ORIGINAL ARTICLE

Temperature Stress Effect on the Survival of *Aedes albopictus* (Skuse) (Diptera: Culicidae) Adult Mosquito: An Experimental Study

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ABSTRACT

Introduction: The survivorship of mosquito is the most important aspect that affects its ability as a pathogen transmitting vector such as *Aedes albopictus* which is a vector of dengue. In this study, temperature stress effect on the survival of adult *Aedes albopictus* were investigated. **Methods:** It was conducted by manipulating different constant temperatures (15°C to 35°C) on juvenile stage of *Ae. albopictus* in natural and artificial container. Then, the development growth of adult mosquito was observed under laboratory condition. The effects of exposed temperature on certain biological parameters of adult mosquito were evaluated in terms of survival rates, longevity of female mosquito, fecundity rate, gonotrophic cycle and wing length of the adult mosquito. **Results:** In higher temperatures, the longevity of adult female was reduced and the highest longevity was found at optimum temperature (25°C) with average of 8.6 ± 0.18 days and 6.7 ± 0.29 days in natural and artificial container respectively. Both sexes showed a clearly tendency towards decreasing survivorship with increasing temperature where the highest survival was found at 15°C. Conversely, survival was lowest at a high temperature (30°C to 35°C). This study also showed that the wing length of both sexes was significantly affected by the rearing temperature where the longest found at 15°C and the shortest at 35°C. **Conclusion:** Thus, this study provide useful information of mosquito ecology in response to variation of temperatures.

Keywords: *Aedes albopictus*, Temperature, Survival, Longevity, Gonotrophic cycle

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INTRODUCTION

Mosquitoes are one of the important vector that attracting major public health attention because of their ways as main vector for various disease transmission which affecting human health globally. The survivorship of mosquito is the most important aspect that affects its ability as a pathogen transmitting vector such as *Ae. albopictus* which is a vector of dengue and also chikungunya (1). *Ae. albopictus* has extended its range throughout the world last decade as their rapid population growth and competent vector for several arboviruses (2-4). The incidence of dengue have increased tremendously and recent statistics indicates about 390 million of dengue infection are reported and 96 million manifest clinically every year (5, 6).

The effect of ecology on *Ae. albopictus* larval habitat and adult development has become a public health concerns because it is highly adaptive and can survive in various environment, therefore it also can spread worldwide in cooler temperate regions due its tolerance to temperatures below freezing, hibernation and ability to shelter in microhabitats (5). Recent studies on climate variability and climate change have affected the geographical distributions of adult mosquito as temperature is considered as the fundamental factor influencing the development of mosquito population includes the nutrition and density which correlated with mosquito life demographic in terms of survival, longetivity, fecundity and gonotrophic cycle (5, 7-12).

The exposure of immature stages under different temperatures affects the development and ability of mosquitoes as changes in physical condition; environmental modification, density and availability of food are some factors which correlated to the adult ability (7, 13). In Asia, *Ae. aegypti* intently connected with the human conditions incorporates indoor and artificial containers, for example, drum, tires, basins, vases for *Ae. aegypti* breeding sites. *Ae. albopictus* are found to prefer natural containers such as bamboo stumps and tree gaps (12).

In general, varying temperature affect *Ae. albopictus* survival directly and indirectly during juvenile and adult stage, as rearing temperature during immature stages may have variable effect on bionomics of adult mosquito and its survival by modifying the activity of mosquito body system (14, 15). Most of the studies stated that increases in temperature will increase the adult emergence and shorten the development duration of Aedes species which directly increase the population density and vector borne transmission, therefore, it also leading to production of smaller adults and reduced the fecundity rate (2, 5 ,7-9, 12, 16-17). In this study, we manipulated the temperatures and the type of water container to represent the mosquito life demographic in order to investigate the temperature stress effect to the survival of *Ae. albopictus*.

MATERIALS AND METHODS

Mosquito strain and experimental condition

Ae. albopictus skuse strain were utilized in this examination are obtained from Puncak Alam, Selangor where the main offspring delivered from mosquito collected through ovitrap surveillance at the dengue outbreak areas. They are colonized in the insectarium of Faculty of Health Sciences, University Teknologi Mara (UiTM) under room temperature of 29 ± 3°C 75±10% relative humidity and with a photoperiod of 13:10 hours (light/dark) (1).

Establishment of mosquito cohort

This study was adopted from a research on temperature towards survival of Aedes mosquito (1, 18). In this experiment, in order to get virgin mosquitoes, the sex separations were done at the pupae stage. A total of 50 pupae were placed individually into plastic container covering with fine netting containing 10 mL of dechlorinated water. Adult mosquito that emerged were designated as F0. Only 10 pairs or virgin mosquitoes were introduced into a standard rearing cage (30 x 30 x 30 cm) supplied with 10% sucrose solution for mating purposes. The mosquito were allowed to mate for 72 hours before given a blood meal using a white mouse for 12 hours to ensure the female had fully engorged.

Two days (48 hours) after blood feeding, an ovitrap lined with filter paper with 225 mL of dechlorinated water was introduced into a cage for oviposition purposes. The water from ovitrap was filtered using No 1 Whatman filter paper. The eggs were allowed to embryonate by air drying at room temperature for seven days. After counting, the filter paper were submerged into a trays containing 150 mL of dechlorinated water with larval nourishment. The water was changed at regular interval to remove and avoid the formation of scum.

Adult maintenance and temperature regimes

Mosquito larvae and pupae are hand separately at various temperatures (15 ± 1°C, 20 ± 1°C, 25 ± 1°C, 30 ± 1°C and 35 ± 1°C) at 75±10% relative humidity by using water bath (GFL: Model 1005) in order to get a continuous exposure day for aquatic stage. Two types of water container had been used in this experiment, namely plastic container (artificial) and coconut shell (natural). Upon emergence into adult, the individual adult were placed at cage under room temperature of 29 ± 3°C with a photoperiod of 13:10 hours (light/dark). The adult mosquitoes were separated into two cages which were natural and artificial containers designated into five different temperatures which maintained under laboratory condition. Figure 1 shows the experimental design used throughout the research. The exposure on development of mosquitoes from aquatic stage into adult stage under room temperature of 29 ± 3°C was also conducted which acts as control. Each cages were monitored daily and mortality was recorded to determine the survivorship of male and female mosquitoes from the day it emerged until they died. The procedures were repeated for different temperatures. The gonotrophic cycle period was recorded and eggs that were laid on the paddle were counted every day for each temperature and the paddle then substituted. On the days where no egg was deposited, the blood meal was reoffered to the surviving females, and this process continued until all females die. All the information gathered through a series of experiments were examine using analysis of variance (ANOVA) and Kaplan-Meirs which use to analysed the life span, mortality, and wing size among five distinctive consistent temperatures.

The wing size measurement of adult mosquito was also conducted. The dead mosquitoes were kept in the freezer to allow a measurement of wing lengths (4). The wing length was characterizes as the straight separation from the axillary cut to the apical edge barring the periphery scales (14, 19-20). To measure the length, the wings were disengaged proximal to the axillary score, and mounted on a magnifying instrument slide and

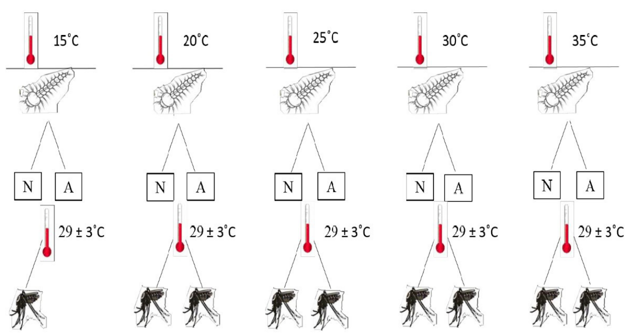

Figure 1: Experimental design. Larvae reared at each temperature (15°C, 20°C, 25°C, 30°C and 35°C) were allowed to develop into imagoes and the adult mosquito were kept and maintained at room temperature. None of the larvae reared at 15°C in artificial container were survived.*Note: (N) represent for natural container and (A) for artificial container.

secured with a spread slip (9, 21). Only one wing of each adult was removed which usually the left wing, unless it damaged (22). The length of wing was measured in cubic value (mm) using a Dino-Lite digital microscope.

RESULTS

Profiling on temperature stress effect on the development growth of adult Ae. albopictus to its survival and longevity

Generally, the data collected throughout the experiment was analysed to determine the adult mosquito development which includes certain biological parameters namely; (i) survival, (ii) longevity, (iii) fecundity, and (iv) gonotrophic cycle of *Ae. albopictus*. From the results tabulated, higher temperature was generally connected with reduced life span (longevity) where adult female longevity was highest at optimum temperature of 25°C with average 8.6 ± 0.18 days and 6.7 ± 0.29 days for natural and artificial container respectively. The lowest longevity was found at 15°C (5.1 ± 0.40 days) in natural container and 20°C (4.1 ± 0.61 days) in artificial container. Reduction in longevity in natural container was bias at 20°C (3.8 ±0.43 days) due to female mosquito died and lost during the experiment. The mean survival of adult males on different temperatures were not significantly different between the natural container (36.70 ± 3.43 days) and artificial container (28.92 ± 2.72 days) as shown in Figure 2A. Thus, there was likewise no noteworthy contrast in the survival of females mosquito between the natural (36.74 ± 3.33 days) and artificial container (30.41 ± 2.51 days). Throughout the study, overall adult females lived longer than the male's mosquito in both containers as shows in Figure 2B & C and the duration of males mosquito averaged about 25 to 59 days in natural container and 31 to 52 days in artificial container. Meanwhile, female mosquitoes averaged about 30 to 62 days and 31 to 57 days in natural and artificial container respectively.

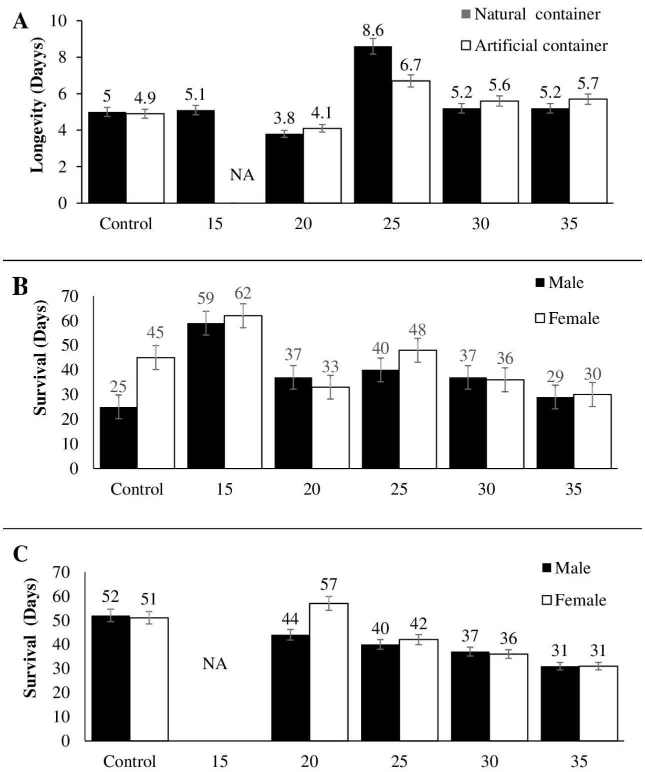

Figure 2: The longevity and survival of Ae. albopictus under different constant temperatures. (A) longevity of adult female mosquito; survival of mosquito in (B) natural container and (C) artificial container. * (NA) indicate the result was not available

In order to understand the life table of *Ae. albopictus* under different constant temperatures, Kaplan-Meir survival plots were performed to compare the cumulative survival for the adult mosquitoes between temperatures as it declined through time for both sexes and containers as shown in Figure 3. The demographic growth parameters of *Ae. albopictus* was developed based on data collected where the male and female adult mosquitoes showed a fluctuating pattern in both containers. Throughout the development period, adult males mosquito have a shorter survival period as compared to the females even though the development period was inconsistent among those temperatures. The result showed the adult mosquitoes lived from the day it emerged until it die. Adult survival was conversely corresponded with temperature, where the most elevated survival rates found at 15°C with 59 days and 62 days for male and female adult mosquitoes respectively in natural container. The shortest survival rate was recorded at temperature 35°C for male (29 days) and female (30 days) adult mosquitoes. Indeed, in artificial container, the shortest survival rates also was found at 35°C with 31 days for both adult mosquitoes and the highest was recorded at temperature 20°C with 44 days for male and 57 days for female mosquito.

Effect of different constant temperatures stress exposure towards the duration of gonotrophic cycle fecundity rate of Ae. albopictus

During the experiment, the duration and number of gonotrophic cycle was varied with temperatures as shown in Figure 4. In natural container, In natural container, the average duration of the first gonotrophic cycle at 30 and 35 °C was 3.5 days after blood feeding, 2 days shorter compared to control, 15, 20, and 25°C.

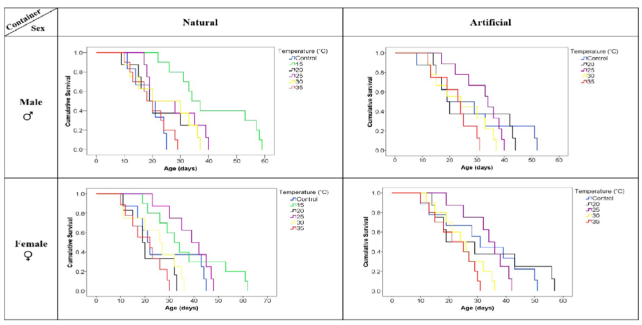

Figure 3: Kaplan-Meir survival plots of Aedes albopictus under different constant temperature in natural and artificial container for male and female adult mosquito. *Note: Survival curves of temperature in the containers for both adult mosquito, control (blue); 15°C (green); 20°C (black); 25°C (purple), 30°C (yellow); 35°C (red).

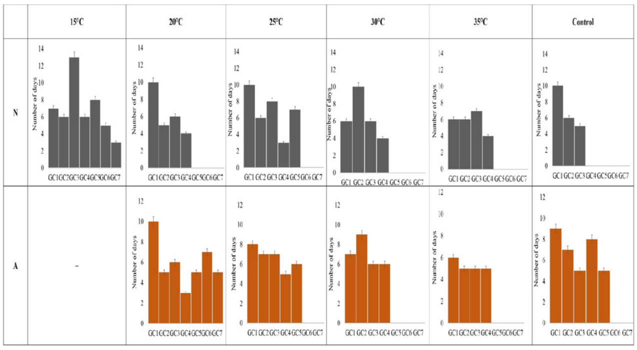

Figure 4: The duration of gonotrophic cycle by females Ae. albopictus after exposed to different constant temperatures. (N): GC in natural container and (A): GC in artificial container.*Note: (-) indicate the result was not available

However, the average mean of 6.9 days was observed at 15°C which had a longest cycle and the shortest average GC was observed at 35°C (5.8 days). At 15°C, the minimum cycle duration was 3 days and the maximum was 13 days compared to other temperatures while at 35°C, was 4 days and 6 days for minimum and maximum duration respectively. These may be used to indicate that mosquito had a longer GC during cold season and shorter GC during dry season.

The number of GC observed per female varied from 3 GC at control (29 ± 3°C) to 7 GC at 15°C. The number of female mosquito during the blood meal affected the duration of gonotrophic cycle at all temperatures. Figure 4 showed the pattern of GC in natural and artificial container under different constant temperatures where the lowest temperatures (15°C) have a longest cycle while shorter cycles at 20, 30 and 35°C. The data for 15°C in artificial container was not available as the egg does not hatching during experiment. There was slightly difference of GC duration between temperatures in both containers as the average duration for 20°C was 5.9 days with the longest GC (seven cycles) in artificial container than 6.3 days (three cycles) for 20°C in natural container. However, the mean duration of GC under 35°C was significantly difference in both containers, 5.3 days and 5.8 days respectively ($p<0.05$).

Meanwhile, the fecundity rate of *Ae. albopictus* mosquito obtained in this study from observing the total number of egg laid during each gonotrophic cycle. The total numbers of eggs laid by female mosquito during each GC were different at each temperature. It was discovered that the length of gonotrophic cycle in this investigation was diminished with the expanding of temperature where the shortest was seen at 30 and 35°C in both containers. The number of eggs deposited by female mosquito was varied between temperatures. The highest total number of eggs laid was observed at 15°C with 1479 eggs and a mean fecundity of 35.4 eggs per female in natural container and 890 eggs with a mean fecundity of 31.3 eggs per female at 30°C in artificial container. Meanwhile, the lowest number of egg laid was observed at 35°C in both artificial with 388 eggs and 540 eggs respectively.

In this study, some of the oviposition begins on 2nd or 3rd day after a blood meal and female mosquito were found to lay their eggs during several days with an average duration of GC was 7 days with an average of 51.6 eggs laid during the first cycle at control temperature. The highest number of eggs laid was 692 eggs at 20°C during the first GC. At higher temperature, the fecundity rate was decreased and varied according to gonotrophic cycle which correlated with the number of female mosquitoes. Indeed, Kruskal-Wallis independence test were used to analyse the relative between temperature and number of egg per gonotrophic cycle, where there were no significant different among group ($p>0.05$) for natural container and artificial container.

Effect of different constant temperatures stress exposure towards the wing size of Ae. albopictus

Further study has been conducted to identify the effect of temperatures towards the wing length which consider as body size of adult mosquitoes. Results obtained in this study indicate that the wings of *Ae. albopictus* females were longer than males even though they were exposed under similar temperature. Table I shows the mean and standard error (SE) of wing length between temperatures in different containers. The wing size of adult female and male averaged from 1.534 mm to 3.130 mm and 1.101 mm to 1.858 mm in natural container respectively. Meanwhile, in artificial container, the wing size of adult female averaged from 1.411 mm to 2.568 mm and 1.010 mm to 1.976 mm for adult male.

The ANOVA has shown that there is no difference between means temperature of adult male in natural container as F (7.735), $p < 0.05$ and adult female F (4.633), $p < 0.05$ in terms of wing size. The result indicates that the increased temperature will decreased the wing size. Meanwhile, the wing size of adult female between temperatures in artificial container were significantly influenced the wing development F (4.040), $p = 0.012$. However, the wing size of adult male in artificial container was not statistically significant F (1.261), $p = 0.311$. Overall, the mean sizes for males' mosquito were shorter than female's mosquito in both containers. The shortest measurement for male was observed at 35°C with 1.46 ± 0.08 mm and 1.47 ± 0.12 mm in natural and artificial container respectively. While the longest measurement was observed at lower temperature which 15°C (1.75 ± 0.03 mm) in natural container and 20°C (1.80 ± 0.05 mm) in artificial container. Meanwhile, the longest wing size for female mosquito was observed at 15°C and 20°C with the value of 2.63 ± 0.12 mm and 2.34 ± 0.10 mm in natural and artificial container respectively. The shortest measurement of female wing size also was found at higher temperature (35°C) in both containers with 1.91 ± 0.10 mm and 1.82 ± 0.08 mm. Thus, the wing length of males and females obtained in this experiment was affected by the rearing temperature.

TABLE I: The wing length of the adult females and males of the *Aedes albopictus* Skuse emerged at different constant temperatures

A

Temperature (°C)	Mean ± Standard Error (SE) (mm)	
	Male	Female
Control	1.84 ± 0.04	2.31 ± 0.11
15	1.75 ± 0.03	2.63 ± 0.12
20	1.74 ± 0.03	1.97 ± 0.12
25	1.72 ± 0.04	2.30 ± 0.12
30	1.72 ± 0.04	2.13 ± 0.16
35	1.46 ± 0.08	1.91 ± 0.10
p-value	0.000	0.03
F	7.735	4.633

B

Temperature (°C)	Mean ± Standard Error (SE) (mm)	
	Male	Female
Control	1.70 ± 0.13	2.14 ± 0.09
20	1.80 ± 0.05	2.34 ± 0.10
25	1.62 ± 0.11	2.04 ± 0.07
30	1.53 ± 0.14	1.97 ± 0.14
35	1.47 ± 0.12	1.82 ± 0.08
p-value	0.311	0.012
F	1.261	4.040

*Note: *p-value* generated from analysis of variance (ANOVA)

DISCUSSION

In this present investigation, the temperature stress had variable influenced on the population development of adult *Ae. albopictus* (Skuse) by altering their life demographic. The exposure of different constant temperature on immature stages influenced the life expectancy of mosquito population which correlated to their survivorship and capability as vectors of pathogens (7). In this investigation, the life table parameters was based on the observational data from the first day of adult emerged until they die. Similar to the previous study by Delatte et al, (2009), he mentioned that the estimated average adult life expectancies were the longest at 15°C for females and males which were 38.59 and 31.31 days, respectively while the shortest at 34°C for females and males were 19.86 and 14.9 days, respectively (5). They also found that the adult female longevity were longer than males at all different constant temperature. In previous study by Phanitchat et al, (2017), he found that from 30 days observation, at high temperature had a lower survival where the higher percentages of survivors were female (18). The average life span of male mosquito was 36.70 days in natural container and 28.92 days in artificial container, shorter than female mosquito in the present study which had 36.74 days and 30.41 days in natural and artificial container respectively.

Indeed, the overall survival of adult females lived longer than the adult male's mosquito in both water containers. Both sexes of adult mosquitoes demonstrated a clearly tendency towards decreasing survivorship with increasing temperature (1, 7, 14) where most elevated survival rates was found at 15°C and least at 35°C comparable with the outcome found in different investigations (5, 23). High temperature in this present investigation resulted shortest period for adults emergence during the examinations, however less grown-ups staying alive toward the finish of the test were recorded and vice-versa (7, 8). Throughout the study, the laboratory conditions was controlled and sufficient food supply was provided because in previous study, they mentioned that both mosquito survivorship was influenced by the nutrition of 10% sucrose solution provided after the blood feeding as it became one of important food resources for male fitness in order to survive, dispersal and mating (1, 24, 25).

In this study, the duration and number of gonotrophic cycle was varied with temperatures. The result from the experiment shows the average mean of 6.9 days which was observed at 15°C, had a longest cycle and the shortest average GC was observed at 35°C (5.8 days) which was similar with the previous study where all the gonotrophic cycles became faster and shorter with increased temperatures (17, 25-27). In artificial container, at the temperature of 20, 25 and 30°C, the gonotrophic cycle lengths were insignificant with temperature and furthermore the quantity of eggs was not significant at each gonotrophic cycle for any temperature variation (5). After all, female Ae. abopictus took 13 days to deposit all their eggs at 15°C in natural container. The number of female mosquito alive during the blood meal affected the duration of gonotrophic cycle at all temperatures as it have positive relationship with offspring turnover (18). Moreover, other study stated the temperatures approximately influenced the vectorial capacity of mosquito in relation of virus transmission that increased if the longevity of adult female mosquito higher, especially at the point when temperatures are low and it might had long enough times to transmit the infection, regardless of whether it takes more time to complete its gonotrophic cycle (28). However, at higher temperatures, the transmission of virus may be less significant if the mosquito mortality is higher although the gonotrophic cycle is shorter as the temperature increased, the length of gonotrophic cycle and mosquito survival decreased. Thus, more research is needed to explore the connection between vectorial limit and temperature.

Besides, temperature stress on mosquito also affected the fecundity rate of *Ae. albopictus*. From this study, it was found that at higher temperature, the fecundity rate was decreased and varied according to gonotrophic cycle, which correlated with the number of female mosquitoes. The number of female mosquito during blood meal in each gonotrophic cycle influenced the number of eggs deposited which varied between temperatures. The result in this study observed that the higher total number of egg laid was at extreme temperature of 15°C and 35°C in natural container and 30°C and 35°C in artificial container. The quantity of eggs oviposited per gonotrophic cyle are regularly estimated from 30 to 80 and afterward decrease with age (26).

The wing length was estimated as the reaction of adult mosquito in different temperature exposed during the development of juvenile stages indicates the mosquito body size (22) which can influence epidemiologically relevant traits. In order to measure the body size of adult mosquito, wing length was frequently used as a marker of body measure, and the association between wing length and weight is significant as the information can be utilized in evaluating the biology of the adult mosquitoes by determining mosquito fitness such as mortality and fecundity (4). There was an indirect relationship between wing length and temperature which the wing size was essentially reduced by increasing temperature and shorter wings are related with lower fecundity (12, 22). There were a few investigations expressed that fecundity is identified associated with the body size of adult *Ae. albopictus*. The mosquito which exposed with lower temperatures show a slower development period and recorded bigger wing size. In contrast, higher temperature may progress more rapidly in terms of time, but the adults mosquitoes are likely to become smaller and simultaneously reduced the fecundity rate (4, 9, 15, 16, 22, 30).

Moreover, study by Gomes et al., (1995) mentioned that wing size also associated with the field fitness where it affected the flight ability of adult mosquitoes (13). Larger adult male are more likely to be fitter than smaller mosquito. The measurement of wing size was assessed to analyse the effect of wing size variation to the flight capacity of mosquito to find a reproducing destinations and blood feed under field conditions. In the study, it stated that larger size of female mosquito may reflect the fact that it have a better flight extend than small mosquito, increased survival rate and host finding, successfulness of blood feeding and improved ability to locate oviposition sites hence, increased the fecundity. So, it concluded that the fecundity rate and flight ability to locate ovipostition site was higher at lower temperature and declines as the temperatures increased.

In this study, the results obtained shows that the wing length decreased as the temperature increased where the largest adult female mosquitoes produced at 15°C (2.63 mm) and the smallest mosquitoes produced at 35°C (1.91 mm) in natural container while in artificial container was largest at 20°C (2.34 mm) and at 35°C with the smallest wing length (1.82 mm). The number of eggs produced varied between temperatures in this study as the body size influenced the fecundity rate which larger female mosquito tend to produce more eggs than the smaller one (4). Female mosquito were found to lay their eggs and produced more eggs in the first gonotrophic cycle (4, 5) as in this study, it proven that highest number of total eggs was during first gonotrophic cycle or second gonotrophic cycle at all temperatures. Notwithstanding, at 35°C, the quantity of eggs oviposited by females were contrasted from every exposed temperature. Larval temperature reared at 35°C made the adult female mosquito laid significantly fewer eggs than those from 15, 20, 25 and 30 °C. It shows that there is an effect of temperature on the population of Aedes mosquitoes through the fecundity rate of the female mosquitoes. This study showed that mosquitoes have the ability to adapt to various environmental condition to survive. Modern society lifestyle which creates a great variety of natural and artificial breeding containers has become the vital impact of the distribution of Aedes mosquitoes. The availability of these containers helps in the maintenance of a great population of vector and consequently favour the interaction between man and vector.

CONCLUSION

In conclusion, the outcomes from this investigation demonstrated that temperature significantly affects the ecology and population dynamics of *Ae. albopictus* (Skuse) by altering their life demographic on their development time, survival rate and adult size where it's directly affected transmission of viruses. The temperature stress exposed to the mosquito during development stages affect the adult survival directly or indirectly. At higher temperatures, *Ae. albopictus* develops more rapidly and its mortality became faster which influenced the decreasing of fecundity and the body mass of mosquito and vice versa at lower temperature. Furthermore, the data in this study such as survival and mortality rate, gonothrophic cycle and fecundity rate, the development and body mass of adult mosquito could be a useful information as a baseline data in order to understand more on the population dynamic especially the life demographic of local strain *Ae albopictus*.

ACKNOWLEDGEMENTS

The authors genuinely thank to every one of the associations engaged with this undertaking particularly those that had given the priceless information. Special thanks to the Faculty of Health Sciences, Universiti Teknologi MARA for the technical assistance rendered.

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