EFFECT OF TEMPERATURE ON THE IMMATURE DEVELOPMENT OF *AEDES ALBOPICTUS* SKUSE

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Abstract. Temperature is often identified as the main environmental factor affecting the growth of mosquito population. It is important to study the effect of temperature on the life parameters of *Aedes albopictus* in the local environment using the local strain, in order to understand more on the biology of the mosquito which will directly affect the transmission of viruses such as dengue to human. In this study, we examined the effect of constant temperatures (15°C to 40°C) on the developmental period of two local *Ae. albopictus* strains using environmental chambers. Based on the developmental data it was determined that increase in temperature reduced the developmental period of the mosquito except for the 1st instar. In addition, the mosquito was able to survive at a higher temperature, which at 40°C the mosquito was able to complete development up to the 3rd instar, indicating ability to survive at the high temperature. This finding provided valuable baseline information on the effects of global warming in Malaysia due to climate change on the bionomics of *Ae. albopictus*.

Keywords: *Aedes albopictus*, temperature, development period

INTRODUCTION

The global climate changes have affected the geographical distributions of mosquito vectors which directly influence the transmission of vector-borne infectious diseases (Farjana *et al*, 2012). Physical environmental factors such as temperature, nutrition and density have been identified to influence the development time and survivorship of mosquitoes (Christophers, 1960; Rae, 1990; Clements, 1992; Atkinson, 1994; Alto and Juliano, 2001; Teng and Apperson, 2000; Tun-lin *et al*, 2000; Delatte *et al*, 2009; Farjana *et al*, 2012). Temperature was identified as the main environmental factor affecting the growth of mosquito population (Clements, 1992; Atkinson, 1994). *Aedes albopictus*, a dengue vector, is a poikilotherm which is susceptible to external temperature variations that directly influence their body temperature (Hawley, 1988). Nowadays, global warming which mainly considers the increase of world temperature have become our main concern. The climate changes projection indicates an increase in the world temperature of 0.85 (0.65 to 1.06) °C, over the period 1880 to 2012, with total increase of 0.78 (0.72 to

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0.85) °C between the average of the 1850-1900 period and the 2003-2012 period (IPCC, 2013).

It has been reported that climate change has expanded the geographical range of *Ae. albopictus* in several continents (Alto and Juliano, 2001; Monteiro *et al*, 2007). *Aedes albopictus* is indigenous in tropical Asia but presently the distribution is worldwide (Gratz, 2004; Rozilawati *et al*, 2005, 2007, 2015). It was known as a rural species (Hawley, 1988) however recently, it was reported that the species is adapted to urban and suburban areas in Malaysia, which overlaps with the distribution of *Ae. aegypti*, the main dengue virus vector with single mixed infestation in the same breeding container (Yap, 1975; Chen *et al*, 2006; Rozilawati *et al*, 2007; Lau *et al*, 2013; Rozilawati *et al*, 2015).

The ability of *Ae. albopictus* to serve as a vector transmitting diseases has been reported for a wide range of arboviruses (Rozilawati *et al*, 2015). Under experimental conditions, *Ae. albopictus* is a competent and capable vector of 26 viruses (Gratz, 2004). It also have been identified as the main chikungunya virus vectors during the recent outbreaks in Malaysia (Rozilawati *et al*, 2011; Yusoff *et al*, 2013).

It was reported that a change of temperature does affect the mosquito life parameters (Mohammed and Chadee, 2011) and the vectorial capacity to certain viruses such as dengue (Yadav *et al*, 2005) and chikungunya viruses (Mourya *et al*, 2004). The life demographic of *Ae. albopictus* such as the survival, longevity, fecundity and gonotrophic cycle are influenced by temperature (Delatte *et al*, 2009). Temperature may affect diverse aspects of mosquito population dynamics, such as the rate and egg viability (Parker, 1986; Hawley, 1988; Impoinvil *et al*, 2007) and development rate (Shelton, 1973; Rueda *et al*, 1990; Lyimo *et al*, 1992; Teng and Apperson, 2000; Alto and Juliano, 2001; Mohammed and Chadee, 2011).

Despite its wide distribution and recent vector status, data on the effect of temperature on the life of *Ae. albopictus* is limited especially in this country. It is very important to study the effect of temperature on the life parameters such as the developmental period of this vector mosquito using the local strain, in order to understand more on the biology of the mosquito which will directly affect the transmission of viruses to human. It is quite difficult though to directly determine the effect of temperature on the mosquito's biological development, therefore a laboratory study can be conducted with control parameters to simulate the situation in nature. In this study the developmental period of two Malaysian strains of *Ae. albopictus* under several constant temperatures was determined using the control conditions in environmental chambers.

MATERIALS AND METHODS

Mosquito strain

Two *Aedes albopictus* strains were used in this study. The field strain (KL) which was originally collected using the oviposition traps from the dengue prone areas in Keramat, Kuala Lumpur was used (Rozilawati *et al*, 2015). The F1 & F2 generation were used for different experiments. A laboratory strain (SEL) originated from Selangor was also employed. This strain has been colonized for 41 generations in the insectarium of Institute for Medical Research, Kuala Lumpur.

The mosquitoes were colonized using the standard procedure in the insectarium of IMR under a room temperature of 25 ± 1°C, 75 ± 10% relative humidity and a photoperiod of 12:12 hours (light / dark).

Sex separation was conducted during the pupal stage. Pupae were then transferred into a glass container with netting, in order to reconfirm the sexes once they emerged as adults. A total of 200 males were put into a standard rearing cage 24 hours after emergence before a total of 100 virgin females (aged 1-2 days) were introduced into the cage (ratio 1:2, female: male). They were allowed to mate for five to seven days, before the females were given a blood meal by introducing a mouse confined in a small screen cage into the mosquito cage, at seven days interval. Sucrose was then provided in the cage. Eggs were collected for seven days by placing an ovitrap containing 225 ml dechlorinated water, and lined with filter paper in the cages 48-hour post-feeding. The eggs were allowed to dry at room temperature for seven days before hatching them for the experiments.

Experimental conditions

Environmental chambers were used in this study. The chambers were programmed with specific temperatures, with a photoperiod of 12:12 hours (07:00 AM-07:00 PM, and vice versa) and controlled relative humidity (75% ± 10%). A thermo-hygrometer was placed in every chamber to monitor and ensure the correct temperature and relative humidity for each temperature tested. Six constant temperatures were employed for this study (15°C, 20°C, 25°C, 30°C, 35°C and 40°C).

Experimental design

For this study, the F1 eggs were used. A total of 200-500 eggs, aged seven days old were allowed to hatch in the chambers. Then, a total of 50 larvae (L1) for each strain were collected and placed into individual glass vials (size: 2.5 cm X 7.5 cm) containing 10 ml dechlorinated water. Larvae were fed daily with 0.2 mg per L1 and L2 and 0.5 mg per L3 and L4 (Farjana *et al*, 2012). Water was changed every two days to remove/avoid scum. The duration of days spent in each stage, the survival and mortality of each stage were recorded every 24 hours. Each instar was determined by the floating moulted skin on the water surface in the vial. The developmental period of each life stage (Larva instar 1 until adult eclosion) were then compared between strains for each temperature using the Mann-Whitney *U* test. In order to compare the developmental period between each temperature for each life stage, the Kruskal-Wallis test was performed using the Bonferroni correction for all possible comparisons within each analysis. The adult emergence was enumerated by date and the sex of emerged adults was also recorded. Chi-square tests were used to determine the sex ratio from 1:1 ratio. The wings were then removed and measured under an imaging system using the DIMAS 5.0 software and compared between strains and temperature using the one-way ANOVA test.

The Kaplan-Meier survival analyses (Log rank tests) were used to assess the effect of temperature on the development and survival time. The post hoc pairwise comparisons were conducted with the Bonferroni correction method for multiple comparisons if any significant differences were detected. These analyses were performed using the SPSS software version 17 for window base system. The survival percentage, which is the apparent mortality is the measured mortality calculated as the numbers dying as a percentage of the numbers entering the stage (d_x as a % of l_x) and the real mortality is calculated on the basis of the population density at the beginning of the generation ($100 \text{ X } d_i / l_c$ = the deaths in the *i*th age interval and l_c the size of the cohort at the commencement

of the generation were also calculated (Southwood, 1978; Suman *et al*, 2011) using the Microsoft Excel 2010 program.

RESULTS

Immature developmental period

Under the constant temperature of 15°C, the larvae only survived at the first instar for a maximum of 12 days and all of them died before moulting to the second instar. Three replicates for each strain (total 150 mosquitoes) were conducted for this temperature. Consequently, the period of development of the entire life cycle could not be determined at this temperature.

Based on the Mann-Whitney *U* test, it was determined that under the constant temperature of 20°C, there was no significant difference between the median developmental period for the first, second and third instar larvae and pupae of both strains. However, the median developmental period of the fourth instar larval stage was significantly longer than the KL strain ($U = 454$, $z = -3.7731$, $p<0.05$). The SEL strains also took significantly longer period from the instar one to the adult stage than the KL strain ($U = 104$, $z = -2.27$, $p<0.05$). There was no significant difference in the male emergence time between both strains, however, the median eclosion time of the female SEL strains took a longer period than the KL strain ($U = 8.00$, $z = -2.012$, $p<0.05$). For the SEL strain, it took a minimum of 21 days and a maximum of 24 days for the male adults to emerge, whereas for the KL strain it took 20 to 24 days for the male adults to emerge. Generally, females emerged one day after males, and in this study it is 24 days for the SEL strain and between 22 and 24 days for the KL strain.

It took a shorter period to complete the development from the first instar larva to the adult eclosion under the constant temperature of 25°C. At this temperature, there was no significant difference of the median developmental period between both strains for every life stage (Mann-Whitney *U* test, $p>0.05$). It took 12 to 15 days for the adult eclosion for the SEL strain and 12 to 14 days for the KL strain. There was also no significant difference of the median developmental period between both strains for each life stage at 30°C and 35°C. At 30°C, the developmental period from the first instar to adult eclosion was between 10 to 12 days for the SEL strain and 10 to 13 days for the KL strain. In comparison, it took only 9 to 12 days for the SEL strain to develop into the adult stage and 9 to 11 days for the KL strain at the 35°C. The larvae only survived up to the third instar at 40°C while the SEL strain survived until third days and KL strains survived until fourth days at this stage. No significant difference was determined between both strains for the first and second instar larvae. No comparison was conducted for the third instar larvae since only six and two larvae survived and completed the stage for the SEL and KL strains, respectively. The median development period of under each constant temperature are presented as in Table 1 for SEL strain and Table 2 for KL strain.

There was significantly a decreasing trend of developmental period with increasing temperature. The overall development period from instar 1 to adult emergence showed significant decreased period with increasing of temperature for both strains. The trend was more obvious at the pupal stage for both SEL and KL strains as shown in Table 1 and Table 2. The longest development period from L1 to the adult eclosion was at 20°C, while the shortest was at 35°C for both strains.

Table 1
The median developmental period (IQR) for the *Aedes albopictus* Skuse SEL strain under the five constant temperatures.

Stage	Temp (°C)	Median (IQR)	Kruskal-Wallis
Instar 1	20	1 (0)	
	25	1 (0)	
	30	1 (0)	χ^2 (4) = 0.00, p = 1.00
	35	1 (0)	
	40	1 (0)	
Instar 2	20	5 (1)	
	25	3 (0)	
	30	3 (0)	χ^2 (4) = 216.209, p = 0.000
	35	2 (1)	
	40	1 (0)	
Instar 3	20	2 (2)	
	25	2 (1)	
	30	3 (1)	χ^2 (4) = 67.890, p = 0.000
	35	1 (0)	
	40	3 (0.25)	
Instar 4	20	11 (2)	
	25	3 (1)	χ^2 (3) = 115.46, p = 0.000
	30	3 (1)	
	35	2 (0)	
Pupa	20	5 (2)	
	25	4 (1)	χ^2 (3) = 102.934, p = 0.000
	30	2 (1)	
	35	2 (1)	
Instar 1 to adult eclosion	20	24 (1.5)	
	25	13 (0)	χ^2 (3) = 114.86, p = 0.000
	30	11 (2)	
	35	9 (1)	

p-value is adjusted by Bonferroni method for each comparison.

The sex ratio of the emerging adult did not differ significantly from 1:1 [χ^2 (1), p>0.05], except at 25°C, where more males emerged significantly than females for the KL strain [χ^2 (1) = 4.891, p<0.05] (Table 3 and Table 4).

Survivorship and mortality of immature stage

The survivorship was determined based on age (from L1 to adult eclosion) as presented in Fig 1 (SEL strain) and Fig 2 (KL strain) and also by Kaplan-Meier survival analysis (Figs 3 and 4). For both strains, the survivorship decreased with an increase in age. The age specific survivorship showed that the mosquitoes survived the longest at 20°C and shortest at 40°C for both strains. This result was supported by the Kaplan-Meier analysis, which according to the log-rank tests, the

Table 2
The median developmental period (IQR) for the *Aedes albopictus* Skuse KL strain under the five constant temperatures.

Stage	Temp (°C)	Median (IQR)	Kruskal-Wallis
Instar 1	20	1 (0)	
	25	1 (0)	
	30	1 (0)	χ^2 (4) = 0.00, p = 1.00
	35	1 (0)	
	40	1 (0)	
Instar 2	20	5 (0)	
	25	3 (0)	
	30	3 (0)	χ^2 (4) = 215.304, p = 0.000
	35	2 (1)	
	40	1 (0)	
Instar 3	20	2 (1.25)	
	25	1 (1)	
	30	3 (1)	χ^2 (4) = 77.002, p = 0.000
	35	1 (0)	
	40	4 (0)	
Instar 4	20	9 (1.75)	
	25	3 (1)	χ^2 (3) = 113.774, p = 0.000
	30	3 (1)	
	35	2 (1)	
Pupa	20	5 (3)	
	25	3 (1)	χ^2 (3) = 130.232, p = 0.000
	30	2 (0)	
	35	2 (1)	
Instar 1 to adult eclosion	20	23 (1)	
	25	13 (0)	χ^2 (3) = 139.756, p = 0.000
	30	11 (3)	
	35	9.5 (1)	

p-value is adjusted by Bonferroni method for each comparison.

temperature did influnce the larval development curves for both the SEL [χ^2 (3) = 253, p<0.05] and KL strains [χ^2 (3) = 245, p<0.05]. The calculated median survival time also decreased with the increasing temperatures for both strains (Table 5 and Table 6). Based on the multiple comparison statistical analysis using the Bonferroni correction, all temperature treatments were statistically different from each other for both strains (Table 7 and Table 8).

The survivorship rates are more clearly explained in Table 9 and Table 10 which indicate the survivorship for each stage until the adult eclosion. Even though the longest survival time was recorded at 20°C for both strains, the lowest survivorship rate was determined at this temperature, during which only 26% survived to the adult stage for the SEL strain and a 56% survivorship rate for the KL strain. The survivorship rate increased

Table 3
The mean (± SE) developmental period for the *Aedes albopictus* Skuse SEL strain under the five constant temperatures.

Temp (°C)	Mean ± SE						% Female
	L1	L2	L3	L4	Pupae	L1-Adult eclosion	
20	1.00 ± 0.00	5.52 ± 0.16	2.83 ± 0.31	10.77 ± 0.25	5.23 ± 0.32	23.31 ± 0.27	30.77
25	1.00 ± 0.00	3.14 ± 0.05	1.66 ± 0.16	2.95 ± 0.12	3.58 ± 0.77	13.14 ± 0.10	34.88
30	1.00 ± 0.00	3.00 ± 0.00	1.15 ± 0.06	2.51 ± 0.10	2.37 ± 0.08	11.11 ± 0.15	57.14
35	1.00 ± 0.00	2.44 ± 0.08	1.19 ± 0.06	2.09 ± 0.09	1.81 ± 0.08	9.60 ± 0.13	41.86
40	1.00 ± 0.00	1.07 ± 0.05	2.83 ± 0.17				

Table 4
The mean (± SE) development period for the *Aedes albopictus* Skuse KL strain under the five constant temperatures.

Temp (°C)	Mean ± SE						% Female
	L1	L2	L3	L4	Pupae	L1-Adult eclosion	
20	1.00 ± 0.00	5.51 ± 0.31	2.81 ± 0.28	9.45 ± 0.25	4.70 ± 0.13	22.50 ± 0.89	39.30
25	1.00 ± 0.00	3.06 ± 0.33	1.58 ± 0.17	3.34 ± 0.12	3.40 ± 0.08	13.13 ± 0.07	32.61[a]
30	1.00 ± 0.00	3.00 ± 0.00	1.10 ± 0.05	2.74 ± 0.09	2.15 ± 0.05	11.00 ± 0.11	50.00
35	1.00 ± 0.00	2.44 ± 0.08	1.18 ± 0.07	2.18 ± 0.17	1.62 ± 0.09	9.53 ± 0.10	52.94
40	1.00 ± 0.00	1.00 ± 0.00	4.00 ± 0.00				

[a]Sex ratio is significantly different from 1:1 (chi- square test).

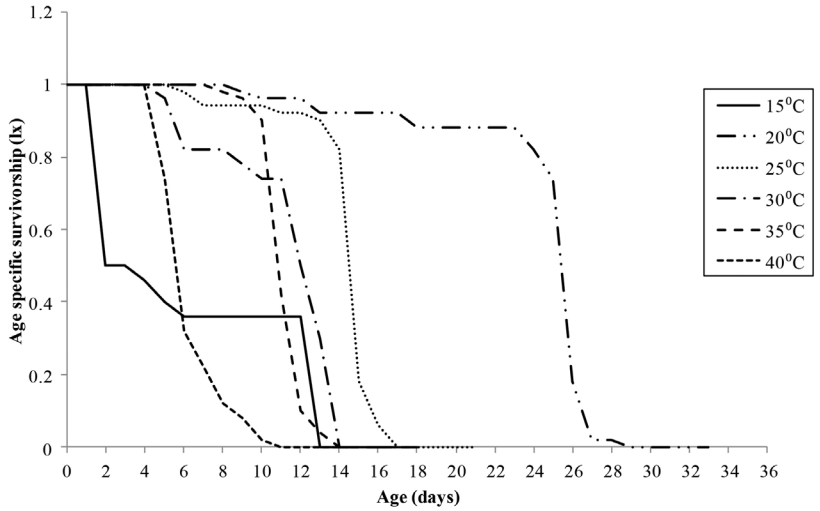

Fig 1–Age-specific survivorship for the immature stages of the *Aedes albopictus* Skuse SEL strain.

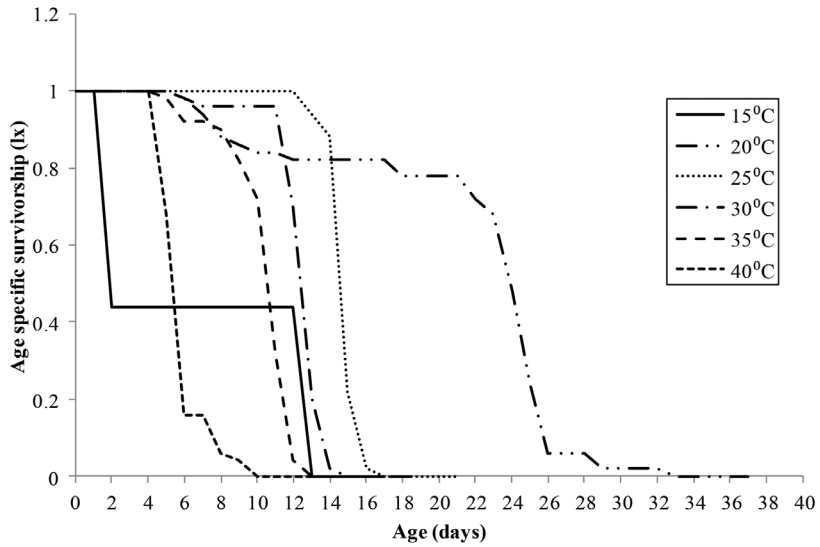

Fig 2–Age-specific survivorship for the immature stages of the *Aedes albopictus* Skuse KL strain.

at the constant temperature of 25°C, with a survivorship rate of 86% for the SEL strain and 92% for the KL strain. The survivorship rate decreased to 70% for the SEL strain at the constant temperature of 30°C. However, for the KL strain the survivorship rate was maintained at 92%. At 35°C, an increase in the survivorship rate was recorded for the SEL strain (86 %) while a decrease was recorded for the KL strain (68%). For each temperature tested, the highest mortality (apparent and real mortality) was recorded at the pupal stage except for 15°C, during which 100% mortality of the first instar larvae occurred and total mortality occurred at the instar three larvae at 40°C. The wing length of the adult females and males generally decreased with an increase in temperature which can be clearly observed at 35°C than the other temperatures. For the SEL strain the female sizes were significantly smaller at 35°C than at other temperatures [$F(3,53) = 8.572$, $p<0.05$], whereas the males at 30°C and 35°C were found to be significantly smaller than at other two lower temperatures, [$F(3,73) = 48.34$, $p<0.05$] (Table 11). For the KL strain, the females were also determined to be significantly smaller at 35°C [$F(3, 36) = 15.16$, $p<0.005$]. The males of the KL strain were also significantly smaller at 35°C than at other temperatures [$F(3,83) = 23.73$, $p<0.05$] (Table 12).

DISCUSSION

Temperature is one of the key factors that affect the insect survival especially

Table 5
The SEL strain *Aedes albopictus* Skuse median survival time at four constant temperatures.

Temp (°C)	Survival time in days[a] Estimate (SE)	95% CI	χ^2 (p-value)[b]
20	25.00 (0.28)	(24.46-25.54)	
25	13.00 (0.09)	(12.82-13.18)	253.83
30	11.00 (0.25)	(10.52-11.48)	(<0.001)
35	9.00 (0.14)	(8.73-9.27)	

[a]Median survival time; [b]Overall log rank test.

Table 6
The KL strain *Aedes albopictus* Skuse median survival time at four constant temperatures.

Temp (°C)	Survival time in days[a] Estimate (SE)	95% CI	χ^2 (p-value)[a]
20	24.00 (0.30)	(23.46-24.58)	
25	13.00 (0.08)	(12.84-13.16)	245.03
30	11.00 (0.10)	(10.80-11.20)	(<0.001)
35	9.00 (0.00)		

[a]Median survival time; [b]Overall log rank test.

Table 7
Multiple comparisons of survival time of the *Aedes albopictus* Skuse SEL strain at three constant temperatures.

Temp (°C)	25 χ^2 (p-value)	30 χ^2 (p-value)	35 χ^2 (p-value)[a]
20	85.33 (<0.001)	77.42 (<0.001)	96.02 (<0.001)
25		72.58 (<0.001)	99.60 (<0.001)
30			39.20 (<0.001)

[a]p-value is adjusted by Bonferroni method.

Table 8
Multiple comparisons of survival time of the *Aedes albopictus* Skuse KL strain at three constant temperatures.

Temp (°C)	25 χ^2 (p-value)	30 χ^2 (p-value)	35 χ^2 (p-value)[a]
20	84.06 (<0.001)	83.99 (<0.001)	66.83 (<0.001)
25		82.77 (<0.001)	83.43 (<0.001)
30			43.11 (<0.001)

[a]p-value is adjusted by Bonferroni method.

Table 9
The survivorship and mortality of the immature stages of the *Aedes albopictus* Skuse SEL strain.

Temp (°C)	Parameter	L1	L2	L3	L4	Pupa	L1-Adult
15	% survival	0.00					
	% apparent mortality	100.00					
	% real mortality	100.00					
20	% survival	100.00	100.00	96.00	91.67	29.55	26.00
	% apparent mortality	0.00	0.00	4.00	8.33	70.45	
	% real mortality	0.00	0.00	4.00	8.00	62.00	
25	% survival	100.00	100.00	94.00	97.87	93.48	86.00
	% apparent mortality	0.00	0.00	6.00	2.13	6.52	
	% real mortality	0.00	0.00	6.00	2.00	6.00	
30	% survival	100.00	96.00	85.42	100.00	85.37	70.00
	% apparent mortality	0.00	4.00	14.58	0.00	14.63	
	% real mortality	0.00	4.00	14.00	0.00	12.00	
35	% survival	100.00	100.00	98.00	97.96	89.58	86.00
	% apparent mortality	0.00	0.00	2.00	2.04	10.42	
	% real mortality	0.00	0.00	2.00	2.00	10.00	
40	% survival	100.00	92.00	13.04			
	% apparent mortality	0.00	8.00	86.96			
	% real mortality	0.00	8.00	80.00			

Table 10
Survivorship and mortality of the immature stages of the *Aedes albopictus* Skuse KL strain.

Temp (°C)	Parameter	L1	L2	L3	L4	Pupa	L1-Adult
15	% survival	0.00					
	% apparent mortality	100.00					
	% real mortality	100.00					
20	% survival	100.00	90.00	93.33	95.24	70.00	56.00
	% apparent mortality	0.00	10.00	6.67	4.76	30.00	
	% real mortality	0.00	10.00	6.00	4.00	24.00	
25	% survival	100.00	100.00	100.00	96.00	95.83	92.00
	% apparent mortality	0.00	0.00	0.00	4.00	4.17	
	% real mortality	0.00	0.00	0.00	4.00	4.00	
30	% survival	100.00	98.00	97.96	97.92	97.87	92.00
	% apparent mortality	0.00	2.00	2.04	2.08	2.13	
	% real mortality	0.00	2.00	2.00	2.00	2.00	
35	% survival	100.00	100.00	92.00	86.96	85.00	68.00
	% apparent mortality	0.00	0.00	8.00	13.04	15.00	
	% real mortality	0.00	0.00	8.00	12.00	12.00	
40	% survival	100.00	92.00	4.35			
	% apparent mortality	0.00	8.00	95.65			
	% real mortality	0.00	8.00	88.00			

Table 11
The wing length of adult females and males of the *Aedes albopictus* Skuse SEL strain emerged at four constant temperatures.

Temp (°C)	Mean ± SE	
	Female	Male
20	2.65 ± 0.05[a]	2.23 ± 0.04[a]
25	2.60 ± 0.02[a]	2.20 ± 0.02[a]
30	2.59 ± 0.03[a]	2.21 ± 0.02[b]
35	2.44 ± 0.03[b]	1.93 ± 0.01[c]

Means within columns with different letters are significantly different (p<0.05).

Table 12
The wing length of the adult females and males of the *Aedes albopictus* Skuse KL strain emerged at four constant temperatures.

Temp (°C)	Mean ± SE	
	Female	Male
20	2.73 ± 0.06[a]	2.33 ± 0.06[a]
25	2.62 ± 0.02[a,b]	2.20 ± 0.02[b]
30	2.58 ± 0.04[b]	2.12 ± 0.02[b]
35	2.40 ± 0.02[c]	1.93 ± 0.04[c]

Means within columns with different letters are significantly different (p<0.05).

mosquitoes which are poikilothermic (Mohammed and Chadee, 2011). Under the constant temperature of 15°C, the larvae remained in the first instar for a maximum of 12 days, after which all of them died before developing into the second instar. This finding differed from the study conducted by Delatte *et al* (2009) who managed to determine the immature development at this temperature using the La Reunion strain of *Ae. albopictus*. On the other hand, it was reported earlier in the study by Delatte *et al* (2009) that at 40ºC, the mosquito did not develop to the immature stage since no eggs were hatched. It was accepted generally that the temperature of 40ºC and above is the limit for mosquito immature survival (Waldock *et al*, 2013). However, in this study, the mosquito larvae could develop into the immature stages until the third instar. This finding can be an indication that our strains are adapted to survive at a higher temperature. Therefore during the dengue / chikungunya outbreak especially at the higher temperature of 35°C to 40°C the vector control operations should be enhanced, since it has been reported that mosquitoes that could withstand higher temperatures would possess the capability to transmit the viruses more effectively due to the increased virus propagation and dissemination (Carrington *et al*, 2013; Waldock *et al*, 2013).

In the present study, both strains showed a reduced developmental period with the increasing temperatures, during which shorter development periods were recorded at each life stage across the temperature except for the first instar, for which only one day was needed for both strains to complete their development. The longest developmental period from L1 to adult eclosion occurred at 20°C, while the shortest was determined at 35°C for both strains. These results concurred with the previous studies which showed that higher temperatures shortened the development duration of the *Aedes* species (Alto and Juliano, 2001; Kakimura e*t al*, 2002; Teng and Apperson, 2000; Ho *et al*, 2005; Yang *et al*, 2009; Farjana *et al*, 2012). On the other hand, lower temperatures slowed the development rate and the period of each stage might be longer than at the higher temperatures (Galliard and Golvan, 1957; Udaka, 1959; Hawley, 1988;

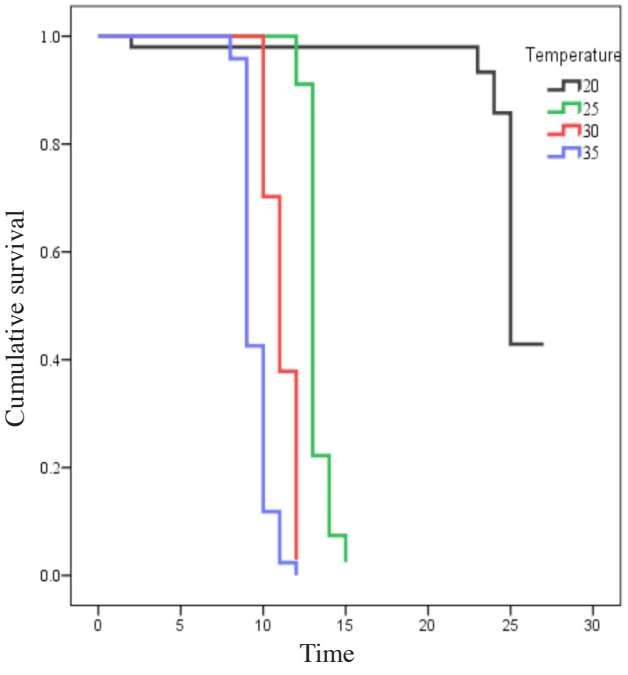

Fig 2–Kaplan-Meier survival plot for the *Aedes albopictus* Skuse SEL strain.

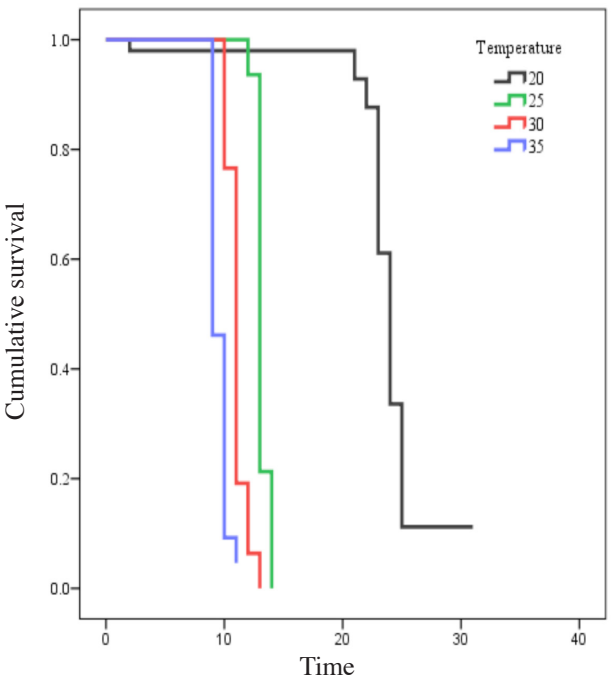

Fig 3–Kaplan-Meier survival plot for the *Aedes albopictus* Skuse KL strain.

Briegel and Timmermann, 2001). Based on this present result, the adults will emerge at the shorter period with increasing temperature, which will directly impact on the population increase and consequently increasing the possibility of disease transmission during an outbreak. It was also shown that the ratio of males and females emerged was not significantly different from one to another in all treatments except at 25°C for the KL strain, in which the proportion of males was signifiantly higher than females, which was similarly found at normal environmental conditions. The same results were reported by studies conducted earlier, which no significant difference of sex ratio observed (Tun-lin *et al*, 2000; Monteiro *et al*, 2007; Delatte *et al*, 2009; Mohammed and Chadee, 2011). The same obeservation was also reported for *Ae. aegypti* (Carrington *et al*, 2013; Lopes *et al*, 2014).

The survivorship of this mosquito decreased with the increasing age as determined in this study. The age specific survivorship also showed that the longevity of the mosquitoes was significantly affected by temperature; with shorter longevity following the increasing temperature, which were similarly reported by Delatte *et al* (2009). Although the longest longevity was recorded at 20°C, the lowest survival rate until the adult emergence was also recorded at this temperature and slightly increased fluctuations in survivorship pattern was deter-

mined across the temperatures for both strains. Based on the results obtained in this study, the species adapted well to the higher temperature in terms of survivorship rate, as more than 50% survived. For each temperature tested the highest mortality (apparent and real mortality) was found at the early pupal stage except for 15°C and 40°C, during which complete mortality occured at the first instar and early fourth instar, respectively. These findings are not so much different from the previous studies which reported the highest mortality was recorded at the last larval stages or pupal stage especially at 35°C (Monteiro *et al*, 2007; Delatte *et al*, 2009). The adaptation of *Ae. albopictus* to higher temperature might be the main factor that contributes to its survival, and directly the abundance in different regions of the world.

The temperatures significantly affect the size of emerged females and males The smaller adults produced from the first progeny of *Ae. albopictus* and *Ae. aegypti* at a higher temperature (mostly at the temperature 35°C and above) were also reported previously (Rae, 1990; Tunlin *et al*, 2000; Farjana *et al*, 2012). It was also determined that the frequency of the adult emergence and also the sex ratio for both first generation of *Ae. albopictus* in both experiments were not significantly different from 1:1, except for experiment one during which at 25°C, more males significantly emerged than females for the KL strain. Similar findings were reported by Delatte *et al* (2009) and Monteiro *et al* (2007) who found that the temperature between 15°C to 35°C did not have any effect on the adult *Ae. albopictus* sex ratio produced. In comparison, for *Ae. aegypti* the sex rate variations due to the temperature changes have been reported (Tun-lin *et al*, 2000) with female domination over males at 30°C and an equal ratio at 25°C. It was also reported by Mohammed and Chadee (2011) that at constant temperatures of 24°C-35°C the sex ratio was not significantly different. In another study by Briegel and Timmermann (2001), *Ae. albopictus* larvae showed a 2:1 ratio of males:females that did not vary with temperature nor density. The data obtained in this present study showed that the probability to produce males and females for the population growth of the species is not affected by temperature. With the same possibility of adult males and females produced, it will contribute to the success of the population growth and distribution of the species worldwide.

Based on the results provided in this study, it can be concluded that constant temperature does not affect the survival capability of our *Ae. albopictus*. A more comprehensive study including the impact of cyclic temperature should be done in the future in order to understand more the influence of temperature on the survivorship of this mosquito species.

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