LABORATORY AND FIELD STUDIES ON THE BIONOMICS OF *Culex quinquefasciatus* SAY (DIPTERA: CULICIDAE) IN PULAU PINANG, MALAYSIA

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by

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KAJIAN MAKMAL DAN LAPANGAN BIONOMIK NYAMUK *Culex quinquefasciatus* SAY (DIPTERA: CULICIDAE) DI PULAU PINANG, MALAYSIA.

ABSTRAK

Kajian bionomik nyamuk *Culex quinquefasciatus* di dijalankan selama 14 bulan bermula dari Februari 2008 sehingga Mac 2009 di Pulau Pinang dengan menggunakan kaedah umpan kaki terdedah (bare-leg catch). Tangkapan 12 jam (jam 1900-0700) dijalankan untuk menentukan kitaran mengigit manakala tangkapan selama 5 jam (jam 2100-0100) pula untuk menentukan kelimpahan bermusim.

Kelimpahan bermusim *Cx. quinquefasciatus* didapati berkait rapat dengan corak curahan hujan di kedua-dua kawasan kajian (Bayan Lepas; r = 0.948 dan Juru; r = 0.591). Terdapat korelasi antara populasi nyamuk ini dengan suhu purata bulanan di Bayan Lepas (r = -0.646) dan Juru (r = -0.513). Populasi nyamuk *Cx. quinquefasciatus* juga didapati berkorelasi dengan purata peratusan kelembapan di kawasan kajian Bayan Lepas (r = 0.749) dan Juru (r = 0.539). Kitar gigitan *Cx. quinquefasciatus* di kedua-dua kawasan kajian mempunyai dua puncak gigitan di mana puncak pertama pada Jam 2345 hingga Jam 0045 dan kedua pada Jam 0345. *Culex quinquefasciatus* menunjukkan kelimpahan populasi tertinggi adalah pada bulan September di kedua-dua kawasan kajian yang berkorelasi dengan curahan hujan.

Bilangan telur bagi setiap rakit telur untuk strain VCRU adalah lebih tinggi berbanding dengan strain Bayan Lepas dan Juru. Apabila diletakkan secara berasingan di dalam bekas didapati hanya 10% hingga 25% nyamuk betina *Cx. quinquefasciatus* berjaya mencapai sehingga kitar gonotrofik yang ke empat. Nisbah nyamuk jantan kepada nyamuk betina yang berjaya muncul ke peringkat dewasa adalah hampir 1:1.

Kaedah suapan bermembran mempunyai potensi yang tinggi dalam pengkulturan nyamuk, 72% to 90% nyamuk yang diberi suapan darah menggunakan kaedah ini berjaya memproses darah tersebut. Suapan darah tidak dipengaruhi oleh darah dari jenis perumah yang berbeza [$F(3,16)=3.549$, $p>0.05$]. Purata peratusan nyamuk yang berjaya bertelur adalah di antara 86.4% hingga 95.9%. Kefekunan dan nisbah jantina yang ditunjukan oleh nyamuk *Cx. quinquefasciatus* yang disuap menggunakan kaedah ini adalah hampir sama dengan kaedah suapan darah secara terus daripada perumah [$F(4,95) = 0.158$, $p>0.05$].

LABORATORY AND FIELD STUDIES ON THE BIONOMICS OF *Culex quinquefasciatus* SAY (DIPTERA: CULICIDAE) IN PULAU PINANG, MALAYSIA

ABSTRACT

The biting cycle and seasonal abundance of *Culex quinquefasciatus* mosquito were studied for 14 months from February 2008 to Mac 2009 in Penang using the bare-leg-catch technique. Twelve hours collections (1900-0700 hours) were conducted to determine the biting cycle of *Cx. quinquefasciatus* whereas five-hour collections (2100-0100 hours) were conducted to determine its seasonal abundance.

The seasonal abundance of *Cx. quinquefasciatus* was closely related to the rainfall pattern in both study sites (Bayan Lepas; r = 0.948 and Juru; r = 0.591). A correlation exists between monthly temperature and number collected in Bayan Lepas (r = -0.646) and Juru (r = -0.513). The population of *Cx. quinquefasciatus* mosquitoes seemed to be influenced by mean monthly relative humidity in Bayan Lepas (r = 0.749) and Juru (r = 0.539). The biting cycle in both study sites showed that the mosquitoes were active throughout the night with two distinct peaks, first between 2344 hour to 0045 hour and the second at 0345 hour. *Culex quinquefasciatus* exhibited a major peak of abundance in September for both study sites which corresponded with high rainfall.

The number of eggs per raft of the VCRU strain was significantly higher than both the Juru and Bayan Lepas strains. When kept individually in cups only 10% to 25% female *Cx. quinquefasciatus* successfully reached their fourth gonotrophic cycle. The male to female ratio of adults successfully emerged was almost 1:1.

The artificial membrane feeding technique showed potential in mosquito culture where 72% to 90% mosquitoes were observed engorged in this study. Blood feeding was not influenced by blood from different host types [$F(3,16) = 3.549$, $p > 0.05$]. The mean percentage of engorged females that laid eggs ranged from 86.4% to 95.9% for all host blood types. An almost similar pattern of fecundity and sex ratio were determined for *Cx. quinquefasciatus* fed through artificial membrane feeding and through direct feeding techniques [$F(4,95) = 0.158$, $p > 0.05$].

1.0 INTRODUCTION

Mosquitoes are among the best known groups of insect, because of their importance to human as pests and vectors of some of the most distressing human diseases. There are about 3100 species of mosquitoes from 34 genera. There are members of a single family, the Culicidae which is divided into three sub-families; Anophelinae, Culicinae and Toxorhynchitinae (Ryan *et al.,* 2004).

Based on the findings of a 1995 study by USM's Vector Control Research Unit, problems caused by mosquitoes are found to be foremost among other pest such as cockroaches and termites in Penang (Lee & Yap, 1999). This proves that mosquitoes are the public number one enemy.

Mosquitoes are distributed widely throughout the world and practically no part of the globe that can support human existence is free from them especially in the tropics, temperate region and even the Arctic. The only region which does not support mosquito population is the Antarctic (Service, 1996). *Culex* is one of the most dominant genera in its family. However, according to Abu Hassan (2004), *Culex* is the second largest mosquito genera in Malaysia after *Aedes*. *Culex* are distributed around the world but is absent on the northern temperate climate zone (Sulaiman, 1990). The southern house mosquitoes, *Cx. quinquefasciatus* is found in tropical and warm or temperate regions. Its dispersal has been aided by human activities such as rapid and long distance transportation.

A number of insects and related arthropods feed on blood, but mosquitoes are one of the most prominent members of this group. Mosquitoes are a vector agent that carries disease-causing viruses and parasites from person to person without catching the disease themselves. Female mosquitoes bite and suck blood from humans and other animals as part of their eating and breeding habits. The female bites are irritating, in some cases causing allergic reaction at the feeding puncture, and it can transmit diseases. The female mosquito that bites an infected person and subsequently bites an uninfected person might leave traces of virus or parasite from the infected person's blood (Allen *et al.,* 2001).

Mosquitoes are estimated to transmit diseases to more than 70 million people annually in Africa, South America, Central America, Mexico and much of Asia with millions resulting in deaths. In Europe, Russia, Greenland, Canada, the United States, Australia, New Zealand, the UK, Japan and other temperate and developed countries, mosquito bites are now mostly an irritating nuisance; but still cause some deaths each year (James *et al.,* 2004). Historically, before mosquito transmitted diseases were brought under control; they caused tens of thousands of deaths in these countries and hundreds of thousands of infections. Mosquitoes were shown to be the vector by which yellow fever and malaria were transmitted from person to person according to Walter Reed, William C. Gorgas and associates in the U.S. Army Medical Corps, first in Cuba and then around the Panama Canal in the early 1900’s (Molly, 2005). Since then other diseases have been shown to be transmitted the same way including the urban filariasis disease which is transmitted by *Cx. quinquefasciatus.*

In Malaysia, *Cx. quinquefasciatus* is the most dominant mosquito species in urban areas making it among the most important species here. At one point, this mosquito species was only recognized as a species which are an annoyance factor to human life.

However, this species has now achieved medical importance for it has been identified as a potential vector for the disease 'Urban filariasis' or 'Lymphatic filariasis'. *Culex quinquefasciatus* is responsible for transmitting *Wuchereria bancrofti* (filariasis parasite) through its bite from one victim to another (Atkinson *et al.,* 2000). Therefore control measures against this mosquito species are important to prevent the spread of this disease in Malaysia.

In 1948 the distribution of the two main species of human filarial parasites had been well mapped and the major mosquito vector identified. *Wuchereria bancrofti* is found in 17 countries and areas: American Samoa, China, Cook Islands, Federated States of Micronesia, Fiji, French Polynesia, Kiribati, Malaysia, Niue, Papua New Guinea, the Philippines, Samoa, Tonga, Tuvalu, Vanuatu and Viet Nam (Wuchun *et al.,* 1997). The first reference to urban filariasis in Malaysia was by Daniels (1908), who found three microfilarial carriers among 100 patients at the Kuala Lumpur Hospital (Mak, 1983).

According to the Ministry of Health Pulau Pinang, in Penang there are 35 filariasis cases reported in the year 2006 and 32 filariasis cases in 2007 involving *W. bancrofti* infection. All patients were recent immigrants form Myanmar, Nepal, India and Indonesia.

Lymphatic filariasis or bancroftian filariasis is a disease caused by the infection of *W. bancrofti*. The important vectors of this disease are *Ae. imitator, Ae. demostes, Ae. anandalei, Ae. harinasuti* and *Mn. dives* (Suvannadabba, 1993). Recently, *Cx. quinquefasciatus* was reported to be a highly efficient insect host for the larval development of nocturnal periodic *W. bancrofti* (Jitpakdi *et al.,* 1998 and Wilawan *et al.,* 2005).

Control measures in filariasis can be directed towards three main approaches; these being, reduction of the reservoir of infection, reduction of human-vector contact and finally vector control. Vector control, theoretically, is of immense attraction as a control measure.

Over the last decade, the rapid changes in the urban environment and demographic structure in the country has undoubtedly influenced changes in the vector ecology and consequently the epidemiology of filariasis. The reasons above suggested more study should be done on *Cx. quinquefasciatus* biology and ecology to understand more about their relation to filariasis transmission.

In the last decade, considerable amount of research on filariasis vector focused on insecticide efficacy, insecticide resistance and its infection and infectivity rate. Lack of up-to-date information on the biology and ecology of *Cx. quinquefasciatus* in Malaysia made it difficult to choose the best method which could be effectively applied in filariasis control.

Service (1993) had emphasized the importance of ecology in the control of vector borne-diseases. Therefore, this study of its bionomics and biology are necessary, in order for control programme to be effective.

The objectives of this research are:

1. To examine the relationship between environmental variables and abundance of *Cx. quinquefasciatus* in selected endemic areas in Penang.

2. To determine the biting cycle of *Cx. quinquefasciatus* female adults in the field.

3. To study the biology of *Cx. quinquefasciatus* in the laboratory including estimating the fecundity and sex ratio.

4. To demonstrate the potential of artificial membrane feeding technique in culturing *Cx. quinquefasciatus* in the laboratory.

5. To determine *Cx. quinquefasciatus* blood type preferrence through artificial membrane feeding and its effect on the mosquito biology.

2.0 LITERATURE REVIEW

2.1 *Culex quinquefasciatus* Say

According to Mustapha *et al.* (2005) the scientific classification of *Cx. quinquefasciatus* is as follows;

Order	:Diptera
Family	:Culicidae
Sabfamily	:Culicinae
Genus	:Culex
Subgenus	: Neoculex
Species	: *Culex quinquefasciatus*

Mosquitoes are among the best known group of insects, because of their importance to man as pests and vectors of some of the most distressing human diseases. Mosquitoes are responsible for causing more human suffering than any other animals. One of the most important mosquito species is *Cx. quinquefasciatus.* Like other mosquitoes, these are small and two-winged insects belonging to the family Culicidae of the order Diptera (two-winged).

According to Dobrotworsky (1965), *Cx. quinquefasciatus* is part of the *Pipiens* group and belongs to the subgenus *Culex.* In 1823, Say named the species *Cx. quinquefasciatus* but in 1828 the named *Cx. fatigans* was introduced by Weidmann (Dobrotworsky, 1965 and Stone, 1956). The name was applied to what appears to be the same species and both names have been used as synonyms since they arose (Stone, 1956).

Until recently this species has been regarded as a subspecies of *Cx. pipiens fatigans* or *Cx. quinquefasciatus.* Due to the inherent confusion this variable naming causes, there was a movement towards returning to use the originally naming of *Cx. quinquefasciatus* (Stone, 1956).

Identification of morphological characteristics based on identification keys is important to differentiate species within *Culex* mosquitoes. The five morphological characteristic introduced by Linnaeus used to identify adult *Cx. quinquefasciatus* were an entirely dark proboscis, mesonotom with well developed acrostichal bristles, presence of lower mesepimeral bristles, upper and lower sternopleuron and middle mesepimeron with distinct scale patches and banded abdomen (Belkin, 1968).

2.2 Biology of *Culex quinquefasciatus*

2.2.1 Life cycle of *Culex quinquefasciatus.*

Like other dipterans, *Cx. quinquefasciatus* undergoes a complete metamorphosis, passing through four distinct stages during their life cycle; the egg, larva, pupa and adult or imago. Therefore, they are members of Holometabola, the large group of insects in which the youngs are very different from their parents in form, structure and habits.

The females usually mate once but produce eggs at intervals throughout their life. In order to be able to do so female mosquitoes require blood-meal, a necessity for the propagation of the species. However, male do not suck blood but rather feed on plant juices because the male mouth part of male are not developed for piercing.

The whole period from the beginning of one blood-meal to the next generally consists of three phases which include the search for a host to obtain a blood-meal, the ingestion of the blood and egg development, and the search for a suitable breeding place and oviposition (Detinova, 1962). This intervening period, between the ingestion of blood and oviposition, is known as the gonotrophic cycle.

If there are no atmospheric disturbances, sunrise and sunset are the preferred times for oviposition for *Cx. quinquefasciatus*. The factors determining the oviposition cycle were demonstrated by De Meillon *et al.* (1967) to have a positive relationship between the date of the blood meal and the date of oviposition. Under natural tropical humidity and temperature conditions, the sunset peak corresponded to females *Cx. quinquefasciatus* which had taken a blood-meal after midnight three nights before, while the sunrise peak corresponded to the females which had taken a blood-meal before midnight two nights earlier.

The duration of digestion of the blood-meal has been studied during the rainy season in the laboratory and in the field in Kenya by Rebekah & Douglas (2005). Although most digestions were completed within 60 hour, some only required 48 hours (Figure 2.1). As soon as oviposition has been completed the mosquito is ready to take another blood meal.

The duration of the gonotrophic cycle of *Cx. quinquefasciatus,* appears to last a minimum of 5 days for the first, 3 days for the second, and the subsequent ones 4 days or more, since old parous females require a longer time than primiparae (Detinova, 1968).

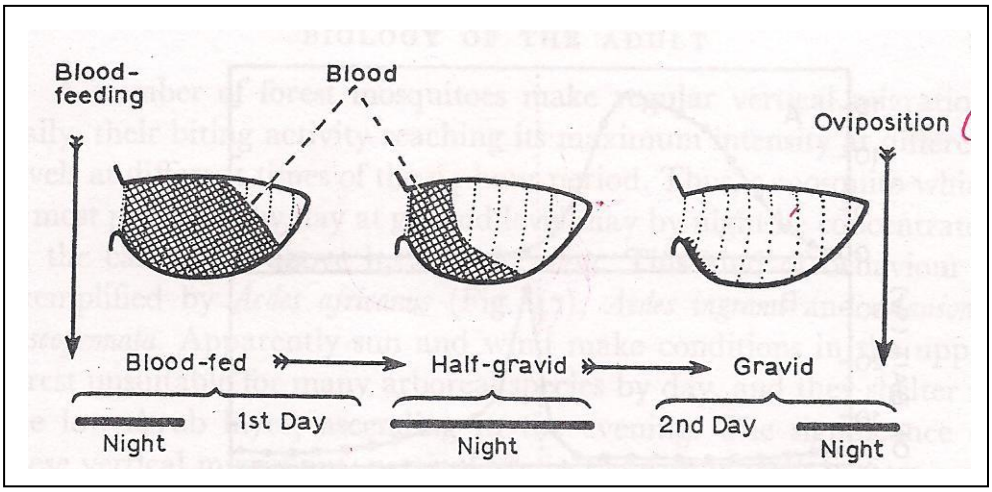

Figure 2.1: Gonotrophic cycle of mosquitoes in the tropics (modified from Muirhead- Thomson, 1951)

2.2.2 Egg Biology

Mosquito species have evolved to seek out and lay eggs in various locations and different conditions. In general, *Cx. quinquefasciatus* mosquito will lay clusters of eggs, often called egg rafts that float on the water surface. This mosquito species usually lay their eggs at night over a period of time sticking them together to form a raft of about 100 to 300 eggs. A raft of eggs looks like a speck of soot floating on the water and its about ¼ inch long and 1/8 inch wide.

The shape of the mosquito eggs vary considerably and characteristically between species. According to Service (1996), eggs of *Cx. quinquefasciatus* mosquitoes are generally elongated-oval shape with the outer shell smooth, brownish and do not have air-floats at the sides like *Anopheles* eggs.

Culex quinquefasciatus eggs have a prominence called corolla. At the anterior pole of the egg is a minute opening known as the micropyle, through which the sperm enters to fertilise the egg. The micropyle is separated from the cytoplasm of the egg by a modified region of the endochorion (an intermediate, hard, thick and opaque layer of the egg shell) known as the micropyle plug. It is considered that sperm penetrates the micropyle during oviposition and that the micropyle plug is traversable by the sperm at that time.

Mosquito distribution is determined by the oviposition habits of the female parents. Consequently, female *Cx. quinquefasciatus* mosquitoes lay their eggs in polluted stagnant water and will die when kept away from water for prolonged periods. The eggs are entirely passive and cannot stand extreme weather (Lee & Yap, 1999). They have no power of mobility and remain where they are until they hatch.

According to Clements (1992), eggs are at first white in colour and are soft, then become white and flexible but after one to two hour after oviposition they turn black, become quite hard and waterproof for mechanical support and protection. *Culex quinquefasciatus* eggs do not go through a diapause period whereas eggs of one batch hatch together when the embryo are fully developed. Eggs require only a few hours or one whole day at the most to hatch under tropical conditions.

Eggs survival may depend on a combination of density dependent and independent selective pressures (Estrada-Franco, 1995). Factors that may endorse egg loss are desiccation, predation and freezing. Predation appears to play an important role in egg mortality in natural populations of *Cx. quinquefasciatus*.

2.2.3 Larval biology

The two main factors regulating mosquito larval growth are nutrition and the temperature of the water in the breeding places (Clements, 1963). In the southern United States during the coldest months, pre-adults can live past 48 days on the average (Hayes, 1975). In the tropics, it is much shorter. In Rangoon, where temperature varies little throughout the year, De Meillon *et al.* (1967) explicated that at a temperature around 28^{o}C, the egg incubation period was approximately 27 hours and the male and female larvae hatch out simultaneously.

However, the duration of the larval stages in male *Cx. quinquefasciatus* (about 118 hour) is shorter than that in females (about 135 hour). In Africa, field study by Subra (1981) at Bobo Dioulasso showed that the average time elapsing between oviposition and hatching rate ranges from 24 hour to 36 hour. The *Cx. quinquefasciatus* larval stage lasted between 6 and 8 days whereas the wide range of the larval period may be due to temperature difference of the larval breeding places in various types of soakage pits.

During its life the larva sheds its cuticle four times. At each of the first three moulds (ecdysis) it comes out a little larger than before, but with little change otherwise. All four instars are similar, varying in size and in number, arrangement and complexity of certain hairs and other surface structures, for example the form and number of pecten spine (Snow, 1990).

The first instar larva, which hatches from the egg, is at once distinguished from subsequent larval instars, not only by its small size, but also by the possession of an egg breaker on the top of the head. At the forth instar, the usual larva reaches a length of almost ½ inch and towards the end of this instar, it ceases to feed.

Culex quinquefasciatus mosquito larvae do not possess legs and their bodies are divided into a well developed head bearing mouth-brushes and antennae, a bulbous thorax which is broader than the head and abdomen, and elongated abdomen which ends in an anal segment bearing four gill-like lobes, the anal papillae.

Mosquito larvae are very active in their aquatic environments and have been called "wriggler" because of their almost constant wriggling movement. They are consistently moving down to the bottom to feed and moving up to breathe because larvae breathe at the water surface through siphon (specialized tube). They hang with their heads down and the brushes by their mouths filtering anything small enough to be eaten toward their mouth to nourish the growing larvae. When disturbed, *Cx. quinquefasciatus* larvae will show whip-like tail movement (DuPonte & Larish, 2003).

Larvae of *Cx. quinquefasciatus* have 10 identifying characteristics to recognize the mosquitoes species. The morphology characteristics are; (i) Pecten teeth not extending to near apex of air tube; (ii) Comb scale of 16 or more, usually in a triangular patches; (iii) Air tubes not swollen at the middle; (iv) Air tube without "false joint" beyond middle; (v) Preclypeal spine tapering or if stout pointed at tip; (vi) Pecten teeth with 1 set denticles; (vii) Median spine of comb scale about as long as lateral spine; (viii) Upper and lower head hairs with 5 or more branch; (ix) Air tube with 4 siphonal tufts and gills not 3 to 4 times longer than saddle; (x) Preclypeal spine not longer than the distance between their bases and gills not rounded at tip Dubose & Curtin (1965).

2.2.4 Pupal biology

The final, non-feeding immature stage is called the pupa. Pupae are less mobile than larvae. However, they are often seen “tumbling” at water surface. The pupae remain at the water surface for most of the time, breathing through their paired respiratory trumpets. When disturbed they dive with a jerking motion, returning to the surface passively (Clements, 1963).

While in the pupal stage, the mosquito larva is changing or metamorphosing into the adult form. The pupa has all the adult organs, though in an incomplete state of development and is clearly a preliminary adult. The pupa of a mosquito is recognised by its distinctive comma shape, the ‘dot’ of the comma representing the combined head and thorax (cephalothorax) and the “tail” representing the abdomen.

The sex of the pupa can be determined by examination of the genital lobe which contains the developing adult external genitalia. In the female this is short and undivided whereas in the male it is long and consists of two lobes (Snow, 1990).

2.2.5 Adult Biology

With a final moult of the pupa the completed adult mosquito appears, equipped for an entirely different life from that of either the larva or the pupa. The adult mosquito emerges from its pupal case on the water surface and immediately rest for several days to allow itself to dry and to harden its outer cuticle or “skin” before it can fly.

Mating does not take place immediately after leaving the larval breeding place, but occur 36 to 48 hour after emergence (Yasuna & Harinasuta, 1967 and De Meillon *et al.*, 1967). Usually, the females are fertilized before their first blood-meal, but sometimes fertilization occur when the female have already taken blood according to Yasuna & Harinasuta (1967).

Soon after emergence both male and female mosquitoes begin to feed on nectar or other plant juices which provides them with sugar for daily energy needs. Within a week, the adult females begin searching for suitable host to bite for a blood-meal. This blood-meal gives her all the proteins and nutrients required to produce the eggs.

After feeding, female mosquitoes will find a cool resting spot in which to convert the blood-meal into eggs. The female seek successive blood-meals for about two weeks throughout their life which lasts for several weeks to several months, depending upon environmental conditions.

According to Snow (1990), there are 4 categories to classify the appearance of the female abdomen as viewed with a hand lens or dissecting microscope. The categories used to report the appearances of the abdomen are; i) Unfed, the midgut (stomach) is empty and the abdomen is therefore thin. The ovaries are small, occupying less than one-third of the abdomen. Newly emerged females which have not fed and females which have passed through a cycle of feeding and egg-laying and are awaiting another blood meal are in this category. ii) Freshly-fed, the midgut is distended with red blood and the ovaries occupy about a quarter of the abdomen. iii) Late fed/ half gravid, the blood in the midgut is dark red and the ovaries occupy about half of the abdomen. iv) Gravid, at most a trace of dark blood is visible. The ovaries occupy almost the entire abdomen.

2.3 Habitat of *Culex quinquefasciatus*

Culex quinquefasciatus usually breeds in organically rich and polluted surface waters or artificial containers (Weinstein *et al.*, 1997). It has been found breeding in shallow pond within streams, phytotelmata (Derraik, 2004), and artificial habitats such as drains and drain sumps, wells, oxidation ponds at sewage treatments plants (Derraik & Slaney, 2005), stock drinking troughs, septic tanks, rain water container, tyres and various other small container (Lee *et al.*, 1989; Laird 1990). It may also be found utilising the same container for breeding as other species (Lee *et al.*, 1989).

Culex quinquefasciatus develops mainly in habitats containing highly polluted water rich in organic matter that the larvae can use for nourishment. Thus food supply is a factor which can be disregarded in most cases, since food is usually available in excess in their numerous breeding habitat (Subra, 1981). The effect of the amount of nourishment available in breeding places are reflected not only in the number of and size of pre-imaginal form but also in the size of the adults, which are larger when a greater amount of nourishment is available (Kurihara, 1963).

Subra (1981) has classified the categories of *Cx. quinquefasciatus* larval habitats: man-made or modified larval breeding places, the most important and dependant on man for their establishment; peridomestic breeding places, drains and gutters designed to take away rain water in towns frequently become breeding places for *Cx. quinquefasciatus*; industrial and agricultural breeding, waste-discharge channels at sisal-processing factory may enable the development of large numbers of the aquatic form of *Cx. quinquefasciatus.* The organic pollution usually found in the breeding places of this species is supplemented here by a chemical pollution to which the larvae seem well adapted; the last category is the natural larval habitats. Natural sites are usually small and *Cx. quinquefasciatus* is often found breeding with other species. By themselves, they doubtlessly contribute to only a number of adults but they cannot be disregarded in campaigns designed to interrupt disease transmission. Tree holes, crab burrows and coral rock-holes are natural breeding places for *Cx. quinquefasciatus* which differ greatly from one another both in terms of origin and structure.

Furthermore, this usually occurs in large villages which are constructed on a town-like pattern, several hundred houses built along streets and lanes. As in most tropical towns where space is limited in the centre of these localities, people have to dig pit latrines and cesspools to eliminate waste and used water.

2.4 Distribution and seasonal abundance

According to MacFie & Ingran (1916), before the Second World War, *Cx. quinquefasciatus* was known only in a few scattered localities, mainly on the coast where it represented only a small percentage of the culicid fauna. After the Second World War, the number of places where it becomes the prevalent species increased considerably. This phenomenon seems to have accelerated during the last decade, and today *Cx. quinquefasciatus* is the dominant if not the sole species in most of the urban areas.

The wide distributions of *Cx. quinquefasciatus* in both the northern and southern hemispheres illustrate this species's capability to survive a variety of climatic challenges. It is able to adjust its seasonal cycle of reproductive activity to the environments ranging from temperate continental climates to humid tropics. *Culex quinquefasciatus* occurs in all climatic zones, ranging from forest to semi-desert (Rochlin *et al.*, 2009).

Previous studies demonstrated the existence of *Cx. quinquefasciatus* in subtropical or temperate continental climates. *Culex quinquefasciatus* was observed surviving in the coolest season in deserts in Alabama (Mullen & Qualls, 2006), in Texas (Odula & Awe, 2006), in Indonesia (Argueta *et al.*, 2004) as well as in the coolest season in a settling lagoon in Carlifornia (Marieta & Ring, 2007). Jagdis & Jagbir (2003) stated that *Cx. quinquefasciatus* was present during the dry season in Burma and Catarina *et al*. (2003) concluded in their studies in Venezuela that *Cx. quinquefasciatus* is present in the wet season in the humid tropic.

Altitude does not seem to limit *Cx. quinquefasciatus* distribution, since it has been found as high as 2770m in India and 2130m in Sri Lanka (Bhat, 1975). However it is not generally found at such a high altitude and the upper limit of its altitudinal distribution in other regions is said to be at about 1680 m in the South Pacific and 1600 m in Reunion (Dobrotworsky, 1967).

In recent decades, epidemiologists have increasingly investigated the relationship between faith-based communities, topography, seasons and disease risk area with the abundance of mosquito. Most previous studies have found that temperature, rainfall and relative humidity are physical factors that influence the abundance of mosquitoes (Warabhorn *et al.,* 2007).

There is no changing season in Malaysia, therefore the significant difference in mosquito abundance throughout the year shows a negative correlation compared to countries that have obvious seasonal changes (Lee, 1991).

The most important abiotic factors that affect *Culex* breeding is rainfall (Muturi *et al.,* 2007). Reproduction of *Cx. quinquefascitus* in tropical and subtropical zones occur all year around and their abundance can either be associated with rainfall regimens (Chow, 1973) or no association is observed (Nayar *et al.*, 2002). Generally, *Cx. quinquefasciatus* breeds after rain, not during raining days. With heavy rainfall, water in breeding sites will overflow, and consequently larvae cannot survive in it (Yasuno *et al.*, 1973).

In a study on female adult *Culex* in Penang, the highest peak was seen in October, the lowest in January of the year, these situations are closely related to rainfall pattern (Jaal *et al.*, 1996). According to Pipitgool *et al.* (1998) in a study in Thailand, the lowest density was observed in winter and the higher densities were in the summer and rainy seasons.

Temperature is the most important variable that influences the biting density and abundance of mosquito (Pipitgool *et al.*, 1998). Mosquitoes are sensitive to temperature changes as immature stages in aquatic environment and as adults. If the water temperature rises, the developmental period of the larvae is shorter (Rueda *et al.*, 1990) and there is a greater capacity to produce more offspring during the transmission period.

According to Mahanta *et al.* (1999), minimum temperature was found to have a limiting effect on the biting density of vector mosquitoes. Adult female mosquitoes digested blood faster and feed more frequently in warmer climate, thus increasing the abundance and transmission intensity (Gillies, 1953). However, warming above 34^{o}C generally has a negative impact on the survival of vectors and parasites (Reuda *et al.*, 1990).

According to Zielke & Kuhlow (1977), the distribution and density of *Cx. quinquefasciatus* in tropical and subtropical countries are therefore two constantly involving phenomena for which different explanations have been suggested. Apart from the development of rapid modes of transportation which promote the dispersal of *Cx. quinquefasciatus* though not its establishment, there are two essential reasons which led to the rapid multiplication of this mosquito; the utilization of insecticide and rapid urbanization (Highton & Van Someren, 1970).

2.5 Host preference

The feeding behaviour of mosquitoes is of paramount importance in the epidemiology of mosquito borne diseases. It is known that a mosquito must feed on a host's blood at least twice to serve as a vector.

Only female mosquitoes require a blood meal and bite animals which are either warm or cold blooded and birds. Mosquitoes are known to perceive visual, thermal and olfactory stimuli which enable them to detect light source, odour and several other volatile chemicals such as carbon dioxide emanating from host skin, breath and waste product (Takken, 1991). According to Davis & Bowen (1994), stimuli that influence biting (blood feeding) include a combination of carbon dioxide, temperature, moisture, smell, colour and movement. Of those female mosquitoes capable of blood feeding, human blood meals are seldom first or second choices. Cattle, smaller mammals or birds are preferred.

The survey reported by Reisen *et al.* (2005) in North America, reported that *Cx. quinquefasciatus* fed predominantly on birds and less than 1% of the time on humans. A more recent report from California found *Cx. quinquefasciatus* fed approximately equally on mammals and birds (Zinser & Willotta, 2004).

Analysis of blood- meal sources from *Cx. quinquefasciatus* in two urban sites and one wooded site in Louisianna suggested that mosquitoes are opportunistic feeders that feed readily on human or bird (Niebylski & Meek, 1992). Mosquito from a site adjacent to a dog kennel had more than 96% dog blood meals. More typical residential areas yielded 65% to 70% dog, 9% to 15% human, and 6% to 30% bird blood. A wooded area had 23% to 33% dog, 13% to 23% human, and 43% to 53% bird blood (Niebylski & Meek, 1992; Zinser & Willotta, 2004).

According to Gillett (1972), *Culex* mosquitoes are more zoophilic than anthropopilic. However Vythilingam *et al*. (1996), in their study on host feeding pattern of JE vectors in Selangor, Malaysia showed that *Cx. quinquefasciatus* had a greater preference for avian host compared to other *Culex* mosquitoes.

Culex quinquefasciatus has achieved medical importance for it is a vector of the disease, filariasis. Due to its role as a vector of filariasis, *Cx. quinquefasciatus* therefore has shown anthropophilic characteristics (Atkinson *et al*., 2000 and Oduala & Awe, 2006).

Most authors also agree that *Cx. quinquefasciatus* is antrophophilic (Heisch *et al.,* 1959; Chandler *et al.,* 1975; Zinser & Willott, 2004). The degree of this anthropophily moreover varies according to the places where fed females are trapped, and *Cx. quinquefasciatus* may be attracted by various types of bait (Lee *et al.,* 1958).

2.6 Biting habits and behaviour

Culex quinquefasciatus is a markedly domestic species and are painful and persistent biters, but prefers to attack at dusk and after dark. They readily enter dwellings for blood meals. It is a common house mosquito and major nuisance, causing sleepless nights.

The adult females bite hosts mostly in the evening and throughout the night to avoid the heat of the day, indoors and outdoors. Only female mosquitoes bite. Not because the males are not as mean as the females, but it is because of the lack of blood-sucking mouthparts in the males. Both sexes feed on nectar. However, females bite because they need blood-containing proteins and nutrient for egg development and egg lying, since the normal mosquito diet consists of nectar and fruit juice, which has no protein (Apperson *et al.,* 2002).

An adult host seeking female *Cx. quinquefasciatus,* can travel up to 10 km in a night and fly for one to four hours continuously at up to 1 to 2 km/h purposely to track the potential pray to complete its life cycle. In Burma, Lindquist *et al.* (1967) estimated the flight range of *Cx. quinquefasciatus* at 1 km.

In uninhabited areas, mosquitoes are able to fly for several kilometres (Afridi & Majid, 1935). Yasuno *et al.* (1973) captured nearly 8% of marked mosquitoes at 1 km from the release point in an uninhabited area near a village in a region in New Delhi. However, in the same region in a rural area Brooks (1976) recorded a capture at a distance of 11 km thus much greater than any known reported.

The mosquitoes flight range may be influenced by the wind and the climatic conditions (Fussell, 1964). According to Fussel (1964) and Yasuno *et al.* (1973) sex does not affect the distance covered, but Lindquist *et al.* (1967) found that females had a greater flight range than males.

Some *Cx. quinquefasciatus* females have an endophagic behaviour while others are exophagic. During the day, inactives are often found resting in dark corners of room, shelters and culverts. However exophilic species rest outdoors on vegetation and in holes in trees in forested areas (Chow & Thevasagayam, 1957).

The mosquito generally spends only a few hours in the vicinity of the breeding places from which it emerged. It then makes one or more flights, which takes it to another shelter either inside a house or outdoors. When they reached the appropriate age, the females take their first blood-meal, mostly inside dwellings where they then pass the first few hours after the blood-meal. Most of them then leave the dwellings and digestion is completed outdoors in shelter.

According to De Meillon *et al.* (1967) exophagic females may even enter inhabited houses after biting man or animals outside. In any case, females whether endophilic or exophilic, will carry out a series of movements by day and night throughout their gonotrophic cycles.

2.7 Public health importance of *Culex quinquefasciatus* as a disease vector.

In nature, *Cx. quinquefasciatus* is an important vector of periodic filariasis in some parts of the world (Belkin, 1968), and it has a more restricted role as a vector of viral diseases of animal and man. *Culex quinquefasciatus* is known to carry and transmit *W. bancrofti* to some degree of efficacy in many regions of the globe (Hawking, 1973). In Asia, lymphatic filariasis caused by *W. bancrofti* remains a public health problem only in Myanmar, Thailand, Nepal, India, Indonesia and some subtropical countries.

According to Wilawan *et al.* (2005), bancroftian filariasis is a disease caused by the infection of *W. bancrofti* and remains a public health problems in Myanmar, especially in the areas near the Thai-Myanmar border. Recently, *Cx. quinquefasciatus* was reported to be a highly efficient insect host for the larval development of nocturnally periodic *W. bancrofti* in Myanmar, India and Sao Paula, Brazil (Jitpakdi *et al.,* 1998; Wilson & Reid, 1951; Kurihara & Oemijati, 1975).