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Research Articles

Malaria vector population dynamics in highland and lowland regions of western Kenya

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ABSTRACT

Background & objectives: Malaria is the major cause of morbidity and mortality in sub-Saharan Africa. A child below five years dies after every 30 min. Highland areas under land use change impact on malaria transmission by altering the microclimate of the immature stages and adult mosquitoes. Adult vector population dynamics is important because it is an indicator of transmission risk of the disease. This study was to investigate the effects of microclimatic changes on the mosquito indoor-resting behavior.

Methods: The study was conducted at a highland site of Marani and at a lowland site of Kombewa where 30 houses were randomly selected at either site. Outdoor and indoor weather conditions were monitored throughout the study period. Indoor mosquitoes were collected using the pyrethrum spray catch method, gonotrophic stage of the females determined and species identified to species level using rDNA polymerase chain reaction method. ELISA was carried out to determine the *Plasmodium* sporozoites in mosquitoes.

Results: Anopheles gambiae s.s. was more abundant at the highland site whereas *An. funestus* at the lowland site. Indoor densities were highest in June 2003 at both the sites: *An. gambiae* at the highland site and *An. funestus* at the lowland site. There was an association between *An. gambiae s.s.* abundance and relative humidity at the highland site. Combined entomological inoculation rate (EIR) for both the vector species was 0.4 infected bite per year (ib/yr) at the highland site and 31.1 ib/yr at the lowland site. Prolonged indoor spraying with insecticide decreased vector indoor abundance.

Key words *Anopheles funestus; Anopheles gambiae;* Kenya; malaria; population dynamics

INTRODUCTION

Malaria continues to be the major cause of morbidity and mortality in sub-Saharan Africa[1]. Higher regions of Africa had infrequent malaria epidemics between 1920s and 1950s[2, 3] that were controlled by malaria campaign[3]. From 1960s to 1980s, highland regions of East Africa were malaria free[3, 4] but in the recent past malaria epidemics had been reported[5–11]. In Kenya, within the last three decades, malaria epidemics have been reported in the western Kenya highlands[6, 9, 12–19].

Malaria infections in the same region are distributed heterogeneously[20–23]. Studies have shown that among other factors that contribute to the differences include land use/land cover types, distance of the houses from the aquatic habitats, house structure and building materials, topography and malaria prevention strategies employed by individual households[9, 24]. In transition zones that are desert fringes and highland regions, malaria transmission is low and intermittent causing instability or epidemics resulting in high mortality due to low immunity in the highland human populations[25, 26]. Highland areas that are under land use change impact on malaria transmission by altering the microclimate of the immature stages and adult mosquitoes[27]. Duration of *Plasmodium* sporogony and gonotrophic cycle of the vector is reduced that increases the risk of malaria transmission.

Adult vector population dynamics is important because it acts as an indicator of transmission risk of the disease[28–31]. Vector abundance is an important determinant of malaria transmission force[32]. Through monitoring the abundance of adult malaria vectors, comparative analyses enable to determine the thresholds of indoor density in cases of outbreak of epidemics. Because epidemics do build up, monitoring adult vector population in epidemic prone regions is useful in models that

detect likelihood of epidemic and, therefore, early warning system. This information can be used to put in place both curative and control measures to counter the epidemic[33–35].

Few studies have been carried out on adult population dynamics in the western highlands of Kenya[36]. Altitude, topography and land use have been shown to affect vector abundance hence malaria transmission[28]. The present study was carried out in a highland region prone to malaria epidemics with reference to a low holoendemic region to compare the differences in malaria vector population dynamics and transmission intensities that will elucidate epidemics in the region. Monitoring is also of paramount importance in determining whether control measures put in place are effective or not. In both regions, insecticide-impregnated bednets are in use though transmission rate is still high[36].

MATERIAL & METHODS

Study site

The study was conducted in Marani and Kombewa. Marani is located at 34°48′ East and 0° 35′ South of Kisii district in western Kenya. It is prone to malaria epidemics. Marani is a highland area with an altitude between 1508 and 1703 m above sea level (Fig. 1) on undulating land drained by Marani permanent river and streams. The slopes are steep. Kombewa is a low-lying region located 34°30′ East and 0° 07′ South. Altitude ranges between 1100 and 1300 m above sea level. It is located within the Lake Victoria basin. The study area covered 16 km^2 at both the sites. Marani is a densely populated area that has undergone land-use changes over a long period of time. The major land-use changes include: conversion of forest land to crop-farming, mainly tea growing; conversion of grassland into sugarcane or maize growing; draining of natural swamps and settlement in the former natural forests and grassland areas. Artificial forests of *Eucalyptus* trees located along river valleys are used for timber production. Main farming activities include maize, millet, banana, tea and coffee, vegetable growing and dairy farming. Kombewa is characterized by maize, mango and sorghum growing and animal keeping. The area has no permanent river. Marani has two rainy seasons that are not well-defined and one dry season. During the study period (2003 to 2005) the monthly rainfall was 221.4 mm while mean minimum and mean maximum temperatures

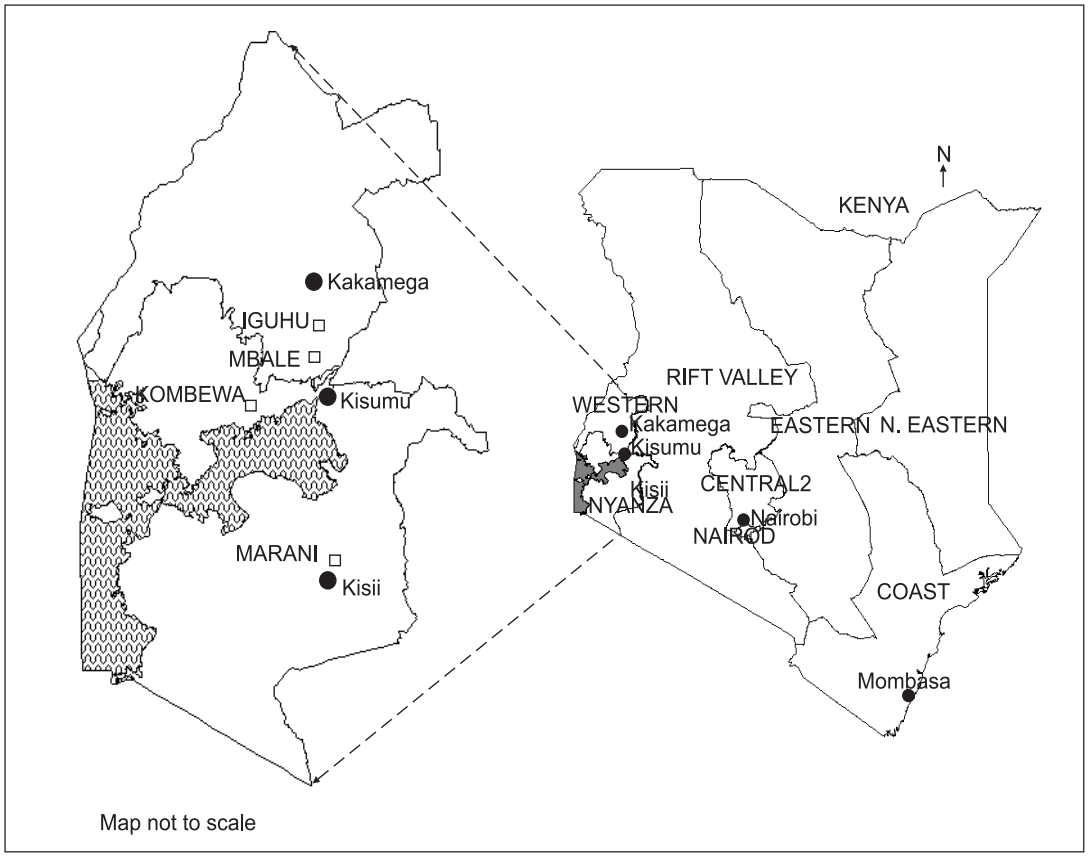

Fig. 1: The map of Kenya showing relative positions of study sites (Kombewa and Marani).

were 14.4 and 26.7°C respectively. Kombewa has two rainy seasons and one dry season. Average monthly rainfall was 120.7 mm; mean minimum and maximum temperatures were 18.5 and 29.3°C respectively. Kombewa was warmer than Marani having holoendemic malaria.

Mosquito sampling

In all 30 houses were randomly selected at either Marani or Kombewa. Coordinates and altitude of each house were taken using GPS system in differential mode[37]. Among 30 houses at each site, 10 houses were further randomly selected where weather stations were constructed (Stevenson's screens). Weather stations were built 10 feet far from the main houses to avoid the reflection of light. Hobos data loggers devices to record temperature and relative humidity were placed both inside and outside the houses. The number of human sleepers in the house of the previous night was noted. Mosquitoes were collected using the pyrethrum spray catch method[38]. Female malaria vectors gonotrophic status was determined microscopically and classified as unfed, blood fed, half gravid, and gravid[39], mosquitoes were then put in vials and stored in refrigerator for species determination with PCR. Sampling was carried out in 30 houses on monthly basis from February 2003 to February 2005 at Marani, and from June 2003 to January 2005 at Kombewa.

Species identification

Individual species were identified to species level using rDNA polymerase chain reaction method[40] (n= 95 from Marani; and n=92 from Kombewa). Individuals from *An. funestus* complex were identified according to Koekemoer *et al*[41] (n=24 from Marani; and n=122 from Kombewa).

Plasmodium sporozoite rates and entomological inoculation rates

The head and thorax of each malaria vector were removed from abdomen, the later was put in 1.5 ml eppendorff micro-centrifuge tube, processed and tested for the presence of *Plasmodium* circumsporozoite antigen (CSP1)[42, 43]. A total of 1775 female mosquitoes (649 *An. gambiae s.l.* and 1055 *An. funestus*) were assayed and EIR calculated according to WHO[44].

Data analysis

The monthly average house abundance was computed for both the sites. The abundance of vector species was determined among houses and between months and sites. Analysis of variance (ANOVA) was used to determine among site variation and Tukey-Kramer honestly significant difference (HSD) tests were used to compare the mean densities of *An. gambiae s.l.* and *An. funestus* among both the sites. ANOVA was also used to compare monthly variation of house abundance.

RESULTS

Malaria vector abundance and density

Anopheles gambiae s.s. was more abundant than *An. funestus* at the highland site of Marani. At Kombewa (lowland), *An. funestus* was more abundant than *An. gambiae s.l.* (Fig. 2a).

At the highland site, *An. gambiae* indoor population in June 2003 was significantly different from the rest of the months ($t = 16$, $p < 0.05$). At the lowland site of Kombewa, the population of *An. gambiae* (*s.l.*) in June 2003, April 2004, and May 2004 was significantly different ($t = 9.5$, $p < 0.05$; $t = 6.7$, $p < 0.05$; and $t = 8.6$, $p < 0.05$ respectively) than the rest of the months. At the highland site, indoor density was generally low (<0.02) for the rest of the months. *Anopheles gambiae s.s.* indoor density was highest in June 2003. No *An. gambiae s.s.* was collected during few months. One peak was attained in June 2003, with density of 1.5 females per house. At

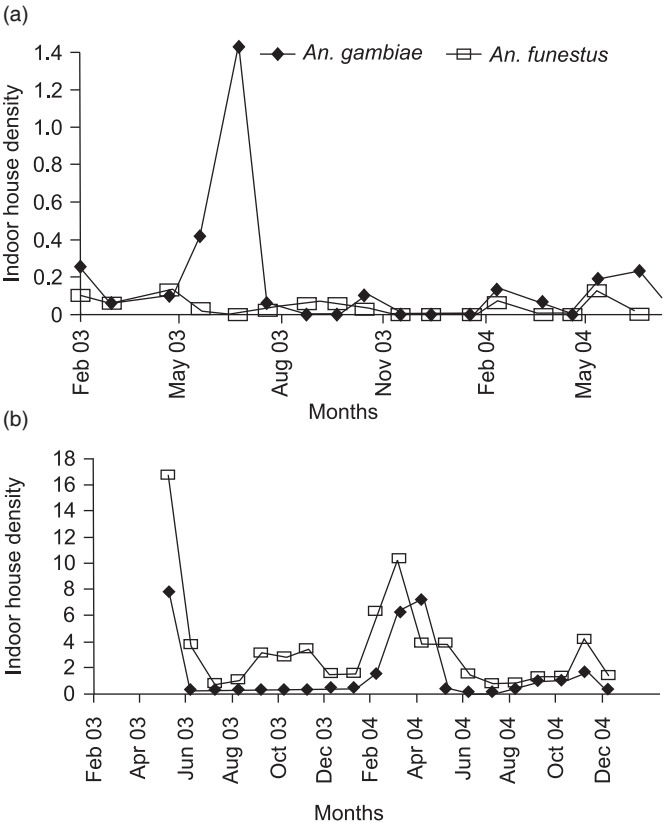

Fig. 2: Mean numbers of *An. gambiae* and *An. funestus* per house at (a) Marani; and (b) Kombewa.

the lowland site of Kombewa indoor house densities of the malaria vectors were generally high (Fig. 2b). *Anopheles gambiae s.l.* attained two peaks in June 2003 and May 2004. The highest peak was attained in June 2003 with a mean indoor density of 8.1. Indoor density was significantly different over the months.

At the highland site, over 70% of houses sampled in June 2003 had *An. gambiae s.s.* (Fig. 3a). There was a significant difference in the number of houses positive with *An. gambiae s.s.* over the months (F = 12.6, df = 24, p <0.05). The number of houses with *An. gambiae s.s.* for the following months was significantly different from the rest; February, May and June 2003 (t = 4.5, p <0.05, t = 3.6, p <0.05, and t = 15. 3 respectively). The month of June 2003 had the highest number of houses with *An. gambiae s.s.*, followed by February and May 2003. At the lowland site over 80% of houses sampled in June 2003, April and May 2004 had *An. gambiae s.l.* present (Fig. 3b). The number of houses positive for *An. gambiae* (*s.l.*) was significantly different over the sampling period (F = 11.9, df = 19, p <0.05). For the months of June, July, August, October, November 2003, and March, April, May, July, August and December 2004, the number of houses with *An. gambiae* (*s.l.*) were significantly higher than the rest (t = 6.7, p <0.05; t = 3.3, p <0.05; t = 2.8, p <0.05; t = 3.3, p <0.05; t = 3.2, p <0.05; t = 2.5, p <0.05; t = 6.9, p <0.05; t = 6.8, p <0.05; t = 3.7, p <0.05; t = 4.2, p <0.05; and t = 3.2, p <0.05 respectively).

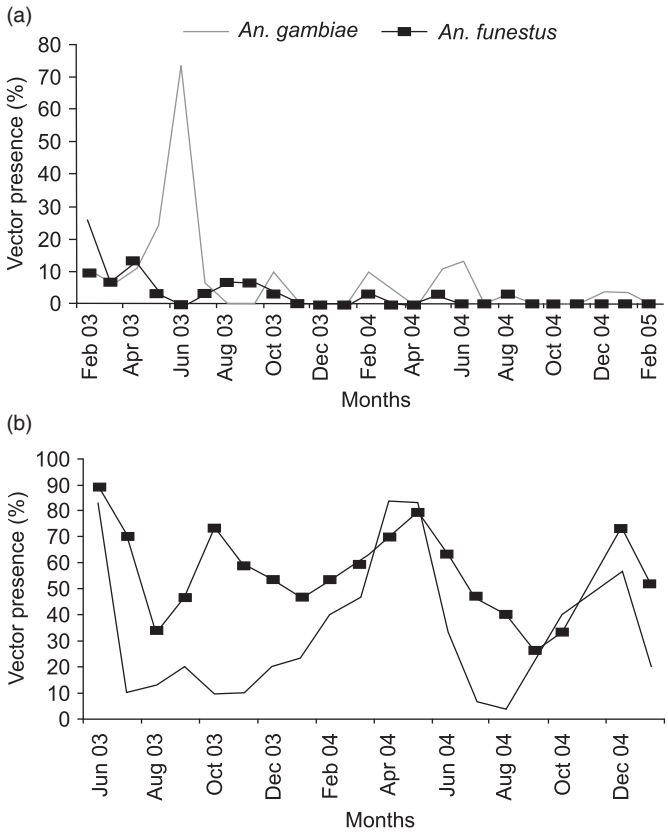

Fig. 3: Abundance of houses with malaria vectors at: (a) Marani; and (b) Kombewa.

At the highland site, the indoor density of *An. funestus* was generally low for the whole sampling period. No peak was observed (Fig. 2a). There was no significant difference in population of *An. funestus* over different months (F = 1.1, df = 24, p >0.05). The highest percentage of positive houses with *An. funestus* was 13.3%. The number of positive houses for February and April 2003 was significantly higher than the rest (t = 2.7, p <0.05; t = 3.9, p <0.05 respectively). At the lowland site, indoor density of *An. funestus* was generally high (Fig. 2b) with two peaks. The highest peak was attained in June 2003 with indoor density of 17.3 female mosquitoes per house. The population of *An. funestus* was significantly different over sampling months (F = 9.8, df = 19, p <0.05). The population was highest in June 2003.

At the lowland site of Kombewa, over 80% of the houses were infested with *An. funestus* in June 2003 (Fig. 3b). Over 70% of the houses were infested in October 2003, May and December 2004, whereas in July 2003, 70% of the houses were infested. There was a significant difference in the number of houses infested with *An. funestus* over the months (F = 4.1, df = 19, p <0.05). The following months had significantly high number of houses infested: June, August, October 2003, and May, August, September, October, November and December 2004 (t = 4, p <0.05; t = 2.6, p <0.05; t = 2.2, p <0.05; t = 2.8, p <0.05; t = 2.6, p <0.05; t = 3.1, p <0.05; t = 2.6, p <0.05; t = 2.6, p <0.05; and t = 2.2, p <0.05, respectively).

Gonotrophic status

At both sites, the proportion of blood fed malaria vectors in houses was found to be highest followed by gravid, half gravid and finally empty vectors (Fig. 4). The population of *An. gambiae* empty for lowland and highland sites was significantly different over different months (F = 5.9, df = 19, p <0.05 and F = 1.9, df = 24, p <0.05 respectively). At the highland site of Marani, the population in June 2003 and May 2004 was significantly higher than the rest of the months (t = 4.2, p <0.05). The density of empty females in the houses was high during the two months. For the lowland site, the population of empty females for June 2003, April and May 2004 was significantly higher than the rest of the months (t = 6.3, p <0.05; t = 4.9, p <0.05; and t = 5.0, p <0.05 respectively). For blood-fed *An. gambiae* at both sites, in house population over months was significantly different (F = 9.8, df = 19,

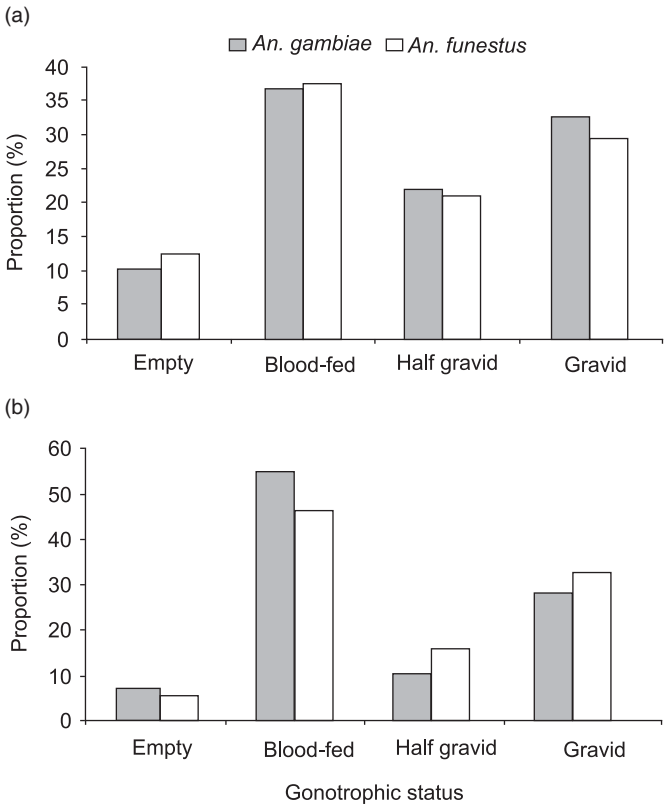

Fig. 4: Proportion of gonotrophic status of malaria vectors at: (a) Marani; and (b) Kombewa.

p <0.05 for lowland; and F = 6.2, df = 24, p <0.05 for highland). At the highland site, the population of indoor blood-fed female *An. gambiae* in May, June 2003 and June 2004 was significantly high (t = 3.4, p <0.05; t = 10.9, p <0.05 respectively). The mean densities were high, 0.2, 0.5 and 0.3 for the months of May, June 2003 and June 2004 respectively. The trend for half gravid and gravid female *An. gambiae* population at both the sites was similar to what was observed earlier for the other gonotrphic status. However, at the highland site, half gravid female *An. gambiae* population for February and June 2003 was significantly higher (t = 2.5, p <0.05; and t = 12.4, p <0.05 respectively). The density of half gravid was high during February and June 2003.

For *An. funestus* the trend was different. The population was very low comparatively. The gonotrophic composition for the highland was as follows: 12.5% empty, 37.5% blood-fed, 20.8% half gravid and 29.2% gravid while the lowland comprised 46.5% for blood-fed, 31.8% gravid, 16% half gravid and 5.7% empty. At the highland site, empty female population was not different (F = 0.9, df = 24, p >0.05) over the months.

At the lowland site, the population of empty females in houses was different over months (F = 4.9, df = 19, p <0.05). In June 2003, April and May 2004 had significantly high population (t = 7.2, p <0.05; t = 2.2, p <0.05; and t = 4.6, p <0.05, respectively). No difference was found for the blood fed *An. funestus* at the highland site (F = 0.8, df = 24, p >0.05). But for the lowland site over months the populations were different (F = 9.8, df = 19, p <0.05). June, August, September 2003, April, May and September 2004 had significantly high population (t = 10.8, p <0.05; t = 2.2, p <0.05; t = 2.0, p <0.05; t = 2.5, p = 0.05; t = 6.2, p <0.05; and t = 2.3, p <0.05, respectively). At both the sites, *An. funestus* half gravid and gravid female population was different during different months. At the highland site, February and April 2003 had half gravid population higher (t = 4.2, p <0.05 each) than the rest of the months. While for gravid females, May 2004 population was significantly higher (t = 2.9, p <0.05).

Association of weather and malaria vectors

The monthly rainfall, mean relative humidity and indoor mean temperatures were computed for both the sites. The highland site received rains during all the months, although February, March 2003 and March 2004 had the least. There was no direct association between rainfall and indoor vector density. The indoor mean relative humidity was 59.9% and indoor mean temperature was 21.4°C. Indoor and outdoor temperatures were lower at the highland of Marani than Kombewa lowland. For the lowland site, the indoor mean relative humidity was 65.5% and indoor mean temperature 23.5°C.

Regression analysis showed that there was an association between *An. gambiae s.s.* abundance and relative humidity of the same month and one month earlier at the highland site (Table 1). At the low land site, no association was detected. At the highland site, three peaks of rainfall anomalies were observed in March, November 2003 and July 2004 while two peaks were attained that coincided with maximum temperature at the lowland.

Plasmodium falciparum sporozoite rates and entomological inoculation rates

The mean sporozoite rate at Marani was 1.5% for *An. gambiae* and 5.6% for *An. funestus*. The EIR was 0.2 ib/yr from *An. gambiae* and 0.2 ib/yr from *An. funestus*. Combined EIR for both the vector species was 0.4 ib/yr. Malaria transmission is likely to have been high during May when mosquito was found infected with the sporozoite. However, at the lowland site, the mean sporozoite rate was 3.4% for *An. gambiae* and 3.9% for *An. funestus*. High proportion of *An. funestus* (67.8%) was found infected with the sporozoites as compared to *An. gambiae*

Table 1. Association between vector species abundance and minimum, maximum relative temperature, relative humidity and rainfall at Marani and Kombewa

Period	Species	Marani				Kombewa			
		Min temp (°C)	Max temp (°C)	RH	Rainfall (mm)	Min temp (°C)	Max temp (°C)	RH	Rainfall (mm)
Same month	*An. gambiae*	0.3	2.7	9.4*	0.1	0	0.5	1	0.4
	An. funestus	1.7	0	1.3	0	1.3	0.4	0.6	0.2
one month earlier	*An. gambiae*	0.3	2.7	9.4*	0	0.7	2.1	1.6	2.1
	An. funestus	1.7	0	1.2	0.2	3.3	0.4	1.8	3.2

* F-values are significant association at $p = 0.05$ level of regression analysis.

(32.8%). The EIR was 10.1 ib/yr from *An. gambiae* and 21 ib/yr from *An. funestus*. Combined EIR for both the vector species was 31.1 ib/yr.

DISCUSSION

During the study period, two anopheline species were collected resting indoors at both the Kombewa and Marani sites. *Anopheles gambiae s.s*. (80.5%) was the most abundant anopheline species at the highland site while at the lowland, *An. funestus* (71.2%) was the most abundant. The mean indoor density of *An. gambiae* and *An. funestus* increased by 11.4 and 122.4 fold at the farmer site respectively at the lowland as compared to the highland. Each of the two sites has specific ecological conditions arising from the difference in elevation, temperature and rainfall. As a result of ecological variability, malaria vector population was very low at highland and high at lowland sites.

Anopheles gambiae is an r-strategist[45] species thus able to thrive in both the ecological conditions. *Anopheles funestus* thrives best in lowland conditions due to higher temperature that enhance its growth and development since this species takes a longer period to emerge[46]. The low temperatures in the highland lengthen the developmental period[27] and most likely affect larval survival and emergence thus few adults.

The low sporozoite rates at the highland site (1.5%) may indicate the low levels of endemicity in highland regions. At the lowland, the high (3.4%) sporozoite rate indicates the holoendemic status of the area. The highland site is a hypoendemic area, which if microclimatic conditions are changed, local epidemic may occur. The EIRs from *An. gambiae* and *An. funestus* of the highland site were low (0.2 bites/ person/ year). However, malaria transmission increases during the long rain season in the month of May. This may be due to more breeding habitats made available by the rains. The highland land cover/ land use is dynamic. Natural swamps are being converted into cultivated swamps by draining. Thus suitable conditions for vector growth and development are created. The main malaria vector was *An. gambiae s.s.* This species thrives best in sun-lit open temporary pools that are created by draining of the natural swamps.

EIR for the lowland site was high (31.1 ib/yr). Malaria transmission in lowland is continuous throughout the year with peaks during the rainy season. The main malaria vectors were *An. gambiae s.l.* and *An. funestus.* Vector populations were high in most of the months. Abundance of *An. funestus* was higher than *An. gambiae* suggesting that the former species is ecologically suited in lowland areas. This is because the site is located within the lake basin where many permanent water bodies (ponds and swamps) and high temperature provide suitable habitats for its breeding. Of the infected mosquitoes, 32.8% comprised *An. gambiae* whereas 67.2% were *An. funestus*. Though both the species transmitted malaria, the latter one played a more important role.

During the transmission season, clustering of houses with vectors occurred near the valley in the highland site. This phenomenon can be utilized in malaria control or management whereby targeted indoor spraying can be done instead of amorphous spraying. It was also noted that some houses were consistently with higher vectors density over the transmission months. Thus such houses can be targeted.

Temporal population dynamics and abundance of the malaria vectors was heterogeneous over the time and space in both the lowland and highland regions. This knowledge is important and partly explains the occurrence of local malaria epidemics. Since indoor vector density increases during the transmission season, target control measures before onset of rains can likely mitigate against an otherwise severe epidemic. For designing malaria control measures, such climate and entomological data are required for designing models that can be used for fore-

casting epidemics hence early warning systems. In cases of very low EIR it is indicative that malaria can be managed through indoor spraying.

The proportion of female blood-fed malaria vectors indoors was highest. Once female malaria vectors are blood-fed and fully engorged, their flight ability reduces and they rest indoors. Gravid females rest indoors until the eggs develop and are mature for laying then they move out early in the evening to look for appropriate breeding sites[47]. Unfed females leave the houses early in the morning to rest in nearby bushes.

In conclusion, vector abundance and dynamics at the two sites showed great variations probably due to the varied elevation, hence temperature and rainfall. The lowland experiences high temperatures since it is a low elevated area. The highland on the other hand has lower temperatures because it is highly elevated. Temperatures affect growth, development and survival of the malaria vectors[27]. The highland site has high rainfall but its topography favours good drainage thus vector breeding sites limited to lower areas in the valley bottom which has been disturbed by man. The trend of population dynamics of vector species was different at both the sites. In the lowland, population of *An. funestus* remained relatively high whereas at the highland, *An. gambiae* was the predominant species. Though the two species are sometime found sympatric, *An. gambiae* probably is more habitat condition specific.

This study has shown that there are variations in abundance and spatial dynamics of malaria vectors in lowland and highland regions of western Kenya. At the beginning of the study, the abundance of malaria vector species was high at both the lowland and highland sites. At the end of the study period, the abundance had drastically reduced at both the sites suggesting that prolonged indoor spraying had effect on the abundance of the vector mosquitoes.

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