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HABITAT CHARACTERIZATION OF *ANOPHELES* SP. MOSQUITO LARVAE IN MALARIA RISK AREAS

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ABSTRACT

Objective: The purpose of this research was to evaluate the habitat features of Anopheles sp. mosquito larvae in Terengganu county disease threat regions. **Methods:** Felda Selancar 4 (Malaria danger regions) is the research region for this research. The testing was performed using a conventional 350 ML mosquito dipper with three larval environments (Slow flowing stream (SFR), Ditches (DT) and Animal foots (AF)) present within a research zone radius. During this research, environmental data included physical characteristics. Physical features include canopy cover, water depth, and turbidity, emerging crop tissue and heat, pH, complete dissolved materials, dissolved oxygen, and conductivity. **Result:** All Anopheles sp. for characterizations of physical environment in this research. Habitats of reproduction favor more in transparent water (100 %). When the water was turbid, the probabilities of *An. maculatus* larvae to be present were decreased. For characteristics of physiochemical habitats, the average water temperature values of the slow-flowing river (SFR) and animal foot (AF) spectrum are in the range of 24 ± 26.8^{o}C. **Conclusion:** Water temperature significantly influenced the difference in Anopheles sp. Habitats for reproduction. No statistically important difference in water temperature, pH, complete dissolved materials, dissolved oxygen and electrical conductivity was noted between the favorable and negative specimens from slow-flowing stream, ditches and animal foots.

Keywords: Slow- flowing river (SFR), Ditches (DT), Animal foots (AF), physical characteristics, physicochemical characteristics.

1. Introduction

In many tropical and subtropical areas, malaria is a common and life-threatening disease (Tuyishimire, 2013; Soleimani-Ahmadi et al., 2014; Dida et al., 2018). A large proportion of cases of malaria occur in central mountains and forest parts of the country in Peninsular Malaysia. Malaysia's incidence has declined substantially; from over 200,000 cases in the 1950s and 243,870 cases in 1961 to 7,390 cases in 2008. Malaria occurs in confined endemic areas with less accessible and hilly forest land with inadequate transportation and communication facilities such as tribal villages and people working in agriculture and land development (Rohani et al., 2010). Malaria is triggered by Plasmodium genus protists who introduce the bite from an infected Anopheles tree into the circulatory structure (WHO, 2012; Tuyishimire, 2013; Duka, 2017). Four distinct Plasmodium genera cause human disease: P*. vivax, P. ovale, P. falciparum and P. malariae* (WHO, 2012; Yong et al., 2018). Sometimes humans get infected with Plasmodium organisms that usually infect livestock like P. knowlesi (WHO, 2012).

The Plasmodium is transferred by the bites of female mosquitoes from Anopheles The common Anopheles sp. responsible as vectors in the transmission of malaria in Malaysia is *An. maculates, An. sundaicus, An. balabacensis, An. dirus, An. letifer, An. camperstris, An donaldi, An. leucophyrus and An. flavirostris* (Yong et al., 2018). Several amounts of environmental, socio-demographic, financial and human variables affect malaria transmission. Increased precipitation and living in regions where there was flow or stagnant water are favorably correlated with the incidence of tuberculosis because water offers a nice breeding place for Anopheles mosquitos. Land-use habits and farming methods coupled with climate shift and economically oriented habitats are human activities that have supported malaria vector reproduction, subjected communities to disease, and supported malaria parasite motion (Tuyishimire, 2013).

The vector control operations concentrate primarily on the use of remaining indoor spraying and bed nets treated with insecticide. Key action in the eradication of poverty in many areas of the globe is larval control by reducing the origin and regular larvicide implementation (Rohani *et al*., 2010; Soleimani-Ahmadi *et al*., 2014). Larval control interventions are designed to decrease malaria transmission by stopping the spread of mosquito species and consequently decreasing human vector disease connections. The eggs are generally focused, comparatively immobile, and often easily available, so it is often advantageous to regulate larval insect populations. Also, unlike adults, mosquitos larvae cannot alter their environment to prevent avoid control activities. The malaria vector larvae's growth and survival rate was influenced by the various environmental features that affect larva size. These features include marine habitat environment, physical and chemical circumstances, soil sort, and biological features. Finding local changes in anopheline larval habitat economic features can assist to carry out appropriate vector control programs (Soleimani-Ahmadi *et al*., 2014).

A significant reason for studying mosquito larval ecology is to gather data about variables that may determine lay eggs (oviposition), survival, and significant malaria illness in term of spatial and temporal allocation. The quality of breeding water is an significant determinant of whether female mosquitoes will lay their eggs and the subsequent immature phases will finish their growth to adulthood effectively (Konradsen, 2016). In Malaysia, Anopheles sp. research on environmental characteristics. The larvae of mosquitoes are very restricted. A Rohani (2010) research on Anopheles sp. demonstrates that,the most prevalent larval environments were 5.0-15.0 cm profound shallow ponds with transparent air, sand substratum and crops or float. The mosquito also favored environments that were open or partly obscured. Breeding habitats from the closest human colony were usually situated at 100-400 m. Changes in breeding features have also been noted. Instead of growing in fast moving rivers, the majority of larvae were grown along the stream bank in tiny water pockets. Thus, the purpose of this research is to evaluate Anopheles sp's habitat features. The research outcome could provide data about the characteristics of the Anopheles mosquito environment. This data is essential for the development of efficient, viable and cost effective vector control policies

2. Materials and Method

This research used the layout of a sampling survey to explore Anopheles sp environment description. Mosquito larvae in Felda Selancar 4 (danger regions for malaria) with respect to various larval environments. The testing was performed using a conventional 350 mL mosquito dipper with three larval environments (Slow flowing stream (SFR), ditches (DT) and animal foots (AF)) present within a research zone radius. Three kinds of breeding locations (Slow flowing stream (SFR), ditches (DT) and cattle foots (AF)) and distribution of animals were independent factors. ANOVA has tabulated and evaluated the information gathered for each test using descriptive and statistical scores.

Felda Selancar 4 (Malaria danger regions) is the research region for this research in stage one. In stage two, the sampling was performed using a conventional 350 mL mosquito dipper with three larval environments (Slow flowing stream (SFR), ditches (DT) and animal foots (AF)) present within a research zone radius. At intervals along the border of the insect beds, 3-10 dips were drawn based on the size of each larval environment. Larval sampling was carried out using plastic pipettes in tiny breeding sites where dippers were not efficient. The water was gathered in a black plastic container and the existence of mosquito larvae was closely noted. In each larval environment, sampling and in-situ analysis were always performed by the same person between 10:00-18:00 hrs. for approximately 30 minutes. During the larval com-pilation, environmental features of each larval environment were evaluated and registered. Physical and physiochemical features were included in the environmental information determined in this research Physical features include canopy cover, water depth, turbidity, emergent plant coverage and distant to nearest house and physiochemical characteristics include temperature, pH, total dissolved solids, dissolved oxygen and conductivity.

3. Results

Previous surveys observed the significance of habitat characterizations heavily regulate Anopheles sp's amount and geographic distribution. The biggest task is to determine the reproduction of mosquito vectors as the danger of revival of tuberculosis depends on the existence of a mosquito vector. Malaria vector control operations in Malaysia concentrate on the use of bed nets treated with insecticide and remaining indoor spraying. Measures targeting Anopheles sp larval phases as a supplement to these vector control operations and timely access to therapy. Another successful approach for endemic fields of disease is mosquitoes (Rohani *et al*., 2010).

Several environmental features affect larval size, which can affect the malaria vector larvae's growth and survival frequency. These features include marine habitat environment, physical and chemical circumstances, soil sort, and biological features. Finding local changes in anopheline larval habitat environmental features can assist to conduct appropriate vector control programs. Knowledge of larval vector ecology is the main variable in risk assessment and efficient control interventions, as the most efficient way to regulate plant populations is to manage eggs in their aquatic environments before they become adults (Soleimani-Ahmadi, Vatandoost, & Zare, 2014).

3.1. Larval habitat characterizations of *Anopheles* sp.

The physical habitat characteristics were observed and identified as soon as the breeding habitat of *Anopheles* sp. were found. The physical habitat characteristics that was observed and identified is canopy cover, water depth, turbidity, emergent plant coverage and distant to nearest house. The canopy cover and emergent plant coverage of *Anopheles* sp. breeding habitat can be classified as L (a lot), M (moderate) and S (a few). The turbidity of *Anopheles* sp. breeding habitat can be classified as C (clear), T (turbid) and VT (very turbid). The water depth and distant to nearest house was measure by unit of centimeter (cm) for water depth and meter (m) for distance to nearest house.

Figure 1 highlights the absence of the physical habitat characterizations. Out of 17 breeding habitats, for canopy cover (10 presence in a lot, 6 presence in moderate and 1 presence in a few) and for emergent plant coverage (6 presence in a lot, 1 presence in moderate and 10 presence in a few). For water depth, from 17 breeding habitats (10 presence in water depth <5 cm and 7 presence in water depth >5 cm). For distant to nearest house, from 17 breeding habitats (4 presence in distant to nearest house <150 m and 13 presence in distant to nearest house >150 m). For turbidity, all 17 breeding habitats presence in clear water.

Based on the study done by Rohani *et al.,* (2010), all the *An. maculatus* larvae were found in natural habitats, either in stagnant or slow flowing water, with emergent vegetation or floating debris. *An. maculatus* bred in rather clean, clear water. The probability of *An. maculatus* larvae to be present was reduced when the water was turbid. Outcomes by Dida *et al.,* (2018) state that mosquito abundance showed a negative significant association with water turbidity, suggesting that mosquitoes prefer clearer water. Almiron and Brewer (1996) pointed out that different types of habitats, the nature of vegetation, water movement and water depth are the main characteristics explaining the variations in mosquito species.

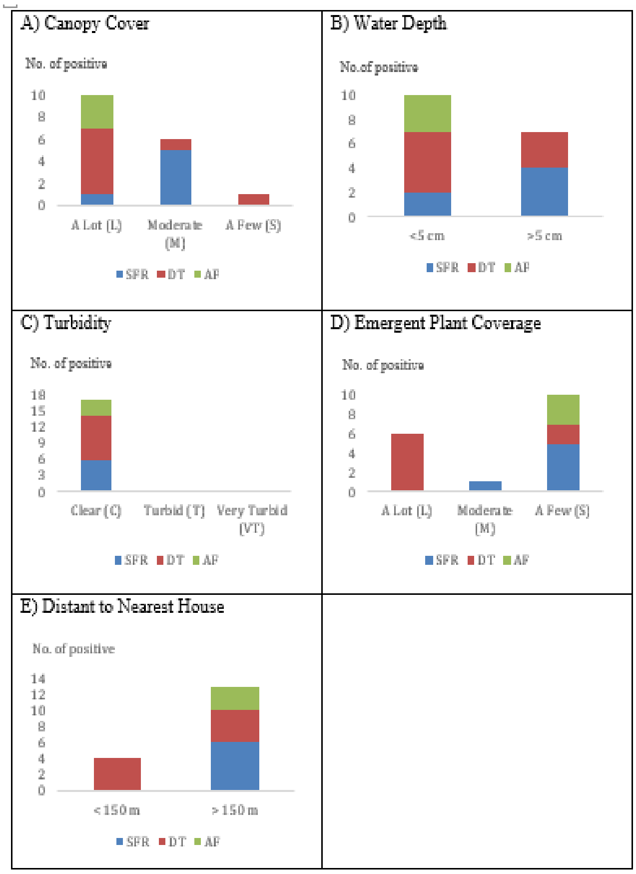

Fig. 1: Physical habitat characterization; (A) Canopy cover, (B) Water depth, (C) Turbidity, (D) Emergent plant coverage and (E) Distance to nearest house

3.2. Physiochemical characteristics of different breeding habitat of *Anopheles* species

In this objective, focus will be more on physiochemical habitat characteristics. The variable being measured includes water temperature (°C), pH of water, total dissolved solids (ppm), dissolved oxygen (mg/L) and electric conductivity (mS/cm). Table 2 shows the summarization of physiochemical habitats characteristics of different types of *Anopheles* sp. breeding habitats. The ranges of key factors measured were observed based on Slow-flowing river (SFR), Ditches (DT) and Animal foots (AF). Variation in habitat type among different parameter was analyzed for normality test. As the habitat type was not normally distributed, a *Kruskal Wallis* test was being used in this study.

For physiochemical habitat characterizations, the mean values for water temperature of Slow-flowing river (SFR) and Animal foots (AF) are in the range 24 ± and 26.84 °C for Ditches (DT). The average value for pH of water are almost in the same in the entire water samples which ranges from the 5.77 to 5.88. For total dissolved solids, the lowest mean is Animal foots (31.67 ppm), followed by Slow- flowing river (35.33 ppm) and Ditches (37.13 ppm). Meanwhile, for the lowest dissolved oxygen is Animal foots (AF) which are 656.67 mg/L and the highest is for Ditches (DT) which are 725.00 mg/L. The average value for electric conductivity are same in the entire water samples which are 0.10 mS/cm.

Table 2: Mean values (± SE) of the physicochemical characteristics of the habitat types

Variables	Mean values (± SE)/ Habitat types			*p*-value*
	Slow- flowing river (SFR)	Ditches (DT)	Animal foots (AF)	
Water temperature (°C)	24.88 ± 0.06	26.84 ± 0.21	24.67 ± 0.23	0.002*
pH	5.88 ± 0.19	5.85 ± 0.15	5.77 ± 0.20	0.828
Total dissolved solids (ppm)	35.33 ± 2.94	37.13 ± 2.15	31.67 ± 2.60	0.461
Dissolved oxygen (mg/L)	669.83 ± 58.31	725.00 ± 41.10	656.67 ± 76.86	0.638
Electric conductivity (mS/cm)	0.10 ± 6.21E-18	0.10 ± 5.25E-18	0.10 ± 9.81E-18	1.000

**p*-value by Kruskal-Wallis test

The *Kruskal Wallis* test showed that water temperature ware statistically significant with *p*-value 0.002 respectively. However it was found that there were no significant difference in pH, total dissolved solids, dissolved oxygen and electric conductivity between the habitat types (p>0.05).

3.3. Physiochemical habitat characteristics of positive and negative samples based on different types of *Anopheles* sp. breeding habitats

Larval environments are critical to determining the existence and concentration of various vector organisms, including immature phases, and the abundance and distribution of adult mosquitoes. It is therefore critical to know the dynamics and abundance trends of mosquito larval environments if present attempts to model and comprehend the distribution and abundance of adult mosquitoes are to be successful. For this subject, the focus will be more on favorable and negative sample physiochemical habitat features depending on distinct kinds of Anopheles sp. Habitats for reproduction. Table 3 demonstrates a summary of the features of favorable and bad samples of physiochemical environments based on various kinds of Anopheles sp. Habitats for reproduction. Based on favorable and negative specimens from Slow-flowing creek (SFR) habitat, favorable and negative specimens from Ditches (DT) habitat, and favorable and negative specimens from animal foots (AF) habitat, the ranges of main variables tested were noted. For the normality exam, variation in favorable and negative specimens was evaluated between distinct parameters. Because the favorable and bad results were usually spread, this research used an autonomous sample t-test.

For characterizations of physiochemical habitat, the mean water temperature values for positive samples from the Slow-flowing River (SFR) and Animal Foot (AF) range from Ditches (DT) are 24 ± and 26.84 ° C. The mean water temperature values for Slow-flowing River (SFR) and Animal Foot (AF) negative samples are from Ditches (DT) in the range 25 ± and 26.97 ° C. In all water specimens ranging from 5.77 to 5.88 for favorable specimens and 5.65 to 5.90 for adverse specimens, the median pH value of favorable specimens and adverse specimens from Slow-flowing River (SFR), Ditches (DT) and Animal Foots (AF) is almost the same. The smallest average for beneficial specimens for complete dissolved materials is from Animal foots (31.67 ppm), followed by Slow-flowing stream (35.33 ppm) and Ditches (37.13 ppm).

Animal foots (35.47 ppm), followed by Ditches (41.50 ppm) and Slow-flowing stream (41.93 ppm) are the smallest average for adverse specimens. Meanwhile, for favorable specimens, the smallest natural water is from 656.67 mg / L animal foots (AF) and the highest is from 725.00 mg / L Ditches (DT). For adverse specimens, the smallest dissolved water is from 549.29 mg / L animal foots (AF) and the highest is from 746.00 mg / L Ditches (DT).The median electrical conductivity value for beneficial specimens from Slow-flowing creek (SFR), Ditches (DT) and Animal foots (AF) is the same for all soil specimens of 0.10 mS / cm. The autonomous test t-test found that there was no statistically important distinction in air temperature, pH, complete dissolved materials, dissolved oxygen and electrical conductivity between Slow-flowing creek, Ditches and Animal foots (p>0.05) favorable and negative specimens.

Table 3: Mean values (± SE) of the physicochemical characteristics for positive and negative samples

Variables	Mean values (± SE)								
	Slow-flowing river (SFR)			Ditches (DT)			Animal foots (AF)		
	Positive	Negative	*p*-value*	Positive	Negative	*p*-value*	Positive	Negative	*p*-value*
Water temperature (°C)	24.88 ± 0.06	25.16 ± 0.13	0.271	26.84 ± 0.21	26.97 ± 0.22	0.690	24.67 ± 0.23	25.01 ± 0.14	0.323
pH	5.88 ± 0.19	5.89 ± 0.16	0.973	5.85 ± 0.15	5.90 ± 0.26	0.885	5.77 ± 0.20	5.65 ± 0.08	0.599
Total dissolved solids (ppm)	35.33 ± 2.94	41.93 ± 4.27	0.433	37.13 ± 2.15	41.50 ± 5.60	0.908	31.67 ± 2.60	35.47 ± 3.11	0.873
Dissolved oxygen (mg/L)	669.83 ± 58.31	666.29 ± 35.75	0.958	725.00 ± 41.10	746.00 ± 51.83	0.774	656.67 ± 76.86	549.29 ± 24.47	0.120
Electric conductivity (mS/cm)	0.10 ± 6.21E-18	0.12 ± 0.01	0.231	0.10 ± 5.25E-18	0.11 ± 0.01	0.414	0.10 ± 9.81E-18	0.11 ± 0.01	0.674

*p-value by independent sample paired *t-test*

4. Discussion

Larval densities are controlled by interactions between abiotic (hydrology, temperature, light/ shade, pH, salinity, nutrient availability) and biotic (predation, competition) factors (Washburn, 1995; Chase & Knight, 2003; Stresman, 2010). For comprehensive analyses of patterns in the productivity of larval habitats the studies should incorporate a landscape context, because presence and abundance of mosquito larvae in aquatic habitats and consequently the number of adults capable of malaria transmission are regulated by a variety of ecosystem processes operating and interacting at several organizational levels and spatial/temporal scales (Rejmankova *et al*., 2006). Humans can affect habitat availability and quality through ecosystem and landscape changes such deforestation/ reforestation, desertification, irrigation and other hydrological changes, and agricultural practices (Oku, 2016).

Temperature impacts all major procedures such instances are the rate of larval development and survivorship, pupation rates, larval-to-adult survivorship and larval-to-adult development time (Bayoh & Lindsay, 2004; Paaijmans, 2008; Wamae *et al*., 2010; Ndenga *et al*., 2011). Water temperatures are affected by different parameters such as local climate, water depth and movement, habitat size and geometry, land cover type or canopy overgrowth, presence of vegetation and/or algae, soil properties and turbidity (Paaijmans, 2008).

For physical habitat characterizations in this study, *Anopheles* sp. breeding habitats more prefer presence in a lot canopy cover (58.9%) and in a few emergent plant coverage (58.9%). For water depth, *Anopheles* sp. breeding habitats more prefer presence in water depth <5 cm (58.9%) and for distant to nearest house, *Anopheles* sp. breeding habitats more prefer presence in distant to nearest house >150 m (76.5%). For turbidity, all *Anopheles* sp. breeding habitats more prefer presence in clear water (100%). Based on the study done by Rohani *et al.,* (2010), *An. maculatus* bred in rather clean, clear water. The probability of *An. maculatus* larvae to be present was reduced when the water was turbid.

For characteristics of physiochemical habitat in this study, the mean water temperature values for Slow-flowing River (SFR) and Animal Foot (AF) for Ditches (DT) are within 24 ± and 26.84 ° C. The median pH level of water in all water specimens ranging from 5.77 to 5.88 is almost the same. Animal foots (31.67 ppm), accompanied by Slow-flowing creek (35.33 ppm) and Ditches (37.13 ppm) are the smallest average for complete dissolved materials. Meanwhile, the smallest natural water is the 656.67 mg / L animal foots (AF) and the highest for 725.00 mg / L Ditches (DT). In all water samples, the average value for electrical conductivity is the same that is 0.10 mS / cm. Rohani et al. (2010) study indicates soil pressure ranging from 21.9 oC to 30.9 oC for breeding environments. Due to the distinct sampling occasions, the differences in water temperature were owing to reduced morning temperature and greater winter temperature. The pH of the environments for reproduction ranged from slightly acidic (pH 4.4) to slightly alkaline (pH 7.8).

In this study, physiochemical habitat characteristics of positive and negative sample based on different types of *Anopheles* sp. breeding habitats were also observed. The ranges of key factors measured were observed based on positive and negative samples from Slow- flowing river (SFR) habitat, positive and negative samples from Ditches (DT) habitat and positive and negative samples from Animal foots (AF) habitat. The independent sample paired t-test showed that there were no statistically significant difference in water temperature, pH, total dissolved solids, dissolved oxygen and electric conductivity between the positive and negative samples from Slow- flowing river, Ditches and Animal foots (p>0.05) in this study.

4. Conclusion

A major reason for the study of mosquito larval ecology is to glean information on factors that may determine oviposition, survival, and the spatial and temporal distribution of important disease vector species. Breeding water quality is an important determinant of whether female mosquitoes will lay their eggs, and whether the resulting immature stages will successfully complete their development to the adult stage. This is, however, a somewhat neglected area of research in relation to vectors in general, and malaria vectors in particular. This information is important for widening the knowledge of breeding habitats of potential vectors of human diseases, contributing to their control. A precise knowledge of the geography, biology and ecology of mosquito breeding sites is key to implementing effective larval control measures.

Based on the research conducted by Rohani et al. (2010), there was no important impact of physicochemical parameters (such as air temperature, pH and dissolved oxygen) on bacterial cells (p<0.05). Topography and shade, however, influenced larvae incidence. Topography was a significant component, with locations frequently discovered in foothills where rainwater can collect, adjacent to rivers or ravine beds. The Kruskal Wallis experiment proved in this research that water temperature ware statistically important for distinct kinds of Anopheles sp with p-value 0.002 respectively for reproduction. It was discovered, however, that there was no important distinction between habitat kinds in pH, complete dissolved solids, dissolved oxygen and electrical conductivity (p>0.05). The independent sample test t-test found that there was no statistically important distinction in air temperature, pH, complete dissolved materials, dissolved oxygen and electrical conductivity (p>0.05) between the positive and negative samples from Slow-flowing stream, Ditches and Animal foots.

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