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Research Article

Mosquito Diversity between Logged and Unlogged Forest Areas in Kalabakan Forest Reserve, Sabah

Mohammad Imran bin Ebrahim, Mahadimenakbar Mohamed Dawood*

Institute of Tropical Biology and Conservation, University Malaysia Sabah, 88999, Kota Kinabalu, Sabah, Malaysia.
*Corresponding author: menakbar@ums.edu.my

Abstracts

Mosquitoes were sampled in an undisturbed area within the trail in the forest near the Maliau Basin Conservation Area (MBCA) and in a disturbed area within the Logged Forest Experimental (LFE) area near the SAFE Project camp site. A total of 48 days of sampling was done in both areas in a bi-monthly sampling starting June 2016 to April 2017. The aims of this study were to investigate the species diversity and peak biting hours of mosquitoes in both sites. A total of 807 individuals from 17 species were caught using manual collection method of Human Landing Catch (HLC). 15 species were collected in MBCA while only 9 species were collected in LFE. Based on Generalized Linear Mixed Model (GLMM), there was a significant difference of mosquito abundance between LFE and MBCA ($p<0.05$) and mosquito day biting time ($p<0.05$). Also, based on Independent T-test analysis, there was a significance difference in terms of mosquito diversity level and abundance ($p<0.05$). In this study, LFE had higher mosquito abundance with a total of 563 mosquito individuals caught compared to MBCA with 244 mosquito individuals. In both areas, more species were recorded during day time samplings than night time samplings. *Anopheles balabacensis*, *Aedes albopictus*, *Heizmannia scintillans* and *Culex vishnui* were among the predominant species collected in LFE while in MBCA species collected were *Heizmannia scintillans*, *Anopheles umbrosus*, *Aedes albopictus* and *Armigeres jugraensis*. LFE had peak biting hours around 2:00 p.m., 5:00 p.m., 7.00 p.m. and 9:00 p.m. while for MBCA, the peak biting hours were between 2:00 - 3:00 p.m. and 6.00 p.m.

Keywords: mosquito, Maliau Basin, diversity, logged and unlogged forest, HLC

Introduction

Mosquitoes are classified under family Culicidae in order Diptera. They are mostly found in moderate climate and tropic regions. There are currently about 42 genera and around 3,500 mosquito species that can be found in the world (Service, 2008; Rueda 2008; Harbach & Besansky, 2014).

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Even though there about 3,500 named mosquito species, only a number of this very diverse family are considered medically important and bring nuisance to humans (Fang, 2010). In Malaysia particularly, there are about four medically important mosquito genera which are *Aedes*, *Anopheles*, *Culex* and *Mansonia* (Rahman et al., 1997). Mosquitoes also serve important functions in numerous ecosystems they live in (Fang, 2010). However, mosquito importance is mainly due to its medical reasons towards human health as it has capability in becoming a vector for several dangerous pathogens to humans (Foottit & Adler, 2009). The diseases that are commonly transmitted by these small vectors are malaria, dengue fever, yellow fever, chikungunya, filariasis (Service, 2008) and recently Zika virus (Nhan & Musso, 2015).

The environment plays a major role in affecting mosquito diversity and abundance. Mosquito communities may change across a landscape including when there are changes in habitat which can affect species relative abundance and the invasion of new species (Thongsripong et al., 2013). A changing environment possesses negative impact to human health especially through the transmission of disease especially by a vector mosquito (Vanwambeke et al., 2007).

This study was conducted to determine and compare adult mosquito species diversity and biting activity between disturbed areas within the Logged Forest Experimental (LFE) area near the SAFE Project camp site and undisturbed primary forest in Maliau Basin Conservation Area (MBCA). The objectives of this study were; (a) to investigate the mosquito species that are present in disturbed and undisturbed site; (b) to compare the diversity and abundance in disturbed and undisturbed sites; and (c) to determine the peak biting time of mosquito between disturbed and undisturbed sites. Although previously several mosquito studies had been conducted in the SAFE Project site (Brant et al. 2011; Brant et al., 2016), this study is hopefully able to complement existing data as well as provide new information on mosquito diversity at Kalabakan Forest reserve area.

Materials and Methods

This study was carried out in the 'Stability of Altered Forest Ecosystems' (SAFE) Project area which includes the Kalabakan Forest Reserve at Benta Wawasan and Maliau Basin Conservation Area (Figure 1). SAFE Project is considered as one of the largest ecological study sites in the world. The SAFE Project has been recognized as a Class II Forest Reserve except for the Virgin Jungle Reserve (VJR) which is Class IV Forest Reserve while Maliau Basin Conservation Area is a Class I Forest Reserve (Chung et al., 2010). Maliau Basin Conservation Area is fully protected and has a very broad primary forest area which has never been logged (Hardwick et al., 2015). Samplings were divided into two types of areas; disturbed and undisturbed. The disturbed area was within the Logged Forest Experimental (LFE) area near the SAFE Project camp site while the undisturbed area was within the trail in the forest near the Maliau Basin Conservation Area (MBCA).

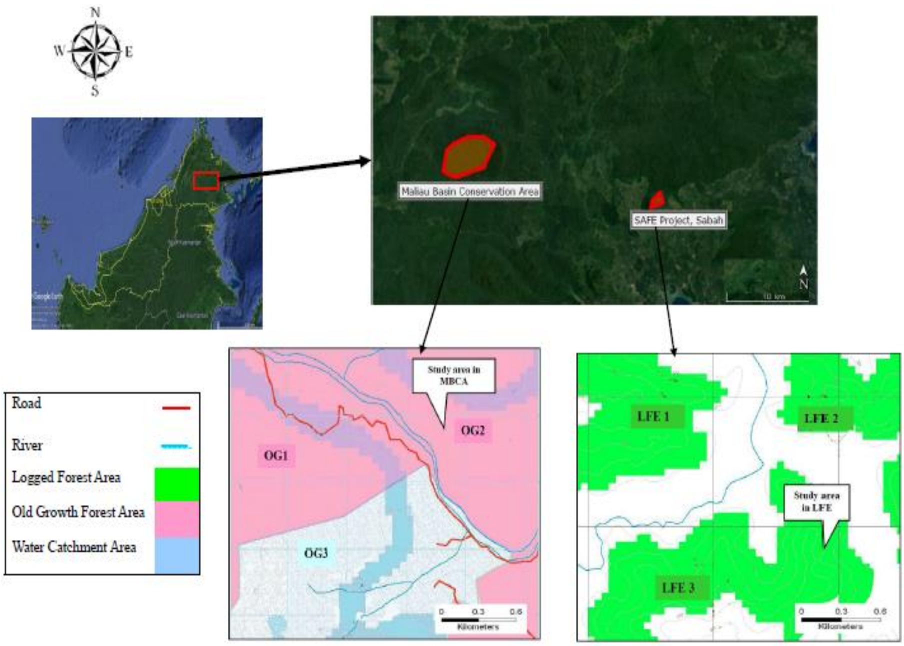

Figure 1 : Location of the study area
(Source: i) Google Earth ii) http://www.safeproject.net/concept/maps/)

Adult mosquitoes were collected using human landing catches (HLC). Anti-malaria pills were taken one week before the sampling started. In this method, legs and hands of the collector were exposed in order to attract the mosquitoes. Mosquitoes that landed on or bit the exposed skin were caught using a vial or Eppendorf tubes. The samplings were divided into two different time frames; day-time and night-time samplings. Each time frame was conducted for two days. Altogether, a total of 48 days of sampling efforts was made in both areas through bi-monthly sampling starting in June 2016 until April 2017. The day-time sampling was conducted from 6 a.m. until 6 p.m. while the night-time sampling was carried out from 6 p.m. until 12 a.m. The purpose of a 12-hours day time mosquito collection in this study was to determine the peak biting time of mosquitoes since different mosquitoes have different peak biting periods (Varnado et al., 2012). Due to logistics and safety issues, night-time sampling usually ended at 12 a.m. Collected mosquitoes were killed using ethyl acetate solution. Mounted specimens were dried for three days in a drying oven. Later, all of the specimens were stored in the specimen box. All mosquitoes were identified using a compound microscope and were identified to genus and up to species level using the Southeast Asia identification key by Ratanarathikul et al., (2005a,b; 2006a,b), Stojanovich & Scott (1966) and Reid (1968). Mosquitoes that could not be identified to species level were written as "sp" after their genus name and if the unidentified species belong to the same genus, a number was written at the end of the 'sp' to represent the species name.

In this study, Generalized Linear Mixed Models were used to detect mosquito abundance differences using sites, sampling time frame (day-time and night-time), month of sampling, average temperature and average humidity of each month in each site as factors. Shannon-Weiner diversity index value was also used to get the diversity value of mosquitoes caught in each site. Then, independent T-test using Statistical Package for Social Science (SPSS) Version 20 was used to compare the mosquito diversity and abundance between the disturbed and undisturbed areas. For peak biting hour record, the numbers of mosquitoes caught were recorded according to the hour of the collection in order to sort and determine the peak biting hour.

Results

A total of 807 mosquito individuals representing 7 genera and up to 17 species were collected using the HCL technique. Figure 2 shows the species of mosquitoes collected. In LFE, the most collected species were *Anopheles balabacensis*, *Aedes albopictus*, *Heizmannia scintillans* and *Culex vishnui* while in MBCA the species were *Heizmannia scintillans*, *Anopheles umbrosus* and *Armigeres jugraensis*. *Aedes albopictus*, *Anopheles balabacensis*, *Heizmannia scintillans* were among the species found in both areas. Fifteen species were recorded in the undisturbed area compared to 9 species in the disturbed area (LFE). Table 1 shows the list of mosquito species caught during day and night sampling in the two study sites.

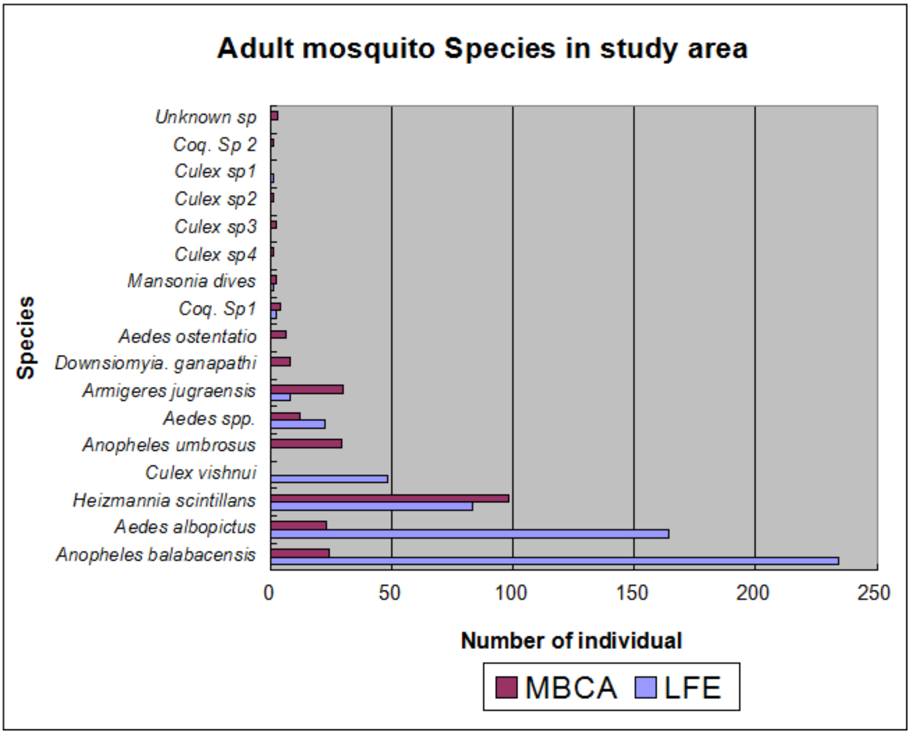

Figure 2: Mosquito species in study sites

Table 1. List of mosquito species caught according to day or night sampling in LFE and MBCA

LFE (Logged Forest)		MBCA (Primary Forest)	
Day	**Night**	**Day**	**Night**
Aedes albopictus	*Culex vishnui*	*Anopheles umbrosus*	*Aedes albopictus*
Aedes spp.	*Aedes albopictus*	*Armigeres jugraensis*	*Anopheles balabacensis*
Coquillettidia sp 1	*Anopheles balabacensis*	*Aedes ostentatio*	*Anopheles umbrosus*
Culex sp1		*Downsiomyia ganapathi*	*Coquillettidia sp2*
Armigeres jugraensis		*Heizmannia scintillans*	*Mansonia dives*
Heizmannia scintillans		*Aedes albopictus*	*Unknown sp.*
Mansonia dives		*Aedes spp*	
		Coquillettidia sp1	
		Culex sp2	
		Culex sp3	
		Culex sp4	

Table 2. Effect of Parameter on Mosquito Abundance using Generalized Linear Mixed Model

Model Term	Coefficient	Std. error	t	Sig.	95% Confidence Interval	
					Lower	Upper
Intercept	427.142	316.448	1.350	.199	-251.570	1,105.855
Site=1	26.620	8.821	3.018	.009*	7.702	45.539
Site=2	0[a]					
Month=1	33.770	16.081	2.100	.054	-0.719	68.260
Month=2	10.744	18.591	0.578	.572	-29.129	50.618
Month=3	3.625	15.176	0.239	.815	-28.924	36.174
Month=4	-1.466	14.857	-0.099	.923	-33.331	30.398
Month=5	-10.178	17.975	-0.566	.580	-48.732	28.375
Month=6	0[a]					
Time=1	39.578	18.259	2.168	.048*	0.416	78.741
Time=2	0[a]					

Probability distribution:Normal, Link Function: Identity
a Coefficient is set to zero because it is redundant
*Significant value <0.05

Mosquito abundance was significantly associated with site factor in this study between LFE and MBCA (GLMM, Site=1, $p<0.05$) (Table 2). Mosquito abundance was much higher in LFE (disturbed site) rather than in MBCA (undisturbed area). Also, day biting activity collection was significantly different than night biting collection (GLMM,Time=1, $p<0.05$). From the biting activity graph (Figure 4 & Figure 5) we can see that the mosquito biting activity was significantly active during the day time compared to night time.

Based on Independent T-test analysis, there was a significant difference between mosquito diversity value in disturbed area and undisturbed area (t(10)= -2.88 p=0.017, d=0.19, 95% [-0.96, -0.12]. The mean for disturbed area (M=1.07 SD=0.34) was significantly different compared to the undisturbed area (M=1.61 SD=0.32). These results show that mosquito diversity was much higher in the undisturbed area than in the disturbed area. Using the same analysis, mosquito abundance was also significantly different in disturbed and undisturbed areas (t(22)=2.8 p=0.01, d=8.8, 95% [6.43, 43.07]. The mean for disturbed area (M=45.75 SD=26.22) was significantly different to the undisturbed area (M=21.0 SD=15.79). These results show that mosquito abundance was much higher in the disturbed area than in the undisturbed area.

Figure 3 shows the graph for bi-monthly mosquito collection data. Based on Figure 3, the highest collection was recorded in June 2016 while the lowest collection made was recorded in August 2016 where the total collection was only 80 individuals.

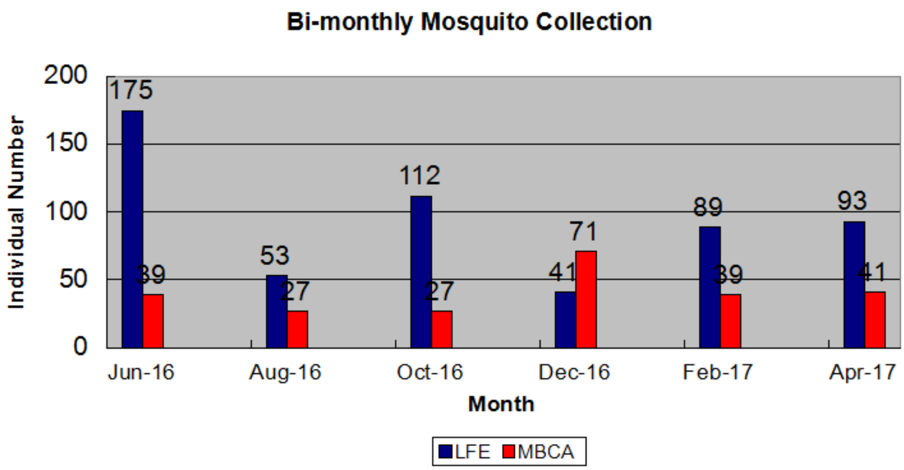

Figure 3. Mosquito Bi-monthly Collection

Table 3. Mean temperature and relative humidity for day and night sampling in study site.

Site	Mean temperature (°C)		Mean relative humidity (%)	
	Day	Night	Day	Night
LFE	26.9	23.5	84.4	96.6
MBCA	25.9	24.2	88.9	97.1

Table 3 shows the total mean temperature and relative humidity for day-time and night-time sampling using one-way ANOVA analysis. LFE had slightly higher temperature and low humidity for day-time and night-time sampling compared to MBCA. However, the temperature in LFE area at night during this study was lower than in the MBCA area, which may have been caused by canopy gap presence in the logged forest area.

For peak biting hour during the sampling periods, LFE had peak biting hours around 2:00 p.m., 5:00 p.m., 7.00 p.m. and 9:00 p.m. while for MBCA, the peak biting hours were between 2:00 - 3:00 p.m. and 6.00 p.m. (Figure 4 & Figure 5).

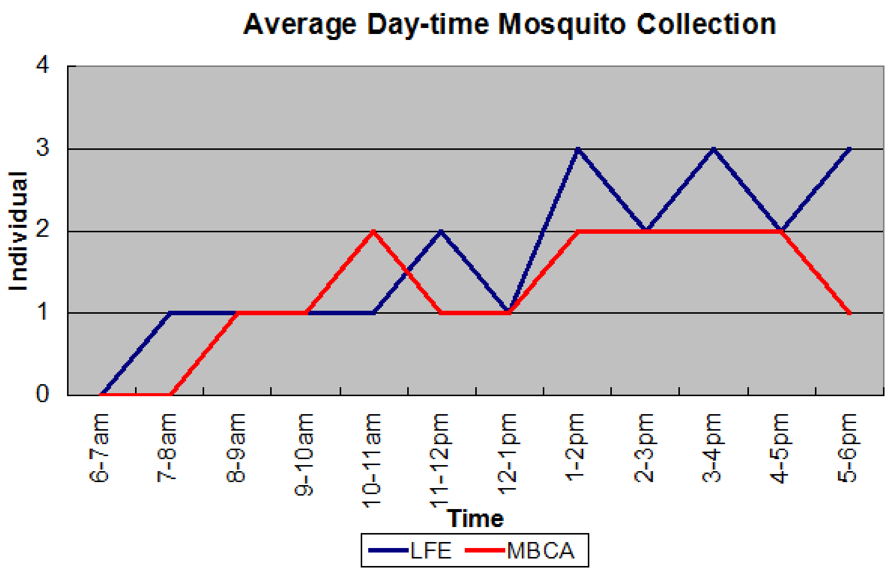

Figure 4. Mosquito biting graph during day sampling

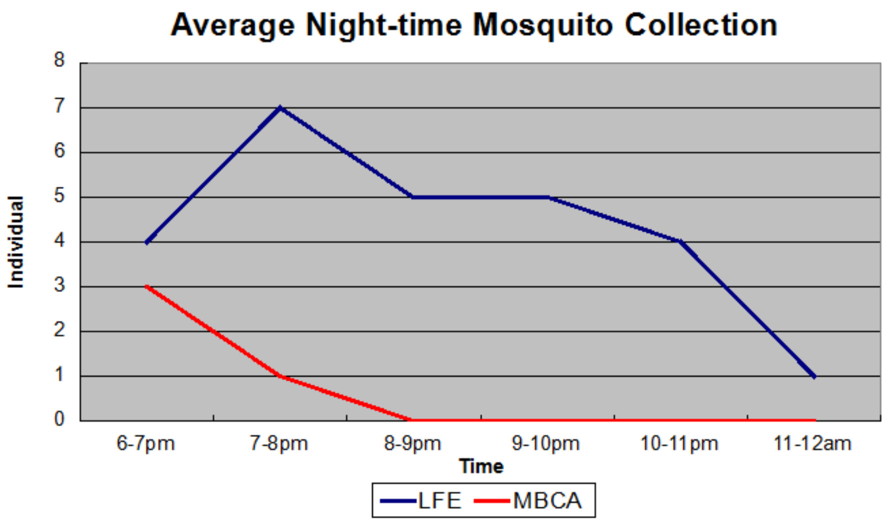

Figure 5. Mosquito biting graph during night sampling

Discussion

Habitat disturbance such as forest modification inevitably caused ecological disturbance in the forest ecosystem and has become a great threat to forest biodiversity (Fitzherbert et al., 2008). Changing environments pose negative impact on human health especially through the transmission of disease by a vector-borne mosquito (Vanwambeke et al, 2007). Mosquito communities may change across landscapes including changes in habitat which can affect species relative abundance and the invasion of new species (Thongsripong et al., 2013). In this study, mosquitoes were much more diverse in the undisturbed area (MBCA) than in the disturbed area (LFE). The possible reasons are undisturbed forests have lots of suitable natural habitat for the mosquitoes to breed and feed on hosts. Also, the old growth forest site in MBCA was cooler compared to the logged forest area (Brant et al., 2011). This was probably due to canopy height differences between LFE and MBCA where in MBCA it consists of lots of higher primary forest trees than in LFE. Canopy height can be the contributing factor that influences forest microclimate (Hardwick, et al., 2015). Previous studies by Chen et al. (1993) and Williams-Linera et al. (1998) show that the air temperature within a forest canopy has a higher relative humidity compared to air in the open area. The open canopy area usually has higher air temperature due to low sunlight cover (Fayle et al., 2010).

MBCA is a protected area and still has lots of primary forest areas which have never been logged before (Hardwick et al., 2015). The difference in terms of number of mosquito species present are related to the disturbance level in an area. The higher the intensity of disturbance, the lower the diversity of organisms in an area as only certain species are able to tolerate the surrounding environment (Haddad et al., 2008; Moretti & Legg, 2009). Loss of natural habitat in a disturbed area due to high intensity of disturbance can lead to loss of certain species and food resources (McCabe & Gotelli, 2000). According to Schowalter (2011), individuals or species that are not tolerant to certain changes in the habitat environment will face a decrease in number and then undergo extinction. Instead, tolerant species will gain benefits in terms of reducing the number of predators.

In this study, the number of mosquito species was lower in the disturbed forest area compared to the undisturbed area. Habitat in the disturbed area usually has been damaged or destroyed which causes the area to be unsuitable for species survival. Human activities such as deforestation and development have changed habitats which result in declining mosquito species numbers in the disturbed area at the SAFE Project. Diversity level in a certain area will increase if the disturbance occurs in low spatial scale while diversity level will decrease if the disturbance occurs in high spatial scale (Dumbrell et al., 2008). In this study, mosquito abundance was higher in logged over forests compared to primary forests. The results seem to complement the previous study by Brant et al. (2016) which found the abundance of landing mosquitoes were higher in a logged forest compared to primary forest area. However, in terms of the number of possible vector mosquito presence, both LFE and MBCA had almost the same vector species. *Aedes albopictus*, *Anopheles balabacensis,* and *Culex vishnui* can be found in LFE while for MBCA, only *Culex vishnui* was not present. Both *Aedes albopictus* and *Anopheles balabacensis* were present in both study sites. *Aedes albopictus* population seems to spread very widely due to its ability to live in all ecotypes in towns, villages, forest fringes and coastal areas. *Ae. albopictus* is also known as dengue vector in Malaysia (Rohani et al., 2008). *Anopheles balabacensis* which was the most common and the most predominant *Anopheles* species found has been incriminated as a malaria vector in Sabah (Reid, 1968; Wong et al., 2015).

The biting activity of mosquito was studied as it provides to our understanding of the biting cycles of some mosquito species, nuisance level determination and possible disease transmission detection (Rohani et al., 2013). In this study, we can see two different biting time behaviour between day-time and night-time samplings. For day-time sampling usually dominated by *Aedes* and *Heizmannia* mosquito groups showed their biting activity was around 2:00 p.m and near dusk which was around 5:00 p.m. This result was almost similar to a previous study where *Aedes* mosquito usually peaks at dusk 5:00-6:00 p.m. (Rogozi et al., 2012; Sahani et al., 2012). For night-time samplings, the biting activity peaked at 7:00-8:00 p.m and 9.00-10:00 p.m. when *Anopheles* mosquitoes were active. Only few *Aedes* mosquito were collected during night-time sampling. Similar to the previous study by Brant et al. (2016), *Anopheles balabacensis* started biting as early as 6:00-7:00 p.m. Since *Anopheles balabacensis* live within forested area of Sabah and readily bite human and monkey hosts, it is no wonder that it is considered as one of the dangerous simian malaria vectors (Vythillingam, 2010). Biting time of mosquitoes in this study showed decreasing biting activity as the night progressed. This situation was almost similar to the study by Chen et al. (2014) and Mahanta et al. (1999) that showed a reduction in mosquito activities towards midnight.

For day sampling in LFE, the peak biting hours were at 2:00 p.m. and 5:00 p.m. and usually were made up of mosquito species from *Heizmannia scintillans* and *Aedes albopictus*. However, *Aedes* mosquito tends to be more active at dusk than other mosquito species caught in this study. A study by Marques & Gomes (1997) reported that the biting activity of *Aedes albopictus* usually peaked at 6:00-7:00 a.m., 1:00-2:00 p.m. and highest activity during 4:00-5:00 p.m.. For the night sampling in LFE, biting activity was highest at 7:00 p.m. and 9:00 p.m., dominated by *Anopheles balabacensis* and *Culex vishnui*. A study by Wong et al. (2015), showed that the biting activity of *Anopheles balabacensis* can be as early as 6:00 p.m. up to 8:00 p.m. On the other hand, the day sampling in MBCA had a peak biting hour at 2:00-3:00 p.m where mosquito species like *Heizmannia scintillans* and *Armigeres jugraensis* were caught during this hour. However, for the night sampling in MBCA, the biting activity only peaked at 6:00 p.m. and the species collected during the hour were *Anopheles umbrosus*, *Anopheles balabacensis* and a few other species from genus *Aedes* and *Mansonia*. The reasons for different peak biting hours between the two sites were probably due to different environment conditions that the mosquito live in and type of species present in the areas. Based on the mean temperature and humidity data, the environment in MBCA was cooler compared to LFE. In terms of abundance of mosquito, LFE has higher abundance of mosquito and vector species from Anopheles, Aedes and Culex groups compared to MBCA which lead to the biting activity in LFE to be more active than in MBCA.

Changes in land use such as deforestation and other development activities have a direct impact on mosquito abundance, species biodiversity, biting behaviour and vector competency (Rohani et al., 2016). In addition, the effects of land modification also cause changes in temperature and moisture which in turn result in increasing vector population and transmission rates (Geist, 2006). MBCA in this study was a good example of the effect of forest modification towards mosquito biodiversity and biting behaviour. Also, the transition of forest land from its previous primary forest state can result in environmental stress due to microclimatic changes (Edwards et al., 2013). For example, a study by Kweka et al., (2016) showed that deforestation affects microclimate conditions and mosquito survivorship where an increase in malaria vector reproductive rate was associated with an increase in temperature. The influence of landscape change on microclimate condition of an area can be the key to determining the effect on diversity, abundance and survivorship of the mosquitoes (Patz & Olson, 2006). In a nutshell, changes in mosquito diversity can affect the risk of infectious diseases in a system by disrupting their normal host and pathogen relationships. By understanding the vector community that lives in an area that has undergone anthropogenic changes, a basis could be formed for understanding the emergence and persistence of mosquito-borne diseases (Thongsripong et al., 2013).

Conclusion

There are differences in diversity of mosquitoes between disturbed and undisturbed areas. Undisturbed area possesses a higher number of mosquito species than disturbed areas. Based on this study, we can see that *Anopheles balabacensis* was the most predominant species for night-time catch and *Aedes albopictus* was the most predominant species for day-time catch. Overall, a good understanding on the ecology and behaviour of these mosquitoes would give us an advantage in managing and controlling the vectors.

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