CHAPTER 2

REVIEW OF LITERATURE

The scopes of this review cover the description of Micronectidae, mosquito as virus transmitting vector and its life cycle, the mosquito borne disease, the mode of transmission of the diseases, the environment of mosquito breeding and practices in vector management which includes the physical, chemical and biological approaches. The experiments on biological approaches that were conducted by other researchers were discussed briefly.

2.1 Family Micronectidae

Micronectidae, commonly known as pygmy water boatmen, are among the most common truly aquatic Heteroptera in Singapore and Peninsular Malaysia. In West Malaysia, the first taxonomic study of the Malayan Corixidae (Hemiptera and Heteroptera) with the description of *Micronecta malayana* was first described by Leong, (1966) from Singapore. A list of 85 species of aquatic Heteroptera including 9 species of Micronectidae was collected from Xishuangbanna Tropical Botanic Garden in China, a recognized UNESCO Biosphere Reserve in 1993 (Cheng *et al.*, 2006).

Micronectidae is known as an aquatic insect of pristine environment. The size of Micronectidae populations fluctuates in such aquatic environments. Thus, in the presence of abundant resources, populations of insect like Micronectidae can grow at geometric or exponential rates as shown in the general growth curve by Molles (2005) (Figure 2.1). Eventually, populations run up against environmental limits to further increase.

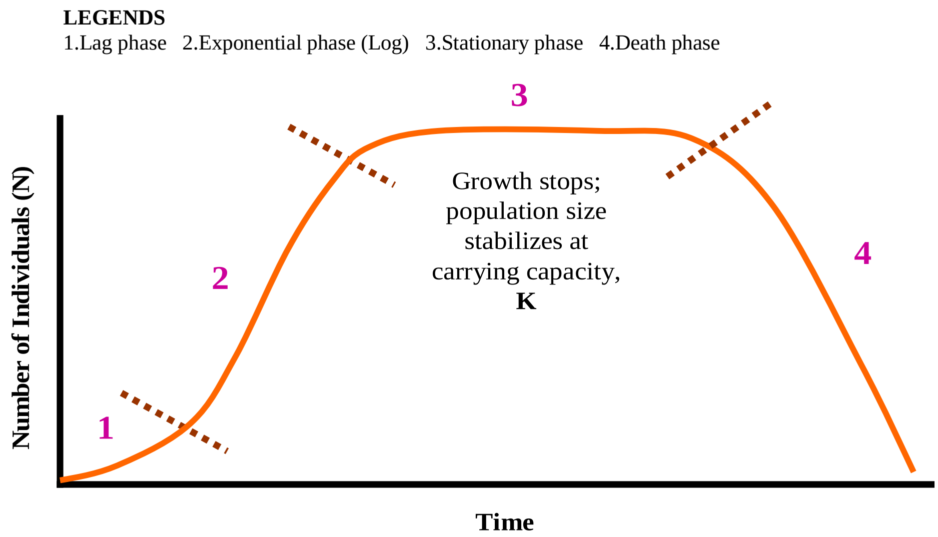

Figure 2.1 The population growth curve results from environmental limitations (Source: Molles, 2005)

The effect of the environment on population growth is reflected in the shapes of population growth curves. This pattern of growth produces a sigmoidal or S-shaped, population growth. The population size at which growth stop is generally called carrying capacity, or K, which is the number of individuals of a particular population that the environment can support (Figure 2.1). Some *Micronecta* species are only or predominantly known in the macropterous form while others are only or predominantly known in the brachypterous form. Some of the predominantly macropterous species are often found in stagnant, sometimes small, pools of water (Daly *et al.*, 1998). A few of these are very widespread e.g *Micronecta scutellaris* which occurs in large parts of Africa, through Arabia, India, Sri Lanka, South East Asia and Indonesia to Hong Kong, Taiwan, the Philippines and North Australia (Wroblewski, 1968). The predominantly brachypterous species tend to be associated with quiet parts of streams or larger stagnant water bodies (Daly *et al.*, 1998).

2.1.1 Taxonomy and Morphological Appearances

Taxonomy is the scientific classification (Figure 2.1.1) of organisms according to a pre-determined system, with the resulting catalog used to provide a conceptual framework or discussion, analysis or information retrieval (Merritt and Cummins, 1995). Latin and Greek words are used in the scientific classification.

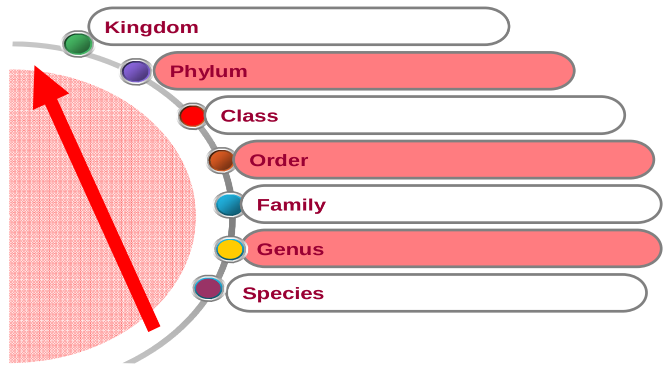

Figure 2.2 The scientific classification for organisms
(Source: Merritt and Cummins, 1995)

Species classification has a two-part of scientific name. Most of these names come from Greek or Latin words namely the binomial system of nomenclature or binomial nomenclature. An 18th century Swede, Carl Linnaeus, originated the binomial system. Seven chief groups make up a system in scientific classification (Daly *et al.*, 1998). The groups are: kingdom, phylum or division, class, order, family, genus and species (Figure 2.2).

The Corixoidea are represented in the Malay Peninsular by two families, the Corixidae (water boatmen) and Micronectidae (pygmy water boatmen) (Leong, 1966). They were characterized by having the antennae shorter than the head in combination with well developed eyes. The antennae are inserted under the eyes and are either concealed or with only their apices visible in dorsal view.

Corixidae and Micronectidae are closely related morphologically as shown in Figure 2.3 and Figure 2.4. However, there are many differences which support the elevation to family rank of these taxa.

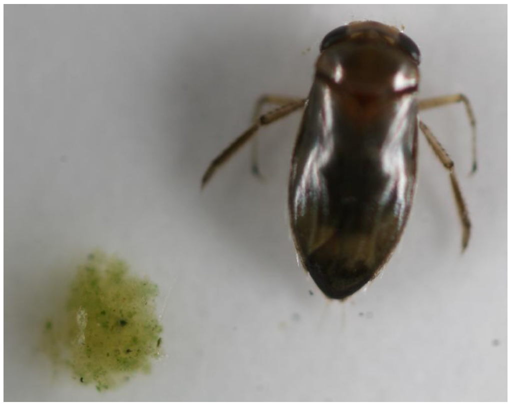

Figure 2.3 Micronectidae

Figure 2.4 Corixidae (Source: CALS, 2009)

Nieser (2002.a) has distinguished several key characters between Micronectidae and Corixidae based on their morphological characteristic (Table 2.1). Pala refers to a part of insect's leg that is spade shaped and can be used as scoop for feeding as well as pedal for swimming (Nieser, 2002.a).

Table 2.1 Key characters distinguishing between Micronectidae and Corixidae

Micronectidae	Corixidae
Scutellum exposed	Scutellum covered by hemielytra
Female fore tibia and pala fused	Female fore tibia and pala separate
Male claw of pala modified and able to fold in the pala without pegs	Male pala claw not modified pala with pegs
Male right paramere at base usually with a field of ridges (for stridulation)	Male right paramere without such ridges
Stridulation in males only by movement of the genital capsule	Stridulation by rubbing peg fields on the anterior femur against the side of the head, females of some species also able to stridulate
Antennae three-segmented	Antennae four-segmented
Middle tibia shorter than tarsus	Middle tibia longer than tarsus

(Source: Nieser, 2002.a)

2.1.2 Key characteristic of Micronectidae from West Malaysia and Singapore

The key characteristics of Micronectidae contain species collected in Singapore or Peninsular Malaysia, and other few species recorded were from Sumatra and Thailand (Niesser, 2002.b). Below is the description for Micronectidae under study, *Micronecta polhemusi* Niesser 2000 (*MpN2000*).

Diagnosis. –Length macropterous 2.3-2.9mm. A dark grey elongate species. Head light brown, eyes grey, frons with a distinct castaneous mark extending onto clypeus and rostrum. Hemielytra grey, embolium and an ill-defined transverse band on corium darker brown, these darker marks little contrasting, well visible with light shining through the hemielytra only. The castaneous mark on the frons and the dark color of the hemielytra without longitudinal stripes are characteristic.

Distribution. –Described from Thailand and Vietnam. There are two samples from Singapore: Pulau Ubin, 28 May 1997, in ZRC, new record for Singapore. In view of the general distribution the species may occur in West Malaysia only in macropterous form.

2.2 Mosquito

Many researchers have studied mosquito in great detail (Baerg and Boreham, 1974). Generally, mosquitoes are regarded as undesirable in both rural and urban areas. Not only is their biting activity a nuisance, mosquitoes also vector transmitting pathogens that cause human and animal diseases (Pitts *et al.*, 2000).

Mosquitoes belong to the Class Insecta, Order Diptera and Family Culicidae. They are thin brown, sized 6.4 to 12.7 mm, long legged winged insects; adults have three pairs of long, slender legs, and elongate ‘beak’ or piercing proboscis. Female mosquito uses its long forward-pointing proboscis to suck blood. The male is distinguished by its bushy antennae and feeds on nectar. Male’s palps are long and hairy; female’s palps are always slender. The wing veins and margins clothed with scales (Baerg and Boreham, 1974).

Mosquitoes are mainly nocturnal, although some woodland species are active by day (Baerg and Boreham, 1974). There are approximately 3000 species of mosquitoes worldwide and all require water to complete their life cycle. The life cycle of mosquito has four distinct stages: egg, larva, pupa and adult mosquito. Mosquito larvae are aquatic. Female mosquitoes lay eggs on or near water. The eggs hatch into aquatic larvae that feed on organic material and grow through four stages before becoming pupae. Winged adults emerge from pupae, mate and begin the cycle again. Only female mosquitoes feed on blood, which provides the nutrients needed for the development of eggs. Males are more short-lived and feed on plant juices or nectar (Pitts *et al.,* 2000).

Most mosquitoes remain close to the lake, pond or clogged gutter. Rainy season provides plenty of breeding places for them. Mosquito breeding depends on various factors which include temperature, relative humidity and rainfall pattern. While certain characteristics are common to all mosquitoes, there are differences among different genus and species. Habitat and climate conditions determine the dominance of a particular species in any given area. Larval requirement can also be quite specific to the species (Baerg and Boreham, 1974).

2.2.1 Virus Transmitting Vector

The numbers of mosquito species under 4 major genera are listed in Table 2.2 which are major disease vectors (Vasudevan *et al.,* 2000).

Table 2.2 Mosquito Species

Genus	Nos of Species	Nos of Major Vector Species
Anopheles	422	60
Aedes	888	25
Culex	715	12
Mansonia	23	7

(Source: Vasudevan *et al.,* 2000)

Anopheles, *Aedes* and *Culex* are the most common genus and are known to cause various diseases. The diseases are transmitted through mosquito bites (Figure 2.5). The major diseases caused by these various species belonging to the sub-family: Anolphelinae (*Anopheles*) and Culicine (*Culex*, *Aedes*) (Pitts *et al.*, 2000).

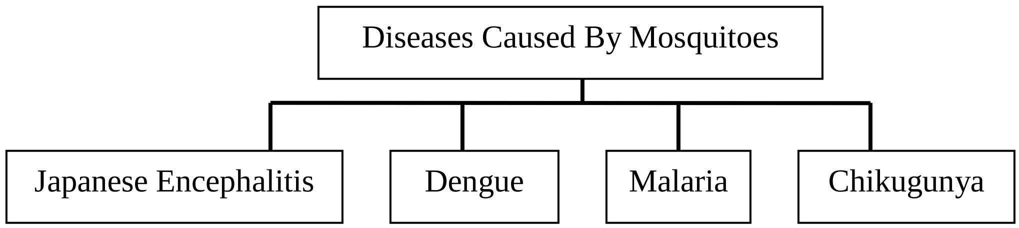

Figure 2.5 The major mosquito borne diseases
(Source: Pitts *et al.*, 2000)

During the past five decades, the population of the world has more than doubled, with the most rapid growth taking place in developing countries in the tropics and subtropics where dengue viruses are spread by mosquitoes. Several factors were combined to produce epidemiological conditions that is highly favorable for viral transmission by the main mosquito vector; population growth, rural-urban migration, the inadequacy of basic infrastructure (e.g unreliable water supply, which may lead householders to collect and store water close to homes) and the huge increase in volume of solid waste resulting from the new habit of consumers. For example, discarded plastic containers and other abandoned items can become larval habitats in urban areas. The magnitude of the public health problem will continue to grow unless more effective measures are taken to reduce viral transmission (WHO, 2002).

2.2.2 Mosquito Borne Diseases

These mosquito borne diseases are widespread throughout the world. It has become a global health concern in vector management program for mosquito control. There are three types of pathogenesis by mosquito. The common type of pathogenesis of mosquito is viral type (Table 2.3 and Table 2.4).

Viral type can cause many diseases such as yellow fever (WHO, 2001), dengue fever (Petersen and Guebler, 2006), Eastern Equine Encephalitis (EEE) (Shope *et al,* 1966), Saint Louis Encephalitis (SLE) (Shope *et al,* 1966), West Nile Encephalitis (WNE) (Mackay, 2007), Japanese Encephalitis (JE) (Yoshito, 1994) and Chikugunya (Chua *et al.,* 2000). The regions of origin for this type of pathogenesis are widely distributed particularly in Africa, Europe and Asia (WHO, 2001; Petersen and Guebler, 2006; Shope *et al,* 1966; Mackay, 2007; Yoshito, 1994; Chua *et al.,* 2000).

Other types of pathogenesis are filarial nematodes and protozoan type (Table 2.3). Filarial nematodes are a group of worms that reliant upon arthropod vector like mosquito. It can cause lymphatic filiaris or heart failure because of the invasion of these worms. The common name for the disease is dog heart worm because the worms usually can be found in the infected dog and pig. It became a serious health threat when the worms are transmitted by mosquito vector to human. The regions of origin for the disease are Africa, Europe and Asia (WHO, 2001).

Meanwhile, pathogenesis of protozoan type is caused by protozoan parasites from the Plasmodium family. It can be transmitted by the sting of mosquito. It can cause malaria. The regions of origin for the disease are America, Asia and Africa (Cheong *et al.*, 2008).

The variety of mosquito borne diseases, the region of origin and the details on their type of pathogenesis are summarized in Tables 2.3 and 2.4.

Table 2.3 The details on mosquito borne diseases

Disease	Pathogenesis	The region of Origin	References
Lymphatic filiarisis (Dog heart worms)	Filarial nematodes	Africa, Europe and Asia	(WHO, 2001)
Yellow fever	Viral	Africa, Europe and Asia	(WHO, 2001)
Dengue fever	Viral	Africa, Europe and Asia	(Petersen and Guebler, 2006)
Eastern Equine Encephalitis (EEE)	Viral	Africa, Europe and Asia	(Shope *et al,* 1966)

Table 2.4 The details on mosquito borne diseases

Disease	Pathogenesis	The region of Origin	References
Saint Louis Encephalitis (SLE)	Viral	Africa, Europe and Asia	(Shope *et al*, 1966)
West Nile Encephalitis (WNE)	Viral	Africa, Europe and Asia	(Mackay, 2007)
Japanese Encephalitis (JE)	Viral	Japan and spread to Asia.	(Yoshito, 1994)
Chikugunya	Viral	Sub-Saharan, Africa, southern India and Pakistan, Southeast Asia, Indonesia and Philippines. Malaysia reported first outbreak in 1999 which was traced from Myanmar immigrants.	(Chua *et al.*, 2000)
Malaria	Protozoan	America, Asia and Africa.	(Cheong *et al.*, 2008)

2.3 Vector Transmitting Disease and Vector Control in Malaysia

The Vector Borne Diseases Control Program (VBDCP) was a sequel implementation from Malaria Eradication Program (MEP) which was focusing in Peninsular Malaysia, Sabah and Sarawak for 32 years ago (Ministry of Health Malaysia, 2009).

A statistics of malaria survey from 1965 to 1966 showed that there were about 300 000 malaria cases annually in Peninsular Malaysia (Ministry of Health Malaysia, 2009). The MEP in Peninsular Malaysia then was launched in 1967 in order to eradicate malaria from Peninsular Malaysia by 1982. Since then and up to 1980, there was a reduction in the number of reported malaria cases from 160 385 in 1966 to 9110 cases. However, the Malaria Eradication Program was changed to Malaria Control Program in 1980 due to administrative, technical and operational problems.

Likewise, the MEP in Sarawak which was started in 1952 was changed to Malaria Control Programme in 1972. There were many problems being encountered through the Eradication Program in Sarawak, such as the influx of malaria carriers from the neighbouring Provinces/States, the nomadic groups of the population in some areas, difficult terrain and communication. It was impossible to eliminate the malaria transmission. The MEP in Sabah was launched in 1961 and continued for ten years. However, it was changed to Malaria Control Programme in 1971 due to the same problems (Ministry of Health Malaysia, 2009).

In 1980, the Ministry of Health converts the existing Malaria Eradication Programme (MEP) into Vector Borne Diseases Control Programme (VBDCP). The basic strategies and activities of the MEP are also focusing on the control of diseases transmitted by the mosquito vectors such as; Malaria by Anopheline mosquitoes, Filariasis by Anopheline and Mansonia mosquitoes, Dengue Fever or Dengue Hamemorrhagic Fever by *Aedes* mosquitoes, *Japanese Encephalitis* by Culicine mosquitoes, Plague by fleas (*Xenopsylla cheopis),* Yellow Fever by *Aedes* mosquitoes, Murine Typhus by fleas and Scrub Typhus caused by mites as the vector (Ministry of Health Malaysia, 2009).

The development of the Vector Borne Diseases Control Programme (VBDCP) is being implemented in three phases (Ministry of Health Malaysia, 2009);

Phase I - Formation of the Anti Malaria Programme (1981 - 1982).

Phase II - Formation of the Vector Borne Diseases Control Programme (VBDCP) 1983-1984

Phase III - Further Expansion of the Scope of the VBDCP (1985 Onwards) The VBDCP took over the control of other vector borne diseases.

Although the concept of eradication has changed to control in 1980, the anti-malaria activities have remained the same. However, additional supplementary activities such as the use of impregnated bed-nets, and the Primary Health Care approach, have been introduced in malaria-prone areas. Focal spraying activity is instituted in localities with outbreaks in both malaria-prone and non-malarious areas. Passive case detection has been maintained in all operational areas (Ministry of Health Malaysia, 2009).

In 1990, 50,500 cases of malaria were reported of which 69.7% (35,190) were from Sabah, 27.8% (14 066) from Peninsular Malaysia and 2.5% (1244) from Sarawak. Until June 1991 a total of 18,306 cases were reported for the country. The case fatality rate for 1990 was 0.09% (Ministry of Health Malaysia, 2009).

Meanwhile, the number of Dengue Fever and Dengue Hemorrhagic Fever reported cases also has been steadily increasing from below 1,000 cases per year in 1980s to more than 5,000 cases in 1991. There were outbreaks in 1974 and 1982, followed by major outbreak in 1998 with 27,381 cases reported (Teng and Singh, 2001).

The situation in the past three years in 2006 and 2007 as reported by Health Ministry Disease Control Division, dengue cases are increasing from 26,738 to a total of 48,185 cases and 64 to 98 deaths in 2007 (Abdul Rahman, 2008). In year 2008, the total number of dengue cases reported in the country was 46,909. Although it gives a slight reduction compare to the previous year, the number of fatal cases was increasing to 106 in 2008 (Abdul Rahman, 2009).

2.4 Mosquito Breeding Problems

Rapid industrial and economic development over the last two decades has brought about massive infrastructure development and a very active construction sector for commercial development (Kolsky, 1999). The development conveyed most opportunity of wealth and wisdom to people. However, the world is currently facing a global health concern which there was more than 80% of the dengue cases occurred in urban areas (Teng and Singh, 2001).

Aedes aegypti, the mosquito vector, has proliferated in most areas of rapid and unplanned urbanization, water storage areas and areas with inadequate waste disposal services (Ira and Bhushan, 2004). Stagnation of water in engineering structures is due to accumulation of silt or garbage which blocked the drains and may cause profuse breeding of mosquito. Drain that receives wastewater from commercial areas are likely to be polluted thus may become potential breeding for mosquito which leads to major health concerns (Mariappan, 2000).

Nevertheless, not all of engineering structures that have water stagnation provide a breeding place for mosquito. The findings suggested that in order to control mosquito breeding in engineering structures, the water need to meet the quality that can support natural predators of mosquito to survive (Abd Manan and Sapari, 2008).

According to Eisenberg *et al.,* (2006), there was a significant relation between the impacts of road construction on mosquito borne disease incidence in Trans-Amazon Highway. It was statistically recorded that the building of the highway was associated with an increase in malaria. The road construction practices create many water pools that provide breeding places for Anopheline mosquito. Another research in the Peruvian Amazon indicated that mosquito biting rates are significantly higher in areas that have undergone deforestation and development associated with road development.

One common purpose and consequences of a new road is increased logging. Deforestation causes major changes in watershed characteristics and local climate, both of which can affect the transmission of enteric pathogens (Metzger, 2004). Other than ecological processes, social processes facilitated by roads such as migration, creation of new communities, and increased density of existing communities can affect pathogen transmission (Eisenberg *et al.,* 2006).

Mosquito breeding in storm water drains was reported to be fluctuating depending on the combination of seasonal variation from summer to winter. Subodh, (2008) signified this statement on his research in India. The higher temperature and humid environment were found to be more conducive for the breeding.

Mosquito borne viruses exaggerated seasonally in most areas of Asia. The seasonal patterns of viral transmission is correlated with the abundance of vector mosquitoes and vertebrate amplifying hosts (Surendran *et al.,* 2007). Although the abundance of vector mosquitoes fluctuates with the amount of rainfall and with the impact of the rainy season, in some tropical locations, irrigation associated with engineering structures is an important factor affecting vector abundance and transmission (Wearing and Rohani, 2006). Thus, the periods of greatest risk for virus transmitting vector abundances vary regionally and within countries from year to year.

Overall, global warming, pollutions, lack of public awareness and ineffective mosquito management practices are among the many factors that have likely contributed to dengue resurgence.

2.5 Practices in the Vector Management

The global strategy enunciated in 1995 recommended the application of integrated vector control measures, with community and inter-sectoral participation. An informal WHO consultation on strengthening implementation of the global strategy for prevention and control of dengue fever/dengue hemorrhagic fever, the subsequent inclusion of dengue in the disease portfolio of the UNDP/World Band/WHO special program for Research and Training in Tropical Diseases in June 2000, and advances in regional strategy formulation in the Americas, South-East Asia and the Western Pacific during the 1990s, have facilitated identification of the following four main priorities; Source reduction, Health education, Law enforcement and Accelerating the research program in this areas for sustainable prevention and control of vectors at individual, household and community, institutional and political levels (WHO, 2002).

Mosquito controls most effective when appointed at immature stages in standing water rather than at adults. It is also best conducted using a combination of techniques. Biological control uses or enhances natural enemies of mosquitoes such as fish; physical control makes habitats less suitable for mosquito production; chemical control uses insecticides that target immature or adult; and legal control can force uncooperative parties to eliminate breeding habitats on their property or face financial penalties (Kay and Nam, 2005).

2.5.1 Physical and Chemical Approach

Many indices were used to estimate the mosquito population density in one particular area such as House Index (HI) (Eq.2.1), Container Index (CI) (Eq.2.2), Bruteau Index (BI) (Eq.2.3) and Pupae Index (PI) (Eq.2.4) (WHO, 1972; WHO, 2002). These indices are base on the aquatic immature stages only. The larvae are collected from different water holding containers infested with larvae and pupae.

$$HI = \frac{Nos\ of\ \ Infested\ \ Houses}{Nos\ \ of\ \ Inspected\ \ Houses} \times 100 \qquad \text{Eq.2.1}$$

$$CI = \frac{Nos\ of\ \ Infested\ \ Containers}{Nos\ \ of\ \ Inspected\ \ Containers} \times 100 \qquad \text{Eq.2.2}$$

$$BI = \frac{Nos\ of\ \ Infested\ \ Containers}{Nos\ \ of\ \ Inspected\ \ Houses} \times 100 \qquad \text{Eq.2.3}$$

$$PI = \frac{Nos\ of\ \ Pupae}{Nos\ \ of\ \ Inspected\ \ Houses} \times 100 \qquad \text{Eq.2.4}$$

Other vector surveillances are man-landing / biting collection, resting collection and ovitrap method (Service, 2008). However, these conventional indices are mainly practiced for the surveillance of immature stages of the mosquito for vector management programs. They do not reflect adult productivity and various container types. These types of surveillance also consumed labor works to make it efficient (Chen *et al.*, 2005).

The WHO standards for mosquito density are shown in Table 2.5 (WHO, 1972).

Table 2.5 WHO standards for mosquito density

WHO Density	House Index	Container Index
1	1-3	1-2
2	4-7	3-5
3	8-17	6-9
4	18-28	10-14
5	29-37	15-20
6	38-49	21-27
7	50-59	28-31
8	60-76	32-40
9	≥77	≥41

(Source: WHO, 1972)

According to the WHO standards for world mosquito density, the highest index is 9 (Table 2.5) with House index more than 77 and Container index more than 41. However, in Malaysia the limit of mosquito density index is around 10 for both House index and Container Index which represent WHO density of 3. It shows that the index is practically not applicable for Malaysia index to represent an alarming condition of mosquito problem.

On 2007, a statistically based index for adult mosquito population has been reported by using ABURAS index (Aburas, 2007). The approach is based on the Poisson distribution. Although the actual density with good prediction correlation can be obtained, the vector management program is only applied in the dengue hot spot locations followed by conventional methods.

According to Garcia (1972), the primary means of mosquito control in earlier days consisted of source reduction, oiling water surfaces and the application of a few inorganic chemicals. These methods formed the back bone of control programs until the modern synthetic insecticides were invented in the late 1940s. Increasing awareness of the necessity to control mosquito population particularly in the larval stage has coincided the widespread use of pesticides (Garcia, 1972). However, environmental scientists warn that pesticides most frequently used, carry their own toxicity risks, and repeated use of the chemicals may reduce their effectiveness. It also contributes to water pollution and kills not targeted organisms (Chua *et al.*, 2005). Thus, these chemical agents must be used as a last effort (Ooi *et al.*, 2006).

Mosquito have a very short life-cycle. However, it can be escalating rapidly if ecological control diminished due to chemical-control of mosquito itself (Adli Muhamed, 2005). The phenomenon shows that the use of biological organisms to control pests is one of the most important weapons in the war against mosquito-born disease. Biological control may become important tool and play a major role in mosquito management strategies in the future.

Some other approach is via cooperation within communities continuously to provide a clean and safe living hood from *Aedes* mosquito (Washington State Department of Health, 2008). However, this approach needs a sharp rule and regulation to make it works eloquently.

2.5.2 Biological Approach

Management of mosquitoes through the manipulation of plants and animals has been practiced since the initiation of vector control programs (Garcia, 1983). One of the approach is by using a high technology like genetic engineering (Barrero and Mistchenko, 2008; Poopathi and Tyagi, 2006) to change the genotype of mosquito free from viruses. However, it needs time and money.

Biological control in mosquito management can be defined as the introduction or manipulation or both of plants, animals and microorganisms in breeding habitats which can act as predators and pathogens, or an organisms that can eliminate a required resource necessary for completion of the mosquitoes life cycles (Garcia, 1983) due to the huge diversity of mosquito species and the wide range of aquatic habitats. Thus, the use of biological organisms for mosquito control must also be broad and wide ranging.

Biological controls for mosquito such as the used of spiders (Breene *et al.,* 1988), beetles and mosquito larvae (Gautam *et al.,* 2006), fish (Wu *et al.,* 1987; West *et al.,* 1995; Webb *et al.,* 2007; Chandra *et al.,* 2008), Copepods, Mesocyclops and Bacillus (Kosiyachinda *et al.,* 2003), planarians (Suprakash and Aditya, 2003), dragonfly nymphs (Pandian *et al.,* 1979) and Micronectidae (Suphapathom *et al.,* 2002) indicated certain degree of efficiency (Sapari and Abd Manan, 2008). Although the application of biological controls is restricted by regions, it can provide an environmental friendly approach in controlling mosquito vectors in the future. Details and elaborations on these biological controls are explained below;

2.5.2.1 Spiders

In this study, a pisaurids, *Dolomedes triton* (Walckenear), and two lycosids, *Pirata sedenterius* (Montgomery) and *Pardosa deliculatula* (Gertsch & Wallace), were evaluated as predator of *Culex pipiens*. These species common in grassy areas, Texas. The mode of predation; individual larvae were grasped from beneath the surface of water, pulling their bodies through surface tension. The results of these evaluations showed the number of these three species that consumed mosquito larvae were of 76.7%, 73.8% and 30.4% respectively. Predation potential: 12 mosquito larvae per day. The average percentage of predation potential of these spiders was 40.7% only. This research provides laboratory evidence that spiders tested will prey readily upon mosquito larvae. However, it is suggested to have an aquatic type of predator to increase the efficiency of predation (Breene *et al.,* 1988).

2.5.2.2 Beetles and mosquito larvae

Predation potential of the dytiscid beetle, *Rhantus sikkimensis* and the mosquito larvae of *Toxorhynchites splendens* towards mosquito larvae of *Culex* sp were found in the annual lentic water bodies in Darjeeling Hills, India (Gautam *et al.*, 2006). The predatory natures of these predators were tested in the laboratory under simulated natural conditions using the fourth instars larvae of *Culex quinquefasciatus.* The research applied two indices of predation as its predation potential for these biological controls agent. The indices are predatory impact (PI) and clearance rate (CR). PI represents the predatory rate of the predators on mosquito larvae. Meanwhile, CR represents the total consumption of the predators on mosquito larvae.

The predation rate for *Rhantus sikkimensis* ranged between 21.56 and 86.89 larvae per day, PI value ranged between 18.67 and 35.33 larvae/day depending on prey densities and CR ranged between 2.21 and 2.23 larvae/litres/day/predator. Predation rate for *Toxorynchites splendens* larvae ranged between 0.67 to 34.22 larvae per day, PI value ranged between 7.67 and 11.33 larvae/day and CR value ranged between 1.422 and 1.76 larvae/litres/day/predator

Although the rate consumption of these predators varied due to difference in traits and features, *R. sikkimensis* and larvae of *Toxorhychites splendens* can consume a good number of mosquito larvae. It can be assumed that these predators play an important role in larval population regulation of mosquitoes and therefore impart an effect on species composition and interactions in the aquatic insect communities (Gautam *et al.*, 2006).

2.5.2.3 Fish

Fish of the genus *Gambusia* for the control of the larvae of *Anopheles* and *Culex* species that breed in water environment such as paddy fields, ponds and canals (West *et al.*, 1995).

Gambusia holbrooki was introduced for mosquito controls in Queensland, Australia (Webb *et al.*, 2007). However, the Australian native species such as rainbow fish and blue eyes are found to be more efficient than the introduced species.

Guppy has the larvivorous capability as viewed by the position of its mouth. This fish has been used as biological controls of *Anopheles culicifacies* and *Anopheles fluviatilis*, which are the main vectors of Malaria in Param, India in year 1993. It has also been found to tolerate pollution more than *Gambusia* species and prefers mosquito larvae than other food (Chandra *et al.*, 2008).

In China, the approach involved of the use of the indigenous edible fish, *Clarias fuscus* or Chinese cat fish to control *Aedes aegypti* larvae breeding in household water containers. *Clarias fuscus* is widely distributed in the tropical and subtropical regions of the world. The adult Chinese cat fish was 50 to 100 g in weight and able to tolerate unfavorable environmental conditions as it can survive in polluted water having a free chlorine concentration of 4 mg/l and low free oxygen content. Three of the villages, located in Fangchen and Hepu counties and Baihai city (consisting of a total of 3022 families and a total population of 14655), were surveyed within this period to evaluate the efficacy of the control program. The results indicated that the Breteau index for *Aegypti larvae* in these villages decreased thoroughly soon after the water containers were stocked with young *Clarias fuscus*.

The population of *Aedes aegypti* vectors remained low throughout the 5 year period. In the remaining five fishing villages where the vector control program was conducted, no case of dengue fever has been reported since 1981 although in the neighboring province of Guangdong there was an outbreak of the disease in 1985. For a period of five years on continued application of the fish species, no dengue outbreak occurred (Wu *et al.*, 1987). Although no predation rate was indicated in the report, the efficiency simulated on the number of dengue cases in particular research areas.

The study suggested a model ecosystem that contains *Clarias fuscus* that can be a biological control of mosquito at aquatic phase (Wu *et al.*, 1987). A modification on this approach has been made in this thesis by using the application of model ecosystems or containers that contained of aquatic insects namely *Micronecta polhemusi Nieser 2000.*

2.5.2.4 Copepods, Mesocyclops and Bacillus

Copepods are small aquatic crustaceans. Observations of copepod predation on first instar mosquito larvae led to the first investigation of their potential as biological control agent in 1981. Since then, various species of predacious copepods have been tested for their potential to control mosquitoes. Most of them are omnivorous and prey on immature mosquitoes, especially first instar larvae, but rarely on later stages.

Several species of copepods including *Mesocyclops aspericornis, M. thermocyclopoides, M. guangxiensis* and *M. longisetus,* have been reported as potential biological control agents of Aedes aegypti. These copepods species have wide distribution in freshwater sources. It is a dominant species found in wells and peridomestic containers in Thailand.

Entomopathogenic bacteria is an alternative to chemical insecticides. It is *Bacillus thuringiensis var. israelensis* (*Bti*). The endotoxins produced by *Bti* are lethal to mosquito larval (Culicidae) upon ingestion and also towards black flies (Simuliidae). As for copepods, they prey mostly on the newly hatched mosquito larvae due to its size limitation (Kosiyachinda *et al.,* 2003).

In comparison of the efficiency level, the average numbers of mosquito larvae in containers with *Bti* and copepods were 10% and 33% respectively. Application of *Bti* for mosquito control at larval stage is relatively safe. It is ecologically acceptable to non-target organisms and has no health effects on humans. However, its limitation is the high sensitivity of *Bacillus* spp. to environmental factors such as water pollutions and it needs reapplication after a certain period for its efficiency (Kosiyachinda *et al.,* 2003).

The findings of the study by Kosiyachinda *et al.,* (2003) reported that an effective control of mosquito larvae was attained when *M. aspericornis* and *Bti* were combined. Reapplication of *Bti* is needed to achieve satisfactory control levels because the controlling efficacy of *Bti* decreases a few days after application under natural settings. In addition, effectiveness of using copepods to control mosquito larvae greatly depends on the availability of their food. The natural feeding types of copepods are either omnivorous (feed on any larval from other insects and also feed on the smaller copepods) or herbivorous (feed on phytoplankton) types. The copepods under the study were reported as omnivorous type because of its predatory behavior on mosquito larvae. Addition of food for the copepods is important to supplement their natural food source, to sustain the copepod population and to enhance the efficacy of the combination to achieve a practical long term control of *Aedes aegypti* vector (Kosiyachinda *et al.*, 2003).

2.5.2.5 Planarians

The present work demonstrates how the larvae of the mosquitoes can be destroyed by planarians, *Dugesia bengalensis* which feed on them. These organisms can be cultured very easily in the stagnant water with other microorganisms and also in ponds and lakes. They breed by both asexual processes and these organisms are carnivorous in habit and can tolerate a temperature range up to 32 ºC. Out of the different stages of mosquito larvae, the planarians mostly preferred second and third stage of larval form of mosquitoes (Anopheles and Culex) where the exoskeleton does not get as hardened as an adult mosquito (Suprakash and Aditya, 2003).

Consumption rate is the numbers of mosquito larvae consumed by planarian over a change of time. The consumptions rate towards *Anopheles* larvae was higher (52.08%) than the Culex larvae (20.83%). In the experimental set up, it is noticed that five starved planarian can feed 25 larvae of *Anopheles* mosquito larvae within first 6 hours and the second 6 hours these planaria feed only 8 number of *Anopheles* larvae. In other hand, it has been found that 5 starved planaria can feed only 10 number of *Culex* larvae within first 6 hours which is less than the intake of *Anopheles* larvae and as the time increases the feeding rate is also declines.

The planarians have a distinct choice for the *Anopheles* larvae than *Culex* larvae. This type of food preference might be due to the larval posture (parallel to the water surface) which helps the planaria to hold or attack and capture *Anopheles* larvae easily when the hungry planaria float on the water surface. The mosquito larva is captured by the planarian which encircles the larva strongly and inserts the proboscis through any terminal end (mouth or tail end) of the larva and sucks the entire fluid.

The fasting planarians move very fast along the floor margin of the containers and very often float to the surface of the water in search of any prey. After the meal, the planarian leaves the exoskeleton and goes down the floor of the container. Therefore, it will not be unlikely that the planarians in large number will be of great help in eradicating the mosquitoes from the locality effectively.

The planarians were selective against the different stages of mosquito imatures and the species of mosquitoes. It has been observed that in both cases for *Anopheles* and *Culex,* the larval forms were palatable to the planarians than the other stages such as pupa and eggs. It also has been reported that the planarians generally avoid very small mosquito larvae (first stage of larval form) because of their fast movement (Suprakash and Aditya, 2003).

2.5.2.6 Dragonfly nymphs

According to Pandian *et al.,* (1979), the dragonfly nymph under study namely *Mesogomphus lineatus* is well suited for controlling mosquito at aquatic stages particularly mosquito larva and pupa. *Mesogomphus lineatus* lives in streams and channels, which are commonly known to have a wide fluctuation in temperature. Thus, with the high temperature, many different instars of *Mesogomphus lineatus* occur together and feed on mosquito larvae almost throughout the year (Pandian *et al.,* 1979).

The implemented procedure was based on the observations on predation of *Mesogomphus lineatus* towards mosquito larvae under the different temperatures. Subsequently, the nymphs were acclimated at 25 ºC or 40 ºC for a period of 7 days and the rest were acclimated to 10 ºC, 15 ºC or 20 ºC by lowering the temperature by 1 ºC for every 2 days until the desired temperature was reached. During acclimation to lower temperatures, there was no mortality. At 35 ºC, 20% of the test nymphs died within the first two days. Over 80% of the nymphs succumbed, as the temperature was raised over 37 ºC. At 40 ºC, no individual survived which is also a fatal temperature condition for most aquatic organisms (Pandian *et al.*, 1979).

Statistical analysis of the data reveals that the maximum food intake of the nymph is significantly dependent on weight as well as temperature. Temperature and body weight were two limitations found on dragonfly nymphs as biological control of mosquito. It will affect its predation efficiency. The more body weight it has, the more mosquito larvae consumed (Pandian *et al.*, 1979).

2.5.2.7 Micronectidae and Aedes aegypti larvae

A survey on the prevalence of *Micronecta* spp., predating on Aedes aegypti larvae was carried out in wide range of regions in Thailand. The survey involved inspection of various water storage jars outdoors. The mosquito particularly *Aedes aegypti* bred in the water storage jars. The presence of *Micronecta* spp. species approximately 2-3.5mm in size was observed. They were found in water containers especially in the rural areas. The research stated that *Micronecta* spp. is not only consumed *Aedes aegypti* larvae but also other weaker organisms by sucking the haemolymph of the prey until it is dead. The study reported that 74% of the containers inspected contained *Micronecta* spp. (Suphapathom *et al.*, 2002). With such a widespread aquatic predator of *Aedes aegypti* larvae, the findings promoted the use of this predator as a component in the vector management programs. However, the details of this predator, the species classification and its effectiveness were not yet determine.

The innovative methods using biological controls were summarized and listed in Table 2.6 and Table 2.7.

Table 2.6 Innovative methods to control mosquito using bio-controls

Bio-control / References	Research Summary	Comment & Response
Spiders (Breene, *et al.,* 1988).	Predation potential: 12 mosquito larvae per day.	The average percentage of predation potential of these spiders were 40.7% only.
Beetles and Mosquito larvae (Gautam *et al.,* 2006).	Predation Potential: *Rhantus sikkimensis* Predation rate ranged between 21.56 and 86.89 larvae per day. *Toxorynchites splendens* larvae Predation rate ranged between 0.67 to 34.22 larvae per day.	The rate consumption of these predators varied due to difference in traits and features, *R. sikkimensis* and larvae of *Toxorhychites splendens*.
Fish (West P. *et al.,* 1995).	Fish of the genus *Gambusia* for the control of the larvae in water environment.	*Gambusia holbrooki* was introduced for mosquito controls in Queensland, Australia (Webb *et al.,* 2007). However, the Australian native species such as rainbow fish and blue eyes are found to be more efficient than the introduced species.
Fish (Wu Neng *et al.,* 1987).	In China, the approach involved of the use of *Clarias fuscus* or Chinese cat fish to control *Aedes aegypti* larvae breeding in household water containers.	For a period of five years on continued application of the fish species, no dengue outbreak was occurred.
Copepods, *Mesocyclops* and *Bacillus* (Kosiyachinda *et al.,* 2003).	The average numbers of mosquito larvae in containers with Bti only, or copepods only, were approximately 10% and 33% of those in containers with no control agents, respectively.	An effective control of mosquito larvae was attained when *M aspericornis* and Bti were combined.

Table 2.7 Innovative methods to control mosquito using bio-controls

Bio-control / References	Research Summary	Comment & Response
Planarians (Suprakash and Aditya, 2003).	As the time increases the feeding rate declines.	The research stated that the planarians have selective choice for the eggs/larval/pupal stages and at the same time for the different groups of mosquitoes.
Dragonfly Nymphs (Pandian *et al.*, 1979).	The maximum food intake of the nymph is significantly dependent on weight as well as temperature.	There were two limitations found on dragonfly nymphs as biological control of mosquito: temperature and body weight.
Micronectidae and *Aedes aegypti* larvae in Thailand (Suphapathom *et al.*, 2002).	A survey on the prevalence of *Micronecta* species, predaceous on *Aedes aegypti* larvae was carried out in wide range of regions in Thailand. The research stated that *Micronecta spp.* is not only consumed *Aedes aegypti* larvae but also other weaker organisms by sucking the haemolymph of the prey until it is dead. The average being for the presence of *Micronecta* sp was 74 percent.	The details of this predator and its effectiveness were not yet determine.