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**Research Article**

**Species Composition of *Anopheles* Mosquitoes in Danum Valley, Lahad Datu, Sabah**

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**ABSTRACT**

Malaria continues to be a public health concern globally, while in Malaysia, cases remain high among interior communities in Borneo, including in Sabah. We studied the *Anopheles* species in Danum Valley, Lahad Datu, by random sampling of mosquitoes in the virgin forest of the Danum Valley Conservation Area (VF), low-ground regenerated forest (100m above sea level) (LRF) and high-ground regenerated forest (400m above sea level) (HRF). Over 12 trap nights, a total of 839 individuals of *Anopheles* mosquitoes belonging to nine species were collected with mosquito magnet: *Anopheles asiaticus* (94), *An. balabacensis* (12), *An. barbumbrosus* (7), *An. fragilis* (640), *An. interruptus* (38), *An. jamesii* (9), *An. latens* (5), *An. maculatus* (17) and *An. montanus* (17). Among them, are vectors for zoonotic malaria in Malaysia namely *Anopheles balabacensis* (1.43%), *An. maculatus* (2.03%) and *An. latens* (0.60%), albeit relatively low in numbers. HRF had the highest number of *Anopheles* mosquitoes collected (670), followed by LRF (130) and VF (39). Both Simpson's (D) and Shannon-Wiener (H) diversity indices were highest at LRF (D = 2.07; H = 1.2 with highest Species Evenness, E = 0.58), followed by HRF (D = 1.62; H = 0.8; E = 0.39) and VF (D = 1.31; H = 0.47; E = 0.43). Greater numbers of the malaria vectors were found in LRF and HRF, compared to VF suggesting that there may be greater exposure to vectors and vector-associated diseases when entering these regenerated forests. Significant differences ( $p < 0.05$ ) for different forest types were detected for the total number of mosquitoes, total *Anopheles* and *An. fragilis* between different forest types.

**Keywords:** *Anopheles*; tropical rainforest; malaria vectors; DVFC.

## INTRODUCTION

The World Health Organization (WHO) (2015) estimated that about 4.3 million deaths were due to malaria vector mosquitoes between the years 2001 and 2013, making these insects the most dangerous animals in the world. In 2017 alone, approximately 435,000 malaria deaths were recorded worldwide (WHO, 2018).

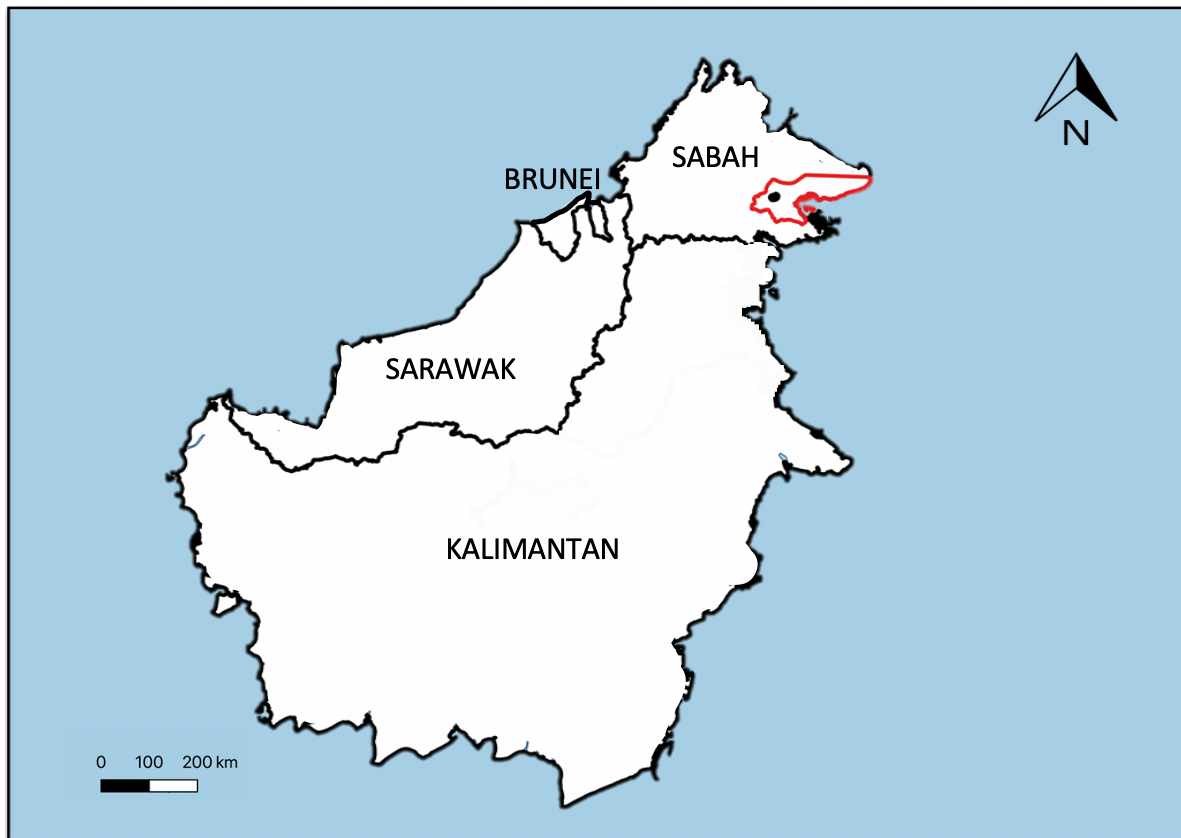
In Malaysia, 16,500 malaria cases were recorded from 2013 to 2017. Sabah (7150 cases; 43.3%) and Sarawak (5684 cases; 34.4%) were the major contributors of malaria cases (Hussin, 2020). The malaria parasites transmitted between humans in Malaysia are *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax*, and *Plasmodium ovale*. While the cases of *P. falciparum* and *P. vivax* have decreased, zoonotic malaria originating from monkeys caused by *Plasmodium knowlesi* continues to increase (Cooper et al., 2019). *Plasmodium knowlesi* is the most common malaria causing parasite species in East Malaysia near forest regions, meanwhile more cases of *P. vivax* were recorded in West Malaysia.

Forests in Sabah have long been heavily logged (or exploited) due to economic globalisation and local socio-political dynamics (Gunggut et al., 2014). This has resulted in rise of *P. knowlesi* incidence in humans, and strong linkage between deforestation and *P. knowlesi* cases has been demonstrated (Fornace et al., 2016). The main vectors of *P. knowlesi* belong to the Leucosphyrus group of mosquitoes (Tan et al., 2008; Vythilingam et al., 2008; Hii et al., 1985) with high biting rates for vector *Anopheles* mosquitoes being recorded at farm edges bordering forest and forest areas.

The main agent of forest disturbance in Sabah has been the timber industry and whose logging activities has left relatively small undisturbed lowland dipterocarp forest areas (Marsh & Greer, 1992). Habitats that have been fragmented create transition areas with increased spatial overlap among humans, mosquitoes, and wildlife populations or altered vector ecology can also increase the frequency of disease transmission (Hutchings et al., 2011; Overgaard et al., 2003). The detrimental effects of these environmental alterations at forest edges have been documented for malaria and other vector-borne zoonotic diseases (Lambin et al., 2010; Vanwambeke et al., 2007). Effects of deforestation and land cover changes for agricultural purposes contributed to temperature rise, vector species dynamics and abundance changes with time strongly linked to land use changes (Kweka et al., 2016).

The diversity and species composition of *Anopheles* mosquitoes dwelling in Danum Valley Conservation Area (DVCA) is poorly studied. With monkey malaria cases caused by *P. knowlesi* transmitted by *An. balabacensis* rising in Sabah and becoming a threat to the planned malaria elimination programme in Malaysia (William et al., 2013), more information on the mosquitoes fauna will be needed. Therefore, this study was conducted to determine the composition and diversity of *Anopheles* mosquitoes in the DVCA based on different forest types and hopefully such information will help formulate control strategies.

## METHODOLOGY



**Figure 1:** Map of Borneo Island. Danum Valley Conservation Area is demarcated in red and located within Lahad Datu, Sabah.

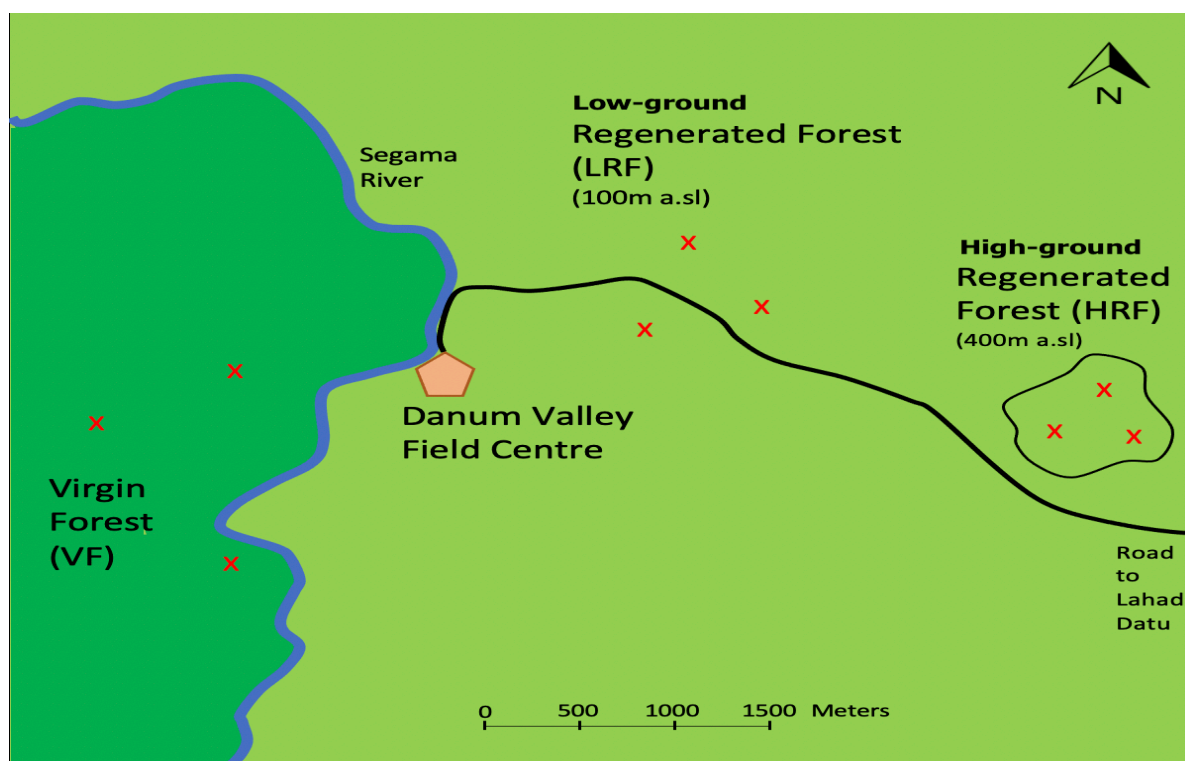
The Danum Valley Conservation Area (DVCA) (N 04°57.829' E 117°48.055') situated in Lahad Datu was selected for this study. The DVCA is located 81.1km from Lahad Datu Airport and it takes approximately 2 hours 30 minutes by four-wheel drive to reach Danum Valley Field Centre (Fig. 1).

The DVCA is a well-known Class 1 Forest Reserve managed by Yayasan Sabah and Royal Society's Southeast Asia Rainforest Research Programme (SEARRP). The virgin forest area includes the uninhabited 43,800 ha of lowland forest (Marsh & Greer, 1992). The DVCA presents opportunities for ecological research to obtain scientific understanding of ecological processes and evolutionary mechanisms operation within tropical rainforests ( Marsh & Greer, 1992) .

Danum Valley comprises dense, undisturbed lowland forests with mostly dipterocarp species, mangroves, montane forest, swamps and transitional forest and re-logged forest at least once (Marsh & Greer, 1992; Reynolds et al., 2011). During rainy periods, animal tracks in the lowland forest easily get flooded; becoming temporary breeding grounds for mosquitoes, long enough for larvae to become adults. Dense tree canopies in the forest minimise sunlight exposure to the ground, which makes the environment moist and humid; perfect for mosquitoes to breed.

The influence of the weather on mast fruiting is reportedly strongest in the eastern part of Borneo (Ashton et al., 1988). During the transition months following the equinoxes (May–June and October–November), the study area experiences highest rainfall, and at the height of the northerly monsoon in December and January (as cited by Clarke & Walsh, 2006). The months of August and September are drier as the south-westerly monsoon reaches its height, and rainfall is also occasionally lower in March and April, which are the months most vulnerable to low rainfall in El Niño Southern Oscillation (ENSO) atmospheric conditions (Walsh & Newbery, 1999).

*Anopheles* mosquitoes were collected with Mosquito Magnet® Independence MM3200 traps (MMTs) using the mosquito attractants, Lurex3 and Octenol. The cartridge of the Lurex3 contained lactic acid, a chemical compound found in human skin odour (Steib et al., 2001), whereas Octenol mimics human breath (Chaiphongpachara et al., 2018). The MMTs were powered by four AA batteries and the combustion process was facilitated by a 30kg propane gas cylinder. During the combustion of propane, MMTs produce carbon dioxide. Supplemented with a cartridge of Lurex3 and Octenol for each trap, MMTs give off heat, humidity, lactic acid provided by the cartridge of Lurex3, human breath from Octenol and carbon dioxide gas simulating the human presence (Sant’Ana et al., 2014).



**Figure 2:** The symbols, X, indicate the trap sites within different forest types of Danum Valley: Virgin Forest (DVCA) = VF, Low-ground Regenerated Forest = LRF, High-ground Regenerated Forest = HRF (400m a.s.l.).

The MMTs were set at random sites within the virgin forest (VF), low-ground regenerated forest (LRF) and 400m above sea level high-ground regenerated forest (HRF) (Fig. 2); approximately 1 km apart from each trap. MMTs were set up at 8.00 am on Day 1 and remained switched on until 8.00 am of Day 2. The MMT nets containing mosquitoes were collected every 24 hours and new fresh nets were placed daily for a total of 12 trap nights at each site. In the sampling months of May and January, three MMT were used, but in October only one

MMT was used due to mechanical issues. Mosquito sampling was carried out in the first week of May 2019, October 2019 and January 2020. A total of six trap nights for the month of May 2019 sampling, and three trap nights for October 2019 and January 2020 were carried out at each site.

MMT nets with *Anopheles* mosquitoes were taken to the Danum Valley Field Centre (DVFC) Scientific Laboratory for further processing. The mosquitoes were killed in a  $-30^{\circ}\text{C}$  freezer, mounted individually and placed into 1.5  $\mu\text{l}$  microcentrifuge tubes and stored in the  $-30^{\circ}\text{C}$  freezer temporarily prior to being transferred out from Danum Valley. They were later carefully packaged and transported to the Faculty of Medicine and Health Sciences Entomology Laboratory in Universiti Malaysia Sabah for identification process.

*Anopheles* mosquitoes were identified to the species level using the identification keys of Rattananarithikul et al., (2006) and Sallum et al. (2005). Species richness, along with species diversity, were assessed through the application of Simpson's Reciprocal Diversity Index (D), Shannon-Wiener Diversity Index (H), and Evenness (E). Statistical analysis was done using R programming language for statistical analysis (version 3.5.2). A generalized linear mixed-effect model (GLMM, with R glmmTMB package) was used to analyse mosquito abundance (*An. fragilis*, the most abundant species and total *Anopheles*) as a function of the dependent variables (VF, LRF and HRF), using day and site as random factors, and Poisson distribution as the underlying distribution. The numbers for the other species were too low and too zero inflated for meaningful analysis. In all analyses, different forest types were considered as a fixed effect. Month of sampling was fit alternatively as a fixed (to predict monthly values) or random effect (to test for differences between forest types while controlling for seasonal variation). Poisson distribution was used in the analysis of mosquito abundance.

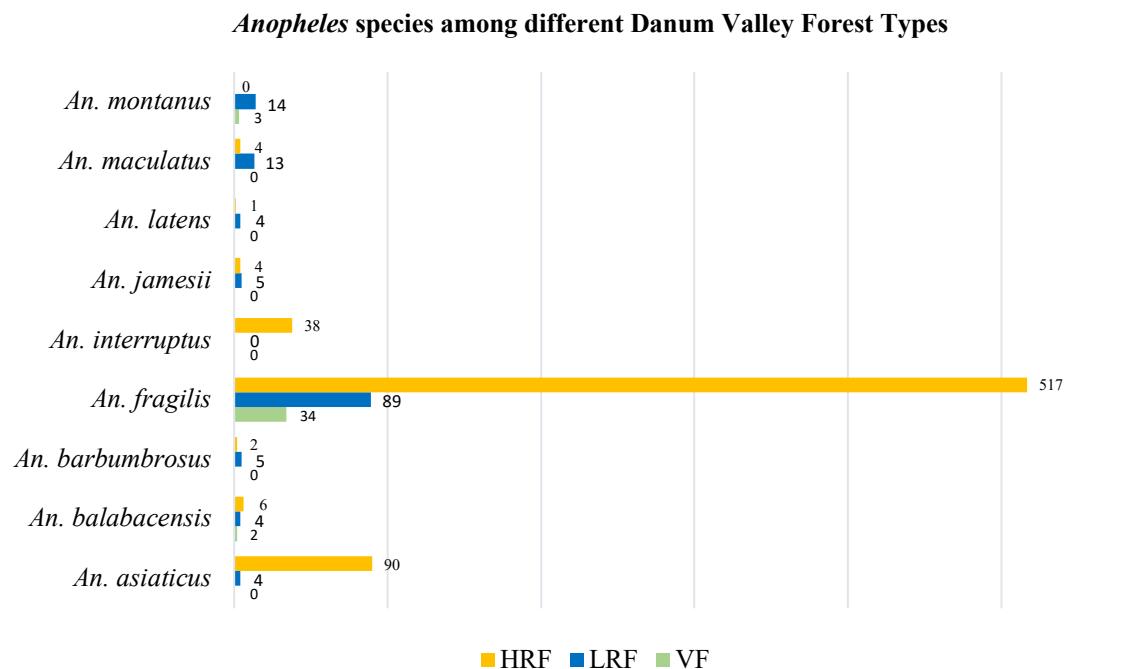
Models testing associations between response variables (mosquito abundance) explanatory variables (forest types and month of sampling) were assessed through comparison on the basis of having higher log-likelihood and lower Akaike information criterion (AIC) values. Tukey post hoc contrasts were used to differentiate the nature of statistical differences between forest types.

## RESULTS

A total of 839 female *Anopheles* mosquitoes belonging to nine different *Anopheles* species were collected from all sites (Fig. 3). The dominant species was *Anopheles fragilis* (76.28%), followed by *An. asiaticus* (11.20%), *An. interruptus* (4.53%), *An. maculatus* and *An. montanus* 2.03% respectively, *An. balabacensis* (1.43%), *An. jamesii* (1.07%), *An. barbumbrosus* (0.83%) and *An. latens* (0.60%). The total *Anopheles* recorded in HRF constituted 78.9% of all mosquitoes caught, while in VF it was only 4.65%. Mosquito traps were set up in the various sampling sites using similar amounts of the same baits. Thus, the catch should reflect mosquito abundance. It is unlikely that under sampling has occurred on one site and not another.

Three species of vectors, namely *Anopheles balabacensis*, *An. latens* and *An. maculatus* were recorded at LRF and HRF (Fig. 1). These vectors were collected in small numbers at LRF: *Anopheles balabacensis* (4), *An. latens* (4), and *An. maculatus* (13). The numbers of the same three species from HRF were 6, 1 and 4, respectively, while at VF, only two individuals of *An. balabacensis* were collected.

Higher numbers of *Anopheles* mosquito at HRF were caught in all three sampling dates in May, October 2019 and January 2020. The number of *Anopheles* mosquitoes trapped at VF was the lowest with 39 individuals. Furthermore, *An. balabacensis* was caught in all three sites whereas *An. latens* and *An. maculatus* were only caught in LRF and HRF (Fig. 3).



**Figure 3:** The number of individuals for each *Anopheles* sp. collected from different forest types. VF = virgin forest; LRF = low-ground regenerated forest; HRF = high-ground regenerated forest.

*Anopheles* species richness,  $S$  at HRF and LRF are eight and three species found in VF (Table 1). Both Simpson's and Shannon Wiener Diversity Indices were highest at LRF ( $D = 2.29$ ,  $H = 1.28$ ). HRF ( $D = 1.58$ ,  $H = 0.76$ ) and lowest at virgin forest ( $D = 1.31$ ,  $H = 0.47$ ) (Table 1). The three *Anopheles* species found at VF are *An. balabacensis*, *An. fragilis* and *An. montanus* (Fig. 3).

**Table 1:** *Anopheles* species richness and diversity indices by forest types. VF = virgin forest; LRF = low-ground regenerated forest; HRF = high-ground regenerated forest.

| Species richness and diversity Index | VF   | LRF  | HRF  |
|--------------------------------------|------|------|------|
| Species richness, $S$                | 3    | 8    | 8    |
| Simpson Reciprocal Index, $D$        | 1.31 | 2.29 | 1.58 |
| Shannon-Weiner Index, $H$            | 0.47 | 1.28 | 0.76 |
| Evenness, $E$                        | 0.43 | 0.62 | 0.36 |

Significant differences in the total number of *Anopheles* mosquitoes and the most abundant species, *An. fragilis* were detected for different forest types (Table 2). HRF had the highest

mean catch per trap per night ( $46.5 \pm 10.24$ ), but LRF had the lowest mean catch per trap per night ( $16.2 \pm 3.64$ ) although it had significantly higher predicted mean catch per trap per night than VF ( $22.6 \pm 5.03$ ) for total *Anopheles* mosquitoes. Similarly, significant differences were observed in mosquito catch across the months ( $P < 0.1$ ). January 2020 had a significantly higher number of total mosquitoes caught per trap per night ( $50.6 \pm 11.20$ ) than May 2019 ( $30.4 \pm 6.65$ ) and October 2019 ( $11.0 \pm 2.56$ ).

The dominant *Anopheles* species is *An. fragilis* (78.9% of all *Anopheles*) (Figure 3) and the lowest predicted mean catch per trap per night for *An. fragilis* at VF was ( $1.35 \pm 0.42$ ). A formal comparison of this dominant species in all three types of forest revealed this species has a significant preference in HRF with highest predicted mean per trap per night ( $20.55 \pm 5.34$ ) followed by LRF ( $3.54 \pm 0.98$ ) and VF ( $1.35 \pm 0.42$ ).

**Table 2:** Fitting GLMM models for total *Anopheles* and *An. fragilis* trapped at Danum Valley. The predicted means and 95% standard error were calculated from the model.

| Forest type               |                                | Predicted mean catch/trap/night | p-value for difference of 2 means | AIC    |
|---------------------------|--------------------------------|---------------------------------|-----------------------------------|--------|
| Total <i>Anopheles</i>    | High-ground regenerated forest | $26.96 \pm 6.75^a$              | a-b <0.0001                       | 424.81 |
|                           | Low-ground regenerated forest  | $5.62 \pm 1.47^b$               | a-c <0.0001                       |        |
|                           | Virgin forest                  | $1.59 \pm 0.47^c$               | b-c <0.0001                       |        |
| <i>Anopheles fragilis</i> | High-ground regenerated forest | $20.55 \pm 5.34^a$              | a-b <0.0001                       | 397.92 |
|                           | Low-ground regenerated forest  | $3.54 \pm 0.98^b$               | a-c <0.0001                       |        |
|                           | Virgin forest                  | $1.35 \pm 0.42^c$               | b-c =0.001                        |        |

## DISCUSSION

### *Anopheles* mosquitoes in Danum Valley

This study presents initial findings regarding the species composition and diversity of *Anopheles* species across different forest types within Danum Valley. To our knowledge, this is the first study conducted in Danum Valley concerning the diversity of *Anopheles* mosquitoes. Our analysis showed that the highest diversity of *Anopheles* mosquitoes was found in HRF whereas VF has the lowest diversity of *Anopheles* mosquitoes. Only three species of *Anopheles* mosquitoes were recorded from VF. These suggest that human disruptive activities in regenerated forests gives rise to more mosquito species than that of an undisturbed forest. The Intermediate Disturbance Hypothesis (IDH) provides an explanation for the role of disturbance coexistence of climax and colonist species (Loaiza et al., 2017). Assuming that, in undisturbed VF, disturbance-intolerant species (climax species) tend to monopolise resources (e.g., space and food sources), driving less competitive species to local extinction and reducing overall species diversity.

In our study *An. fragilis* is the predominant species found in all forest types and the highest number caught was in HRF despite being a more disturbed area. According to Reid (1965),

*An. fragilis* (Aitkeni group) is known to be distributed on lowland in Malaya, Indonesia, Borneo and the Philippines. The Aitkeni group has not been found to be involved in disease transmission yet, but more information is needed on their biology and ecology. Forest land alteration at HRF environment seems to be more favourable to *An. fragilis* than LRF and VF. There is limited knowledge on why *An. fragilis* is found in abundance in HRF. However, the current knowledge by Byrne et al. (2021) shows that land use change is creating more suitable habitats for *Anopheles* vector larvae and this may be contributing to the abundance of *An. fragilis* in the regenerated forest of Danum Valley. The forest floor of the virgin forest tends to be shaded and covered with thick leaf litter and acidic water environments whereas logged lands tend to have sunlit neutral water environments (Brant, 2011). Logged lands are also prone to form temporary breeding puddles. Habitat requirements vary across different mosquito species and this gives a succession of vector species through different forest types (Patz et al., 2000; Norris, 2004). Landscape changes strongly provide significant affect to microclimate of a habitat, such as temperature, runoff, evapotranspiration (Patz & Olson, 2006). Hence, these factors are key in determining the abundance, survivorship and diversity of *Anopheles* mosquitoes (Patz & Olson, 2006).

In May and January, higher numbers of mosquitoes were collected because these months coincide with Sabah's two main rainy seasons (December to February and May to July). The increase in mosquito number could be due to more temporary breeding grounds for mosquitoes such as road puddles, rain pools, animal prints in the forest, potholes and tyre prints in human settlements, resulting from the rain (Hamza & Rayah, 2016; Hawkes et al., 2019). The number of trap nights for the first sampling trip at the DVCA was longer compared to the other two sampling trips in October and January. Three MMT were used in the first and third sampling trips which may be one of the reasons why the number of *Anopheles* mosquitoes was the highest in the month of May.

In October, the number of *Anopheles* mosquitoes collected was the lowest; a possible reason may be due to less rain as compared with May and January. The fruiting season in Danum Valley roughly corresponds to the period from the end of the rainy to the dry season, and the period at the beginning of the rainy season (Tomoko et al., 2010). The abundance and richness of mosquitoes highly depends on biotic factors (oviposition, presence of hosts for food and plants as shelter) and to abiotic factors (relative humidity, seasonality and temperature) (Abella-Medrano et al., 2020). *Plasmodium knowlesi* has been shown to be very infectious, with rhesus monkeys (*Macaca mulatta*) developing blood stage infections if one or more *P. knowlesi*-infected female *Anopheles* mosquitoes successfully feed on them (Murphy et al., 2014). Our temporal variation data to show the correlation between rainforest types and the effect on mosquito abundance is still lacking. There are other limitations such as difficulties in identifying microscopically incomplete or damaged mosquitoes resulting from over handling and transportation of fragile mosquitoes from DVCA to the UMS faculty laboratory, and loss of characters in specimens stored over an extended period of time. There was also limited equipment for the molecular screening of parasites in the mosquitoes.

### **Relationship between *Anopheles* mosquitoes and forest types**

GLMM model (Table 2) fitting showed that in general, the highest number of mosquitoes caught were from the high-ground regenerated forest. The INFAPRO Tower was built around HRF for the purpose of tourism and meteorological study station for scientists. The construction of a gravel road up to the tower and the clearing of trees to build the tower had changed the environment of the ecosystem. Other alterations of the forested environment include logging of the rain forest with notably abandoned logging tracks, gullies, heavily



disturbed or compacted areas causing detrimental effects on the terrain components and leading to soil erosion (Clarke & Walsh, 2006). Landscape features largely control the ability of female vectors to spread from their breeding sites to hosts, increases host-vector contacts (Raffy & Tran, 2005). Land cover alterations, such as deforestation and agricultural expansion had been associated with altered dynamics and geographical distribution of malaria and other vector borne diseases (Lambin et al., 2010) caused by *P. knowlesi* in Sabah.

### **Forest habitats and the linkage of emerging diseases**

Some of the *Anopheles* species caught are confirmed vectors of zoonotic malaria. In Sabah, *An. balabacensis* (Wong et al., 2015; Ang et al., 2020) and *An. latens* (Vythilingam et al., 2006) are *P. knowlesi* malaria vectors belonging to the Leucosphyrus group. *Anopheles maculatus* is a vector of human malaria in Peninsular Malaysia (Vythilingam et al., 1995). The majority of these *Anopheles* vectors are found in LRF and HRF in the DVCA where there was a degree of man-initiated disturbances to the forest. People will have a greater risk of exposure to zoonotic diseases when living at the forest fringe and working or entering the forest, reason being their proximity with the malaria parasite reservoir monkeys and the *Anopheles* vectors (Singh & Daneshvar, 2013; Anstey & Grigg, 2019). Our findings are consistent with recent findings where *An. balabacensis* were found to increase in abundance in disturbed forests (Brant et al., 2016; Chua et al., 2019; Wong et al., 2015).

*Plasmodium knowlesi* is responsible for approximately 70% of the reported malaria cases affecting humans in Malaysian Borneo (William et al., 2013). This could be due to the vector *An. balabacensis* which transmits the malaria parasite, being frequently found in disturbed forest habitats of regenerated forests as a result of human activities involving land-use changes. Such alterations create a pathway for both macaque hosts and highly adaptable vectors to migrate closer to human settlements, increasing interactions between man and mosquitoes. At night, when female mosquitoes generally feed, most of the hosts like humans and animals are immobile (Lambin et al., 2010). Logging and deforestation create a strong bridge, in which it increases interactions between humans, mosquito vectors and the macaque hosts of *P. knowlesi*. Evidence suggests that agricultural expansion and forest fragmentation affect *P. knowlesi* exposure, which supports linkages between land use change and *P. knowlesi* transmission (Fornace et al., 2019).

Factors that influence the diversity, abundance and range of host and *Anopheles* vector species are socio ecological processes such as man-made land use change, globalization of agricultural settings, population growth, and climate change (Davidson et al., 2019). The effect of land cover changes according to Fornace et al. (2019) are associated with the increased risks of *Plasmodium knowlesi* exposure and disease transmission. Alterations of landscapes have massive potential to threaten any future malaria eradication efforts (Fornace et al., 2021). Our findings indicate that land use alteration creates more favourable habitats for *Anopheles* vector larvae, thus contributing to more diverse *Anopheles* mosquitoes in the regenerated forest of Danum Valley. Byrne et al. (2021) working elsewhere in Sabah had similar finding.”

## **CONCLUSIONS**

This study focused on mosquito diversity within different forest types of Danum Valley and their medical importance. We believe this study has provided significant baseline data on vector mosquitoes within the pristine forest of Sabah that can be used for future research directions. The findings will be useful to plan a strategic vector control programme for villagers

staying near forest fringes. More research is needed to gather data on the effects of wind, rainfall and feeding time of mosquitoes for effective fogging treatments for *Anopheles* in Sabah. Molecular work for pathogen screening will reveal more about the *Plasmodium* parasite species and uncover newly emergent malaria parasites carried by the *Anopheles* mosquitoes found in the pristine rainforest of Borneo

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## REFERENCES

- Abella-Medrano CA, Roiz D, Islas CGR, Salazar-Juárez CL, Ojeda-Flores R. (2020) Assemblage variation of mosquitoes (Diptera: Culicidae) in different land use and activity periods within a lowland tropical forest matrix in Campeche, Mexico. *Journal of Vector Ecology*, 45(2): 188–196. <https://doi.org/10.1111/jvec.12389>.
- Ang JXD, Kadir KA, Mohamad DSA, Matusop A, Divis PCS, Yaman K, Singh B (2020) New vectors in northern Sarawak, Malaysian Borneo, for the zoonotic malaria parasite, *Plasmodium knowlesi*. *Parasites and Vectors*, 13(1): 1–13. <https://doi.org/10.1186/s13071-020-04345-2>.
- Anstey NM, Grigg MJ (2019) Zoonotic malaria: the better you look, the more you find. *Journal of Infectious Diseases*, 219(5): 679–681. <https://doi.org/10.1093/infdis/jiy520>.
- Ashton PS, Givnish TJ, Appanah S (1988) Staggered flowering in the Dipterocarpaceae: New insights into floral induction and the evolution of mast fruiting in the aseasonal tropics. *American Naturalist*, 132(1): 44–66. <https://doi.org/10.1086/284837>.
- Brant HL (2011) Changes in abundance, diversity and community composition of mosquitoes based on different land use in Sabah, Malaysia. MSc Thesis. Imperial College, London. 58 pp
- Brant HL, Ewers RM, Vythilingam I, Drakeley C, Benedick S, Mumford JD (2016) Vertical stratification of adult mosquitoes (Diptera: Culicidae) within a tropical rainforest in Sabah, Malaysia. *Malaria Journal*, 15(1): 1–9. <https://doi.org/10.1186/s12936-016-1416-1>.
- Byrne I, Aure W, Manin BO, Vythilingam I (2021) Environmental and spatial risk factors for the larval habitats of *Plasmodium knowlesi* vectors in Sabah, Malaysian Borneo. *Scientific Reports*, 1–11. <https://doi.org/10.1038/s41598-021-90893-1>.
- Chaiphongpachara T, Padidpoo O, Chansukh KK, Sumruayphol S (2018) Efficacies of five edible mushroom extracts as odor baits for resting boxes to attract mosquito vectors: A field study in Samut Songkhram Province, Thailand. *Tropical Biomedicine*, 35(3): 653–663.
- Chua TH, Manin BO, Vythilingam I, Fornace K, Drakeley CJ (2019) Effect of different habitat types on abundance and biting times of *Anopheles balabacensis* Baisas (Diptera: Culicidae) in Kudat district of Sabah, Malaysia. *Parasites & Vectors*, 12(1): 364. <https://doi.org/10.1186/s13071-019-3627-0>.
- Clarke MA, Walsh RPD (2006) Long-term erosion and surface roughness change of rain-forest terrain following selective logging, Danum Valley, Sabah, Malaysia. *Catena*, 68(2–3): 109–123. <https://doi.org/10.1016/j.catena.2006.04.002>.
- Cooper DJ, Rajahram GS, William T, Jelip J, Mohammad R, Benedict J, Grigg MJ, William T, Yeo TW, Drakeley CJ, Ta TM, Barber BE (2019) *Plasmodium knowlesi* malaria in Sabah, Malaysia, 2015–2017: Ongoing increase in incidence despite near-elimination of the human-only *Plasmodium* Species. *Clinical Infectious Diseases*, 70(3): 1–7. <https://doi.org/10.1093/cid/ciz237>.
- Davidson G, Chua TH, Cook A, Speldewinde P, Weinstein P (2019) Defining the ecological and evolutionary drivers of *Plasmodium knowlesi* transmission within a multi-scale framework. *Malaria Journal*, 18(1): 1–13. <https://doi.org/10.1186/s12936-019-2693-2>.
- Fornace KM, Abidin TR, Alexander N, Brock P, Grigg MJ, Murphy A, William T, Menon J, Drakeley CJ, Cox J (2016) Association between landscape factors and spatial patterns of *Plasmodium knowlesi* infections in Sabah, Malaysia. *Emerging Infectious Diseases*, 22(2): 201–208. <https://doi.org/10.1097/00004770-200106000-00017>.
- Fornace KM, Brock PM, Abidin TR, Grignard L, Herman LS, Chua TH, Daim S, William T, Patterson CLEB, Hall T, Grigg MJ, Anstey NM, Tetteh KKA, Cox J, Drakeley CJ

- (2019) Environmental risk factors and exposure to the zoonotic malaria parasite *Plasmodium knowlesi* across northern Sabah, Malaysia: A population-based cross-sectional survey. *The Lancet Planetary Health*, 3(4): e179–e186. [https://doi.org/10.1016/S2542-5196\(19\)30045-2](https://doi.org/10.1016/S2542-5196(19)30045-2).
- Fornace KM, Diaz AV, Lines J, Drakeley CJ (2021) Achieving global malaria eradication in changing landscapes. *Malaria Journal*, 1–15. <https://doi.org/10.1186/s12936-021-03599-0>.
- Gunggut H, Saufi DSNSAM, Zaaba Z, Liu MSM (2014) Where have all the forests gone? deforestation in land below the wind. *Procedia - Social and Behavioral Sciences*, 153: 363–369. <https://doi.org/10.1016/j.sbspro.2014.10.069>.
- Hamza AM, Rayah EAEl (2016) A qualitative evidence of the breeding sites of *Anopheles arabiensis* Patton (Diptera: Culicidae) in and around Kassala Town, Eastern Sudan. *International Journal of Insect Science*, 8, IJIS.S40071. <https://doi.org/10.4137/ijis.s40071>.
- Hawkes FM, Manin BO, Cooper A, Daim S, Homathevi R, Jelip J, Tanrang H, Chua TH (2019) Vector compositions change across forested to deforested ecotones in emerging areas of zoonotic malaria transmission in Malaysia. *Scientific Reports*, 9: 13312. <https://doi.org/10.1038/s41598-019-49842-2>
- Hii JLK, Kan S, Vun YS, Chin KF, Lye MS, Mak JW, Cheong WH (1985) *Anopheles flavirostris* incriminated as a vector of malaria and Bancroftian filariasis in Banggi Island, Sabah, Malaysia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 79(5): 677–680. [https://doi.org/10.1016/0035-9203\(85\)90189-0](https://doi.org/10.1016/0035-9203(85)90189-0).
- Hussin N, Lim YAL, Goh PP, William T, Jelip J, Mudin RN (2020) Updates on malaria incidence and profile in Malaysia from 2013 to 2017. *Malaria Journal*, 19(1): 1–14. <https://doi.org/10.1186/s12936-020-3135-x>.
- Hutchings RSG, Sallum MAM, Hutchings RW (2011) Mosquito (Diptera: Culicidae) diversity of a forest-fragment mosaic in the Amazon rain forest. *Journal of Medical Entomology*, 48(2): 173–187. <https://doi.org/10.1603/ME10061>.
- Kweka EJ, Kimaro EE, Munga S (2016) Effect of deforestation and land use changes on mosquito productivity and development in Western Kenya Highlands: Implication for malaria risk. *Frontiers in Public Health*, 4: 238. <https://doi.org/10.3389/FPUBH.2016.00238>.
- Lambin EF, Tran A, Vanwambeke SO, Linard C, Soti V (2010) Pathogenic landscapes: interactions between land, people, disease vectors, and their animal hosts. *International Journal of Health Geographics*, 9(5): 54. <https://doi.org/10.1186/1476-072X-9-54>.
- Loaiza JR, Dutari LC, Rovira JR, Sanjur OI, Laporta GZ, Pecor J, Foley DH, Eastwood G, Kramer LD, Radtke M, Pongsiri M (2017) Disturbance and mosquito diversity in the lowland tropical rainforest of central Panama. *Scientific Reports*, 7(1): 1–13. <https://doi.org/10.1038/s41598-017-07476-2>.
- Marsh CW, Greer AG (1992) Forest land-use in Sabah, Malaysia: An introduction to Danum Valley. *Philosophical Transactions - Royal Society of London, B*, 335(1275): 331–339. <https://doi.org/10.1098/rstb.1992.0025>.
- Murphy JR, Weiss WR, Fryauff D, Dowler M, Savransky T, Stoyanov C, Muratova O, Lambert L, Orr-Gonzalez S, Zeleski KL, Hinderer J, Fay MP, Joshi G, Gwadz RW, Richie TL, Villasante EF, Richardson JH, Duffy PE, Chen J (2014) Using infective mosquitoes to challenge monkeys with *Plasmodium knowlesi* in malaria vaccine studies. *Malaria Journal*, 13(1): 1–11. <https://doi.org/10.1186/1475-2875-13-215>.
- Norris DE (2004) Mosquito-borne diseases as a consequence of land use change. *EcoHealth*, 1(1): 19–24. <https://doi.org/10.1007/s10393-004-0008-7>.
- Overgaard HJ, Ekbohm B, Suwonkerd W, Takagi M (2003) Effect of landscape structure on

- Anopheles* mosquito density and diversity in northern Thailand: Implications for malaria transmission and control. *Landscape Ecology*, 18(6): 605–619. <https://doi.org/10.1023/A:1026074910038>.
- Patz JA, Graczyk TK, Geller N, Vittor AY (2000) Environmental changes & parasitic diseases. *International Journal for Parasitology*, 30(12–13): 1395–1405. [https://doi.org/10.1016/S0020-7519\(00\)00141-7](https://doi.org/10.1016/S0020-7519(00)00141-7)
- Patz JA, Olson SH (2006) Malaria risk and temperature: Influences from global climate change and local land use practices. *Proceedings of the National Academy of Sciences of the United States of America*, 103(15): 5635–5636. <https://doi.org/10.1073/pnas.0601493103>.
- Raffy M, Tran A (2005) On the dynamics of flying insects populations controlled by large scale information. *Theoretical Population Biology*, 68(2): 91–104. <https://doi.org/10.1016/j.tpb.2005.03.005>.
- Rattanaarithikul R, Harrison BA, Harbach RE, Panthusiri P, Coleman RE (2006) Illustrated keys to the mosquitoes of Thailand IV. *Anopheles*. *Southeast Asian Journal of Tropical Medicine and Public Health*, 37(SUPPL. 2): 1–26.
- Reid JA (1965) A revision of the *Anopheles aitkenii* group in Malaya and Borneo. *Annals of Tropical Medicine & Parasitology*, 59(1): 106–125. <https://doi.org/10.1080/00034983.1965.11686289>.
- Reynolds G, Payne J, Sinun W, Mosigil G, Walsh RPD (2011) Changes in forest land use and management in Sabah, Malaysian Borneo, 1990-2010, with a focus on the Danum Valley region. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366(1582): 3168–3176. <https://doi.org/10.1098/rstb.2011.0154>.
- Sallum MAM, Peyton EL, Harrison BA, Wilkerson RC (2005) Revision of the Leucosphyrus group of *Anopheles* (Cellia) (Diptera, Culicidae). *Revista Brasileira de Entomologia*, 49(Supplement 1): 1–152. <https://doi.org/10.1590/S0085-56262005000500001>.
- Sant'Ana DC, de Sá ILR, Sallum MAM (2014) Effectiveness of Mosquito Magnet® Trap in rural areas in the southeastern tropical Atlantic Forest. *Memorias Do Instituto Oswaldo Cruz*, 109(8): 1021–1029. <https://doi.org/10.1590/0074-02761400297>.
- Singh B, Daneshvar C (2013) Human infections and detection of *Plasmodium knowlesi*. *Clinical Microbiology Reviews*, 26(2): 165–184. <https://doi.org/10.1128/CMR.00079-12>.
- Steib BM, Geier M, Boeckh J (2001) The effect of lactic acid on odour-related host preference of yellow fever mosquitoes. *Chemical Senses*, 26(5): 523–528. <https://doi.org/10.1093/chemse/26.5.523>.
- Tan CH, Vythilingam I, Matusop A, Chan ST, Singh B (2008) Bionomics of *Anopheles latens* in Kapit, Sarawak, Malaysian Borneo in relation to the transmission of zoonotic simian malaria parasite *Plasmodium knowlesi*. *Malaria Journal*, 7: 1–8. <https://doi.org/10.1186/1475-2875-7-52>.
- Tomoko K, Kuze N, Bernard H, Malim TP, Kohshima S (2010) Feeding ecology of Bornean Orangutans (*Pongo pygmaeus morio*) in Danum Valley, Sabah, Malaysia: A 3-year record including two mast fruitings. *American Journal of Primatology*, 72(9): 820–840. <https://doi.org/10.1002/ajp.20848>.
- Vanwambeke SO, Lambin EF, Eichhorn MP, Flasse SP, Harbach RE, Oskam L, Somboon P, Van Beers S, Van Benthem BHB, Walton C, Butlin RK (2007) Impact of land-use change on dengue and malaria in northern Thailand. *EcoHealth*, 4(1): 37–51. <https://doi.org/10.1007/s10393-007-0085-5>.
- Vythilingam I, Foo LC, Chiang GL, Chan ST, Eng KL, Mahadevan S, Mak JW, Singh KI (1995) The impact of permethrin impregnated bednets on the malaria vector *Anopheles maculatus* (Diptera: Culicidae) in aboriginal villages of Pos Betau Pahang, Malaysia.

- The Southeast Asian Journal of Tropical Medicine and Public Health, 26(2): 354–358.
- Vythilingam I, Tan CH, Asmad M, Chan ST, Lee KS, Singh B (2006) Natural transmission of *Plasmodium knowlesi* to humans by *Anopheles latens* in Sarawak, Malaysia. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100(11): 1087–1088. <https://doi.org/10.1016/j.trstmh.2006.02.006>.
- Vythilingam I, Noorazian YM, Huat TC, Jiram AI, Yusri YM, Azahari AH, NorParina I, NoorRain A, Lokmanhakim S (2008) *Plasmodium knowlesi* in humans, macaques and mosquitoes in Peninsular Malaysia. Parasites and Vectors, 1(1): 1–10. <https://doi.org/10.1186/1756-3305-1-26>.
- Walsh RPD, Newbery DM (1999) The ecoclimatology of Danum, Sabah, in the context of the world's rainforest regions, with particular reference to dry periods and their impact. Philosophical Transactions of the Royal Society B: Biological Sciences, 354(1391): 1869–1883. <https://doi.org/10.1098/rstb.1999.0528>.
- WHO (2015) Global technical strategy for malaria 2016-2030, 2021 update. Geneva: World Health Organization; 2021. Licence: CC BY-NC-SA 3.0 IGO. United Kingdom. 32 pp.
- WHO (2018) World Malaria Report 2018. Geneva: World Health Organization; 2018. Geneva: World Health Organization; 2018. Licence: CC BY-NC-SA 3.0 IGO. Luxembourg. 210 pp.
- William T, Rahman HA, Jelip J, Ibrahim MY, Menon J, Grigg MJ, Yeo TW, Anstey NM, Barber BE (2013) Increasing incidence of *Plasmodium knowlesi* malaria following control of *P. falciparum* and *P. vivax* malaria in Sabah, Malaysia. PLoS Neglected Tropical Diseases, 7(1): e2026. <https://doi.org/10.1371/journal.pntd.0002026>.
- Wong ML, Chua TH, Leong CS, Khaw LT, Fornace K, Wan-Sulaiman WY, Vythilingam I (2015) Seasonal and spatial dynamics of the primary vector of *Plasmodium knowlesi* within a major transmission focus in Sabah, Malaysia. PLoS Neglected Tropical Diseases, 9(10): e0004135. <https://doi.org/10.1371/journal.pntd.0004135>.