

Factors influencing the seasonal abundance of *Aedes (Stegomyia) aegypti* and the control strategy of dengue and dengue haemorrhagic fever in Thanlyin Township, Yangon City, Myanmar

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Abstract. From June 2006 to May 2007, mosquito surveys were conducted in Thanlyin Township, Yangon City, Myanmar, to determine factors influencing the abundance of *Aedes aegypti* (*Stegomyia aegypti*) during the rainy season. Both the biological and environmental factors were included in this study. Increase in the hatchability of egg, larval survival rate, the shortened larval life-span and increased pupation rates supplemented by rainfall (i.e. continuous flooding of the containers, stimulate the continuous hatching of eggs) were observed for correlation with the increase in population density of *Ae. aegypti* during the rainy season in the study area. Control strategy of *Ae. aegypti* to analyze the infestation in the community (study area) with larval *Ae. aegypti*, integrated management measures including health education, attitudes and practices regarding dengue and dengue haemorrhagic fever, transmission of the disease and possible preventive measures, reduction of breeding sites and testing the efficacy of *Bacillus thuringiensis israelensis* (*B.t.i.*) with respect to the reduction level of *Ae. aegypti* larvae in breeding sources, were taken into consideration.

INTRODUCTION

Several countries continue to experience endemic and re-emerging dengue fever (DF) and dengue haemorrhagic fever (DHF) (Gubler, 1997). In Myanmar, outbreaks of DHF were first recognized in the Yangon Division in 1970. A marked seasonal peak had occurred during the monsoon months of June, July and August in the 35 years.

The principal vector is *Aedes aegypti* in urban areas and the secondary vector is *Aedes albopictus* (*Stegomyia albopicta*) in the suburban/rural areas. *Aedes aegypti* has spread throughout the tropics and into susceptible human populations especially in urban areas. In spite of continued efforts in

control measures, dengue cases in Myanmar recently increased between 2003–2006 (5621 cases in 2005 and 11,049 cases in 2006, Health Ministry, Annual Report). This may be most likely due to a combination of factors including the increase in economic activities in the urban and suburban zones. In addition, traditional water storage practices contribute to the increase in the availability of breeding sites for *Ae. aegypti*.

Aedes aegypti, a day biting mosquito, is highly anthropophilic and often resides in and near human dwellings (Christophers, 1960; Yasuno & Tonn, 1970; Gubler, 1997; Thavara *et al.*, 2001). This mosquito has been found to be highly adapted to all man-made and natural environments and is extremely

efficient as a primary vector of dengue (Rodhain & Rosen, 1997; Chareonviriyaphap *et al.*, 2003; Vazeille *et al.*, 2003).

Design of a control strategy for vector mosquitoes included the sampling of ecological data with special emphasis on the breeding sites of *Ae. aegypti* which is of major importance. These data combined with the available epidemiological data, may provide an emerging concept of the relation between occurrence of vector mosquitoes and frequency of disease incidences. As DF and DHF cases are correlated to the density of *Ae. aegypti* present in the locality (Yangon Child Hospital Annual Report 2002 to 2005), factors influencing the seasonal abundance of the vector need further investigation. This research project (June 2006-May 2007) was initiated to assist the control strategy of *Ae. aegypti*, by conducting detailed studies of all the factors influencing the seasonal fluctuations in the developing stages of larval densities.

The objectives of this study were: To determine the individual factors that could influence the seasonal abundance; to analyze the larval *Ae. aegypti* infestation in the community; to understand the people's knowledge, attitudes and practices regarding DF and DHF, the transmission of the disease and possible preventive measures and to test the efficacy of *Bacillus thuringiensis israelensis* (*B.t.i.*) tablets against the larvae of *Ae. aegypti*.

MATERIALS AND METHODS

Study Area

The southern part of Thanlyin township (Daragar ward) was selected as the study area (Figure 1), a township in Yangon City having a high prevalence of DHF cases (Yangon-Child Hospital, 2002). The study area, ~8.5km² with ~4,500 people living in 550 homes, contains various types of housing depending upon the socio-economic status of the community.

Three types of housing units can be categorized in the study area: (a) bungalows with separate compounds, (b) single buildings

without compounds and (c) squatter settlements-mostly common living units, comprising of huts built close to each other.

The houses in the first two types consist of (i) brick, (ii) brick and wood or (iii) mostly wooden. In the squatter settlements, most of the houses are made of bamboo and wooden planks with thatch roofs.

Almost all the family units occupying these houses have either metal drums or glazed clay jars to store water. These domestic water storage containers are found even in houses which have piped water systems, as the water supply at its best, is very unreliable.

Selection of family units for the study

Of the total number of family units within the study area, 150 were selected on the basis that these units are not concentrated into a particular sector of the study area. The selection was based on proportional allocation (population-to-sites method) in the strata characterized by socio-economic status and types of housing.

Study methods

Survey of mosquito breeding sites (larval indices)

All potential breeding sites within the selected 150 family units, were examined monthly to observe the increase or decrease in potential breeding sites by season. The breeding sites were then categorized as "major" and "minor" sources. Metal drums (55 gallon capacity: either ½ or full sized), glazed or unglazed clay jars (up to 30 litres) were considered as "major" breeding sites. Other sources such as flower vases, discarded utensils, ant-guards, old car tyres and batteries were collectively considered as "minor" breeding sites. Seasonal comparison of numbers of both the major and minor breeding sites were made.

The infestation rate of mosquito larvae were determined by visual larval survey techniques (Service, 1976) and 3 indices were computed per sampling area, namely: House Index (HI), Container Index (CI) and Breteau Index (BI).

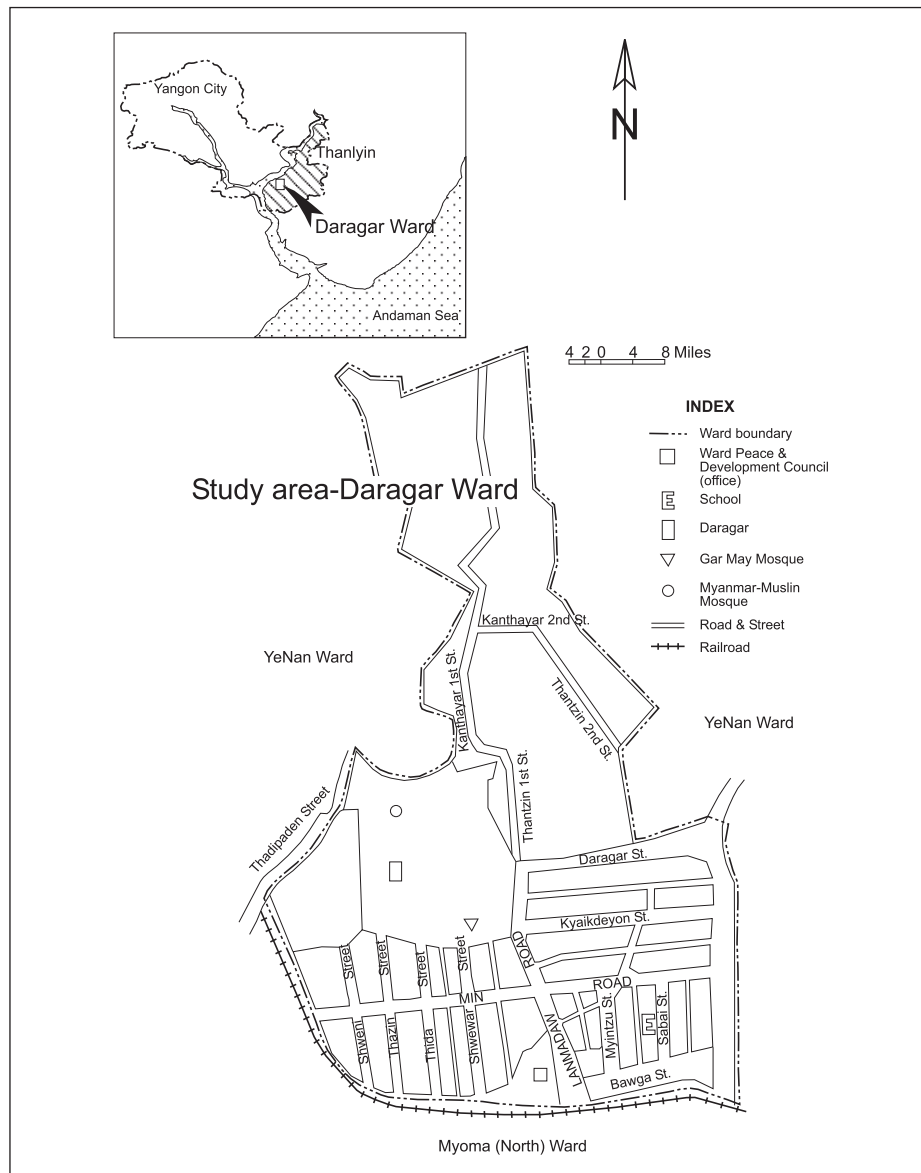


Figure 1. Location map of the study area (Thanylin Township) in Yangon City, Myanmar

Larval life span

The average larval life span was calculated seasonally from 5 randomly selected metal drums. After emptying the drums, the insides were carefully wiped to destroy all eggs previously laid. The drums were filled with water and 500 *Ae. aegypti* eggs were added (to estimate hatching). The drums were covered with cloth to prevent further oviposition. All pupae were collected and the number of days as larvae were compared seasonally.

Assessment of chemical quality of breeding sites

Examination of breeding sites was conducted seasonally from 10 randomly selected drums. One hundred cc of water was collected in sterile screw-capped bottles and examined the same day to determine levels of pH, nitrates, dissolved oxygen, sodium chloride, sulphate, chlorine, ammonia, iron and water hardness.

Larval food supply

Larval food supply was studied by collecting water samples from drums with varying degrees of larval infestation. Samples of 100cc were collected after stirring the water and centrifuging for 20 min at 2000 rpm. The sediments were examined under a compound microscope for micro-fauna. The sediment was also diluted with 10cc of distilled water and placed in a Red Blood Cell counter to obtain quantitative data on the micro-fauna. A guide for the identification of micro-fauna (Edward D. Ruppert: Invertebrate Zoology 1994, Saunders College, U.S.A.) was used.

Recording of meteorological data

Meteorological data such as rainfall, relative humidity, maximum and minimum temperatures and daily sunshine hours were obtained from the Department of Meteorology and Hydrology, Yangon, throughout the study. In the study area, maximum rainfall was recorded during the Southwest monsoon, peaking in August. The maximum daily temperatures ranged between 27° and 30° during the Southwest monsoon, and 31° and 32° during the Northeast monsoon seasons.

Adult mosquito collections

Adult collections were made bi-weekly. To undertake the adult landing catches, 5 catching stations were randomly selected according to the ratio of types of housing in the study area. Twenty-minute collections of landing and resting mosquitoes were conducted at each station between the peak *Ae. aegypti* biting hour of 08:00-11:00 a.m. All mosquitoes collected from the landing stations were identified according to the "Illustrated keys to the medically important mosquitoes of Myanmar" (1994) and Becker *et al.* (2003, 2010).

Control of dengue vectors

Fifteen metal drums (3 test series, each with 5 water-storage containers) were selected as artificial breeding containers for semi-field tests in the study area. These water containers were filled with 25 liters of tap water and 50

early fourth-instar larvae of *Ae. aegypti* were placed into each container. The larvae used in the semi-field tests were obtained from major breeding sites of the study area. First test series of 5 water containers were treated with half of Culinex/Vectobac DT-*B.t.i.* tablet (0.192g/half tablet), the 2nd test series of 5 containers, each with one tablet (0.384g/tablet) and the 3rd series of 5 containers served as control. The weight of each tablet was 0.384g/tablet (activity is 3000 ITU/mg; lot-no: 0601, Culinex GmbH, Germany).

After 24 hours, all of the still live or dead larvae were collected with a pipette to determine the mortality rate. Afterwards, 5 liters of water were taken from each container and replaced with fresh water to simulate normal conditions. Finally, 50 fourth-instar larvae were added again to each container. Mortality rate of the larvae was evaluated daily, starting from the application of the tablets until a mortality rate of <50% was reached.

RESULTS

Entomological and ecological results

The results indicated that all *Ae. aegypti* larval indices increased during the rainy season as compared with cool-dry and hot-dry seasons (Table 1). As is also shown in Figure 2, there were no significant differences in the number of major breeding sources between each month or between different seasons. The larval life span was shortened and the pupation rate was increased during the rainy season (Table 2).

On detailed examination of sediments and organic debris occurring in the breeding sources, *Rotifer vulgaris*, *Paramecium* spp. and *Vorticella* spp. were found to be the predominant micro-fauna. Other organisms such as *Euglena* spp., *Volvox auscus*, *Selpina macracantha*, *Eucharis dilata*, *Diglena forcipata*, *Monostyla bulla* and few nematode larvae were also observed. In those drums where the *Ae. aegypti* larval density was high, the density of *R. vulgaris* and *Paramecium* spp. were always found to be

Table 1. Entomological indices of the study area compared with major breeding sources

Season	Container Index	House Index	Breteau Index	Adult density (man/hour) Mean & standard deviation	Larval Density Mean & standard deviation	Average number of major sources Mean & standard deviation
Rainy	48.68	77.27	132.7	15.8 ± 3.51	97.9 ± 5.42	258.4 ± 10.95
Cool/dry	35.51	63.0	72.7	9.5 ± 1.10	69.8 ± 0.94	158.25 ± 68.92
Hot/dry	15.8	25.7	33.7	3.4 ± 0.49	50.1 ± 2.82	73.66 ± 14.72

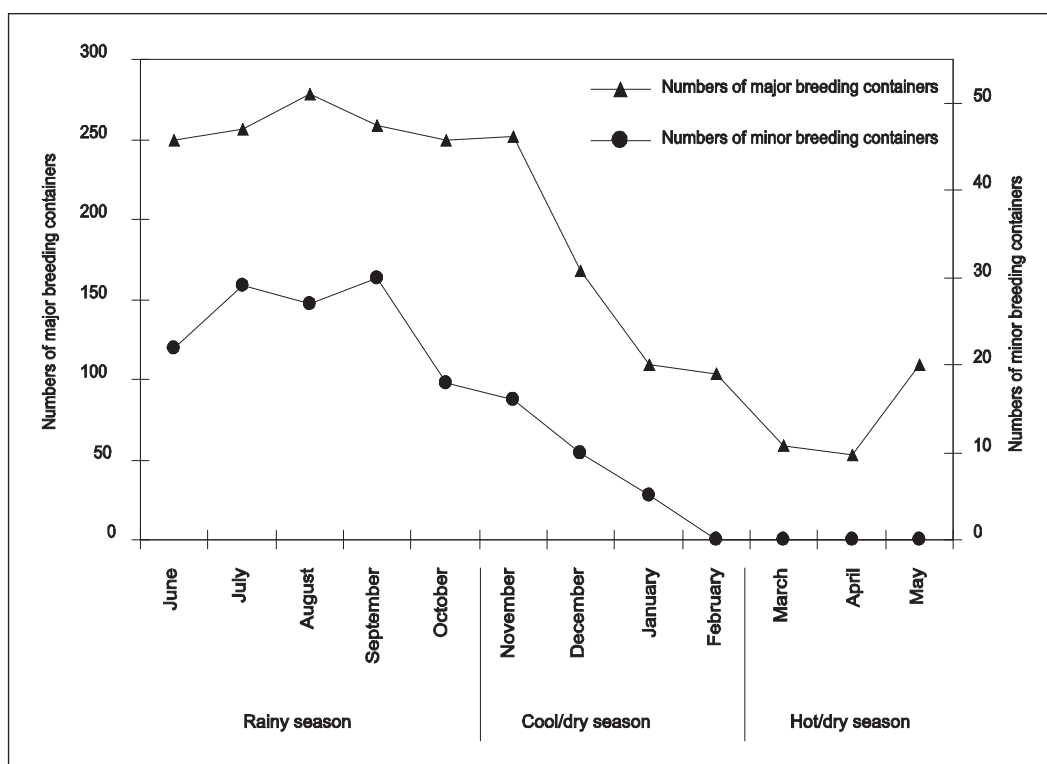


Figure 2. Containers most commonly found positive for *Aedes aegypti* larvae in the study area

Table 2. Seasonal comparison of larval life span and pupation rate in the study area

Season	Types of container	Numbers of eggs tested	Pupated on (duration)	Numbers of pupated Mean & standard deviation	Percentage of pupation rate Mean & standard deviation
Rainy	5 metal drums	500x5	9.5 days	126 ± 2.92	25.2 ± 2.33
Cool/dry	5 metal drums	500x5	17.5 days	71.2 ± 4.09	14.24 ± 0.84
Hot/dry	5 metal drums	500x5	14.5 days	72 ± 5.96	14.4 ± 1.19

correspondingly high. The presence of abundant food supply probably supports a correspondingly abundant larval population.

Water chemistry of the breeding sources was studied seasonally, no significant difference was noted and no correlation was determined between water chemistry and larval breeding (Table 3).

Control Results

Average mortality rate of *Ae. aegypti* larvae in the semi-field tests are summarized in

Figure 3 and Table 4. Nearly 100% mortality rate was obtained during the 3rd week of monitoring. During the 4th week after the application, some larvae (16%) survived the treatment. During the 5th and 6th week following application, the efficacy of *Culex/Vectobac DT-B.t.i.* tablets became less effective. On the 7th week after the application, the mortality rate was <50% and the survey had to be terminated. In the control tests, the mortality rates were between 0 and 1.2% (Table 4).

Table 3. Chemical analysis of breeding - water samples in the study area

Season	M.D of L/dip	Iron	Sulphate	Nacl (ppm)	Chlorine (ppm)	Nitrates (ppm)	Dissolved O2	Ammonia	Total Hardness	pH
Rainy										
July	30.8	0.08	1	100	0.05	0.5	0.7	0.065	17.16	7
September	20.8	0.08	1	100	0.05	0.5	0.8	0.07	17.16	7
Cool-dry										
November	1.7	0.07	2	150	0.05	0.5	0.72	0.054	17.16	7
January	1.5	0.08	1	100	0.05	0.5	0.66	0.065	17.16	7
Hot-dry										
March	0.5	0.08	1	150	0.05	0.6	0.65	0.07	17.16	7
April	0	0.08	2	250	0.05	0.6	0.75	0.05	17.16	7

M.D of L/dip = Mean Density of Larvae - pupae /dip

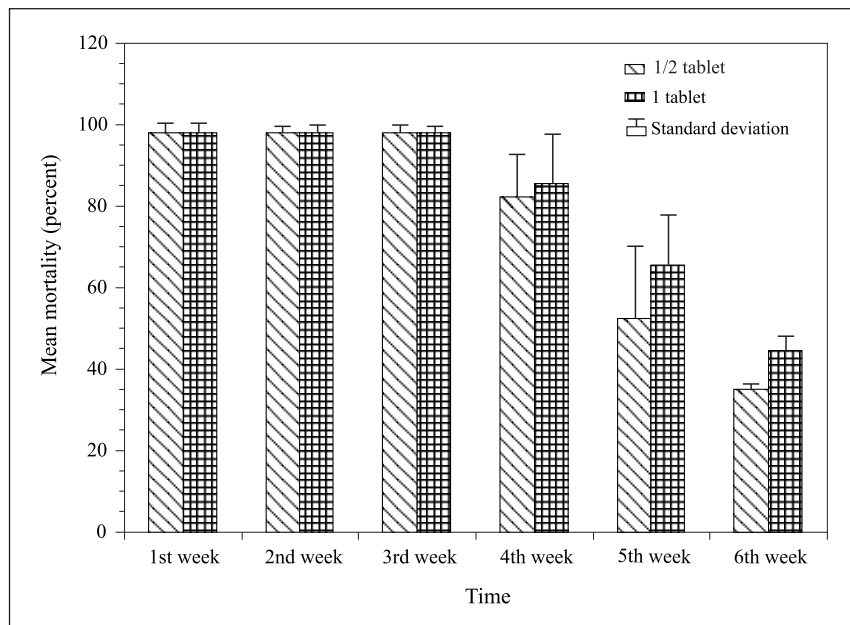


Figure 3. The efficacy of *Culex -B.t.i.*-tablets against *Aedes aegypti* larvae in the study area (dosages: 1/2 tablet = 576,000 ITU/container; 1 tablet=1,152,000 ITU/container).

Table 4. The efficacy of Culinex- *B.t.i.*-tablets against *Aedes aegypti* larvae in the study area

Tablet dosage	Mean mortality in percent and standard deviation					
	1st week	2nd week	3rd week	4th week	5th week	6th week
1/2 tablet	98.0 ± 2.45	98.0 ± 1.45	98.0 ± 2.45	82.4 ± 9.45	52.4 ± 18.19	35.0 ± 1.35
1 tablet	98.0 ± 2.25	98.0 ± 2.10	98.0 ± 1.45	85.6 ± 12.10	65.6 ± 12.67	44.7 ± 3.75
Control	0.2 ± 1.10	0.2 ± 1.75	1.2 ± 1.75	1.0 ± 1.10	1.2 ± 1.75	1.2 ± 2.25

DISCUSSION

Epidemics of DF and DHF occurred typically during rainy season when the *Ae. aegypti* population increased. In the study area, *Ae. aegypti* indices increased during the rainy season (see Table 1 and Figure 2) as compared to the cool/dry and hot/dry seasons (Breteau Index, larval density, etc.). Our results correlated with the findings of various researchers (Christophers, 1960; Tonn *et al.*, 1970; Myat Myat Thu, 1975). The increase in major breeding sources, such as metal drums and glazed pots, during the monsoon was also observed by Myat Myat Thu (1975) and Tonn *et al.* (1970). Tonn's study in Bangkok stated that "any increase in population size should result in an increased number of positive habitats and not just an increase in the density of larvae in the habitats already occupied".

In the study area, the type of containers investigated, were classified as a major breeding containers (primary breeding sites) and minor breeding containers (secondary breeding sites), seasonality and different months of the year (Figure 2). The primary breeding sources during pre-monsoon season were metal drums, cement tanks and non-removable glazed pots which served as breeding foci for *Ae. aegypti*. With the onset of monsoon *Ae. aegypti* larvae breed not only in these foci but also in "secondary foci" such as discarded tyres, etc. During the post-monsoon period when the secondary foci dried-up, the breeding of *Ae. aegypti* shrank to original foci (various containers). This finding was in agreement with Katyal *et al.* (1996).

The larval density of *Ae. aegypti* increased from April through August (during the rainy season) and the Breteau indices

(house and container) remained very high. This increased the likelihood of transmission of DF and DHF in this area. It was found that during the monsoon, more positive containers were found harbouring more larvae. The reasons for the increased larval population could be explained by the following factors (see also Figure 4).

(i) *Larval life span and population rate:* The larval life span during the monsoon season was found to be 9-10 days and was much shorter than the cool/dry and hot/dry season (Table 2). Pupation rate which determines the survival of larvae during different seasons also showed a much clearer picture of the seasonal difference. The pupation rate during monsoon was 25.2% and this rate was found to be nearly twice as that of the other two seasons, *this was one of the most important factors*. Southwood *et al.* 1972 (WHO "Ae. Research Unit"), working on the life budget of *Ae. aegypti* in Bangkok, also found that population changes depended more on the mortality rates of immature stages rather than on the variation in the number of eggs laid. This finding of increased larval survival influencing the adult population, is in agreement with the results of this study.

(ii) *Larval food supply:* Upon detailed examination of sediments and organic debris (main larval food) in containers harbouring different larval concentrations, it was found that availability and abundance of food has a marked influence on the larval density. The containers with high counts of micro-fauna, harbour the highest number of larvae. The main larval foods identified were, unicellular organisms such as *Paramecium* spp. and *R. vulgaris*. Southwood *et al.* (1972) working

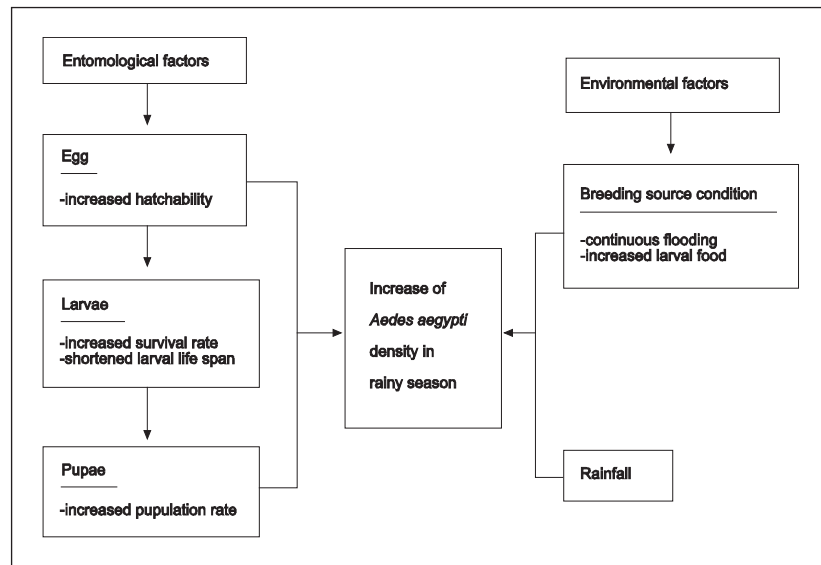


Figure 4. Factors influencing or associated with seasonal density of *Aedes aegypti* in Thanlyin township, Yangon City

in Bangkok, found that larval life span and survival rates were determined by availability of food. This is agreement with the results obtained in this study.

In summing up the results obtained, the main factors influencing the seasonal abundance of *Ae. aegypti* in the study area are the increased survival rate of larvae, hence the resultant increase in pupation rate and the shortened larval life span during the monsoon.

Increase in the hatchability of eggs during the monsoon season is also another important factor that could contribute to the increase in density of *Ae. aegypti*. In addition, we noted that the major breeding sources (metal drums) which are usually placed under roof gutters just outside the houses, are usually replenished by rainfall. During the peak monsoon months of June, July and August, it rains almost daily and these drums placed under the roof gutters are continuously replenished. The eggs of *Ae. aegypti* laid on the insides of these major breeding sources are in contact with water most of the time and the stimulus for egg laying and hatching is prevalent.

Likewise, most of the breeding sites that were identified in the study are the ones

classified as artificial breeding sites which are usually provided by the inhabitants, *Ae. aegypti* breed primarily in metal drums (65.5%), glazed clay jars (20.06%), and earthen pots (4.98%), all containing stored water for daily domestic use. Seasonal surveys of all indoor and outdoor breeding habits of *Ae. aegypti* in the Thanlyin area, indicated >90% breeding in domestic water-storage containers and the remaining in miscellaneous receptacles. Thus, the mosquito breeding sites can be controlled and prevented by the local community governed by strictly following appropriate sanitation and hygienic practices.

The main methods of controlling dengue vectors are: (1) reduction of breeding sites by means of environmental sanitation. This refers to the drastic reduction of all non-essential water-storage receptacles that serve as breeding sites of *Ae. species*. This is the most effective method in terms of longterm reduction of the mosquito population; (2) protection of water containers, for example, by placing tightly-covered lids to prevent egg laying by mosquitoes; (3) introduction of larvivoracious fish; (4) observation of a “Weekly Dry Day”; meaning that the containers are to be emptied and cleaned (removal of eggs) at least once/

week; (5) cleaning the containers before and after the rainy season can also contribute in reducing mosquito populations; and lastly, (6) space application with Malathion against adult mosquitoes and larviciding with Temphos, the most common methods practiced in Myanmar.

Ultra low volume (ULV) application of adulticides, an additional method recommended for emergency control during disease outbreaks, is usually conducted by health service personnel. Apart from the numerous advantages, however, the application of chemicals also has risks which should not be underestimated. Health service personnel have to enter the premises in order to apply the insecticides, whilst the residents have to leave their houses for the duration of the control operation. The continued use of traditional insecticides should be discouraged. One reason for this is that it is irksome for people to have to take additional measures to avoid insecticide contamination, having to cover up all foodstuffs (Becker, 1992) and the development of insecticide resistance.

Other important reasons are increased environmental awareness and the frequently negative attitude towards the unpleasant odour of many of the products used for control. Moreover, because of their toxicity, these chemicals must be dispensed with additional precautionary measures.

Therefore, in conclusion, there is a need to develop new approaches such as "Integrated Vector Management (IVM)" practices for controlling mosquitoes in the vicinity of humans. Products based on microbial agents such as *B.t.i.* and Culinex Tablets might be promising in the fight against dangerous diseases such as DHF in the tropics (WHO, 1983; Becker *et al.*, 1991). Community education and active participation by the community can result in significantly decreasing mosquito populations as well as reducing the potential for disease transmission.

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