# ENVIRONMENTAL VARIABLES ASSOCIATED WITH IMMATURE STAGE HABITATS OF CULICIDAE COLLECTED IN ABORIGINAL VILLAGES IN PAHANG, MALAYSIA

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**Abstract.** Many of the most widely spread vector-borne diseases are water related, in that the mosquito vectors concerned breed or pass part of their lifecycle in or close to water. A major reason for the study of mosquito larval ecology is to gather information on environmental variables that may determine the species of mosquitoes and the distribution of larvae in the breeding habitats. Larval surveillance studies were conducted six times between May 2008 and October 2009 in Pos Lenjang, Kuala Lipis, Pahang. Twelve environmental variables were recorded for each sampling site, and samples of mosquito larvae were collected. Larval survey studies showed that anopheline and culicine larvae were collected from 79 and 67 breeding sites, respectively. All breeding sites were classified into nine habitat groups. Culicine larvae were found in all habitat groups, suggesting that they are very versatile and highly adaptable to different types of environment. Rock pools or water pockets with clear water formed on the bank of rivers and waterfalls were the most common habitats associated with *An. maculatus*. Environmental variables influence the suitability of aquatic habitats for anopheline and culicine larvae, but not significantly associated with the occurrence of both larvae genera ($p>0.05$). This study provides information on mosquito ecology in relation to breeding habitats that will be useful in designing and implementing larval control operations.

**Keywords:** mosquito larvae, environmental variables, distribution, breeding habitat, Malaysia

## INTRODUCTION

Vector-borne diseases continue to result in high morbidity and mortality, placing severe limitations on attempts to improve the quality of life (WHO, 1987). Some examples of diseases transmitted to humans by mosquito are dengue, filariasis, Japanese encephalitis, and

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malaria (WHO, 1972). There are many species of mosquito that serve as vectors for various vector-borne diseases. Some even transmit more than one disease. It is very important to note that one species of mosquito that is not a vector in one area may be an important species to transmit diseases in another area. The principal vector of dengue is the *Aedes* mosquito; *Anopheles*, *Culex,* and *Mansonia* mosquitoes transmit filariasis; *Culex* mosquitoes for Japanese encephalitis; and *Anopheles* mosquitoes for malaria.

The lack of mosquito control activities by mosquito control agencies contribute to high mosquito populations and directly expose communities to vector-borne diseases. Mosquito surveillance should be a routine activity of any mosquito control program. A good surveillance program provides information on mosquito species, abundance, distribution, and effectiveness of the control strategies being used. Without a proper and systematic mosquito control program, there can be no effective control of mosquitoes. Entomological, parasitological, and clinical studies provide useful information on the characteristic of disease transmission in an area, as well as the habits and habitats of the specific vector species.

Adult collections are most frequently conducted because adult mosquitoes are generally easier to survey, collect, and identify than the immature stages. The detection of adults in routine surveys does not provide an immediate indication of the related breeding sites (Tham, 2000). Mosquitoes were quite discriminate in selecting sites for egg deposition (Rohani *et al*, 2010). Although species overlap in habitat preference, oviposition site selectivity is considerably species dependent (Bently and Day, 1989). The occurrence of different sibling species can explain part of the heterogeneity in behavior. However, differences between individuals of the same species underline the major role of environmental factors in determining the occurrence, distribution, seasonality, behavior, and vectorial statutes for each species (Obsomer *et al,* 2007). Oyewole *et al* (2009) demonstrated that prevailing physicochemical in breeding habitats are important factors for survival and development of mosquitoes.

Larval surveillance refers to the determination, by whatever means, of the presence or absence of immature mosquitoes within a given site, and its collection and identification (Gaines, 2007). The aim of mosquito larval surveillance is to determine the breeding sites of vectors and for control measures (WHO, 1975). Routine mosquito larval surveys should be an on-going activity of every mosquito control agency, but the importance of larval surveillance is often overlooked (O'Malley, 1989). Before beginning a survey, information about the general breeding behavior and habitats of the species known or suspected present in the area must be obtained. Determining the specific breeding sites and establishing permanent larval sampling stations require a more detailed inspection (Reed and Husbands, 1969).

This study aims to determine the correlation between environmental variables and the distribution of larvae in the breeding habitats. It is hoped that the information gathered from this study will help broaden our understanding regarding the biology and ecology of mosquito breeding sites, and thus effective and efficient larval control measures can be applied.

## MATERIALS AND METHODS

The study was carried out in Pos

Lenjang (N4º15.413' E101º32.843), Kuala Lipis, Pahang, which is located about 240 km East of Kuala Lumpur. Pos Lenjang consists of 17 aboriginal villages, carved out of a secondary forest and situated on hilly terrain. The vegetation of the areas consists of tall grasses, shrubs, tapioca, patches of rubber trees of different varieties, banana trees, and hill-paddy. The inhabitants live in bamboo huts with *attap* roofs. The houses are scattered about, usually in the clearing of the foothills. The Pos Lenjang population continues to practice a semi-nomadic lifestyle. Felling and burning of forests for farming purposes is a practice adopted by tradition. Pos Lenjang is one of the low-malaria endemic areas reported by Ministry of Health (2007).

Larval collections were conducted on six occasions between May 2008 and November 2008. All possible bodies of water were sampled in and around all 17 villages and along rivers that are around Pos Lenjang. A habitat was inspected for the presence of mosquito larvae. When mosquito larvae were present, a standard mosquito dipper was used to collect the larvae by lowering it gently into the water at an angel of about 45º, and allowing one side of the dipper to be below the surface. Three to 18 dips were performed with the dipper at each site (Minakawa *et al*, 1999). For a habitat that was found to be too small, water was dipped as many times as possible (Minakawa *et al*, 2005). The larvae for each habitat were pipetted and placed separately in labeled bottle. Identifications were confirmed by rearing immature to adult stage (Piyaratne *et al*, 2005) in an insectarium, in white plastic trays on a diet of ground ox liver. A number of taxonomic keys were used in identification (Delfinado, 1966; Reid, 1968; Sallum *et al*, 2005).

During the larval survey, environment variables recorded for each habitat were habitat type, substrate type, percentage of emergent plants, canopy cover, percentage of surface debris coverage, distance to the nearest house, water depth, area size, dissolved oxygen, water temperature, pH, conductivity, and turbidity. Habitat types were classified into rock pool, water pocket, and ground pool. Substrate types were classified into clear, cloudy, and muddy. Canopy covers was classified into open, shaded, and partially shaded. Emergent plants included both aquatic and immersed terrestrial vegetation. Debris coverage was defined as the amount of terrestrial vegetation and other objects above the habitat. Area size was measured by placing square frame (1 $m^2$) with grid (100 $cm^2$) above the habitat. At the same time, plant and debris coverage of a habitat were measured in percentages of water surface covered.

Water depth for each breeding site was obtained by lowering a 100 cm ruler to the bottom of the pool at three locations, and the mean depths were recorded (Rohani *et al*, 2010). Distances to the nearest house were measured using a hand-held Geographic Positioning System (GPS) (GPSMAP® 60CSx, Garmin® International, Olathe, KS). Hand-held portable meters were used to determine dissolved oxygen (CyberScan DO300, Eutech Instruments®, Thermo Fisher Scientific, Rockford, IL), water temperature and pH (CyberScan, Eutech Instruments®, Thermo Fisher Scientific), conductivity (EcoScan con6, Eutech Instruments®, Thermo Fisher Scientific) and turbidity (TN-100, Eutech Instruments®, Thermo Fisher Scientific) on site during the surveys.

The environmental variables were classified into three categories: continuous, ordinal, and nominal (Minakawa *et al,* 1999). Continuous variables include dissolved oxygen, temperature, pH, water depth, area size, coverage of debris, and conductivity. The ordinal variable was turbidity, while nominal variables included habitat type and substrate type. Correlations between pairs of continuous or ordinal variables using Pearson's correlation coefficients were examined, while associations between nominal variables and the continuous or ordinal variables were evaluated.

Multiple regression analysis by the backward elimination method was employed to obtain the best predictor variables contributing to the occurrence of mosquito larvae for each genus. Density of larvae was categorized as low, median, and high corresponding to the number of larvae from 1-5, 6-10, and over 10, respectively. The weather station consisted of automated rain gauge (Oregon Scientific®, ExploraTrack, Cannon Beach, OR) was installed at key location in the study sites. Association between number of breeding sites and mean rainfall was analyzed by correlation coefficient. SPSS for Windows® (version 13, IBM, Armonk, New York) was used for the analysis.

## RESULTS

**Population of mosquito larvae in Pos Lenjang, Pahang**

A total of 291 of anopheline and 164 of culicine larvae were collected during the study period. Immature *Anopheles* mosquitoes were taxonomically identified as *An. maculatus,* and *An. macarthuri*. Culicine mosquitoes were taxonomically identified as *Ae. butleri*, *Ae. (Finlaya) macfarlanei*, *Ae. pseudoalbopictus*, *Armigeres* sp, *Cx. fuscanus*, *Cx. mimeticus* sg, *Cx. nigropunctatus*, *Tripteroides* sp, and *Uranotaenia* sp (Table 1).

**Breeding type**

This study characterized and identified key environmental factors for mosquito breeding habitats. A total of 120 breeding habitats were sampled and classified into 9 groups combining 3 substrate types and 3 habitat types, namely clear ground pool, cloudy ground pool, muddy ground pool, clear rock pool, cloudy rock pool, muddy rock pool, clear water pocket, cloudy water pocket, and muddy water pocket.

Fig 1 shows the distribution of anopheline and culicine larvae in nine habitat groups. Anopheline larvae were found in 79 habitats, of which 53 (67.1%) of these had only anopheline. Culicine larvae were found in 67 habitats, and 41 (61.2%) of these habitats had only culicine. Both anopheline and culicine larvae were found in 26 habitats, suggesting the mosquito larvae from the subfamilies Culicinae and Anophelinae coexisted in some of the habitats surveyed. The highest number of breeding site for anopheline mosquito larvae was clear rock pool, while the most common breeding site for culicine larvae was cloudy rock pool. The most common breeding site where anopheline and culicine mosquito larvae co-existed was clear rock pool.

Our survey showed that the primary malaria vector, *An. maculatus,* was present in 6 out of 9 habitat groups (Fig 2). The most common larval habitats for *An. maculatus* were clear rock pool (12 habitats), followed by clear water pocket (10 habitats), muddy water pocket (4 habitats), and muddy rock pool (2 habitats). *An. maculatus* was not found in cloudy ground pool, muddy ground pool, or cloudy rock pool. We also found that 82.3% of anopheline breeding sites were positive with *An. macarthuri,* which was found in all the group types except cloudy ground pool.

Table 1
Total number of mosquitoes larvae from larval survey trip in Pos Lenjang, Pahang.

| Mosquito species | Year 2008 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | May | Jun | Jul | Aug | Oct | Nov | Total | (%) |
| *Ae. butleri* | - | 1 | 1 | 3 | - | 7 | 12 | 2.6 |
| *Ae. (Finlaya) macfarlanei* | - | 2 | - | - | - | 10 | 12 | 2.6 |
| *Ae. pseudalbopictus* | - | - | 3 | 10 | - | 6 | 19 | 4.2 |
| *An. macathuri* | 44 | 40 | 37 | 36 | 6 | 2 | 165 | 36.3 |
| *An. maculatus* | 44 | 76 | 3 | 3 | - | - | 126 | 27.7 |
| *Armigeres* sp | - | - | 5 | - | - | 15 | 20 | 4.4 |
| *Cx. fuscanus* | - | - | 2 | 2 | 3 | 7 | 14 | 3.1 |
| *Cx. mimeticus* sg | - | - | 3 | - | 1 | 10 | 14 | 3.1 |
| *Cx. nigropunctatus* | - | 11 | 7 | 2 | - | 18 | 38 | 8.3 |
| *Tripteroides* sp | - | - | - | - | - | 10 | 10 | 2.2 |
| *Uranotaenia* sp | - | - | - | - | - | 25 | 25 | 5.5 |

Table 2
The range of environmental variables of habitat with only anopheline, only culicine and both anopheline and culicine larvae in Pos Lenjang, Kuala Lipis, Pahang.

| Larvae breeding habitat | pH | Dissolved oxygen (%) | Temp (ºC) | Debris (%) | Turbidity (NTU) | Conductivity (µS) |
|---|---|---|---|---|---|---|
| Anopheline | 4.11-8.74 | 14.2-136.0 | 21.7-32.6 | 0-100 | 1.17-125 | 9.0-171.4 |
| Culicine | 5.16-12.0 | 20.3-164.8 | 23.0-31.5 | 0-100 | 2.09-97.8 | 28.3-188.3 |
| Both anopheline and culicine | 4.82-7.55 | 16.8-110.5 | 22.9-25.7 | 9.21-100 | 1.45-156 | 20.6-41.7 |

Table 3
Correlation coefficient between continuous and ordinal environmental variables of 120 larval habitats sampled in Pos Lenjang, Pahang.

| | Dissolved oxygen | Temperature | pH | Water depth | Area size | Turbidity | Conductivity |
|---|---|---|---|---|---|---|---|
| Temperature | 0.459[a] | | | | | | |
| pH | 0.386[a] | 0.331[a] | | | | | |
| Water depth | -0.015 | 0.007 | -0.038 | | | | |
| Area size | -0.041 | -0.146 | 0.007 | 0.147 | | | |
| Turbidity | 0.028 | 0.017 | 0.008 | -0.086 | -0.112 | | |
| Conductivity | -0.072 | 0.164[b] | 0.370[a] | -0.120 | -0.127 | 0.037 | |
| Debris coverage | -0.272[a] | -0.091 | 0.000 | -0.175[b] | -0.187[b] | -.0183 [b] | 0.300[a] |

[a]$p< 0.01$; [b]$p< 0.05$

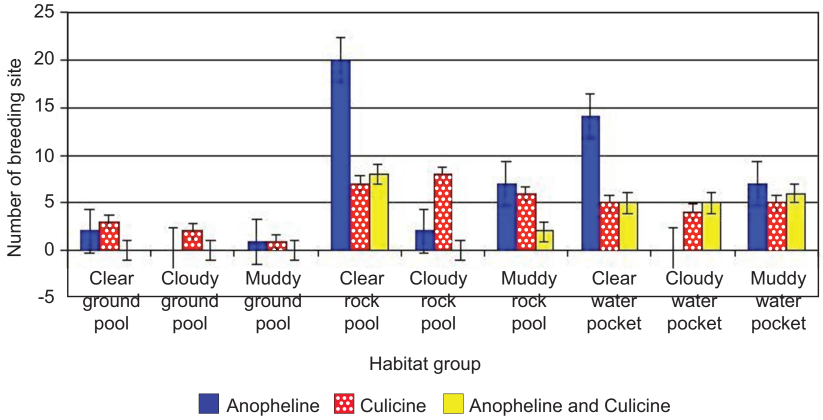


Fig 1–Distribution of anopheline and culicine larvae in nine habitat groups.

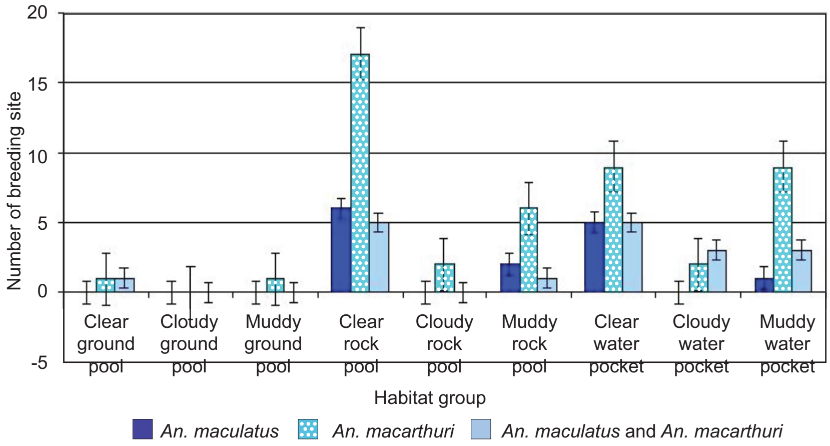


Fig 2–Distribution of *Anopheles* species in nine habitat groups.

Our study showed that as the area size increased, the number of breeding sites reduced (Fig 3). Culicine larvae were present in all habitats of different sizes except the one with 4.1-5.0 $m^2$. Anopheline larvae were more selective and detected mainly in habitat with 1.1-2.0 $m^2$ and smaller. Fig 4 shows the distribution of anopheline and culicine larvae in habitats with different water depth. The range of 0-4.99 cm and 5-9.99 cm were probably the most suitable depth in supporting the survival of both tribes. As the water depth gets deeper (25->30 cm), only anopheline mosquitoes were found.

Regardless of the canopy coverage, all habitats were found to harbor anopheline, culicine, or anopheline, together with culicine larvae. Looking at only the *Anopheles* species (Fig 5), the number of habitats harboring *An. maculatus* reduced as the canopy coverage changed from open to shade. Based on the number of habitat harboring *An. macarthuri* larvae, the *An. macarthuri* mosquito might have special preference towards partially shaded habitat over open and shade habitats.

The distribution of anopheline and

**Breeding habitat characterization**

Table 2 shows the range values of environmental variables from the nine habitat groups. The pH of the breeding habitats varied from slightly acidic (pH 4.11) to strongly basic (pH 12.00). The dissolved oxygen of the water breeding habitats ranged from 14.2% to 164.8%. The water turbidity for the breeding habitats ranged from 1.157 NTU to 156 NTU, while the water conductivity for the breeding habitats ranged from 9.0 µS to 188.3 µS.

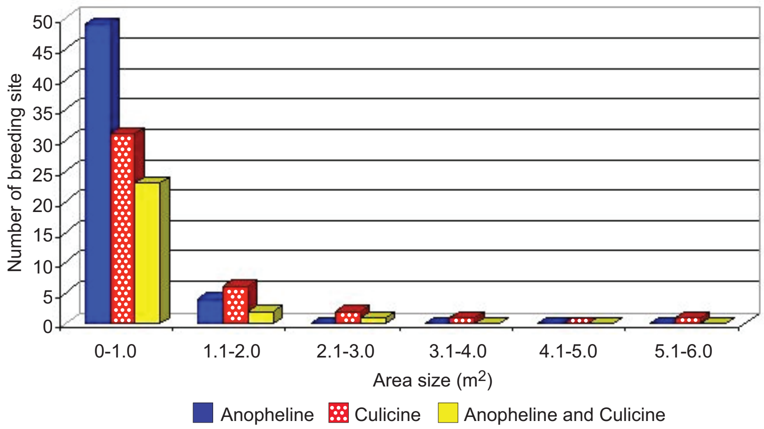


Fig 3–Distribution of anopheline and culicine larvae in habitat with different area sizes.

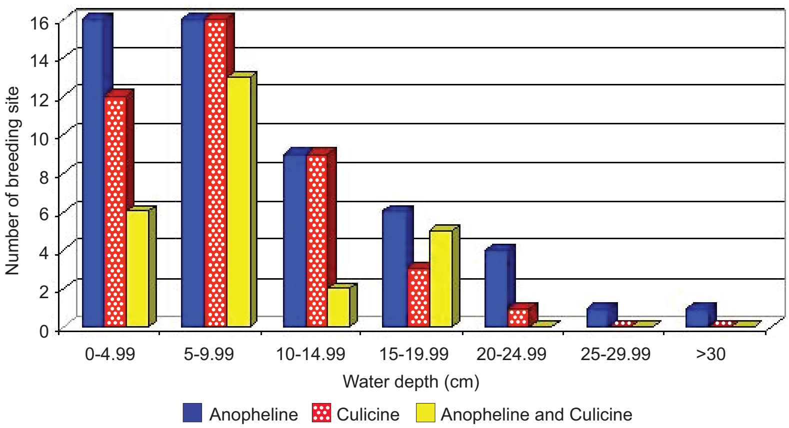


Fig 4–Distribution of anopheline and culicine larvae in habitat with different water depths.

culicine larvae in habitats at different distances from nearby village is shown in Fig 6. The highest numbers of breeding habitat were recorded in the areas with distances between 200 m to 400 m from the nearest house, and very few habitats were found in the 100 m distance range. Only culicine was found breeding in area more than 800 m away from the village.

**Statistical analysis of environment variables**

Correlation coefficients among continuous and ordinal environmental variables are shown in Table 3. Ten of 28 correlation coefficients (35.7%) were statistically significant, suggesting non-random association between some pairs of variables. Table 4 shows associations between nominal and continuous, or ordinal variables. Only water depth was significantly correlated with habitat type. Non-random associations suggested that the variables examined were not independent. Multiple logistic regressions did not detect any variable that was significantly correlated with the occurrence of anopheline and culicine (Table 5). Interaction between number of breeding sites and mean rainfall for each trip were pooled (Fig 7). The number of breeding sites was observed significantly and negatively correlated with the mean rainfall ($r$=-0.868, $p$<0.05).

## DISCUSSION

In the study area, our baseline results suggested that natural larval habitats constitute the bulk of vector sources in this hilly topographic context. River margins, waterfalls, and stream pools were seen as potential habitats for the development of anopheline and culicine larvae. Environmental variables influence the suitability

Table 4
Association between nominal variables and continuous or ordinal environmental variables for anopheline larval habitats sampled in Pos Lenjang, Pahang.

| | | Substrate type | | Habitat type | |
|---|---|---|---|---|---|
| | df | $\chi^2$ | $p$ | $\chi^2$ | $p$ |
| Dissolved oxygen | 152 | 148.1 | 0.574 | 153.8 | 0.443 |
| Temperature | 96 | 83.2 | 0.821 | 106.0 | 0.228 |
| pH | 122 | 121.1 | 0.506 | 104.2 | 0.876 |
| Water depth | 86 | 77.6 | 0.731 | 111.1 | 0.036[a] |
| Area size | 94 | 85.4 | 0.726 | 101.8 | 0.273 |
| Turbidity | 156 | 158.0 | 0.440 | 158.0 | 0.440 |
| Conductivity | 138 | 146.7 | 0.291 | 131.6 | 0.638 |
| Debris coverage | 124 | 120.4 | 0.575 | 142.6 | 0.121 |

[a]$p<0.05$

Table 5
Chi-square statistic from the multiple logistic regression analyses to measure associations between mosquito larval occurrence and environmental variables.

| | Occurrence of mosquito subfamily | | | | | |
|---|---|---|---|---|---|---|
| | Anopheline | | | Culicine | | |
| | $\chi^2$ | df | $p$ | $\chi^2$ | df | $p$ |
| Dissolved oxygen | 154.3 | 152 | 0.432 | 102.1 | 104 | 0.535 |
| Temperature | 108.9 | 96 | 0.174 | 70.3 | 66 | 0.334 |
| pH | 118.0 | 122 | 0.585 | 93.4 | 92 | 0.440 |
| Water depth | 91.1 | 86 | 0.333 | 45.8 | 54 | 0.780 |
| Area size | 87.0 | 94 | 0.682 | 79.3 | 82 | 0.563 |
| Turbidity | 158.0 | 156 | 0.440 | 110.0 | 108 | 0.428 |
| Conductivity | 140.9 | 138 | 0.415 | 95.4 | 98 | 0.554 |
| Debris coverage | 121.0 | 124 | 0.560 | 81.2 | 78 | 0.379 |

of aquatic habitats for two anopheline larvae species and nine culicine larvae species. However, statistical analysis did not found significant associations between environmental variables with the occurrence of both larvae genera.

Culicine larvae were found in all nine habitat groups suggesting that they are very versatile and highly adaptable to different types of environment found in the sampling areas. Rock pools or water pockets with clear water formed on the bank of rivers and waterfalls were the most common habitats associated with *An. maculatus*. Larvae of *An. maculatus* were collected from various habitats, either alone or in association with *An. macarthuri.*

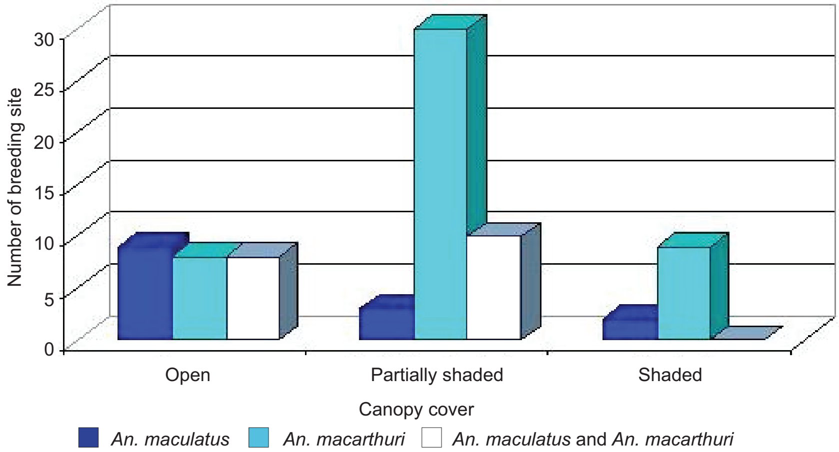


Fig 5–Distribution of *Anopheles* species in habitat with different canopy coverage.

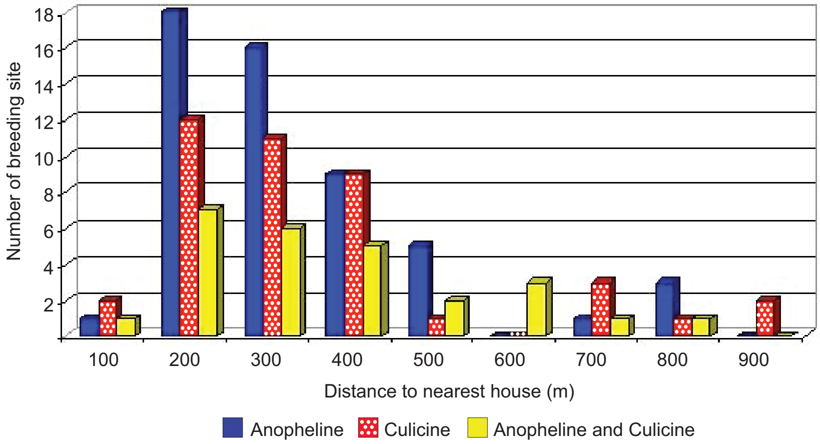


Fig 6–Distribution of anopheline and culicine larvae in habitat with different distance from nearby village.

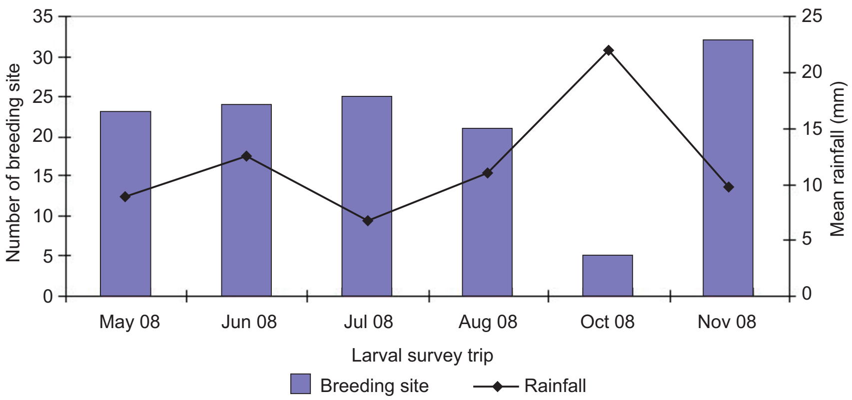


Fig 7–Correlation between total numbers of breeding sites per trip and mean rainfall in Pos Lenjang, Pahang.

The wide pH range (from pH 4.11 to pH 12.00) established at the breeding site during larval survey is an indication that mosquitoes can breed in both acid and alkaline habitats. The mean pH values for habitat having only anopheline, only culicine and anopheline in combination with culicine hovered from slightly acidic to weak basic, weak acidic to strong basic and slightly acidic to basic in nature, respectively. These results support findings reported by other previous studies (Sandosham and Thomas, 1982; Adebote *et al*, 2008). Sandosham and Thomas (1982) suggested that *Anopheles* species breeding in cleared jungle, such as *An. maculatus*, *An. karwari*, and *An. tessellates* have a preference for acidic water with a pH range from 5.5 to 6.6. Adebote *et al* (2008) reported that *An. ardensis*, *An. distinctus*, and *An. wilsoni* were associated with pools of acidic nature (pH

5.86-6.55); however, *Cx. ingrami* occurred in partly acidic and partly alkaline pools (pH 5.86-9.85). This study was also supported by Sattler *et al* (2005); they reported that culicine larvae favored a neutral environment.

*Anopheles maculatus* breeds in habitats with slow moving water and exposed to sunlit (Reid, 1968; Rahman *et al,* 1997) or partially shaded (Rohani *et al*, 2010). A similar finding was observed during this study. A study by Minakawa *et al* (2005) reported the importance of canopy cover as the one factor significantly associated with the occurrence of the *An. gambiae* complex and *An. funestus* larvae. The abundance of the aquatic stages of *An. dirus* was also reported to be influenced by shade, vegetation, and debris on the surface of well water (Oo *et al,* 2002).

We recorded a wide range of the water temperature for larvae habitats of both anopheline and culicine breeding sites during the survey. Supported by the findings of Paaijmans *et al* (2008), our results indicated that wide temperature ranges under natural conditions support mosquito development. Warm temperatures in small and open habitats during daytime hours help accelerate larval and pupal development, therefore, hasten adult fly emergence (Suhaiza, 2009). Water temperature has an effect on the diversity, densities, and activity of other aquatic organisms including algal matter for food resources of the mosquito larvae (Minakawa *et al*, 1999, 2005; Paaijmans *et al*, 2008).

Water temperature also affects dissolved oxygen (DO) levels. DO is essential to healthy streams and lakes. The DO values from 14.2% to 136% indicated the different levels of pollution encountered in the study area environment. Muturi *et al* (2008) demonstrated that the sum of all dissolved organic, inorganic, and suspended solids in water resulted in higher concentration of dissolved oxygen, thereby promoting *Cx. quinquefasciatus* productivity.

Organic compounds from decayed plants and fallen leaves were typical sources of turbidity to mosquito breeding habitats in the tropical rain forest area. For a long time, it was assumed that the probability of larvae present in water with high turbidity was low (mainly in the case of anopheline mosquito larvae). This conventional thinking about *Anopheles* species only breeding in rather clean and clear water is not necessary true since larvae were also found in habitats organically polluted. A study by Sattler *et al* (2005) showed that as long as turbidity of water is due to edible particles, it would favor the larvae production of *Anopheles* species. These findings from Pos Lenjang, together with other studies, could indicate a change of *Anopheles* breeding habitat preference. In South Punjab, Pakistan, *Anophele*s species were found in the wastewater system (Mukhtar *et al,* 2003), while In Dar es Salaam, Tanzania, *An. gambiae* was found in sewage pond (Sattler *et al,* 2005).

It is interesting to note that the numbers of mosquito larval habitats are generally related to rainfall. Low amounts of rainfall greatly reduced the availability of aquatic habitats (Minakawa *et al,* 2005). The probability of finding anopheline larvae during the dry season was reduced by 75% compared with the rainy season (Majambere *et al,* 2008). Excessive rains on the contrary cause flushing effects that kill immature stages (Sattler *et al,* 2005; Rohani *et al,* 2010). Thus, the larval and pupal density was found to be directly proportional to rainfall (Oo *et al*, 2002).

Excessive rains with prolonged rainfall however, will fill rock holes and ground pool with rainwater and contribute to availability of temporary breeding sites, as well as increasing the size and depth of permanent breeding sites.

Regarding habitat size, most anopheline larval habitats (91.1%), principally *An. maculatus* larval habitats (87.5%), were found with area size less than 1.0 $m^2$. A previous study reported that bigger habitat size has been associated negatively with the density of larvae (Fillinger *et al*, 2009). However, in this study we did not measure the actual density of the larvae but categorized it as low, median, or high.

Water volumes are contributed by rainfall (Oo *et al*, 2002). Previous studies, although not defining the optimum water depth required being potential breeding habitats, *An. maculatus* and *An. macarthuri* preferred shallow water (Reid, 1968; Sandonsham and Thomas, 1982; Rohani *et al*, 2010). Water depth below 10.0 cm was determined as the most favorable for both *Anopheles* species in Pos Lenjang. Culicine larvae were found only in water depth less than 25.0 cm. These findings clearly defined the term "shallow" as it has been used by previous studies.

More than 80% *An. maculatus* breeding sites were found from breeding sources within 400 m from the nearest villages. Thus, this study has managed to give a clear association between the distance to potential breeding sites and the variability in *An. maculatus* larvae. Compared with a distance of 750 m recommended by Hoek *et al* (2003) in his study in Sri Lanka, this study placed a much shorter distance (400 m within the rivers) as a cut-off point for developing a risk map of malaria in Pos Lenjang. As a consequence, this finding brought us to the conclusion that all villages studied were situated in malaria risk transmission area.

This study has suggested what were the factors that influence the natural habitats of anopheline and culicine larvae. The ecology of this principal malaria vector and other potential vectors was described. Information on the abundance of mosquito species, breeding habitat characterization, and other characteristics could be used to guide intervention measures and set targets for larval control operation at specific sites or time periods. Additionally, to achieve a satisfactory result, exhaustive targeting of all potential vector species is necessary.

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