LIFE TABLES STUDY OF IMMATURE *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE) DURING THE WET AND DRY SEASONS IN PENANG, MALAYSIA

Nur Aida Hashim, Abu Hassan Ahmad, Che Salmah Md Rawi, Nurita Abu Tahir and Norsamah Basari

School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia

Abstract. Life tables were constructed for twelve cohorts of immature stages of the dengue vector *Ae. albopictus* in a wooded area of Penang, Malaysia. The development time of *Ae. albopictus* ranged from 6 to 10 days depending on the mean environmental temperature ($r=-0.639$, $p<0.05$). Total cohort mortality was correlated with total development time ($r= 0.713$, $p<0.05$) but not temperature ($r=-0.477$, $p>0.05$). Rainfall was correlated with neither development time ($r= 0.554$, $p>0.05$) nor mortality ($r=0.322$, $p>0.05$). There was a significant difference among the total mortality that occurred in the twelve cohorts ($H=119.783$, $df=11$, $p<0.05$). There was also a significant difference in mortality among the different stages ($H=274.00$, $df=4$, $p<0.05$).

INTRODUCTION

Before any integrated approach to the control and management of disease vector species of mosquitoes can be implemented, it is important to understand their population dynamics and how they exist in nature. Development times and rates of survival for various stages of mosquitoes are of particular importance. Once these parameters are established, we can better understand their populations dynamics. Models can be constructed to predict growth in certain conditions and the feasibility of the different approaches to control.

Larval size and duration of larval development are influenced by temperature, food supply, crowding and sex (Estrada-Franco and Craig, 1995). In many insects, mortality rate varies according to the developmental stage (Southwood, 1978). Knowledge of the immature stages and the mortality factors affecting each stage may assist in control measures (Harcourt, 1969).

Correspondence: Abu Hassan Ahmad, School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.
E-mail: aahassan@usm.my

Numerous studies have been carried out on *Ae. albopictus* biology, but few studies have attempted to evaluate survival of the immature stages of *Aedes albopictus* in the field. Understanding population dynamics requires considerable quantitative data, such as life table statistics, which have been largely lacking for this species. The present study is an attempt to fill this need. The objective of this study was to construct a seasonal life table for *Ae. albopictus* immature stages and to determine their relationship with environmental conditions, such as temperature and rainfall, under field conditions.

MATERIALS AND METHODS

Study site

Aedes albopictus eggs used in this experiment were collected from a wooded area, Lurah Burung, Universiti Sains Malaysia, Penang by using ovitraps. The life table experiments were carried out in Lurah Burung to ensure that all cohorts developed under natural conditions.

Sampling method

Ten 2.5 liter glass jars with a diameter of 15.0 cm and height of 27.0 cm were used to construct the *Ae. albopictus* life table in the field. The glass jars were filled with 500 ml tap water in order to provide a water medium, suitable for the growth of larvae until the pupal stage.

Aedes albopictus eggs on paddles were submerged in dechlorinated water in a large plastic container measuring 37 x 27 x 10 cm. The container was then covered with a thin cloth to prevent other mosquitoes from ovipositing on the water and placed outside in the field for hatching. The eggs on the paddles hatched in 24 hours and the number of dead and live larvae were recorded. The paddles were immediately removed from the plastic container to ensure that all larvae were uniform first instar larvae.

The 1,000 first instar larvae that hatched were separated into 10 glass jars containing 500 ml water. A maximum of 100 larvae were placed into each glass jar, along with some larval feed. Growth of the larvae was followed until adult emergence.

This study was carried over a period of 12 months, from January 2003 until December 2003. The second week of each month was chosen to conduct the experiments. In each experiment, 1,000 larvae were separated into 10 glass jars that served as replicates. In the pupal stage, all individuals were removed from their containers and brought back to the laboratory. These containers were placed in cages measuring 30 cm x 30 cm x 30 cm. All pupae were reared until they reached the adult stage. The sex ratio was determined by counting the number of adults that emerged.

Meteorological data, such as total daily rainfall, relative humidity and air temperature, were obtained from the Bayan Lepas Meteorological Service station.

The larval feed used was a culture medium consisting of cow liver, dog biscuits, skim milk powder and yeast in a ratio of 1:1:1:1. A small portion of the feed, about 1.0 mg, was added to the larvae in each glass jar daily since too large portions of feed in the jars may effect the survival of the larvae. A large amount of feed may also cause a disturbing odor and coat the surface of the water with oil.

Data analysis

All jars containing the larvae were placed in the field and covered with a thin cloth to avoid other mosquitoes ovipositing in the jars. The development of each immature stage from the 1st instar to the 4th instar and up to the pupa stage were observed. Observations on larvae development was conducted daily from 09:00 AM to 10:00 AM. The number of larvae that survived (l_x) were counted and recorded. From this data, the number of dead larvae (d_x) can be calculated. Other life table parameters, such as q_x, p_x, L_x, T_x and e_x, can also be determined.

Formulations for these parameters are:

$$q_x = \frac{d_x}{l_x}$$

$$p_x = \frac{l_{x+1}}{l_x}$$

$$L_x = \frac{l_{x+1} + l_x}{2}$$

$$T_x = e_x\, l_x \text{ or } L_{x+1} + L_x$$

$$e_x = \frac{T_x}{l_x}$$

The results were analyzed with Kruskal-Wallis and Correlation tests using SPSS 12.0 for windows.

RESULTS

Studies of the seasonal patterns of mortality and survival of immature *Ae. albopictus* were conducted by following the progress of 12 cohorts of *Ae. albopictus*; each cohort started with 1,000 first instar larvae.

Larval development time

Development times from first instars to adults varied among cohorts. Cohort 8 (August) and cohort 10 (October) took the longest time (10 days) to complete their development (Fig 1). The shortest development time of 6 days occurred in cohorts 3 (March), 4 (April) and 5 (May) (Fig 1). Seasonal patterns were detected in the duration of instar stages and total development times for the different cohorts. There were significant differences among the pre-adult development times, stages (H=274.00, df=4, $p<0.05$) and cohorts (H=119.783, df=11, $p<0.05$).

During this study, the daily mean temperature varied from 22.2ºC to 27.2ºC. The development time decreased as temperature increased. The mean development time for each cohort was significantly correlated with temperature (r=-0.639, $p<0.05$) (Fig 1), indicating temperature influenced development time in immature *Ae. albopictus* in the field.

Total rainfall during the study varied from month to month. The seasons were divided into dry and wet seasons based on the total monthly rainfall in the study area (Fig 2). The dry seasons were in January, February, April, May and December, 2003. The wet seasons were in March, and June to November, 2003.

Total rainfall during the study varied from month to month. However, total rainfall did not influence the time immature *Ae. albopictus* took to complete their development (Spearman's rho correlation test , r = 0.554, $p>0.05$) (Fig 3).

Table 1 shows the average time needed for each immature *Ae. albopictus* to complete their development. The first larval instar took the shortest time of 1 day to complete its development during both dry (January, February, April, May and December) and wet (March and June to November) seasons. A period of

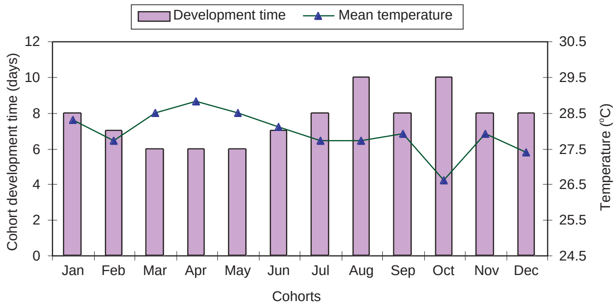

Fig 1–Larval development time for 12 cohorts of *Ae. albopictus* with the mean average temperature during development for each cohort in the field in the year 2003.

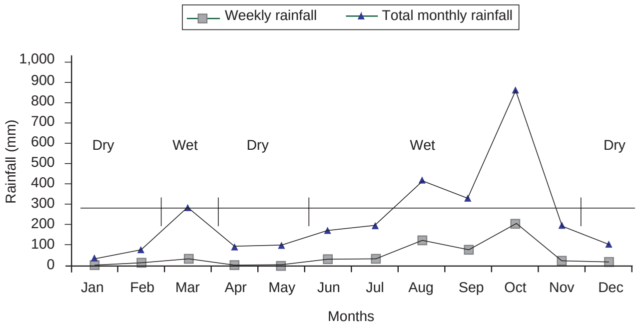

Fig 2–Total weekly rainfall and total monthly rainfall during the life table study.

Table 1
Average time in days needed by immature *Ae. albopictus* to complete their development in the field during the dry and wet seasons.

Stage	Average in days ± SD	
	Dry (n=5)	Wet (n=7)
1st instar	1.00 ± 0.00	1.00 ± 0.00
2nd instar	1.00 ± 0.00	1.42 ± 0.53
3rd instar	1.40 ± 0.55	1.86 ± 0.90
4th instar	1.42 ± 0.55	1.86 ± 0.38
Pupa	2.00 ± 0.00	2.00 ± 0.00
Total development time	6.82 ± 1.10	8.14 ± 1.81

n = Number of months

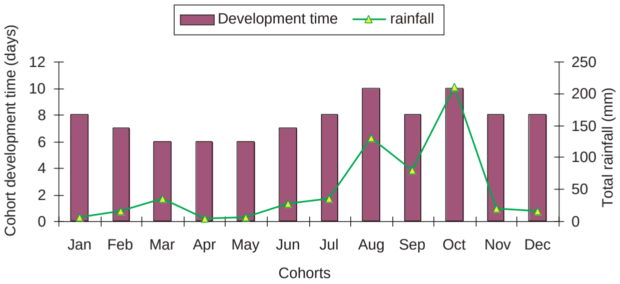

Fig 3–Development time for 12 cohorts of *Ae. albopictus* in relation to total rainfall during development of each cohort.

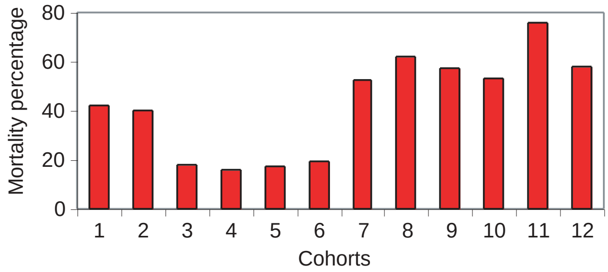

Fig 4–Mortality percentage (%) throughout development of 12 cohorts of immature *Ae. albopictus*.

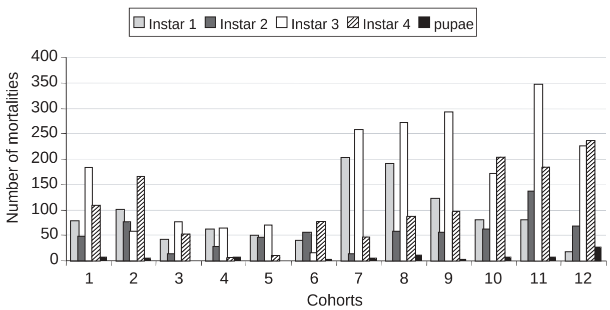

Fig 5–Total number of mortalities according to different stages in 12 cohorts of immature *Ae. albopictus*.

one day was needed for the second instar to complete its development during the dry season and 1.42 ± 0.53 days were needed during the wet season. The third instar needed 1.40 ± 0.55 days in the dry season and 1.86 ± 0.90 days during the wet season to complete their development. The fourth instar took 1.42 ± 0.55 days and 1.86 ± 0.38 days during the dry and wet seasons, respectively. The pupal stage took the longest time, with an average of 2.0 days to complete their development during both seasons. Immature *Ae. albopictus* took a total of 6.82 days ± 1.10 days and 8.14 ± 1.81 days during the dry and wet seasons respectively to complete their development.

Larval mortality

The mortality percentages for *Ae. albopicus* from first instar to adulthood was 41.8% for cohort 1, 39.5% for cohort 2, 17.8% for cohort 3, 15.8% for cohort 4, 16.8% for cohort 5, 18.4% for cohort 6, 52.0% for cohort 7, 61.2% for cohort 8, 56.4% for cohort 9, 51.8% for cohort 10, 74.9% for cohort 11, and 56.9% for cohort 12 (Fig 4). The highest mortality was observed for the development of cohort 11 (November). The lowest mortality occurred for cohort 4 (April).

There were significant differences in mortality between immature stages (H=254.627, df=4, p<0.05) and in mortality among the different cohorts (H=109.395, df=11, p<0.05). The highest mortality for immature *Ae. albopictus* mosquitoes occurred in the 3rd instar stage for all cohorts with the exception of cohorts 2, 6, 10 and 12, in which the mortality was highest in the 4th instar stage (Fig 5). The lowest mortality occurred in the pupae stage for all cohorts (Fig 5).

The daily mean temperature did not affect the mortality percentage in any of the cohorts, (r=-0.477, p>0.05) (Fig 6) and the mortality percentage did not correlate significantly with rainfall (r=0.322, p>0.05) (Fig 7). Total development time showed a positive correlation with total mortality (r= 0.713, p<0.05) (Fig 8). Longer development time was associated with higher total mortality.

Life expectancy

The life expectancy (e_x) for the *Ae. albopictus* cohorts progressively decreased

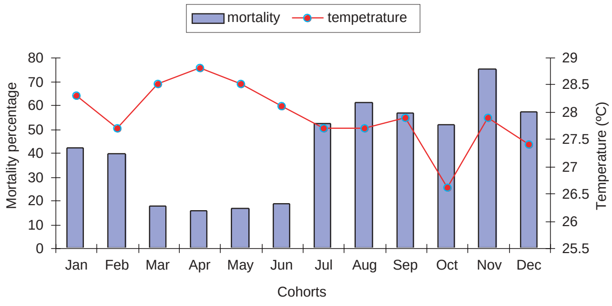

Fig 6–Mortality percentage for 12 cohorts of *Ae. albopictus* and the mean temperature during development of each cohort.

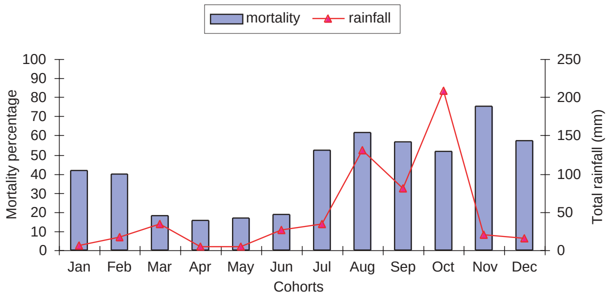

Fig 7–Mortality percentage for 12 cohorts of *Ae. albopictus* with total weekly rainfall during development of each cohort.

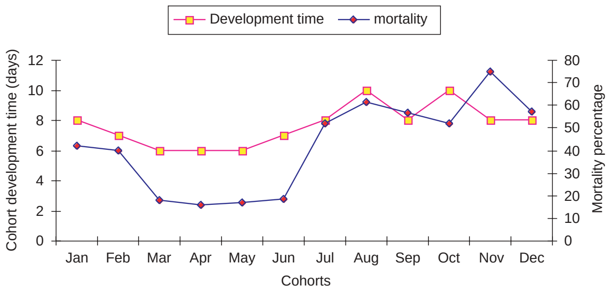

Fig 8–The cohort development time and mortality percentages which occurred during the development of immature *Ae. albopictus*.

from the early larval stage until they begin to pupate, except for cohorts 7 (July) and 8 (August), which showed an increased e_x on the second day (Fig 9).

Lowest value for e_x was observed in the pupal stage for all cohorts ranging from 1.441 days in cohort 12 (December), to 1.500 days in cohorts 3 (March) and 5 (May). The highest e_x values were observed during the 1st instar stage for all cohorts, which ranged from 10.015 days in cohort 10 (October) to 5.884 days in cohort 11 (November), except for cohorts 7 (July) and 8 (August) in which the highest e_x values were observed in the 2nd instar stage.

DISCUSSION

Aedes albopictus is of importance in its capacity as a vector of disease and its nuisance to human (Turell *et al*, 2001). It has also been known to cause negative ecological effects on resident mosquito species. The *Ae. albopictus* life table study has provided valuable information that can be used in vector control programs. Construction of the *Ae. albopictus* life table provides the time frame that is needed for a mosquito to develop from one stage to another. Therefore this information enables vector control programs to be carefully planned by selecting the optimum time to act.

In this study, the time needed for *Ae. albopictus* mosquitoes to develop from the egg stage to the pupal stage before becoming an adult varied from 6 to 10 days in the field, with an average of 6.82 ± 1.10 days during the dry season and 8.14 ± 1.81 days during the wet season.

This time frame is within the *Ae. albopictus* development range as documented by Abu Hassan and Yap (1999). In their study, it was discovered that the time taken for the *Ae. albopictus* mosquito to develop from the egg stage to an adult was 6 to 8 days. According to Nor Adzliyana (2003), development time for *Ae. albopictus* was 8 to 16 days, depending on the availability of food supply. She also found that the size of the container that served as the breeding site effected the development time for immature *Ae. albopictus*. Wijeyaratne

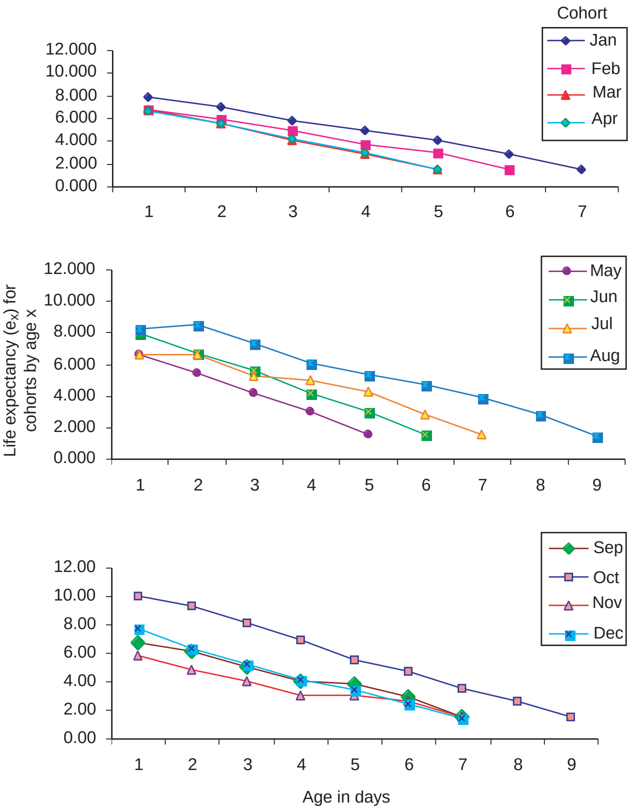

Fig 9–Life expectancy (e_x) plotted as a function of age in days for 12 cohorts of *Aedes albopictus* in the year 2003.

et al (1974) found the development time of larvae in the field is longer. They also discovered that the survival of immature stages and the percentage of eggs that hatched were reduced. The availability of larval food appeared to be the major factor responsible for the differences. When food was added to breeding water in the field, the time for development was reduced from 39 to 13 days. However, temperature had some effect and was probably the major reason for the differences in development times between larvae reared in the laboratory and those reared in the field with extra larval food provided. Alto and Juliano (2001) showed experimentally that population growth of *Ae. albopictus* was directly proportionate to temperatures ranging from 22 to 26ºC, although vector capacity (which may be more relevant to competitive ability) for this species was lower at higher temperatures.

According to Halcrow (1955), the time to hatching and the duration of the life cycle are thus dependent upon the time taken for the egg to mature after oviposition and also on the prevailing temperature during its development. Hatching time and health of the larvae are also affected by temperature. Immature eggs (1-12 hours old) hatch unevenly on contact with water and are followed by a prolonged developmental period. Six hour old eggs, for example, hatched over a period of 4 days and the adults emerged after 25 days (Halcrow, 1955). Mature eggs (4-7 days old), however, hatched evenly and almost immediately when immersed in water. Under natural conditions, eggs of the same age and laid in a single container required 3 to 12 days to hatch (Ho *et al* 1972).

In this study, no remarkable variations in development time among the instars were observed. However, variations occurred in the total development time among cohorts, where development time decreased when environmental temperatures increased. Higher temperatures favor earlier, more rapid production of adults and yield an increase in the rate of spread of *Ae. albopictus* to new sites.

Development time from egg hatch until pupation may be as long as 3 weeks at temperatures ranging from 14 to 18ºC (Lounibos *et al,* 2002). In the immature stages of a vector, prolonging the development time will have many negative effects on the mosquitoes. The immature mosquitoes have a longer exposure to mortality factors, such as parasitism, predation, desiccation and starvation. According to Boots *et al* (2002) in the field an increase in development time tends to result in higher cumulative mortality. With an extended development period, larvae are more exposed to possible parasites and predators as well as increased desiccation as habitats dry up prior to adult emergence.

Longer development times produce smaller adults which effects fertility and fecundity of female adults. Size is often correlated with fitness in the field, because it can affect both reproductive potential and feeding behavior of adult mosquitoes (Boots *et al,* 2002). Smaller adult females tend to have less fitness, which can effect the ability of the vector to find hosts and transmit disease. In *Ae. albopictus* males, small size can cause delayed spermatogenesis (Smith and Hartberg, 1974). Larger females are thought to have greater vector potential, because they may be more successful at finding a second blood meal (Nasci,1986).

The highest mortality was observed for the egg stage in previous life table studies of *Ae. albopictus* (Nur Aida, 2002, Cik Mohd Rizuan, 2004). In our study, mortality of eggs was not observed. In each experiment, 1,000 first instar larvae were collected from the field 24 hours after they hatched to ensure all the larvae were uniform in age. In this study, mortality during the egg stage was excluded due to many factors. Mortality during the egg stage may be due to embryogenesis failure and desiccation. There could have been mistakes in counting the eggs used in the experiments, since the size of *Ae. albopictus* eggs is very small and the color is dark. Egg counting has to be done under a dissecting microscope. Thus, it was easier to start the life table study during the first instar stage.

From our life table study it was discovered that the mosquito development stage with the highest mortality was the third instar stage for all cohorts except in 2, 6 10 and 12, where the highest mortality occurred during the fourth instar stage. This indicates the third instar stage is the most sensitive stage for this vector and a high mortality rate occurred in late instars in this species. The stage with the lowest mortality was the pupal stage. Chan (1971), in his study in Singapore, found that immature *Ae. albopictus* survival was 10.7% and the total mortality rate was 89.3%, with the highest rate in the fourth instars and the lowest in the pupal stage. The pupal mortality rate was only 1% under field conditions and 0.6% to 10.6% under laboratory conditions. There was no proportionate increase in pupal mortality with decreasing food supply at the beginning of the larval stages (Chan, 1971). Decreased survival may have resulted in part from the transfer of larvae from one rearing medium to another (Wijeyaratne *et al,* 1974).

The number of immature forms per breeding site was low in this study, and only 100 larvae were placed in the glass jars that contained 0.5 liters of water (20 larvae/100 ml). If larvae were in crowded conditions, the survival of these stages could be much lower. The high survival of immature stages in nature indicates that the impact of density-regulating factors at this period were minimal in this population. However, in a tropical area, such as Malaysia, *Ae. albopictus* breed continuously, and the regulation of a population is most likely relate to the effect of density-dependent factors on the survival of immature stages due to food.

The mortality and developmental times of immature stages of mosquitoes are influenced by food availability. In this study, food was provided throughout the experiments. Barrera (1996) suggested that larvae of *Ae. albopictus* resisted starvation longer grown on senescent leaf litter because of higher stored energy. Yee *et al* (2004) suggested the larvae exploiting the food resources was a major contributor to interspecific differences in survival. Access to resources and leaf surfaces is an important determinant of mosquito growth and development (Leonard and Juliano, 1995). In this case, larvae of *Ae. albopictus* had an advantage due to food availibility.

The conclusion can be made that third and fourth instar stages are key stages in determining population dynamics of *Ae. albopictus*, since mortality occurred more commonly in these stages. This weakness gives an advantage in controlling this vector. Application of chemical or biological control measures during these stages would increase the mortality of this vector before it reaches an adult stage.

This study demonstrated that there were significant differences in total mortality among cohorts. Temperature and rainfall were not factors influencing mortality. Correlation between mortality and development time was observed. This shows prolonging of development time is a factor that contributes to mortality. Southwood *et al* (1972) in their studies of *Ae. aegypti* in Wat Samphaya, Bangkok, Thailand, stated there was no clear association between mortality and season except for a decrease in larval mortality in April-May preceding the increase in the annual incidence of hemorrhagic fever, which usually occurs in June.

Since the rearing jars were covered and food was provided, predation and starvation can be ruled from being a mortality factor in this study. Under natural conditions parasitism may contribute to higher mortality in immature stages. Wijeyaratne *et al* (1974) indicated that decreased survival may have resulted in part from the transfer of larvae from one rearing medium to another.

In this study, the development time for immature *Ae. albopictus* was related to temperature. The development time varied from 6 to 10 days depending on the environmental temperature during study. The ability to forecast development time, the most sensitive stage and the seasonal life cycle of this vector may improve management programs against *Ae. albopictus*. This knowledge could make the use of insecticides to manage this vector more efficient when applied at a suitable time during specific stages.

Predictions of population peak times and developmental times in various life stages of insects may facilitate the use of culture practices. In addition, sanitation can be reinforced before outbreaks occur, contributing to the elimination of vector breeding sites, since the breeding sites for *Ae. albopictus* included waste, consisting of artificial and natural containers.

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