ORIGINAL ARTICLE

Effects of Artificial Lights on the Biology of Adult Aedes aegypti (Diptera: Culicidae) Mosquitoes

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ABSTRACT

Introduction: The purpose of this research is to evaluate the effects of artificial light exposures on the survivals and life traits of adult *Aedes aegypti*. **Methods:** This is an experimental research where triplication of each cage filled with a ratio of 20 male : 20 female mosquitoes were exposed under three different light artificial light which are; yellow light, white light and blue light along with three different photoperiod regimes (light:dark) – (14h:10h, 12h:12h and 8h:16h). During this study, life traits data observed were duration of the females to complete a gonotrophic cycle, mean number of days was calculated as well as fecundity rate each cycle was also observed by calculating its number of eggs. **Results:** In general, gonotrophic cycle of this species is significantly affected by the exposure of the artificial lights where the white light has allowed this species to complete a gonotrophic cycle faster than yellow and blue light with post hoc Tukey's HSD p = 0.026, however the gonotrophic cycle is found not affected by the different photoperiod regimes. The fecundity of this species is found not to be affected by the artificial light exposure but is affected by the different photoperiod regimes with p = 0.049. **Conclusion:** In conclusion, artificial light can significantly affect the gonotrophic cycle of *Aedes aegypti* species while on the other hand, the fecundity of this species is affected by the different photoperiod instead of different artificial light exposure.

Keywords: Aedes, Artificial light, Photoperiod, Gonotrophic cycle, Fecundity

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INTRODUCTION

Aedes aegypti (Linnaeus) and Aedes albopictus (Skuse) are common mosquitoes species being studied regionally due to their ability in transmitting dengue virus (1). Comprehending how the biotic and abiotic factors affects the life history and population dynamics of mosquitoes are vital either in terms of ecological or medical aspects (2). This is because, studying the interactions of these factors towards the mosquito traits can impact the disease transmission caused by the mosquitoes. Usual biological life traits that have been studied by the past researchers either in experimental or field settings are the gonotrophic cycle and fecundity of mosquitoes as these can provide critical insights to interrupt from completing the vector-borne disease transmission triangle (3).

Factors like temperature, seasonal changes, use of chemicals and other organisms were often being studied its interactions towards the life traits of the mosquitoes (3-11). Few studies have explored on the effect of light on the biological life traits of mosquitoes. Yee et al., 2012 have studied on effects of photoperiod to life history traits in Aedes albopictus, however the study only discover the impacts of exposure towards the development time and size of the mosquito (12). Another study conducted by Leinsham et al., 2008 also have conducted on photoperiodic response on Aedes albopictus, nevertheless only to study its geographical distribution (13). Most of other photoperiod studies were focusing on effects of natural photoperiod on Aedes albopictus and there are no studies to date that have investigated photoperiodic sensitivity in *Aedes aegypti* especially for local strain.

Study conducted by Burkett & Butler (2005) had used artificial white light in a chamber with different wavelength of light, the results from this study showed the tested mosquito species were found to be highly attracted to certain wavelengths (350 and 550 nm). These species were found to be sensitive to this wavelength which happened to be dominated by green and blue spectrum which may serve discriminatory in nature. This explained the relatively small numbers captured of both species in light trap, indicating the insignificance of the light exposure preference. At these spectrum, however there was no difference on the duration of feeding times recorded for Aedes albopictus, nevertheless the higher spectrum (>500 nm) would lengthen the feeding duration (14).

A study conducted by Neto & Navarro-Silva (2004) had used artificial light under a chamber with different cyclic temperature. From this study, it is found that the longevity of both male and female mosquitoes are quite the same. Furthermore, the study also has found that the gonotrophic cycle of Aedes mosquito has increase (15). This is verified by other study conducted by Briegel (1990) mentioned that with increased the temperatures will make the gonotrophic cycles became faster. Adult longevity was very similar under cyclic temperatures and constant temperature of 20 C, and this suggests that the lowest temperature of the cyclic regime is a limiting factor for survival and distribution. This fact may determinate the probable areas where Aedes albopictus can inhabit. Male and females longevity did not differ under cyclic temperature condition of 27 C. The mortality rate of adults was constant through the time (16). It is believed that the changes of temperature have affected the reproduction activity by elongating the gonotrophic cycles.

There are major gap on the study of influence of artificial light on development and survival of Aedes mosquito. The data on the effect of the artificial light on the life development of Aedes mosquito is still limited in this country. It is very important to study the effect of artificial light on the development of this vector mosquito in the local environment using local strain in order to understand on the biology of mosquito. Therefore, this study were evaluated the action of photoperiod and artificial lights on the life cycle of Aedes mosquito species on the survival of adult Aedes mosquito.

MATERIALS AND METHODS

Study design

An experimental study design under laboratory setting was applied. The experiment was completely randomized in factorial 4 x 3 designs (16 treatments). The independent variables in this study include four different types of excessive artificial light exposure namely yellow light, white light, ultraviolet and no light as control and three different duration on Light: Dark Ratios (8 : 16; 14 : 10; 12 : 12). The experiments were triplicated for reliable results which then tabulated and analysed. Figure 1 show an overview of the light and cage settings for survival and life traits studies of adult *Aedes aegypti*.

Colonization of Aedes aegypti mosquitoes.

In this study, mosquito strain of *Aedes aegypti* was obtained from Institute for Medical Research, Kuala Lumpur. The colonization of *Aedes aegypti* was carried out in the controlled laboratory conditions in insectarium at Universiti Teknologi MARA (UiTM), Puncak Alam. Before the rearing process, preparation of larval and adult mosquito food was conducted. Larval food was made from chicken liver (17). Fresh chicken liver was oven-dried and grinded to fined form and kept inside capped bottle for storage. Adult mosquito food was made from common table sugar mixed with other additional ingredients to form sucrose solution. Sucrose solution was made from the mixture of one tablet of vitamin B complex, 10 grams of sugar and 100 millilitre of distilled water. The mixture solution was heated until all the ingredients are dissolved completely. The mixture solution of sucrose was let to cool before kept inside capped bottle for storage.

Laboratory strain of *Aedes aegypti* second instar larvae were undergone for mass rearing. The rearing process was conducted under controlled environment in insectarium at 29 ± 3°C with 75±10% humidity and photoperiod 14:10 hours of dark: light cycle. The larvae were reared in a plastic container (20 x 20 x 6 cm) filled with dechlorinated tap water and were left few days until adult mosquito emergence. The larvae were fed daily with the prepared larval food. The water was daily maintained at optimum condition for larval growth until the emergence of pupae. The emerged pupae were separated from the rest of larvae into new container

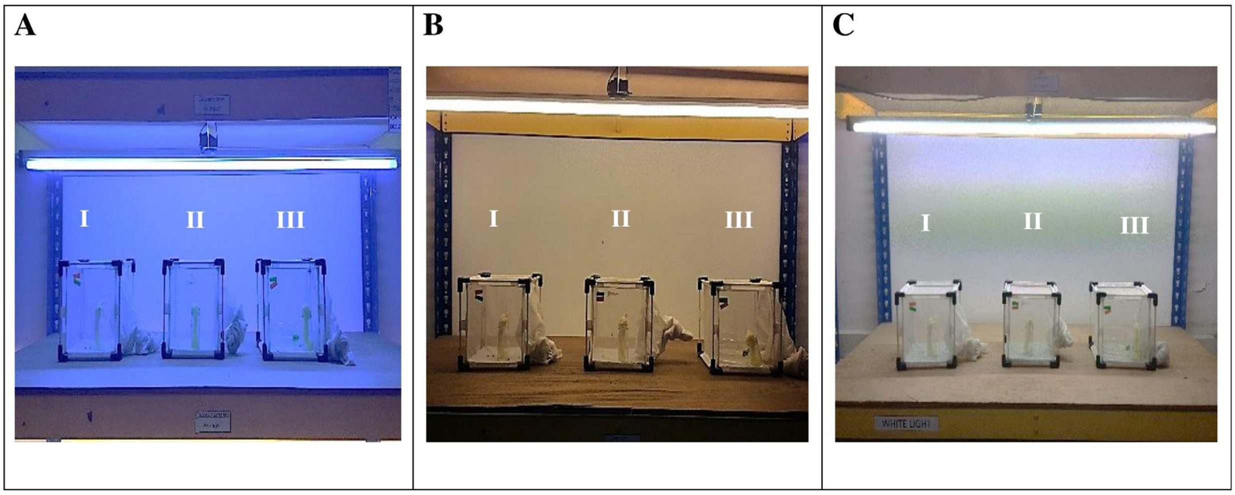

Figure 1: Overview on the light and cage settings for survival and life traits studies of adult Aedes aegypti

and put inside the standard rearing cage (30 x 30 x 30 cm) until emergence of adult mosquito. The emerged adults were fed with the prepared sucrose solution. After random mating was occurred, blood feed on restrained laboratory mouse were introduced to gravid female mosquito for eggs development. Small and black in colour of oviposition container was introduced into the cage for gravid female mosquito to lay eggs. The laid eggs on the oviposition paddle were collected as much as possible and dried under insectarium condition. Dried eggs were kept in covered plastic container for stock purpose.

Data Collection and Analysis

The process started the day when the pupae emerged into adults and immediately being introduced into the different artificial light exposure and different photoperiod. Visual observation was conducted daily from 3.00 pm until 6.00 pm to monitor the survival period and number of eggs deposited. Data on the emergence date, number of eggs deposited, frequencies of blood meals within a gonotrophic cycle and remaining mosquitoes that survive were recorded daily. Survival rate of each treatment was measured by recording the survival of male and female mosquitoes after two weeks being exposed to the artificial lights. Dead mosquitoes were removed and stored in a vial in refrigerator at 4^{o}C. The number of eggs deposited was calculated with the magnification of stereo microscope. The gonotrophic cycles of the females were determined after no eggs were deposited after the second blood meal (18). The fecundity rate per female is calculated by dividing the total numbers of eggs deposited by the total number of numbers of females laying eggs (19). In this study, the data collected on the effects of artificial lights exposure with different photoperiod on the survival of adult *Aedes aegypti* were analyses with analysis of variance (ANOVA) through Statistical Package of Social Science (SPSS) software.

The survival and life traits of the *Aedes aegypti* on the exposure of different artificial lights according to different photoperiod is determined by observing starting from adult emergence until the adults died. Several aspects of survival and life traits were monitored during the observation process in order to determine the impacts of artificial light exposures according to different photoperiod which are (i) duration to complete one gonotrophic cycle, (ii) frequency of blood-meal within a cycle, (iii) total number of eggs collected within a cycle, and (iv) fecundity rate – number of eggs laid by one female.

RESULTS

Profiling on artificial light exposure and different photoperiod on gonotrophic cycle of Aedes aegypti

Generally, at three different artificial light exposure (white, yellow and UV) and three different photoperiod (8h : 16h, 12h :12h and 14h : 10h), the duration of gonotrophic cycle (GC) of the adult *Aedes aegypti* was observed to be deteriorated uniformly from the first gonotrophic cycle until the third gonotrophic cycle while at fourth and fifth GC, the duration of the cycle was just the same. Five consecutive weeks of GC duration were recorded, each cycle showed decreasing in number of days which is to two days from the 1st GC until the 3rd GC while no substantial different from 4th GC to 5th GC as from Table IA. The mean of both duration and blood-meal frequency per GC is not meaningfully different from each cycle.

Moreover, based on the Table IB shows the means duration of each GC and means of frequency of blood-meal per cycle exposed to three different artificial light, both of the means duration of GC and blood-meal frequency from the white light exposure is lesser compared to the yellow and UV light exposure. The mean of GC duration from the white light exposure is 3.73 ± 1.11 days while the yellow and UV light exposures are 6.93 ± 0.57 days and 6.87 ± 0.48 days respectively. On the other hand, the frequency of blood-meal per GC from those three exposures are quite similar with only 1.27 ± 0.28 times at white light exposure while 1.73 ± 0.15 times for both yellow and UV light exposures. In general, the mean duration to complete a cycle and blood-meal frequency at different photoperiod is just the same and has no substantial different between various photoperiod regimes (Table IC).

Effect of exposure from different artificial lights with different photoperiod towards gonotrophic cycle (GC) of the adult Aedes aegypti

The adults under white light exposure has the least mean duration to complete each GC which is only 4.20 ± 0.09 days on 14h:10h (D:L) photoperiod, 3.80 ± 0.31 days on 12h:12h and 3.20 ± 0.44 days on 8h:16h photoperiod. Meanwhile the highest mean is under yellow light exposure with the mean of 7.20 ± 2.49 days on 14h:10h, 7.00 ± 1.41 days on 12h:12h and 6.60 ± 1.97 days on 8h:16h photoperiod. On the other hand, the mean duration to complete a cycle taken by the females in control is 8.60 ± 0.55 days. Thus, the white light exposure has allowed the females to reach faster into a full GC compared to other lights (Table II).

Further study has been conducted by using univariate analysis of variance (ANOVA) in order to investigate the effect of exposure from different photoperiod with different artificial light exposure towards GC of the adult *Aedes aegypti*. The ANOVA has shown that the artificial light exposure has significantly affected the duration needed for the females to complete a GC which is F (5.44), p = 0.03. Post hoc analysis with Tukey's HSD using an α of 0.05 revealed that the exposure of white light (mean = 3.73, SD = 4.32) has allowed the females to reach faster in completing a GC followed by blue / UV light with mean difference 3.13 days and lastly yellow

Table I: Summary of survivorship profile (days) of Aedes aegypti exposed with excessive light in terms of; (A) gonotrophic cycle, (B) types of artificial light and (C) photoperiod (L:D) regimes

A

Gonotrophic Cycle	Mean ± Standard Error (days)	
	Duration of each cycle	Frequency blood-meal per cycle
1	9.89 ± 0.35	2.22 ± 0.15
2	7.44 ± 0.18	2.11 ± 0.11
3	5.11 ± 1.01	1.89 ± 0.20
4	3.56 ± 0.93	1.00 ± 0.24
5	3.22 ± 0.81	0.67 ± 0.17

B

Artificial Light	Mean ± Standard Error (days)	
	Duration of each cycle	Frequency blood-meal per cycle
White	3.73 ± 1.11	1.27 ± 0.28
Yellow	6.93 ± 0.57	1.73 ± 0.15
Blue / UV	6.87 ± 0.48	1.73 ± 0.15

C

Photoperiod (L:D)	Mean ± Standard Error (days)	
	Duration of each cycle	Frequency blood-meal per cycle
8h:16h	5.80 ± 0.79	1.67 ± 0.21
12h:12h	5.93 ± 0.97	1.53 ± 0.24
14:10h	5.80 ± 0.84	1.53 ± 0.19

TABLE II: Tendencies of lab strain Aedes aegypti to complete a gonotrophic cycle under the exposure of artificial lights with different photoperiods

Light / Photoperiod	Mean duration each cycle (days) ± standard deviation			*p-value
	14h : 10h	12h : 12h	8h : 16h	
Yellow	7.20 ± 2.49	7.00 ± 1.41	6.60 ± 1.97	0.030
Blue / UV	7.20 ± 1.64	6.80 ± 2.49	6.60 ± 1.67	0.998
White	4.20 ± 0.09	3.80 ± 0.31	3.20 ± 0.44	0.026

light with mean difference of 3.20 days (Figure 2).

Effect of exposure from different artificial lights with different photoperiod towards the fecundity of adult Aedes aegypti

In this study, the fecundity of the adult *Aedes aegypti* are also being measured in terms of the total number of eggs deposited in one GC and the fecundity rate per single female *Aedes aegypti*. The total numbers of eggs collected for five GC are 4632 eggs with the mean 102.9 ± 14.23 eggs per cycle. The total fecundity rate in which each female mosquito within these five GC oviposited with the average of 20.59 ± 2.85 eggs.

Generally, the most total number of eggs deposited under all three different artificial light exposures (white, yellow and UV) is during photoperiod 14h: 10h with a sum of 380 eggs, 951 eggs and 758 eggs respectively. Meanwhile, the least total amount of eggs deposited is the photoperiod 8h:16h with only 267 eggs under white light exposure, 286 eggs under yellow light exposure and 295 eggs under ultraviolet exposure for five GC. The highest fecundity rate recorded at both artificial light exposure is at photoperiod 14h: 10h with 50.4 eggs laid by one female mosquito under white light exposure and 60.8 eggs laid by each females under yellow light exposure while under UV light exposure, 12h: 12h photoperiod regime is recorded to has the highest fecundity rate with 50.2 eggs laid per female mosquito (Table III).

A Shapiro-Wilk test ($p>0.05$) shows that the number of eggs accumulated are normally distributed. Moreover in terms of its skewness and kurtosis, where the z-value should be in between -1.96 to +1.96. The z-values for number of eggs are Skewness; 0.580 and Kurtosis; 1.121. Therefore, one way ANOVA on both number of eggs and fecundity rate has shown that different photoperiod significantly influenced the fecundity of the females with both F (3.242), $p = 0.049$. Since the photoperiod treatment shows significant effect, the

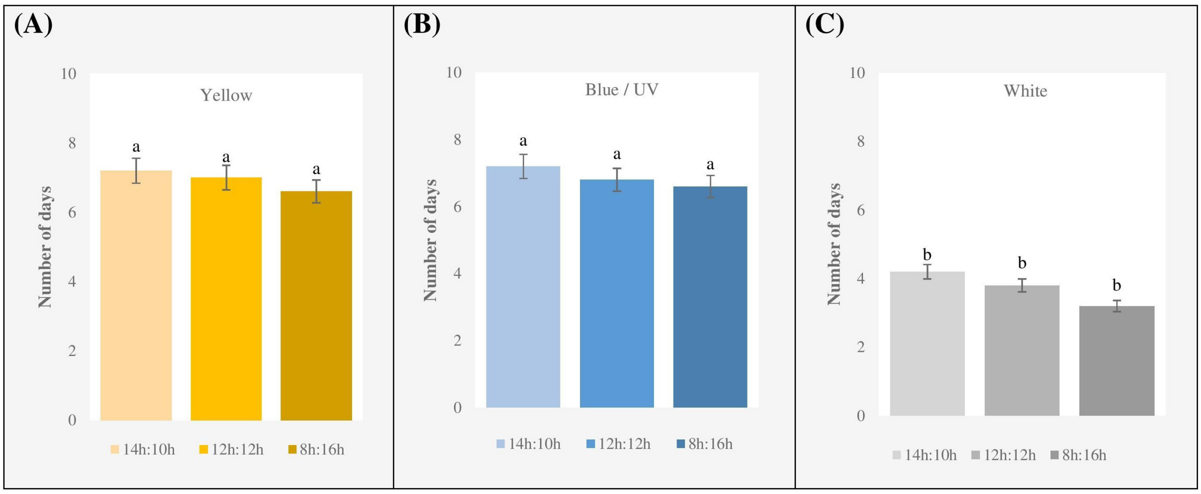

Figure 2: Average of duration of days needed by the females to complete a full gonotrophic cycle that exposed to three different artificial lights (A) represents under yellow light exposure, (B) represents under blue / UV light exposure while (C) represents under white light exposure with three different photoperiods (L:D).

TABLE III: Fecundity of Aedes aegypti under the exposure of artificial lights with different photoperiods

Light / Photoperiod	Fecundity rate (no of eggs) ± standard deviation		
	14h : 10h	12h : 12h	8h : 16h
Yellow	951 ± 105.92	648 ± 109.60	286 ± 66.76
Blue / UV	758 ± 93.18	722 ± 88.11	295 ± 51.67
White	380 ± 112.92	325 ± 93.08	267 ± 75.48

mean number of eggs collected is compared. *Aedes aegypti* laid significantly more eggs when under 14h: 10h photoperiod treatment, and decreasing as the photoperiod treatment decreases to 8h: 16h. Post hoc analysis with Tukey's HSD (using α of 0.05) shows that photoperiod treatment with lowest light duration (M = 56.53, SD = 15.64) has significantly lowered the fecundity of *Aedes aegypti*.

The highest mean number of eggs collected within five GC when the colony was exposed under yellow light which is 125.67 ± 5.25 eggs. The lowest mean number of eggs collected when the colony was exposed under white light which is 64.80 ± 8.53 eggs. On the other hand, in terms of photoperiod, the highest mean number of eggs collected when the colony was under 14h:10h (L:D) exposure with 139.27 ± 8.35 eggs while the least mean number of eggs collected was under 8h:16h (L:D) which is only 56.53 ± 6.58 (Figure 3). Further studies on the number of eggs by using multivariate ANOVA (MANCOVA) between different photoperiod and artificial light. Statistically from the multivariate test, the Pillai's trace of photoperiod's effect towards the fecundity of females is significant with F(3.264), p = 0.050. However, when covariate with light exposure, the Pillai's trace is at F (0.564), p = 0.690 showing less significant effects.

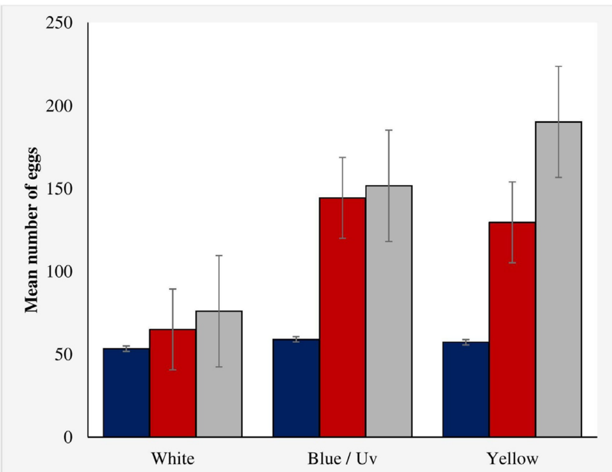

Figure 3: The effects of artificial light exposures with different photoperiods towards the fecundity of Aedes aegypti. Photoperiod (L:D); 8h:16h (Blue bar), 12h:12h (red bar) and 14h:10h (grey bar).

DISCUSSION

This study investigate the impacts of artificial light exposure with different photoperiod treatment on *Aedes aegypti* life traits and its survivals. Even though, the potential impacts of these variables were not studied in natural settings, it is hoped that this would portray ample support that the adult *Aedes aegypti* unveil photoperiodic sensitivity towards artificial lights exposure.

Artificial light or other visual stimuli could attracted some of mosquitoes species and others important diptera. However, the degrees of response of the adult mosquitoes are different from species to species. There is a study claimed that different wavelength were found physiologically more attractive as compared to colors (14). This explained the relatively small numbers captured of both species in light trap, indicating the insignificance of the light exposure preference. However, there is a group of researcher found that gravid *Aedes aegypti* have spectral sensitivities with sensitivity peaks ranging from ultraviolet (323 nm) to orange-red (621 nm) in both the ultraviolet (345 nm) and green (523 nm) wavelengths respectively (20, 21). Therefore, in this study, experiments have been conducted to investigate the response of the following variables; artificial lights and photoperiod treatments with the life traits and longevity of adult *Aedes aegypti*.

This study has shown tendencies of *Aedes aegypti* life traits in terms of its fecundity and gonotrophic cycle and also its longevity when exposed to artificial lights (UV, yellow and white) with different photoperiod treatments (14h:10h, 12h:12h and 8h:16h) (light:dark). In terms of gonotrophic cycle, generally the population of *Aedes aegypti* used in this experiment were significantly influenced when exposed to artificial lights after five gonotrophic cycles were recorded. The duration needed for the mosquito to complete a cycle in the ultraviolet and yellow lights were longer as compared to the white light exposures. Furthermore, the fecundity rate and the number of eggs collected over five cycle of the *Aedes aegypti* is also high when being exposed to yellow and ultraviolet lights while has lower rate when exposed to white lights. Thus these findings have supported the Muir et al., (1992) study that found the gravid *Aedes aegypti* has sensitivities towards yellow-green and ultraviolet lights as well as also from in another study found that this species prefer low ambient light levels, even though it is diurnally active (20, 21). This is due to the interommatidial angle of the *Aedes aegypti* is relatively large, hence has low acuity (21). However, it doesn't mean that it is insufficient for *Aedes aegypti*, as many nocturnal and seminocturnal insects have a large eye parameter, so more photons are captured per sample and contrast sensitivity is improved at low ambient light levels (22). Therefore, it shows that *Aedes aegypti* have an eye parameter optimal for the dim light conditions like the yellow-green and ultraviolet lights which they prefer.

In terms of different photoperiod treatment, this study has shown that it does not significantly affect the gonotrophic cycle of this species, conversely it affects the fecundity trait of the species. The highest total number of eggs and fecundity rate recorded in this study is under 14h: 10h (light:dark) photoperiod treatment, while the lowest is under 8h:16h (light:dark) photoperiod treatment. There is a few studies conclude that in the insectary, a cycle of 14h of light and 10h of darkness appears to allow the best and most uniform development (23-25). Nevertheless, Costanzo et al., (2015) in their studies concluded that different photoperiod treatment was not significantly influenced the development time, size, and fecundity of blood-fed females suggesting that a photoperiod fluctuation does not affect these traits in this species. However, this study differs with the current study as they were only exposed the species under sunlight while in this study, various artificial lights were exposed towards the mosquitoes (1).

From the result shown, it is found that the exposure of ultraviolet light, yellow light and white light has no significant effects on the fecundity of the *Aedes aegypti* mosquito. There are several factors which are based on other studies that influence the fecundity of this species in aspects of its i) body size, (ii) amount of blood ingested, (iii) diet and (iv) temperature. Blackmore & Lord (2000) stated that the female body size is strongly associated with the fecundity trait where larger females tend to lay more eggs compared to the smaller ones [26]. This is because large females can blood fed more than twice that of small females which can provide adequate nutrients supply for the eggs development (16). Maciel-de-Freitas et al., (2011) stated that frequency and availability of diet regimes like blood and sugar, have a direct impact on both survival and reproductive capacity of the adult mosquito. For instance, females that were frequently fed with blood and sugar were observed to have higher fecundity rate and longevity respectively.

CONCLUSION

The study of life traits and longevity of this species under artificial light exposure has shown discoveries on how artificial light affected the gonotrophic cycle, fecundity and the longevity of this species. From this study, gonotrophic cycle of *Aedes aegypti* is influenced by the artificial light exposure as this species tend to complete a complete cycle faster when under high intensity exposure (white light exposure) while tend to have longer gonotrophic cycle when exposed to the ultraviolet / blue light exposure. Statistically the fecundity of this species does not being affected from these artificial light exposure, meanwhile different photoperiod treatment may has affected the fecundity of this species. In this study, *Aedes aegypti* has shown increasing rate of fecundity under longer daylight period ratio which is 14h: 10h (light : dark) compared to shorter daylight period 12h:12h and 8h:16h photoperiod treatments. Further studies can be conducted by including the measurement of the light intensity and wavelength so that optimise light intensity can wavelength can be identified. The findings of this study also reveal that white light has significant effect in reducing the number of eggs laid by gravid female *Aedes aegypti*. This indicate that the use of white light is one of the control measures to overcome and manage the infestation of *Aedes aegypti* in the premises.

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