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Population analysis of *Aedes albopictus* (Skuse) (Diptera:Culicidae) under uncontrolled laboratory conditions

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Abstract. A semi laboratory experiment using 3 cohorts of *Aedes albopictus* adults was performed to obtain age-specific mortality and fecundity information and to derive statistical estimates of some population growth parameters. Life expectancy was calculated for both males and females. The following population parameters were estimated: intrinsic rate of increase (r_m= 0.21), net reproductive (replacement) rate (R_o= 68.70), age at mean cohort reproduction (T_o=10.55 days), birth rate (B=0.23), death rate (D=0.02) and generation time (G=20.14 days). The high r_m/B (0.91) and B/D (11.50) ratios indicated the high colonizing ability of *Ae. albopictus* in nature.

INTRODUCTION

Survival is an important parameter when estimating the vectorial capacity of arthropod vectors of pathogens (Dye, 1992). The ability to determine the age structure and the survival rate of female mosquitoes is of paramount ecological importance because longevity affects net reproduction rates and dispersal distance (Service, 1993). According to Dye (1992), a critical analysis of the age composition of a population is also crucial in epidemiological studies. Furthermore, knowledge of survival rates can help in assessing the impact of control measures (Molineaux *et al.*, 1979). Determination of population increase from reproductive capacity is another crucial component in the study of insect populations. Population increase can be described by a fertility table presenting the potential reproductive ability of females at different times (Harari *et al.*, 1997).

Most of the previous life-table studies were concerned mainly with estimating mortalities of the immature stages. However, after emergence mosquitoes must live sufficiently long for mating, blood feeding, maturation of eggs and oviposition before there is any new population input from the emerging generation. During all these stages there will be further mortalities. If sufficient field data were available on fecundity, number and duration of gonotrophic cycles and survival rates, then it may possible to construct life and fertility tables for adults (Service, 1993).

Age-specific horizontal life tables present a succinct tabular summary of mortality and reproductive schedules (Reisen & Mahmood, 1980). When constructed under relatively benign insectary conditions, with ample resources the value obtained may approach a maximum and may be used to study inherent differences in the survivorship and reproductive strategies of populations of different species evolving under different ecological regimes.

Recently, the life tables and reproductive strategies of colonized culicine mosquitoes including domestic *Aedes aegypti* (L) (Crovello & Hacker, 1972), *Culex quinquefasciatus* Say (Walter & Hacker

1974), *Culex tritaeniorhynchus* Giles (Reisen *et al.*, 1979) and anopheline mosquitoes, *Anopheles albimanus* and *Anopheles vestitipennis* (Greico *et al.*, 2003) were compiled. However, complete survivorship and fecundity-fertility schedules have not yet been compiled for *Aedes albopictus*.

Because of the interest in the ability of this species to survive long enough to become both infected and infective with dengue virus, studies on longevity have previously been conducted in the laboratory under controlled temperature and humidity regimes and under field conditions using mark-release-recapture techniques (Gubler, 1970; Gubler & Bhattacharya, 1971; Lee, 2000, Honorio *et al.*, 2003). Difficulty in colonization has however, precluded a tabulation of the age-specific reproductive efforts of this species.

Greater longevity increases the probability of transmitting diseases (Estrada-Franco & Craig, 1995). Temperature and relative humidity are among the factors that play a vital role in adult survival (Estrada-Franco & Craig, 1995). However few studies to estimate longevity of *Ae. albopictus* adult in the field or under uncontrolled condition (but not directly in the field) have been attempted.

The goal of this study is to determine the survival of adult *Ae. albopictus* and to determine the variables of life and fertility tables to be used as a basis for understanding the population dynamics of *Ae. albopictus* under uncontrolled laboratory conditions.

MATERIALS AND METHODS

Study site

Aedes albopictus adults used in this experiment were produced from field-collected eggs in Lurah Burung, Universiti Sains Malaysia (USM), Penang. These eggs were collected by using ovitraps. This experiment was conducted in the insectarium located in the School of Biological Sciences, USM. Throughout the study, temperature and relative humidity in the insectarium were allowed to fluctuate with the weather outside (uncontrolled conditions). Photoperiod was also unregulated and changed with the surrounding environment. Windows were opened and no temperature controller or air conditioner was used during this study. The study was carried out from 10 March to 31 May 2006.

Sampling technique

Adults were allowed to mate freely and were provided with continuous access to 10% sucrose solution and daily blood meal from laboratory mice from the first day of their emergence. After determining the sex, the adult mosquitoes were promptly placed in adult rearing cages measuring 30cm x 30cm x 30cm. Thirty females and 30 males were placed simultenously in each cage, 3 cages of 60 *Ae. albopictus* were established to quantify adult longevity.

The cages were examined daily in order to record and remove dead mosquitoes. An opportunity to blood feed was provided daily to all females between 1800h-1900h by providing them with laboratory mice confined in a narrow and tight-fitting fine wire-mesh cage for 30 minutes. Everyday after feeding, ovipaper (filter paper Whatman No. 1, folded into a cone and moistened, served as the ovipositon subtract) were changed and eggs counted. These data were recorded daily until all mosquitoes were dead.

All eggs that were produced from this experiment were hatched and larvae were reared until adults emerged in the same insectarium in the School of Biological Sciences, USM. The sex ratio of female to male adult mosquitoes was determined by counting the number of adults.

Data analysis

The formulae used in this study essentially followed Reisen *et al.* (1979).

1. Age-specific survivorship, $l_x = y_x/y_o$ where y_x = the number of mosquitoes that were alive on day x, and y_o = the starting number of mosquitoes in the population.

2. Age specific life expectancy $e_x = T_x/l_x$,

   where $T_x = \sum_{x=1}^{w} L_x$ and $L_x = (l_x=l_{x+1})/2$

   with w = the day the last individual died.

3. Net reproductive rate per cohort,

   $R_o = \sum_{x=1}^{w} L_x m_x$ where, m_x = the number

   of female offspring produced per female per age interval. m_x were observational data.

4. Age of mean cohort reproduction,

   $T_o = \sum_{x=1}^{w} x L_x m_x / R_o$ starting at x = 1, the

   day of adult emergence.

5. The instantaneous rate of increase in females per female was estimated by using Price (1997) equation $r_m = \frac{\log_e R_o}{T_o}$,

   and then to get the actual r_m value,

6. Dobzhansky *et al.* (1964) modification of the original Lotka-Euler equation was used, where e is the base of the natural logarithms

   $$1 = \sum_{x+1}^{w} L_x m_x e^{-rm(x+To)}$$

7. Mean generation time in days, G, could then be calculated as

   $G = \log_e R_o / r_m$

8. To calculate the instantaneous birthrate (B), the equation used was B = ln(1+b)

   where $1/b = \sum_{x=1}^{w} L_x e^{-rm(x+1)}$

   The death rate (D) was then calculated as $D = B - r_m$.

RESULTS

During this study, mean daily temperatures in the insectarium ranged from 26.1 °C to 32.5 °C in accordance with the outside temperature. Highest relative humidity recorded was 86.6% and the lowest was 68.0%.

Figure 1 shows the age specific survivorship for male and female *Ae. albopictus* reared under uncontrolled laboratory conditions. The average longevity for male and female *Ae. albopictus* obtained from the daily survival curve were 11.29 days (1-27 days) and 18.86 days (2-36days) respectively. The males of *Aedes albopictus* demonstrated a shorter adult life span than the females.

Figure 2 represents the reproductive effort or net reproductive rate of 30 *Ae. albopictus* females throughout their life span. A plot of the number of female offspring produced by a female of a particular age (in days) for *Ae. albopictus* showed that the curve peaked within the fifth and seventh day of life and declined as the age of the female increased.

Table 1 shows the life-history characteristics of *Ae. albopictus*. An average of 221 eggs (220.65) per female was laid throughout its life span. The R_o value representing the mean number of female offsprings produced by a single female from a cohort during the course of its lifespan was calculated to be 69 females (68.7). The age of the mean cohort reproduction (T_o) calculated for *Ae. albopictus* females was 10.55 days. The T_o was also known as the time of mean reproductive effort. Higher T_o decreased the innate rate of increase (r_m). The intrinsic rate of increase (r_m) for *Ae. albopictus* was calculated to be 0.21 per female per day of life. Longer T_o caused not only a slower r_m, but also a longer generation time (G) in *Ae. albopictus* female which was 20.14 days. The birth and death rate of *Ae. albopictus* were 0.23 and 0.02 respectively. Here, it is apparent that there is a huge difference in the rate. This is also true for the r_m/B and B/D ratio, which were 0.91 and 11.50, respectively.

Figure 3 represents the life expectancy curve for *Ae. albopictus* populations obtained from 3 cohorts. The life expectancy value of adult females decreased consistently with age, except for a small increase on the ninth day of female

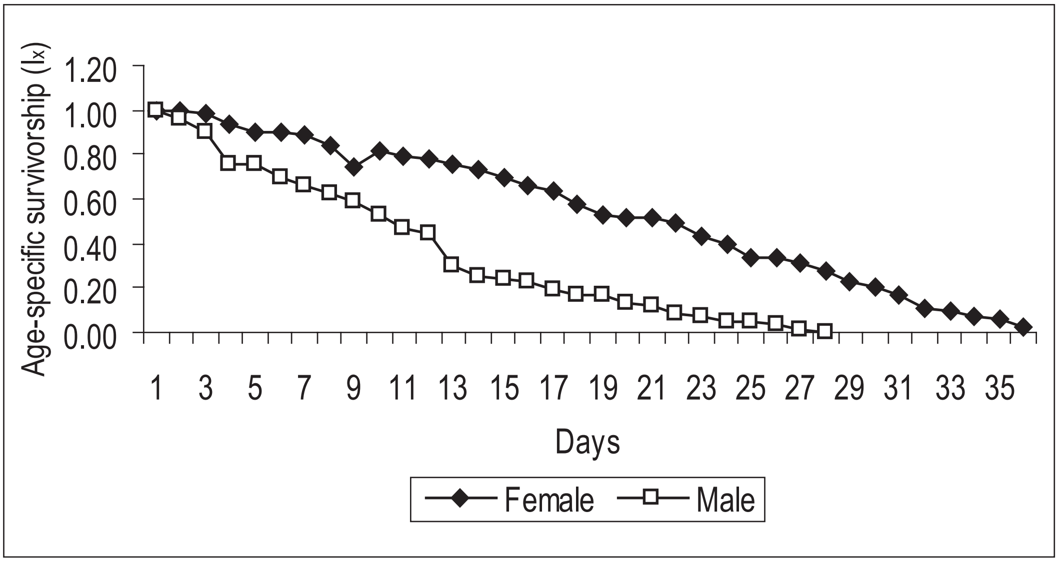

Figure 1. Age-specific survivorship (l_x) for colony adult *Aedes albopictus* under uncontrolled conditions.

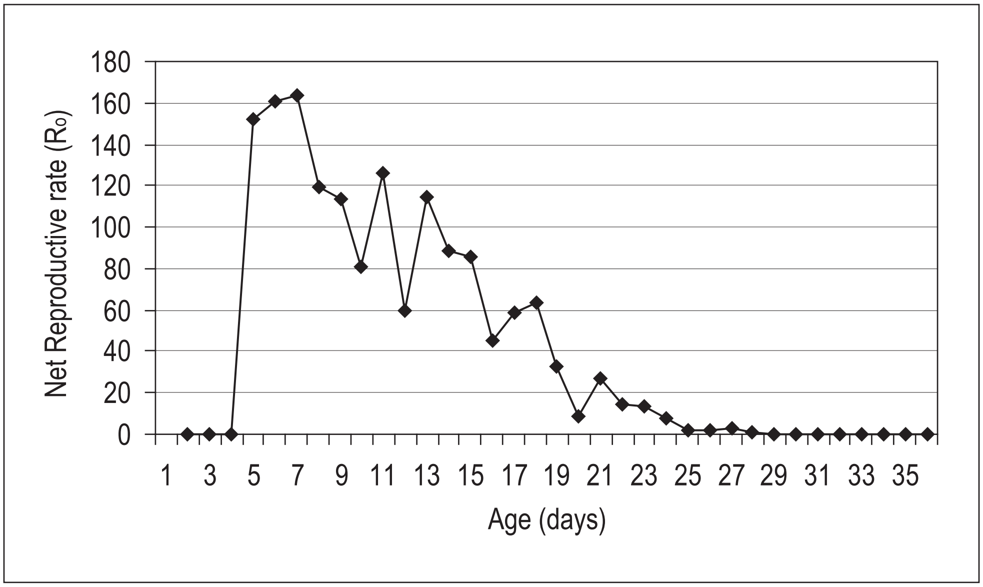

Figure 2. Number of female offspring (R_o) produced by 30 female per generation of *Ae. albopictus* in Penang.

emergence. Immediately after emergence as adults, the females had an average life expectancy of 19.47 days. However, the e_x value for males did not decrease consistently. The average life expectancy at the time of emergence as adults for male *Ae. albopictus* was 10.17 days.

Table 1: Adult life table characteristics of *Aedes albopictus*

Attribute	*Aedes albopictus* ♀♀
e_1: ♀	19.47
♂	10.17
T_p	220.65
R_o	68.70
T_o	10.55
r_m	0.21
G	20.14
B	0.23
D	0.02
r_m/B	0.91
B/D	11.50

Attributes: e_1 = mean life expectancy from emergence in days; T_p = Total lifetime eggs production; R_o = net reproductive rate in living female progeny per female per generation; T_o = age in days at mean cohort reproduction; r_m = instantaneous rate of in living female per female; G mean generation time in days; B = instantaneous birth and D = death rate.

DISCUSSION

In the present study, the mean number of eggs laid per female during the course of a life span of *Ae. albopictus* was 221 (220.65). This value is lower than that observed by Gubler & Bhattacharya (1971) where the total lifetime egg production was from 0 to 784 eggs (total average 283.1 eggs). According to Del Rosario (1963), the number of eggs laid per female *Ae. albopictus* fed only with human blood was 46 eggs (range 6 to 124). However, comparisons between our study and the studies by Gubler & Bhattacharya (1971) and Del Rosario (1963) would be inaccurate because their studies were conducted under controlled laboratory conditions. In the field, egg production is dependent on environmental factors such as temperature and relative humidity, since these factors indeed influence the survival of adult mosquitoes. The number of eggs laid by *Ae. albopictus* also depends on the physiological age, the body weight after emergence and particularly the size of the blood meal (Hien, 1976). In addition, egg production also depends on the development of the immature stages. An optimum condition during the growth of immatures will result in larger and healthier adults who can consume more blood from the hosts to produce eggs.

In this study, females were placed with males in the cage and this enabled them to mate freely but flight was limited. Although the mosquitoes were reared under uncontrolled conditions, food and an oviposition site was provided and predators were nonexistent. Based on the number of females produced per female per day, almost

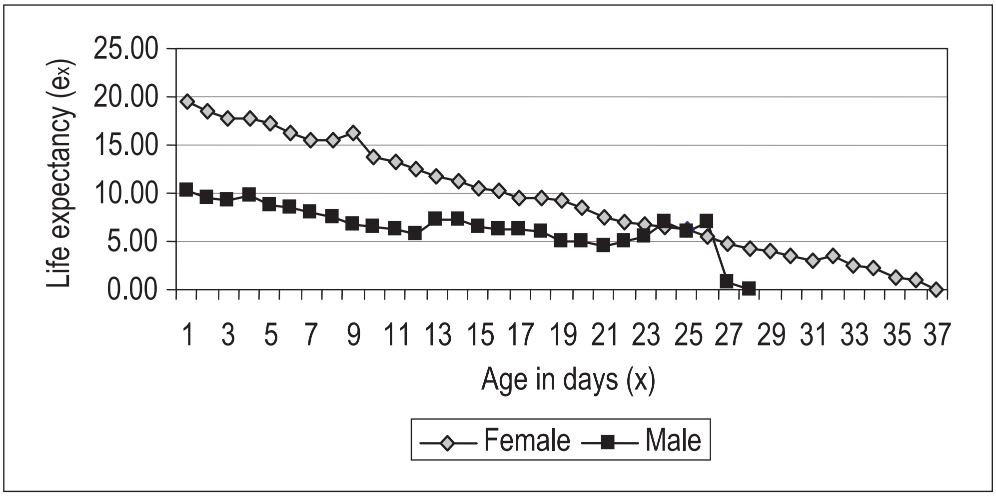

Figure 3. Life expectancy of male and female *Ae. albopictus* from time of emergence.

68% of *Aedes albopictus* egg production occurred within the first two weeks of the adult female's life span. With female *Ae. albopictus* living an average of 18.86 days, the last weeks of life are not as critical in sustaining population density as the first two weeks. It is anticipated that there will be a reduction in the length of the adult life span in nature due to predation and adverse environmental factors relative to the estimates in this study. Therefore, high reproductive activity during the first week of life is necessary for this species to be successful.

According to Mahmood (1997), for multivoltine species with overlapping generations such as *Ae. albopictus*, the r value is a much better reflection of the growth potential per unit of time than the R_o value. The r_m value is more representative of this growth because it takes into account both the number of progeny produced and the times at which these progeny were produced (Hacker, 1972). The r_m value calculated in this study for *Ae. albopictus* was 0.21 females per day. This value is higher than the r_m value estimated by Costanzo *et al.* (2005) which was 0.17. However lower r_m value would be expected in the present study if it was conducted with the presence of competitors as shown in Costanzo *et al.* (2005) study.

Compared to other species, the r_m value in the present study was similar. Study by Costero *et al.* (1998) indicated the r_m values for *Ae. aegypti* were 0.25 during cool season and 0.24 during hot season. Whereas Greico *et al.* (2003) found that *An. albimanus* and *An. vestitipennis* have r_m value of 0.34 and 0.27 respectively. However Reisen & Mahmood (1980) obtained lower r_m value for *Anopheles culicifacies* and *Anopheles stephensi* which was 0.53 and 0.19 respectively. This low r_m value of 0.21 for *Ae. albopictus* indicates low mortality in nature and would lead to a search for adaptive methods for avoiding mortality.

Studies of longevity in different populations of vector mosquitoes often provide important epidemiologic information, because greater longevity increases the probability of transmitting diseases (Estrada-Franco & Craig, 1995). Adult females of *Ae. albopictus* are capable of living long enough to become infected and subsequently transmit infectious agents. This capability is well documented by Shroyer (1986) and Rezza *et al.* (2007).

In the present study, survivorship and longevity estimated for *Ae. albopictus* under uncontrolled laboratory conditions is lower than previous laboratory studies conducted by Gubler & Bhattacharya (1971). They found that blood-nourished *Ae. albopictus* females maintained at a temperature of 26°C and relative humidity of 50% - 60% lived an average of 38.0 days as compared to 30.3 days for males. According to Lee (2000), for *Ae. albopictus*, the life span was 10-22 days for males and 12-40 days for females (mean: 16 and 26 days respectively).

Longevity must be sufficiently long to allow for the development of pathogens in their bodies to be completed. Under experimental conditions, the dengue virus first appeared in the salivary glands of *Ae. albopictus* 10 days after an infective blood meal (Ho *et al.*, 1971). Thus it can be assumed that under tropical conditions, only females of about 10 days old and above can transmit dengue viruses. Gubler & Bhattacharya (1971) demonstrated that in the laboratory, the *Ae. albopictus* gonotrophic cycle was 3.5-4.5 days each. Therefore, at 10 days old, *Ae. albopictus* females may have gone through 1 or 2 gonotrophic cycle. Life expectancy for *Ae. albopictus* female at 10 days was estimated to be 13.73 days and probability of survival is 0.82 . Taking all available data into consideration, *Ae. albopictus* females would live for an additional 13 days or 3 gonotrophic cycles, therefore increasing their vectorial efficiency.

The oldest female *Ae. albopictus* of natural populations in Singapore collected from both human baits and houses had only undergone three gonotrophic cycles, indicating that they were not much older than 11 days (Chan, 1971). In India, wild females were found to oviposit only once or twice in a lifetime (Gubler & Bhattacharya, 1971).

Although their survival appears to be shorter in the field, *Ae. albopictus* females have been known to take multiple blood meals during the individual gonotrophic cycles (Gould *et al.*, 1970; Tempelis *et al.*, 1970). Hien (1976) observed that *Ae. albopictus* females were ready to take blood meals several times during a single gonotrophic cycle. This incident has also been observed by Gubler & Bhattacharya (1971). According to Burkot *et al.* (1988), multiple feeding behaviour during one gonotrophic cycle could be an important component in the epidemiology of vector-borne diseases. Multiple feeding behaviour could increase the ability of *Ae. albopictus* to acquire and deliver infections from and to different hosts thereby spreading pathogens to a larger susceptible human population.

In the present study, the net reproductive rate (Ro) for *Ae. albopictus* was 68.7. The mean cohort reproduction, T_0 occurred later in life for *Ae. albopictus* which was 10.6 days. The intrinsic rate of increase, r_m, birth rate, B, and death rate, D, value for *Ae. albopictus* were low. According to Reisen *et al.* (1979), the birth rate and death rate estimated from a stable age distribution was found to closely correlated with r_m ($r= 0.996$, $p<0.01$). Lower birth rates and death rates accompanied by a lower r_m would suggest a trend towards greater population stability.

In this study, the calculation of life table parameters was based on observational data of m_x value or the number of female offspring produced per female per age interval. All the eggs produced by the females in this experiment were hatched and reared until adults emerged. Therefore the m_x value was more accurate than from others. In previous studies, the m_x value was estimated based on the number of eggs produced by females and the estimated sex ratio for the female progeny was 0.5 (Costero *et al.*, 1998; Crovello & Hacker, 1972; Walter & Hacker, 1974, Lansdowne & Hacker, 1975; Reisen & Mahmood, 1980; Reisen *et al.*, 1979; Greico *et al.*, 2003). Therefore in these cases, they might have overestimated the m_x value resulting in a higher R_0, r, D and B value.

In this study the value of R_0 was low at 68.7 but this value was higher than the R_0 value observed by Costero *et al.* (1998) in *Ae. aegypti* that were fed with blood plus sugar (R_0= 23) during the hot season in Puerto Rico. They also found that *Ae. aegypti* that were fed with blood plus sugar laid less eggs than *Ae. aegypti* fed with only human blood. In this study, the diet provided for *Ae. albopictus* females were consistent with the diet they would get in the field (blood plus sugar). They were given this diet because in natural conditions they would not so easily get frequent blood meals and therefore they would have to take sugar as their energy source.

The calculated r_m value was 0.21. This value is lower than the r_m value observed on *Ae. aegypti* fed only with human blood, but higher than r_m value for *Ae. aegypti* fed on blood plus sugar during hot season in Puerto Rico (Costero *et al.* 1998).

The birth rate calculated for this species was very low, 0.23 but the death rate was lower still resulting in a high r_m/B and B/D ratio. The r_m/B was calculated to be 0.91 while the B/D ratio was 11.50. High r_m values are generally considered to be evolutionary adaptation to existing or colonizing variable environments (Reisen *et al.*, 1979). The r_m/B and B/D ratios, which is an indication of theoretical colonizing ability for *Ae. albopictus* was relatively high. Based on these values, *Ae. albopictus* shows a very high potential for colonization. These value also indicate that *Ae. albopictus* population is subject to very rapid growth. The increases in mortality due to a variety of problems still could be tolerated by the population.

Although this study was conducted under natural simulated conditions, the results obtained provide insight into population dynamics of *Ae. albopictus* that potentially would occur under field conditions. In light of these result, the reduction of this vector populations to below disease transmission threshold by using biological and chemical controls may be unsuccessful, unless an efficient source-reduction program is effective.

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REFERENCES

Burkot, T., Graves, P., Paru, R. & Lagog, M. (1988). Mixed blood feeding by the malaria vectors in the *An. punchulatus* complex (Diptera: Culicidae). *Journal of Medical Entomology* **25(4)**: 205-213.

Chan, K.L. (1971). Life table studies of *Aedes albopictus* (Skuse). In : *Proceedings of a symposium on the sterility principle for insect control or eradication*. International Atomic Energy Agency, p. 131-144.

Costanzo, K.S., Mormann, K. & Juliano, S.A. (2005). Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology* **42(4)**: 559-570.

Costero, A., Edman, J.D., Clark, G.G. & Scott, T.W. (1998). Life table study of *Aedes aegypti* (Diptera: Culicidae) in Puerto Rico fed only human blood versus blood plus sugar. *Journal of Medical Entomology* **35(5)**: 809-813.

Crovello, T.J. & Hacker, C.S. (1972). Evolutionary strategies in life table characteristics among feral and urban strains of *Aedes aegypti* (L.). *Evolution* **26**: 185-196.

Del Rosario, A. (1963). In: Ho BC, Chan YC. & Chan KL. 1973. Field and laboratory observation on landing and biting periodicities of *Aedes albopictus* (Skuse). *Southeast Asian Journal of Tropical Medicine and Public Health* **4 (2)**: 238-243.

Dye, C. (1992). The analysis of parasite transmission by bloodsucking insects. *Annual Review of Enomology* **37**: 1-19.

Estrada-Franco, J.G. & Craig, G.B., Jr. (1995). *Biology, Disease Relationships and Control of Aedes albopictus*. Washington D.C.: Pan American Health Organization. 49 p.

Gould, D.J., Mount, G.A., Scanlon, J.E., Ford, H.R. & Sullivan, M.F. (1970). Ecology and control of dengue vectors on an island in Gulf of Thailand. *Journal of Medical Entomology* **7**: 499-508.

Grieco, J.P., Achee, N.L., Briceno, I., King, R., Andre, R., Roberts, D. & Rejmankova, E. (2003). Comparison of life table attributes from newly established colonies of *Anopheles albimanus* and *Anopheles vestitipennis* in northern Belize. *Journal of Vector Ecology* **8(2)**: 200-207.

Gubler, D.J. (1970). Comparison of reproductive potentials of *Aedes (Stegomyia) albopictus* Skuse and *Aedes (Stegomyia) polynesiensis* Marks. *Mosquito News* **30**: 201-208.

Gubler, D.J. & Bhattacharya, N.C. (1971). Observations on the reproductive history of *Aedes (Stegomyia) albopictus* in the laboratory. *Mosquito News* **31(3)**: 356-359.

Hacker, C.S. (1972). Measuring reproductive potential in populations. *Mosquito News* **32**: 193-196.

Harari, A.R., Ben-Yakir, D., Chen, M. & Rosen, D. (1997). Life- and Fertility-Tables of *Maladera matrida* (Coleptera: Scarabaeidae) *Environmental Entomology*. **26(5)**: 1073-1078.

Hien, D.S. (1976). Biology of *Aedes aegypti* (L., 1762) and *Aedes albopictus* (Skuse, 1895) (Diptera: Culicidae). IV. The feeding of females. *Acta Parasitologica Polonica* **24(6)**: 37-55.

Ho, B.C., Chan, K.L. & Chan, Y.C. (1971). *Aedes aegypti* (L) and *Aedes albopictus* (Skuse) in Singapore City. 3. Population fluctuations. *Bulletin of the World Health Organization* **4**: 636-641.

Honorio, N.A., Da Costa Silva, W., Leite, P.J., Goncalves, J.M., Lounibos, L.P. & De-Olievera, R.L. (2003). Dispersal of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in an urban endemic dengue area in the state of Rio de Janeiro, Brazil. *Memorias Do Instituto Oswaldo Cruz* **98(2)**: 191-198.

Lansdowne, C. & Hacker, C.S. (1975). The effect of fluctuating temperature and humidity on the adult life table characteristics of five strains of *Aedes aegypti*. *Journal of Medical Entomology* **11(6)**: 723-733.

Lee, H.L. (2000). *Aedes*: mosquitoes that spread dengue fever. In: *Mosquitoes and Mosquito-Borne Diseases* (Editors F.S.P. Ng & H.S. Yong) pp45-62.

Mahmood, F. (1997). Life table attributes of *Anopheles albimanus* (Weidemann) under controlled laboratory conditions. *Journal of Vector Ecology* **22**: 103-108.

Molineaux, L., Shidrawi, G.R., Clarke, J.L., Boulzaguet, J.R. & Ashkar, T.S. (1979). Assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the man-biting rate and age composition. *Bulletin of the World Health Organization* **7**: 265-74.

Reisen, W.K. & Mahmood, F. (1980) Horizontal life table characteristics of the malaria vectors *Anopheles culicifacies* and *Anopheles stephensi* (Diptera: Culicidae). *Journal of Medical Entomology* **17(3)**: 211-217.

Reisen, W.K., Siddiqui, T.F., Aslam, Y. & Malik, G.M. (1979). Geographic variation among life table characteristics of *Culex tritaeniorhynchus* Giles from Asia. *Annals of Entomology Society of America* **72**: 700-709.

Rezza, G., Nicoletti, L., Angelini, R., Romi, R., Finarelli, A.C., Panning, M., Cordioli, P., Fortuna, C., Boros, S., Magurano, F., Silvi, G., Angelini, P., Dottori, M., Ciufolini, M.G., Majori, G.C. & Cassone, A. (2007). Infection with chikungunya virus in Italy: an outbreak in a temperate region. *Lancet* **370**: 1840-1846.

Service, M.W. (1993). *Mosquito Ecology: Field Sampling Methods* United Kingdom: Elsevier Science Publishers LTD.

Shroyer, D.A. (1986). *Aedes albopictus* and arboviruses: A concise review of the literature. *Journal of the American Mosquito Control Association* **2**: 424-428.

Tempelis, C.H., Hayes, R.O., Hess, A.D. & Reeves, W.C. (1970). Blood-feeding habits of four species of mosquito found in Hawaii. *American Journal of Tropical Medicine and Hygiene* **19**: 335-341.

Walter, N.M. & Hacker, C.S. (1974). Variation in life table characteristics among three geographic strains of *Culex pipiens quinquefasciatus*. *Journal of Medical Entomology* **11(5)**: 541-550.