POPULATION ABUNDANCE OF *Aedes albopictus* AND *Culex quinquefasciatus* IN 24 HOURS CYCLE IN RESIDENTIAL AREAS, PENANG USING DIFFERENT TRAPPING METHODS

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ABSTRACT

The increasing concern on the current activity of *Aedes albopictus* and *Culex quinquefasciatus* mosquitoes as pest and diseases vector in the residential areas require thorough and immediate evaluation. Therefore, 24 hours trapping was conducted at the urban Sungai Dua and sub-urban Batu Maung residence area, Penang, Malaysia, using two different traps; BG- Sentinel Trap and CDC Fay-Prince Light Trap under two different light conditions to investigate the possibility effect of light to both mosquito species. CDC Fay-Prince light trap was effective in trapping *Cx. quinquefasciatus* than BG–Sentinel traps. The results indicated that both traps were ineffective to capture *Ae. albopictus*. In this study, the peak activity of *Ae. albopictus* was not following the diel host-seeking activity which this species active at daylight (6:00am–8:00am) and during night time (6:00pm–8:00pm). Whereas, *Cx. quinquefasctus* was captured mostly during the nocturnal time (12:00am–6:00am) with a greater number of *Cx. quinquefasciatus* mosquitoes captured under no light conditions. Meteorological parameter recorded within experiment such as temperature, relative humidity, and wind speed did not correlate with the abundance of both mosquito species ($p > 0.05$). However, light intensity parameters significantly affected *Cx. quinquefasciatus* mosquito abundance in both residential areas ($p = 0.002$), but not the *Ae. albopictus* mosquitoes ($p = 0.086$ Sungai Dua; $p = 0.803$ Batu Maung) as light is not a primary cue attraction factor to any host source towards this species. Ovitraps seem to be better tools for collecting and surveillance population of *Ae. albopictus* mosquitoes as compared to BG- Sentinel trap (Paired t-test; $p = 0.019$) and CDC Fay-Prince light trap (Paired t-test; $p = 0.025$). As a conclusion, BG-Sentinel trap and CDC Fay-Prince light traps is a not suitable tool for monitoring the activities of *Ae. albopictus* in the residential area, but CDC Fay-Prince light traps still found effective to use to capture *Cx. quinquefasciatus*. This study has therefore established immature sampling as the most effective method of capturing *Ae. albopictus* in a residential area.

Keywords: *Aedes albopictus*, biting cycle, BG-Sentinel trap, CDC Fay-Prince trap, *Culex quinquefasciatus*

ABSTRAK

Peningkatan kebimbangan terhadap aktiviti semasa oleh nyamuk *Aedes albopictus* dan *Culex quinquefasciatus* sebagai perosak dan vektor penyakit di kawasan kediaman memerlukan penilaian yang segera dan kukuh. Oleh itu, penangkapan menggunakan perangkap 24 jam telah dilakukan di kawasan kediaman bandar Sungai Dua dan kawasan subbandar Batu Maung, Pulau Pinang, Malaysia menggunakan dua jenis perangkap; perangkap BG- Sentinel dan perangkap cahaya CDC Fay-Prince di bawah dua keadaan cahaya berbeza untuk mengkaji kebarangkalian kesan cahaya terhadap kedua-dua spesies nyamuk. Perangkap cahaya CDC Fay-Prince adalah berkesan untuk memerangkap *Cx. quinquefasciatus* berbanding perangkap BG-Sentinel. Kedua-dua perangkap didapati tidak berkesan untuk memerangkap *Ae. albopictus.* Dalam kajian ini, aktiviti puncak oleh *Ae. albopictus* adalah tidak mengikut aktiviti pencarian-perumah harian yang mana spesies ini aktif pada waktu siang (6:00am–8:00am) dan semasa malam hari (6:00pm–8:00pm). Sementara itu, *Cx. quinquefasctus* kebanyakannya ditangkap pada malam hari (12:00am–6:00am) dengan bilangan yang tinggi pada sewaktu ketiadaan cahaya. Parameter meteorologi semasa kajian mencatatkan bahawa suhu, kelembapan dan kelajuan angin tidak berkorelasi dengan kelimpahan kedua-dua spesies nyamuk ($p>0.05$). Walau bagaimanapun, parameter intensiti cahaya mempengaruhi kelimpahan nyamuk *Cx. quinquefasciatus* di kedua-dua kawasan kediaman secara signifikan ($p= 0.002$), tetapi tidak bagi nyamuk *Ae. albopictus* ($p= 0.086$ Sungai Dua; $p= 0.803$ Batu Maung) disebabkan intensiti cahaya bukan merupakan faktor tarikan utama terhadap perumah bagi spesies ini. Ovitrap merupakan alat yang lebih baik untuk mengumpul dan pengawasan populasi nyamuk *Ae. albopictus* berbanding perangkap BG-Sentinel (Paired t-test; $p= 0.019$) dan perangkap cahaya CDC Fay-Prince (Paired t-test; $p= 0.025$). Sebagai kesimpulan, perangkap BG-Sentinel and perangkap cahaya CDC Fay-Prince adalah alatan yang tidak sesuai untuk memantau aktiviti *Ae. albopictus* di kawasan kediaman, tetapi perangkap cahaya CDC Fay-Prince masih berkesan untuk memerangkap *Cx. quinquefasciatus.* Kajian ini mendapati pensampelan peringkat tidak matang adalah cara yang lebih berkesan untuk memerangkap *Ae. albopictus* di kawasan kediaman.

Kata kunci: *Aedes albopictus*, *Culex quinquefasciatus*, kitar gigitan, perangkap BG-Sentinel, perangkap cahaya CDC Fay-Prince Trap

INTRODUCTION

Human global demographic population, distribution, societal changes, urbanization and movement via modern transportation known as the main behavioural factors that related to the global spread of Mosquito-Borne Disease (MBD) in most of the invasive mosquito species. This is because these factors not just increase the exposure to infected mosquitoes bite, but also provides mechanisms for arboviruses to breakout from their natural area. Therefore, the establishment in new locations enhances and allow the major outbreak occur (Kow et al. 2001; Petrić et al. 2014). Two common *Aedes* species mosquitoes in Malaysia mainly acted as vectors of dengue virus transmission, which are *Aedes aegypti* and *Aedes albopictus*. The dengue fever is caused by four serotypes (virus DENV-1, DENV-2, DENV-3 and DENV-4) of the genus Flavivirus (Mia et al. 2013). The dengue disease transmitted by these mosquitoes has been declared as one of the national health threat to the public in Malaysia (Er et al. 2010). Over the past year, a major cause of hospitalization and death among children in most of the Asian countries have been reported mainly by dengue hemorrhagic fever (DHF) disease (Potts & Rothman 2008). The difference in distribution between these two common *Aedes* species in Malaysia is significantly related to the behaviour of blood feeding, resting, host preference, and preference for vegetation (Higa 2011).

Aedes albopictus commonly found throughout the vegetation of rural and urban areas which may affect by urbanization (Chan et al. 1971; Honorio et al. 2009). Petrić et al. (2014) found most of *Ae. albopictus* prefers to feed at 89% outdoor (exophagic) and 87% rest outdoor (exophilic). Usually, mosquitoes rest outdoor in the vegetation and natural shelter (Almeida et al. 2005). Most researchers refer *Ae. albopictus* as resting and feeding outdoors, but a recent study showed geographical variation in this behaviour with gravid females being captured indoor in Rome, Italy (Bonizzoni et al. 2013). Higa (2011) studied the reasons of this changing of behaviour still unclear, however, it look-like the possible of connection with society developments where some of parks and vegetation are constructed, and people cultivate gardens at home areas causes this normal behaviour to change. *Aedes albopictus* feeds on various hosts and can breed in a wide range of habitat, especially with vegetation such as gardens in the urban cities. *Aedes albopictus* is diurnal species, which active during the day and biting cycle of *Ae. albopictus* has found normally bite during sunset and dawn (Hassan et al. 1996). There are times when some of meteorological parameters such as low temperature that could inhibit their feeding or heavy cloud and high humidity that promote levels of their feeding during a normal hour (Burkot et al. 2013). In the tropical countries with a warm climate including Malaysia, the dengue fever incidence is high (Jeefoo et al. 2010). In Thailand and Vietnam, a large peak of dengue incidence occurred between June to November where the rainfall and temperature are the highest of the year and shows a positive association with the dengue incidence (Wu et al. 2009; Hu et al. 2013). The dengue cases were at a low level during the cold season and reach a peak during the summer and rainy season in Thailand (Jeefoo et al. 2010). In Bangkok, the biting activity of *Ae. aegypti* changed slightly depending on the seasons. Such as the afternoon peak in the cool season was between 2:00 pm to 3:00 pm, and then shifted to 1 hour later in the hot season and the rainy season. However, the highest peak was found mostly between 2 - 3 hours before sunset (Yasuno & Tonn 1970).

Studies found out mosquitoes are successful at breeding in drainage and sewage systems (Mogi & Okazawa 1990) where this system completely builds either in the urban and sub-urban housing area. *Culex quinquesfasciatus* can be one of major nuisance mosquito species in urban areas (Calhoun et al. 2007) and one the most abundant tropical house mosquito (Abu Hassan & Che Salmah 1990) which can breed in various water sources. It is also vectors for human lymphatic filariasis, caused by nematode *Wuchereria bancrofti* (Harwood & James 1990). It is also capable of achieving high larval densities in water with high organic content, such as sewage treatment ponds, drains (Sunahara et al. 1998) and pit latrines (Cutris et al. 2002). The streams under the influence of nutrient pulses from drainage or sewage systems have increased the presence of *Cx. quinquesfasciatus* (Calhoun et al. 2007). *Culex quinquesfasciatus* is a nocturnal biter (Mahanta et al. 1999) with two peak times of appearance during nocturnally period, which between 10:00 pm and 11:00 pm and after midnight at 1:00 am and 4:00 am (Sucharit et al. 1981).

The study of the current activity of mosquitoes such as resting and feeding behaviour of mosquito population is crucial for the evaluation of disease control measures and nuisance response. Changing either in resting or biting behaviour could give a problem for future control efforts (Pombi et al. 2014). Therefore, in this study, we investigated the 24 hours' current activity pattern of two common mosquitoes; *Ae. albopictus* and *Cx. quinquefasciatus* mosquitoes in the urban and sub-urban areas with an addition of the environmental effects.

We also investigate the effectiveness of two selected adults trapping methods; BG-Sentinel trap and CDC Fay-Prince Light trap in trapping various mosquito species with response to light conditions. We also compared with the conventional ovitrap method on the collection of *Ae. albopictus* mosquitoes.

MATERIALS AND METHODS

Site Selection

The study was conducted in two selected areas; urban and sub-urban areas which located at the North East District of Penang, Malaysia. The urban areas site was located at Sungai Dua (5º.349981N, 100º.300060E) and the sub-urban area was at Batu Maung (5º.281821N, 100º.275745E), Penang Island, Malaysia. Sungai Dua is an urban area with the high development of high rise condominiums as well as commercial properties. In Sungai Dua urban area, the Flat Hamna which consisted of few blocks of apartments was selected as sampling sites due to the common recurring dengue hotspot in Pulau Pinang (Daily, 2010; idengue, 2014).

Whereas, Batu Maung is classified under suburban areas because it is known as a small town with less development in Penang that consists of several smaller fishing villages namely Kampung Seronok, Kampung Binjai, Permatang Damar Laut, and many more. The sampling was done in a few small blocks of an apartment in Taman Muhibbah, Batu Maung. Batu Maung was also listed as dengue hotspot since 2005 (Daily 2010). Penang experiences a uniform tropical climate with the consistent temperature around the year that falls between 29°C to 35°C and high relative humidity (75-95%) which is suitable for mosquito reproductions.

Study Design

At 24 hours trapping of *Ae. albopictus* and *Cx. quinquefasciatus* mosquitoes, two different traps were used which is CDC Fay-Prince Light Trap and BG-Sentinel Trap. At each location, a BG-Sentinel trap and CDC Fay-Prince Light trap was placed at a distance of 15 meter from each other (Kroeckel et al. 2006). The traps were set under two different light conditions; with present and no present of light to investigate the possibility effect of light on the activities of *Ae. albopictus* and *Cx. quinquefasciatus* mosquitoes. The light was provided by the fluorescent lamp (MCF Model, 240V, 50Hz, 40W) situated inside the building which was switched on approximately from 7:00 pm to 7:00 am (next day). The traps were set at ground level, which a conducive place for mosquitoes to harborage, resting and make contact with the host. The sampling was conducted for three consecutive months from January until March 2016 at urban Sungai Dua and sub-urban Batu Maung areas. At one-time sampling, three BG-Sentinel traps and three CDC Fay-Prince Light traps were set up under two conditions; with the presence of light and no lights. The traps were set up in the different blocks of the apartment under these two conditions to give a clear differentiation of light condition which one trap was placed under the stair at each block which provides no light, while another one placed at the block with the presence of light (Fig. 1).

This experiment was repeated three times (with three replicate traps each time), resulting in a total nine replicates of BG-Sentinel trap and CDC Fay-Prince Light trap for each location and light conditions. The position of the traps was alternated after every 24 hour sampling periods to account for positional effects (Kroeckel et al. 2006). The traps were operated continuously for 24 hours with BGs lure continuously available for BG-Sentinel trap and dry ice provided four times in a day for CDC Fay-Prince Light trap (Russell 2004). The mosquitoes trapped inside the catch bag of each trap were collected every hour starting from 11:00 am for 24 hours. Trapped mosquitoes were placed separately in the test tube and brought back to the laboratory for identification.

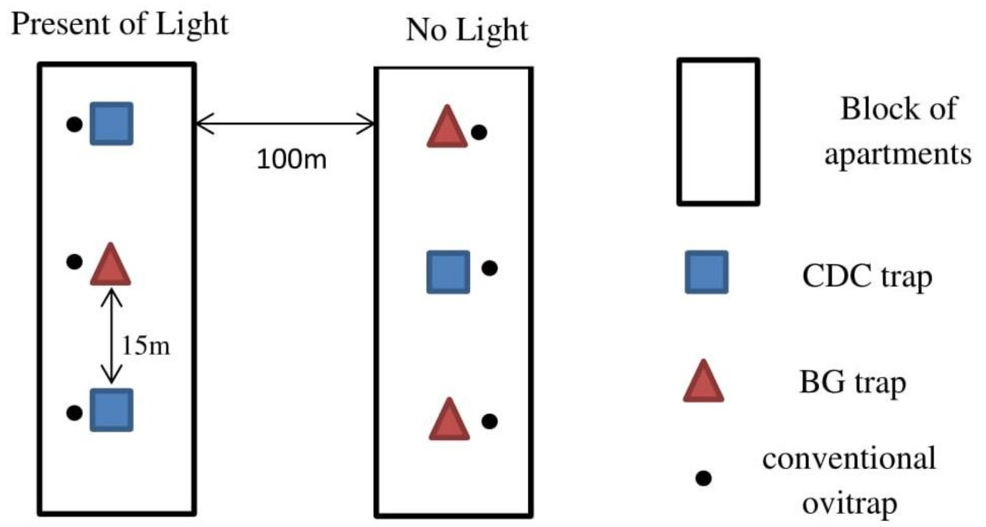

Figure. 1. The placement of traps at the sampling site. Traps were positioned alternated after every 24-hour sampling periods to account positional effects. Traps were placed at a distance 15 meters from each other and 150 meters from each block of apartments.

Trap Settings

The BG-Sentinel trap (Bioagents, Regensburg, Germany) is designed to attract anthropophilic mosquito species such as *Ae. Aegypti* and *Ae. Albopictus* (Williams et al. 2006; Geier et al. 2004). The BG-Sentinel trap attempts to mimic convection currents created by human body heat with a fan and mimics odours through the BG lure (ammonia, caproic acid and lactic acid) (Meeraus et al. 2008) which lasted for five months (Farajollahi et al. 2009). The BG-Sentinel trap is a black cylindrical 'busket' with 0.40 m height, 0.36 m in diameter with a white gauze top. In the top centre of the trap is a black 0.10 m diameter opening to a catch bag. A 12V DC fan draws air (and mosquitoes) in through the black opening and exhausts it through the perimeter of white gauze at the top of the trap (Williams et al. 2006), operated using a 12V battery. The BG lure is placed in the designated pocket for the lure inside the trap (Bhalala & Arias 2009) and the odour is blow out of the top exterior of the trap. The BG-Sentinel traps were set in the ground with a clear space directly above them to allow the air plume above the trap to flow freely (Williams et al. 2006).

The CDC Fay-Prince Light trap (Fay & Prince 1970) is specifically designed, and field tested for the collection of *Ae. aegypti* by scientists from the Communicable Disease Center (CDC), United States. The design of the trap is based upon the attractiveness of contrasting gloss black and white panel, which employs a wind-orienting cover and cylinder that houses a suction motor with a suspending collection bag. The suction motor is powered by 12 volt batteries and was placed at 1.5 meters above ground with 2 kg dry ice was placed within an insulated small bucket with ventilation and served as the source of carbon dioxide (CO_2) (Farajollahi et al. 2009). The carbon dioxide given off by animals is used by mosquitoes such as both in activation (take off and sustained flight) as well as in host location (Gillies 1980). However, for this trap, carbon dioxide basically represent as live hosts (as in human bare leg catch) (Service 1993).

As a comparison, we used the conventional technique which is the ovitrap tins were set up at the same time for 24 hours along with each BG-Sentinel and CDC Fay-Prince Light traps. The ovitrap tins were set up as a secondary confirmation technique in the present of *Aedes* mosquitoes in the sampling area.

Meteorological Parameter

The meteorological parameter for humidity and temperature were recorded using temperature and humidity measuring device (Digital Temperature Humidity Meter HTC-1). Whereas, the wind speed data was recorded using digital anemometer wind speed air flow (Benetech GM816 Model). While the light intensity was recorded using a digital light Lux meter (Dr. Meter LX1010B 100,000 Light Meter with LCD Display Model). This meteorological parameter was recorded each hour from six different locations for 24 hours. No rain was recorded during the experiments time.

Statistical Analysis

Data were analyzed using SPPS statistical software version 20.0. Data were transformed prior to parametric tests. Significance abundance of mosquito species in 24 hours-time experiment in two locations was tested using multinomial analysis of variance (MANOVA). The number of captured mosquitoes served as the dependent variable, whereas captured time, location, and type of trap as the independent variables. Data were run separately for species tested; *Ae. albopictus* and *Cx. quinquefasciatus*.

In order to understand the influence of environmental factors on the abundance of mosquito species within 24-hour experiment, the Pearson Correlation test was used. The environmental factors tested were temperature, humidity, wind speed, light intensity and wind speed. As no rainfall during sampling, therefore rainfall data do not include in the analysis.

To understand the effectiveness of the BG-Sentinel and CDC Fay-Prince Light traps to attract mosquitoes with the influence of the presence of light, data were analyzed using multinomial analysis of variance (MANOVA). The number of captured mosquitoes was served as the dependent variable, while the type of mosquito traps, species (*Cx. quinquefasciatus* and *Ae. albopictus*) and condition of light (presence of light and no light) served as independent variables. Data for each location (urban Sungai Dua and sub-urban Batu Maung) were analyzed separately.

To find out a significant of means between two selected traps in trapping *Ae. albopictus* mosquitoes, we ran the comparison using paired t-test between conventional ovitrap methods, BG-Sentinel and CDC Fay-Prince Light trap for both urban Sungai Dua and sub-urban Batu Maung.

RESULTS

Abundance and Activities of *Aedes qlbopictus* and *Culex quinquefasciatus* Mosquitoes Based On The 24 Hours' Experiment

The abundance of both mosquito species in the urban Sungai Dua and sub-urban Batu Maung residence area was monitored started from 11:00am until 11:00am the next day which approximately 24-hours duration of the experiment. During the 24-hour collection, the overall collection data show a total of only three *Ae. albopictus* and six *Cx. quinquefasciatus* individuals were collected using the BG-Sentinel trap (Fig. 2) from both locations. Whereas, more numbers were collected using CDC Fay-Prince Light Trap with three *Ae. albopictus* and 65 *Cx. quinquefasciatus* in both locations after 24-hour collection (Fig. 3).

Culex quinquefasciatus mosquitoes were trapped in the BG-Sentinel trap at urban Sungai Dua area at 12:00 am in light condition (Fig. 2a). Whereas, in no light condition, *Cx. quinquefasciatus* was trapped at nearly dawn around 4:00am (Fig. 2c). The situation in sub-urban Batu Maung area is a little bit different with *Cx. quinquefasciatus* were captured during daylight time at 9:00am and 3:00pm in no light areas (Fig. 2d). However, *Cx. quinquefasciatus* was captured at 11:00pm in the present of light (Fig. 2b).

By using CDC Fay-Prince Light trap, more number of *Cx. quinquefasciatus* was collected in both urban and sub-urban areas. In response to presence of the light condition in urban Sungai Dua, *Cx. quinquefasciatus* were collected at the nocturnal hour (12:00am–6:00am) and the early daylight time at 10:00 am (Fig. 3a). Response of *Cx. quinquefasciatus* almost the same for the no light condition in urban Sungai Dua area, where *Cx. quinquefasciatus* mosquitoes attracted to CDC Fay-Prince Light trap majorly at the nocturnal hour (12:00 am till 5:00 am) (Fig. 3c). The abundance of *Cx. quinquefasciatus* decreased after reaching a peak activity at 3:00 am. The situation in sub-urban Batu Maung a little bit different where the biting activity of *Cx. quinquefasciatus* started a few hours early during the night time. In the presence of light, *Cx. quinquefasciatus* collected at nearly midnight (10:00 am and 12:00 am) (Fig. 3b). Whereas at no light condition, *Cx. quinquefasciatus* were trapped started at dusk (7:00 pm) and reach a peak at 11:00 pm, and then slowly shows fluctuated number of *Cx. quinquefasciatus* captured till dawn. As a comparison for both traps, CDC Fay-Prince Light traps were significantly showed more efficient in trapping *Cx. quinquefasciatus* mosquitoes as compared to BG-Sentinel traps (F= 17.65; df= 1, 192, P= 0.00; Table 1). Although, *Cx. quinquefasciatus* showed an abundance mostly during the nocturnal time in this study, however no significant differences were detected between captured time within 24 hours in either urban Sungai Dua and sub-urban Batu Maung (P > 0.05, Table 2).

Table 1. Results of two-way ANOVA for the abundance of *Culex quinquefasciatus* mosquito species based on 24 hours' time experiment trapped using BG-Sentinel trap and CDC Fay-Prince Light Trap in the urban Sungai Dua and sub-urban Batu Maung.

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	0.867	1	0.867	33.412	0.000
Captured Time	0.616	23	0.027	1.032	0.427
Trap	0.458	1	0.458	17.651	**0.000**
Location	0.070	1	0.070	2.680	0.103
Captured Time*Trap	0.565	23	0.025	0.947	0.537
Captured Time*Location	0.736	23	0.032	1.233	0.221
Trap*Location	0.049	1	0.049	1.903	0.169
Captured Time*Trap*Location	0.452	23	0.020	0.758	0.780
Error	4.983	192	0.026		
Total	8.796	288			
Corrected Total	7.929	287			

*****Significant values are in bold.

Aedes albopictus mosquitoes were trapped in the BG-Sentinel trap at urban Sungai Dua area at 12:00am in light condition (Fig. 2a). Whereas, in no light condition, no *Ae. albopictus* was captured (Fig. 2c). The situation in sub-urban Batu Maung area is a little bit different, where the *Ae. albopictus* was captured during daylight time at 9:00 am at no light areas (Fig. 2d). However, *Ae. albopictus* was captured at 11:00 pm in the present of light (Fig. 2b).

In response to no light condition, *Ae. albopictus* were collected at midnight (12:00am) and nearly dawn (5:00am) at urban Sungai Dua by using CDC Fay-Prince Light trap (Fig. 3c). However, no *Ae. albopictus* was trapped to CDC Fay-Prince Light trap in present of light condition (Fig. 3a). The condition of mosquitoes trapped in sub-urban Batu Maung is also the same as in urban Sungai Dua where *Ae. albopictus* was absent trapped in response to the presence of light condition using CDC Fay-Prince Light trap (Fig. 3b). While only one *Ae. albopictus* was trapped at nearly midnight (11:00pm) at no light areas (Fig. 3d).

Although *A. albopictus* mosquitoes have their specific peak hour of abundance, but results from this study showed the non-significant difference in number of mosquitoes from urban Sungai Dua and sub-urban Batu Maung within 24-hour collection either using BG-Sentinel or CDC Fay-Prince Light trap (P= 0.938; Table 2). Thus, suggested both mosquito traps tested have no significant different influence in collecting *Ae. albopictus* mosquitoes and less effective based on the collection number of both residential areas of urban Sungai Dua and sub-urban Batu Maung.

Table 2. Results of two-way ANOVA for the abundance of *Aedes albopictus* mosquito species based on 24 hours' time experiment trapped using BG-Sentinel trap and CDC Fay-Prince Light Trap in the urban Sungai Dua and sub-urban Batu Maung.

Variable	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Intercept	0.011	1	0.011	6.000	0.015
Captured Time	0.049	23	0.002	1.130	0.316
Trap	0.001	1	0.001	0.667	0.415
Location	0.000	1	0.000	0.000	1.000
Captured Time * Trap	0.029	23	0.001	0.667	0.874
Captured Time * Location	0.060	23	0.003	1.391	0.118
Trap*Location	0.005	1	0.005	2.667	0.104
Captured Time*Trap*Location	0.025	23	0.001	0.580	0.938
Error	0.362	192	0.002		
Total	0.544	288			
Corrected Total	0.532	287			

*Significant values are in bold.

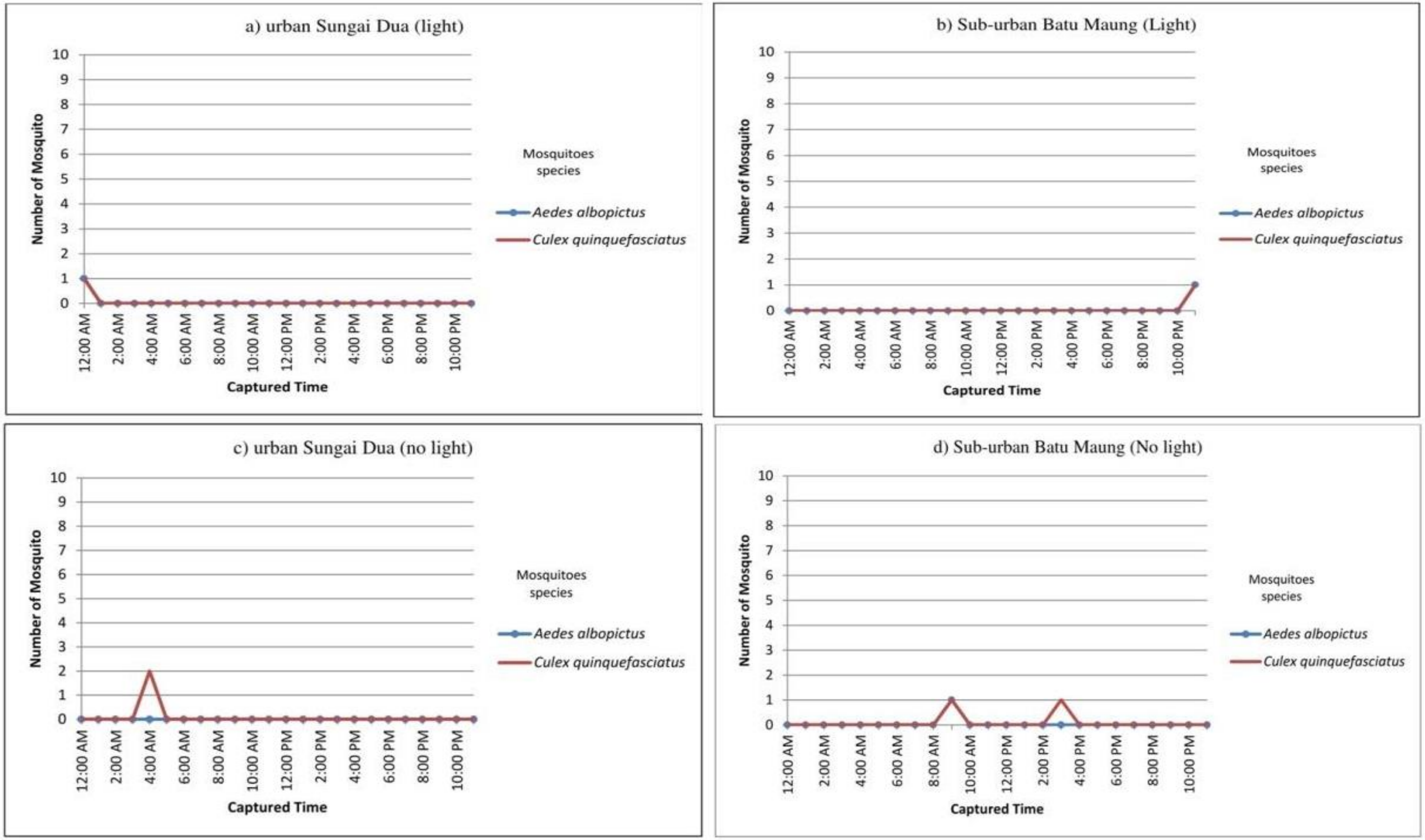

Figure. 2. The abundance of *Aedes albopictus* and *Culex quinquefasciatus* mosquitoes caught using BG-Sentinel trap in the presence of light in (a) urban Sungai Dua, (b) sub-urban Batu Maung and, in the absence of light in (c) urban Sungai Dua, and (d) sub-urban Batu Maung

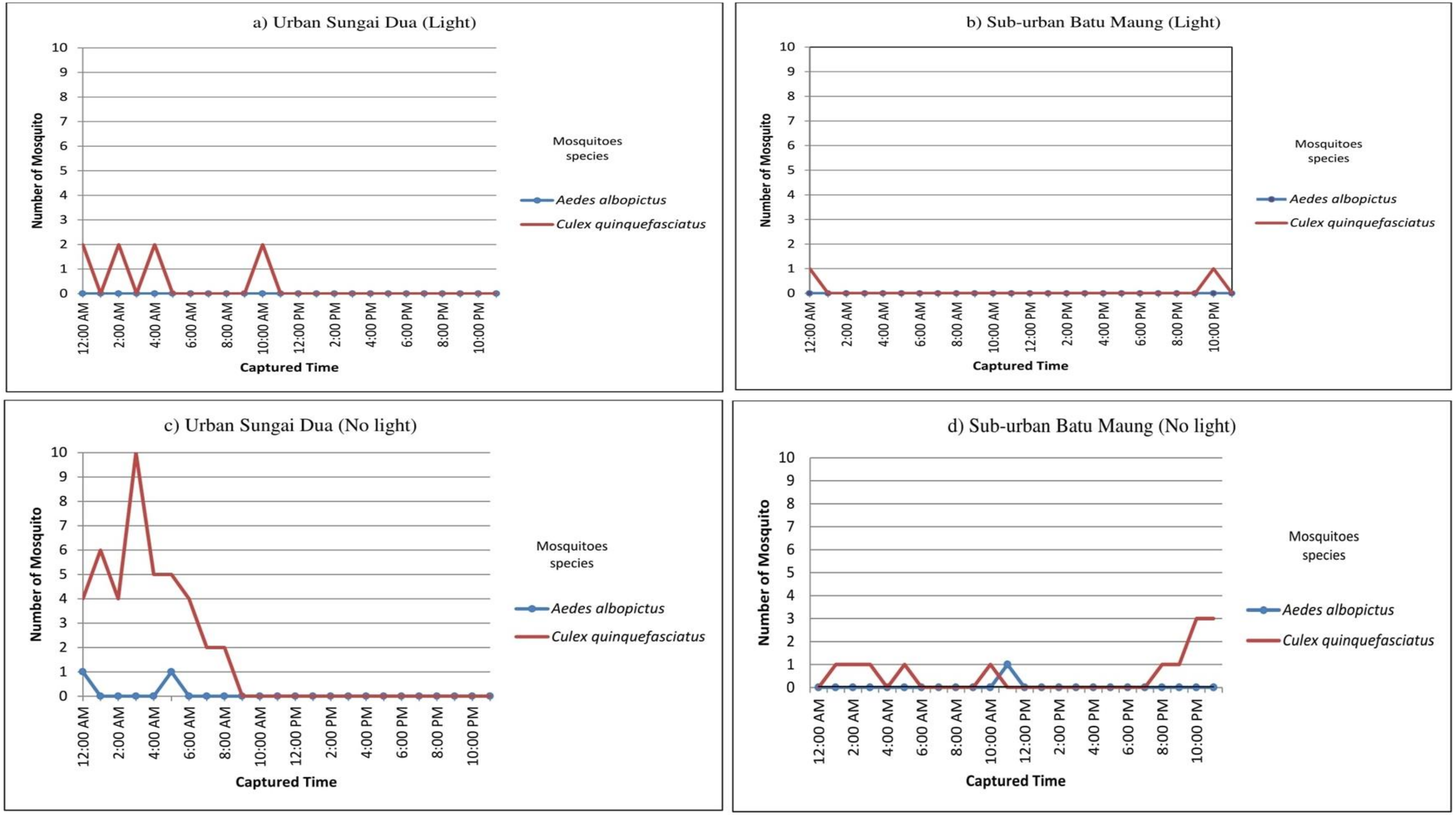

Figure. 3. The abundance of *Aedes albopictus* and *Culex quinquefasciatus* mosquitoes caught using CDC Fay-Prince Light trap in the presence of light in (a) urban Sungai Dua, (b) sub-urban Batu Maung and, in the absence of light in (c) urban Sungai Dua, and (d) sub-urban Batu Maung.

Influence of Meteorological Parameters on the Abundance of *Aedes albopictus* and *Culex quinquesfasciatus* Mosquitoes in the Urban Sungai Dua and Sub-Urban Batu Maung

Most of *Cx. quinquefasciatus* were captured during nocturnal hours when the light intensity was below than 100 Lux which between 12:00am to 07:00am and at 7:00pm and 11:00pm (Fig. 4 and Fig. 5). The light intensity variable was significantly negative correlated with *Cx. quinquefasciatus* abundance in both urban Sungai Dua (r= -0.361, n= 72, p= 0.002; Table 3) and sub-urban Batu Maung (r= - 0.352, n=72, p= 0.002; Table 3) which suggested low light intensity caused more captured a number of *Cx. quinquefasciatus*. The Pearson correlation analysis indicated a significant positive association between captured time and *Cx. quinquefasciatus* abundance in the urban Sungai Dua (r=- 0.529, n= 72, p= 0.00; Table 3), but not in the sub-urban Batu Maung (r= 0.155, n= 72, p= 0.193; Table 3). Temperature and humidity revealed not significantly affected the *Cx. quinquefasciatus* abundance in both locations (p > 0.05; Table 3).

However, for *Ae. albopictus*, light intensity gave no correlation effects towards the abundance of *Ae. albopictus* in both residential areas (p> 0.005; Table 3). Besides that, Pearson correlation analysis revealed there was significant between the abundance of *Ae. albopictus* with captured time (r= 0.296, n= 72, p= 0.012; Table 3) in the urban Sungai Dua, but not at sub-urban Batu Maung (r= 0.075, n= 72, p= 0.530; Table 3). Temperature and humidity parameters also have no significance in affecting the *Ae. albopictus* abundance in both locations (p> 0.05; Table 3; Fig. 6 and Fig. 7).

Table 3. Pearson correlation analysis on the influence of meteorological parameters on the abundance of *Aedes albopictus* and *Culex quinquefasciatus* mosquitoes in the urban Sg. Dua and sub-urban Batu Maung.

Variable	Location	Mosquito Species	Pearson Correlation	N	Sig. (2-tailed)
Captured Time	Urban Sg. Dua	*Aedes albopictus*	-0.296	72	**0.012**
		Culex quinquefasciatus	-0.529	72	**0.00**
	Sub-urban Batu Maung	*Aedes albopictus*	0.075	72	0.530
		Culex quinquefasciatus	0.155	72	0.193
Humidity	Urban Sg. Dua	*Aedes albopictus*	-0.037	72	0.755
		Culex quinquefasciatus	-0.11	72	0.352
	Sub-urban Batu Maung	*Aedes albopictus*	-0.184	72	0.123
		Culex quinquefasciatus	0.041	72	0.731
Temperature	Urban Sg. Dua	*Aedes albopictus*	-0.52	72	0.665
		Culex quinquefasciatus	-0.221	72	0.62
	Sub-urban Batu Maung	*Aedes albopictus*	-0.023	72	0.851
		Culex quinquefasciatus	-0.135	72	0.259
Light Intensity	Urban Sg. Dua	*Aedes albopictus*	-0.204	72	0.086
		Culex quinquefasciatus	-0.361	72	**0.002**
	Sub-urban Batu Maung	*Aedes albopictus*	0.030	72	0.803
		Culex quinquefasciatus	-0.352	72	**0.002**

*Correlation is significant at the 0.05 level (2-tailed).
*Significant values are in bold.

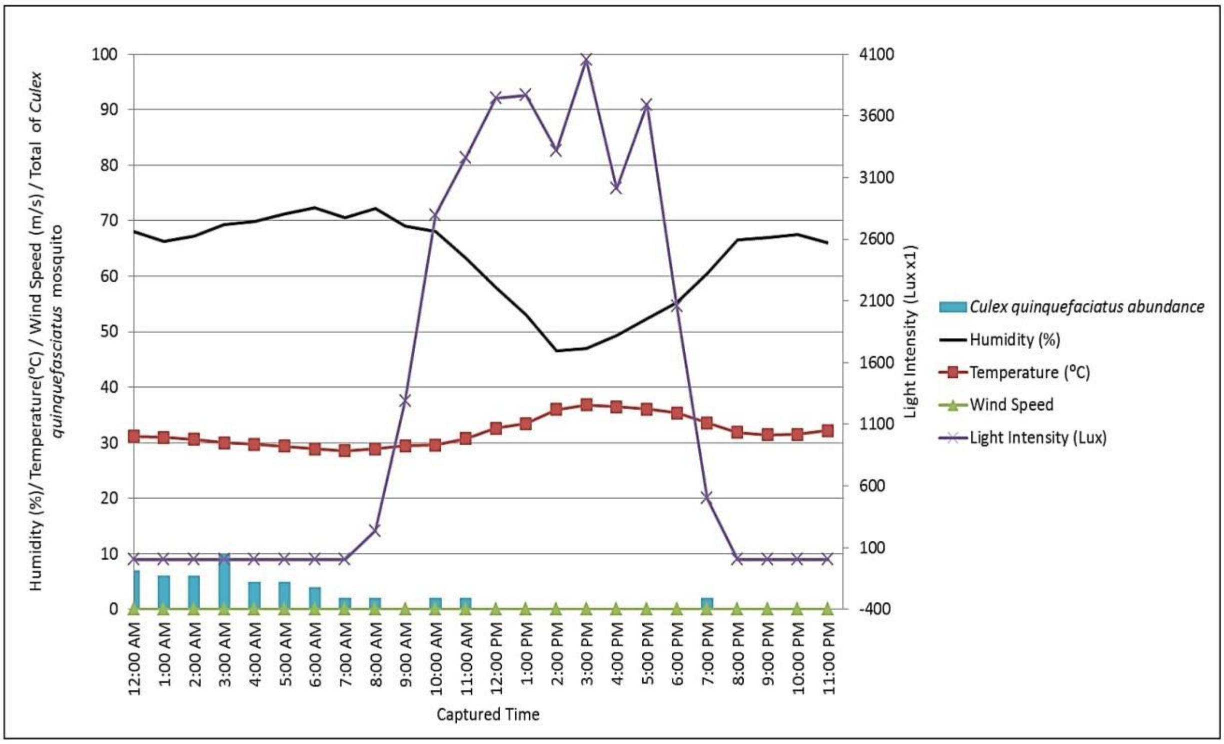

Figure. 4. The influence of meteorological parameters on the abundance of *Culex quinquefasciatus* mosquitoes in the urban Sungai Dua

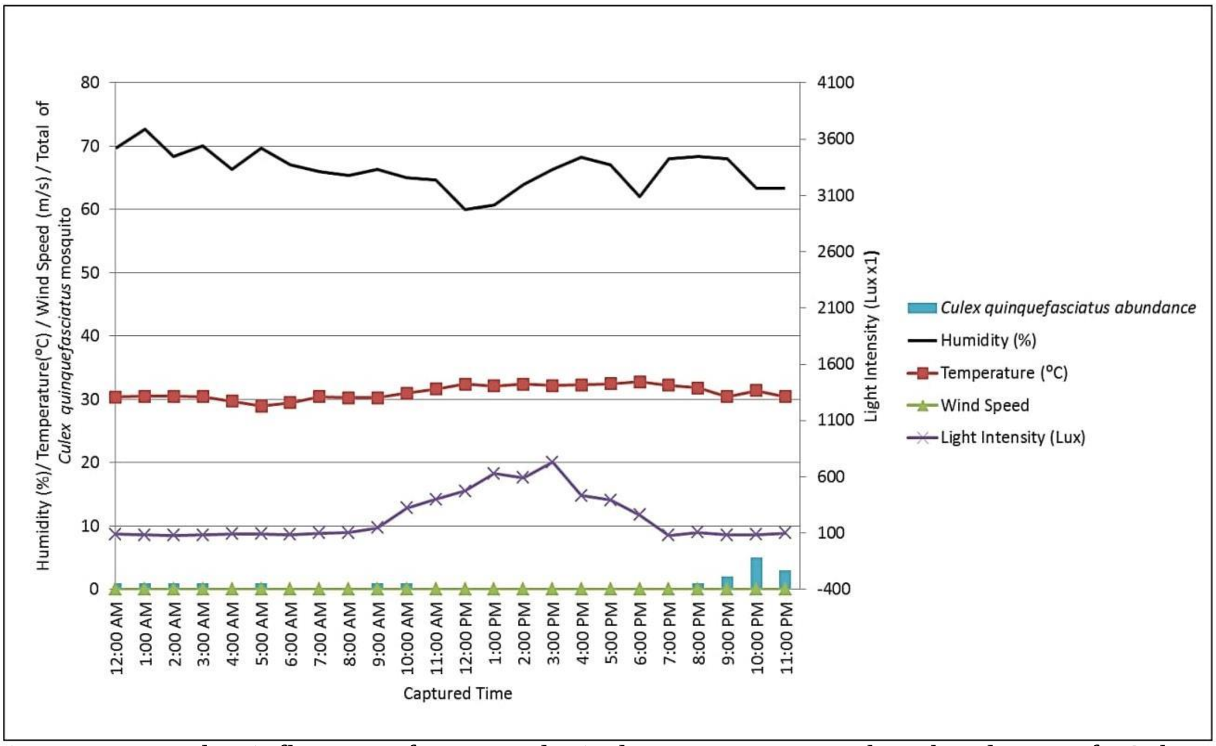

Figure. 5. The influence of meteorological parameters on the abundance of *Culex quinquefasciatus* mosquitoes in the sub-urban Batu Maung

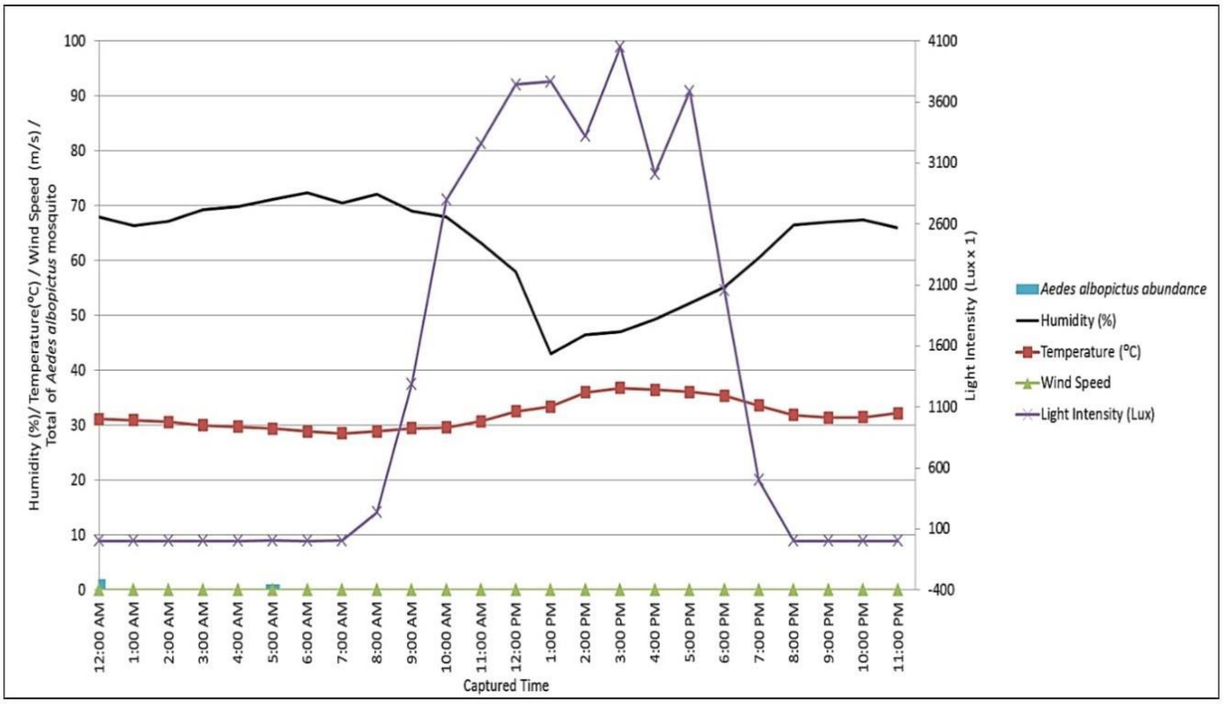

Figure. 6. The influence of meteorological parameters on the abundance of *Aedes albopictus* mosquitoes in the urban Sungai Dua.

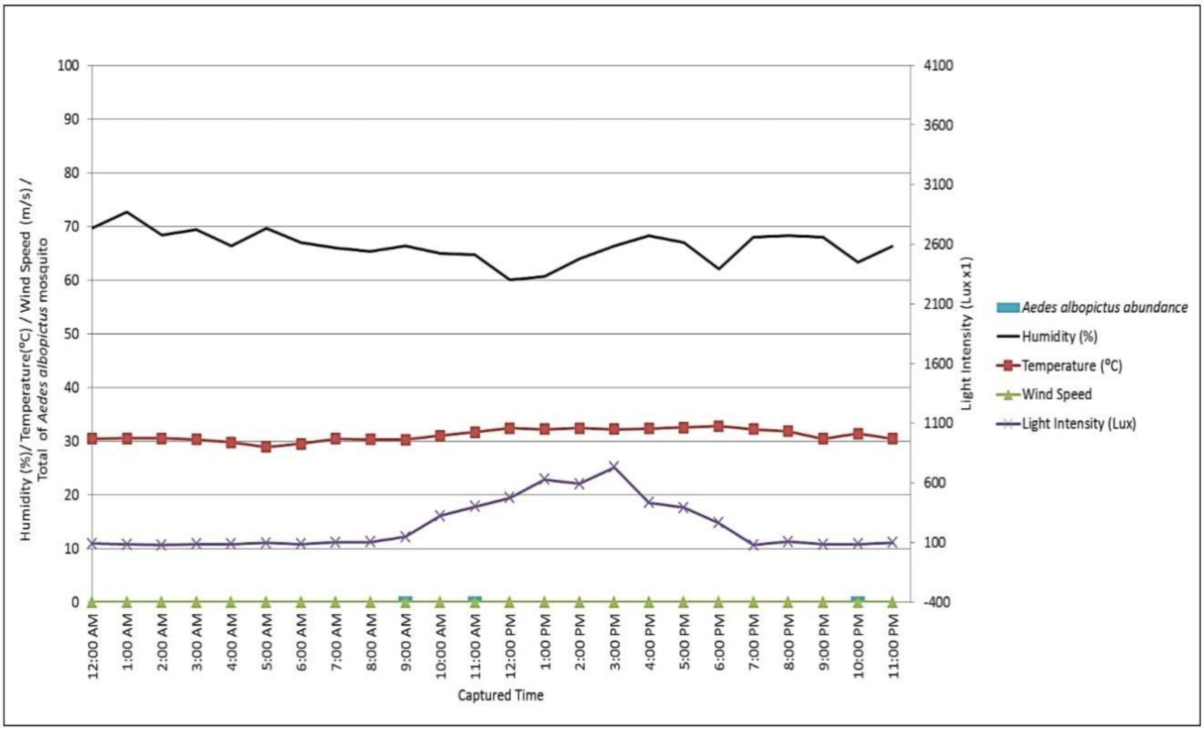

Figure. 7. The influence of meteorological parameters on the abundance of *Aedes albopictus* mosquitoes in the sub-urban Batu Maung

The Effectiveness of Traps to Attract Mosquitoes Based on the Presence of Light
Multivariate analysis was performed to investigate the effectiveness of the BG-Sentinel and CDC Fay-Prince Light trap in the influence of light as we predicted the traps would be more effective to attract mosquitoes when located in no light condition. Unfortunately, the results show no significant differences between the number of both collected mosquito species in both light conditions at urban Sungai Dua (*p*= 0.490; Table 4) and sub-urban Batu Maung (*p*= 0.087; Table 5). More *Cx. quinquefasciatus* mosquitoes were trapped compared to *Ae. albopictus* mosquitoes. Significantly more number of *Cx. quinquefasciatus* were captured as compared to *Ae. albopictus* in both urban Sungai Dua (*p*= 0.029; Table 4) and sub-urban Batu Maung (*p*= 0.08; Table 5) residential areas. Our results revealed non-significant differences between the interaction of traps (BG-Sentinel and CDC Fay-Prince Light trap), mosquito species and condition (light and no light) that was located in both residential areas of the urban Sungai Dua (*p*=0.992; Table 4), but significant interaction in the sub-urban Batu Maung (*p*=0.021; Table 5).

Table 4. Results of two-way ANOVA between mosquito species collected from the urban Sungai Dua on the effectiveness of traps (BG-Sentinel and CDC Fay-Prince Light trap) in response to light conditions (light and no light).

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	5.339	1	5.339	9.000	0.006
Trap	1.229	1	1.229	2.072	0.161
Light Condition	0.436	1	0.436	0.734	0.399
Mosquito Species	3.156	1	3.156	5.319	**0.029**
Trap*Light Condition	0.277	1	0.277	0.467	0.500
Trap*Mosquito Species	0.976	1	0.976	1.645	0.210
Light Condition* Mosquito Species	0.291	1	0.291	0.490	0.490
Trap*Light Condition* Mosquito Species	6.037E-05	1	6.037E-05	0.000	0.992
Error	16.611	28	0.593		
Total	29.015	36			
Corrected Total	23.332	35			

* a. R squared = 0.288 (Adjusted R Square = 0.110)
*Significant values are in bold.

Table 5. Results of two-way ANOVA between mosquito species collected from the sub-urban Batu Maung on the effectiveness of traps (BG-Sentinel and CDC Fay-Prince Light trap) in response to light conditions (light and no light).

Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	0.661	1	0.661	19.503	0.000
Trap	0.102	1	0.102	3.008	0.094
Light Condition	0.046	1	0.046	1.355	0.254
Mosquito Species	0.272	1	0.272	8.017	**0.008**

Trap * Light Condition	0.029	1	0.029	0.846	0.365
Trap * Mosquito Species	0.186	1	0.186	5.494	**0.026**
Light Condition * Mosquito Species	0.107	1	0.107	3.145	0.087
Trap * Light Condition * Mosquito Species	0.056	1	0.056	1.653	**0.021**
Error	0.949	28	0.034		
Total	2.318	36			
Corrected Total	1.680	35			

* a. R squared = 0.288 (Adjusted R Square = 0.110)
*Significant values are in bold.

Effectiveness of Different Mosquito Traps to Attract *Aedes albopictus* Mosquitoes

The selection of our study sites is based on the regular occurring of dengue cases in which the virus is transmitted by the *Aedes* mosquitoes. However, results in the previous section found that the BG-Sentinel trap and CDC Fay-Prince Light trap was less effective to trap *Ae. albopictus* mosquitoes. Therefore, a comparison between these two modern traps with conventional ovitrap was made. *Aedes albopictus* mosquitoes collected from conventional ovitrap in the urban Sungai Dua residence areas showed a significantly higher number of collected *Ae. albopictus* mosquitoes (4.67 ± 1.80) as compared to BG-Sentinel trap (0.11 ± 0.12; Ovitrap – BG-Sentinel trap, P= 0.019) and CDC Fay-Prince Light trap (0.22 ± 0.22; Ovitrap – CDC Fay- Prince Light Trap, P= 0.025; Table 6). Thus, suggested that the conventional ovitrap still and more effective in trapping *Ae. albopictus* mosquitoes at the urban Sungai Dua residence area as compared to the modern traps.

The same results were given after using an ovitrap, with 1.67 ± 0.88 collected *Ae. albopictus* mosquitoes at the sub-urban Batu Maung residence area which a number of mosquitoes collected seven times more as compared to BG-Sentinel (0.22 ± 0.15) and CDC Fay-Prince Light trap (0.11 ± 0.11). However, no significant on the number of *Ae. albopictus* mosquitoes detected among the traps (*P*> 0.05; Table 6).

Table 6. The effectiveness of different mosquito traps in capturing *Aedes albopictus* mosquitoes in the urban Sungai Dua and sub-urban Batu Maung, Penang.

Area	**Paired Test**	**Mean ± SE**	**Standard Deviation**	**t**	**df**	**Sig (2-tailed)**
Urban	Ovitrap – BG trap	1.118 ± 0.380	1.140	2.943	8	**0.019**
	Ovitrap – CDC trap	1.073 ± 0.390	1.170	2.751	8	**0.025**
	BG trap – CDC trap	- 0.451 ± 0.152	0.457	-0.296	8	0.775
Sub-urban	Ovitrap – BG trap	0.435 ±. 0.286	0.858	1.521	8	0.167
	Ovitrap – CDC trap	0.512 ± 0.326	0.977	1.572	8	0.155
	BG trap – CDC trap	0.077 ± 0.139	0.417	0.555	8	0.594

*Significant values are in bold.

DISCUSSION

Our study found that *Ae. albopictus* mosquitoes were less trapped using CDC Fay-Prince Light trap or BG-Sentinel trap in urban Sungai Dua and sub-urban Batu Maung as compared to *Cx. quinquefasciatus* with fluctuation hour of activities in 24-hour duration of the experiment. In this study, mostly biting time of *Cx. quinquefasciatus* in urban Sungai Dua and sub-urban Batu Maung is during the nocturnal time with a peak between 12:00 am to 3:00 am. A different pattern has been discovered from the previous study, which biting cycle of *Cx. quinquesfasciatus* to be nocturnal which active throughout the night but with peak activity from 10:00 pm to 11:00 pm (Gowda & Vijayan 1993; Mahanta et al. 1999). However, the biting activity showed gradually declines as the night proceeded till 6:00 am (Pipitgool et al. 1998; Mahanta et al. 1999). While in this study, the *Cx. quinquefasciatus* biting activity showed gradually decreased after 3:00 am.

Aedes albopictus reported having a clear bimodal activity pattern in their diel host-seeking activity (Hassan et al. 1996; Kawada & Takagi 2004). Based on previous studies, the peak activity of *Ae. albopictus* was discovered at crepuscular dawn (6:00 am to 8:00 am) and at dusk (6:00 pm to 8:00 pm) (Hassan et al. 1996; Almeida et al. 2005). In this study, *Ae. albopictus* was found trapped in both modern traps during daylight (9:00 am, and 11:00 am), night (10:00 pm, 12:00 am) and nearly dawn (5:00 am). It was found that this species is not following the diel-host seeking activity as suggested by previous researchers. Biting activity in the sub-urban Batu Maung started a few hours early during capture time at early night compared in the urban Sungai Dua.

In our study, both traps captured less number of *Ae. albopictus* mosquitoes, although previous studies said these both traps were the most effective for this species. The BG-Sentinel trap has been shown most effective to capture *Aedes* mosquito species such as *Ae. aegypti* and *Ae. albopictus* (Kroekel et al. 2006; Meeraus et al. 2008; Obenauer et al. 2010; Drago et al. 2012), but less effective in capturing mosquito species such as *Cx. quinquefasciatus* (Mercer et al. 2012). It is probably a low number of adults *Ae. albopictus* trapped in both traps due to the fact that the study area located in the middle of an urban area in Sungai Dua surrounding with concrete buildings, and fewer vegetation areas, as new as preferred habitat for adult mosquitoes. A lot of man-made containers within the study area are suitable for breeding places for mosquitoes, but not a suitable place for adult mosquitoes to rest.

While, the Batu Maung resident consistently did communal work monthly on the sanitation which eradicates most of the breeding site for *Aedes* larvae and their area have less presence of vegetation that makes adult mosquitoes less favour to disperse and distribute in the residence area (Muir & Kay 1998; Hayden et al. 2010). In which, vegetation is highly preferred for *Ae. albopictus* resting habitat (Takagi & Tsuda 1995; Higa 2011). Most previous studies investigated the effectiveness of traps in wooded areas (Meeraus et al. 2008; Bhalala & Arias 2009) with the presence of vegetation (heavily tree) in well-shaded areas (Mahanta et al. 1999; Hoel et al. 2009) which differ from ours focuses on the residential areas. Even though, the unusual low dengue vector density was found in this study area, but high dengue cases are still reported. This proven that that both traps in incapable to traps *Ae. albopictus* populations. However, in this study, *Ae. albopictus* seems to be more attracted to conventional ovitrap compared to BG-Sentinel and CDC Fay-Prince Light trap. Thus, proving the existence of *Ae. albopictus*, the vector of dengue viruses in both locations, but failed to be captured by the modern traps. Ovitrap represents a sampling technique that widely used to collect indirect indices of adult abundance of container-breeding *Aedes* species, derived from the number of eggs laid by adult female mosquitoes (Facchinelli et al. 2007).

Various *Culex* species seems to attract to light and carbon dioxide from CDC Fay-Prince Light trap which manifest as the most effective trap to capturing this nocturnal mosquito (Hoel et al. 2009; Drago et al. 2012), unlike *Aedes* mosquitoes didn't attract too much to CDC Fay-Prince Light trap (Tiiurman & Thurman 1955; Silver 2008; Hoel et al. 2009). Differ from *Ae. albopictus*, more number of *Cx. quinquefasciatus* were trapped from both study areas; urban Sungai Dua and sub-urban Batu Maung. Our observation on the study sites revealed a blockage and polluted drainages surrounding the areas located about 500 m from the trap positions which may contribute the high number of *Cx. quinquefasciatus*. Larvae of *Cx. quinquefasciatus* habitat was found have a close distance with human habitation (Burke et al. 2010), abundant in polluted drains (Hassan et al. 1993) in Penang, Malaysia, roadside drainage in a residential area (Marten et al. 2000).

Climate plays a vital role in the activity of mosquitoes, such as temperature variations become one of the important factors in biting activity (Yasuno & Tonn 1970). High dengue outbreaks and vector abundance occurred related to the high temperatures (Wu et al. 2009; Unlu et al. 2011) and low rainfall (Nakhapakorn & Tripathi, 2005). However, in this study, temperature, wind speed and relative humidity seem did not affect the abundance of *Ae. albopictus* and *Cx. quinquefasicatus*. The lack of significance of these factors may be due to that mosquitoes were sampled in the temperate period (Jian et al. 2014). When the meteorological factors are consistently within the favourable range, the mosquito populations will be at the sustaining level. For examples, *Cx. quinquefasciatus* mosquitoes are well adapted to human dwelling, where the outside temperature relatively low influences them. Female mosquitoes may have a wider response to microclimatic variations than to outside rainfall and temperature (David et al. 2012).

In this study, light intensity parameters were correlated with the abundance of *Cx. quinquefasciatus* mosquitoes, but not to *Ae. albopictus* mosquitoes. There are a small number of *Ae. albopictus* captured in light traps indicates the light is relatively unimportant cue in the host searching (Burkett & Butler 2005) for *Ae. albopictus*. The most of successful *Ae. albopictus* adult traps do not use light, but rely on strategic placement and low dark reflective colours (Fay & Prince 1968; Freier & Francy 1991). *Aedes albopictus* trapped in the trap was mostly found in night time during in this experiment. An unusual nocturnal host-seeking activity by *Aedes* mosquitoes was positive correlated with increasing light intensity (Kawada et al. 2005; Kawada et al. 2007). *Aedes albopictus* does not often prefer sunny areas; this is because possibly the condition of air temperature that high or too low that not favourable to them. Crepeau et al. (2013) find out the possibility of the effectiveness of bright and contrasting trap results from its visibility in low light settings. *Aedes albopictus* was captured during a nocturnal time might be having an intrinsic reaction to light (Kawada et al. 2005).

As our observations, *Cx. quinquefasciatus* mosquitoes not mainly attracted to UV light colour in CDC Fay-Prince Light traps only, but also the influence by day and night phase. In this study, most *Cx. quinquefasciatus* trapped in the trap during the low light intensity below 100 Lux, which is during the night to dawn times. Though, the light intensity is considered more important as an attraction than the colour of light when using light-baited traps to trap nocturnal mosquitoes (Barr et al. 1963). However, low UV wavelengths at 350 nm may cause the physiologically more stimulating and result in greater behavioural response to CDC Fay-Prince Light trap (Burkett et al. 2005). A spectral sensitivity to light from the ultraviolet and green light spectrums attracted the mosquitoes (Muir et al. 1992; Burkett et al. 1998; Hoel et al. 2007). In addition, in natural conditions, moonlight may have influences to the level of activity (Veronesi et al. 2012), however our study was not influenced by the moonlight as the traps were located inside of the building compartments.

CONCLUSION

Modern trap BG Sentinel trap and CDC Fay-Prince Light traps were not suitable tools for monitoring the activities of *Ae. albopictus* in residential areas, but CDC Fay-Prince Light traps still relevant and effective to use to capture *Cx. quinquefasciatus*. Ovitraps seem to be better tools for collecting and surveillance population of *Ae. albopictus* mosquitoes. This study has therefore established immature sampling as the most effective method of capturing *Ae. albopictus* in a residential area. Monitoring densities and activity patterns of adult mosquito populations are a major challenge in efforts to evaluate the epidemiology of mosquito borne-diseases and their respond to vector control interventions.

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