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DIVERSITY OF MOSQUITO SPECIES (DIPTERA: CULICIDAE) IN THE RESIDENTIAL AREA AND RICE FIELD OF KAMPUNG REMBUS, SAMARAHAN DIVISION, SARAWAK, MALAYSIA

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ABSTRACT

The information on the distribution and occurrence of mosquito species is crucial for anticipating the prevalence of mosquito borne diseases. The surveillance data of mosquitoes for Sarawak continues up to recently. Anthropogenic activities provide breeding grounds for mosquitoes. For example, a flooded wetland rice field created an artificial aquatic habitat for mosquitoes. In Sarawak, Malaysia no data is available on the occurrence of mosquito species in wetland rice fields so far. The objective of this study was to document the diversity of mosquitoes in the rice field and residential area of Kampung Rembus, Sarawak, Malaysia during the early stages of the rice planting season. Ten plastic containers containing one litre of rainwater were placed permanently in the residential area and rice field, respectively. All mosquito larvae and pupae were collected from every plastic container once a week and identified. The water temperature, ambient temperature and relative humidity were monitored during the study. A total of four BG-Sentinel trap baited with BG-Lure were also set in the residential area and rice field. The trapped adult mosquitoes were identified and counted. Eight mosquito species from six genera were recorded in the present study namely Aedes albopictus, Culex quinquefasciatus, Culex fuscocephala, Culex pseudovishnui, Coquillettidia sp., Uranotaenia maxima, Toxorhynchites indicus and Lutzia fuscanus. A total of 4,756 individuals were encountered in residential areas and 1,390 individuals from rice field. The highest number of mosquito species collected was Ae. albopictus with 4,515 individuals at the study sites. The two mosquito species with the lowest number of individuals were Cx pseudovishnui and Coquillettidia sp. with only one individual collected during a three-month study. Aedes albopictus, Cx quinquefasciatus, Cx fuscocephala and Cx pseudovishnui are recorded for the first time in the rice field of Sarawak. Aedes albopictus and Cx. guinguefasciatus showed a weak correlation with ambient temperature and relative humidity. However, both species demonstrated a strong correlation with water temperature. Our findings are the first report for the study area and for the rice field of Sarawak. There were mosquito species which are vectors

of mosquito borne diseases. To prevent the spread of mosquito borne diseases among residents in Kampung Rembus, it is crucial to continuously monitor and manage mosquito breeding sites.

Keywords: Mosquitoes, rice field, Sarawak, Malaysia

ABSTRAK

Maklumat mengenai taburan dan kehadiran spesies nyamuk adalah penting untuk menjangkakan kelaziman penyakit bawaan nyamuk. Kajian pengawasan nyamuk bagi Sarawak dilaporkan sehingga kini. Aktiviti antropogenik akan menyediakan tempat pembiakan nyamuk. Sebagai contoh, sawah padi mewujudkan habitat akuatik buatan untuk nyamuk. Di Sarawak, Malaysia tidak ada data mengenai spesies nyamuk yang boleh didapati di sawah padi. Objektif kajian ini adalah untuk mendokumentasikan kepelbagaian nyamuk di kawasan sawah dan kawasan perumahan Kampung Rembus, Sarawak, Malaysia pada peringkat awal musim penanaman padi. Sepuluh bekas plastik berisi satu liter air hujan diletakkan secara kekal di kawasan perumahan dan sawah padi. Semua larva dan pupa nyamuk dikumpulkan daripada setiap bekas plastik seminggu sekali dan spesies nyamuk dikenal pasti. Suhu air, suhu persekitaran dan kelembapan relatif dipantau semasa kajian. Sebanyak empat perangkap BG berumpan dengan B-Lure turut dipasang di kawasan perumahan dan sawah padi. Nyamuk dewasa yang terperangkap dikenal pasti spesiesnya dan dikira. Lapan spesis nyamuk daripada enam genus telah direkodkan dalam kajian ini iaitu Aedes albopictus, Culex quinquefasciatus, Culex fuscocephala, Culex pseudovishnui, Coquillettidia sp., Uranotaenia maxima, Toxorhynchites indicus and Lutzia fuscanus. Sebanyak 4,756 individu ditemui di kawasan perumahan dan 1,390 individu dari sawah. Bilangan spesies nyamuk tertinggi yang dikumpul ialah Aedes albopictus dengan 4,515 individu di tapak kajian. Dua spesies nyamuk dengan bilangan individu paling rendah ialah spesies Culex pseudovishnui dan Coquillettidia sp. dengan hanya satu individu telah dikumpul semasa kajian selama tiga bulan. Aedes albopictus, Cx quinquefasciatus, Cx fuscocephala dan Cx pseudovishnui adalah rekod pertama untuk sawah padi Sarawak. Aedes albopictus dan Cx. quinquefasciatus menunjukkan korelasi lemah dengan suhu persekitaran dan kelembapan relatif. Walau bagaimanapun, kedua-dua spesies menunjukkan korelasi kuat dengan suhu air. Penemuan ini adalah laporan pertama untuk kawasan kajian dan juga untuk sawah padi Sarawak. Terdapat spesise nyamuk yang merupakan vektor kepada penyakit bawaan nyamuk. Bagi mencegah penularan penyakit bawaan nyamuk dalam kalangan penduduk di Kampung Rembus, adalah penting untuk memantau dan mengurus tempat pembiakan nyamuk secara berterusan.

Kata Kunci: Nyamuk, sawah padi, Sarawak, Malaysia

INTRODUCTION

Mosquitoes belong to the family Culicidae, in the order Diptera. A mosquito is a small, twowinged insect and undergoes complete metamorphosis. There are approximately 56 species of mosquitoes reported from various habitats in Sarawak, Malaysia from 2005 to 2008 (Miyagi et al. 2009). Survey was continuously conducted in residential areas across different divisions in Sarawak (Ali et al. 2020; Lau et al. 2017). Several common mosquito species in the genus *Aedes, Culex, Anopheles* and *Mansonia* are known in transmitting viral and parasitic diseases to human, for example, Japanese encephalitis, dengue, malaria and filariasis (Huang et al. 2015; Tandina et al. 2018). Various anthropogenic footprints such as the flooded rice fields and stagnant water around rice field provide breeding grounds for mosquitoes (Chan et al. 2023), and the mosquito larvae abundant had a significant relationship with physical parameters namely pH, salinity, temperature, and humidity (Ratnasari et al. 2023). In rice cultivation, a flooded wetland rice field created an artificial aquatic habitat for insects including mosquitoes. Several studies reported collection of mosquito species from rice fields. A total of the 15 mosquito species from *Anopheles, Culex* and *Aedes* genera were captured in rain-fed and irrigated rice fields in North Sulawesi, Indonesia (Mogi et al. 1995). In Southern region of Sri Lanka, *Culex tritaeniorhynchus* and *Anopheles peditaeniatus* were the two dominant mosquito species collected in the rice field of Habaraluwewa (Yasuoka & Levins 2007). In Northern Vietnam, *Cx. tritaeniorhynchus* and *Culex vishnui* were the two dominant species found rice field (Ohba et al. 2015). In Malaysia, *Cx. vishnui* was the common mosquito species collected in rice field at Sekinchan, Selangor (Leong et al. 2011). In general, *Anopheles* and *Culex* are the two genera commonly found in rice fields.

The occurrence of mosquito species changes across different planting stages, including the seedling stage, transplanting stage, young to maturing stages and harvesting stage in a rice field. *Anopheles rufipes, Ficalbia splendens* and *Culex annulioris* were among mosquito species collected during the post transplanting stage from rice growing areas in Mwea, Kenya (Muturi et al. 2007). In the Philippines, *Anopheles vagus, Cx. vishnui* and *Cx. tritaeniorhynchus* were abundant during the ploughed and nurseries stages in rice growing areas (Mogi & Miyagi 1990). In Malaysia, *Aedes albopictus* and *Culex quinquefasciatus* were recorded during the maturing stage in the rice fields at Padang Serai, Kedah (Wan-Norafikah et al. 2018c) and the rice field in Kuala Pilah, Negeri Sembilan, Malaysia (Wan-Norafikah et al. 2018a).

In Sarawak, the occurrence of mosquito species in rice cultivation areas is unknown. Such data are crucial to assist vector control strategies, which depend on target mosquitoes and their habitats. The objective of this study is to provide firsthand information on the diversity of mosquitoes in the rice field and residential area of Kampung Rembus, Sarawak, Malaysia during the early stages of the rice planting season.

MATERIALS AND METHODS

Sampling Site

Kampung Rembus is situated at 1°29'19"N, 110°29'14"E (Figure 1), Samarahan Division of Sarawak, Malaysia Rice planting began in September and continued until March of the following year. Rainfall was the main water source for the rice field in the study site.



Figure 1. Star indicates the geographic location of sampling site at Kampung Rembus, Samarahan Division Sarawak

> (Sources: https://www.mapquest.com/malaysia/kota- samarahan-364804597) (Map Quest 2019)

Insect Sampling

A larval and adult mosquito survey was carried out from August to October 2019. During this period, larval and adult mosquito samples were collected at different rice planting stages, i.e. land preparation stage, nursery stage (rice at germinating stage to 1 month-old) and seedling stage (rice at 1 - 2-month-old). In the residential area of Kampung Rembus, the vegetation habitat was surrounded by tall trees, tropical shrubs and naturally growing herbs.

Ten plastic containers (20 cm depth x 16 cm diameter) containing one litre rainwater collected from water storage tanks were deployed permanently by local villagers in the residential area and rice field of Kampung Rembus, respectively. The distance between each plastic container was one metre from one another. Larvae and pupae were collected from each plastic container once a week between August and October 2019. Three readings of ambient temperature and relative humidity were recorded at 0900 at the study sites by using Thermo Hygrometer Hygrotherm before the samples were collected from the containers. The three readings were recorded at 5-minute interval at the same locations in the residential area and rice field. The collected samples of larvae and pupae were transported to the laboratory in a cooler box for further identification.

Adult mosquitoes were trapped using BG-Sentinel trap baited with BG-Lure. Two traps were set up in the residential area and rice field of Kampung Rembus, respectively. The mosquito traps were hung 1.5 metres above the ground. All the mosquito traps were operated overnight from 1800 to 0600 of the following day (Wagman et al. 2014). The trapping was performed once a week from August to October 2019 (four weeks each month). Adult mosquito samples from each trap were collected and kept in separate bags. All the samples were transported to the laboratory in a cooler box for further identification.

Insect Collection, Rearing and Preservation

The first and second instar larvae were preserved in 95% ethanol. The third and fourth instars larvae were reared according to Miyagi et al. (2009) in separate mosquito traps using water

from the collection site until the emergence of adult mosquitoes. Adult mosquitoes were reared with a moist cotton ball with 10% sugar solution in a mosquito trap for 24 hours and preserved as dry specimens using micropin (Miyagi et al. 2009).

Species Identification

The identification of mosquitoes using larvae, pupae and adult samples followed the classification by Bram (1967), Sirivanakarn (1976), Rattanarithikul (1982), and Evenhuis and Steffan (1986).

Data Analysis

Diversity was calculated by using the Shannon-Wiener Index (H') and the Simpson's Index (D). Cumulative larvae and pupae frequency of three most common mosquito species from the residential area and rice field was used to perform Pearson correlation and multiple linear regression analysis (Dahiru 2008) against ambient temperature, relative humidity and water temperature of container. The r-value between 0.00 - 0.10 was considered negligible correlation, 0.10 - 0.39 weak, 0.40 - 0.69 moderate, 0.70 - 0.89 strong and 0.90 - 1.00 very strong correlation (Schober et al. 2018). Data was analysed with the Statistical Package for the Social Sciences (SPSS) version 20.0

RESULTS

During the sampling period of this study, the ambient temperature ranged between 28.3°C and 32.9°C and relative humidity ranged from 60.8% to 76.7% were recorded in the residential area. Meanwhile, the water temperature was measured at 25.9°C to 29.6°C in the residential area and 25.2°C to 29.0°C in the rice field.

Cumulatively, there were 6,146 individuals collected from the residential area (4,756 individuals) and rice field (1,390 individuals) of Kampung Rembus (Table 1). There were eight species in cumulative, from six genera. From the larvae and pupae collections, a total of six species from five genera were identified. The species identified were *Aedes albopictus*, *Culex quinquefasciatus*, *Culex fuscocephala*, *Uranotaenia maxima*, *Toxorhynchites indicus* and *Lutzia fuscanus*. All six species were collected in the residential area, but only three species (*Ae. albopictus*, *Cx. quinquefasciatus*, *Cx. fuscocephala*) were also collected in the rice field.

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Mosquito Species	Sample Type –	Residential				Rice Field			Total By	
		Aug	Sept	Oct	Sub-total	Aug	Sept	Oct	Sub-total	Species
Ae. albopictus	L+P	918	1,692	1,018	3,628	249	355	257	861	4,489
	А	1	7	2	10	3	12	1	16	26
	Total	919	1,699	1,020	3,638	252	367	258	877	4,515
Cx. quinquefasciatus	L+P	208	319	279	806	45	24	35	104	910
	А	55	118	62	235	31	91	54	176	411
	Total	263	437	341	1,041	76	115	89	280	1,321
Cx. fuscocephala	L+P	0	1	1	2	70	96	53	219	221
	А	10	5	9	24	3	8	2	13	37
	Total	10	6	10	26	73	104	55	232	258
Cx. pseudovishnui	L+P	0	0	0	0	0	0	0	0	0
	А	0	0	0	0	0	0	1	1	1
	Total	0	0	0	0	0	0	1	1	1
	L+P	0	0	0	0	0	0	0	0	0
<i>Coquillettidia</i> sp.	А	0	1	0	1	0	0	0	0	1
1 1	Total	0	1	0	1	0	0	0	0	1
Ur. maxima	L+P	25	0	0	25	0	0	0	0	25
	А	0	0	0	0	0	0	0	0	0
	Total	25	0	0	25	0	0	0	0	25
Lt. fuscanus	L+P	0	13	0	19	0	0	0	0	19
	А	0	0	6	0	0	0	0	0	0
	Total	0	13	0	19	0	0	0	0	19
Tx. indicus	L+P	0	4	2	6	0	0	0	0	6
	А	0	0	0	0	0	0	0	0	0
	Total	0	4	2	6	0	0	0	0	6
	Total by month	1,217	2,160	1,379	4,756	401	586	403	1,390	6,146

Table 1.The overall mosquito species identified from larvae (L), pupae (P) and adult (A) mosquito samples collected from the residential
area and rice field of Kampung Rembus from August to October 2019

For the adult mosquito sampling using BG-Sentinel traps, five mosquito species were collected belonging to three genera. There were three mosquito species recorded in the residential area and rice field, respectively. Throughout the sampling period, *Cx. quinquefasciatus*, *Cx. fuscocephala* and *Ae. albopictus*, occurred in both residential and rice field areas. *Culex quinquefasciatus* with a total of 411 individuals was recorded as a common mosquito species in the residential area and rice field. The occurrence of *Cx. quinquesfasciatus* species increased from August to September in both residential and rice field areas. However, the number dropped from September to October across the sampling period. *Culex fuscocephala* (37 individuals) and *Ae. albopictus* (26 individuals) were consistently recorded throughout the sampling periods at Kampung Rembus. *Coquillettidia* sp. and *Cx. pseudovishnui* had less number collected in the residential area and rice field area and rice field, respectively. Each species was represented by one individual.

Among the eight species, three species (*Ae. albopictus, Cx. quinquefasciatus* and *Cx. fuscocephala*) had their larvae, pupae and adult collected. The other five species had either their larvae or pupae, or adult collected. Based on cumulative data, *Ae. albopictus*, was the most dominant species with 3,638 individuals collected in the residential area and 877 individuals in a rice field. *Culex quinquefasciatus* was the second most dominant species with 1,041 individuals collected in the residential area and 280 individuals in the rice field. The population of *Ae. albopictus* and *Cx. quinquefasciatus* seemed to peak in September. It is also true for the population of *Cx. fuscocephala* in the rice field with a total 232 individuals.

Over the sampling period, larvae and pupae of *Ur. maxima* were collected with 25 individuals in the residential area only in the month of August. *Lutzia fuscanus* (19 individuals) and *Tx. indicus* (6 individuals) were only collected in the residential area in September and October. These three species were not collected in the rice field during the survey. For *Coquillettidia* species, only one adult mosquito was collected in the residential area in September. *Coquillettidia* species was not found in the rice field. *Culex pseudovishnui* was recorded in October with one individual at the rice field, but not in the residential area.

The average Shannon-Wiener Index for residential area and rice field were 0.6258 and 0.9174, respectively. Analysis of the Simpson Index had an average mean of 0.6331 from residential area and 0.4661 from rice field (Table 2). The correlations between larvae and pupae of the three common mosquito species with recorded ambient temperature, relative humidity and water temperature from August to October 2019 in Kampung Rembus residential and rice field are shown in Table 3. Based on Pearson coefficient correlation and multiple linear regression analysis, ambient temperature showed weak negative correlation to the frequency of Ae. albopictus (r = -0.2374; P = 0.0136), and Cx. quinquefasciatus (r = -0.1234; P = 0.0134). No correlation for ambient temperature was observed with Cx. fuscocephala (r =0.0418; P = 0.2361). Relative humidity showed weak negative correlation to the frequency of sampled Ae. albopictus (r = 0.3164; P = 0.0163) and Cx. quinquefasciatus (r = 0.2076; P =0.0281). No correlation of relative humidity was observed with Cx. fuscocephala (r = 0.1263; P = 0.0891). The water temperature in the container had a strong positive correlation to the frequency of sampled Ae. albopictus (r = 0.9994; P = 0.0115) and Cx. quinquefasciatus (r =0.9286; P = 0.0068) but no correlation of water temperature was observed with Cx. fuscocephala (r = -0.9965; P = 0.1222).

Table 2.Diversity indices of mosquito species in residential area and rice field habitats
in Kampung Rembus

Habitat Type	No. of Species	Shannon-Wiener Index (H')	Simpson's Index (D)
Residential	7	0.6258	0.6331
Rice field	4	0.9174	0.4661

Table 3.Pearson coefficient correlation (r-values) and multiple linear regression (p-values) between the numbers of sampled mosquitoes and the ambient
temperature, relative humidity and water temperature from August to October
2019 in Kampung Rembus (cumulative data of residential and rice field)

Environmental Value		Mosquito Species					
variables		Ae. albopictus	Cx. quinquefasciatus	Cx. fuscocephala			
Ambient	r-value	-0.2374	-0.1234	0.0418			
temperature	p-value	0.0136*	0.0134*	0.2361			
Relative	r-value	0.3164	0.2076	0.1263			
humidity	p-value	0.0163*	0.0281*	0.0891			
Water	r-value	0.9994	0.9286	-0.9965			
temperature	p-value	0.0115*	0.0068*	0.1222			

R = 0.00-0.10 (Negligible Correlation), r = 0.10 - 0.39 (Weak Correlation), r = 0.40 - 0.69 (Moderate Correlation), r = 0.70 - 0.89 (Strong Correlation), r = 0.90 - 1.00 (Very Strong Correlation), P<0.05 (significant)*

DISCUSSION

Mosquito Species Recorded in Kampung Rembus

The field surveys showed that the most commonly collected mosquito species using plastic containers and BG-Sentinel traps in Kampung Rembus were *Ae. albopictus*, *Cx. quiquefasciatus* and *Cx. fuscocephala*. These three mosquitoes were recorded as common domestic species. *Aedes albopictus* are widely distributed in Malaysia (Chen et al. 2009). *Aedes albopictus* immatures were reported as the dominant species in rice fields such as those in Kedah (Wan-Norafikah et al. 2018c) as well as in breeding habitats surrounding rice fields in Negeri Sembilan (Wan-Norafikah et al. 2018a). *Aedes albopictus* are also common in the rice fields in Thailand (Wongkoon et al. 2007).

Culex quinquefasciatus was the second highest number of species collected in the study site. *Culex quinquefasciatus* is commonly found in tropical country like Malaysia (Abagli & Alavo 2019). The adults of this species were also reported in residential area nearby rice field in Selangor (Leong et al. 2011) and the immatures in residential area nearby rice fields in West Malaysia (Wan-Norafikah et al. 2018b). Outside Malaysia, *Cx. quinquefasciatus* immatures were also collected from rice fields in Sri Lanka (Amarasinghe & Weerakkodi 2014) and Bangladesh (Farjana et al. 2015). *Culex fuscocephala* is a common species, however, there is little research on it in Malaysia when compared to *Ae. albopictus* and *Cx. quinquefasciatus*. In Sarawak, adults of *Cx. fuscocephala* was recorded in low number in Balai Ringin (Rohani et al. 2013). The immatures of *Cx. fuscocephala* breed in stagnant water present in residential areas, drains, rice fields and poultry farms (Farjana et al. 2015). In this study, this species was collected more frequently in rice field but also can be found in the residential area nearby. The immatures of this species were also recorded in rice fields of Sri Lanka (Amarasinghe & Weerakkodi 2014) and adults were found in residential area of Indonesia Central Java (Astuti et al. 2016).

Collection of *Ur. maxima*, *Tx. indicus* and *Lt. fuscanus* were found in low numbers in the residential area and none in the rice field. Breeding habitats of these species are closely associated with human activities in residential area. These species prefer the environmental habitat of natural containers such as tree holes and cut or broken bamboo rather than artificial containers (Mogi 2000). For *Lt. fuscanus*, water condition in the containers influenced the breeding ground selection. *Lt. fuscanus* was often collected from ground water habitats with high organic content (Bram 1967).

One *Cx. pseudovishnui* adult was collected from BG-Sentinel traps set in the rice field. This species was recorded in the rice fields in Peninsular Malaysia (Kumar et al. 2018). *Culex pseudovishnui* has a preference to breed in the freshwater ground pools, streams, ponds and rice fields (Amarasinghe & Weerakkodi 2014). There was only one sample obtained for this species possibly because there were no preferred hosts (pigs, cattle and birds) nearby (Maquart et al. 2022).

Environmental Variables Affecting the Occurrence of Mosquito

Environmental variables such as temperature and relative humidity affect mosquito distribution and activity. Temperatures above 28°C are favourable for *Ae. albopictus* (Awang et al. 2019; Phanitchat et al. 2017) and *Cx. quinquefasciatus* (Phanitchat et al. 2017). However, higher temperatures above 32°C will lead to a reduction in the hatching rate of *Cx. quinquefasciatus* (Ciota et al. 2014). During the sampling period of this study, the ambient and water temperature seemed to be favourable for *Ae. albopictus* and *Cx. quinquefasciatus*, where there is significant correlation of ambient and water temperature towards the frequency of the two species were observed. The optimum temperature for *Cx. fuscocephala* is unknown thus far. In this study, the frequency of *Cx. fuscocephala* did not correlate to the ambient or water temperature.

A relative humidity of 65% is suitable for *Aedes* and *Culex* mosquitoes (Jemal & Al-Thukair 2018). The relative humidity recorded in this study ranged from 60 - 77% approximately. This could be one of the reasons for *Ae. albopictus* and *Cx. quinquefasciatus* to be found abundantly in Kampung Rembus. A significant correlation of relative humidity to the frequency of the two species was observed. For *Cx. fuscocephala*, the relative humidity showed no correlation to its frequency.

Potential Mosquito-Borne Diseases in Kampung Rembus

Aedes albopictus, Cx. quinquefasciatus and *Cx. fuscocephala* are found abundant in Kampung Rembus. *Ae. albopictus* is known as a vector of dengue in Asian countries including Malaysia (Ahmad et al. 1997; Teo et al. 2017). *Culex quinquefasciatus* and *Cx. fuscocephala* are potential vector for Japanese encephalitis (Nitatpattana et al. 2005). The authorities may need to continually observe and manage the mosquito's breeding grounds in residential area and rice field. These are essentials to prevent the spread of mosquito-borne diseases among residents in Kampung Rembus.

CONCLUSION

In this study, eight species of mosquito were collected. The two most common mosquito species found in the rice field and residential area are *Ae. albopictus* and *Cx. quiquefasciatus*. *Aedes albopictus*, *Cx quiquefasciatus*, *Cx fuscocephala* and *Cx pseudovishnui* are new records from the rice field of Sarawak. *Aedes albopictus* and *Cx. quiquefasciatus* showed a weak correlation with ambient temperature and relative humidity, these environmental factors had minimal impact on their presence and distribution. In contrast, both species showed a strong

correlation with water temperature, highlighting the significance of water temperature in influencing mosquito populations in these areas.

The findings highlight the ecological complexity and adaptability of mosquito species across diverse habitats. The occurrence of different mosquito species in both residential area and rice field emphasizes the necessity for targeted vector control measures that are adapted to specific environmental conditions. Furthermore, the strong correlation between mosquito species and water temperature may suggest that temperature alteration in water management practices could significantly affect mosquito populations and, consequently, the transmission dynamics of mosquito-borne diseases in the region.

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AUTHORS DECLARATIONS

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Declarations

No ethical issue required for this research.

Data Availability Statement

My manuscript has no associated data.

Authors' Contributions

Siew Fui Wong (SFW) and Freddy Kuok San Yeo (FKSY) conceived this research and designed experiments. SFW, Yee Ling Chong (YLC) and FKSY participated in performing the experiments, data analysis, and interpretation. SFW and FKSY wrote the paper and participated in the revisions. All authors read and approved the final manuscript.

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