and Ashraf (2013), whereby the school children had sufficient knowledge about dengue and its control but such knowledge did not change their behaviour and was not translated into practice. Davis (2009) revealed that search and destroying activities of *Aedes* breeding had been conducted in Malaysia in 2008 and the MOH reported considerable success with an 84% reduction in dengue cases in suburban areas. Jose and Craig (1995) reported that the best approach for controlling *Ae. albopictus* and other *Stegomyia* species is to limit the availability of larval habitat.

According to WHO (2013) the prevention and control of dengue fever have relied on the control of the *Ae. aegypti* mosquito. "Vector control" refers to actions used to control a "vector" (in this case the mosquito), which can transmit a pathogen (the four dengue serotypes). Fogging activity was carried out during outbreak of dengue cases, fogging of insecticides e.g. malathion, reslin and other synthetic pyrethroids to kill adult mosquitoes in the affected area (Yap, 1984). Although fogging has the advantage to kill the adults mosquitoes but at the same time with disadvantages such as large volumes of organic solvents used as diluents, which may have bad odour and result in staining, high cost of diluent and spray application, householders may object and obstruct penetration of fog into houses by closing windows and doors, fire risk from machinery operating at very high temperatures with flammable solvents, and can cause traffic hazards in urban areas (WHO, 2003c). Karunaratne *et al.* (2013) reported that the source reduction and use of insecticdes in space spraying/ fogging and larviciding were the primary means of controlling the vector mosquitoes of *Ae. aegypti* and *Ae. albopictus.*

Other control measures reported by both groups, staff and public were the use of temephos an organophosphate (mosquito larvae insecticide) and using guppy fish. Temephos (ABATE) was given by health personnel to public to control mosquito larvae breeding in container that can not be destroyed. Both groups responded awareness on the use of temephos and use as control measure of mosquito larvae population. Similar results found by Koenraadt *et al.* (2006), where most of the respondent in their study were aware of mosquito control by covering all containers of water storage use temephos and fish. Temephos an organophosphate, is regularly used in containers for the control of *Ae. aegypti* larvae (Chareonviriyahpap *et al.* 1999). Phuanukoonon, *et al.* (2005) summarized that measures that prevent mosquitoes from developing in water-holding containers such as adding temephos to containers, covering containers and or placing larvivorous fish in containers, were effective in reducing mosquito larvae. In Malaysia, the use of temephos larvicide on a large scale in high-risk areas was also initiated in 1998 to reduce *Aedes* larval density (Teng & Singh, 2001).

Both groups of respondent knew the undesirable effects of insecticide besides causing health problem to human such as negative effect to the environment, insecticides will kill the non target organisms, costing and effect on animal. Dynah *et al.* (2010) reported that more than 50% of workers believed that insecticide can also affect the environment. They also stated that the chemical use can cause soil depletion, pollute water and can affect animals found in the community. Pesticide is a term used to describe a range of mixtures used to kill or reduce many types of pests (Fait *et al.* 2001). According to Carson (2002) & Vega (1994) majority of pesticdes is not only specifically targeting the pest but also affecting plants, animals, and contaminate wide range of environment including groundwater and surface water. Jansamood (2013) found that the use of pesticides rated as having high efficiency also had environmental and health impact. Certain insecticides for example DDT, were restricted, because chemicals can build up in the oceans, air, soil, food chain and fresh water supplies (Mansour 2009, Ogata *et al.* 2009, van den Berg 2009).

Survey in Saudi Arabia found that the respondents had the knowledge on the effects of the use of pesticides. They were aware of the fact that pesticdes cause pollution, can affect soil fertility and impose toxic effects on the soil (Al- Zaidi *et al.* 2011). Cornwall *et al.* (1995), also reported the risk of pesticides on the environment and public health in the developing countries. Aktar *et al.* (2009), concluded that the used of pesticides has contaminated almost every part of environment such as impact on food commodities, contaminate soil, surface water, ground water and also non target organisms. Moreover, the economic impact of pesticides in non-target species (including humans) has been estimated at approximately $8 billion annually in developing countries.

9.5.2 Perception of Factors Contributing to Increase of dengue Cases

The perceptions on factors contributing to the increase of dengue case showed that the human behaviour was the most important factors. Patel *et al.* (2011) reported that community participation is essential for control of mosquito-borne diseases. This was also proven by Nam *et al.* (1998) in that the number of *Ae. aegypti* was reduced when the community was involved in community clearing programme. Communication for Behavioural Impact (COMBI) also was implemented in certain locations in Malaysia (Lam, 1993). Most dengue control programs rely on field staffs that go door to door checking homes and surrounding premises for mosquito larvae and pupae of the mosquito in water-holding containers. This process has proven to be ineffective over the long term because communities are not active partners in the control actions but rather passive participants or recipients of the control efforts (Gubler, 2002). Yasuoka *et al.* (2006) suggested that the community-based educational interventions are effective in increasing understanding and active involvement in mosquito control and disease prevention. COMBI is one of the best ways to educate people and at the same time the public was encouraged to participate in the dengue control programme such as source reduction of mosquitoes breeding sites. Other studies found a significant reduction in *Ae. aegypti* infestation index after community based prevention campaign was conducted (Clark *et al.* 1992; Lloyd *et al.* 1992; Fernandez *et al.* 1993; & Sanchez *et al.* 2005). Constant mobilization of huge numbers of volunteers in *Aedes* search and destruction missions in every urban and suburban and indeed rural areas throughout the country would effectively control the spread of dengue.

Other factors that contribute to dengue cases such as lack of knowledge in control of dengue cases, environmental factors, ineffective mosquito control measures, and mosquito resistance to insecticide. Chareonviriyahpap *et al.* (1999) reported that many of the environmental factors or environmental changes such as deforestation, irrigation and urbanization have favoured conditions enhancing vector transmission of diseases. In the review article on dengue prevention and control, Claro *et al.* (2004), the results showed that adequate knowledge of dengue and prevention methods are in close association with high rates of domiciliary infestation by *Ae. aegypti*. Nahida (2007) found the association of knowledge and attitude towards *Aedes* control to be of importance in her study. It is important to make sure the public should have knowledge about mosquito control in order to prevent outbreak of dengue fever. However, she also mentioned that human behaviour did not depend only on attitude and knowledge but also others factors such as motivation, perceived benefits, social factors, and taboos. According to Parks and Lloyd (2004) researchers have noted that, despite growing levels of knowledge and awareness about dengue and mosquitoes, many people are still not taking action. In some countries, people knew that dengue is caused by mosquitoes and that mosquitoes can breed in water containers, yet they still fail to do what is best for them and containers are left unprotected. Different finding was reported by Kyu *et al.* (2005) they found the significant association between knowledge and attitude and they concluded that if the repondents were supplied with correct knowledge through appropriate channels, they may change their attitude and ultimately, their daily practice. Mohanty *et al.* (2013) indicated that there is a significant association between knowledge of the farmers and their practices related pesticides.

As reported by William (2013) routine fogging was ineffective in the control mosquito population. Chua *et al.* (2005) concluded that the usual chemical fogging in natural environment was ineffective in breaking the reproductive lifecycle by eliminating gravid female *Aedes* mosquitoes. Davis (2009) reported that the lack of success with outdoor spraying has been noted worldwide and the Malaysian Ministry of Health's pesticide fogging program for dengue has failed to stop the spread of dengue. Reiter (2009) was quoted by the Malaysian New Strait Times as mentioning that the 'fogging with insecticides from road vehicles has little or no impact in urban areas'.

Both groups of respondents also mentioned that the increase of dengue cases was due to chemical resistance. Andrade (2003) indicated that resistant of *Cx. quinquefasciatus* to organophosphate and prethroid insecticides and the need for evalution and monitoring of the efficiency of insecticides to be used in mosquito control program. Kumar *et al.* (2011) also reported that *Cx. quinquefasciatus* is highly resistance to DDT and malathion. Teng and Singh (2001) reported that in Malaysia, tradisionally malathion was the chemical of choice for dengue control. The use of malathion was stopped in 1996 and replaced with water-based pyrethroid fogging formulations such as resigen and aqua-resigen. Observation and feedback by the fogging teams indicated that the people did not accept fogging inside their houses since malathion was smelly and diesel-solvent ehich left oily residues on floors and walls of houses. In Iran, Vatandoost *et al.* (2005) found that malaria vector which is *An.*

stephensi was resistant to DDT and dieldrin. These two insecticides were commonlly used to control malaria vector and Lak *et al*. (2002) mentioned that malaria vector *An. sacharovi* was resistant to DDT but susceptible to dieldrin. Other researchers also reported the insecticides resistant such as *Cx. quinquefasciatus* resistance to fenthion, temephos, fenitrothion and chlorpyrifos (Bashir *et al.* 2012), *Ae. aegypti* and *Ae. albopictus* highly resistant to DDT (Karunaratne *et al.* 2013), and *Ae. aegypti* resistant to organophosphate and pyrethroids (Pimsamarn *et al.* 2009). Fourty years of intensive use of organic insecticides to control insect pests and disease vector has led to the extensive selection of insecticide resistance in more than 450 species (Georghiou, 1986). Brown (1986) and Neng *et al.* (1993) reported that *Ae. albopictus* is resistant to the organochlorines DDT and HCH in China, India, Japan, Malaysia, Southeast Asia and the Philiphines and resistant to malathion in Singapore and Vietnam, fenthion in Malaysia and fenitrothion in Madagascar.

9.5.3 Perception on Biocontrol Agent Use to Control Mosquito Population

Both groups of respondents knew about biocontrol method in the control of mosquito population. Regarding biocontrol method the awareness of biocontrol method of staff was higher than public and unsure of biocontrol method was higher among public than staff. Other study found that the knowledge concerning biocontrol and natural control was low among respondents in Gaza Strip. The lack of knowledge of biocontrol for vector control was the justification for the continuous use of insecticide (Yassin *et al*. 2002). Biological control measures were commonly used before the introduction of insecticides in the 1940s. Insecticides dominated vector control approaches after their introduction, but damage to the environment, vector resistance to insecticides, and community resistance to their use have resulted in a new focus on biological control measures (WHO 2013). One of the methods suggested by many researchers was use of biocontrol agent to control of vector population (Brown 1981; WHO 1986b; Robert & Andre 1994; Chareonviriyaphap 1995).

Guppy was most famous as a biological control agent by both groups of respondents. Fish are the most extensively used larval biocontrol agent. According to Chakraborty *et al.* (2008) fish have the greatest potential as biocontrol agents against the aquatic stages of mosquitoes and are used as major component of the integrated vector control programme. They also mentioned that the most widely used of fish in India were *G.affinis*, *Aplocheilus panchax* and *P. reticulata*. Most commonly and used biocontrol agents used in mosquito control was guppy, *P. reticulata* (Service, 2000). The use of guppies (*P. reticulata*) to control dengue vector of *Ae. aegypti* in domestic water storage containers in rural areas in Cambodia was proven successful (Chang *et al*. 2008). The use of more than one biological control agent for the suppression of a vector species may prove feasible and should be encouraged wherever possible, since it may lead to an optimum level of vector suppression (WHO, 1982).

9.5.4 Self reported adverse health Symptoms by Respondents in Both Study Areas

In this study, all of staffs experienced more than one symptom of health effects after being exposed to fogging activity or handling of the insecticide and 80% of the public reported health symptoms related to use of insecticide. This percentage was higher than other previous studies for example Pasiani *et al.* (2012) and Faria *et al.* (2009) who showed that only a small percentage of workers exposed to pesticide were reported adverse health symptoms. Khan (2011) found more than (77%) farmers in both districts in their study experienced one or more health effects while spraying and many of them experienced multiple symptoms. Kishi *et al.* (1995) reported that the negative signs occurred significantly more often during spraying than during non-spraying seasons. Spraying activities are important to destroy all breeding sites of the mosquitoes in order to prevent the outbreak of dengue cases. When outbreak happens only fogging activities will be carried out to destroy adult mosquitoes and staffs will be exposed to insecticide. To minimize the exposure of insecticide to the workers, public fogging should be the last resort. Dey *et al*. (2013) reported, among populations, the prevalence of signs and symptoms related to pesticide exposure were higher among the sprayer than non-sprayer. This could be due to their direct exposure to pesticide or due to the previous exposure to pesticide. Besides being ineffective, routine fogging is also harmful to the public health and hazardous to vulnerable and at-risk groups. Thermal fogging uses diesel as a carrier for the insecticide. This makes the constituents of the fog fat-soluble and when absorbed into the body (by contact or inhalation), will accumulate and remain in the fatty tissues of the body - a process known as bio-accumulation. The outcome is an accumulation of the pesticide and diesel, which is toxic and potentially carcinogenic in the long term (William, 2013).

This study reported that the most common symptom experienced by staff was fatigue which is similar reported by Toe *et al.* (2012) & Kishi *et al*. (1995) among workers that were exposed to insecticides. Others symptoms reported were headache, skin itching, diarrhoea, nausea, cough and dizziness. Fatigue is one of the most common symptoms reported among the workers due to the weight of fogging machine during the fogging activity. As mentioned by Kishi *et al.* (1995) in their study workers had to carry the content of five 17-liter back pack tank during the spraying operation. A thermal fog machine weigh 6–11 kg and the workers had to bear this heavy load during fogging activities (WHO, 2003c). The main symptom reported by farmer who was exposed to insecticide was easy fatigability as indicated by Del Prado-Lu, (2007). Other study also reported the common pesticide-related symptoms such as dizziness, headache, nausea/vomiting and fever. However, it is noteworthy that in this study none of the respondents reported fever as one of the symptoms due to insecticides exposure which was also similarly reported by Kachaiyaphum *et al.* 2010.

Many chemical compounds such as organochlorine, organophosphate, carbamates, and pyrethroids, have been used in both agricultural practices and public health programs (Chareonviriyahpap *et al.* 1999). Some studies did suggest that long term effects on the central and peripheral nervous system might be associated with the frequent but low level exposure to organophospate (Williams *et al.* 1997; de Blaquire *et al.* 2000). In this situation the public were exposed to chemical or insecticides frequently but at low level of chemical especially when the outbreak of dengue happened. Blain (2001) suggested that populations that have been exposed to the concentrate should be investigated for changes in neurobehavioural variables and neuromuscular electrophysiology. The long term toxicity of organophosphates is important public and occupational health issues.

Organophosphates are well known toxicants affecting the nervous system through the inhibition of acetylcholinesterase. Most of the health problems due to acute poisoning of organophosphorus compounds on sensitive targets in the human body have been attributed to the inhibition of the enzyme acetylcholinesterase in a range of nerve, neuromuscular and glandular tissues where this enzyme plays a key role in cell to cell communications (Karalliedde *et al.*, 2003). Soomro *et al.* (2008) revealed blood contamination and cholinesterase inhibition among the spray-workers in Sindh, Pakistan and noticed the effect and extent of exposure in the spray-working community. This study found both public and staff groups were exposed to chemical during fogging activity and from the self-reported symptoms showed that they had symptoms of organophosphate poisoning. Other study found that the residue concentrations of some organochlorine and organophosphorus pesticides were also detected in blood samples of school children which prompted the adult studies in the directly exposed spray workers (Mohammed *et al.* 2001).

Mekonnen and Ejigu (2005) measured plasma cholinesterase (PChE) level among the sprayers in both farms in Ethopia and they found that the sprayers were the most affected groups compared to control groups as they had PChE values below 50%, and it is believed that cholinesterase values of 50% or less for plasma represent abnormal depressions in most individuals. Gallo and Lawryk (1991) said that an abnormal reduction in cholinesterase activity of workers exposed to chemical pesticides is almost always a result of absorption of an anti-cholinesterase compound. As a result, the exposure of workers to organophosphate or carbamate pesticides is the main cause for significant depression of cholinesterase activity. Duangchinda *et al.* (2014) indicated that the use of chemical pesticide was related to acetylcholinesterase (AChE) level of farmers with the methods of uses, practice, duration, chemical content, frequency and chemical type. They also reported that the Ache levels were lower than standard, due to the pesticide exposure experienced among farmers. Ntow *et al.* (2009) found that the exposed farmers were the high risk group as the cholinesterase (ChE) results were significantly lower than the control participants. However the results were not significantly correlated with compounding factors of age, sex, body weight and height. In our study, the blood sample to test ChE was not taken, but from the self-reported results of the health effect showed that both groups of the respondents were exposed to chemical. According to the Health officer in both Health office all vector control staffs had to undergo medical check-up every year especially for ChE test. From the questionnaires also most workers mentioned that they have to carry out the ChE test every year. This showed that the authorities are concerned on the health of workers who were exposed to chemical.

Besides being exposed to chemical hazards, workers of vector control also experience the risk of Noise Induce Hearing Loss (NIHL) from the machine fogging. NIHL was significantly associated with the age-group of 40 years and older, length of service of 10 or more years, current occupational noise exposure, listening to loud music, history of firearms use and history of mumps/measles infection as reported by Masilamani *et al.*(2012).

During fogging activity, residents did not use Personal Protective Equipment (PPE) like face mask. Pesticide can enter the human body through inhalation (Ogg *et al.* 2012). The spray-workers are directly exposed to pesticides while mixing, handling and spraying and through contaminated soil, air, drinking water, eating food and smoking at work places. Ultimately these are absorbed by inhalation, ingestion, and dermal contact (Vega, 1994). The purposes of face mask are to prevent direct exposure of insecticide. Booman (2005) suggested that workers should use complete Personal Protective Equipment (PPE) such as gloves, googles, coverall and ear plugs during the use of insecticide such as pyrethroids and DDT to control mosquito vectors, in order to protect from hazards. Study done by Al - Haddad and Al- Sayyad (2013) found majority of workers felt that the proper use of PPE is one of the important factors to promote safe pesticide use. It is because they found that some of the workers in their study area did not use complete PPE while working with pesticide.

There was no correlation between health effect of staffs and residents with age, education level, frequency of exposure with insecticide and length of service (for staff) which is similar to the finding of Pasiani *et al.* (2012). They found that there was no correlation between age, level of education, years of pesticide use, and hours of work and use of PPE. However, a study in Vietnam shows that health problems were positively correlated to the number of years on using insecticide, while training did not have any effect on farmers' health because the IPM Program in Vietnam was implemented only in the recent years (Chi *et al*. 1999). Del Prado-Lu (2007) reported that the RBC cholinesterase levels were positively associated with age, sex, incorrect mix type of pesticides, illness due to pesticides and number of years using pesticides. In this study there was no correlation between age, length of service and frequency of exposure because of the majority of staffs or new workers in the vector control unit service in less than five years. Other factors might be all the staffs and workers wore complete PPE during fogging activities and may practiced the protective measures as reported by Kumari & Reddy (2013). They concluded that workers with low level of education might be at higher risk during the usage of insecticide, possibly due to difficulties in understanding the instructions and safety procedures included the product labels which are printed in English.

9.6 Survey of Natural Predator from Both Study Areas

Six types of natural predators were collected from study areas. However, only the dominant species were used in the predation activity. A study in Thailand conducted by Wongsiri (1982) found twenty non-insect predators and fourteen insect predators in association with various mosquito species. The most abundant predators collected were similar with this study which is *P. reticulata* and dragonfly nymphs. In the field work both adults and nymph of dragonfly were collected at potential breeding habitats. Eight species of adult dragonfly species were found in the suburban areas and seven species in the urban areas. Norma-Rashid *et al*. (2001) reported that the distribution of Odonate species and population differed across the localities. For instance in coastal areas of Malaysia, they found 16 species of Odonata belonging to two families and the predominant species was *Crocothemis servilia* (Drury, 1770) (Norma-Rashid, 2010). A study done in India found 21 species of Odonata (14 species of Anisoptera and seven of Zygoptera) recorded from 13 temporary water bodies and *Pantala flavescens* was most abundant in the temporary water body (Arulprakash & Gunathilagaraj, 2010). The work revealed one predominant Odonata family group of Libellulidae having 8 different species.

Libellulidae being the most dominant family group in samplings had been reported by several authors (Das *et al*. (2012); Sethy & Siddiqi, (2007); Sharma *et al*. (2007); and Wahizatul *et al*. (2006). Nasemman *et al.* (2011) reported that the larvae from family Libellulidae, are usually very abundant in all types of stagnant waters and are able to colonize successfully even in small water bodies with low oxygen where other odonates cannot survive. This is revealed in this study, where only Libellulidae was found in the study locations as the habitat compressed of small water bodies such as drain and marshes.

The eight species of adult dragonflies found within the residential areas included *O. chrysis, O. sabina*, *N. fluctuans, T. aurora, T. festiva, B. chalybea, B. contaminata* and *R. phyilis.* These are common species found by other researchers in Malaysia (Norma-Rashid *et al*. 2001), India (Andrew *et al*. 2008) and Singapore (Norma-Rashid *et al*. 2008). However, only dragonfly nymphs were used in the predation experiment. According to Orr (2005) the adults of *O. sabina* commonly found in degraded, open habitats including drains, ponds and marshes; often forages at forest margins and in the canopy. From this study the results revealed that 6 species of dragonfly nymph were collected in both study areas in urban and suburban. The six species collected included *N. fluctuans, O. sabina*, *O. chrysis*, *T. aurora, T. festiva* and *B. chalybea*. For the predation experiments the predators were selected among the dominant species which are *N. fluctuans, O. sabina* and *O. chrysis.*

9.7 Feeding Experiments

The use of biocontrol agent has become popular recently and many researchers focused on this approach. In Malaysia, the common biological control agents are for example Bti (*Bacillus thuringiensis israelensis*), guppy fish (*Poecilia reticulata*) and *Toxorhynchites* larvae (Nyamah *et al.* 2011). However, the use *Toxorhynchites* larvae in environment setting that involves community participation is still undergoing investigation. The studies of biocontrol against mosquito larvae are well documented all over the world. The predators that were used as biocontrol agents are, for example, *Rhantus sikkimensis* and larvae of *Toxorhynchites splendens* (Aditya *et al*. 2006; Aditya *et al*. 2007) *Diplonychus sp*. and *Anisops sp* (Shaalan *et al.* 2007) Odonate nymphs (Chandra *et al*. 2006a; Mandal *et al*. 2008) *Acilius sulcatus* (Coleoptera: Dytiscidae) (Chandra *et al.* 2008), *Mesocyclops* (Copepoda: Cyclopoida (Marten,1990b; Marten *et al*. 1989; Marten *et al*. 1994b; Soumare & Cilek 2011), planaria (*Dugesia bengalensis*) (Kar & Aditya, 2003), diving beetles (Ohba & Takagi, 2010) and guppy, *P. reticulata* (Chang *et al*. 2008).

The behaviour of predator and mosquito larvae species was influencing the predation activity. For instance, guppy behaviour was active and constantly searching for mosquito larvae. On the other hand, dragonfly nymphs were immobile or motionless. They used a sit wait strategy to ambush the mosquito larvae and capture then consumed. The female guppies are aggressive and active in searching mosquito larvae in predation activities. Most of the times were spent on surface waters and searching for mosquito larvae. Through observation of predator behaviour towards prey in predation experiments the first introduction of mosquito larvae in the aquaria and scored for the very quick for abour a second. This situation occurred in three species of mosquito larvae.

Three species of dragonfly nymphs were used in the experiments. All species showed almost the same behaviour in dealing with the mosquito prey in predation activities. The score for the first attack dragonfly nymphs toward prey was a few second but much slower compared to guppies. This is the first study in Malaysia, reporting dragonfly nymphs as potential biocontrol towards mosquito larvae. However both guppy and dragonfly nymph showed the same efficiency in terms of mosquito consumption. Most of the time, dragonfly nymphs spent at the bottom of water with little movement or motionless. They waited for the mosquito larvae to approaches before attack, ambush and seize them, unlike the guppies that searched and pursued the preys. For example Kweka *et al.* (2011) found *Gambusia affins* was most efficient while tadpoles were the least efficient predators among all in the predation activities. Their study concluded that the most efficient predator was *Gambusia affins* > backswimmer > dragonfly nymph > belestoma > tadpoles was the least efficient. However, in the present study both predator guppies and dragonfly nymphs were most efficient as they are able to consume all mosquito larvae species.

9.7.1 Feeding Experiment of Dragonfly Nymphs

Only certain species of dragonfly nymphs were investigated as biocontrol agents for example *Brachytron pratense* nymphs to control mosquito larvae of *Anopheles subpictus* is efficient in laboratory and field work. *Brachytron pratense* nymphs consumed an averaged of 66 larvae *An. subpictus* during 24 hour in laboratory conditions (Chandra *et al*. 2006), 5 species of odonate were used as biocontrol agents against *Cx. quinquefasciatus* in the experiment and the results showed that the most efficient to least effciect were *I. forcipata* (64 larvae/day), *A. flavifrons* (57), *R. ignipennis* (45), *S. durum* (25), and *C. kashmirum* (14) (Mandal *et al*. 2008), *Mesogomphus lineatus* against *Cx. quinquefasciatus* larvae (Mathavan 1976; Pandian *et al*., 1979), *Crocothemis servilia* (Drury) aginst *Ae. aegypti* larvae (Sebastian, 1990), *Pantala hymenaea* against *Cx. quinquefasciatus* larvae (Quiroz-Martinez *et al*. 2005), *Ceriagrion coromandelianum* and *Brachydiplax chalybea chalybea* against *Cx. quinquefasciatus* larvae (Saha *et al*. 2012). Their cryptic colouration and keen eyesight make dragonfly nymphs as effective predator. Dragonfly nymphs are generally ambush predators that are they wait for their prey to come close before striking (Subramaniam, 2005).

In this study, there was no significant difference of the daily feeding rate of dragonfly nymphs on mosquito larvae species. However, there was a significant difference in species preference among the dragonfly nymph species. It shows that all 3 species of dragonfly nymphs were able to consume a good number of all 3 common mosquito larvae species in Malaysia. For example the dominant species of dragonfly nymph *O. sabina*, *N. fluctuans* and *O. chrysis* ate all mosquito larvae species. However, this was in contrast with Mandal *et al.* (2008) which found that different Odonata species showed different efficiency when exposed to mosquito larvae.

In terms of prey preferences there is a significant difference in number of prey species consumed by predators. The current work reported on specific prey preferences shown by odonate predators where dragonfly nymphs of *O. sabina,* and *N. fluctuans* captured more of the *Ae. aegypti* larvae in contrast to the other 2 mosquito species whereas, *O. chrysis* consumed more of *Cx. quinquefasciatus* larvae. In summary the most preferred prey was *Ae. aegypti* > *Ae. albopictus* > *Cx. quinquefasciatus* larvae. Among 3 dominant species of dragonfly nymph *O. sabina* was the most active predator and also can be a good biocontrol agent for mosquito larvae as they consumed all mosquito species of *Ae. aegypti, Ae. albopictus* and *Cx. quinquefasciatus.* According to Mathavan, (unpublished data) *O. sabina* also consumed other mosquito larvae of *Cx. fatigans*.

Different predators showed different feeding capacity towards prey. Aditya *et al*. (2006) showed that *Rhantus sikkimensis* and larvae of *Toxorhynchites splendens* could consume a good number of *Cx. quinquefasciatus* larvae and *Rhantus sikkimensis* was more efficient than *Toxorhynchites splendens* larvae. Other studies also proved that certain biocontrol agent, could be more efficient than other biocontrol agent for example adults *Diplonychus* spp. was a more efficient predator than *Anisops sp*. (Shaalan *et al*. 2007) and under laboratory condition cyclopoid copepod of *Mesocyclops aspericorni*s was consumed about 33-50 of 1st instars *Ae. aegypti* larvae eating the body portion first and leaving the head capsule behind (Ramanibai & Kanniga, 2008).

9.7.2 Feeding Experiment of *Poecillia reticulata*

Many studies showed that *P. reticulata*, (guppy) is a good predator as they can control mosquito larvae population (Anogwih & Makanjuola, 2010; Ghosh *et al*. 2011, Manna, 2008; Chang *et al*. 2008) but guppy failed to consume *Cx. quinquefasciatus* when other food was available in polluted water or drain water such as plankton (Dua *et al*. 2007). However, *P. reticulata* was reported as the most active predator as they fed on almost all stages of mosquito from eggs to larva than other predator such as copepod and desert pupfish (*Cyprinodon macularius*) (Mian *et al*. 1986). According to Lawal *et al*. (2012) *P. reticulata* fed mainly on algae, organic detritus, diatoms, mosquito larvae parts, protozoan, zooplankton and fish parts as their found these eight categories of food in stomach content of *P. reticulata.* A recent study by Gupta & Banerjee (2013) reported that the predation efficiency in relation to fish size and larval size has revealed significant better predation efficiency of Panchax minnow over guppy in all size groups except for pupae in small sized group fish. They also suggested that Panchax minnow is a better mosquito biocontrol agent in waterbodies whereas guppy can be used for mosquito control in very shallow water depth.

Guppies were also used as predators against 3 common mosquito larvae in Malaysia. Many studies show that *P. reticulata* is a good predator they can control mosquito larvae population (Anogwih & Makanjuola, 2010; Ghosh *et al.*, 2011; Manna, 2008; Chang *et al.*, 2008). However, guppies did not select *Cx. quinquefasciatus* when other food were available in polluted water or in drain water, such as plankton (Dua *et al.*, 2007). In this study, it was observed that female guppies (*P. reticulata*) ate mosquito larvae more than male guppies.

Statistically the present study reported there was significant difference between the number of mosquito larvae consumed between female guppy and male guppy. Manna *et al*. (2008) indicated that in predation pattern of *P. reticulata* (guppies) and *Cx. quinqufasciatus* showed that the predator guppy consumed prey varied between body size of guppy and time interval within 3 hours period. Female fish could consume more mosquito larvae than male fish and the relevant factor is that the female were bigger size than male so that they could consume more mosquito larvae. Elias *et al* (1995) reported that the female fish was more active than males as the female consumed larger number of *Cx. quinquefasciatus* larvae under laboratory conditions. The female was aggressive and active in searching mosquito larvae in predation activities. According to Anyaele and Obembe (2010), adult female fish is more voracious and has higher biocontrol potential compared to the adult male fish.

In terms of prey preference male guppy eat more on *Ae. aegypti* larvae, followed by *Ae. albopictus* and the least preferred was *Cx. quinquefasciatus*. Female guppy also showed a similar result as they consumed more on *Ae. aegypti* larvae, followed by *Ae. albopictus* and *Cx. quinquefasciatus*. *Ae. aegypti* > *Ae. albopictus* > *Cx. quinquefasciatus* larvae, and their tried to avoid pupal stage of mosquito. Both sexes of guppy preferred *Aedes* species larvae than *Culex* species. Female *P. reticulata* (guppy) eat more mosquito larvae with 121 and male guppy 98 larvae of *Ae. aegypti*. The result of this study supported the finding by Chang *et al*. (2008) where female guppies ate more than male guppies with 122.9 and 74 of larvae *Ae. aegypti* per day respectively.

Haq and Yadav (2011) reported the larval feeding propensity of *A. dispar* showed that the fish consumed larvae of all the three mosquito species with varying preference the mean number of larvae consumed per fish per day was in the following order *An. stephensi* > *Ae. aegypti*, > *Cx. quinquefasciatus.* In their opinion, the lower consumption of *Aedes* and *Culex* larvae may have been due to their larger size but also due to *A. dispar*'s which is always found on top of water column *Anopheles* larvae that also tend to occupy the top part of the water column. This situation makes *Anopheles* species can easy capture by *A. dispar*.

Most of the time, male and female guppies were at the water surface actively searching and attacking mosquito larvae; however, they totally avoided mosquito pupae. This behaviour had been observed by Anogwih and Makanjuola (2010). Through observation of predator behaviour toward prey in predation experiment the first introduction of mosquito larvae in the aquaria the first attack of guppies was very fast about a second.

The number of mosquito larvae eaten by male and female guppies showed different results. This is due to the different in the body size of female guppy, the behaviour of predator itself and the behaviour of mosquito larvae species. Body sizes of male and female guppies showed the different ability in searching and consuming mosquito larvae. The prey consumption ability of the *P. reticulata* increases with the body size. It means prey consumption increases with the body size. These results also support finding by Cavalcanti *et al*. (2007) where the efficacy as predators depends on its weight and sex. They used 5 different fish as predators against *Ae. aegypti* larvae and found the most effective predator were the larger fish and female guppies were more capable in eradicating *Ae. aegypti* larvae than male guppy.

According to Neng *et al.* (1987), predation efficiency of *Clarias fuscus* depends on the body weight of the predator. Therefore, larger fish consumed more mosquito larvae than small fish. Other example showed that fish with difference group size also showed the difference in feeding rate, where the big sized fish consume more larvae mosquito than small size. It may be due to the big size fish have a large appetite so that they could devour more mosquito larvae than small size fish. *Clarias gariepinus* ate more mosquito larvae of *Anopheles stephensi* than *Ctenopharyngodon idella, Cryprinus carpio Linnaeus,* and *Oreochromis*. Other finding also reported that the large fish ate more than medium size and small size fish (Lawal *et al*. 2012) and the feeding efficacy of fish was found to increased as size of group also increased (Pemola & Jauhari, 2011; Phukon & Biswas, 2013).

In other study by Ohba and Takagi (2010) where they used 3 different sizes of 14 beetle species and categorized as a small sized, medium sized and large sized. The predation rate of medium size beetle were highest followed by small-sized beetle and large-sized beetle. In the functional response study of 3 species of medium-sized beetle, between

Hydaticus grammicus, Rhantus suturalis and *Eretes griseus* the species of *Eretes griseus* species showed the highest attack rate and shortest prey-handling time. The diving beetle used their foreleg to captured mosquito larvae during the predation activities. The medium-sized beetle species able to grasp the body of 4th instar larvae of *Culex tritaeniorhynchus* better than smaller sized beetle species and large size beetle species.

9.8 Feeding Experiment between Light on and Light off

9.8.1 Dragonfly Nymphs

The results showed all predator species were more active during the light on as they consumed more mosquito larvae during the light on compared to the light off. Other study that found the same results such as the *Brachytron pratense* nymphs are daylight stalkers and active at daytime. The biology of *Brachytron pratense* nymphs itself for example have good vision that actively hunt prey that can be a good predator of mosquitoes (Chandra *et al*. 2006a). In terms of time between photophase with light and scotophase at dark the feeding rate was also different where all species consumed more prey at light or day time compared to the dark time. It depended on the ability of odonate nymphs to search prey at different time. According to Saha *et al.* (2008), the rate of consumption varied between light on and light off was due to differential adaptability of the predators in prey capture under light and dark conditions. They concluded that the vision of predator, aids in prey detection. Odonate nymphs used their vision sensory organ to search prey (Mandal *et al*. 2008). Prey was always detected at a short distance, not exceeding the length of the larva itself. The progressive increase in the importance of the eyes might be expected to have affected the diurnal rhythm of feeding activity (Corbet, 1962).

However, as reported by Chandra, *et al*. (2008) the feeding rate of *Acilius sulcatus* (Coleoptera: Dytiscidae) against *Cx. quinquefasciatus* larvae during day time and night time had no difference which mean that the *A. sulcatus was* active throughout.

9.8.2 *Poecilia reticulata*

The presence of light influenced the feeding rate as the predator can easily search and attack the prey. Some studies indicate that when the water is turbid, fish find it difficult to search for prey because their vision is not clear. Vision is the dominant sense of many fish. It was observed that male and female guppies were more active and consumed more mosquito larvae during light on. Okorie and Abiodun (2010) also found that fish ate more under light than dark time, and they concluded that fish relied on visual ability to search for prey. Another study by Rajaei *et al.* (2012) also found that the fish saw the target in light conditions than dark. In darkness or light off conditions visual receptor did not receive enough light and therefore no reaction shown. They also concluded that the *P. reticulata* as a visual feeder. Previous study by Turesson and Brönmark (2007) revealed that the prey – predator encounter rates was influenced by water transparency. They reported that when water transparency decreases, it will reduce prey detection distance by predator and thus predator search efficiency because it affected the fish vision. Robertis *et al*. (2003) also found that the turbidity or water transparency was the factor that influenced the feeding consumption of fishes. Chatterjee and Chandra (1997) reported that *G.affinis* more active during light on which is between 0.400-10.00h.

Increase turbidity decreased the visibility of prey and decreases the predation activity (Minello *et al*. 1987). Ghosh *et al.* (2004) also revealed that the fish predators were more active during light phase compared to dark phase. However, another study found in the 24 hour evaluation experiments, all predators of tadpole, belestoma, dragonfly nymph, *Gambusia affinis* and backswimmer were more efficient nocturnal predators (Kweka *et al*. 2011). Ghosh *et al*. (2005) this change of behaviour during day time and night time has no practical significance in biocontrol strategy.

9.9 Factors Affecting to Predation Activities

Many factors influence predation activities which are number of predators, prey densities, water volume, size of predator and prey (Aditya *et al.* (2007); Chandra *et al.* (2008)), aquatic vegetation (Savino and Stein (1989); Shaalan *et al.* (2007)), sex (Chang *et al*. 2008) body size of predator, behaviour of predator, and mechanism of prey capture (Ohba and Takagi (2010); Tranchida *et al.* (2009). In this study the variable assessed was the number of predator, prey densities, prey species, and water volume. Clement (1999) states that the rates of prey consumption were affected by a number of external factors including water volume, prey density and prey size.

The factors that influence predation activities were discussed by Griffin and Knight (2012) and these factors were categorised into ecological and behavioural factors. The ecological factors included suitable breeding sites or habitat for predator and prey, prey preference by predators, and developmental stage of both prey and predator. The behavioural factors were for feeding habits of predator and preference for alternative prey. The effective way to use biocontrol agents depended on suitability of the breeding site for predator to eradicate mosquito population and species preference on mosquito larvae. For instance a study reported in French Polynesia shows that covered sites were preferred by *Aedes* spp. and suitable for *Mesocyclops aspericornis* but not suitable for fish due to insufficient light. Therefore, the most effective way to control *Aedes* spp. in covered sites is by using *M. aspericornis.* The advantage of using fish as a biocontrol agent was that fish had a good adaptation to its new environment (Lardeux, 1992).

The number of predator used was one and two predators. The prey densities that were exposed to predator were 100 IV instar and 200 IV instar mosquito larvae. Water volume was 1 liter and 2 liters. When the different predator size was used in the experiment, the feeding rate of two different size of predators also varied. Some study showed that predator with large body size had the ability to search and capture more prey compare to small size predators (Aditya *et al.* 2006; Mandal *et al.* 2008). Large predators might be more energetic than the small ones but study conducted by Ohba & Takagi (2010) proved that medium sized predators had good ability to consume more larvae than large and small predators. It's showed that the medium sized of beetle ate more mosquito larvae than large and small predators. Study by Kar & Aditya (2003) and Chandra *et al*. (2008) found that predation between of beetles and planarian as predator against mosquito larvae of *Culex* spp. The ability and body as of beetle made them as good predators where they consumed more than planarian predators.

9.9.1 Number of Predator

The number of predators used in the experiment influenced the feeding rate. In the experimentation when a single predator in contrast to two predators with the same densities of prey was used it was found that the two predators consumed more than a single predator, this is because when two predators were present there were competition among two predators and they will try to find as many prey as they can (Aditya *et al*. 2006).

Low foraging occurred when only one odonata nymph was exposed to mosquito larvae. But, when two odonata nymphs were exposed to mosquito larvae, there was a competition between the two odonata nymphs. Anogwih and Makanjuola (2010) was conducted a study on fish predatory pattern in the presence of alternative prey and their predatory behaviour. They indicated that the competition between predators is present due to the energy level of two predators to search, attack and capture prey frequently.When only one predator was present, there was no competition and thus the energy level is low (Aditya *et al.* 2006; Chandra *et al.* 2006a; Manna *et al.* 2008). However, in the predation activity of *O. chrysis* there was the inverse relationship, where *O. chrysis* consumed more when alone than when two predators present.

In this study, when two male fish were exposed to *Ae. albopictus* and *Cx. quinquefasciatus* larvae, the number of prey consumed was greater than when only one fish was released. As a result, more mosquito larvae were consumed by these two fish. This was observed especially when two male guppies were released in the aquaria; but, this was not observed when two female guppies were released which was due to high competition between them. Anogwih and Makanjuola (2010) reported low foraging behaviour of guppies when a single fish was exposed to the mosquito larvae, but when two fish were exposed to mosquito larvae, competition between the two fish was present thus increased their foraging behaviour. In addition, in terms of feeding rate, in this study, female guppies had increased feeding rate when the prey densities increased. This result supported the finding by Anyaele and Obembe (2010) and Manna *et al*. (2008) reported that when four guppies were used in the experiment instead of one guppy, the feeding rate of four guppies increased.

9.9.2 Prey Preferences

In terms of prey preference the different predator showed varied prey preference. Many factors could influence the predator's selection for the prey species such as prey behaviour, prey size, nutrition value and the availability of prey or because of presence of alternative preys. Prey behaviour means that how prey try to escape or attract the predator. Some mosquito species move very fast as an anti – predatory strategy such as *Culex* spp.

while others were easily caught without the need to search or attack for *Anopheles* and *Aedes* larvae. In this study the escape behaviour from the predator showed that *Cx. quinquefasciatus* species have very good escape behaviour as they are very active so that predator found them hard to capture and attack. The postures of mosquito larvae species were also different as a results prey the predators to attack and seize them. The attack technique of predator varied among guppy and dragonfly nymphs. Some attacked from frontal and some attacked from behind. The behaviour of predator and prey influenced the number of mosquito larvae consumed by predators.

In the presence of alternative prey for example other than mosquito larvae, like worm. Some predator preferred alternative prey like worm and other predator preferred mosquito larvae. In the experiment when the alternative prey was present predator choosed or preferred other prey like worm but they still consumed a good number of mosquito larvae. However, Manna *et al*. (2011) also found that larvivorous fish, *Aplocheilus panchax* consumed more *Cx. quinquefasciatus* larvae over other alternative prey such as tubificid worms and chironomid larvae in all the habitat conditions. Hurst *et al.* (2006) also reported that fish had strong preferences for mosquito larvae than other alternative prey such as chironomid midge larvae, tadpoles and frog. Similarly in the study done by Bhattacharjee, *et al.* (2009), where in the presence of alternative preys, the consumption for mosquito larvae did not differ significantly for fish.

According to Deacon (2010) a guppy displayed a preference for the *Tubifex* prey than Daphnia when these two types of prey were used in the experiment and the total number consumed throughout all trials was almost double that of *Daphnia* (2630 *Tubifex*, 1377 *Daphnia*). In this study three species of IV instars of mosquito larvae *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* were used to assess the prey or species preference.

The different predator species showed the difference prey preference for example dragonfly nymph of *O. sabina, N. fluctuans* and *P. reticulata* (both male and female*)* preferred or ate more *Ae. aegypti* larvae, but the dragonfly nymph of *O. chrysis* did not show any preference to prey species as they consumed all types of mosquito species. Soumare & Cilek, (2011) reported in their study that the *Mecyclops longisetus* preferred *Ae. albopictus* and *Ae. triseriatus* than *Cx. quinquefasciatus*.

Factors that influenced the selectivity of prey by predator depended on how the prey escaped from the predator and the ability of the predator to chase. The observation of experiment showed that the dragonfly nymph of *O. sabina* was very active and aggressive than other predators. They consumed more *Ae. aegypti* larvae. The activity and position of mosquito prey was one of the factors that influenced the predation activity. There were four activities of mosquito larvae such as resting, browsing, filtering and thrashing. The positions of mosquito larvae also contributed to the predation activities. The four common positions of mosquito larvae within the water medium were surface, bottom, wall and middle. Studies conducted by Juliano *et al.* (1993) and Yee *et al.* (2004) revealed that there were two factors that influenced the predation activity which were position and activity of prey. These two factors varied in terms of normal activity and the presence of predator. Moreover, Juliano *et al.* (1993) also indicated that decreased risk associated with decreased thrashing in hungry larvae was more than offset by increased risk due to decreased resting and increased browsing, an activity with intermediate risk. Risk associated with activity pattern was more consistently related to hunger than was risk associated with positions.

The contributing factor to this high capture rates on *Aedes* compared to *Culex* could be deduced from work done by Yee *et al*. (2004) where they found different strategies in the mosquito larvae feeding behavior; the former prey species for example *Aedes* spp.were spent more of their activity time trashing below the water surfaces and *Culex* spend more time at the surfaces. This evidential stratification in foraging areas made *Aedes* to be the targeted prey for dragonfly predators since dragonflies spent most of the time stalking for preys at bottom levels making *Culex* tendency to escape predation. Additionally, the prey posture could be the contributing factor to the high success rate of capture on *Aedes*, because the *Aedes* larvae spent more time thrashing below the surface whereas the *Culex* spp. spending more time at the surface. This finding was supported by Kesavaraju *et al*. (2007) where the *Corethrella appendiculata* (Grabham) which hunt the prey at bottom level of water and the larvae that trashed on container bottom had a higher risk of being captured than larvae that spend time on the surface water and Marten, *et al*. (1994b) Soumare & Cilek (2011) found that the weaker predation on *Cx. quinquefasciatus* larvae could be due to less contact of *Culex* larvae with the predator and the large size of *Culex* spp than *Aedes* species. However, in terms of species preferences, dragonfly nymph, *N. flactuans* and *O.sabina* preferred and ate more on *Ae. aegypti* larvae over *Ae. albopictus* larvae. As mentioned by Yee *et al*. (2004) the *Ae. aegypti* was most closely associated with non feeding activity which was trashing at bottom, whereas *Ae. albopictus* tended to be at surface.

Kar and Aditya (2003) stated that planarian preferred and consumed *Anopheles* larvae more than *Culex* larvae. It was due to the behaviour of the prey and the predator itself as the larval posture of *Anopheles* larvae was paralleled to the water surface. These postures help planarian to attack the larvae more easily. In addition to that, Kar and Aditya (2003) stated that *Culex* larvae were more active and move faster than *Anopheles* larvae and thus *Culex* larvae were more difficult to be attacked. So, this explained the reason why both sexes of guppy observed in this study found difficulty to attack and consume *Cx.*

quinquefasciatus than the other two species. Okorie and Abiodun (2011), found that larvivorous fish preferred *Anopheles* than *Culex* larvae in their experiment. It is most likely because *Culex* had the ability to escape faster than *Anopheles*. However, a study reported by Louca, (2009) the anopheline and culicines differed behaviourally and in their macrohabitat preferences. The active behaviour of culicines might made them more easily predated upon by visual predators, like fish whereas anophelines often adhere to vegetation, where they lie parallel to the surface water and are relatively motionless.

Culler and Lamp (2009) found that the preference towards certain type of prey is not only due to the ease to capture they prey, but it also depending on availability and profitability to the predator. For example, although ostracods was easy to capture, they lacks nutrition composition needed by predators for their growth, thus predators did not prefer to eat the ostracods (Culler & Lamp, 2009). Anogwih and Makanjuola (2010) stated that guppies preferred alternative preys i.e., *Chironomous* larvae, which were the most preferred prey, only then followed by mosquito larvae and worm larvae. Manna *et al.* (2008) in their study indicated that guppies preferred alternative preys such as tubificid larvae when these alternative preys were present; but, guppies still consumed mosquito larvae nonetheless. Manna *et al.* (2008) mentioned guppies had a wide range of dietary choices. Both studies i.e., Anogwih and Makanjuola (2010) and Manna *et al.* (2008) showed that the guppy preferred alternative prey than mosquito larvae when both are present together; however, in both experiments, it was observed that the guppy also consumed the mosquito larvae. Other larvivorous fish, *Aphyosemion gularis* preferred mosquito larvae than non-mosquito macroinvertebrates such as chironomids larvae (Okorie, & Abiodun, 2011).

As suggested by Kesavaraju *et al.* (2007), the predation rate was influenced by the behaviour of prey species and development stage of prey. This means that different predator attacks different stage of mosquito larvae. But, in this study, the 3rd and 4th instar larvae were used as the trial experiment showed that guppy preferred late-stage larvae than early-stage larvae. The black colour of the *Ae. aegypti* larvae could be the factor that attracted the guppy to attack and consume them, unlike the *Cx. quinquefasciatus* larvae, which were pale in colour. Other than that, Rajasekharan and Chowdaiah (1972) suggested that the preference of Gambusia towards *Ae. aegypti* larvae could be attributed to the larvae's small size, their vertical position in water, and their tendency to clump in groups; these factors facilitated their capture.

The size of prey significantly influenced the predation activities or feeding rate. Most of the predators preferred smaller prey to bigger prey. This situation happens when 2nd and 3rd instars prey used in the experiment the 2nd prey species more vulnerable than 3rd prey (Kesavaraju *et al*. 2007) and the water bugs preferred small prey of 2nd to 4th instars mosquito larvae (Saha *et al*. 2010). Flatworm fed more on 1st instars larvae compared to other stage of mosquito larvae. The small and large flatworm ate mosquito larvae at similar rate (Tranchida *et al*. 2009).

Generally all predators except *O. chrysis* preferred on *Aedes* spp than *Culex* spp. this was due to the size of 4th instar *Aedes* species that is smaller than 4th instar *Culex* species and weighs less than *Culex*. Besides, sizes of prey species, dragonfly nymphs of *O.sabina* and *N. flactuans* ate more on *Aedes* spp. than *Culex* spp. due to the behavior of predators themselves as dragonfly nymph hunt primarily as the bottom of containers. Similar to *C.appendiculata*, (Kesavaraju *et al.* 2007) and *Tx. Rutilus*, (Kesavaraju, *et al.* 2011) and the *Aedes* spp. spent more time at the bottom than *Culex* spp. According to

Kesavaraju, *et al.* (2011) *Cx. pipiens* larvae spent more time motionless at the surface even in the absence of predation-risk cues when compared with the other species, indicating that *Cx. pipiens* larvae are the least vulnerable prey. As compared with the other prey species, *Ae. albopictus* larvae exhibited more high-risk behaviours both in the presence and absence of predation-risk cues, indicating that they are the most vulnerable prey.

This reduced handling time and accelerated successive prey consumption by predator and the same opinion given by Mathavan, (1976) where they found dragonfly nymph preferred and ate more of *Anopheles* spp. than *Culex* spp because of the prey size, and the handling time reduced for attack and captured. However, when both species were released in the aquarium the predator ate more on *Culex* species than on *Anopheles* species because of the posture of the prey. This was due to *Culex* species occasionally moved to the bottom while *Anopheles* still remaining. The *Mesogomphus lineatus* predated less number of pupa stages and consumed more of larvae stages. The predators were more efficient in attack and capture for sinking prey than floating ones. These situations happened when they ate more *Culex* spp than *Anopheles* and preferred on larvae stage than pupa stage. In contrast, all predators try to avoid pupa stages when the larve changed to that stage. The reason was the pupa tended to hang to the surface of the aquarium whereas the larvae moved freely in the water and inability to accommodate the pupa, whose width exceeds the width of the nymph mouth parts. This is supported by Futami, *et al.* (2008) study, where they found that the pupae were less active, which may draw less attention from the predator.

Cyclopoid predated more on *Aedes* spp. than *Culex* spp. in the laboratory study. The 2 species of of *Mesocyclops longisetus* and *Macrocyclops albidus* avoided attacking *Culex* spp because of the bristles on *Culex* larvae gave cyclopoids the false impression they were too large to attack (Marten *et al*. 1994b). Several studies reported that Cyclopoid prefer small prey which was 1st and 2nd instars of mosquito larvae than late stage 3rd and 4th instars (Rey *et al*. 2004; Marten, 1990a; Marten, 1990b).

Kumar and Rao (2003) found the same results that the prey size influenced the predation activities and prey preferences. The handling time in *Mesocyclops thermocyclopoides* increased with increasing prey size. For instance it was significantly longer time for IV instar larvae than I instar mosquito larvae and longer time required for *Cx. quienquefasciatus* larvae than *An. stephensi* larvae. Besides the larger size, the restless thick exoskeleton and heavy setation of *Culex* IV instar would be responsible for the greater handling time.

A previous study by Juliano and Reminger, (1992) reported that the prey size or stages of mosquito larvae was correlated with the mosquito larvae position and activity and indirectly will influence the predation activity. From their study a few results found such as earlier instars (1st and 2nd) spent more time thrashing and less time at the surface than did late instars (3rd and 4th). While browsing activity was significantly greater in late instars (3rd and 4th) than earlier instars (1st and 2nd). The position of early and late instars also differed as the early instars spent most of their time at the bottom and the late instars at the surface of water. Thus, larvae at the surface filtered frequently, but rarely thrashed. Conversely, larvae below the surface (at the wall or the bottom) thrashed frequently, but rarely filtered. They also concluded that the risky activity for *Ae. triseriatus* was thrashing and the risky position was at the bottom when the presence of *Toxorhynchites rutilus.* Thrashing seems to be a risky behaviour, and because *Ae. triseriatus* rarely thrashed at the surface, the surface may appeared to be associated with lower risk of predation. The position and activity of prey were being varied depending on the predator species and behaviour. Because position and activity are so closely correlated, it was difficult to decide whether activity or position was a more important determinant of risk of predation.

Studies by Futami *et al.* (2008) confirmed that the diving frequency and duration decreased with age of mosquito larvae (stages). For example in young larvae, the surface to volume ratio was high and a greater portion of the larval oxygen requirements could be met by surface diffusion through the cuticle. As size increased, the surface to volume ratio decreased and the larva required more oxygen to meet the increasing demand. For this reason, older larvae must spend more time at the surface to draw oxygen through a respiratory siphon. They concluded that the diving duration was significantly longer during first and second instars compared to fourth and pupae.

Kesavaraju, *et al.* (2011) conducted a study to compare the behaviour of *Ae. albopictus, Cx. pipiens* and *Ae. japonicas* larvae in the presence and absence of predation risk cues from *Tx. rutilus* larvae. They found *Cx. pipiens* larvae were least at risk from predation by *Tx. rutilus. Ae. japonicus* larvae spent more time browsing or thrashing near the wall, middle, and bottom of the container in control treatments, but reduced their movements and increased resting near the surface in the presence of predation-risk cues. *Ae. albopictus* larvae browsed near the wall and at the bottom of containers more than the other activities and positions in control treatment, but increased thrashing in the middle of the container and resting near the surface in the presence of predation cues. Kesavaraju *et al.* (2008) also reported that *Ae. albopictus* larvae was more vulnerable to predation *C.appendiculata* than *O.triseriatus.* In this study, *O.chrysis* consumed all three species of mosquito larvae and did not show any prey prerefences. It means all species of mosquito larvae did not change its behavior in the predation activity.

One example of such study done by Aditya *et al*. (2007) found that the *Toxorhynchites splendens* more preferred or consumed on *Cx. quinquefasciatus* larvae compared to *Ar. subalbatus* larvae. As the biomass of *Ar. subalbatus* larvae was bigger than *Cx. quinquefasciatus* larvae, so that more effort or energy needed to search and attack *Ar. subalbatus* larvae. The rate of predation decreased and dropped when the stage instars of *Toxorhynchites splendens* change to pupa.

All fish preferred or consumed more of the 4th larvae than pupal stages (Ghosh *et al*. 2005). Marti *et al*. (2006) indicated that *C. decemmaculatus* ate less than *J. multidentata* fish due to the attack strategy and handling time of *C. decemmaculatus* less were than *J. multidentata* fish. *C. decemmaculatus* took longer time to search and attack mosquito larvae of *Culex pipiens* (Marti *et al*. 2006). The feeding experiment between fish *Pseudomugil signifier* Kner and *Gambusia holbrooki* (Girard) in laboratory trials showed that both fish consumed 100% of 1st, 2nd and 3rd instars of mosquito larvae *Cx. annulirostris* at 50 and 100 densities of larvae. When 200 densities of mosquito larvae exposed to both fish, the mean predation rate for 1st, 2nd and 3rd instars were greater than 90%. On the other hand the predation rate of fish with 4th instars was lower than 45%. It showed that both species prefer 1st, 2nd and 3rd instars of mosquito larvae rather than 4th instars (Willems *et al*. 2005).

The predation of mosquito larvae and other prey taxa by using two species of larval dytiscid beetles (Agabus; Coleoptera: Dytiscidae) was conducted in the laboratory and wetlands (Culler & Lamp, 2009). The two species used were *Agabus punctatus* and *Agabus disintegrates*. Choice test and no choice test were the experimentation protocols. In the choice test both species of beetles were exposed to mosquito larvae. However in the no choice the predator were exposing to three prey taxa of mosquito larvae, copepods and ostracods. Both tests showed that both predator species preferred or consumed more on mosquito larvae than copepods and ostracods. This was due to ease of capture on mosquito larvae. However *Agabus punctatus* was more aggressive than *Agabus disintegrates*. As mentioned by Culler and Lamp (2009) the preference of prey type was due to not only ease to capture but also availability and profitability to the predator. They indicated that in prey preference, although ostracods were easy to capture but both predators preferred them as they lacked of nutrition composition that both predator needed in their growth. For instance *Agabus* grew larger when consumed mosquito larvae rather than copepods than ostracods. This showed that the mosquito larvae and copepod were rich in nutrition. In terms of cannibalism, the mortality rates of preys decreased when the second predator was present. Competition for prey could be strongest between and within *A. punctatus* as they are more active and aggressive than *Agabus disintegrates.*

According to Aditya *et al*. (2006) both predator species *Rhantus sikkimensis* and larvae of *Toxorhynchites splendens* could consumed a high amount of *Cx*. *quinquefasciatus* larvae, where *Rhantus sikkimensis* predated more than *Toxorhynchites splendens.* Many factors contributed to the effectiveness of *Rhantus sikkimensis* compared to *Toxorhynchites splendens,* one of which would be the capability of *R.sikkimensis* expectedly more compared to the larvae of *Tx. splendens*, due to the greater body size and energy requires of *R.sikkimensis.* Time also affected the predatory capability as IV instars larvae *Tx. splendens*, proceeds pupation, the predation rate drops. The effectiveness of *R.sikkimensis* to consume more larvae also depended on the ability of *R.sikkimensis* itself to kill more target preys and the ability to kill preys was lower in *Tx. splendens*. When the number of predator was increased more preys were killed and consumed.

When the alternative prey used with the mosquito larvae in the experiment the predator preferred mosquito larvae than other prey (Kumar & Rao, 2003; Anyaele & Obembe, 2011; Culler & Lamp, 2009) but in contrast studies done by (Anogwih & Makanjuola 2010; Manna *et al*. 2008; Quiroz-Martinez *et al*. 2005) some predators used as biocontrol agents preferred alternative prey to mosquito larvae.

9.9.3 Prey Densities

Prey densities influence the predation activities of prey and predator. For example dragonfly nymph of *Mesogomphus lineatus* consumed more mosquito larvae when the prey density increased (Mathavan, 1976) and *Mesocyclops thermocyclopoides* predated on mosquito larvae of *Cx. quinquefasciatus* and *An. stephensi* (Kumar & Rao, 2003). However, in this work dragonfly nymphs consumed more mosquito larvae when the number of prey densities decreased. This situation was reported in the predation activities of *N. flactuans* (when exposed to *Ae. albopictus* and *Ae. aegypti* larvae), *O. chrysis* (when exposed to *Ae. aegypti* and *Cx. quinquefasciatus* larvae) and *O. sabina* (when exposed to *Cx. quinquefasciatus* larvae). Similar finding was found by Willems *et al.* (2005) where both species of *Pseudomugil signifer* Kner and *Gambusia holbrooki* Girard consumed more larvae at the lowest densities compared to the highest densities. However, both species reached a level of satiation when they were exposed to high densities of larvae and late instars of mosquito larvae.

Female guppies consumed more mosquito larvae when the prey density increased, but in *N. flactuans*, *O. sabina* and *O.chrysis* feeding rate increased when number of prey densities decreased. Guppy consumed all of mosquito larvae when exposed with 100 IV instar mosquito larvae in 24 hours. On the other hand, dragonfly nymph could consume a good number of mosquito larvae but not 100% in certain condition.

Female guppies ate more when the densities of *Ae.aegypti* larvae were increased. This result was supported by the finding of Okorie and Abiodun (2010). They reported that larval consumption increased when the densities of prey increased until satiation level was reached i.e., when the fish became overwhelmed. Prey densities did not influence predation activities of male guppies. Different predator could show different results in feeding experiment when the densities of mosquito larvae increased or decreased. Chatterjee and Chandra (1997) also reported that the feeding rate increased with the increase in prey and predator densities (number of predator).

In experimentation when the vegetation was introduced both species consumed more with no vegetation than medium density vegetation and high density vegetation. This was because fish spent more time searching area of highest larvae densities. In situation, where the two difference species of fish were used, *Pseudomugil signifier* Kner consumed more than *Gambusia holbrooki* (Girard) at medium density vegetation and high density vegetation (Willems *et al*. 2005). The aquatic vegetation would influence the predation activities in both adult and nymph *Diplonychus spp.* and *Anisops spp*. consumed greater amount of smaller instars than larger ones as the smaller preys were easier to catch but provide less nutrition therefore must be consumed in greater quantity (Shaalan *et al.* 2007). Sharma *et al.* (1987); Linden and Cech (1990); Asimeng and Mutinga (1992) also reported that the predation efficacy of some fish species was reduced by aquatic vegetation. In contrast, a study conducted by Hurst *et al.* (2006) revealed the presence of vegetation did not affect the predation rate of predators.

9.9.4 Water Volume

Predatory foraging decisions were also affected by dilution factors, as displayed by *O. sabina* and *N. flactuans*. In the predation experiment water volume influenced the predation activities of *O.sabina* (when exposed to *Ae. aegypti* larvae and *Ae. albopictus* larvae), and *N. flactuans* (when exposed to *Cx. quinquefasciatus*). From the observation made in this study, their attack behaviours decreased when water volume was increased. The tendencies for preys to be able to escape from predators were enhanced with increased water volume and predators were less successful in their attacks. Such finding had also been reported by Mandal *et al*. (2008) in their experiments on dragonfly larvae predating on *Cx. quinquefasciatus*. Although water volume seemed to be a way for the mosquito larvae to escape from being preys such factor also reflected the increased foraging area for the predators (Shaalan *et al.* 2007).

Feeding rate lowered when water volume of water was increased. When the foraging area increased, predators spent more time to search for preys. The tendencies for preys to escape from predators were enhanced with increased water volume as predators will be less successful in their attacks (Shaalan *et al.* 2007; Mandal *et al*. 2008; Chandra, *et al.* 2008; Ghosh *et al.* 2005; Bhattacharjee *et al*. 2009). They revealed that the water volume had an inverse relationship with feeding rate. Ghosh *et al.* (2006) reported that with increment of space, the foraging behaviour of the fishes changed and possibly required more time to capture and consume the mosquito preys. Chatterjee and Chandra (1997) found that the feeding rate decreased with the increase in water volume, e.g. its feeding rate is directly proportional to the prey.

Water volume also influenced the predation activities of male guppy (when exposed to *Ae. aegypti* larvae and *Ae. albopictus* larvae) and female guppy when exposed to all three species of mosquito larvae. When 2 L of water was used in the experiment, the predation activities and feeding rate decreased. Fish spent more time to forage and search for mosquito larvae. The feeding rate decreased when water volume of water was increased, and the feeding rate increased when the number of predators and the densities of preys were increased (Chandra *et al*. 2006; Mandal *et al.* 2008). In another study, they reported that although the mortality was greater in shallow water compared to deep water for the second instar, the statistical analysis revealed insignificance of water depth. Poor diving performance of older instar individuals and pupae might reduce the effect of depth in the statistical analysis. Another possible reason was that the water depth was not enough for older mosquitoes to escape from the spiders (Futami *et al*. 2008).

As discussed by Jacob *et al.* (1983), environmental factors such as temperature and lighting also influence predation efficiency of larvivorous fish but salinities did not affect the predation activities. The predation activities increased when the temperature was increased and the feeding rate under lighting was higher than in dark condition. In addition, Marti *et al.* (2006) suggested that different prey attack strategies and handling time of predator to consumption of prey also influence feeding rate. Bhattacharjee *et al.* (2009) concluded the consumption of mosquito larvae at a particular prey density reduced with increased volume of water possibly due to the evasion tactics of the mosquitoes.

Shaalan *et al*. (2007) used a common predator of *Diplonychus spp*. and *Anisops spp*. found in Towns ville, Australia as a predator against *Cx. annulirostris* mosquito larvae. As a result they found that adult *Diplonychus sp*. was more efficient predator than *Anisops sp*. when they increased water volume or foraging area and introduction of aquatic vegetation caused the predation capacity to be low. With increase of the foraging area, predators spent more time in search of preys. When the predator spent more time in prey search the attack rate of predator decreased but did not affect the predator capacity of adults *Diplonychus spp.* As adults *Diplonychus spp.* were highly active predator and fast attack. The foraging area will affect predatorial capacity but not for all predators.

9.10 Recommendation & Future direction

Dengue education campaign should be conducted at the local community level, primary and secondary school, universities, government sector and also NGOs. The campaign should focus on changing human behaviour and practices towards reducing mosquito breeding places within their residential areas. The main breeding sites were found to be gardening utensils in urban areas and artificial containers in suburban areas which should be destroyed. Therefore physical activities such as search and destruction of any potential breeding habitats, COMBI and educatation to resident to recycle items should be encouraged and campaigned.

The proper solid waste management system should be provided in suburban area for residents to dispose off unused items or discarded items in strategic locations and recycling activities should be implement among communities. In urban areas of Putrajaya, it seem most of the residents are working and they hired maids who should be educated on the health effect of the dengue fever, the potential breeding habitat of mosquito and use larvicides in a proper way. Whereas in Putrajaya the main breeding habitat was gardening utensils such as flower pot, watering can, and flower pots plates. In order to prevent mosquito breeding in Putrajaya, the communities should aware on the mosquito breeding habitat in that areas such daily check on potential breeding habitat. The larval survey activities be should continuously be carried out by local authority and health department as from such activity the useful information can be obtained such as the mosquito density, mosquito species, breeding habitat of mosquito, which can be used to predict the outbreak from the AI, BI and CI indices. The ovitrap surveillance is a best tool to detect the distribution and prevalence of mosquito species both in urban and suburban areas.

Job rotation should be implemented among staff in health district office especially who are involved in fogging activity more than 5 years. Health surveillance and monitoring should be continuously carried out at least once a year among staff and public as the adverse health symptoms as a results of fogging activities were also experienced by both groups.

Integrated Vector Management (IVM) is based on a concept that utilizes several different approaches to vector control such as chemical control, biological control, environmental management and source reduction. Besides, use of chemical control and guppy as biocontrol agent, the use of dragonfly nymph should be taken into consideration as from this study showed their positive potential as novel biocontrol agent in Malaysia. Odonates also consume all the 3 common mosquito species in Malaysia which make them to be efficient predator. The use of dragonfly nymphs as biocontrol agents against mosquito immature stages can be applied to other countries as well since dragonfly nymphs are commonly found all over the world. Dragonfly nymphs can be use as one of the approach in order to mitiges mosquito density in Malaysia, besides existing control measures. This biocontrol method is one of approach that can be support others control measures.

Future direction

This work studied several potential biocontrol agent of dragonfly nymph for eradication of mosquito larvae species. This study found that all 3 species of dragonfly nymphs efficiently consumed mosquito larvae and can be suggested as one of alternative method in controlling mosquito population in Malaysia. Several suggestion for future direction in biocontrol study include the following:

1) Selecting dominant species of dragonfly nymphs in selected areas before pursues the experimentation work.
2) Applying the dragonfly nymphs to the field environment and assess its field impact and operational potential for field control purposes.
3) Using dragonfly nymphs as biocontrol for others mosquitoes for instances *Anopheles* species.

CHAPTER 10

CONCLUSION

1) Three common species of mosquito found in Putrajaya and Kuala Selangor areas were *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus. Ae. albopictus* was the predominant species in both study areas.

2) The main breeding habitats were gardening utensils in Putrajaya and artificial containers in Kuala Selangor. Other habitat were building design, discarded items, rubbish bins, tyres, water storage and natural habitat.

3) In both study areas the predominant mosquito species found in the ovitrap surveillance was *Ae. albopictus*.

4) The ovitrap index was found to be higher in suburban area compared to the urban area.

5) There was no correlation between climatic factors and mosquito density obtained for ovitraps sampling.

6) The staffs involved in vector control unit were (94%) males and (6%) female. Most of them (31%) aged between 30-35. A total of 94% staff were Malay and 6% Indian. All the staffs had completed secondary school and among them (33%) had certificate from MOH and (14%) and (6%) achieved higher education at diploma and degree levels.

7) The public involved in this study were 48% males and 52% female. Most of them (26%) aged between 24-29. A total of 97% were Malay and 3% Indian. All the public had completed the secondary school and among them (29.6%) had achieved higher education at diploma level.

8) The highest control measures reported by both groups of respondents were cleaning up the mosquito breeding sites.

9) The perception on factors contributing to the increase of dengue cases, shows that the human behaviour was the most common factor contributing to this problem, followed by lack of knowledge in controlling dengue fever, environmental factors, less effective control method and chemical resistance.

10) Guppy was reported as the most common biological control agent used by both groups of respondents.

11) Most of the workers reported that they had multiple adverse health symptoms after involved in fogging activities. In our study, fatigue was the most frequently reported symptom (27.3%), followed by dizziness (15%), blurred vision (12%), breathing difficulty (10.6%), and itching (7.6%). Other symptoms reported by workers such as anxiety were 4.5%.

12) Self-reported symptoms of breathing difficulty (26.9%), dizziness (23.5%), nausea (13%) were the higher symptoms reported by public/residents.

13) The dominant species in both study areas was *Neurothemis fluctuans* (Fabricius, 1793) commonly known as (Coppertone velvetwing) with a total of 112. Other species collected were the Sober skimmer, *Orthetrum sabina* (Drurry, 1770) (105), *Orthetrum chrysis* (Selys, 1891) (Redfaced skimmer) (92), *Trithemis aurora* (Burmeistar, 1839) (Down dropwing) (65), *Trithemis festiva* (Rambur, 1842) (Indigo dropwing) (26) and *Brachydilax chalybea* (27).

14) Three common dragonfly nymph species were found which are *O. chrysis*, *O. sabina* and *N. flactuans* with other dragonfly nymph species.

15) There was no significant difference among the three species of dragonfly nymphs in terms of mosquito larvae consumption. However, there was a significant difference in terms of the mosquito species most preferred by the dragonfly nymphs. It was observed that the dragonfly nymphs consumed more on *Ae. aegypti*.

16) In terms of preying preferences, there was a significant difference in the number of prey species between *Ae. aegypti* and *Cx. quinquefasciatus* consumed by the predators. The Odonata predators showed specific preying preference; *N. fluctuans* and *O. sabina* consumed more *Ae. aegypti* larvae than *Ae. albopictus* larvae and *Cx. quinquefasciatus* larvae, while *O. chrysis* does not show any larvae preference as it consumed three of mosquitoes species *Cx. quinquefasciatus* larvae, *Ae. aegypti* larvae and *Ae. albopictus* larvae.

17) The efficiency of dragonfly nymph under experimental studies in laboratory was good as they consume all species of mosquito larvae.

18) The overall feeding rates of female guppies were significantly higher than males for all three species of mosquitoes tested.

19) The number of mosquito larvae consumed by predators is different between light on and light off. All predators consume more larvae during light on than light off.

20) From the regression equation, it was observed that factors such as number of predators, water volume and prey density influenced the feeding consumption of Odonata species and guppies.

21) In the feeding experiment between female guppy and male guppy, there was significant difference in mosquito larvae consumed. Both guppies consumed greater *Aedes* than *Culex*.

22) When two fish were exposed to prey, the number of prey consumed was greater than when only one fish was released.

23) The behaviour of predator and prey influenced the predatory activities. *Poecilia reticulata* (guppy) is more active than dragonfly nymph. They like to follow, search and attack the mosquito larvae, however the dragonfly nymphs are motionless, like to wait for their prey to come nearer and ambush them. Most of their time is spent at the bottom of water and hide back of stone.

24) The attacking behaviours of predator decreased when water volume was increased. Feeding rate decreases when the volume of water is increased.

25) Low foraging happens when only one odonata nymph is exposed to mosquito larvae. However, when two odonata nymphs are exposed to mosquito larvae, there will be a competition between the two odonata nymphs.

26) The feeding rate increases when the prey density is increased. Larval consumption increases when the density of prey is increased until satiation level is reached.

27) In the predatory studies both predators prefer *Aedes* larvae to *Culex* larvae.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

PUBLICATION

1. Saleeza. S.N.R., Norma-Rashid Y, Sofian–Azirun, M. (2013). **Predacious efficacy of three Odonata Nymphs as biocontrols against mosquito larvae in Malaysia.** *Journal of Tropical Biomedicine* (submitted) (ISI journal) – Acknowledge 146/13

2. Saleeza. S.N.R., Norma-Rashid Y, Sofian–Azirun, M. (2013). **Guppies (*Poecilia reticulata*) as predators for 3 common species of mosquito larvae in Malaysia.** *Southeast Asia Journal of Tropical Medicine and Public Health*. **45 (2)** : 299- 309

3. Saleeza. S.N.R., Norma-Rashid Y, Sofian–Azirun, M. (2012). **Studies on mosquito outdoor breeding places and mosquito species in residential areas in Malaysia**. *Southeast Asia Journal of Tropical Medicine and Public Health* **44(6)**: 963-969.

4. Saleeza. S.N.R., Norma-Rashid Y, Sofian–Azirun, M. (2011). **Mosquitoes Larval Breeding Habitat in Urban and suburban areas, Peninsular Malaysia.** *Journal of World Academy of Sciences, Engineering and Technology*. **58** : 569-573

BOOK CHAPTER

1. Norma-Rashid, Y & Saleeza SNR. Eco-friendly control of 3 common mosquito larvae species by Odonata nymphs. In: Biopesticides- Basic and Applied. Sahayaraj, K. (Ed.). 2014, XVII, 384 p. 71 illus., 50 illus. in color. ISBN 978-81-322-1877-7. Springer.

ORAL PRESENTATIONS

1. 15th Biological Sciences Graduate Congress (15th BSGC): Bridging Ideas, Building Talents, 15-17 December 2010, held in University of Malaya.
2. ICEEE 2011: International Conference on Ecological and Environmental Engineering. October 26-28 2011, held in Bali, Indonesia.
3. Candidature Defense Presentation, 29 December 2011, Institute for Biological Sciences (IBS), held in Faculty of Sciences, University of Malaya (UM), Kuala Lumpur.
4. 17th Biological Sciences Graduate Congress (15th BSGC): 8-10 December 2012, held in Chulalongkorn University, Bangkok Thailand.
5. Seminar before submission of thesis on 14 July 2014, Instititute of Biological Sciences, Faculty of Science, University Malaya.

POSTER PRESENTATIONS

1. **Feeding efficiency of Odonate species and *Poecilia reticulata* against 3 mosquito species in Malaysia**. ICE 2012: XXIV International Congress of Entomology. 19-25 August 2012, held in Daegu Korea.
2. **Dragonflies as potential biocontrol against mosquito larvae in Malaysia**. Seminar ZEN 2012. 18 December 2012, held in Faculty Science, University Malaya, Kuala Lumpur

Appendix A (Questionnaire for Staff)

Borang Soal Selidik **INSTITUT SAINS BIOLOGI, FAKULTI SAINS** **UNIVERSITI MALAYA** **KUALA LUMPUR**	**Questionnaire** **INSTITUTE OF BIOLOGICAL SCIENCE, FACULTY OF SCIENCE, UNIVERSITY OF MALAYA** **KUALA LUMPUR**
Tuan/Puan	Sir/Madam
Saya pelajar yang kini sedang menjalankan kajian untuk tesis Ph.D saya di Fakulti Sains, Universiti Malaya. Kajian ini bertujuan untuk mendapatkan maklumat mengenai penggunaan racun serangga dalam kawalan nyamuk dewasa, dan tahap kesedaran terhadap penggunaan racun serangga di Putrajaya dan Selangor.	I am a student currently doing research for my Ph.D thesis in the Faculty of Science, University of Malaya. This research is done to obtain information on the use of insecticide in curbing adult mosquitoes and to assess the awareness level of insecticide usage in Putrajaya and Selangor.
Borang kaji selidik ini mengandungi 4 bahagian (A, B, C, dan D) dan setiap bahagian mempunyai beberapa penyataan. Kepada kakitangan Unit Vektor di Pejabat Kesihatan, saya mohon kerjasama Tuan/Puan untuk menjawab keempat-empat bahagian untuk melengkapkan kajian ini.	This questionnaire contains 4 parts (A, B, C, and D) and each part contains several statements. To the staff of Vector Unit of the Health Office, please answer all the four parts to provide necessary information to complete this study.
Kerjasama anda dalam melengkapkan borang kaji selidik ini amatlah saya hargai. Semua maklumat anda adalah sulit dan	I highly appreciate your cooperation in completing this questionnaire. All of your information will be kept confidential

hanya akan digunakan untuk kajian ini sahaja.	and will be used for the purpose of this research only.
Terima kasih atas kerjasama anda semua.	Thank you for your cooperation.
Siti Nurhafizah Saleeza Bt Ramlee **SHC090022**	**Siti Nurhafizah Saleeza Bt Ramlee** **SHC090022**
BAHAGIAN A: PROFIL RESPONDEN	**PART A: RESPONDENT'S PROFILE**
1. Umur: A. 18–23 tahun B. 24–29 tahun C. 30–35 tahun D. 36–41 tahun E. 42–47 tahun F. Lebih daripada 48 tahun	1. Age: A. 18–23 years old B. 24–29 years old C. 30–35 years old D. 36–41 years old E. 42–47 years old F. More than 48 years old
2. Jantina: A. Lelaki B. Perempuan	2. Gender: A. Male B. Female
3. Bangsa: A. Melayu B. India C. Cina D. Lain-lain (sila nyatakan) ________________________	3. Race: A. Malay B. Indian C. Chinese D. Others (please specify) ________________________

4. Tahap pendidikan: A. Penilaian Menengah Rendah (PMR) B. Sijil Pelajaran Malaysia (SPM) C. Sijil Tinggi Pelajaran Malaysia (STPM) D. Sijil E. Diploma F. Ijazah G. Lain-lain (sila nyatakan) __________________________	4. Education level: A. Lower Secondary Assessment (PMR) B. Malaysian Certificate of Education (SPM) C. Malaysian Higher School Certificate (STPM) D. Certificate E. Diploma F. Degree G. Others (please specify) __________________________
5. Pekerjaan: Sila nyatakan __________________________	5. Occupation: Please specify __________________________
6. Adakah anda merokok? A. Ya B. Tidak 7. Adakah anda mempunyai sebarang masalah kesihatan? A. Ya B. Tidak	6. Do you smoke? A. Yes B. No 7. Do you have any health problems? A. Yes B. No
8. Jika ya, apakah masalah kesihatan yang anda alami sekarang? A. Kanser B. Darah tinggi C. Diabetis D. Sakit jantung E. Lain-lain (sila nyatakan) __________________________	8. If yes, what kind of health problems that you are experiencing currently? A. Cancer B. High Blood Pressure C. Diabetes D. Heart Disease E. Others (please specify) __________________________

BAHAGIAN B: PENGETAHUAN TERHADAP DEMAM DENGGI DAN PENGGUNAAN RACUN SERANGGA	PART B: KNOWLEDGE ON DENGUE FEVER AND INSECTICIDE USAGE
1. Adakah anda tahu mengenai demam denggi? A. Ya B. Tidak	1. Do you know about dengue fever? A. Yes B. No
2. Pada pendapat anda bagaimanakah caranya untuk mengawal demam denggi dan membunuh nyamuk? (Jawapan boleh lebih daripada satu) A. Semburan kabus (*fogging*) B. Penggunaa ABATE (ubat membunuh jentik-jentik) C. Menggunakan ikan gapi D. Membersihkan kawasan-kawasan pembiakan nyamuk E. Lain-lain (sila nyatakan) __________________________	2. In your opinion, how to control dengue fever and kill mosquitoes? (Answer(s) can be more than one) A. Fogging B. The use of ABATE (mosquito larvae insecticide) C. Using the guppy fish D. Cleaning up mosquito breeding areas E. Others (please specify) __________________________
3. Adakah semburan kabus (*fogging*) pernah dijalankan di kawasan rumah anda? A. Ya B. Tidak	3. Has fogging ever been held in your vicinity? A. Yes B. No

4. Adakah anda risau tentang kesan kesihatan anda dan keluarga terhadap penyemburan kabus (*fogging*) yang digunakan untuk membunuh nyamuk dewasa? A. Ya B. Tidak (Jika tidak, sila nyatakan) ____________________	4. Do you worry about how fogging that is used to kill adult mosquitoes will affect you and your family's health? A. Yes B. No (If no, please specify) __________________________
5. Pada pendapat anda, adakah penyemburan kabus (*fogging*) memberi kesan buruk terhadap alam sekitar? A. Ya B. Tidak	5. In your opinion, does fogging negatively affect the environment? A. Yes B. No
6. Pada pendapat anda, apakah kesan penggunaan racun serangga (*fogging*) selain daripada masalah kesihatan kepada manusia? (Jawapan boleh lebih daripada satu) A. Memberi kesan buruk terhadap alam sekitar B. Masalah kesihatan terhadap binatang C. Penggunaan racun serangga melibatkan kos yang tinggi D. Penggunaan racun serangga akan membunuh serangga lain selain daripada nyamuk E. Lain-lain (sila nyatakan) ____________________	6. In your opinion, what are the effects of the use of insecticide apart from causing health problems to human? (Answer(s) can be more than one) A. It causes negative impacts on the environment B. Animal health problems C. The use of insecticide is very costly D. The use of insecticide will also kill other insects besides mosquitoes E. Others (please specify) ____________________
7. Adakah penyemburan kabus (*fogging*) sangat berkesan mengawal nyamuk dan mengawal kes demam denggi? A. Ya Tidak (sila nyatakan) ____________________	7. Is fogging highly effective in controlling mosquitoes and dengue fever cases? A. Yes B. No (please specify) ____________________

8. Pada pendapat anda, adakah semburan kabus (*fogging*) perlu ditukar dengan cara yang lain? A. Ya B. Tidak	8. In your opinion, should fogging be replaced by other methods? A. Yes B. No
9. Jika tidak, sila nyatakan kenapa. ___________________	9. If not, please state why. ___________________
10. Pada pendapat anda, apakah faktor yang mendorong kepada peningkatan kes demam denggi? (Jawapan boleh lebih daripada satu) A. Faktor persekitaran B. Tingkah laku manusia C. Kurang pengetahuan terhadap pencegahan demam denggi D. Langkah kawalan kurang berkesan E. Racun serangga yang digunakan tidak memberi kesan terhadap nyamuk (*chemically resistant*) F. Lain-lain (sila nyatakan) ___________________	10. In your opinion, what factors lead to increased cases of dengue fever? (Answer(s) can be more than one) A. Environmental factor B. Human behaviour C. Lack of knowledge in curbing Dengue Fever D. The curbing method is less effective E. Ineffective insecticide use against mosquitoes (chemically resistant) F. Others (please specify) ___________________
BAHAGIAN C: PENGETAHUAN TERHADAP KAWALAN BIOLOGI	**PART C: KNOWLEDGE ON BIOLOGICAL CONTROL**
1. Adakah anda tahu mengenai kaedah kawalan biologi (*biocontrol*) untuk mengawal pembiakan nyamuk? A. Ya B. Tidak C. Tidak pasti	1. Are you familiar with biological control methods (biocontrol) in controlling mosquito breeding? A. Yes B. No C. Not sure

2. Jika ya, apakah kaedah biologi yang biasa digunakan untuk mengawal pembiakan nyamuk? A. Serai wangi B. Ikan gapi C. Nyamuk gergasi (*Toxo mosquito*) D. Pepatung E. Kumbang F. Garam G. Lain-lain (sila nyatakan) ___________________	2. If yes, what is the biological method commonly used in controlling mosquito breeding? A. Lemon grass B. Guppy fish C. Elephant mosquito (*Toxo mosquito*) D. Dragonfly E. Beetle F. Using salt G. Others (please specify) ___________________
3. Adakah anda rasa kaedah biologi selamat dan berkesan digunakan untuk mengawal nyamuk? A. Ya B. Tidak C. Tidak pasti	3. Do you think that biological method is safe and effective in controlling mosquitoes? A. Yes B. No C. Not sure
4. Adakah anda rasa kaedah biologi selamat dan tidak mencemarkan alam sekitar? A. Ya B. Tidak C. Tidak pasti	4. Do you think that the biological method is safe and not polluting the environment? A. Yes B. No C. Not sure
5. Adakah anda rasa kaedah biologi selamat kepada kesihatan manusia? A. Ya B. Tidak C. Tidak pasti	5. Do you think that the biological method is safe for human health? A. Yes B. No C. Not sure

6. Adakah anda mengamalkan kaedah biologi ini di persekitaran tempat anda? A. Ya (sila nyatakan) ___________________ B. Tidak	6. Do you use this biological method within your vicinity? A. Yes (please specify) ___________________ B. No
BAHAGIAN D: SILA ISI BAHGAIAN INI JIKA ANDA TERLIBAT DALAM AKTIVITI PEMYEMBURAN KABUS (*FOGGING*) – KAKITANGAN PEJABAT KESIHATAN	**PART D: PLEASE FILL IN THIS SECTION IF YOU ARE INVOLVED IN FOGGING ACTIVITY – HEALTH OFFICE STAFF**
1. Berapa lamakah anda bekerja di Unit Vektor? A. 1–2 tahun B. 2–3 tahun C. 3–4 tahun D. 5 tahun E. Lebih daripada 5 tahun	1. How long have you been working in Vector Unit? A. 1–2 years B. 2–3 years C. 3–4 years D. 5 years E. More than 5 years
2. Berapa jamkah anda bekerja semasa aktiviti penyemburan kabus (*fogging*) dalam sehari? A. Sejam B. 1–2 jam C. 2–3 jam D. 3–4 jam H. Lain-lain (sila nyatakan) ___________________	2. How many hours per day that you spent during fogging activity? A. An hour B. 1–2 hours C. 2–3 hours D. 3–4 hours H. Others (please specify) ___________________

3. Apakah kaedah kawalan nyamuk Aedes yang biasa dijalankan oleh jabatan anda di kawasan wabak demam denggi? (Jawapan boleh lebih daripada satu) A. Kawalan menggunakan racun seperti ABATE (ubat membunuh jentik-jentik) and semburan asap (*fogging*) B. Kawalan menggunakan kaedah biologi (menggunakan ikan gapi) C. PPA (cari dan musnah tempat pembiakan nyamuk Aedes) D. Pemberian ABATE (ubat membunuh jentik-jentik) kepada penduduk di kawasan wabak	3. What is the Aedes mosquito controlling method(s) commonly used by your department at the dengue fever epidemic areas? (Answer(s) can be more than one) A. Control by insecticides such as ABATE (antilarva insecticide) and fogging B. Biological method (using guppy fish) C. PPA (search and destroy all Aedes mosquito breeding areas) D. Providing ABATE (antilarvae insecticide) to the residents within the affected areas
4. Apakah jenis racun yang biasa digunakan untuk membunuh nyamuk dewasa? (Jawapan boleh lebih daripada satu) A. Malathion B. Aqua resigen C. Sumithion L40 D. Actellic 50EC E. Gokilahts F. Mospray I. Lain-lain (sila nyatakan) ____________________	4. What type(s) of insecticide is/are commonly used to kill adult mosquitoes (Answer(s) can be more than one) A. Malathion B. Aqua resigent C. Sumithion L40 D. Actellic 50EC E. Gokilahts F. Mospray I. Others (please specify) ____________________
5. Adakah anda tahu tentang kandungan bahan kimia yang digunakan? A. Ya B. Tidak	5. Do you know about the content of the chemicals used? A. Yes B. No

6. Jika ya, bagaimanakah cara anda mendapat maklumat mengenai bahan kimia yang terkandung di dalam racun serangga? A. Televisyen B. Rakan-rakan C. Majikan D. Radio E. Surat khabar F. Poster G. Risalah H. Internet J. Lain-lain (sila nyatakan) ____________________	6. If yes, how do you obtain the information about the chemicals contained in the insecticide? A. Television B. Friends C. Employer D. Radio E. Newspaper F. Poster G. Pamphlet H. Internet J. Others (please specify) ____________________
7. Adakah anda mengikuti kursus yang berkaitan dengan penggunaan bahan kimia (racun serangga) untuk kawalan nyamuk? A. Ya B. Tidak	7. Do you attend trainings related to the use of chemicals (insecticide) for controlling mosquitoes? A. Yes B. No
8. Di manakah tempat anda membancuh sukatan racun serangga sebelum penyemburan kabus (*fogging*) dijalankan? A. Stor B. Pejabat C. Kawasan lapang D. Lain-lain (sila nyatakan) ____________________	8. Where do you prepare the insecticide mixture before the fogging starts? A. Store B. Office C. Open air areas D. Others (please specify) ____________________

9. Adakah anda terdedah kepada racun serangga? (Sila tandakan (✓) pada kotak yang disediakan)

Statement	Ya	Tidak
Melalui pernafasan		
Resapan oleh kulit		
Termakan atau terminum		

8. Are you exposed to the insecticide? (Please tick (✓) in the boxes provided)

Statement	Yes	No
Through respiration		
Through skin contact		
Through oral contact		

9. Adakah anda menggunakan alat perlindungan diri (*Personal Protective Equipment*, PPE) semasa penyemburan kabus (*fogging*)?
   A. Ya (sila terus ke soalan no.12)
   B. Tidak

10. Did you use personal protective equipment (PPE) while fogging?
   A. Yes (please continue to question 12)
   B. No

10. Jika tidak, sila nyatakan ___________________

11. If no, please specify ___________________
12.

13. Sila tandakan (✓) di kotak jawapan yang disediakan

No.	Kenyataan
1.	Adakah anda membaca label sukatan racun serangga sebelum membancuh racun tersebut?
2.	Adakah anda memakai alat pelindung mata (*goggle*) semasa penyemburan kabus (*fogging*)?
3.	Adakah anda memakai alat pelindung mulut (*respiratory mask*) semasa penyemburan kabus (*fogging*)?
4.	Adakah anda memakai sarung tangan (*gloves*) semasa penyemburan kabus (*fogging*)?
5.	Adakah anda memakai alat pelindung telinga (*ears plug*) semasa penyemburan kabus (*fogging*)?
6.	Adakah anda memakai baju yang sesuai semasa penyemburan kabus (*fogging*)?
7.	Adakah anda memakai kasut yang sesuai (*safety*

12. Please tick (✓) in the provided answer box.

No.	Statement
1.	Do you read the insecticide measuring label before mixing it?
2.	Do you use goggle eyewear while fogging?
3.	Do you wear respiratory mask while fogging?
4.	Do you wear gloves while fogging?
5.	Do you wear ear plugs while fogging?
6.	Do you wear overalls while fogging?
7.	Do you wear safety boots while fogging?
8.	Do you drink or eat while fogging?
9.	Do you smoke while fogging?
10.	Do you change your clothes after the fogging ends?
11.	Do you wash your hands after fogging?
12.	Do you clean yourself after the fogging ends?

	boots) semasa penyemburan kabus (*fogging*)?
8.	Adakah anda minum atau makan semasa penyemburan kabus (*fogging*)?
9.	Adakah anda merokok semasa penyemburan kabus (*fogging*)?
10.	Adakah anda menukar pakaian selepas penyemburan kabus (*fogging*)?
11.	Adakah anda mencuci tangan selepas penyemburan kabus (*fogging*)?
12.	Adakah anda membersihkan diri selepas selesai penyemburan kabus (*fogging*)?

14. Di manakah anda membuang sisa racun serangga (bahan kimia) selepas penyemburan kabus (*fogging*)? A. Di kawasan lapangan B. Bawa balik pejabat dan buang di tempat yang disediakan C. Buang ke dalam sungai D. Tanam E. Bakar	14. Where do you dispose the insecticide waste (chemicals) after fogging? A. In an open field B. Bring it back to workplace and dump it in allocated area C. Throw it into the river D. Bury it E. Burn it
15. Adakah anda menjalani sebarang pemeriksaan kesihatan? A. Ya B. Tidak	15. Did you undergo any medical checkups? A. Yes B. No
16. Jika ya, berapa kerap pemeriksaan kesihatan yang dijalankan oleh jabatan anda? A. Setiap tahun B. 1–2 tahun	16. If Yes, how often do medical checkups conducted by your Department? A. Every year B. 1–2 years

C. 2–3 tahun D. Lebih daripada 5 tahun E. Lain-lain (sila nyatakan) ____________________	C. 2–3 years D. More than 5 years E. Others (please specify) ____________________
17. Apakah jenis pemeriksaan kesihatan yang dijalankan? ____________________	17. What type of medical checkups is conducted? ____________________
18. Di manakah anda melalui pemeriksaan kesihatan? ____________________	18. Where do you take your medical checkup? ____________________
19. Adakah anda tahu tentang kesan penggunaan racun serangga terhadap kesihatan anda? A. Ya B. Tidak	19. Do you know about the effect of insecticide usage on your health? A. Yes B. No
20. Jika Ya, bagaimanakah anda mendapat maklumat mengenai masalah kesihatan daripada penggunaan racun serangga yang digunakan? A. Televisyen B. Rakan-rakan C. Majikan D. Radio E. Surat khabar F. Poster G. Risalah H. Internet I. Lain-lain (sila nyatakan) ____________________	20. If Yes, how do you get the information on health problems caused by the use of insecticide? A. Television B. Friends C. Employer D. Radio E. Newspaper F. Poster G. Pamphlet H. Internet I. Others (please specify) ____________________

21. Selepas melakukan aktiviti penyemburan kabus (*fogging*), adakah anda mengalami tanda-tanda berikut:

Sila tandakan (✓) di kotak yang disediakan. Jawapan boleh lebih daripada satu.

	Rasa loya
	Pening kepala
	Muntah-muntah
	Sukar bernafas
	Dada rasa sesak/padat
	Rasa gatal/tompok merah atau putih pada kulit
	Hidung berdarah
	Penglihatan kabur
	Terketar-ketar/menggigil
	Sakit pada bahagian bawah perut
	Rasa bahang ketika membuang air kecil
	Letih
	Sakit belakang
	Sendi lutut bengkak
	Lain-lain ___________________

21. After performing fogging activities, do you experience the following symptoms:

Please tick (✓) in the box provided. Answer(s) can be more than one.

	Nausea
	Dizziness
	Vomiting
	Hardness in breathing
	Chest feels tight/stuffed
	Itch/red or whitey spots on the skin
	Bloody nose
	Blurry vision
	Shivering/shaking
	Abdominal pain
	Heat sensation while urinating
	Fatigue
	Back pain
	Swollen knee joints
	Others ___________________

Appendix A (Questionnaire for Public)

Borang Soal Selidik	**Questionnaire**
INSTITUT SAINS BIOLOGI, FAKULTI SAINS UNIVERSITI MALAYA KUALA LUMPUR	**INSTITUTE OF BIOLOGICAL SCIENCE, FACULTY OF SCIENCE, UNIVERSITY OF MALAYA KUALA LUMPUR**
Tuan/Puan	Sir/Madam
Saya pelajar yang kini sedang menjalankan kajian untuk tesis Ph.D saya di Fakulti Sains, Universiti Malaya. Kajian ini bertujuan untuk mendapatkan maklumat mengenai penggunaan racun serangga dalam kawalan nyamuk dewasa, dan tahap kesedaran terhadap penggunaan racun serangga di Putrajaya dan Selangor.	I am a student currently doing research for my Ph.D thesis in the Faculty of Science, University of Malaya. This research is done to obtain information on the use of insecticide in curbing adult mosquitoes and to assess the awareness level of insecticide usage in Putrajaya and Selangor.
Borang kaji selidik ini mengandungi 3 bahagian (A, B, dan C) dan setiap bahagian mempunyai beberapa penyataan. Kepada penduduk di kawasan Putrajaya dan Kuala Selangor, saya mohon kerjasama Tuan/Puan untuk menjawab keempat-empat bahagian untuk melengkapkan kajian ini.	This questionnaire contains 3 parts (A, B, and C) and each part contains several statements. To the public in Putrajaya and Kuala Selangor please answer all the four parts to provide necessary information to complete this study.
Kerjasama anda dalam melengkapkan borang kaji selidik ini amatlah saya hargai. Semua maklumat anda adalah sulit dan	I highly appreciate your cooperation in completing this questionnaire. All of your information will be kept confidential

hanya akan digunakan untuk kajian ini sahaja.	and will be used for the purpose of this research only.
Terima kasih atas kerjasama anda semua.	Thank you for your cooperation.
Siti Nurhafizah Saleeza Bt Ramlee **SHC090022**	**Siti Nurhafizah Saleeza Bt Ramlee** **SHC090022**
BAHAGIAN A: PROFIL RESPONDEN	**PART A: RESPONDENT'S PROFILE**
1. Umur: A. A.18–23 tahun B. 24–29 tahun C. 30–35 tahun D. 36–41 tahun E. 42–47 tahun F. Lebih daripada 48 tahun	1. Age: A. 18–23 years old B. 24–29 years old C. 30–35 years old D. 36–41 years old E. 42–47 years old F. More than 48 years old
2. Jantina: A. Lelaki B. Perempuan	2. Gender: A. Male B. Female
3. Bangsa: A. Melayu B. India C. Cina D. Lain-lain (sila nyatakan) __________________________	3.Race: A. Malay B. Indian C. Chinese D. Others (please specify) __________________________

4. Tahap pendidikan: A. Penilaian Menengah Rendah (PMR) B. Sijil Pelajaran Malaysia (SPM) C. Sijil Tinggi Pelajaran Malaysia (STPM) D. Sijil E. Diploma F. Ijazah G. Lain-lain (sila nyatakan) __________________________	4.Education level: A. Lower Secondary Assessment (PMR) B. Malaysian Certificate of Education (SPM) C. Malaysian Higher School Certificate (STPM) D. Certificate E. Diploma F. Degree G. Others (please specify) __________________________
5. Pekerjaan: Sila nyatakan __________________________	5.Occupation: Please specify __________________________
6. Adakah anda merokok? A. Ya B. Tidak 7. Adakah anda mempunyai sebarang masalah kesihatan? A. Ya B. Tidak	6. Do you smoke? A. Yes B. No 7. Do you have any health problems? A. Yes B. No
8. Jika ya, apakah masalah kesihatan yang anda alami sekarang? A. Kanser B. Darah tinggi C. Diabetis D. Sakit jantung E. Lain-lain (sila nyatakan) __________________________	8. If yes, what kind of health problems that you are experiencing currently? A. Cancer B. High Blood Pressure C. Diabetes D. Heart Disease E. Others (please specify) __________________________

BAHAGIAN B: PENGETAHUAN TERHADAP DEMAM DENGGI DAN PENGGUNAAN RACUN SERANGGA	PART B: KNOWLEDGE ON DENGUE FEVER AND INSECTICIDE USAGE
1. Adakah anda tahu mengenai demam denggi? A. Ya B. Tidak	1. Do you know about dengue fever? A. Yes B. No
2. Pada pendapat anda bagaimanakah caranya untuk mengawal demam denggi dan membunuh nyamuk? (Jawapan boleh lebih daripada satu) A. Semburan kabus (*fogging*) B. Penggunaa ABATE (ubat membunuh jentik-jentik) C. Menggunakan ikan gapi D. Membersihkan kawasan-kawasan pembiakan nyamuk E. Lain-lain (sila nyatakan) ________________________	2. In your opinion, how to control dengue fever and kill mosquitoes? (Answer(s) can be more than one) A. Fogging B. The use of ABATE (mosquito larvae insecticide) C. Using the guppy fish D. Cleaning up mosquito breeding areas E. Others (please specify) ________________________
3. Adakah semburan kabus (*fogging*) pernah dijalankan di kawasan rumah anda? A. Ya B. Tidak	3. Has fogging ever been held in your vicinity? A. Yes B. No
4.Adakah anda risau tentang kesan kesihatan anda dan keluarga terhadap penyemburan kabus (*fogging*) yang digunakan untuk membunuh nyamuk dewasa? A. Ya B. Tidak (Jika tidak, sila nyatakan)	4. Do you worry about how fogging that is used to kill adult mosquitoes will affect you and your family's health? A. Yes B. No (If no, please specify) ________________________

____________________	
5.Pada pendapat anda, adakah penyemburan kabus (*fogging*) memberi kesan buruk terhadap alam sekitar? A. Ya B. Tidak	5. In your opinion, does fogging negatively affect the environment? A. Yes B. No
6.Pada pendapat anda, apakah kesan penggunaan racun serangga (*fogging*) selain daripada masalah kesihatan kepada manusia? (Jawapan boleh lebih daripada satu) A. Memberi kesan buruk terhadap alam sekitar B. Masalah kesihatan terhadap binatang C. Penggunaan racun serangga melibatkan kos yang tinggi D. Penggunaan racun serangga akan membunuh serangga lain selain daripada nyamuk E. Lain-lain (sila nyatakan) ____________________	6. In your opinion, what are the effects of the use of insecticide apart from causing health problems to human? (Answer(s) can be more than one) A. It causes negative impacts on the environment B. Animal health problems C. The use of insecticide is very costly D. The use of insecticide will also kill other insects besides mosquitoes E. Others (please specify) ____________________
7.Adakah penyemburan kabus (*fogging*) sangat berkesan mengawal nyamuk dan mengawal kes demam denggi? A. Ya B. Tidak (sila nyatakan) ____________________	7. Is fogging highly effective in controlling mosquitoes and dengue fever cases? A. Yes B. No (please specify) ____________________
8. Pada pendapat anda, adakah semburan kabus (*fogging*) perlu ditukar dengan cara yang lain? A. Ya B. Tidak	8. In your opinion, should fogging be replaced by other methods? A. Yes B. No

9. Jika tidak, sila nyatakan kenapa. ___________________	9. If not, please state why. ___________________
10. Pada pendapat anda, apakah faktor yang mendorong kepada peningkatan kes demam denggi? (Jawapan boleh lebih daripada satu) A. Faktor persekitaran B. Tingkah laku manusia C. Kurang pengetahuan terhadap pencegahan demam denggi D. Langkah kawalan kurang berkesan E. Racun serangga yang digunakan tidak memberi kesan terhadap nyamuk (*chemically resistant*) F. Lain-lain (sila nyatakan) ___________________	10. In your opinion, what factors lead to increased cases of dengue fever? (Answer(s) can be more than one) A. Environmental factor B. Human behaviour C. Lack of knowledge in controlling Dengue Fever D. The controlling method is less effective E. Ineffective insecticide use against mosquitoes (chemically resistant) F. Others (please specify) ___________________
11. Selepas aktiviti penyemburan kabus (*fogging*), adakah anda mengalami tanda-tanda berikut: Sila tandakan (✓) di kotak yang disediakan. Jawapan boleh lebih daripada satu	11. After fogging activities, do you experience the following symptoms: Please tick (✓) in the box provided. Answer(s) can be more than one.

	Rasa loya
	Pening kepala
	Muntah-muntah
	Sukar bernafas
	Dada rasa sesak/padat
	Rasa gatal/tompok merah atau putih pada kulit
	Hidung berdarah
	Penglihatan kabur
	Terketar-ketar/menggigil
	Sakit pada bahagian bawah perut
	Rasa bahang ketika membuang air kecil

	Nausea
	Dizziness
	Vomiting
	Hardness in breathing
	Chest feels tight/stuffed
	Itch/red or whitey spots on the skin
	Bloody nose
	Blurry vision
	Shivering/shaking
	Abdominal pain
	Heat sensation while urinating
	Fatigue

<table><tr><td></td><td>Letih</td></tr><tr><td></td><td>Sakit belakang</td></tr><tr><td></td><td>Sendi lutut bengkak</td></tr><tr><td></td><td>Lain-lain ___________________</td></tr></table>	<table><tr><td></td><td>Back pain</td></tr><tr><td></td><td>Swollen knee joints</td></tr><tr><td></td><td>Others ___________________</td></tr></table>
BAHAGIAN C: PENGETAHUAN TERHADAP KAWALAN BIOLOGI	**PART C: KNOWLEDGE ON BIOLOGICAL CONTROL**
1.Adakah anda tahu mengenai kaedah kawalan biologi (*biocontrol*) untuk mengawal pembiakan nyamuk? A. Ya B. Tidak C. Tidak pasti	1. Are you familiar with biological control methods (biocontrol) in controlling mosquito breeding? A. Yes B. No C. Not sure
2.Jika ya, apakah kaedah biologi yang biasa digunakan untuk mengawal pembiakan nyamuk? A. Serai wangi B. Ikan gapi C. Nyamuk gergasi (*Toxo mosquito*) D. Pepatung E. Kumbang F. Garam G. Lain-lain (sila nyatakan) ___________________	2. If yes, what is the biological method commonly used in controlling mosquito breeding? A. Lemon grass B. Guppy fish C. Elephant mosquito (*Toxo mosquito*) D. Dragonfly E. Beetle F. Using salt G. Others (please specify) ___________________
3. Adakah anda rasa kaedah biologi selamat dan berkesan digunakan untuk mengawal nyamuk? A. Ya B. Tidak C. Tidak pasti	3. Do you think that biological method is safe and effective in controlling mosquitoes? A. Yes B. No C. Not sure

4. Adakah anda rasa kaedah biologi selamat dan tidak mencemarkan alam sekitar? A. Ya B. Tidak C. Tidak pasti	4. Do you think that the biological method is safe and not polluting the environment? A. Yes B. No C. Not sure
5. Adakah anda rasa kaedah biologi selamat kepada kesihatan manusia? A. Ya B. Tidak C. Tidak pasti	5. Do you think that the biological method is safe for human health? A. Yes B. No C. Not sure
6. Adakah anda mengamalkan kaedah biologi ini di persekitaran tempat anda? A. Ya (sila nyatakan) ___________________ B. Tidak	6. Do you use this biological method within your vicinity? A. Yes (please specify) ___________________ B. B. No

Appendix B

Table for determining sample size from a given population

TABLE 1

Table for Determining Sample Size from a Given Population

N	*S*	*N*	*S*	*N*	*S*
10	10	220	140	1200	291
15	14	230	144	1300	297
20	19	240	148	1400	302
25	24	250	152	1500	306
30	28	260	155	1600	310
35	32	270	159	1700	313
40	36	280	162	1800	317
45	40	290	165	1900	320
50	44	300	169	2000	322
55	48	320	175	2200	327
60	52	340	181	2400	331
65	56	360	186	2600	335
70	59	380	191	2800	338
75	63	400	196	3000	341
80	66	420	201	3500	346
85	70	440	205	4000	351
90	73	460	210	4500	354
95	76	480	214	5000	357
100	80	500	217	6000	361
110	86	550	226	7000	364
120	92	600	234	8000	367
130	97	650	242	9000	368
140	103	700	248	10000	370
150	108	750	254	15000	375
160	113	800	260	20000	377
170	118	850	265	30000	379
180	123	900	269	40000	380
190	127	950	274	50000	381
200	132	1000	278	75000	382
210	136	1100	285	1000000	384

Note.—*N* is population size.
S is sample size.