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THESIS

MOVEMENT PATTERNS OF *AEDES AEGYPTI* (L.) (DIPTERA: CULICIDAE) INTO AND OUT OF THE EXPERIMENTAL HUTS

NANTAWAN SUWANNACHOTE

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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Nantawan Suwannachote 2010: Movement Patterns of *Aedes aegypti* (L.) (Diptera: Culicidae) Into and Out of the Experimental Huts. Master of Science (Entomology), Major Field: Entomology, Department of Entomology. Thesis Advisor: Associate Professor Somnuk Wongtong, Ph.D. 101 pages.

Mark – release – recapture experiments with *Aedes aegypti* were performed using experimental huts equipped with entrance and exit traps to evaluate the movement patterns of *Aedes aegypti* during a two – year period in Thailand. Results indicated that there was no significant differences in both entry and exit patterns between the two years of observation. Movement into the huts occurred during the early morning period (0600 – 1100 hour) with a peak at 0700 hour in the summer and rainy season and 0900 hour in the winter. In contrast, the exit pattern was observed during the late morning (0900 – 1200 hour) and early afternoon (1200 – 1500 hour), with a peak at 0900 hour in the winter, 1100 hour in the summer and 1400 hour in the rainy season. Multiple regression analysis indicated that movements of *Ae. aegypti* females into and out of the huts were partially influenced by relative humidity and ambient temperature during the day.

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Nantawan Suwannachote

January 2010

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INTRODUCTION

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are two of the most important mosquito-borne viral disease syndromes in the tropic and subtropical world. Each year, 50 – 100 million cases are reported as being infected with dengue viruses (Gubler and Kuno, 1997; World Health Organization (WHO), 1999). In Southeast Asia, the disease is of paramount importance with approximately 50,000 – 200,000 cases annually (Lederberg *et al.*, 1992). Dengue viruses are transmitted from person to person by the bite of *Aedes aegypti* (L), a notoriously efficient mosquito vector that invariably breeds near or inside human dwellings and preferentially feeds on humans even when other warm blooded animals are freely available (Edman *et al*., 1992; Xue *et al*., 1995; WHO, 1999). A diet of from human blood has been found to be a proximate benefit in the synthesis of energy reserves and an ultimate advantage in mosquito fitness, especially in a house – haunting mosquito like *Ae. aegypti* (Christopher, 1960; Harrington, 2001; Polawat and Harrington, 2005; Polsomboon *et al*. 2008). As no acceptable vaccine or antiviral agent is yet available for the prevention and treatment of dengue infection, the control of the mosquito vector remains the most important method to prevent dengue virus transmission and averting dengue epidemics (Reiter and Gubler, 1997; Perich *et al*., 2001). Control of the vector by chemicals remains the most significant means to interrupt transmission potential and preventing mosquito bites (Roberts *et al*., 1997; WHO, 1999).

Chemicals protect humans from the bite of mosquitoes through three different ways: irritation after making physical contact, repelling prior to contact with chemicals, or by killing the insects (Grieco *et al*., 2007). Most research has focused on the toxic function of chemicals whereas comparatively few have concentrated on non-toxic chemical characteristics. Non-toxic actions can be categorized into two different mechanisms, contact irritancy and noncontact repellency (Roberts *et al*.,

1997; Chareonviriyaphap *et al*., 1997). To facilitate the study of true responses of mosquitoes to chemicals, several studies have utilized experimental huts to investigate the natural movement of mosquito populations. Most work has concentrated on the entrance and exit behaviors of malaria vectors in response to indoor insecticide treated surfaces of house walls (Kennedy, 1947; Roberts and Alecrim, 1991; Grieco *et al*., 2000). However, little is known about the movement patterns for natural populations of *Ae. aegypti* into and out of huts in the presence of a human host. Recently, the effect of host type on the movement patterns of *Ae.aegypti* using the experimental huts was conducted in Thailand (Suwonkerd *et al*., 2006). The same huts were further used to investigate the three actions of chemicals against *Ae. aegypti* mosquitoes (Grieco *et al*., 2007). However, this study failed to compare the movement patterns of natural population of *Ae. aegypti* into and out of the experimental huts between seasons and under different environmental conditions. This study was the first attempt to investigate the ingress and egress movements of *Ae. aegypti* in response to environmental factors, relative humidity, and temperature during a two years period.

Mosquito behavior is one of the most important factors to help better understand vectorial capacity and the role it plays in disease transmission. Movements of *Ae. aegypti* into and out of the human structures are considered a significant factor in disease transmission and this subject remains poorly understood. Relatively little is known about the pattern of entrance and exit movements of *Ae. aegypti*. Knowledge of temporal host – seeking patterns of mosquitoes is important for understanding mosquito – host contact, as mosquitoes will only bite hosts that are available during the period of their host – seeking activity (Williams, 2005).

OBJECTIVES

The objective of the study was to observe the movement patterns of *Aedes aegypti* into and out of experimental huts in response to human host stimuli during the three different seasons.

LITERATURE REVIEW

1. *Aedes aegypti*

Aedes aegypti is a medium – sized blackish mosquito easily recognized by a silvery-white 'lyre-shaped" pattern of scales on its scutum. Segments 1 to 4 of the hind tarsi possess broad basal white rings, segment 5 is white (Figure 1). The coloration of both sexes is somewhat similar but females are generally larger than males. Males have plumose antennae, whereas females have sparse short hairs.

Aedes aegypti is widespread in tropical and subtropical areas of South – East Asia, and is common in most urban setting (WHO, 1999). *Aedes aegypti* is highly anthropophilic, although it will feed on other available warm blooded animals. Females seem to have two biting activity periods, one in the morning for several hours after day break and the other in the afternoon for several hours before dark (Lumsden, 1957; Sheppard *et al.*, 1969; Nelson *et al.*,1978; Lehane, 2005).

Aedes aegypti is called a holometabolous insect. This means that the insect goes through a complete metamorphosis by beginning with an egg, larvae, pupae, and adult stages. The adult life span can range from two weeks to a month, depending on surrounding environmental conditions. The life cycle of *Ae. aegypti* can be completed within one and a half to three weeks (Figure 2). After taking a complete blood meal, females produce on average of 100 to 150 eggs per batch. Eggs are laid on damp surfaces in areas likely to temporarily flood, such as tree holes and man – made containers, and are laid singly, rather than in a mass. Eggs of *Ae. aegypti* are long, smooth, ovoid shaped, and approximately one millimeter long. When first laid, eggs appear white but within minutes turn a shiny black. In warm climates, such as the tropics, eggs may develop in as little as two days. Larval *Ae. aegypti* consumes oxygen through a posteriorly located siphon, which is held above the water surface while the rest of the body hangs vertically. Larvae feed on the aquatic microbiota, such as algae and other microscopic organisms (Nelson, 1986). The total time for development through all 4 instars is dependent upon water temperature and nutritional

supply, and typically ranges from 4 to 10 days. After the fourth instar, *Ae. aegypti* goes to pupal stage. Pupae, is called "tumblers," and do not feed. It takes approximately two days to become adult.

Aedes aegypti is a domestic species found not far from human dwellings. This species is particularly abundant in urban setting. The *Ae. aegypti* females lay her eggs in various natural and artificial containers such as tree holes, rock holes, barrels, water pots and discarded automobile tyres located outdoors, some of the sites being sheltered, others exposed to rainfall (Haddow, 1945; Garnham *et al.*, 1946; Fox *et al.*, 1960; Trpis and Hausermann, 1975). The water in which females of domestic populations oviposit is usually clean and clear (Clemens, 1999).

Aedes aegypti has a cosmopolitan range extending from 40° N to 40° S latitude (Womack, 1993) (Figure 3) and altitude is considered to be one of the most important factors in limiting the distribution of *Ae. aegypti*. In India, *Ae. aegypti* was found at 1000 meters above sea level. Lower elevations (less than 500 meters) have moderate to heavy mosquito population densities (Kalra *et al.*, 1997) while mountainous areas (greater than 500 meters) have low population densities. In countries of South – East Asia, 1000 to 1500 meters appears to be the limit for *Ae. aegypti* distribution. In other regions of the world, it is found at even higher altitudes, i.e. up to 2200 meters (Christopher, 1960) in Columbia.

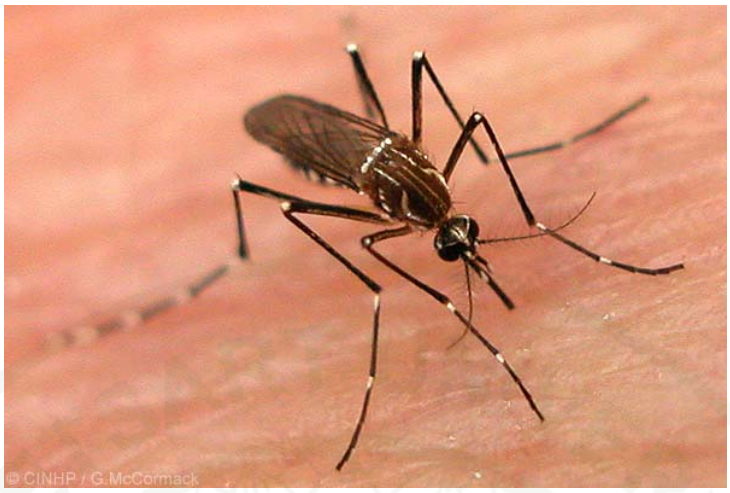

Figure 1 *Aedes aegypti* female.

Source: McCormack (2005)

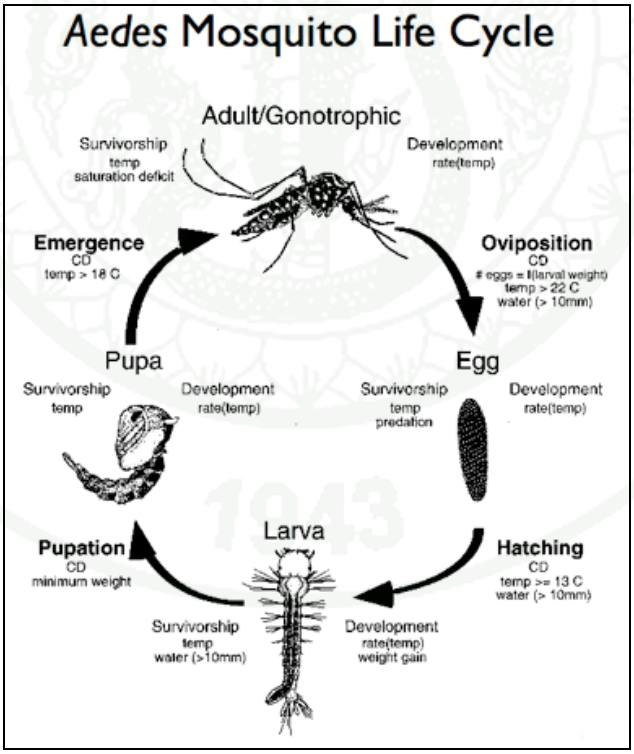

Figure 2 Life cycle of *Aedes aegypti*.

Source: Hopp and Foley (2001)

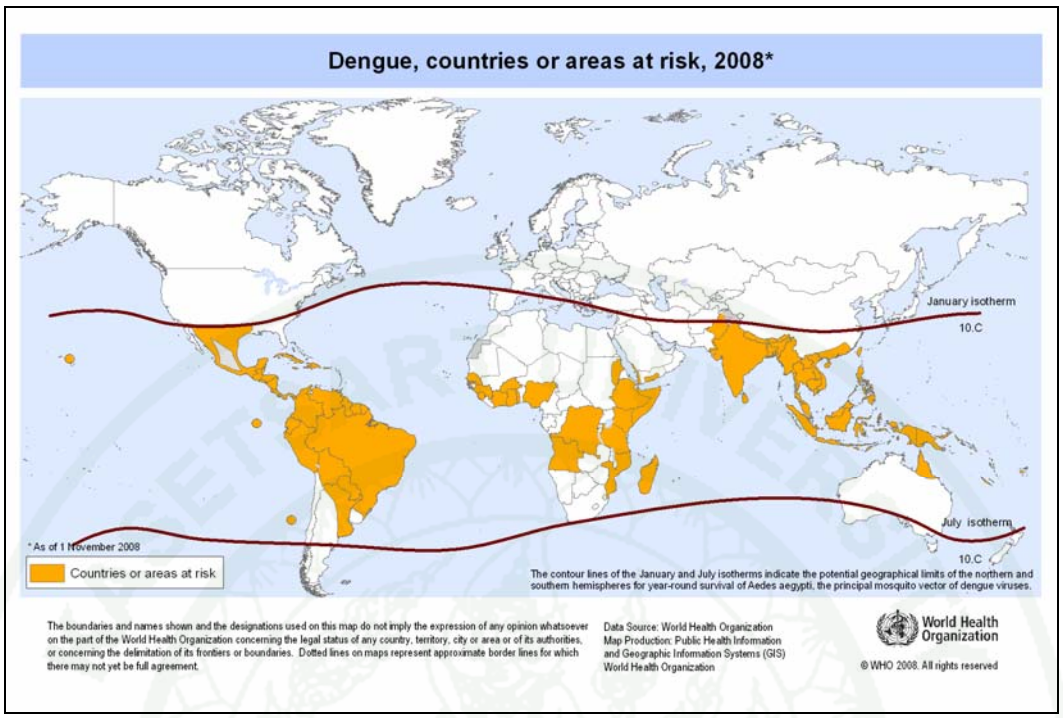

Figure 3 World distribution map with dengue transmission areas.

Source: World Health Organization, WHO (2008)

2. Dengue and dengue haemorrhagic fever

Dengue outbreaks have occurred over the last three centuries in tropical, subtropical and temperate areas of the world. The first epidemic of dengue was documented in 1635 (Howe, 1977) in the French West Indies, although a similar disease to dengue had been reported in China as early as 992 AD (Gubler and Kuno, 1997).

The first recorded outbreak of a dengue compatible with DHF occurred in Australia in 1897. A similar haemorrhagic disease was recorded in 1928 during an epidemic in Greece and again in Taiwan in 1931. The first confirmed epidemic of DHF was recorded in the Philippines in 1953-1954. Since then, major outbreaks of DHF with significant mortality have occurred in most countries of South – East Asia, including India, Indonesia, Maldives, Myanmar, Sri Lanka, and Thailand, as well as in Singapore, Cambodia, China, Laos, Malaysia, New Caledonia, Palau, Philippines, Tahiti and Vietnam in the Western Pacific Region. Over the past 20 years, there has been a dramatic increase in the incidence and geographical distribution of DHF, and epidemics now occur each year in several South – East Asia countries.

In Thailand, the first outbreak of dengue hemorrhagic fever (DHF) was recognized in 1958 with 2,500 cases in the Bangkok metropolitan area. Since then its incidence has increased periodically (Chareonsuk *et al.*, 1999). Several factors play into its complex epidemiology, including mosquito, virus, man, climate and environmental factors (WHO, 1997). *Aedes aegypti* (L) and *Aedes albopictus* (Skuse) are regarded as the primary and secondary vectors in Thailand. At present, the patterns of outbreaks appear different from the past five decades, and thus are more difficult to predict (Charoensuk *et al.*, 1999; Cummings *et al.*, 2004). There are many areas that have re-ported experiences of re-epidemics of DHF with a high incidence during the dry season (MoPH, 2005).

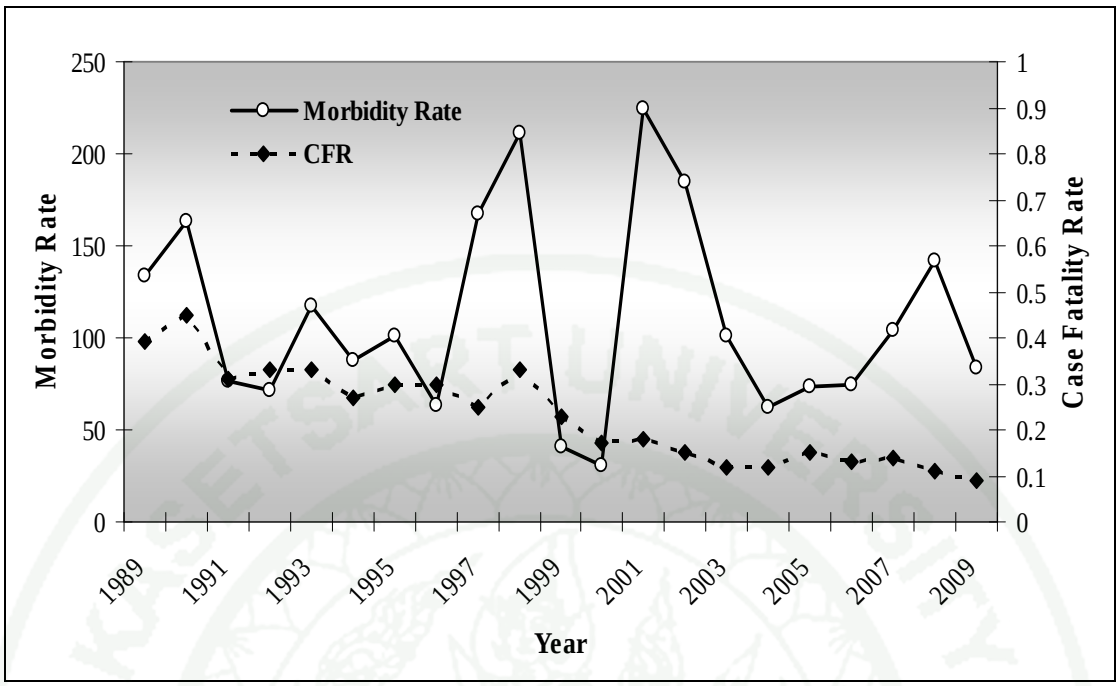

Figure 4 Dengue situation in Thailand, 1989 – 2009.

Source: Ministry of Public Health, MoPH (2010).

3. Host – seeking behavior of mosquitoes

Mosquitoes process a variety of senses to locate potential sources of blood. In general, host-location behavior is separated into 3 different phases, which include long – range, middle – range, and short – range orientations (Sutcliffe, 1986; Gibson and Tort, 1999). Long – range orientation usually involves the reception and evaluation of olfactory and visual cues (Takken, 1991). Many mosquitoes species fly upwind as they search for a host. Olfactory cues, especially host – produced carbon dioxide (CO_2) and secondary cues, such as octanol and lactic acid, are found in host – produced odor plumes that mosquitoes detect in the course of their searching flights. Odor receptors are located on the antennae and palps of all female mosquitoes. Recent evidence suggests that genes encode species – specific odor receptors and that the temporal sensitivity to selected host odors may be regulated by the gonotrophic condition of the female mosquito (Fox *et al.*, 2001). Host – derived odor plumes that are carried on light winds help guide mosquitoes toward the host. Once a mosquito enters an odor plume, it executes a series of 90 degree turns, in much the same way that a hunting dog searches for a hidden pheasant. The concentration of the olfactory cues diminishes on the edge of the plume and increases toward its center and its proximity to the host. As the mosquito approaches a host, vision may become important for host identification and recognition (Allan *et al.*, 1987). Mosquitoes are sensitive to contrast, motion, and color. Vision may act as both a middle – and a short – range cue. Olfaction may also serve as a middle – range cue when secondary attractants, such as lactic acid and octanol, become detectable within the odor plume in close proximity to the host (Kline *et al.*, 1991).

Short – range cues include vision, heat, sound, and olfaction and generally play a significant cue when the mosquito comes closely enough to touch the host (Khan *et al.*, 1966). Host temperature and tactile chemical cues on the host's skin, including odors associated with host – produced microflora, which help the mosquito to identify the host, initiate probing, and start blood feeding (De Jong and Knols, 1995).

Successful host location and selection by a mosquito may depend on the relative abundance of individual hosts. This is especially true for mosquitoes that exhibit opportunistic blood – feeding behavior. In general, abundant hosts will be fed on more frequently than rare hosts (Hess *et al.*, 1968). Sometimes, artificial manipulation of a host population will alter blood – feeding dynamics (Nasci, 1984). Eventually, the normal circadian activity patterns of a vertebrate host may determine the mosquito species that are most likely to blood feed on that host (Day and Edman, 1984).

4. The modification of mosquito behavior by climatic factors

All mosquitoes show periodicities of activity and inactivity that are correlated well with the climatic factors such as temperature and relative humidity (RH) (Clements, 1999). One example of climatic factors is the variation in the saturation vapour pressure of water with change of air temperature. Because a rising air temperature lowers RH, decreases in cloud cover lead not only to increases in temperature but also to increases in RH

Temperature and relative humidity affect insect behavioral periodicities as permissive factors. The effects of extreme weather conditions on activity levels are obvious, but under more equable conditions, it is not easy to establish the nature of relationships between ambient temperature or humidity and levels of activity. Statistically significant correlations obtained in the field may not reflect cause and effect. Diel fluctuations of physical factors will necessarily parallel some biological rhythms that are entrained to diel light cycles. Many of the early studies of the effects of physical factors on mosquito flight activity were reviewed by Clements (1963). Unfortunately, most laboratory experiments are of little value in interpreting behavior in the field, and early field studies were conducted in ignorance of the role of circadian rhythms and the effects of moonlight.

The climatic conditions to which insects are exposed differ substantially at different latitudes. Microclimate, by definition, differs between different habitats at

any latitude. In fact, within single habitats microclimatic conditions can vary greatly during the day, as was shown by investigations in three mosquito habitats in Western Province, Uganda, during June and September 1943. Instruments were exposed in Stevenson screens in dense rainforest, in banana plantation with moderately dense undergrowth, and on open ground. By day, the climate in the open was characterized by fairly high temperature, rather low relative humidity (RH), and high light intensity; in the banana plantation temperature was lower, RH distinctly higher, and light intensity lower; in forest, temperature was relatively low, RH very high, and light intensity much reduced. At night, in contrast, all three environments showed a similarity, with slowly falling temperatures and high and very stable RH At sunrise all conditions changed rapidly in the open, but in the banana plantation and forest both temperature and RH changed only after a lag of 2 hr, although light intensity immediately began to increase. Biting collection indicated that some species of mosquitoes that found only in forest, flew into the surrounding open country at night when surrounding conditions were more uniform (Haddow, 1945a). In Uganda, mosquitoes that formed swarms over open grassland and above the forest canopy at dusk and dawn were considered to have entered climatically marginal zone that were, beyond the boundaries of a similar environment (Corbet, 1964).

4.1 Temperature

Flight activity in many insect depends mainly upon body temperature. Their flight muscles cannot function well at low temperatures, and various methods are used to raise thoracic temperature. As would be expected, mosquito species adapted to colder climates are active at lower temperatures than those adapted to warmer climates. The flight activity of *Aedes nigripes* in an arctic area was inhibited when air temperatures was below 5°C (Corbet and Danks, 1973). In Greenland, the lower temperature threshold that initiated swarming by *Aedes impiger* and *Ae. nigripes* was 6°C (Nielsen and Nielsen, 1966). In Sweden, *Aedes punctor* were strongly active and responded to their vertebrate hosts at 4°C (Jaenson, 1988). In Wisconsin, mosquitoes fed on nectar at dusk at temperatures of 11°C. At localities where the temperature at sunrise was 2 – 8 °C, there was no nectar feeding as

occurred further south (Grimstad and DeFoliart, 1974). At a location in central Argentina, no females of *Aedes albifasciatus* were collected at human bait at temperatures below 6°C, and they were not captured in substantial numbers below 8°C (Ludueña Almeida and Gorla, 1995). In Florida, for most species, the lower temperature threshold for flight was 12 or 13°C (Bidlingmayer, 1974), while the swarming by *Psorophora confinnis* was 11°C (Provost, 1958). Even close to the equator a 'cold nights' usually yielded a smaller biting catch of *Anopheles bwambae* (Haddow and Ssenkubuge, 1973).

4.2 Relative humidity

The most commonly used measure of humidity is relative humidity (RH), which is the amount of water vapour in the air divided by the amount that would be present, if the air were completely saturated. Usually, RH is expressed as a percentage. Because the saturation vapour pressure of water increases sharply with increase in temperature, any change in temperature has a marked effect on RH Ambient RH tends to rise through the night due to the fall in temperature; around dawn RH usually is high and can approach 100% (Bidlingmayer, 1985; Braack *et al.*, 1994). In lowland rainforest, the air approaches saturation at all elevations during the night, but during the day, as the temperature rises, the RH falls. In the middle of the day the RH may be less than 60% in the upper canopy, where the temperature is highest, but close to the forest floor it remains above 90% throughout the day (Haddow, 1945b; Haddow *et al.*, 1947; Richards, 1996).

Laboratory experiments indicated that mosquitoes could detect temperature gradients of 0.05°C cm^{-1} or less, and humidity gradients of 0.05% RH cm^{-1}. By varying temperature and humidity, it was shown that mosquitoes respond to RH rather than saturation deficit (Muirhead Thomson, 1938; Platt *et al.*, 1957).

Circumstantial evidence from the field suggested that nectar-feeding activity was completely inhibited at 97% RH (Grimstad and DeFoliart, 1974). Provost (1974)

suggested that in regions where night time temperatures do not fall low enough to have an appreciable effect on flight activity. Under these conditions humidity may become a dominant regulating factor.

MATERIALS AND METHODS

Materials

1. Study Site

This study was conducted at Pu Teuy Village, Sai Yok District, Kanchanaburi Province (14° 20'N, 98° 59'E, 304 m above sea level), western Thailand, approximately 150 kilometers northwest of Bangkok. The site is located in a hilly area and is largely surrounded by primary dense forest. The study site belongs to the Armed Forces Development Command, Department of Royal Thai Armed Forces, Ministry of Defence. The nearest home is approximately 800 meters away from the field site where the experimental huts were located (Figure 5).

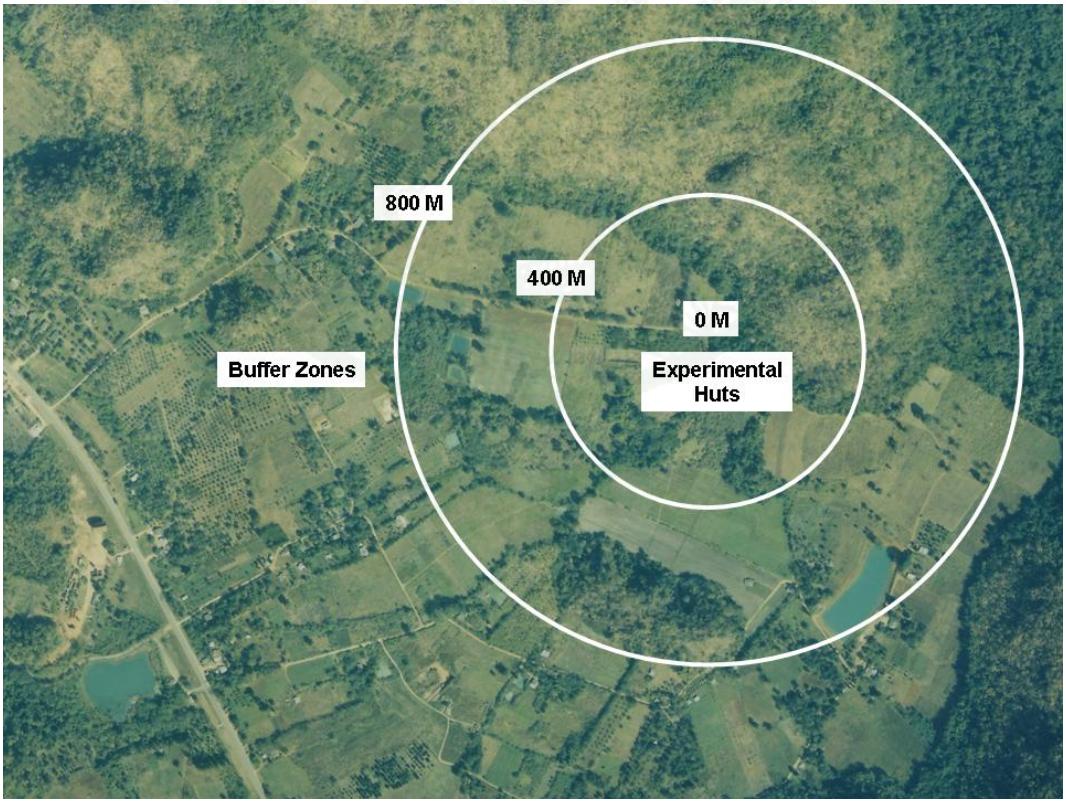

Figure 5 Map of Pu Teuy Village, Sai Yok District, Kanchanaburi Province, Western Thailand.

2. Mosquito Population

An initial population of *Ae. aegypti* was collected from immature stages from Pu Teuy Village, Sai Yok District, Kanchanaburi Province. Approximately 200 – 300 pupae and larvae were brought back to the insectary at the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. Wild population was introduced into a colony once every two months. Morphological identification was made by using the conventional key of Rattanarithikul *et al.* (1994). Subsequent colonization was performed at the same insectary, following the method of Kongmee *et al.* (2004).

3. Mosquito Rearing

All life stages of *Ae. aegypti* were maintained under insectary controlled-conditions. Larvae and adults were reared under a 12:12 hour light: dark photophase regime, a 25±5°C controlled temperature and a 80±10% controlled relative humidity. All larvae and pupae were reared in plastic trays measuring 22.5 cm wide x 32 cm long x 8.5 cm high. Each tray contained 150 – 200 larvae and pupae in approximately 2.5 liters of fresh water (Figure 6). All larvae were provided fish food. Upon emergence, all adults were maintained in 30 cm x 30 cm x 30 cm screened cages and provided cotton pads soaked with a 10% sucrose solution (Figure 7). Female mosquitoes were provided a guinea pig blood meal on the fourth day post – emergence. Two days post blood feeding, oviposition dishes with filter paper were placed in the cage with the gravid females for collecting eggs.

Figure 6 Larval and pupal were rearing tecnique in the insectary at the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok.

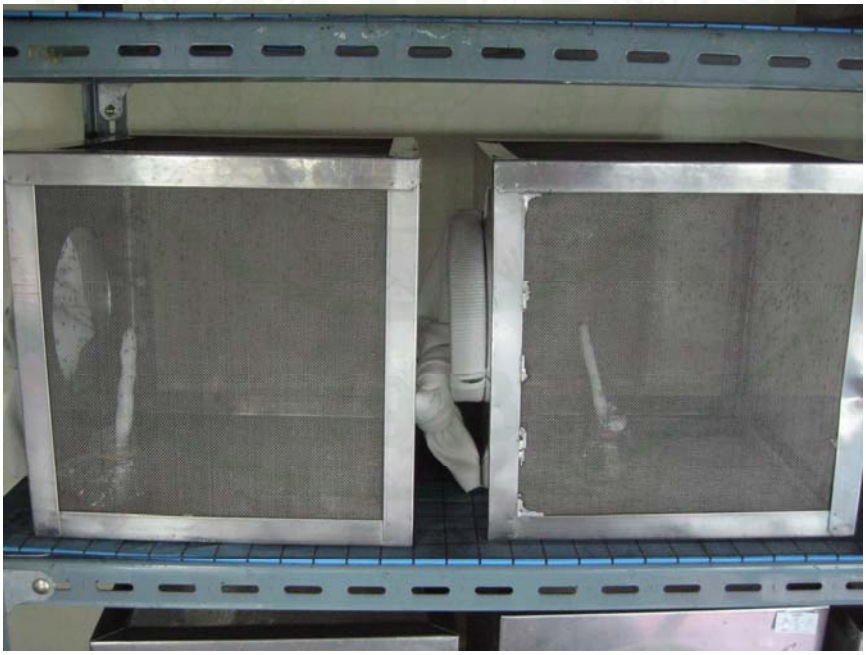

Figure 7 Adults screened cages with cotton pad soak with 10% sucrose solution in the insectary.

4. Experimental Huts

The experimental huts used in this study were previously described (Chareonviriyaphap *et al.*, 2005; Grieco *et al.*, 2007). Two identical huts were constructed in an isolated area (Figures 8 and 9). Each hut, measuring 4 m wide x 5 m long x 3.5 m high, and each hut had three windows and one door. The dimensions of the windows and door were 1.125 m x 1.175 m and 0.8 m x 2 m, respectively. Each portal was constructed in such a way as to allow them to be affixed with entrance and exit traps. Huts were constructed of similar material and in a similar fashion to the indigenous Thai homes. Huts were constructed from pieces of untreated plank wood of 1 m x 2.5 m in length and pieces of zinc roofing of 0.75 m x 3 m in size. Hut frames used to support the walls were made from galvanized iron pipe measuring 1 m x 2.5 m in length and was custom – welded to accommodate each wall. The apex of the angled roof measured 3.5 m from level ground. The huts consisted of three windows, one on each of three sides, and a northward – facing door which were all affixed with either entrance or exit traps.

The dimensions of the window traps were 0.84 m long, 1.065 m wide, and 1.065 m high and were constructed using an iron frame (Figure 10). Louvers made of 3/8-in non-treated plywood and fixed vertically at 60 degree angles were placed over the front opening of each of the three window traps, 1.065 m x 1.065 m, with a horizontal row of 10-cm wide slit openings made of 3/8-in non-treated plywood fixed vertically to 60 degrees. The louvers were placed in the open position producing a series of horizontal, 10-cm wide openings through which mosquitoes could enter. A door trap, measuring 1.2 m long x 0.845 m wide x 2.10 m high, was fixed to the door opening (Figure 11). Twenty plywood louvers identical to those used in the window traps were installed over the front opening and were again fixed at 60 degree angles to the vertical. These were arranged to facilitate the movement of mosquitoes from the hut into the trap. Both trap types were covered by nylon insect netting. Cotton sleeve material was sewn over several holes in both types of trap to facilitate the removal of mosquitoes.

Additional details pertaining to the experimental huts were given in Suwonkerd *et al.* (2006) and Grieco *et al.* (2007).

Figure 8 The experimental hut.

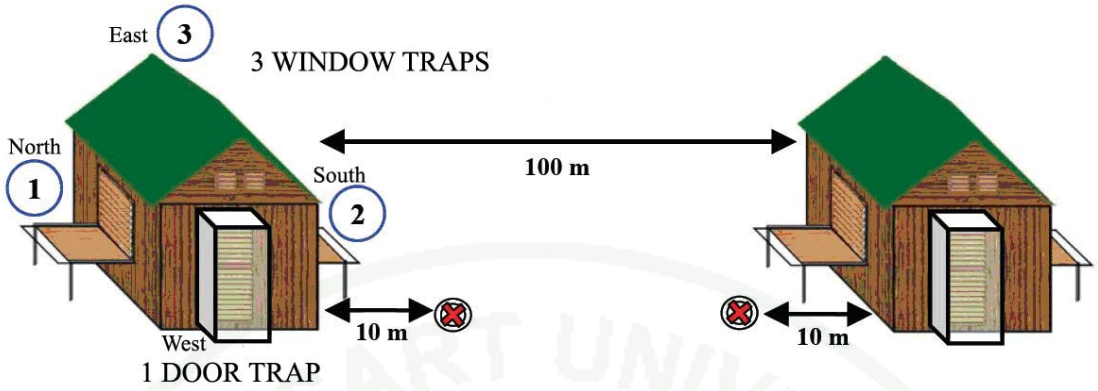

Figure 9 Two experimental huts positioning 100 meters apart.

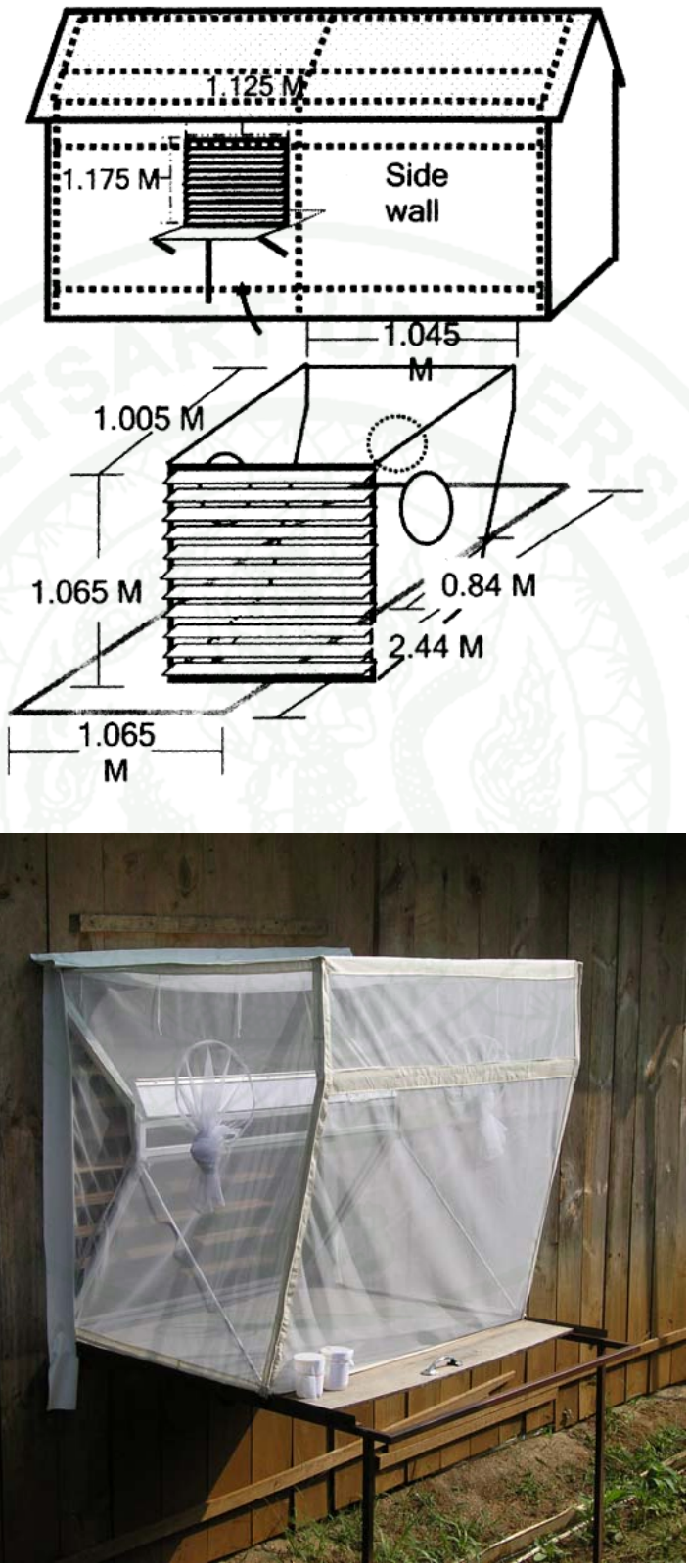

Figure 10 Removable window traps with a support platform.

Figure 11 Door trap fixed to the door opening.

Methods

1. Mosquito Marking and Releasing Technique

The F1 adult generation was used in this study. Two groups of 3 – 5 day old, nonbloodfed *Ae. aegypti* females were marked with fluorescence marking powder (BioQuip Products, Rancho Dominquez, CA) following the method of Achee *et al*. (2005). Both groups of 125 females each were used. This consisted of one population of 100 females that were used as a release population and 25 females that were used as controls. Marked mosquitoes were sugar starved for 24 hour, placed in a humidified chamber that was kept moist using water soaked towels, and were provided with water soaked cotton pads until the time of release.

For the entrance experiment, 100 marked mosquitoes were released 10 meters outside of each hut. For the exit collections, 100 marked mosquitoes were released inside of each hut. The released time was at 0500 hour, approximately 1 hour before the start of the collection.

Figure 12 Marking technique.

2. Recapture collections

All experiments were replicated two times in both huts and in each month. One human hosts were covered by mosquito nets to protect them from being bitten during the study. Entrance and exit traps were sampled every 20 minutes between 0600 – 1800 hour. The collections were made by 2 collectors per hut. Mosquitoes collected from the traps were placed into plastic cups, which were topped with mesh netting affixed with rubber bands. The location, collector and time of collection were labeled aside. Collectors were alternated between huts every 20 minutes to control for collectors bias. All mosquitoes from the traps were examined for fluorescent powder using a UV light and a stereomicroscope. The ambient temperature and relative humidity were recorded by the collector inside the hut every 20 minutes.

3. Data Analysis

Mean numbers of recaptured mosquitoes were analyzed by a one – way analysis of variance (ANOVA). Fisher's Least Significant Differences (LSD) was used to compare the difference in average number of recaptured *Ae. aegypti* in traps. Differences in number of mosquitoes recaptured from entrance and exit traps over four hourly intervals (0600 – 0900 hour, 0900 – 1200 hour, 1200 – 1500 hour, and 1500 – 1800 hour) were analyzed using regression analysis that included the following independent variables: ambient temperature, relative humidity and precipitation. Multiple regression was performed to investigate the association between the two types of movement behaviors (hut entry and exit) of *Ae. aegypti* and the environmental variables of temperature and relative humidity. All data were analyzed using the SAS program package (SAS Release 6.10, SAS Institute, Cary, NC). The discriminating level for all tests was set at 0.05%.

RESULTS AND DISCUSSION

Results

The average numbers of *Ae. aegypti* females recaptured from entrance and exit traps during a two – year period are given in Table 1. A comparison of the number of mosquitoes recaptured from entrance and exit traps during the same months between the first and second year was performed. Results reveal that the number of *Ae. aegypti* females recaptured from entrance traps in the month of April was the only statistical different sample period during the two – year collection (T = -6.602, P = 0.007) (Table 1). However, there was no significant difference in the total number of mosquitoes recaptured from entrance traps during the two – year period (T = 0.235, P = 0.818). For the exit regime, statistical differences in the average number of *Ae. aegypti* recaptured during the first two years of the study were found for the months of February (T = -13.118, P = 0.001), March (T = -20.000, P = 0.000), June (T = 4.078, P = 0.027), July (T = 3.211, P = 0.049), and August (T = 4.422, P = 0.021). No statistical difference in the total number of exit specimens recaptured between the first and second years was found (T = 0.196, P = 0.848).

Table 1 Average numbers of *Aedes aegypti* recaptured in traps during a two year period.

Month	Average number of *Ae. aegypti* recaptured from entrance traps		Average number of *Ae. aegypti* recaptured from exit traps	
	Year 1	Year 2	Year 1	Year 2
January	8.25 ± 2.87	27.25 ± 10.87	70.50 ± 9.47	44.25 ± 10.31
February	21.00 ± 2.94	16.25 ± 8.30	30.50 ± 3.79*	65.00 ± 3.83*
March	36.25 ± 18.26	19.25 ± 3.86	18.75 ± 4.03*	58.75 ± 7.41*
April	3.75 ± 2.06*	39.25 ± 9.95*	72.75 ± 7.27	71.75 ± 15.41
May	35.00 ± 13.29	29.25 ± 2.87	47.50 ± 8.19	55.50 ± 10.28
June	45.00 ± 5.35	29.25 ± 5.85	67.50 ± 7.14*	37.75 ± 12.92*
July	18.75 ± 12.87	24.75 ± 9.43	53.50 ± 14.64*	35.75 ± 12.82*
August	32.50 ± 14.20	24.25 ± 9.32	69.25 ± 16.15*	25.25 ± 7.93*
September	26.75 ± 7.04	22.50 ± 7.94	48.00 ± 26.34	54.50 ± 6.61
October	15.00 ± 7.02	26.50 ± 12.87	69.75 ± 4.99	66.75 ± 14.64
November	41.75 ± 24.35	11.50 ± 0.58	53.00 ± 11.14	56.50 ± 5.54
December	22.00 ± 7.30	21.75 ± 9.88	40.50 ± 25.16	53.00 ± 6.93
Total	306.00 ± 13.00	291.75 ± 7.07	641.5 ± 17.42	624.75 ± 13.83

* Statistical differences in average number of *Ae. aegypti* recaptured in traps between year 1 and 2 of the study ($P < 0.05$).

The average number of *Ae. aegypti* recaptured in traps tabulated by time of collection and season are shown in Table 2. The average temperature and relative humidity for the three seasons are provided in Table 3. Overall, the highest proportion of *Ae. aegypti* females were recaptured from both entrance and exit traps (29.63% from entrance and 53.78% from exit traps) during the summer months. For entrance experiment, the highest total number *of Ae. aegypti* were recaptured in the summer (29.63%) whereas the lowest number was recorded during the winter (21.22%). There was no significant difference in the number of *Ae. aegypti* recaptured during a single days collections conducted during the winter and rainy seasons ($P > 0.05$). Significantly higher numbers of *Ae. aegypti* females were recaptured in traps during the summer as compared to the other seasons ($P = 0.003$ and 0.036, respectively). During the summer, a higher proportion of mosquitoes was recaptured between 0600 – 0900 hour compared to any other time period during the day ($P = 0.000$).

For the exit experiment, the total number of *Ae. aegypti* recaptured from the traps during all three seasons was quite similar, ranging from 51.66% in the winter to 53.78% in the summer (Table 2). In general, higher numbers of *Ae. aegypti* females were recaptured during the late morning (0900 – 1200 hour), with the exception of winter when a higher proportion of specimens was recaptured between 1200 – 1500 hour (16.09%). Specifically, the proportion of *Ae. aegypti* recaptured in exit traps from 0900 – 1200 hour and 1200 – 1500 hour was significantly different from those of the other two periods (0600 – 0900 hour and 1500 – 1800 hour) ($P < 0.05$) (Table 2).

Table 2 Average number of *Aedes aegypti* recaptured in traps during a 12 hour sampling period in all seasons.

Experiment	Times				Total
	0600 – 0900	**0900 – 1200**	**1200 – 1500**	**1500 – 1800**	
Entrance					
Winter	10.53 ± 7.81	8.06 ± 5.93	1.56 ± 1.24	1.06 ± 0.53	21.22 ± 10.32
Summer	24.19 ± 11.44	3.44 ± 1.95	1.34 ± 1.08	0.66 ± 0.60	29.63 ± 12.98*
Rain	15.69 ± 5.19	5.00 ± 2.62	1.84 ± 0.79	1.34 ± 0.78	23.88 ± 5.31
Exit					
Winter	4.84 ± 3.03	15.84 ± 10.05	16.09 ± 6.18	14.88 ± 5.92	51.66 ± 13.02
Summer	11.78 ± 8.90	24.47 ± 11.45	10.28 ± 5.41	7.25 ± 2.33	53.78 ± 18.60
Rain	7.06 ± 6.41	20.13 ± 8.44	18.59 ± 5.43	7.06 ± 2.98	52.84 ± 16.16

* Statistical differences in average number of *Ae. aegypti* recaptured in traps ($P < 0.05$).

Table 3 The average temperature and relative humidity by period during a 12 hour sampling period in all seasons.

Factors	Seasons		
	Winter	**Summer**	**Rain**
Inside Temperature (°C)			
0600 – 0900	21.17 ± 2.84	24.88 ± 1.65	24.74 ± 0.98
0900 – 1200	26.41 ± 2.35	28.37 ± 1.59	27.21 ± 1.10
1200 – 1500	29.77 ± 2.15	29.92 ± 2.65	28.62 ± 1.23
1500 – 1800	28.86 ± 2.64	28.93 ± 2.99	27.35 ± 1.40
Min	12.83	19.00	22.33
Max	34.50	36.67	31.83
Outside Temperature (°C)			
0600 – 0900	20.05 ± 3.43	24.78 ± 2.38	24.99 ± 1.86
0900 – 1200	29.75 ± 3.19	31.61 ± 2.62	30.00 ± 1.97
1200 – 1500	34.18 ± 3.01	32.72 ± 4.12	31.37 ± 2.23
1500 – 1800	30.13 ± 3.80	29.83 ± 4.56	27.69 ± 2.54
Min	12.00	18.00	20.67
Max	41.00	43.00	35.67

Table 3 (Continued)

Factors	Seasons		
	Winter	**Summer**	**Rain**
Relative Humidity (%)			
0600 – 0900	79.67 ± 5.28	80.59 ± 5.59	79.09 ± 3.79
0900 – 1200	59.16 ± 9.15	66.84 ± 9.47	67.20 ± 6.37
1200 – 1500	47.23 ± 9.20	62.50 ± 13.47	62.87 ± 6.77
1500 – 1800	54.01 ± 10.25	67.29 ± 13.91	68.64 ± 7.23
Min	28.33	28.00	40.00
Max	89.00	96.00	88.67

Time trends for entering and exiting of *Ae. aegypti* females were also recorded in the three different seasons (Figures 13 and 14). For the entrance experiment, the majority of entering behavior was seen during the morning period (0700 – 1100 hour) with a peak at 0700 hour in summer, at 0900 hour in the winter and a prolonged peak from 0700 to 0900 hour in the rainy seasons. A very distinct peak was seen in the summer as compared to what was observed in either the winter or rainy seasons. Very few mosquitoes tended to enter the hut during the afternoon period, regardless of season, with fewer than 1 mosquito entering at each hour (Figure 13). During exit collections, a very distinct exiting period was observed in the winter (1200 – 1700 hour) with a peak in activity occurring at 1600 hour. The duration of exiting was considerably longer during the summer and rainy season (0800 – 1600 hour), with a peak at 1100 hour in the summer and 1400 hour in the rainy season (Figure 14).

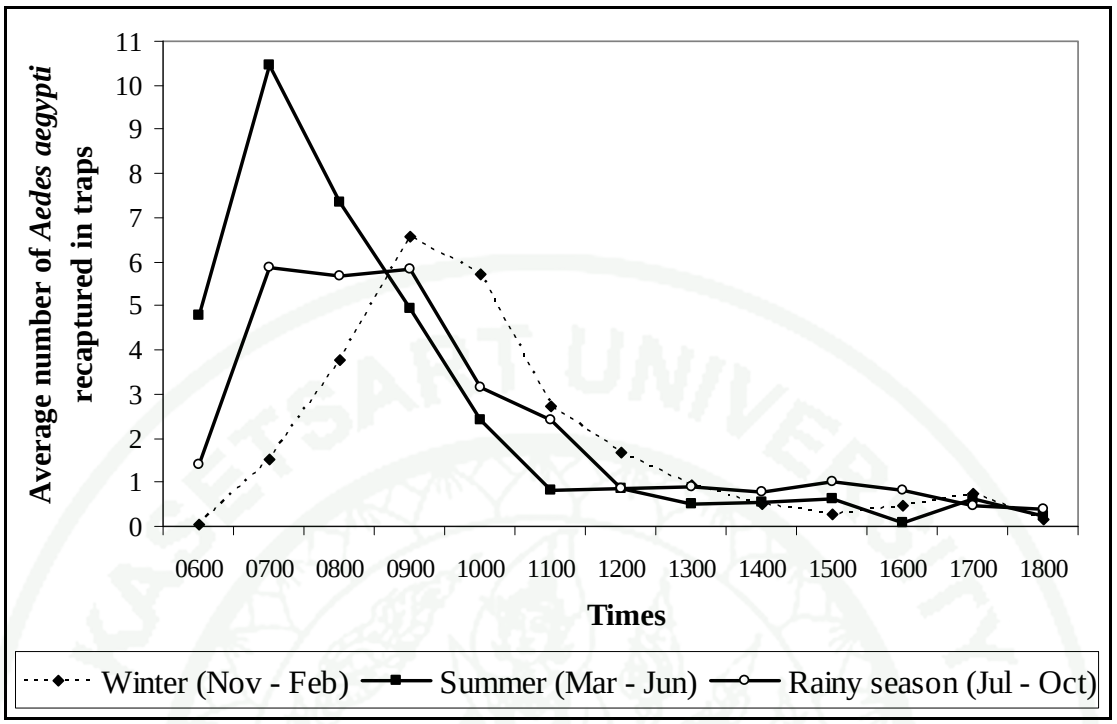

Figure 13 Time of entry of *Aedes aegypti* into traps during a 12 hour sampling period.

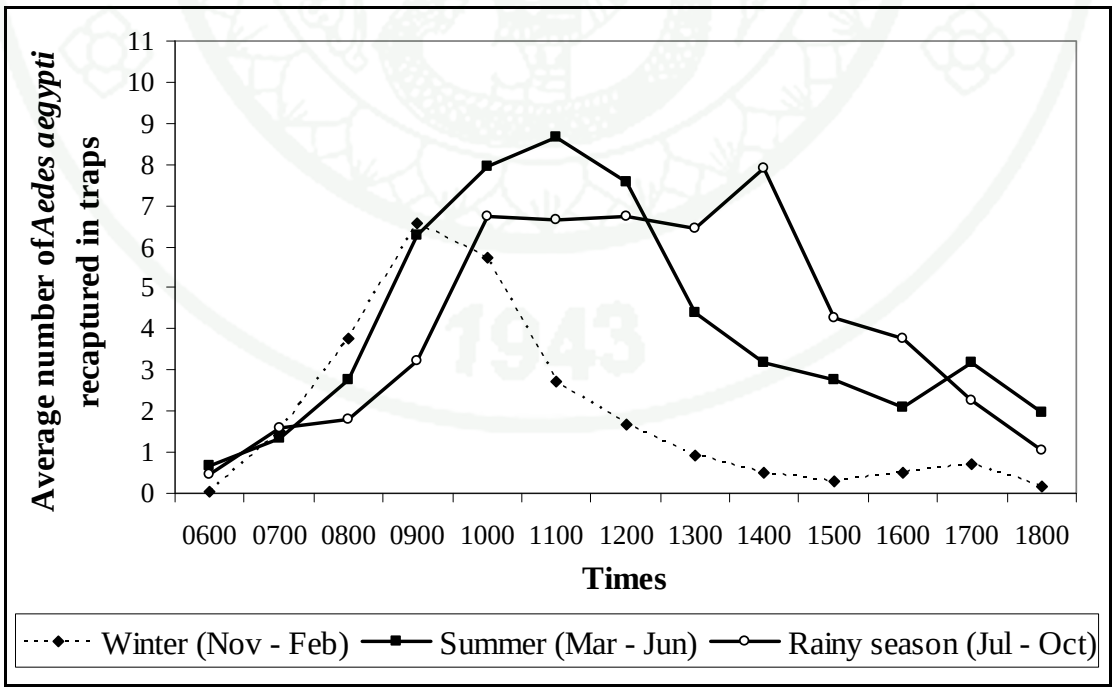

Figure 14 Time of exit of *Aedes aegypti* into traps during a 12 hour sampling period.

The average number of *Ae. aegypti* recaptured in each trap tabulated by time of collection are shown in Table 4. Overall, the highest proportions of *Ae. aegypti* females were recaptured in door trap from both entrance and exit experiments (9.63% from entrance experiment and 21.01% from exit experiment). For entrance experiments, there was statistical difference in total number of recaptured *Ae. aegypti* female in each traps (F = 12.415, *P* = 0.000). The highest total number of *Ae. aegypti* were recaptured in door trap (9.63%) whereas the lowest number were recaptured in window trap 1 (3.42%). There was no significant difference in the number of *Ae. aegypti* recaptured during a single days collections in window trap 1 and 3 (*P* > 0.05). Significantly higher numbers of *Ae. aegypti* females were recaptured in door traps as compared to the other traps (*P* = 0.000, 0.006 and 0.000, respectively). From window trap 1, 3 and the door trap, the highest number of *Ae. aegypti* were recaptured between 0600 – 0900 hour compared to any other time period during the day (*P* < 0.05) (Figure 15).

For exit experiments, there was statistical difference in total number of recaptured *Ae. aegypti* female in each traps (F = 22.561, *P* = 0.000). The highest total number of *Ae. aegypti* were recaptured in the door trap (21.01%) whereas the lowest number were recaptured in the window trap 3 (6.37%). Significantly higher numbers of *Ae. aegypti* females were recaptured in the door trap as compared to the other traps (*P* = 0.000, 0.000 and 0.000, respectively). From window trap 2 and the door trap, the highest number of *Ae. aegypti* were recaptured between 0900 – 1200 hour compared to any other time period during the day (*P* < 0.05). From window trap 3, the lowest number of *Ae. aegypti* were recaptured between 0600 – 0900 hour compared to any other time period during the day (*P* = 0.000, 0.001 and 0.003, respectively) (Figure 16).

Table 4 Average number of *Aedes aegypti* recaptured in each trap during a 12 hour sampling period in all seasons.

Experiment	Times				Total
	0600 – 0900	0900 – 1200	1200 – 1500	1500 – 1800	
Entrance					
Window trap 1 (South)	2.34 ± 0.37	0.75 ± 0.18	0.19 ± 0.17	0.14 ± 0.07	3.42 ± 0.58
Window trap 2 (East)	2.85 ± 0.98	2.42 ± 0.41	0.94 ± 0.14	0.89 ± 0.37	7.09 ± 0.74
Window trap 3 (North)	2.98 ± 1.21	1.39 ± 0.48	0.29 ± 0.08	0.29 ± 0.09	4.95 ± 0.60
Door trap (West)	7.79 ± 2.05	1.41 ± 1.00	0.24 ± 0.06	0.19 ± 0.05	9.63 ± 1.01*
Exit					
Window trap 1 (South)	1.39 ± 0.35	3.61 ± 1.04	1.59 ± 0.67	1.23 ± 0.13	12.12 ± 2.31
Window trap 2 (East)	1.21 ± 0.39	4.22 ± 0.26	5.43 ± 0.95	3.91 ± 1.72	14.72 ± 1.37
Window trap 3 (North)	0.50 ± 0.15	3.34 ± 0.89	2.55 ± 0.79	2.39 ± 1.14	6.37 ± 0.67
Door trap (West)	3.34 ± 1.06	9.60 ± 1.13	5.14 ± 0.52	2.97 ± 0.04	21.01 ± 1.81*

* Statistical differences in average number of *Ae. aegypti* recaptured in traps ($P < 0.05$).

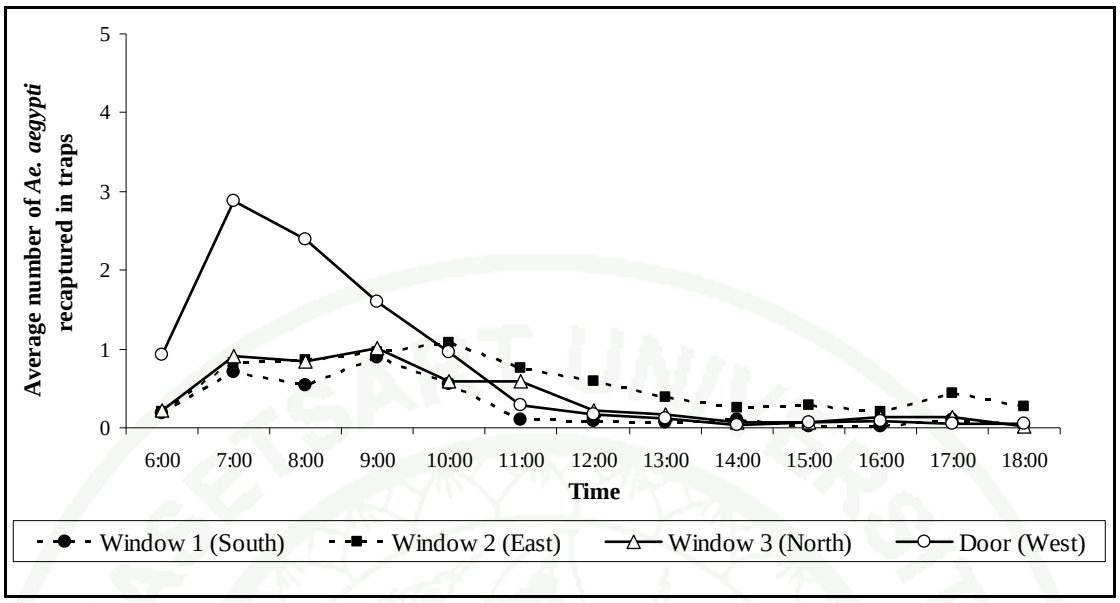

Figure 15 Average number of *Aedes aegypti* collected in each entrance trap during a 12 hour sampling period.

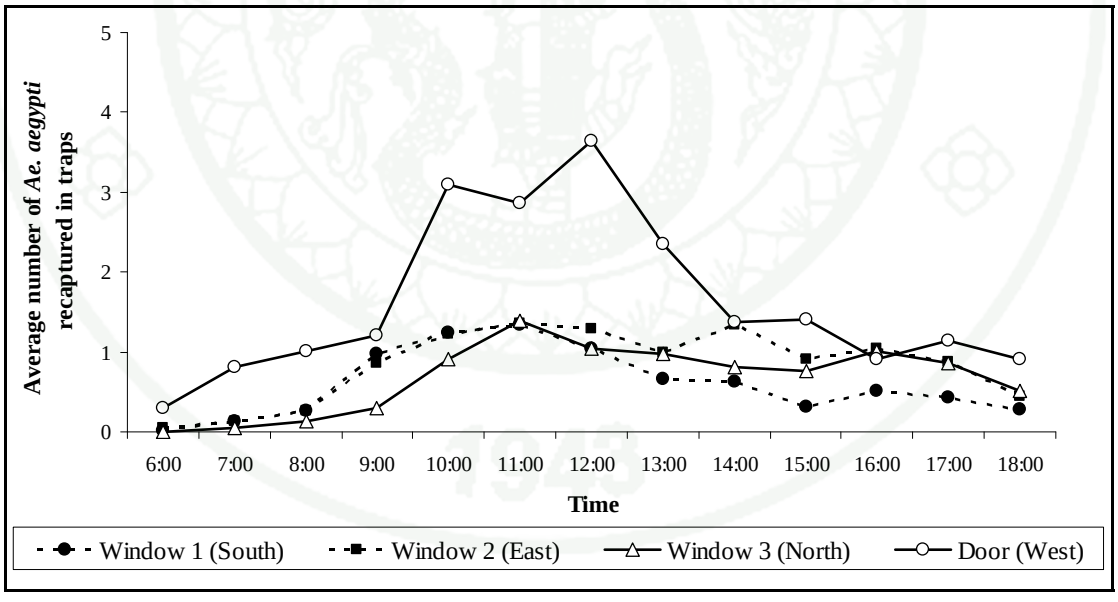

Figure 16 Average number of *Aedes aegypti* collected in each exit trap during a 12 hour sampling period.

Entrance and exit behaviors of *Ae. aegypti* into and out of experimental huts were evaluated in relation to ambient temperature and relative humidity using regression analysis. Entrance movement of *Ae. aegypti* was found negatively associated with temperature but positively correlated with relative humidity (Figures 17 – 25). The average number of *Ae. aegypti* recaptured in traps in response to the ambient temperature and relative humidity are shown in Tables 2 and 4, respectively. The results of the multiple regression analysis on the number of *Ae. aegypti* recaptured in both traps in relation to temperature and relative humidity indicate that these two variables strongly influence the entrance and exit patterns of this mosquito species ($r^2 = 0.585$; $F = 25.391$; $P = 0.000$).

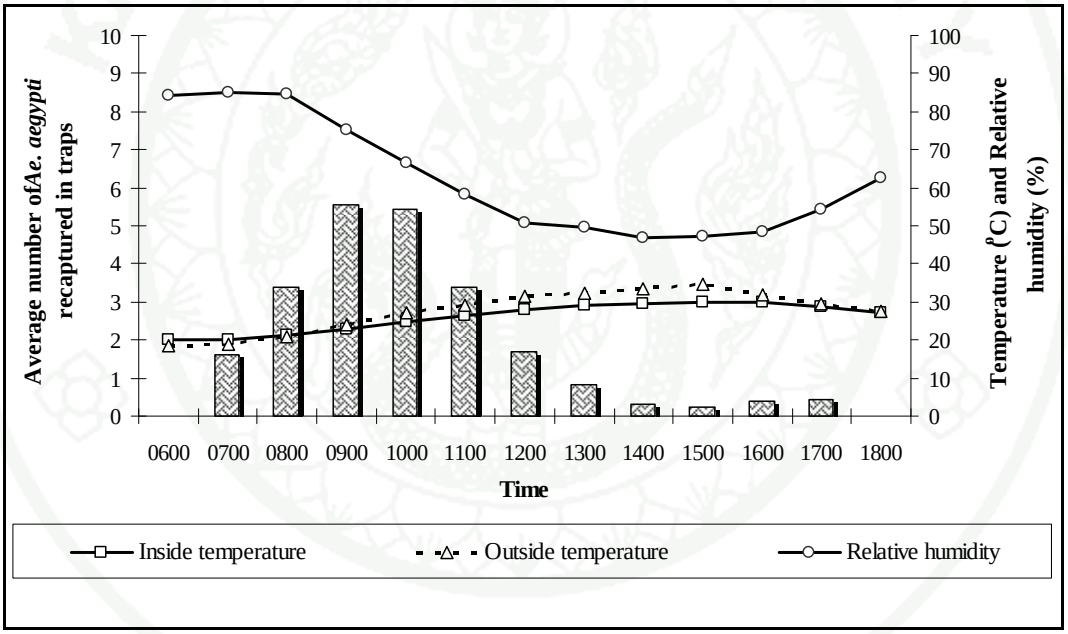

Figure 17 Average number of *Aedes aegypti* collected in entrance traps compared to temperature and relative humidity in winter (November – February) during the first year.

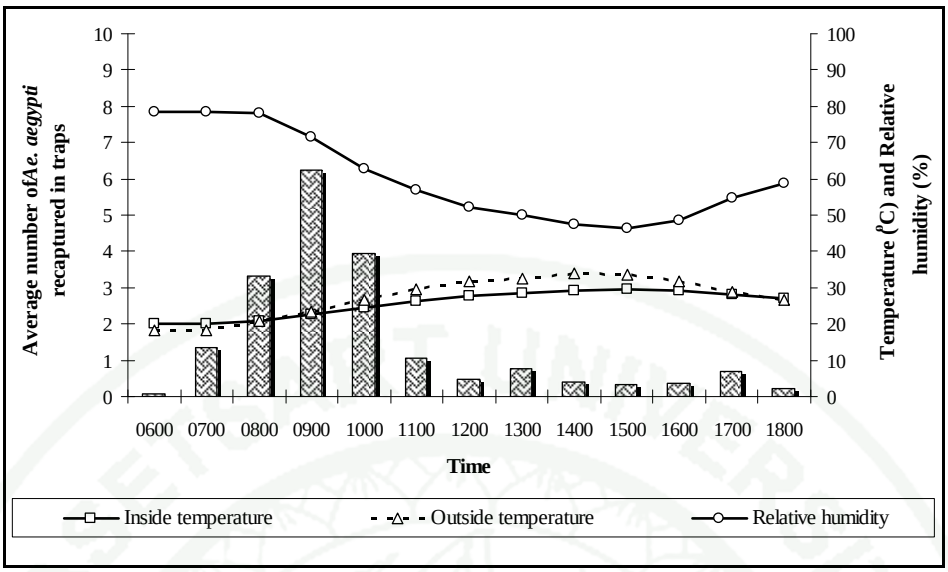

Figure 18 Average number of *Aedes aegypti* collected in entrance traps compared to temperature and relative humidity in winter (November – February) during the second year.

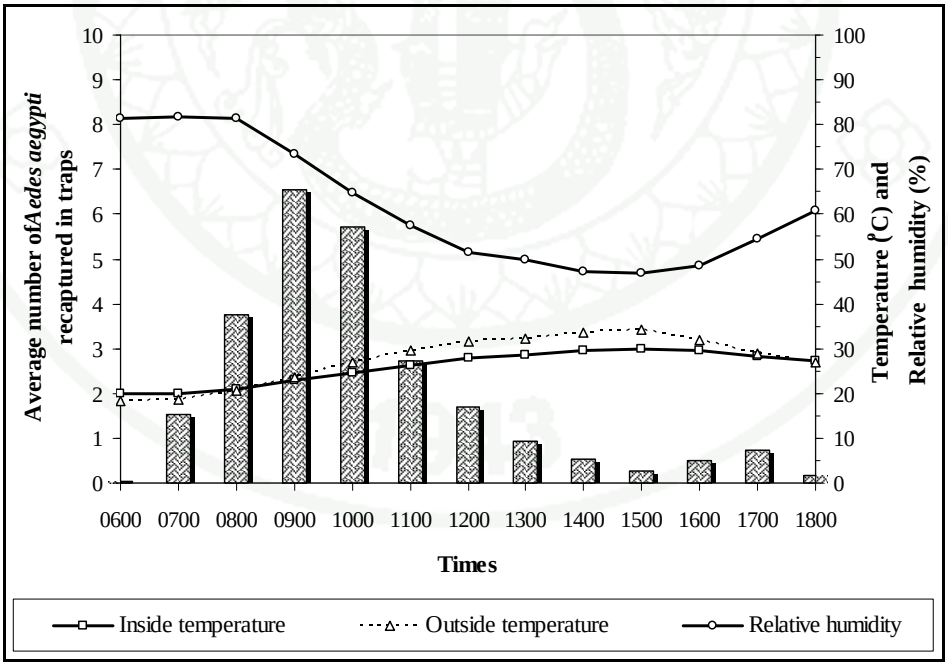

Figure 19 Average number of *Aedes aegypti* collected in entrance traps compared to temperature and relative humidity in winter (November – February) during the two year period.

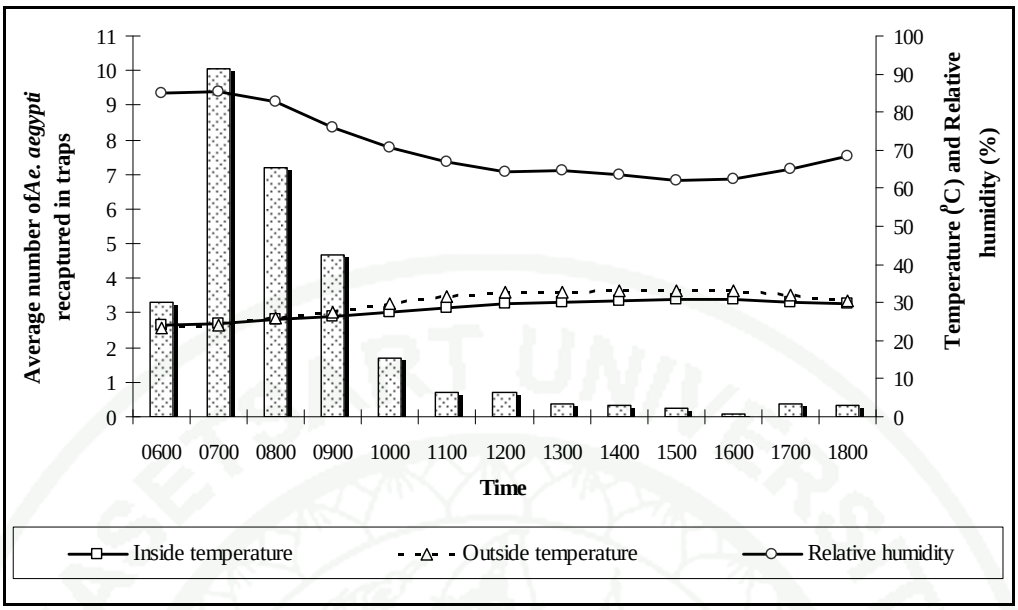

Figure 20 Average number of *Aedes aegypti* collected in entrance traps compared to temperature and relative humidity in summer (March – June) during the first year period.

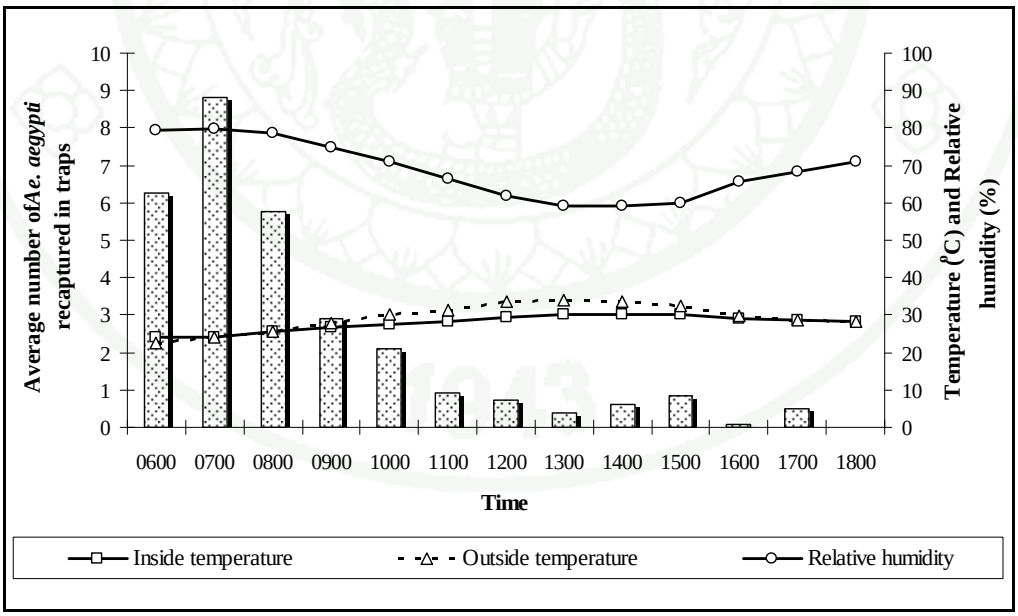

Figure 21 Average number of *Aedes aegypti* collected in entrance traps compared to temperature and relative humidity in summer (March – June) during the second year period.

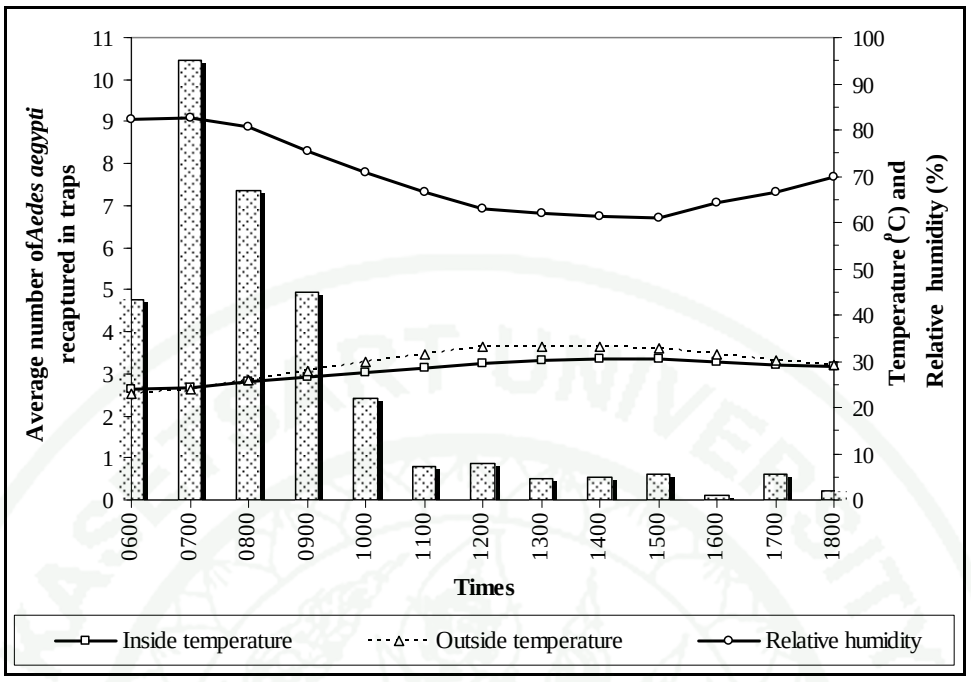

Figure 22 Average number of *Aedes aegypti* collected in entrance traps compared to temperature and relative humidity in summer (March – June) during the two year period.

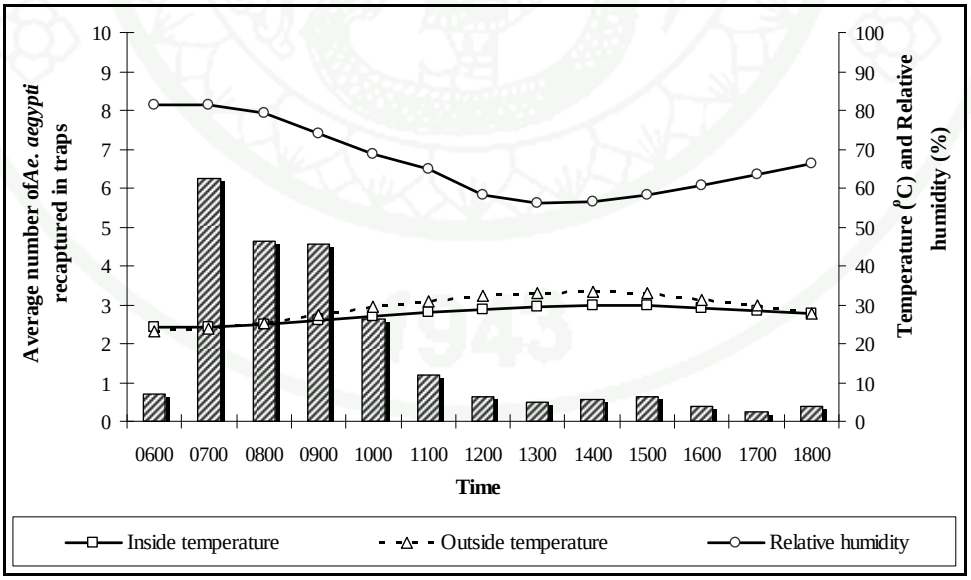

Figure 23 Average number of *Aedes aegypti* collected in entrance traps compared to temperature and relative humidity in rainy season (July – October) during the first year period.

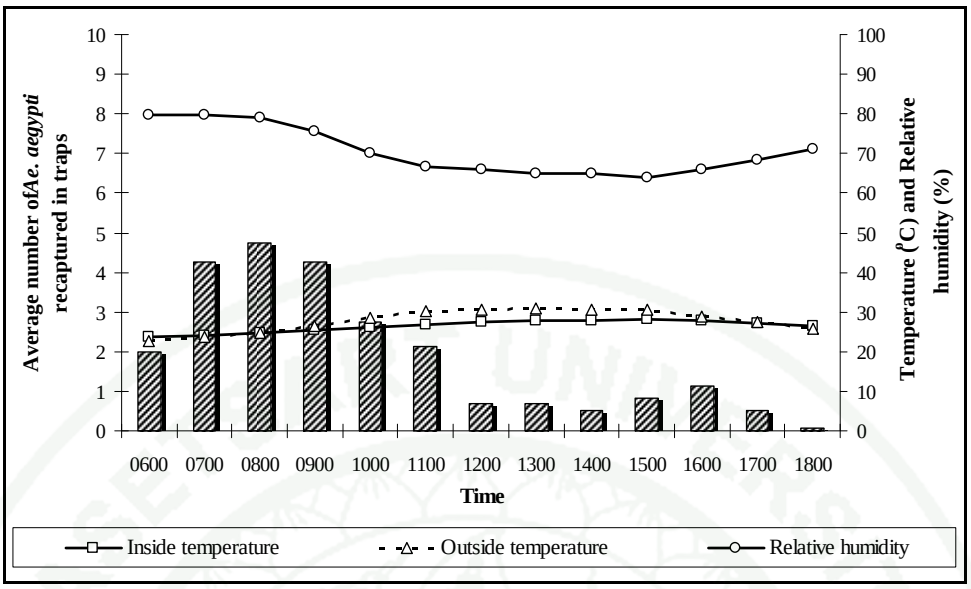

Figure 24 Average number of *Aedes aegypti* collected in entrance traps compared to temperature and relative humidity in rainy season (July – October) during the second year period.

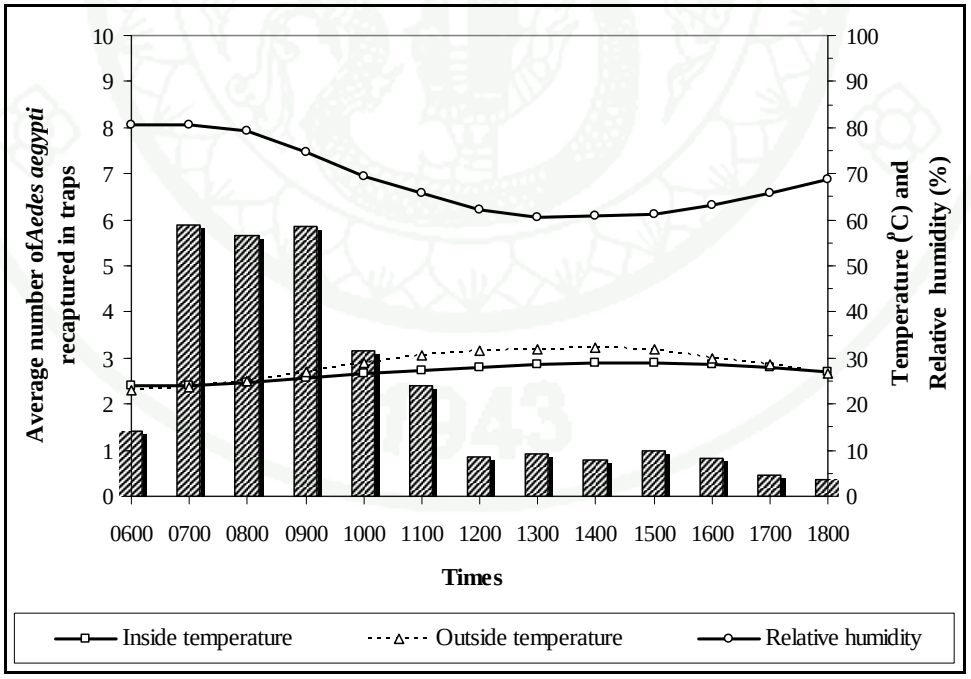

Figure 25 Average number of *Aedes aegypti* collected in entrance traps compared to temperature and relative humidity in rainy season (July – October) during the two year period.

The exiting patterns of *Ae. aegypti* from the experimental hut were found positively associated with temperature but negatively correlated with relative humidity (Figures 26 – 34). Results of the multiple regression analysis on the number of *Ae. aegypti* recaptured in traps suggested that these two environmental factors, have a strong impact on the movement patterns of *Ae. aegypti* females into and out of the hut ($r^2 = 0.335$; $F = 9.077$; $P = 0.000$).

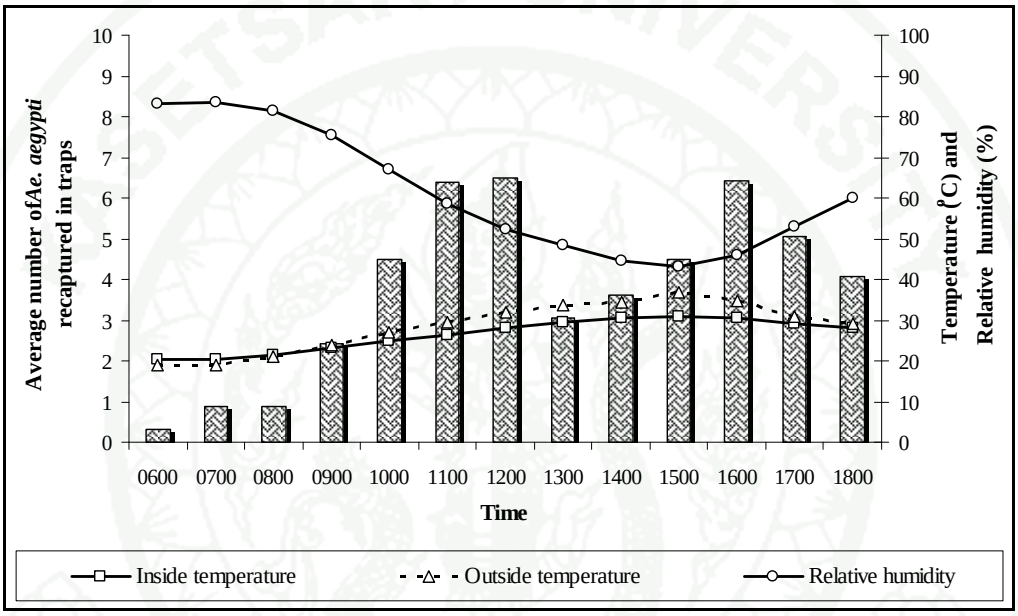

Figure 26 Average number of *Aedes aegypti* collected from exit traps compared to temperature and relative humidity in winter (November – February) during the first year period.

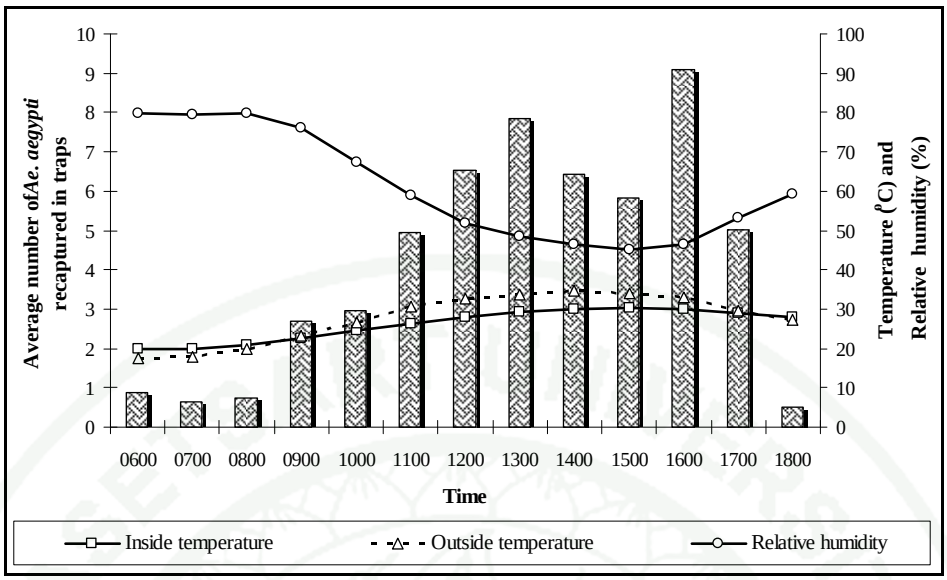

Figure 27 Average number of *Aedes aegypti* collected from exit traps compared to temperature and relative humidity in winter (November – February) during the second year period.

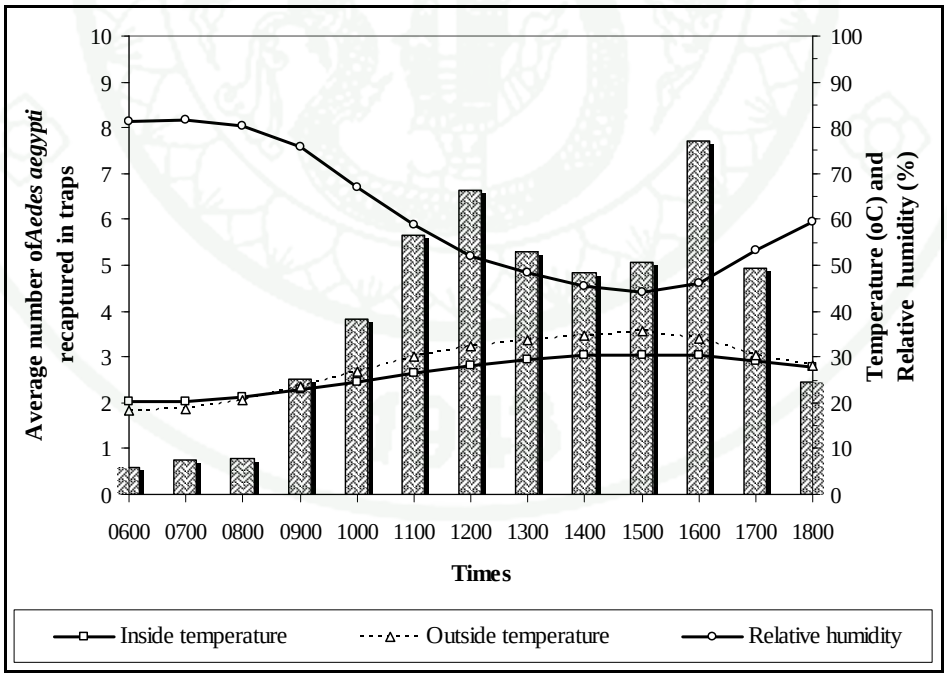

Figure 28 Average number of *Aedes aegypti* collected from exit traps compared to temperature and relative humidity in winter (November – February) during the two year period.

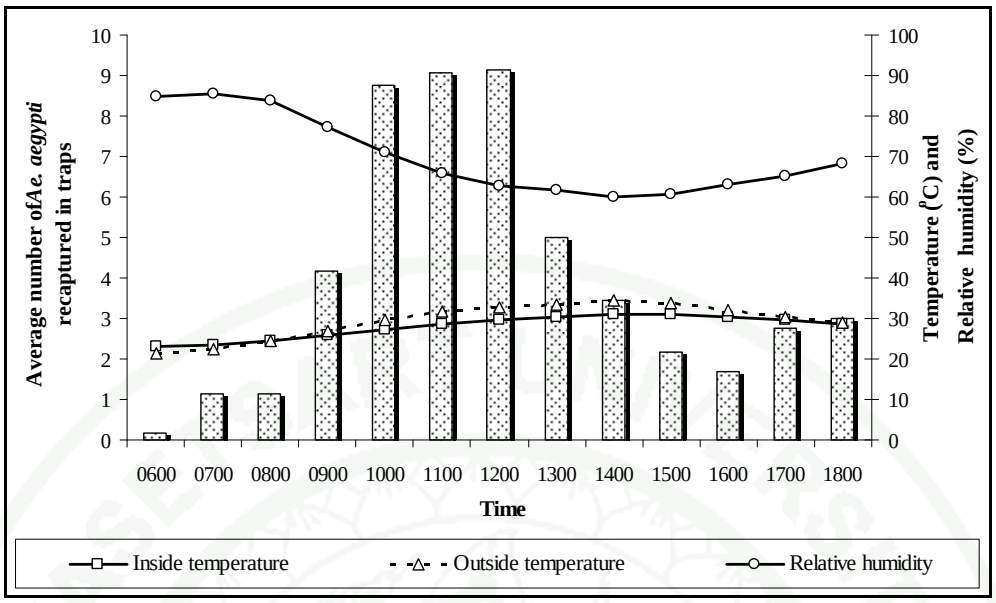

Figure 29 Average number of *Aedes aegypti* collected from exit traps compared to temperature and relative humidity in summer (March – June) during the first year period.

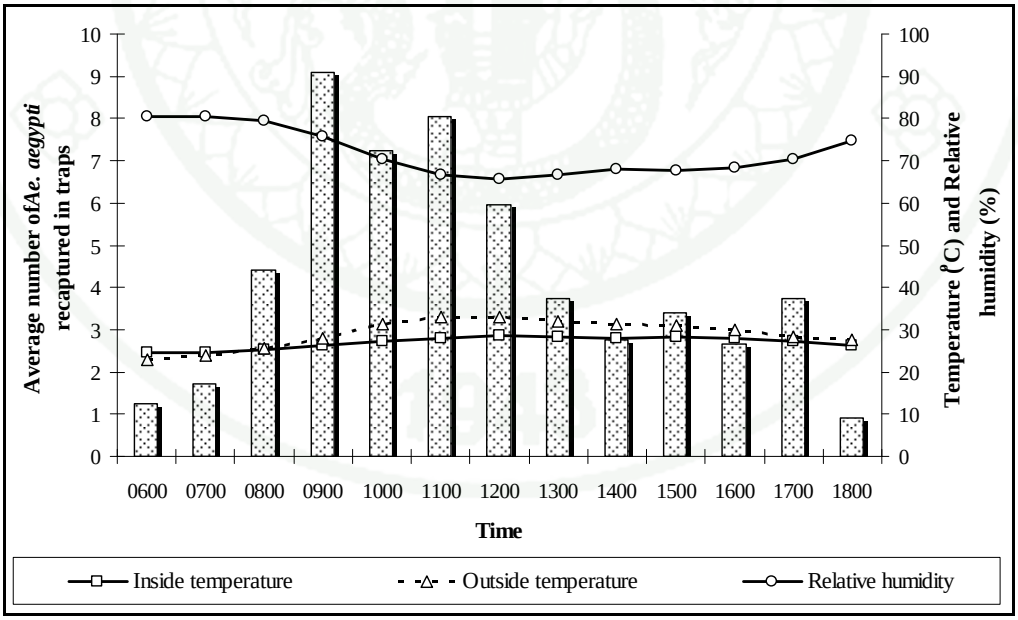

Figure 30 Average number of *Aedes aegypti* collected from exit traps compared to temperature and relative humidity in summer (March – June) during the second year period.

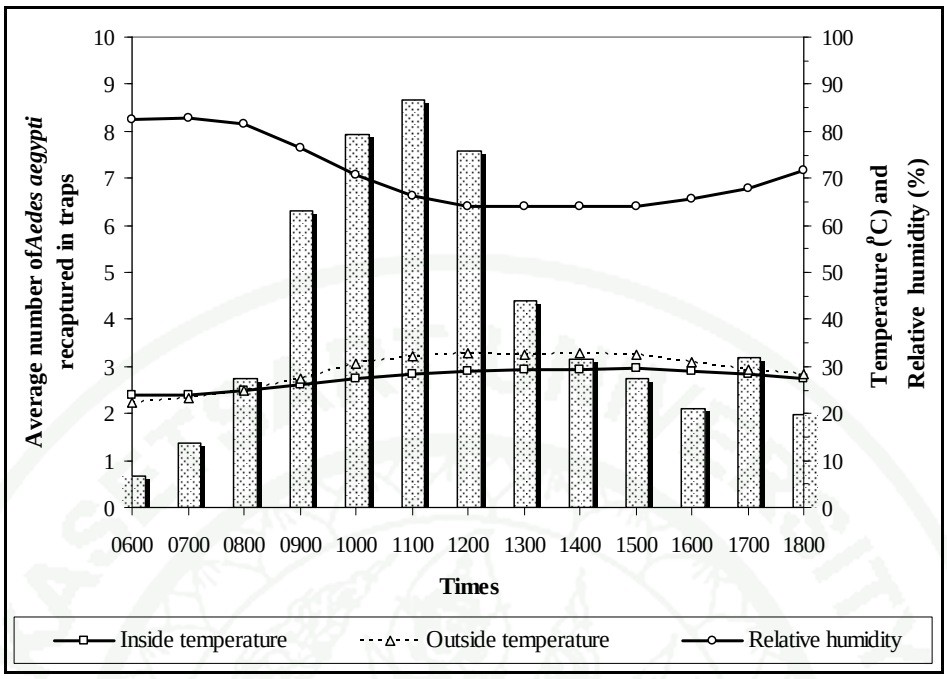

Figure 31 Average number of *Aedes aegypti* collected from exit traps compared to temperature and relative humidity in summer (March – June) during the two year period.

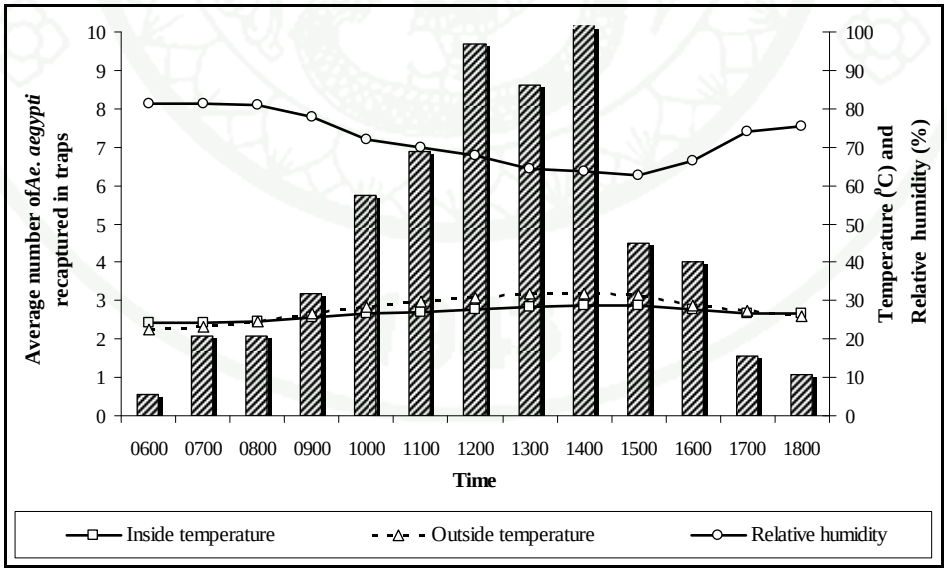

Figure 32 Average number of *Aedes aegypti* collected from exit traps compared to temperature and relative humidity in rainy season (July – October) during the first year period.

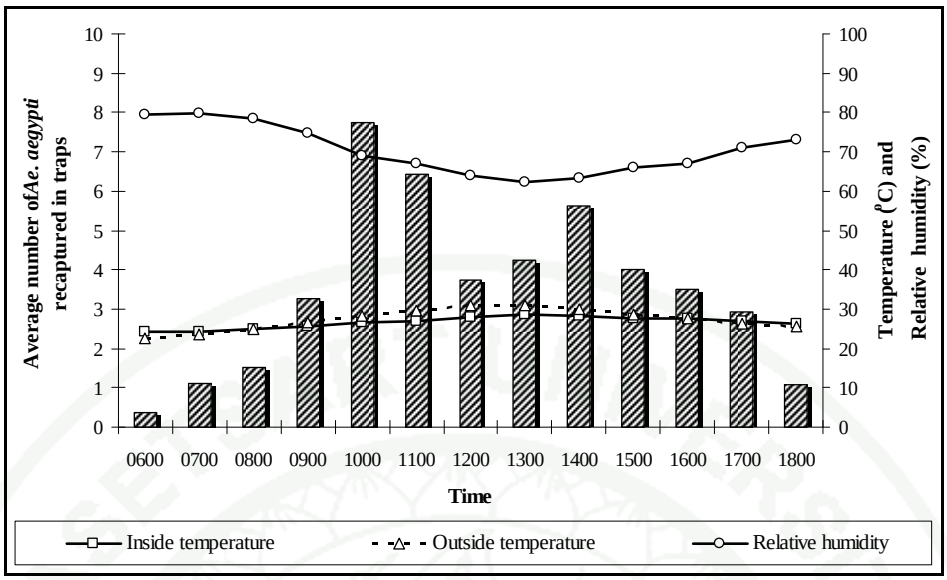

Figure 33 Average number of *Aedes aegypti* collected from exit traps compared to temperature and relative humidity in rainy season (July – October) during the second year period.

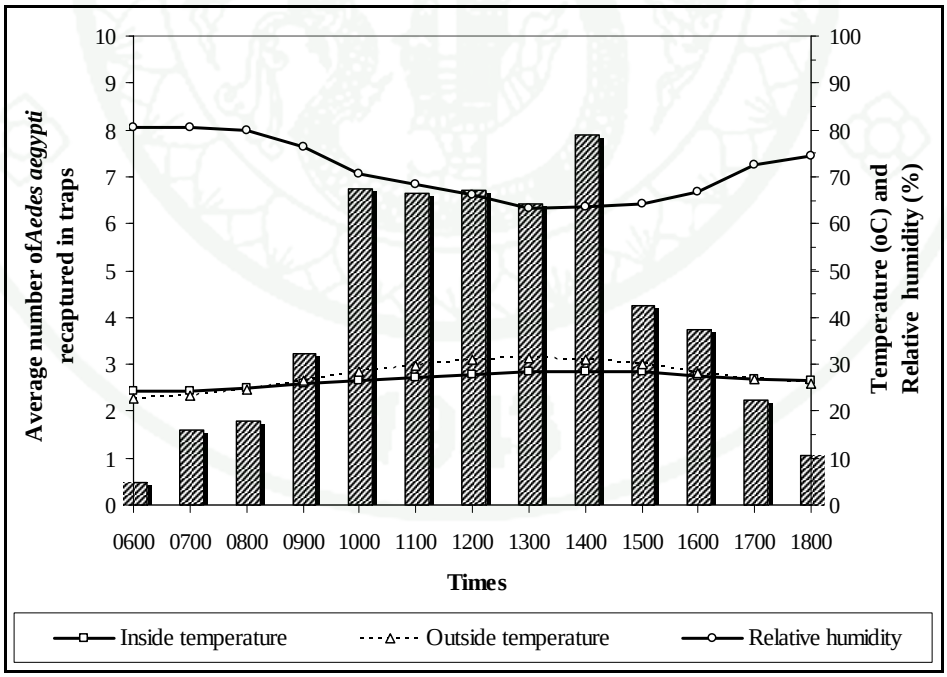

Figure 34 Average number of *Aedes aegypti* collected from exit traps compared to temperature and relative humidity in rainy season (July – October) during the two year period.

Discussions

Most works on the movement patterns of mosquitoes into and out of experimental huts have been conducted on malaria vectors (Roberts *et al.*, 2000; Grieco *et al.*, 2000; Smith, 1965). Relatively few studies have been performed on the movement patterns of *Aedes aegtpti* into and out of the experimental huts (Chareonviriyaphap *et al.*, 2005; Suwonkerd *et al.*, 2006; Grieco *et al.*, 2007). This study represents the first attempt to evaluate the normal movement patterns of *Ae. aegypti* females, a day biting mosquito, into and out of experimental huts in Thailand. An understanding of the factors that influence the movement of *Ae. aegypti* into and out of homes is of great importance in evaluating the role of environmental conditions in disease transmission. Such information helps to define the vector capacity, relative risk for disease transmission, as well as, supports the appropriate vector prevention and control strategies. A better understanding of vector biology and behavior is needed to guide vector control programs and evaluate chemical control strategies. In this study, we utilized the mark-release-recapture technique to evaluate the movement patterns of *Ae. aegypti* into and out of experimental huts fitted with entrance and exit traps.

Many studies conducted on the host preference of *Ae. aegypti* suggested that this mosquito species has a high propensity for feeding on humans inside houses, thus characterizing it as strongly anthropophagic (Gubler and Kuno, 1997; Harrington *et al.*, 2001; Polawat and Harrington, 2005). It would seem that an endophagic and endophilic species such as *Ae. aegypti* would congregate in distinct locations and thus help vector control personnel to easily control them. However, various internal and external factors seem to impede the success of controlling *Ae. aegypti* inside homes. Mosquito behavior is one of these factors that plays a significant role and it in turn is generally affected by changes in the environmental and biological conditions. The natural environment imposes a number of pressures that will impact mosquito behavior including ambient temperature and relative humidity (Kennedy, 1947; Busvine, 1964; Drobozina *et al.*, 1984). This study has increased our knowledge of

how temperature and humidity effect the movement patterns of *Ae. aegypti* into and out of homes.

Previous reports suggest that entering behavior was much stronger when a human host was present in the hut compared with a dog or no host at all (Suwondkerd *et al.*, 2006). In addition, the movement patterns of *Ae. aegypti* females vary according to outdoor temperature as reported by Chareonviriyaphap *et al.* (2005) and Suwonkerd *et al.* (2006). Vector control professionals need to carefully design and improve methods to effectively reduce mosquito populations and risk of disease transmission. In this study, two years of observations on the normal movement patterns of *Ae. aegypti* females were evaluated and it was found that temperature and relative humidity play a major role in altering these patterns.

This study showed that the entering and exiting patterns of *Ae. aegypti* followed a uni – modal periodicity with the peak of entering taking place between 0900 and 1200 hour in winter and between 0600 to 0900 hour in both the summer and the rainy season. These results are quite similar to those reported by Chareonviriyaphap *et al.* (2005) and Suwonkerd *et al.* (2006). In contrast, *Ae. aegypti* human landing collections result in either a bimodal or trimodal periodicity (Corbet and Smith, 1974; Chadee, 1988; Thavara, 2001; Atmosoedjono *et al.*, 1972; Chadee and Martinez, 2000).

The time of entering recorded in winter was delayed compared to what was found in either the summer or the rainy season. This delay is most likely the result of the colder temperatures that occur during the winter which have been found to negatively impact the flight activity of *Ae. aegypti* (Clements, 1999; Rowley and Graham, 1968). In this study, it is quite clear that lower temperatures (as low as 12°C) and lower relative humidity in the morning are impacting the flight behavior. Therefore, the peak of entering the huts during the winter was shifted to later in the morning. The peak of exiting the hut in this study occurred between 1000 and 1400 hour for all seasons. This result is in agreement with those reported by Chareonviriyaphap *et al.* (2005) and Suwonkerd *et al.* (2006). The extreme

temperature and decreased relative humidity inside the hut in the afternoon, seems to force the *Ae. aegypti* females to seek suitable outdoor resting sites.

Despite considerable progress in our understanding of vector biology, there remains much to understand about the biology and behavior of *Ae. aegypti* and how external factors influence disease transmission. The behavior of mosquitoes is impacted by several factors including climatic, environmental and physiological factors. This study demonstrates how the entering and exiting behaviors of *Ae. aegypti* are vulnerable to ambient environmental factors such as temperature and humidity. It is clear from this study that conditions in the peridomestic environment play a critical role in where the mosquitoes will be found throughout the day. If the conditions are unsuitable inside the house (high temperature and low humidity), *Ae. aegypti* will seek more conducive resting sites outdoors. Likewise, the outdoor conditions may delay movement inside if temperatures are too cold for flight. Knowing where the mosquitoes are likely to be in the peridomestic environment based on environmental conditions may aid in control efforts by more precisely directing particular interventions. This information on how climatic factors influence mosquito behavior will also be useful when designing future studies and serve as a baseline for the natural movement patterns of *Ae. aegypti* when evaluating this vectors response to test compounds.

CONCLUSION AND RECOMMENDATION

Conclusion

From the experimental results and discussion of this study, the conclusion can be drawn as follow:

1. The peak of time to entering occurred between 0700 – 1000 hour in the summer and rainy season but in winter the peak of entering occurred between 0900 – 1100 hour. Lower temperature and lower relative humidity in the morning influenced the flight behavior. The delay in time of entering of *Aedes aegypti* is most likely the result of the colder temperatures that occurred during the winter.

2. The peak of exiting occurred between 0800 – 1700 hour during the summer and rainy season but in winter the peak of exiting occurred between at 1100 – 1700 hour. The extreme temperature and decreased relative humidity inside the hut in the afternoon forced the *Aedes aegypti* females to seek the suitable outdoor resting sites.

3. The entering behavior was negatively associated with temperature and positively associated with relative humidity. The exiting behavior was positively associated with temperature and negatively associated with relative humidity.

4. Results from this study is completely useful for the future researches and seem as a baseline for the natural movement patterns of *Aedes aegypti* when characterizing the response to any test compounds.

Recommendation

Collectors in the experimental huts should be rotated throughout the collection in order to obtain a clear picture of the movement patterns. Each host produces different attractant cues and in different levels, which might affect the host seeking and movement patterns of *Aedes aegypti* females.

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APPENDICES

Appendix A

Data collection form

Appendix Table A1 Data collection form for marked *Aedes aegypti* recaptured in entrance window traps.

ENTRANCE DAY……..*Aedes aegypti*																										
Date of release							Host								Treatment											
Marking color							Time of release								Age of females											
Time	**WINDOW TRAP 1: south**								**WINDOW TRAP 2: east**								**WINDOW TRAP 3: north**									
Sample	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed
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Appendix Table A2 Data collection form for marked *Aedes aegypti* recaptured in entrance door trap and environmental data.

ENTRANCE DAY……..*Aedes aegypti*																
Date of release							Host							Treatment		
Marking color							Time of release							Age of females		
Time	**DOOR TRAP: west**								**Environment Data**							
									Humidity		Temp.					
Sample	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	In	Out	In	Out	Rain	Wind Speed (KPH) / Direction		Comments outdoor collection
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Appendix Table A3 Data collection form for other mosquito species recaptured in entrance window traps.

ENTRANCE DAY……..Other Culicines																								
Date of release							Host							Treatment										
Marking color							Time of release							Age of females										
Time	WINDOW TRAP 1: south								WINDOW TRAP 2: east								WINDOW TRAP 3: north							
Sample	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed
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Appendix Table A4 Data collection form for other mosquito species recaptured in entrance door trap.

ENTRANCE DAY…….. Other Culicines															
Date of release							Host						Treatment		
Marking color							Time of release						Age of females		
Time Sample	**DOOR TRAP: west**								**Environment Data**						
									Humidity		Temp.				
	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	In	Out	In	Out	Rain	Wind Speed (KPH) / Direction	Comments outdoor collection
6.00															
6.20															
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17.00															
17.20															
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18.00															

Appendix Table A5 Data collection form for marked *Aedes aegypti* recaptured in exit window traps.

EXIT DAY…….*Aedes aegypti*																								
Date of release						Host						Treatment												
Marking color						Time of release						Age of females												
Time	**WINDOW TRAP 1: south**								**WINDOW TRAP 2: east**								**WINDOW TRAP 3: north**							
Sample	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed
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6.20																								
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16.40																								
17.00																								
17.20																								
17.40																								
18.00																								

Appendix Table A6 Data collection form for marked *Aedes aegypti* recaptured in exit door trap and environmental data.

EXIT DAY……..*Aedes aegypti*															
Date of release							Host						Treatment		
Marking color							Time of release						Age of females		
Time Sample	**DOOR TRAP: west**								**Environment Data**						
									Humidity		Temp.				
	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	In	Out	In	Out	Rain	Wind Speed (KPH) / Direction	Comments outdoor collection
6.00															
6.20															
6.40															
7.00															
7.20															
7.40															
8.00															
8.20															
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16.40															
17.00															
17.20															
17.40															
18.00															

Appendix Table A7 Data collection form for other mosquito species recaptured in exit window traps.

EXIT DAY……..Other Culicines																										
Date of release							Host								Treatment											
Marking color							Time of release								Age of females											
Time Sample	WINDOW TRAP 1: south								WINDOW TRAP 2: east								WINDOW TRAP 3: north									
	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed		
6.00																										
6.20																										
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17.40																										
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Appendix Table A8 Data collection form for other mosquito species recaptured in exit door trap and knockdown mosquito inside hut.

EXIT …….. Other Culicines																			
Date of release								Host							Treatment				
Time Sample	**DOOR TRAP: west**								**GROUND**										**Comments outdoor collection**
	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	Fed	Unfed	
6.00																			
6.20																			
6.40																			
7.00																			
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Appendix B

Statistical analysis

Appendix Table B1 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in entrance traps between the first and second year study.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Nov1 - Nov2	30.25000	24.51360	12.25680	-8.75661	69.25661	2.468	3	.090
Pair 2	Dec1 - Dec2	.25000	14.66004	7.33002	-23.07739	23.57739	.034	3	.975
Pair 3	Jan1 - Jan2	-19.00000	11.97219	5.98609	-38.05043	.05043	-3.174	3	.050
Pair 4	Feb1 - Feb2	4.75000	9.63933	4.81966	-10.58832	20.08832	.986	3	.397
Pair 5	Mar1 - Mar2	17.00000	22.03028	11.01514	-18.05510	52.05510	1.543	3	.220
Pair 6	Apr1 - Apr2	-35.50000	10.75484	5.37742	-52.61336	-18.38664	-6.602	3	.007
Pair 7	May1 - May2	5.75000	11.52895	5.76447	-12.59513	24.09513	.997	3	.392
Pair 8	Jun1 - Jun2	15.75000	10.04573	5.02286	-.23500	31.73500	3.136	3	.052
Pair 9	Jul1 - Jul2	-6.00000	15.42725	7.71362	-30.54820	18.54820	-.778	3	.493
Pair 10	Aug1 - Aug2	8.25000	22.99819	11.49909	-28.34525	44.84525	.717	3	.525
Pair 11	Sep1 - Sep2	4.25000	12.17580	6.08790	-15.12441	23.62441	.698	3	.535
Pair 12	Oct1 - Oct2	-11.50000	8.26640	4.13320	-24.65368	1.65368	-2.782	3	.069

Appendix Table B2 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in exit traps between the first and second year study.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Nov1 - Nov2	-3.50000	15.19868	7.59934	-27.68450	20.68450	-.461	3	.676
Pair 2	Dec1 - Dec2	-12.50000	25.00000	12.50000	-52.28058	27.28058	-1.000	3	.391
Pair 3	Jan1 - Jan2	26.25000	18.00694	9.00347	-2.40306	54.90306	2.916	3	.062
Pair 4	Feb1 - Feb2	-34.50000	5.25991	2.62996	-42.86969	-26.13031	-13.118	3	.001
Pair 5	Mar1 - Mar2	-40.00000	4.00000	2.00000	-46.36489	-33.63511	-20.000	3	.000
Pair 6	Apr1 - Apr2	1.00000	20.80064	10.40032	-32.09846	34.09846	.096	3	.929
Pair 7	May1 - May2	-8.00000	17.30125	8.65063	-35.53015	19.53015	-.925	3	.423
Pair 8	Jun1 - Jun2	29.75000	14.59166	7.29583	6.53141	52.96859	4.078	3	.027
Pair 9	Jul1 - Jul2	17.75000	11.05667	5.52834	.15637	35.34363	3.211	3	.049
Pair 10	Aug1 - Aug2	44.00000	19.89975	9.94987	12.33506	75.66494	4.422	3	.021
Pair 11	Sep1 - Sep2	-6.50000	28.73442	14.36721	-52.22287	39.22287	-.452	3	.682
Pair 12	Oct1 - Oct2	3.00000	18.56520	9.28260	-26.54138	32.54138	.323	3	.768

Appendix Table B3 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in entrance traps by period of time in winter.

(I) Time	(J) Time	Mean Difference (I-J)		Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
1	2	9.87500		6.95128	.228	-9.4249	29.1749
	3	35.87500	*	6.95128	.007	16.5751	55.1749
	4	37.87500	*	6.95128	.006	18.5751	57.1749
2	1	-9.87500		6.95128	.228	-29.1749	9.4249
	3	26.00000	*	6.95128	.020	6.7001	45.2999
	4	28.00000	*	6.95128	.016	8.7001	47.2999
3	1	-35.87500	*	6.95128	.007	-55.1749	-16.5751
	2	-26.00000	*	6.95128	.020	-45.2999	-6.7001
	4	2.00000		6.95128	.788	-17.2999	21.2999
4	1	-37.87500	*	6.95128	.006	-57.1749	-18.5751
	2	-28.00000	*	6.95128	.016	-47.2999	-8.7001
	3	-2.00000		6.95128	.788	-21.2999	17.2999

* The mean difference is significant at the .05 level.

Appendix Table B4 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in entrance traps by period of time in summer.

(I) Time	(J) Time	Mean Difference (I-J)		Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
1	2	83.00000	*	3.39807	.000	73.5654	92.4346
	3	91.37500	*	3.39807	.000	81.9404	100.8096
	4	94.12500	*	3.39807	.000	84.6904	103.5596
2	1	-83.00000	*	3.39807	.000	-92.4346	-73.5654
	3	8.37500		3.39807	.069	-1.0596	17.8096
	4	11.12500	*	3.39807	.031	1.6904	20.5596
3	1	-91.37500	*	3.39807	.000	-100.8096	-81.9404
	2	-8.37500		3.39807	.069	-17.8096	1.0596
	4	2.75000		3.39807	.464	-6.6846	12.1846
4	1	-94.12500	*	3.39807	.000	-103.5596	-84.6904
	2	-11.12500	*	3.39807	.031	-20.5596	-1.6904
	3	-2.75000		3.39807	.464	-12.1846	6.6846

* The mean difference is significant at the .05 level.

Appendix Table B5 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in entrance traps by period of time in rainy season.

(I) Time	(J) Time	Mean Difference (I-J)		Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
1	2	42.75000	*	2.28104	.000	36.4168	49.0832
	3	55.37500	*	2.28104	.000	49.0418	61.7082
	4	57.37500	*	2.28104	.000	51.0418	63.7082
2	1	-42.75000	*	2.28104	.000	-49.0832	-36.4168
	3	12.62500	*	2.28104	.005	6.2918	18.9582
	4	14.62500	*	2.28104	.003	8.2918	20.9582
3	1	-55.37500	*	2.28104	.000	-61.7082	-49.0418
	2	-12.62500	*	2.28104	.005	-18.9582	-6.2918
	4	2.00000		2.28104	.430	-4.3332	8.3332
4	1	-57.37500	*	2.28104	.000	-63.7082	-51.0418
	2	-14.62500	*	2.28104	.003	-20.9582	-8.2918
	3	-2.00000		2.28104	.430	-8.3332	4.3332

* The mean difference is significant at the .05 level.

Appendix Table B6 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in exit traps by period of time in winter.

(I) Time	(J) Time	Mean Difference (I-J)		Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
1	2	-44.00000	*	14.69880	.040	-84.8104	-3.1896
	3	-45.00000	*	14.69880	.038	-85.8104	-4.1896
	4	-40.12500		14.69880	.052	-80.9354	.6854
2	1	44.00000	*	14.69880	.040	3.1896	84.8104
	3	-1.00000		14.69880	.949	-41.8104	39.8104
	4	3.87500		14.69880	.805	-36.9354	44.6854
3	1	45.00000	*	14.69880	.038	4.1896	85.8104
	2	1.00000		14.69880	.949	-39.8104	41.8104
	4	4.87500		14.69880	.757	-35.9354	45.6854
4	1	40.12500		14.69880	.052	-.6854	80.9354
	2	-3.87500		14.69880	.805	-44.6854	36.9354
	3	-4.87500		14.69880	.757	-45.6854	35.9354

* The mean difference is significant at the .05 level.

Appendix Table B7 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in exit traps by period of time in summer.

(I) Time	(J) Time	Mean Difference (I-J)		Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
1	2	-50.75000	*	16.20740	.035	-95.7489	-5.7511
	3	6.00000		16.20740	.730	-38.9989	50.9989
	4	18.12500		16.20740	.326	-26.8739	63.1239
2	1	50.75000	*	16.20740	.035	5.7511	95.7489
	3	56.75000	*	16.20740	.025	11.7511	101.7489
	4	68.87500	*	16.20740	.013	23.8761	113.8739
3	1	-6.00000		16.20740	.730	-50.9989	38.9989
	2	-56.75000	*	16.20740	.025	-101.7489	-11.7511
	4	12.12500		16.20740	.496	-32.8739	57.1239
4	1	-18.12500		16.20740	.326	-63.1239	26.8739
	2	-68.87500	*	16.20740	.013	-113.8739	-23.8761
	3	-12.12500		16.20740	.496	-57.1239	32.8739

* The mean difference is significant at the .05 level.

Appendix Table B8 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in exit traps by period of time in rainy season.

(I) Time	(J) Time	Mean Difference (I-J)		Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
1	2	-52.25000	*	14.94077	.025	-93.7322	-10.7678
	3	-46.12500	*	14.94077	.037	-87.6072	-4.6428
	4	.00000		14.94077	1.000	-41.4822	41.4822
2	1	52.25000	*	14.94077	.025	10.7678	93.7322
	3	6.12500		14.94077	.703	-35.3572	47.6072
	4	52.25000	*	14.94077	.025	10.7678	93.7322
3	1	46.12500	*	14.94077	.037	4.6428	87.6072
	2	-6.12500		14.94077	.703	-47.6072	35.3572
	4	46.12500	*	14.94077	.037	4.6428	87.6072
4	1	.00000		14.94077	1.000	-41.4822	41.4822
	2	-52.25000	*	14.94077	.025	-93.7322	-10.7678
	3	-46.12500	*	14.94077	.037	-87.6072	-4.6428

* The mean difference is significant at the .05 level.

Appendix Table B9 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in entrance window trap 1 by period of time.

(I) period	(J) period	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	6.042 *	1.299	.000	3.46	8.62
	3	8.208 *	1.299	.000	5.63	10.79
	4	8.417 *	1.299	.000	5.84	11.00
2	1	-6.042 *	1.299	.000	-8.62	-3.46
	3	2.167	1.299	.099	-.41	4.75
	4	2.375	1.299	.071	-.21	4.96
3	1	-8.208 *	1.299	.000	-10.79	-5.63
	2	-2.167	1.299	.099	-4.75	.41
	4	.208	1.299	.873	-2.37	2.79
4	1	-8.417 *	1.299	.000	-11.00	-5.84
	2	-2.375	1.299	.071	-4.96	.21
	3	-.208	1.299	.873	-2.79	2.37

* The mean difference is significant at the .05 level.

Appendix Table B10 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in entrance window trap 2 by period of time.

(I) period	(J) period	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	3.167	1.849	.090	-.50	6.84
	3	9.083 *	1.849	.000	5.41	12.75
	4	10.417 *	1.849	.000	6.75	14.09
2	1	-3.167	1.849	.090	-6.84	.50
	3	5.917 *	1.849	.002	2.25	9.59
	4	7.250 *	1.849	.000	3.58	10.92
3	1	-9.083 *	1.849	.000	-12.75	-5.41
	2	-5.917 *	1.849	.002	-9.59	-2.25
	4	1.333	1.849	.473	-2.34	5.00
4	1	-10.417 *	1.849	.000	-14.09	-6.75
	2	-7.250 *	1.849	.000	-10.92	-3.58
	3	-1.333	1.849	.473	-5.00	2.34

* The mean difference is significant at the .05 level.

Appendix Table B11 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in entrance window trap 3 by period of time.

(I) period	(J) period	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	6.458 *	1.797	.001	2.89	10.03
	3	10.500 *	1.797	.000	6.93	14.07
	4	10.500 *	1.797	.000	6.93	14.07
2	1	-6.458 *	1.797	.001	-10.03	-2.89
	3	4.042 *	1.797	.027	.47	7.61
	4	4.042 *	1.797	.027	.47	7.61
3	1	-10.500 *	1.797	.000	-14.07	-6.93
	2	-4.042 *	1.797	.027	-7.61	-.47
	4	.000	1.797	1.000	-3.57	3.57
4	1	-10.500 *	1.797	.000	-14.07	-6.93
	2	-4.042 *	1.797	.027	-7.61	-.47
	3	.000	1.797	1.000	-3.57	3.57

* The mean difference is significant at the .05 level.

Appendix Table B12 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in entrance door trap by period of time.

(I) period	(J) period	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	24.792 *	2.963	.000	18.91	30.68
	3	29.125 *	2.963	.000	23.24	35.01
	4	29.292 *	2.963	.000	23.41	35.18
2	1	-24.792 *	2.963	.000	-30.68	-18.91
	3	4.333	2.963	.147	-1.55	10.22
	4	4.500	2.963	.132	-1.38	10.38
3	1	-29.125 *	2.963	.000	-35.01	-23.24
	2	-4.333	2.963	.147	-10.22	1.55
	4	.167	2.963	.955	-5.72	6.05
4	1	-29.292 *	2.963	.000	-35.18	-23.41
	2	-4.500	2.963	.132	-10.38	1.38
	3	-.167	2.963	.955	-6.05	5.72

* The mean difference is significant at the .05 level.

Appendix Table B13 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in exit window trap 1 by period of time.

(I) period	(J) period	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-8.333 *	2.235	.000	-12.77	-3.89
	3	-.875	2.235	.696	-5.31	3.56
	4	1.167	2.235	.603	-3.27	5.61
2	1	8.333 *	2.235	.000	3.89	12.77
	3	7.458 *	2.235	.001	3.02	11.90
	4	9.500 *	2.235	.000	5.06	13.94
3	1	.875	2.235	.696	-3.56	5.31
	2	-7.458 *	2.235	.001	-11.90	-3.02
	4	2.042	2.235	.363	-2.40	6.48
4	1	-1.167	2.235	.603	-5.61	3.27
	2	-9.500 *	2.235	.000	-13.94	-5.06
	3	-2.042	2.235	.363	-6.48	2.40

* The mean difference is significant at the .05 level.

Appendix Table B14 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in exit window trap 2 by period of time.

(I) period	(J) period	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-10.250 *	2.727	.000	-15.67	-4.83
	3	-8.958 *	2.727	.001	-14.37	-3.54
	4	-1.458	2.727	.594	-6.87	3.96
2	1	10.250 *	2.727	.000	4.83	15.67
	3	1.292	2.727	.637	-4.12	6.71
	4	8.792 *	2.727	.002	3.38	14.21
3	1	8.958 *	2.727	.001	3.54	14.37
	2	-1.292	2.727	.637	-6.71	4.12
	4	7.500 *	2.727	.007	2.08	12.92
4	1	1.458	2.727	.594	-3.96	6.87
	2	-8.792 *	2.727	.002	-14.21	-3.38
	3	-7.500 *	2.727	.007	-12.92	-2.08

* The mean difference is significant at the .05 level.

Appendix Table B15 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in exit window trap 3 by period of time.

(I) period	(J) period	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-10.958 *	2.252	.000	-15.43	-6.49
	3	-7.667 *	2.252	.001	-12.14	-3.19
	4	-6.917 *	2.252	.003	-11.39	-2.44
2	1	10.958 *	2.252	.000	6.49	15.43
	3	3.292	2.252	.147	-1.18	7.76
	4	4.042	2.252	.076	-.43	8.51
3	1	7.667 *	2.252	.001	3.19	12.14
	2	-3.292	2.252	.147	-7.76	1.18
	4	.750	2.252	.740	-3.72	5.22
4	1	6.917 *	2.252	.003	2.44	11.39
	2	-4.042	2.252	.076	-8.51	.43
	3	-.750	2.252	.740	-5.22	3.72

* The mean difference is significant at the .05 level.

Appendix Table B16 The statistic analysis of the difference in average numbers of *Aedes aegypti* recaptured in exit door trap by period of time.

(I) period	(J) period	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-24.500 *	4.202	.000	-32.85	-16.15
	3	-6.958	4.202	.101	-15.30	1.39
	4	1.167	4.202	.782	-7.18	9.51
2	1	24.500 *	4.202	.000	16.15	32.85
	3	17.542 *	4.202	.000	9.20	25.89
	4	25.667 *	4.202	.000	17.32	34.01
3	1	6.958	4.202	.101	-1.39	15.30
	2	-17.542 *	4.202	.000	-25.89	-9.20
	4	8.125	4.202	.056	-.22	16.47
4	1	-1.167	4.202	.782	-9.51	7.18
	2	-25.667 *	4.202	.000	-34.01	-17.32
	3	-8.125	4.202	.056	-16.47	.22

* The mean difference is significant at the .05 level.

Appendix Table B17 The regression analysis of the relationship between average number of *Aedes aegypti* recaptured in entrance traps and climatic factors.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.765	.58	.56	25.15	.58	25.391	5	90	.00

Appendix Table B18 The regression analysis of the relationship between average number of *Aedes aegypti* recaptured in exit traps and climatic factors.

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.579	.335	.298	29.496	.335	9.077	5	90	.000

Appendix C

Thesis publication

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Effects of environmental conditions on the movement patterns of *Aedes aegypti* (Diptera: Culicidae) into and out of experimental huts in Thailand

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ABSTRACT: Mark-release-recapture experiments with *Aedes aegypti* were performed using experimental huts equipped with entrance and exit traps to evaluate their movement patterns during a two-year period in Thailand. Results indicate no significant differences in the patterns of movement between the two years of observation. Movement into the huts occurred during the early morning period (06:00-11:00) with a peak at 07:00 in the summer and rainy season and 09:00 in the winter. In contrast, the exit pattern was observed during the late morning (09:00-12:00) and early afternoon (12:00-16:00), with a peak at 16:00 in the winter, 11:00 in the summer, and 14:00 in the rainy season. Multiple regression analysis indicated that movements of *Ae. aegypti* females into and out of the huts were impacted by humidity and temperature during the day. ***Journal of Vector Ecology* 34 (2): 267-275. 2009.**

Keyword Index: *Aedes aegypti*, movement patterns, behavior, experimental hut, Thailand.

INTRODUCTION

Dengue viruses are transmitted from human to human by the bite of infected *Aedes aegypti* (L), a day-biting mosquito that breeds in or near human dwellings and preferentially feeds on humans even when other warm-blooded animals are freely available (Edman et al. 1992, Xue et al. 1995, WHO 1999). Because no commercial vaccine or successful drug therapy is yet available for the prevention and treatment of dengue infection, the control of the vector remains the most effective method for preventing dengue virus transmission and averting epidemics (Reiter and Gubler 1997, Perich et al. 2001). Control of the mosquito vector by chemical means remains the most successful method for reducing disease transmission and preventing human vector contact (Roberts et al. 1997, WHO 1999).

Chemicals may protect humans from the bite of insects through three different actions; irritancy, repellency, or toxicity (Grieco et al. 2007). Recently, it has been proposed that some chemicals, such as DEET, elicit a fourth action by effectively masking the presence of a host through the inhibition of odor-activated receptors (Ditzen et al. 2008). Most studies have concentrated on the toxic actions of these chemicals, whereas little focus has been placed on non-toxic properties of these compounds. Non-toxic actions can be placed into two different categories, contact irritancy and non-contact repellency (Roberts et al. 1997, Chareonviriyaphap et al. 1997). These two types of behavioral response can be evaluated through the use of laboratory and field assay systems (Roberts et al. 1997, Smith 1965, Chareonviriyaphap et al. 2005, Grieco et al. 2007). In order to truly quantify these behaviors in response to chemical treatments, a baseline of natural behaviors and movement patterns must first be established in the absence of the chemical. Recently, the effect of host types on movement patterns of *Ae. aegypti* using experimental huts was conducted in Thailand (Suwonkerd et al. 2006). These same experimental huts were also used to investigate the three actions of chemicals against *Ae. aegypti* (Grieco et al. 2007). As seen with these previous studies, most experimental hut work evaluates the impact of some type of intervention on mosquito behavior but little has been done to evaluate the movement patterns of *Ae. aegypti* in response to monthly changes in environmental factors. This study was the first attempt to investigate the ingress and egress movements of *Ae. aegypti* in response to changes in seasonal environmental parameters, i.e., relative humidity and ambient temperature, during a two-year period using experimental huts fitted with entrance and exit traps.

MATERIALS AND METHODS

Study site

This study was conducted at Pu Teuy Village, Sai Yok District, Kanchanaburi Province (14° 20'N, 98° 59'E, 304 m asl), western Thailand, approximately 150 km northwest of Bangkok. The site is located in a hilly area and is largely surrounded by primary dense forest. The study site belongs to the Armed Forces Development Command in the Ministry of Armed Forces. The nearest home is approximately 800 m away from the field site where the experimental huts were located.

Mosquito population

An experimental release population of *Ae. aegypti* was established from immature stages collected from Pu Teuy Village, Sai Yok District, Kanchanaburi Province. Approximately 200-300 pupae and larvae were brought back to the insectary at the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. Morphological identification was made by using the conventional key of Rattanarithikul and Panthusiri (1994). Subsequent colonization was performed at the same insectary, following the method of Kongmee et al. (2004).

Mosquito rearing

All life stages of *Ae. aegypti* were maintained under insectary controlled conditions. Larvae and adults were reared under a 12:12 h light: dark photophase regime, a 25±5° C controlled temperature and a 80±10% controlled relative humidity. Upon emergence, all adults were provided cotton pads soaked with a 10% sucrose solution. Female mosquitoes were starved for at least 12 h prior to testing.

Experimental huts

The experimental huts used in this study were previously described (Chareonviriyaphap et al. 2005, Grieco et al. 2007). Two identical huts were constructed in an isolated area adjacent to Pu Teuy village. Each hut measured 4 m wide x 5 m long x 3.5 m high, and had three windows and one door. The dimensions of the windows and door were 1.125 m x 1.175 m and 0.8 m x 2 m, respectively. Each portal was constructed in such a way as to allow them to be affixed with entrance and exit traps. Huts were built of similar materials and in a similar fashion to the indigenous Thai homes. Huts were constructed from pieces of 1 m x 2.5 m untreated wood and pieces of 0.75 m x 3 m zinc roofing. Hut frames used to support the walls were made from galvanized iron pipe measuring 1 m x 2.5 m in length and were custom-welded to accommodate each wall. The apex of the angled roof measured 3.5 m from leveled ground. The hut had three windows, one on each of three sides, and a northward-facing door, and all were affixed with either entrance or exit traps.

The dimensions of the window traps were 0.84 m long, 1.1 m wide, and 1.1 m high and were constructed using an iron frame. Louvers made of 9.5 mm non-treated plywood and fixed vertically at 60° angles were placed over the front opening of each of the three window traps, 1.1 m x 1.1 m, with a horizontal row of 10-cm wide slit openings made of 9.5 mm non-treated plywood fixed vertically to 60°. The louvers were placed in the open position producing a series of horizontal, 10-cm wide openings through which mosquitoes could enter. A door trap, measuring 1.2 m long x 0.85 m wide x 2.1 m high, was fixed to the door opening. Twenty plywood louvers identical to those used in the window traps were installed over the front opening and were again fixed at 60° angles to the vertical. These were arranged to facilitate the movement of mosquitoes from the hut into the trap. Both trap types were covered by nylon insect netting. Cotton sleeve material was sewn over several holes in both types of traps to facilitate the removal of mosquitoes. Additional details pertaining to the experimental huts were given in Suwonkerd et al. (2006) and Grieco et al. (2007).

Mosquito marking and release technique

Only the F1 adult generation was used in this study. Two groups of three- to five-day-old, non-bloodfed *Ae. aegypti* females were marked with luminous marking powder (BioQuip Products, Rancho Dominquez, CA) following the method of Achee et al. (2005). Combined, both groups numbered 125 females. This consisted of one population of 100 females that was used as a release population and 25 females that were used as controls. Marked mosquitoes were sugar starved for 24 h, placed in a humidified chamber that was kept moist using water soaked towels, and provided with water soaked cotton pads until the time of release.

For the entrance experiment, 100 marked mosquitoes were released 10 m outside of each hut. For the exit collections, 100 marked mosquitoes were released inside of each hut. The released time was set at 05:00, approximately one h before the start of the collection.

All experiments were replicated two times in both huts and in each month. Human hosts were covered by mosquito nets to protect them from being bitten during the study. Entrance and exit traps were sampled every 20 min between 06:00-18:00. The collections were made by two collectors per hut. Collectors were alternated between huts every 20 min to control for collectors bias. All mosquitoes from the traps were examined for fluorescent powder using a UV light and a stereomicroscope. The ambient temperature and relative humidity were recorded by the collector inside the hut every 20 min.

Data analysis

Differences in the number of mosquitoes recaptured from entrance and exit traps over four hourly intervals (06:00-09:00, 09:00-12:00, 12:00-15:00, and 15:00-18:00) were analyzed using regression analysis that included the following independent variables: ambient temperature, relative humidity, and precipitation. Multiple regression was performed to investigate the association between the two types of movement behaviors (hut entry and exit) of *Ae. aegypti* and the environmental variables of temperature and relative humidity. All data were analyzed using the SAS program package (SAS Release 6.10, SAS Institute, Cary, NC). The discriminating level for all tests was set at 0.05%.

RESULTS

The average number of *Ae. aegypti* females recaptured from entrance and exit traps during a two-year period is given in Table 1. A comparison of the number of mosquitoes recaptured from entrance and exit traps in the same months between the first and second year was performed. Results reveal that the number of *Ae. aegypti* females recaptured from entrance traps in the month of April was the only statistical different sample period during the two-year collection (T = -6.602, P = 0.007) (Table 1). However,

Table 1. Average numbers of *Ae. aegypti* recaptured in traps during the two-year study period.

Month	Average number of *Ae. aegypti* recaptured from entrance traps		Average number of *Ae. aegypti* recaptured from exit traps	
	Year 1	Year 2	Year 1	Year 2
January	8.25 ± 2.87	27.25 ± 10.87	70.50 ± 9.47	44.25 ± 10.31
February	21.00 ± 2.94	16.25 ± 8.30	30.50 ± 3.79*	65.00 ± 3.83*
March	36.25 ± 18.26	19.25 ± 3.86	18.75 ± 4.03*	58.75 ± 7.41*
April	3.75 ± 2.06*	39.25 ± 9.95*	72.75 ± 7.27	71.75 ± 15.41
May	35.00 ± 13.29	29.25 ± 2.87	47.50 ± 8.19	55.50 ± 10.28
June	45.00 ± 5.35	29.25 ± 5.85	67.50 ± 7.14*	37.75 ± 12.92*
July	18.75 ± 12.87	24.75 ± 9.43	53.50 ± 14.64*	35.75 ± 12.82*
August	32.50 ± 14.20	24.25 ± 9.32	69.25 ± 16.15*	25.25 ± 7.93*
September	26.75 ± 7.04	22.50 ± 7.94	48.00 ± 26.34	54.50 ± 6.61
October	15.00 ± 7.02	26.50 ± 12.87	69.75 ± 4.99	66.75 ± 14.64
November	41.75 ± 24.35	11.50 ± 0.58	53.00 ± 11.14	56.50 ± 5.54
December	22.00 ± 7.30	21.75 ± 9.88	40.50 ± 25.16	53.00 ± 6.93
Total	306.00 ± 13.00	291.75 ± 7.07	641.5 ± 17.42	624.75 ± 13.83

*Statistical differences in average number of *Ae. aegypti* recaptured in traps between year 1 and year 2 of the study ($P < 0.05$).

there was no significant difference in the total number of mosquitoes recaptured from entrance traps during the two- year period ($T = 0.235$, P = 0.818). For the exit regime, statistical differences in the average number of *Ae. aegypti* recaptured during the first two years of the study were found for the months of February ($T = -13.118$, $P = 0.001$), March ($T = -20.000$, $P = 0.000$), June ($T = 4.078$, $P = 0.027$), July ($T = 3.211$, $P = 0.049$), and August ($T = 4.422$, $P = 0.021$). No statistical difference in the total number of exit specimens recaptured between the first and second years was found ($T = 0.196$, $P = 0.848$).

The average number of *Ae. aegypti* recaptured in traps tabulated by time of collection and season is shown in Table 2. The average temperature and relative humidity for the three seasons are provided in Table 3. Overall, the highest proportion of *Ae. aegypti* females were recaptured from both entrance and exit traps (29.63% from entrance and 53.78% from exit traps) during the summer months. For the entrance experiment, the highest total number *of Ae. aegypti* were recaptured in the summer (29.63%), whereas the lowest number was recorded during the winter (21.22%). There was no significant difference in the number of *Ae. aegypti* recaptured during a single day's collections conducted during the winter and rainy seasons ($P > 0.05$). Significantly higher numbers of *Ae. aegypti* females were recaptured in traps during the summer as compared to the other seasons ($P = 0.003$ and 0.036, respectively). During the summer, a higher proportion of mosquitoes was recaptured between 06:00-09:00 compared to any other time period during the day ($P = 0.000$).

Table 2. Average number of *Ae. aegypti* recaptured in traps during a 12-h collection in all seasons.

Experiment	Times				Total
	06:00 – 09:00	09:00 – 12:00	12:00 – 15:00	15:00 – 18:00	
Entrance					
Winter	10.53 ± 7.81	8.06 ± 5.93	1.56 ± 1.24	1.06 ± 0.53	21.22 ± 10.32
Summer	24.19 ± 11.44	3.44 ± 1.95	1.34 ± 1.08	0.66 ± 0.60	29.63 ± 12.98
Rainy season	15.69 ± 5.19	5.00 ± 2.62	1.84 ± 0.79	1.34 ± 0.78	23.88 ± 5.31
Exit					
Winter	4.84 ± 3.03	15.84 ± 10.05	16.09 ± 6.18	14.88 ± 5.92	51.66 ± 13.02
Summer	11.78 ± 8.90	24.47 ± 11.45	10.28 ± 5.41	7.25 ± 2.33	53.78 ± 18.60
Rainy season	7.06 ± 6.41	20.13 ± 8.44	18.59 ± 5.43	7.06 ± 2.98	52.84 ± 16.16

Table 3. The average temperature and relative humidity by period during a 12 h collection in all seasons.

Factors	Seasons		
	Winter	Summer	Rainy season
Inside Temperature (°C)			
06:00 – 09:00	21.2 ± 2.84	24.9 ± 1.65	24.7 ± 0.98
09:00 – 12:00	26.4 ± 2.35	28.4 ± 1.59	27.2 ± 1.10
12:00 – 15:00	29.8 ± 2.15	29.9 ± 2.65	28.6 ± 1.23
15:00 – 18:00	28.9 ± 2.64	28.9 ± 2.99	27.3 ± 1.40
Min	12.8	19.0	22.3
Max	34.5	36.7	31.8
Outside Temperature (°C)			
06:00 – 09:00	20.1 ± 3.43	24.8 ± 2.38	25.0 ± 1.86
0900 – 12:00	29.7 ± 3.19	31.6 ± 2.62	30.0 ± 1.97
12:00 – 15:00	34.2 ± 3.01	32.7 ± 4.12	31.4 ± 2.23
15:00 – 18:00	30.1 ± 3.80	29.8 ± 4.56	27.7 ± 2.54
Min	12.0	18.0	20.7
Max	41.0	43.0	35.7
Relative Humidity (%)			
06:00 – 09:00	79.7 ± 5.28	80.6 ± 5.59	79.1 ± 3.79
09:00 – 12:00	59.2 ± 9.15	66.8 ± 9.47	67.2 ± 6.37
12:00 – 15:00	47.2 ± 9.20	62.5 ± 13.47	62.9 ± 6.77
15:00 – 18:00	54.0 ± 10.25	67.3 ± 13.91	68.6 ± 7.23
Min	28.3	28.0	40.0
Max	89.0	96.0	88.7

For the exit experiment, the total number of *Ae. aegypti* recaptured from the traps during all three seasons was quite similar, ranging from 51.7% in the winter to 53.8% in the summer (Table 2). In general, higher numbers of *Ae. aegypti* females were recaptured during the late morning (09:00-12:00), with the exception of winter when a higher proportion of specimens was recaptured between 12:00-15:00 (16.1%). Specifically, the proportion of *Ae. aegypti* recaptured in exit traps from 09:00-12:00 and 12:00-15:00 was significantly different from those of the other two periods (06:00-09:00 and 15:00-18:00) ($P < 0.05$) (Table 2).

Time trends for entering and exiting of *Ae. aegypti* females were also recorded in the three different seasons (Figures 1A, 1B). For the entrance experiment, the majority of entering behavior was seen during the morning period (07:00-11:00) with a peak at 07:00 in summer, at 09:00 in the winter, and a prolonged peak from 07:00 to 09:00 in the rainy seasons. A very distinct peak was seen in the summer as compared to what was observed in either the winter or rainy seasons. Very few mosquitoes tended to enter the hut during the afternoon period, regardless of season, with less than one mosquito entering each hour (Figure 1A). During exit collections, a very distinct exiting period was observed in the winter (12:00-17:00) with a peak in activity occurring at 16:00. The duration of exiting was considerably longer during the summer and rainy season (08:00-16:00), with a peak at 11:00 in the summer and 14:00 in the rainy season (Figure 1B).

Entrance and exit behaviors of *Ae. aegypti* into and out of experimental huts were evaluated in relation to ambient temperature and relative humidity using regression analysis. Entrance movement of *Ae. aegypti* was found to be negatively associated with temperature but positively correlated with relative humidity (Figures 2A, 2B, 2C). The average number of *Ae. aegypti* specimens recaptured in traps in response to the ambient temperature and relative humidity are shown in Tables 2 and 3, respectively. The results of the multiple regression analysis on the number of *Ae. aegypti* recaptured in both traps in relation to temperature and relative humidity indicate that these two variables strongly influence the entrance and exit patterns of this mosquito species ($R^2 = 0.585$; $F = 25.391$; $P = 0.000$).

The exiting patterns of *Ae. aegypti* from the experimental hut were found to be positively associated with temperature but negatively correlated with relative humidity (Figures 3A, 3B, 3C). Results of the multiple regression analysis on the number of *Ae. aegypti* recaptured in traps suggested that these two environmental factors also have a strong impact on the movement patterns of *Ae. aegypti* females into and out of the hut ($R^2 = 0.335$; $F = 9.077$; $P = 0.000$).

DISCUSSION

This study is the first attempt to evaluate the normal movement patterns of *Ae. aegypti* females, a day-biting mosquito, in response to changes in seasonal environmen-

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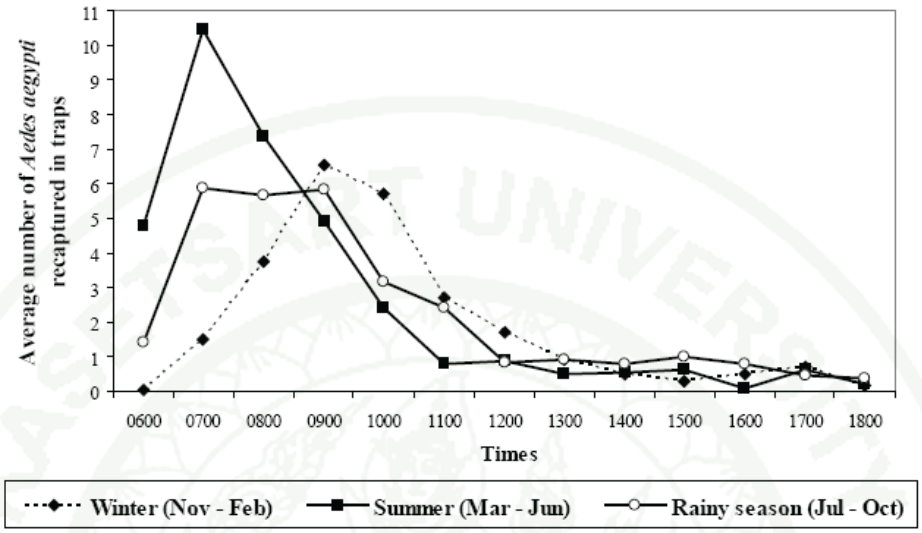

Figure 1A. Time of entry of *Ae. aegypti* into traps during a 12-h collection.

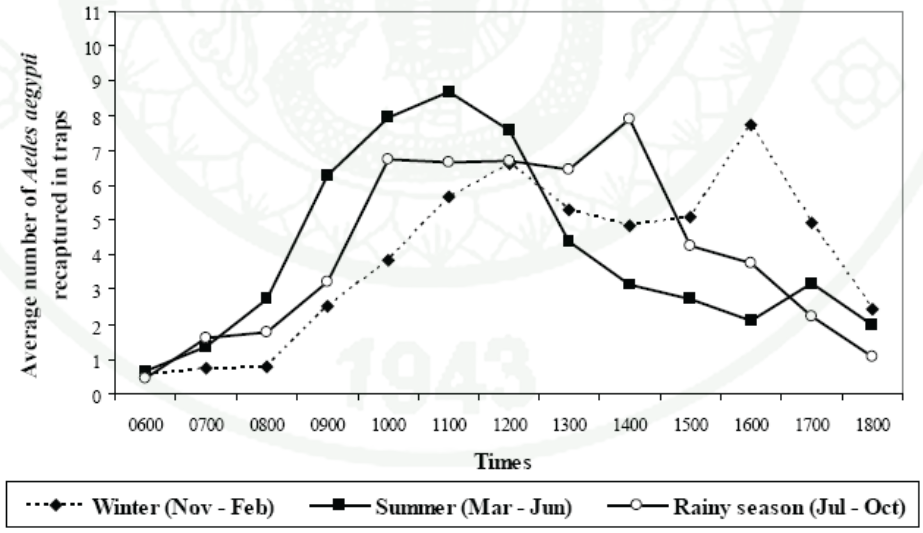

Figure 1B. Time of exit of *Ae. aegypti* into traps during a 12-h collection.

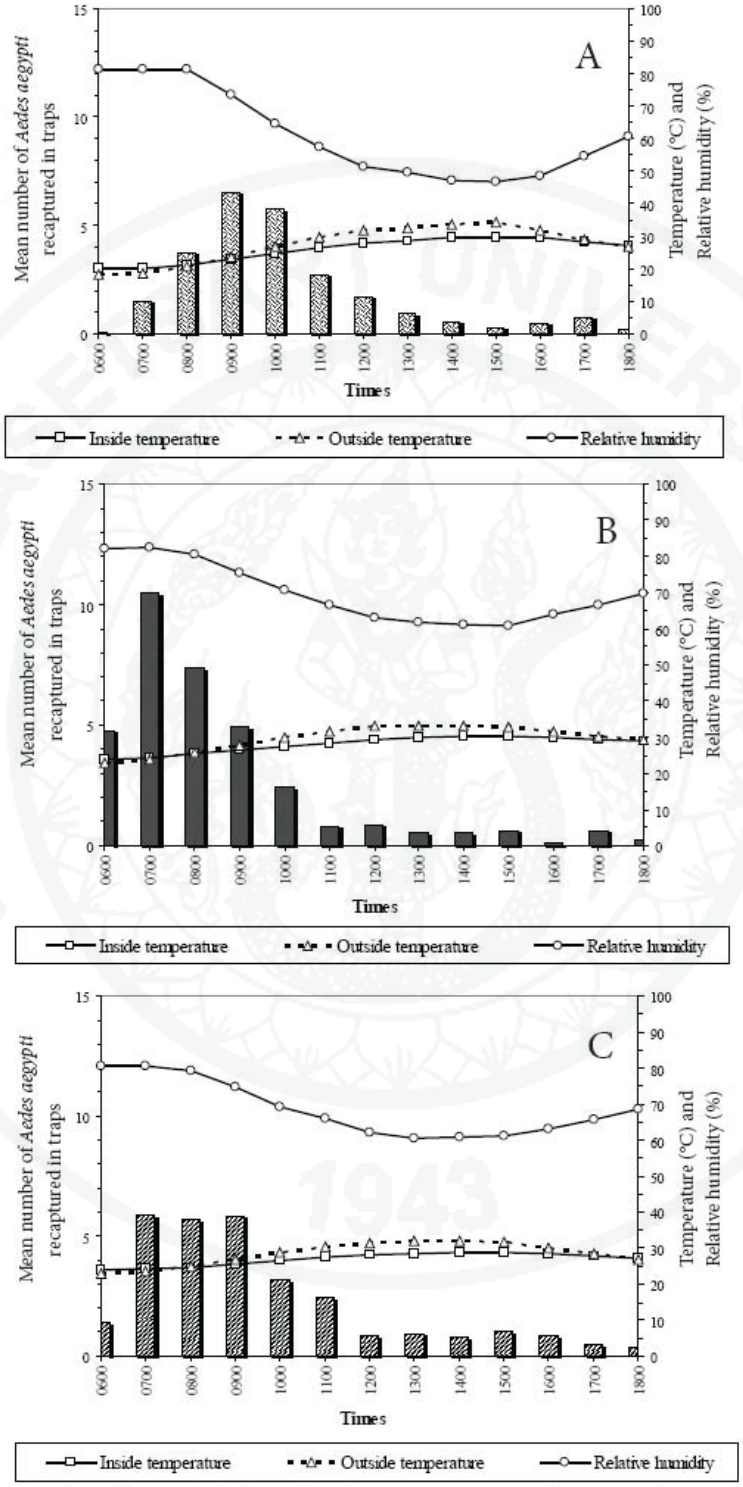

Figure 2. Average number of *Ae. aegypti* collected in entrance traps compared to temperature and relative humidity during a 12-h collection in winter (A), summer (B), and rainy season (C).

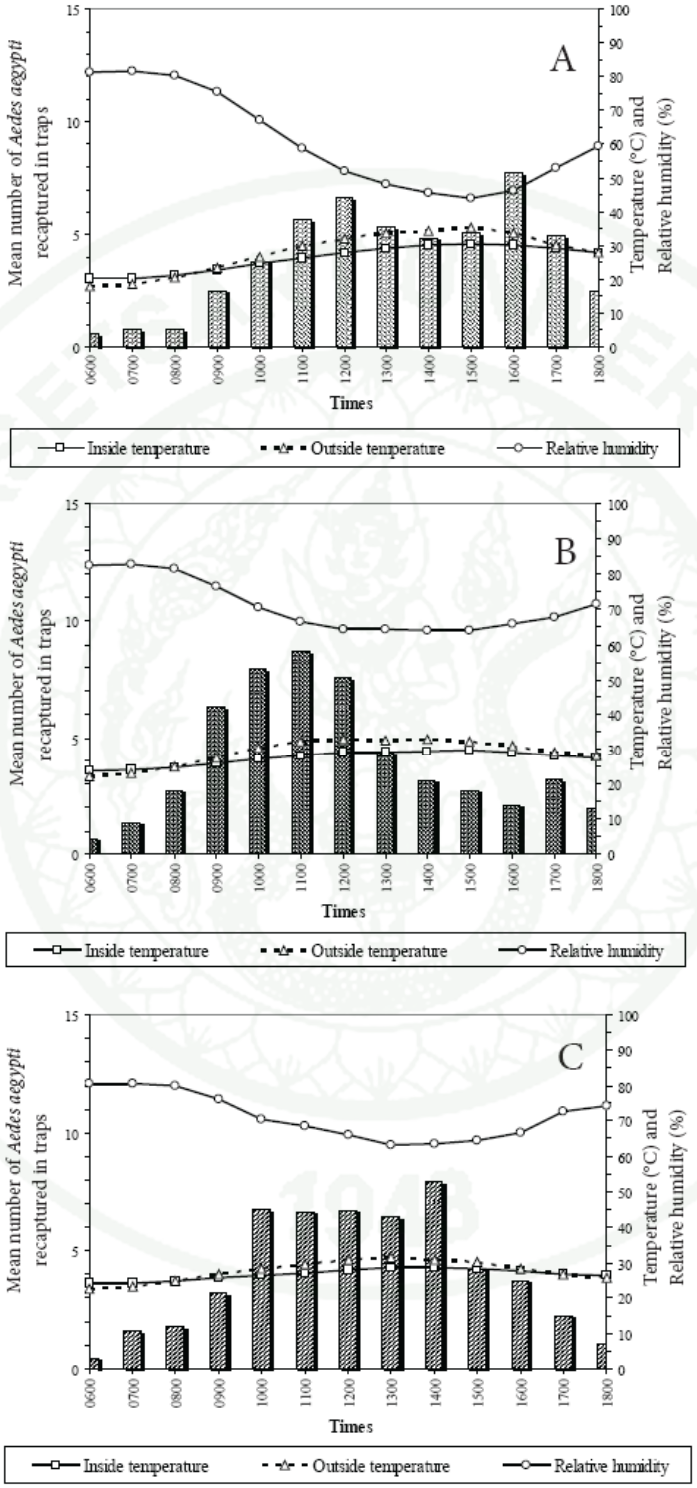

Figure 3. Average number of *Ae. aegypti* collected from exit traps compared to temperature and relative humidity during a 12-h collection in winter (A), summer (B) and rainy season (C).

tal parameters into and out of experimental huts in Thailand. Such information helps to define the vector capacity and relative risk for disease transmission, as well as supports the appropriate vector prevention and control strategies. A better understanding of vector biology and behavior is needed to guide vector control programs and evaluate chemical control strategies. In this study, we utilized the mark–release–recapture technique to evaluate the movement patterns of *Ae. aegypti* into and out of the experimental huts fitted with entrance and exit traps.

Many studies conducted on the host preference of *Ae. aegypti* suggest that this species has a high propensity for feeding on humans inside houses, thus characterizing it as strongly anthropophagic (Gubler and Kuno 1997, Harrington et al. 2001, Polawat and Harrington 2005). It would seem that an endophagic and endophilic species such as *Ae. aegypti* would be congregated in distinct locations and thus make it easier for vector control personnel to control them. However, various internal and external factors seem to impede the success of controlling *Ae. aegypti* inside homes. Mosquito behavior is one of those factors that plays a significant role, and it in turn is generally affected by changes in the environmental and biological conditions. The natural environment imposes a number of pressures that will impact mosquito behavior, including ambient temperature and relative humidity (Kennedy 1946, Busvine 1964, Drobozina et al. 1984). This study has increased our knowledge of how temperature and humidity affect the movement patterns of *Ae. aegypti* into and out of homes.

Previous reports suggest that entering behavior was much stronger when a human host was present in the hut compared with a dog or no host at all (Suwondkerd et al. 2006). In addition, the movement patterns of *Ae. aegypti* females vary according to outdoor temperature as reported by Chareonviriyaphap et al. (2005) and Suwonkerd et al. (2006). Vector control professionals need to carefully design and improve methods to effectively reduce mosquito populations and risk of disease transmission. In this study, two years of observations on the normal movement patterns of *Ae. aegypti* females were evaluated and it was found that temperature and relative humidity play a major role in altering these patterns.

This study showed that the entering and exiting patterns of *Ae. aegypti* followed unimodal periodicities with the peak of entering taking place between 09:00 and 12:00 in winter and between 06:00 to 09:00 in both the summer and the rainy season. These results are quite similar to those reported by Chareonviriyaphap et al. (2005) and Suwonkerd et al. (2006). In contrast, *Ae. aegypti* human landing collections result in either a bimodal or trimodal periodicity (Corbet and Smith 1974, Chadee 1988, Thavara et al. 2001, Atmosoedjono et al. 1972, Chadee and Martinez 2000).

The time of entering in winter was delayed compared to what was found in either the summer or the rainy season. This delay is most likely the result of the colder temperatures that occur during the winter which have been found to negatively impact the flight activity of *Ae. aegypti* (Clements 1999, Rowley and Graham 1968). In this study, it is quite clear that lower temperatures (as low as 12° C) and lower relative humidity in the morning impact flight behavior. Therefore, the peak of entering the huts during the winter was shifted to later in the morning. The peak of exiting the hut in this study occurred between 10:00 and 14:00 for all seasons. This result is in agreement with those reported by Chareonviriyaphap et al. (2005) and Suwonkerd et al. (2006). The extreme temperature and decreased relative humidity inside the hut in the afternoon appears to force the *Ae. aegypti* females to seek suitable outdoor resting sites.

Despite considerable progress in our understanding of vector biology, there remains much to understand about the biology and behavior of *Ae. aegypti* and how external factors influence disease transmission. The behavior of mosquitoes is impacted by several factors including climatic, environmental, and physiological factors. This study demonstrates how the entering and exiting behaviors of *Ae. aegypti* are affected by ambient environmental factors such as temperature and humidity. It is clear from this study that conditions in the peridomestic environment play a critical role in where mosquitoes will be found throughout the day. If the conditions are unsuitable inside the house (high temperature and low humidity), *Ae. aegypti* will seek more conducive resting sites outdoors. Likewise, the outdoor conditions may delay movement inside if temperatures are too cold for flight. Knowing where the mosquitoes are likely to be in the peridomestic environment based on environmental conditions may aid in control efforts by more precisely directing particular interventions. This information on how climatic factors influence mosquito behavior will also be useful when designing future studies and serve as a baseline for the natural movement patterns of *Ae. aegypti* when evaluating the response of this vector to test compounds.

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