HABITAT CHARACTERIZATION AND MAPPING OF *ANOPHELES MACULATUS* (THEOBALD) MOSQUITO LARVAE IN MALARIA ENDEMIC AREAS IN KUALA LIPIS, PAHANG, MALAYSIA

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Abstract. In Peninsular Malaysia, a large proportion of malaria cases occur in the central mountainous and forested parts of the country. As part of a study to assess remote sensing data as a tool for vector mapping, we conducted entomological surveys to determine the type of mosquitoes, their characteristics and the abundance of habitats of the vector *Anopheles maculatus* in malaria endemic areas in Pos Senderot. *An. maculatus* mosquitoes were collected from 49 breeding sites in Pos Senderot. *An. maculatus* preferred to breed in water pockets formed on the bank of rivers and waterfalls. The most common larval habitats were shallow pools 5.0-15.0 cm deep with clear water, mud substrate and plants or floatage. The mosquito also preferred open or partially shaded habitats. Breeding habitats were generally located at 100-400 m from the nearest human settlement. Changes in breeding characteristics were also observed. Instead of breeding in slow flowing streams, most larvae bred in small water pockets along the river margin.

Key words: *Anopheles maculatus*, mosquito larvae, distribution, habitat, Malaysia

INTRODUCTION

The incidence of malaria in Malaysia is declining significantly; from more than 200,000 cases a year in 1950's and 1960's (243,870 in 1961) to a record low of 7,390 cases in 2008 (VBDCP, 2009). Malaria endemic areas are now confined to less accessible and hilly forested land with inadequate transport and communication facilities. Malaria is under control in most parts of Peninsular Malaysia, but persists in a number of problematic foci, such as in arboriginal areas, tribal villages found in cleared hilly jungles, and in communities working in agricultural and land development. *Anopheles maculatus* is the principal vector of human malaria in Peninsular Malaysia (Loong *et al*, 1988; Vythilingam *et al*, 1995).

The malaria control program in Malaysia has been so successful, program managers are contemplating elimination of indigenous cases by 2015. However, there are several challenges unique to low endemic malaria that need to be

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addressed. The greatest challenge is to determine mosquito vector breeding since the risk of malaria resurgence is dependent on the presence of a mosquito vector. Thus mapping of the vector is the most important component of entomological surveillance in this situation.

Malaria vector control activities in Malaysia focus mainly on the use of insecticide-treated bed nets and indoor residual spraying. As a complement to these vector control activities and prompt access to treatment, measures targeted against the larval stages of *Anopheles* sp mosquitoes are also a promising strategy for malaria endemic areas. A precise knowledge of the geography, biology and ecology of mosquito breeding sites is key to implementing effective larval control measures. This is the first report to combine a Geographical Information System (GIS), Remote Sensing (RS) and entomological data in Malaysia.

This paper reports mosquito fauna breeding in malaria endemic areas in Kuala Lipis, Pahang, Malaysia. The physicochemical conditions affecting mosquito occurrence and abundance in these microhabitats are also presented. This information is important for widening the knowledge of breeding habitats of potential vectors of human diseases, contributing to their control.

MATERIALS AND METHODS

Study sites

Study sites in Pos Senderot (N4º10.181' E101º34.758') were chosen because they exhibited documented consistent and recurring endemic malaria transmission in Peninsular Malaysia. The villages had been experiencing dramatic land use changes, such as deforestation and cultivation for subsistence agriculture, growing cash crops and firewood acquisition. Larval collection and site mapping were conducted on 10 occasions between April 2007 and April 2008.

Larval surveys

Routine larval collections were carried out in and around each of the study areas to determine the type and abundance of habitats where anopheline mosquitoes occurred. Collections consisted of systematically dipping with dippers (350 ml). A habitat was first inspected for the presence of mosquito larvae. If *Anopheles* mosquito larvae were present, 3-10 dips were taken, with a greater sampling effort in areas of low mosquito density. The water was collected in a white plastic tray and carefully observed for the presence of mosquito larvae. Habitats containing mosquito larvae were numbered. Mosquito larvae were collected alive in bottles and reared in an insectarium in a white plastic tray on a diet of ground ox liver. Mosquito larvae were reared to adults and only Anopheline mosquitoes were identified to species (Reid, 1968; Harison and Scanlon, 1975). The coordinates of each sampling site were recorded with a hand-held GPS, and later integrated into a GIS database. For each site, the distance to the nearest village was estimated using GPS. ArcView 9.1 software (ESRI, 2006) was used to develop a map of the village and breeding sites.

Larval habitat characterization

Environmental variables recorded for each habitat were size, water depth, water temperature, pH, dissolved oxygen, water surface area, water turbidity, distance to the nearest house, canopy coverage, surface debris coverage, emergent plant coverage, habitat type and substrate type. Canopy cover was defined as the amount of terrestrial vegetation and other objects above the habitat. Emergent plants included both aquatic and immersed terrestrial vegetation. Plant and debris coverage of a breeding habitat were measured in percentage of water surface covered by placing a square frame (1 m^2) with a grid (100 cm^2) above the habitat. The depth of water in breeding habitats was obtained by lowering a meter rule to the bottom of the pools at three locations and the mean of the depths recorded. Substrate type was classified as muddy, sandy with gravel and soil and artificial substrate without soil. Water pH and temperature, conductivity, dissolved oxygen, turbidity and light intensity of larval habitat were determined by means of a portable meter.

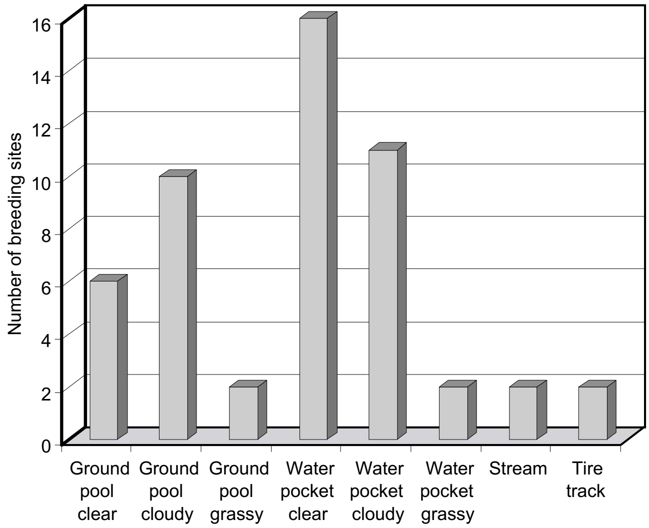

Fig 1–Distribution of breeding habitats of *An. maculatus* in Pos Senderot.

RESULTS

In total, 12 villages in Pos Senderot were systematically searched for mosquito breeding sites. Possible larval habitats for mosquitoes were surveyed; the species and sites positive for mosquito larvae were noted (Table 1). A total of 191 mosquito-positive larval habitats were sampled. Anopheline larvae were found in 78 habitats, of which 67 of these (35.0%) had only anophelines. Culicine larvae were found in 124 sites, and 113 of these habitats (58.5%) had only Culicines. Both anopheline and culicine larvae were found in 11 habitats (5.7%), suggesting the mosquito larvae from the subfamilies Culicinae and Anophelinae coexisted in some of the habitats surveyed. Only two *Anopheles* species (*An. maculatus* and *An. macrathuri*) were found during the survey and these species coexisted in 19 breeding habitats.

An. maculatus is the main malaria vector species in Pos Senderot and its breeding sites are particularly associated with river beds. A total of 273 *An. maculatus* larvae were collected and mapped from 49 larval habitats, which were categorized into 8 types: clear ground pool, grassy ground pool, cloudy ground pool, clear water pocket, grassy water pocket, cloudy water pocket, shallow stream and tire track. The most common larval habitats for *An. maculatus* in Pos Senderot were clear water pocket, cloudy water pocket, cloudy ground pool and clear ground pool (Fig 1). Our survey also showed a high density of *An. maculatus* larvae were associated with the surrounding vegetation around ground pools or water pockets formed on the banks of rivers and waterfalls. Most breeding sites in Pos Senderot were encountered along the Jelai River and breeding in tire tracks occured close to the Jelai Kecil River. Slow flowing streams were also a main breeding source. Fig 2

Table 1
Distribution of Anopheline and Culicine mosquito larvae in 191 aquatic habitats sampled in Pos Senderot, Kuala Lipis, Pahang.

	Number of habitats (percentages)	Larval habitat types							
		Clear ground pool	Grassy ground pool	Cloudy ground pool	Clear water pocket	Grassy water pocket	Cloudy water pocket	Tire track	Shallow stream
Anopheline *vs* culicine									
Presence of anopheline larvae only	67 (35.0%)	9	2	7	33	0	13	0	3
Presence of culicine larvae only	113 (59.2%)	0	17	54	2	3	36	0	0
Presence both of anopheline and culicine larvae	11 (5.8%)	0	0	5	0	0	5	1	0
Total	191 (100%)	9	19	66	35	3	54	1	3
Anopheline									
Presence of *An. maculatus* larvae only	30 (38.5%)	6	0	4	8	0	8	1	3
Presence of *An. macarthuri* larvae only	30 (38.5%)	2	0	3	17	0	8	0	0
Presence of both *An. maculatus* and *An. macarthuri* larvae	18 (23.0%)	1	2	5	8	0	2	0	0
Total	78 (100%)	9	2	12	33	0	18	1	3

shows the distribution of different types of *An. maculatus* larval habitats in Pos Senderot, Kuala Lipis, Pahang.

An. maculatus also showed a clear preference for small and temporary pools (14.3%). The breeding pools occur after rainy days. Breeding sites less than 1 m^2 were more likely to contain *An. maculatus* larvae (Fig 3). Most of these habitats were fresh, shallow water habitats with a water depth of 5.0 - 15.0 cm (Fig 4). *An. maculatus* preferred open and partially shaded water habitats (Fig 5).

The distance from the centers of villages to the nearest larval habitats are shown in Fig 6. More than 80% of *An. maculatus* larvae were found breeding within 400 meters from a village. Larvae in habitats close to houses had a higher proportion of *An. maculatus* than habitats at a greater distance from houses. Fig 7 shows a map of the distribution of *An. maculatus* breeding habitats in relation to the distance from the nearest villages in Pos Senderot.

Table 2 shows the range of values of environmental variables from the water at the breeding sites. The pH of the breeding habitats varied from slightly acidic (pH 4.4) to mildly alkaline (pH 7.8). The water temperature for the breeding habitats ranged from 21.9ºC to 30.9ºC. The variations in water temperature were due to the different sampling times, with a lower temperature in the

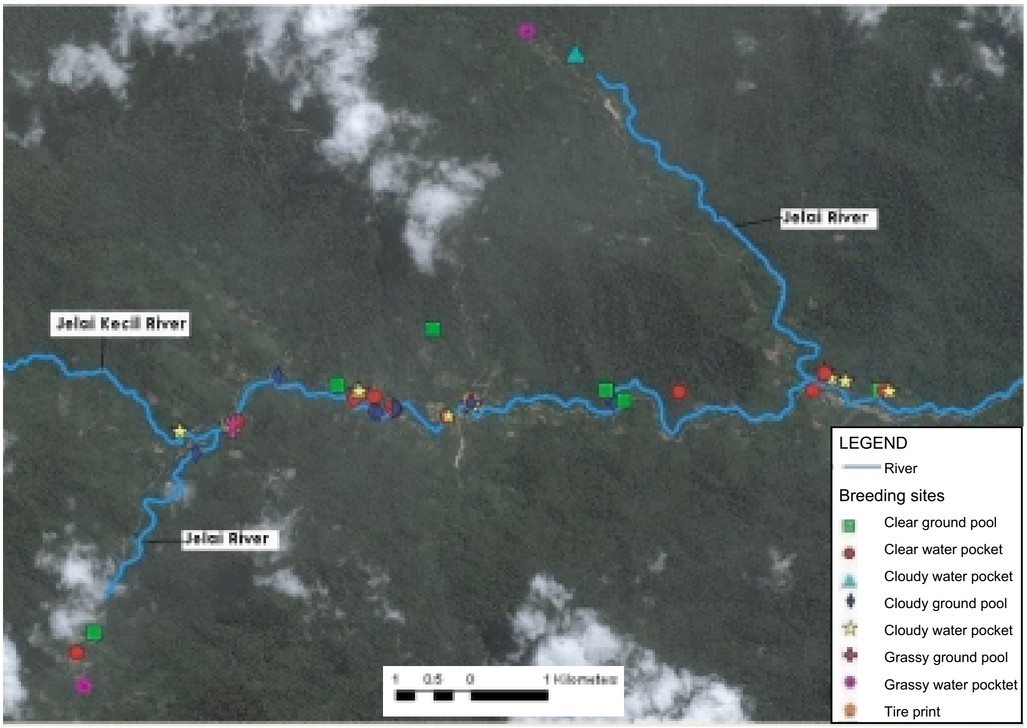

Fig 2–The distribution of *An. maculatus* larval habitats in Pos Senderot.

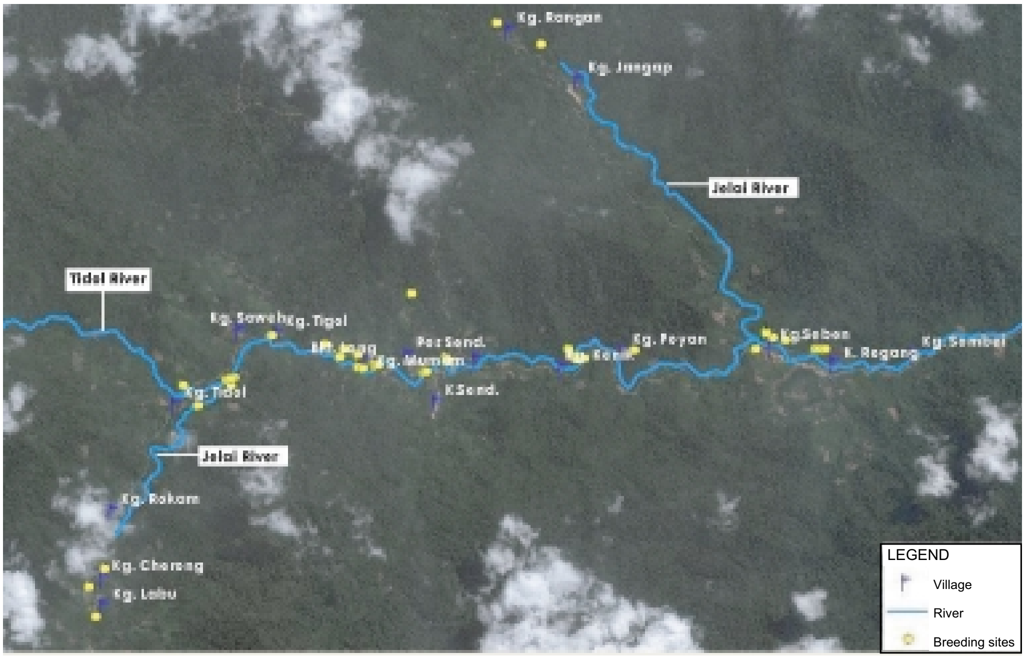

Fig 7–The distribution of *An. maculatus* larval habitats and villages in Pos Senderot.

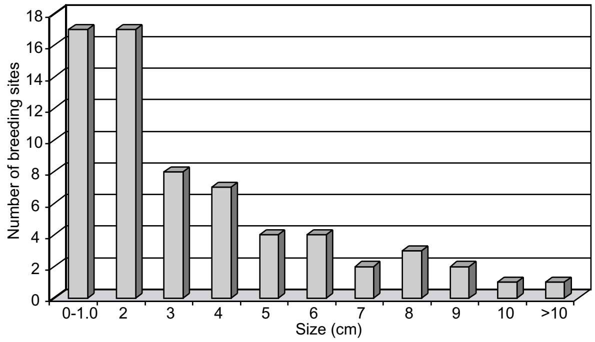

Fig 3–Abundance of different breeding area sizes.

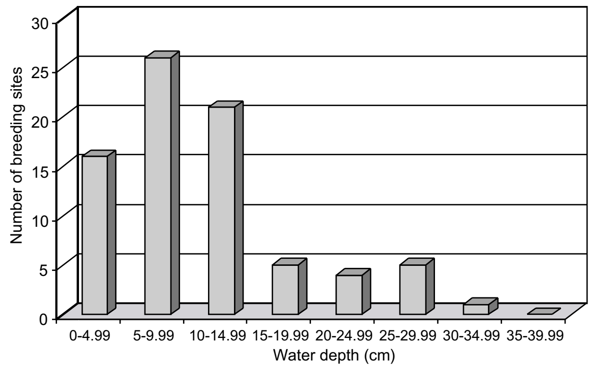

Fig 4–Water depth of breeding sites.

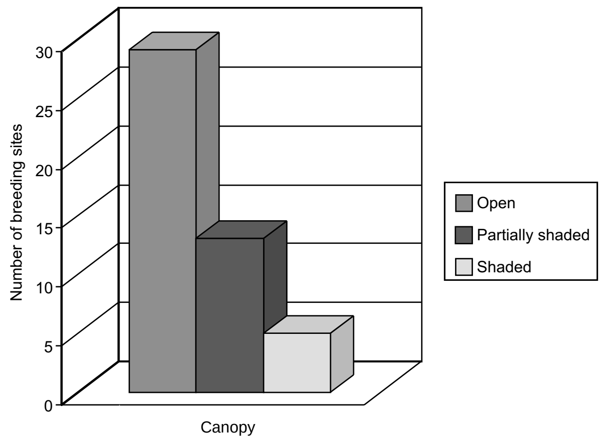

Fig 5–Canopy cover over *An. maculatus* breeding habitats in Pos Senderot.

morning and a higher temperature at noon. All the *An. maculatus* larvae were found in natural habitats, either in stagnant or slow flowing water, with emergent vegetation or floating debris (Table 2). *An. maculatus* bred in rather clean, clear water. The probability of *An. maculatus* larvae to be present was reduced when the water was turbid. The overall range of turbidity varied from 1.08 to 107.0 NTU, while the mean dissolved oxygen ranged between 5.6-90.4%.

DISCUSSION

The Pos Senderot area arboriginal settlement consisted of 280 houses with a population of 784 individuals. The villages of Pos Senderot were carved out of a secondary forest situated on hilly terrain, with most villages being riverine. The vegetation of the areas consists of tall grasses, shrubs, patches of rubber trees of different varieties and hill-padi. The houses are scattered about, mostly in the clearing of the foothills. Villages in Pos Senderot are not static, since houses and sometimes whole villages may be relocated to remain within convenient walking distance of garden crops which are periodically moved to different places according to agricultural practices. Clearing of jungle cover for cultivation may contribute to suitable breeding habitats for malaria vectors such as *An. maculatus*.

Our field studies show mosquitoes are quite discriminating in selecting sites for egg deposition. Although species overlapped in habitat preference, oviposition site selectivity was considerably species dependent (Bently and Day, 1989). The study explored the dis-

Table 2
Environmental variable range for *Anopheles maculatus* breeding habitat type.

Habitat type	pH	Dissolved oxygen (%)	Temperature (ºC)	Debris (%)	Turbidity (NTU)
Clear ground pool	5.8 - 7.1	30.7 - 93.2	22.2 - 28.9	33.3 - 87.5	1.3 - 16.4
Cloudy ground pool	4.9 - 6.9	5.6 - 89.0	22.5 - 28.4	6.7 - 100	1.3 - 86.9
Grassy ground pool	5.7 - 5.9	45.7 - 59.7	25.1 - 27.2	45.8 - 75.0	6.8 - 23.0
Clear water pocket	54.3 - 7.8	15.3 - 100.0	21.7 - 30.9	4.0 - 95.8	0.5 - 169.0
Cloudy water pocket	4.8 - 7.1	19.0 - 83.3	21.5 - 27.6	0 - 66.7	1.1 - 78.0
Grassy water pocket	5.6 - 5.7	55.8 - 82.9	21.9 - 26.7	0 - 22.6	2.3 - 37.0
Stream/drain	5.8 - 6.1	26.9 - 57.3	23.3 - 24.7	0 - 42.1	1.9 - 22.5
Tire track	5.6	46.6	25.7	0	1.6

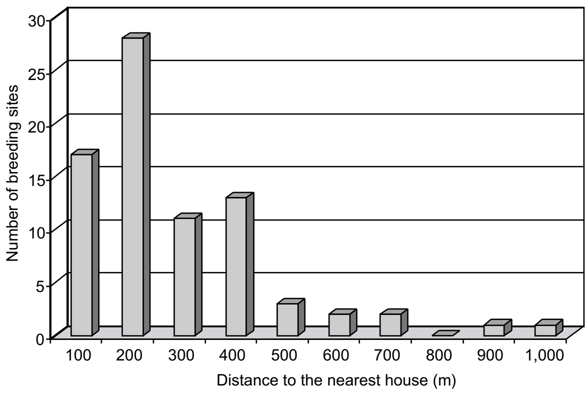

Fig 6–Distance from *An. maculatus* breeding habitats to the nearest house in Pos Senderot.

tribution of the immature stages of *An. maculatus* mosquitoes, considered to be important vectors of *Plasmonium vivax* and *P. falciparum* in Peninsular Malaysia. Rivers and streams within villages were mapped. Distance from the nearest river to the center of the village was calculated and correlated with the breeding sites.

The potential of combining ground-based sampling approaches with modern remote sensing technologies may guide the targeting of specific localities. A clear association was observed in the study areas between the distance to potential breeding sites and the variability in *An. maculatus* density. *An. maculatus* was identified from breeding sources within 400 meters from the villages of the study areas in Pos Senderot, suggesting *An. maculatus* prefers to lay eggs in habitats near houses. This may be explained by the limited flight distance of the gravid *An. maculatus* females. Our results also show a strong association between distance from breeding sites and the presence of *An. maculatus* larvae. One of the prime considerations for malaria risk mapping is the association between malaria incidence and distance from houses to breeding sites, which has been documented from different parts of the world where different vectors play a role in malaria transmission (Oesterholt *et al*, 2006; Saxena *et al*, 2009). Minakawa *et al* (2002) reported that 90% *An. gambiae* were found breeding within 300 meters of human habitation. Similar findings were reported by Sattler *et al* (2005) for *Anopheles* mosquito larvae in Dar es Salaam, Tanzania. Hoek *et al* (2003) suggested the use of a distance of 750 meters as a cut-off point for developing a risk map of malaria in Sri Lanka.

The study showed that stream margins and stream pools were identified as potential habitats for development of *An. maculatus*. Larvae of *An. maculatus* were collected from various habitats either alone or in association with *An. macathuri*. Small ground pools or water pockets with clay substrate, with plants or floating debris that form on the banks of rivers and waterfalls, were the most common habitats associated with *An. maculatus* in Pos Senderot. These habitats, mostly encountered along the Jelai River, may increase after rainy days. Rain water often collects in depressions in rocks along the river margin to form pools that are ideal breeding sites for *An. maculatus*. It was common to find holes and/or depressions in rocks filled with water yielding several mosquito larvae in a single dipping. However, the number of breeding sites is generally related to the amount of rainfall, because excessive rains cause flushing, thus killing immature stages. The principal effect of rainfall may be to increase the number and size of these sites and maintain the observed association of breeding sites and distance to the river.

Breeding habitats of *An. maculatus* in the study areas can be characterized into primary and secondary larval habitats. Both primary and secondary larval habitats of *An. maculatus* have some constant characteristics: temporary, standing or slow moving water exposed to sunlight or partial shade. Primary sites occured year round and tended to be associated with rivers in the deep forest. They varried in nature according by season with drying pools in river bed, slopes on riverbeds and pools connected to the river. Secondary larval habitats occurred during the rainy season and could be found closer to human settlements at the forest fringe. These are commonly small, shallow, temporary, exposed to sunlight or partially shaded water found in depressions.

An. maculatus in our study exhibited a clear preference for small pools and shallow water habitats. Breeding was primarily found in either stagnant or slow flowing water, exposed to sun-light or partly shade with floating debris or submerged plants along river margins. 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.

The abundance of *An. maculatus* larvae seemed to be influenced by water turbidity. In turbid water *An. maculatus* larvae were much more likely to be absent. Robert *et al* (1998) reported a similar finding with *An. ambiensis* breeding more in clear water; however, Gimning *et al* (2001) found higher *An. gambiae* larval densities with increasing turbidity. The influence of physicochemical parameters (such as water temperature, pH and dissolved oxygen) on mosquito larvae was not significant ($p< 0.05$) in our study. However, topography and shade did influence the occurrence of larvae. Topography was a major element, with sites found commonly in foothills where rain water can accumulate, next to streams or in beds of ravines.

When all the villages were considered in the analysis, proximity to a river was significantly correlated with breeding sites. The study data suggest riverine breeding sites are a major source of vectors through out the year. It is expected the association between distance to the river and malaria episodes would be strongest during the dry season when the river is the only available breeding site, and would become weaker after the rains when alternative breeding sites are more abundant (Oesterholt *et al*, 2006). One might conclude simple maps depicting rivers could serve to identify high probability breeding sites for this vector.

Malaria transmission depends on the availability of competent mosquito vectors (Omumbo *et al*, 1998) and is constrained only by the flight distance of mosquitoes. The flight range of the vector (*Anopheles* mosquitoes) from a suitable habitat is limited to a maximum of 2 km (WHO/RBM, 2005). Land use changes have been suggested as one of the causes for malaria epidemics in the highland forest, such as the rainforest area in Pos Senderot. Assuming malaria transmission occurs within the village and is not imported, environmental factors, such as forest type, cultivation, rainfall and proximity of rivers, are useful proxy measures to identify the presence of vector species and its role in malaria transmission (Hakre *et al*, 2004). The strong relationship between the arborigine habitation in Pos Senderot and the river suggests focal malaria control is possible, either by larval control near the river or by selectively targeting households close to breeding sites with transmission reducing interventions.

In this study GIS-based field information provided a graphical representation of the spatial variability in the density of the vector population and allowed generation of a new variable, the distance to potential breeding grounds. Application of GIS considerably improves the management of information obtained from field surveys and facilitates a study of the distribution patterns of vector species.

In conclusion, a clear association was observed in the study between distance to potential breeding sites and *An. maculatus* density. Until reliable, practical and affordable means of targeting particular habitat locations or types are developed, comprehensive coverage is essential if larval control is to achieve substantial reductions in malaria transmission and disease burden.

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