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Title

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Journal

Journal of Vector Borne Diseases, 49(2)

ISSN

0972-9062

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Publication Date

2012-06-01

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Peer reviewed

Published in final edited form as:

J Vector Borne Dis. 2012 June ; 49(2): 61–71.

Effect of swamp cultivation on distribution of anopheline larval habitats in Western Kenya

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Abstract

Background & objective—Malaria resurgence in highland regions of East Africa has been on increase. The spatio-temporal distribution of larval habitats of malaria vectors determines the distribution of adult vectors, hence, disease transmission. Vector's ecology is necessary for strategic vector control through effective plan for source reduction. Mapping of the larval habitats is necessary for targeted control measures. The purpose of this study is to assess and compare the spatial and seasonal variations in anopheline larval habitats in Western Kenya.

Methods—A comparative study was conducted on spatial distribution of GPS geo-located anopheline larval habitats in relation to highland and lowland environments. Land use types were categorized and all potential aquatic habitats of malaria vectors were examined in February, May, August and November 2004. Data analyses were performed using SAS JMP software.

Results & discussion—Results showed a higher percentage of *Anopheles gambiae s.s.* (70.9%) than *An. Funestus* (29.1%) in highland. In the lowland, *An. gambiae s.l.* comprised 60.1% while *An. funestus* represented 39.9%. The distribution of larval breeding is confined to the valley bottom in the highland while it was dispersed in the lowland. Land use type influenced the occurrence of positive breeding habitats in the highland. In the lowland, distribution was due to seasonality. We found high proportion of potential and positive breeding sites in cultivated swamps and farmlands at the highland site. These results suggest that swamp cultivation increases the availability and suitability of larval breeding habitats of malaria vectors, thus malaria transmission in the Western Kenya highlands environment.

Keywords

Habitats; land use change; malaria vector

INTRODUCTION

Highland regions of Africa initially considered malaria-free until the last two decades have experienced episodes of malaria epidemics¹⁻⁶, that are reported to have spread from 3 to 15 districts in Western Kenya within the last few years. These epidemics have caused high morbidity and mortality^{7,8}, that is of immense concern. Indeed, highland communities lack functional immunity^{9,10}, thus, are more vulnerable to malaria infection unlike their lowland endemic region counterparts. Health facilities have not been sufficient to cope up with the epidemics because of different reasons that include: their sudden strike that takes off-guard medical planners; human population in the region has increased rapidly^{11,12} since family planning has not been fully embraced; drug and personnel short-age. Several hypotheses proposed to explain the increased malaria transmission in the highlands include: global climate change, demographic patterns, land use change and drug resistance¹³⁻¹⁸. *Anopheles gambiae* Giles, *An. arabiensis* Patton and *An. funestus* Giles are the primary vectors of human malaria in sub-Saharan Africa. Coetzee¹⁹ observed that *An. gambiae* complex members were sympatric. There have been reports where *An. gambiae sensu stricto* has been observed in the highland regions with high precipitation. It is characteristic of small temporary open sun-lit pools²⁰⁻²². *Anopheles arabiensis* is mainly found in low-lying arid areas^{19,23,24}. *Anopheles funestus* is determined by the presence of permanent breeding habitats and seasonality²³. This implies that malaria transmission is likely to occur throughout the year and hence control strategies should be put in place especially at the time when the risk of transmission is highest. A good understanding of the fluctuations in adult population of vectors is in part determined by factors that affect larval abundance. Larval habitats are an important determinant for adult abundance and distribution.

Lowland areas have stable and endemic malaria. Due to low elevation, with most of the surface almost flat, water accumulates on the ground surface especially in the flood plains, man-made or natural pools, man and animal footprints on farms that provide breeding sites for *An. gambiae s.l.* This vector species, an r-strategist, breeds very fast, thus, population increases rapidly within a short time. In the highland region, on the other hand, the topography is undulating sometimes with many valleys and plateau. The soils are well-drained thus water does not accumulate easily in pools unless directed. Due to pressure on land for agricultural production and human settlement, wetlands (natural swamps) have been drained for crop production. Studies by Lindblade¹⁶ in a Ugandan highland area suggest that cultivation of natural swamps increase malaria transmission. In the same study, the maximum and minimum temperatures were higher in the cultivated swamps than the natural swamps. Minakawa²⁵ found significant association between occurrence of *An. gambiae s.s.* larvae and habitat size and vegetation type in a highland area of Western Kenya. The same study revealed that warmer daytime temperatures characterized larval habitats for *An. gambiae s.s.* These studies suggest that in the highland area, the cultivation of natural swamps increase the microhabitat temperature, which in turn shortens the development time for the vector mosquitoes. This has profound effect on the adult population density because more generations will be attained in a shorter time. A change in land use may lead to microclimate change that may cause local malaria upsurge.

In the past, most control measures have been directed at the adult stages. But source reduction through modification of larval habitats was the key to malaria eradication in the United States, Israel and Italy²⁶. For any effective control through source reduction, an understanding of the dynamics of the larval habitats is required if efforts to model and predict adult abundance has to succeed. The purpose of this study is to assess and compare the spatial and seasonal variations in anopheline larval habitats in both the lowland and highland sites of Western Kenya.

MATERIAL & METHODS

Study sites

The study was conducted at two sites: Marani and Kombewa. Marani is a highland area that suffered from malaria epidemics. It is located at 34°48' east and 0°35' south in Kisii county, Western Kenya, on undulating land, 17 km northeast of Kisii town (Fig. 1). The altitude ranges from 1508 to 1703 m. This highland is drained by several permanent rivers and streams and is characterized by the presence of springs during the long rainy season. The study area is 16 km² wide. It is characterized by a high population density and has undergone land-use changes that occurred 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 farms; draining of natural swamps and settlement in the former natural forests and grassland areas. Plantations of Eucalyptus forests are located along river valleys for timber production. The main farming activities include maize, millet, banana, tea and coffee, vegetable growing and dairy farming. The area has two rainy seasons (April–July and August–October) that are not well-defined and one dry (November–February) season. The annual rainfall is about 2538 mm while the mean maximum, mean average and mean minimum temperatures are 26.7, 19.8 and 14.4°C respectively.

Kombewa study site is lowland where malaria is holoendemic. It is located at 34°30' east and 0° 07' south in Kisumu county, about 30 km from Kisumu town. The altitude ranges between 1100 and 1200 m. It covers 16 km² and population is lower than in the Marani highland. The main farming activities are maize, mango and sorghum and animal husbandry. The area has one permanent and several seasonal rivers. Kombewa has two rainy (April–July and August–October) seasons and one dry (November–February) season. The annual rainfall is about 1200 mm; the mean minimum, mean average and mean maximum temperatures are 18.5, 29.3 and 35°C respectively.

Weather data

Weather stations had been set up at both Kombewa and Marani for collecting daily minimum and maximum temperatures, relative humidity and rainfall. The average monthly temperature, maximum and minimum had been calculated. The amount of rainfall was estimated as the sum of daily rainfall.

Land use types

Different land use types of the study areas had been categorized as: farmland referring to area under cultivation; cultivated swamp corresponding to reclaimed land under cultivation; pasture that is characterized by the presence of grass or shrubs for animal grazing; swamp, land characterized by water logging; road; river referring to river bank and forest that is an area with either indigenous or exotic tree stands with >75% of canopy cover. Larval breeding habitats include drainage ditches, foot prints (human and animal foot prints), man-made pools, tyre tracks, natural pools, roadside ditches and rock pools.

Larval sampling

Both the study sites were surveyed and all potential aquatic habitats were geo-located using a Trimble GPS (Trimble Inc.) connected to iPAQ PDA (IPAQ Inc.). Malaria vectors were examined. For this purpose, a sample of 40 dips (maximum, depending on the amount of water) was collected from each potential breeding habitat using a standard 350 ml plastic dipper. The presence or absence of anopheline larvae was noted and if no larva was present, the habitat was considered negative. Samples of mosquito larvae and pupae were collected²⁷. The larvae were counted, put in vials and taken to the laboratory where they were identified morphologically to species level using the microscope according to Gilles

and De Meillon²⁸. The following environmental variables were recorded: length and width of the habitat, land use type, habitat type, height above the sea level and coordinates. The distance to the nearest river was calculated using the Geographical Information System (GIS), particularly ArcGIS 9.3.1 (ESRI, Inc). *Anopheles gambiae s.l.* and *An. funestus* larvae were further identified using polymerase chain reaction (PCR)²⁹. The samples were collected during the months of February (dry season), May (long rainy season), August (short rainy season), and November (dry season) 2004 at both the study sites.

Data analysis

JMP SAS software (SAS Institute Inc.) was used for data analysis. The statistical distribution of data was determined and the natural log transformation was applied, in case the data were not normally distributed. Yates Chi-square test was performed to determine the occurrence of anopheline larvae in different land use/land cover types over different sampling periods and same land use/land cover type. Generalized linear model was used to identify the variables associated with the occurrence (presence or absence) of *An. gambiae* larvae during the sampling months at both the study sites. The effect of seasonality was determined through an ANOVA test done using generalized linear model for dry and long rain seasons at both Kombewa and Marani. The occurrence of malaria vectors was used as a dependent variable. The multiple comparisons using Tukey-Kramer tests were performed to determine statistical differences in proportions of positive breeding habitats between different land use/land cover types for the same month (season). However, the comparisons were not done in cases where the sample size was 10 and below. The distance of each larval habitat from the nearest river/stream was estimated using ArcGIS 9.3.1 (ESRI, Inc). The *t*-test was carried out to determine association between anopheline occurrence and elevation, distance from the nearest river /stream and surface area. In case of distance, Chi-square test was further performed to determine the critical distance at which the aggregation of positive larval habitats occurred from the nearest river/stream. The statistical significance was at 0.05 level.

RESULTS

Species composition

A total of 5023 anopheline larvae were counted for the months of February, May, August and November at both the sites—Kombewa (1973 larvae) and Marani (3050 larvae). In Kombewa lowland, *An. gambiae s.l.* was the most abundant (49.1%) in anopheline larvae collected while in the highland of Marani, it was *An. marshallii* (51.3%). Of the four species found in November, only two were present in August (*An. coustani* and *An. marshallii*). Two major malaria vector species were found, *An. gambiae* and *An. funestus*. Of the vector larvae collected, *An. gambiae s.s.* from Marani, and *An. gambiae s.l.* from Kombewa were the most abundant (94.6 and 56.9% respectively).

The proportions of vector species in each land use/land cover type during dry and rainy seasons at both the sites are shown in Figs. 2 and 3. The results revealed that higher proportions of malaria vectors occurred mainly on farmlands and cultivated swamps (82.2% *An. gambiae s.s.* and 5.4% *An. funestus*) in the highland of Marani. However, in the lowland of Kombewa, the farmland had higher proportions of malaria vectors (27.2 and 24.6% *An. gambiae* and *An. funestus* respectively) followed by pastures during the rainy season.

Larval habitat dynamics

A total of 1122 potential breeding habitats were sampled among which 579 (51.6%) were found positive with anopheline larvae. The highland site of Marani comprised 887 potential breeding habitats with 360 (40.6%) positive for anopheline larvae. However, the lowland

site of Kombewa comprised only 235 breeding habitats with 219 (93.2%) positive habitats for anopheline larvae.

In the highland site, sampling period significantly affected the presence of anophelines in the potential breeding habitats ($\chi^2 = 17.8$, $df = 3$, $p < 0.05$). There was a significant statistical difference in the presence of anophelines during the months of February, May and August ($\chi^2 = 13.2$, $df = 4$, $p < 0.05$; $\chi^2 = 11.9$, $df = 4$, $p < 0.05$; $\chi^2 = 23.1$, $df = 4$, $p < 0.05$ respectively). At the lowland site of Kombewa, the occurrence of potential breeding habitats in different land use/land cover types was significantly different for August ($\chi^2 = 10.9$, $df = 2$, $p < 0.05$). However, no significant difference was found in potential breeding habitats during February, May and November ($\chi^2 = 0.3$, $df = 2$, $p > 0.05$; $\chi^2 = 0.9$, $df = 2$, $p > 0.05$ and $\chi^2 = 0.01$, $df = 1$, $p > 0.05$ respectively).

The results show that in the highland of Marani, the number of positive breeding habitats in the farmland was significantly different over the sampling periods of August, February and May ($t = 2.5$, $p < 0.05$, $t = 2.5$, $p < 0.05$ and $t = 2.4$, $p < 0.05$ respectively). A significant statistical difference in the number of positive breeding habitats was also observed in pastures over these sampling periods of August, February and May ($t = 1.5$, $p < 0.05$; $t = 2.8$, $p < 0.05$ and $t = 2.6$, $p < 0.05$ respectively). In addition, the numbers of positive breeding habitats were significantly different over sampling period ($F = 2.9$, $df = 3$, $p < 0.05$) in swamps. However, there was no statistical difference of positive breeding habitats in different land use/land cover types in November ($\chi^2 = 0.82$, $df = 4$, $p > 0.05$) (Table 1). No analysis was done for the road and forest land use types due to less data. In the lowland site, Chi-square showed no statistical difference in the number of positive breeding sites in the same land use/land cover type over the sampling period for all the three land use/land cover types.

At the highland site of Marani, there was significant difference in occurrence of anophelines in habitat types in February ($\chi^2 = 12.7$, $df = 4$, $p < 0.05$). There was, however, no significant difference in proportions of positive breeding habitats in different habitat types during May, August and November ($\chi^2 = 7.44$, $df = 5$, $p > 0.05$; $\chi^2 = 4.5$, $df = 3$, $p > 0.05$; $\chi^2 = 8.5$, $df = 6$, $p > 0.05$ respectively). For both rainy (May) and dry (February) seasons, drainage-ditch habitat type had the highest proportion (50% and 31.6% respectively) of the positive breeding sites (Table 2).

At Marani highland site during the wet season, we surveyed 705 habitats and found 360 (51.1%) positive with anopheline larvae. At the lowland site during the same season we sampled 133 potential habitats and found 124 (93.2%) positive with anopheline larvae. In the dry season at the highland site, the total number of potential habitats reduced to 182 habitats and 53 (29.1%) were found positive, whereas at the lowland site in the dry season, potential habitats dropped to 102, of which 95 (93.1%) were positive with anopheline larvae. At the highland site, farmlands had the highest proportion of positive breeding habitats during both the rainy and dry seasons (79.2 and 88.7% respectively). Most positive anopheline breeding habitats were confined to river valley (>100 m from river) during both dry and rainy seasons (Fig. 4). At the lowland site, most breeding habitats were found in pockets of water pools on the riverbed during the dry season. Natural pool habitat type predominated during both the rainy and dry seasons (47.4 and 95.1% respectively). Distribution of positive breeding sites was dependent on the season. In the dry season (February), these were found in the river beds whereas during the rainy season, most were associated with roads (Fig. 4).

Environmental effects

Distance to the stream: At the highland site of Marani, there was a significant association between the distance from the nearest river/stream and the occurrence of anopheline larvae ($F=1.3$, $df=747$, $p<0.05$). In the lowland site of Kombewa, there was no association between distance from the nearest stream to the occurrence of anopheline larvae ($F=0.14$, $df=1$; $p>0.05$). In both Marani and Kombewa, seasonality influenced the occurrence of anopheline larvae in relation to distance to the nearest river/stream ($F=6.97$, $df=3$, $p<0.05$ and $F=24.5$, $df=3$, $p<0.05$ respectively). In Marani, distance from the river/stream was important during the dry season and long rainy season whereas at Kombewa during the dry season and short rain season. In the highland of Marani during February (dry season), 61 and 83% of breeding habitats were located within 50 and 100 m from the river respectively (Fig. 5). At Kombewa, in February (dry season), 92.2% of the positive breeding habitats were located within a distance of 120 m from the stream (Fig. 6). During the rainy season (May) at Marani, 72% of breeding habitats were located within 50 m from the stream and 93% within 100 m. At the lowland, 61.8% positive breeding sites were located within 520 m, 82.4% within 800 m and 94.1% within 1080 m from the nearest stream. In August (the short rain season) at Marani, 75 and 98% of the positive breeding habitats were located within 50 and 100 m respectively from the river/stream while at Kombewa, 92.2% of positive breeding habitats were located within 280 m from the stream. In November at the highland site, 71.4% positive breeding habitats were located within a distance of 120 m from the river/stream. At the lowland site, 85.3% positive breeding habitats were within a distance of 160 m from the stream.

Fisher's exact test was done to determine aggregation. The results showed that at Marani, positive breeding sites were aggregated within a distance of 5 m from the stream in August ($p<0.05$). However, there was not any aggregation of positive breeding habitats during the months of February, May or November ($r = 1.4$, $df = 1$, $p>0.05$; $r = 1.0$, $df = 1$, $p>0.05$ and $r = 1.4$, $df = 1$, $p>0.05$ respectively). At Kombewa, aggregation of positive breeding habitats was detected within a distance of 100 m (Fisher's exact test, $p<0.05$) in February and within 5 m from the river in August. In May and November, no aggregation was found.

Elevation

Marani: The results revealed that at Marani, the occurrence of anopheline breeding habitats was associated with elevation during all sampling periods except in November while at Kombewa, there was association in August and November but not in February and May (Table 3). At the highland site it was also observed that in February (dry season), 65 and 90% of positive breeding sites were within an elevation of 1580 and 1620 m respectively whereas at the lowland site, 96% of positive breeding sites were within an elevation of 1220 m. In May (long rainy season) at the highland site, 59.5 and 96% of positive breeding sites were within an elevation of 1580 and 1620 m while 80% of positive breeding habitats were located within 1220 m at the lowland site. In August, 70.9 and 95.2% of positive breeding habitats were located within an elevation of 1580 and 1620 m respectively at the highland site and 82.4% positive breeding habitats within an elevation of 1220 m at the lowland site. In November, 72.5 and 96.7% positive breeding habitats were located within 1580 and 1620 m at the highland site and 78.3% positive breeding habitats located within an elevation of 1220 m at the lowland site (Fig. 7).

We found an association between surface area of positive breeding habitats and occurrence of anopheline larvae in February and May; we, however, found no association in August and November. The mean surface area for positive breeding habitats was largest in May and smallest in August.

DISCUSSION

The study has demonstrated that topography, seasonality and land cover/land use types play an important role in the spatial distribution of anopheline breeding habitats in the highland environment. Indeed, at the highland site, anopheline breeding habitats were mostly found in the valley bottom throughout the sampling period. During the dry season, larval breeding habitats were exclusively confined to the river valley. Topography and man-made features affect the formation of larval breeding habitats at highland site. The highland is characterized by hills interspersed with valleys. The surface run-off water always drains downstream due to gravity thus accumulates in the valley bottoms creating potential mosquito breeding habitats.

In the lowland site, seasonality was the main determinant of spatial distribution of breeding habitats. In fact, because the land is generally flat in the lowland, during the rainy season, water mainly accumulates alongside roads. Breeding habitats are scattered all over the study area. During the dry season, water dries up in most seasonal streams while the water level tremendously recedes in the permanent river leaving small puddles of water on the riverbed, which becomes colonized by anopheline mosquitoes. The larval breeding sites are aggregated on the riverbed.

The results suggest that land-use types affect the availability and suitability of anopheline breeding habitats at the highland site. Farmlands land cover/land-use types had the highest proportions of potential and positive anopheline breeding habitats during both dry and rainy seasons. In the farmlands, *An. gambiae s.s.* larvae proportion was the highest suggesting better conditions for *An. gambiae s.s.* survival. The removal of vegetation cover of natural swamps increases the microhabitat temperature, which reduce developmental period of immature aquatic stages and improve the nutrient quality of the habitat. Land under high vegetation cover has low water temperature but the cultivation enables the penetration of direct sunlight which increases water temperature. Minakawa *et al*³⁰ in another study demonstrated that development of *An. gambiae s.s.* to adults was shorter in cultivated swamps than natural swamps. In the same study, the survival of immature aquatic stages of *An. gambiae s.s.* was higher in the cultivated swamps than natural swamps. Munga *et al*³¹ demonstrated that emergence of adult *An. gambiae s.s.* only occurred in farmlands and not in natural swamps. These findings concur with the hypothesis that land use change increases the availability of potential larval habitats and improves their quality making them more suitable for the breeding of malaria vector mosquito in the highland regions. In the lowland environment, factors that catalyze surface water collection were the main determinants.

The study has also demonstrated that *An. gambiae s.s.* known to colonize small open sun-lit temporary pools²⁰⁻²² can also thrive in semi-permanent breeding habitats. In Marani, the highest proportion of *An. gambiae s.s.* was found in drainage ditches that are characteristic of cultivated swamps. The water level of these habitats highly fluctuates with seasons. *Anopheles gambiae* being an opportunistic species³² colonizes the habitats once they have been created during cultivation. It is important to note that the main malaria transmission season coincides with the crop-planting season, when most of the land is under cultivation. The opening of drainage ditches during this cultivation period favours the proliferation of algae that constitute food nutrients for mosquito larvae²². From these results we suggest that there could be other major paramount factors that determine the suitability of habitats to be colonized by *An. gambiae* other than size and nature (temporary, semi-permanent or permanent). In the lowland site *An. gambiae s.l.* was always found in permanent habitats.

The results suggest that targeted larval control activities can be undertaken during the dry and short rainy seasons because larval breeding habitats are just confined to small areas in

the valley bottoms. This makes the operation of larval control cost-effective. Furthermore, during these seasons, at both the highland and lowland sites, the breeding habitats are stable and that enhances the effectiveness of larvicides.

CONCLUSION

The study has demonstrated that cultivation of swamps increase the number of breeding habitats and improves their quality making them more suitable for anopheline larval colonization and survival on farmlands. The outcome is increased malaria transmission in areas where the initial conditions did not favour the development of malaria vectors.

Acknowledgments

This paper is published with the permission of the Director, Kenya Medical Research Institute. We acknowledge Ben Omboko and Paul Ochiele for technical assistance. The work was supported by NIH grants R01A150243 and D43 TW01505.

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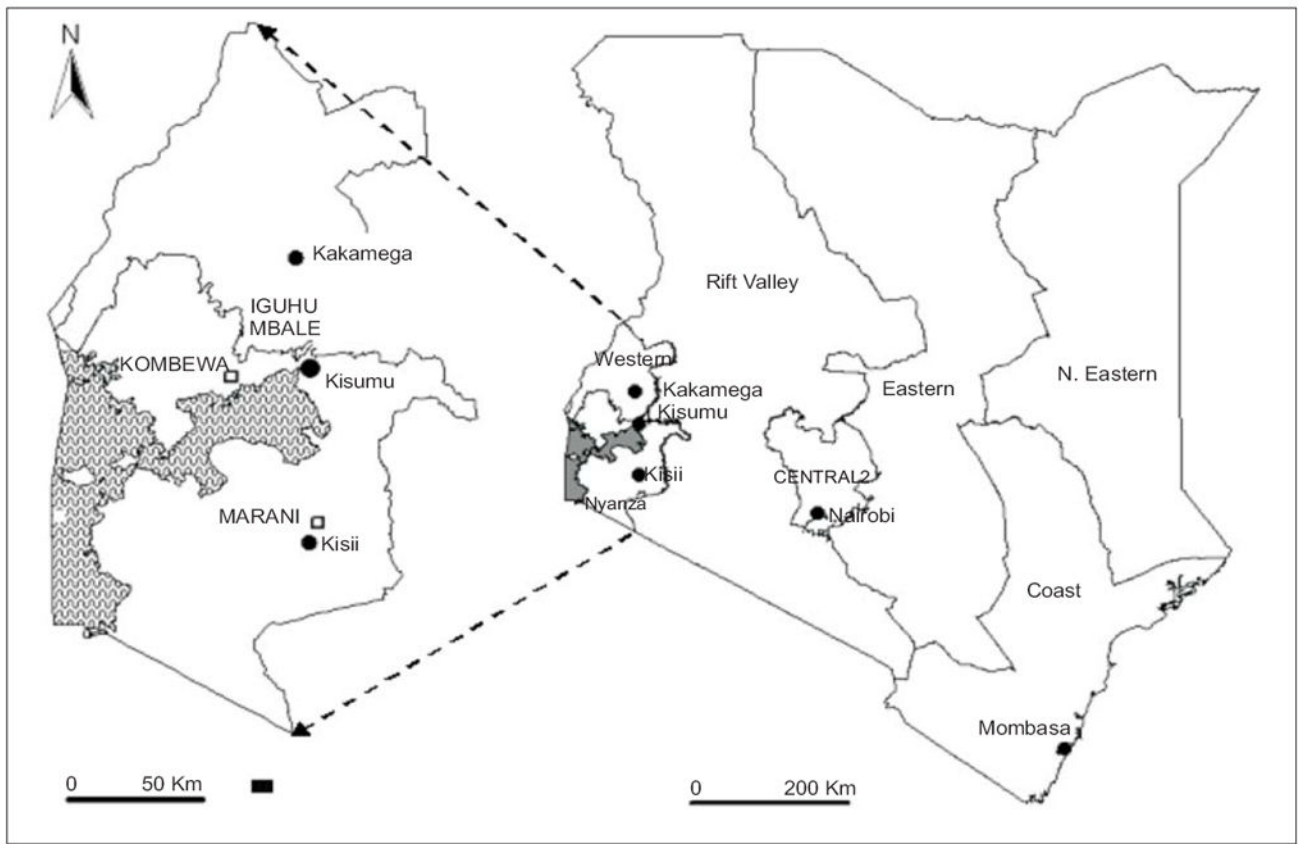


Fig. 1.
Map showing the study sites.

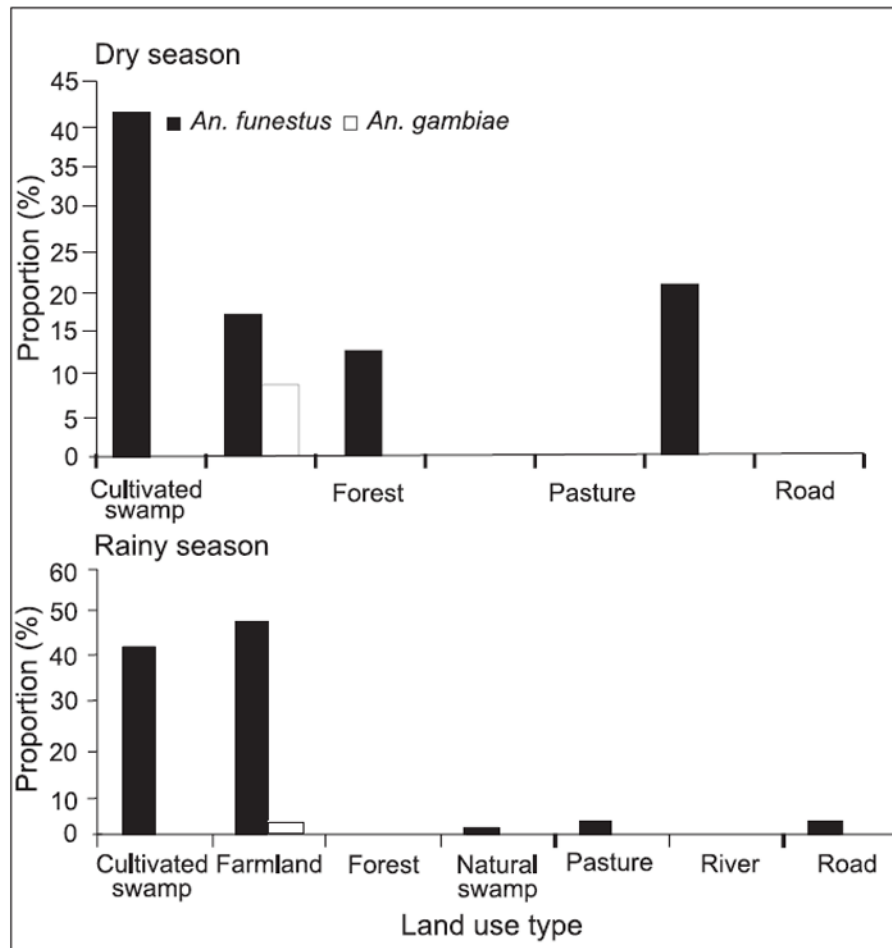


Fig. 2. Proportion of malaria vectors in different land use/land cover types at Marani during dry season.

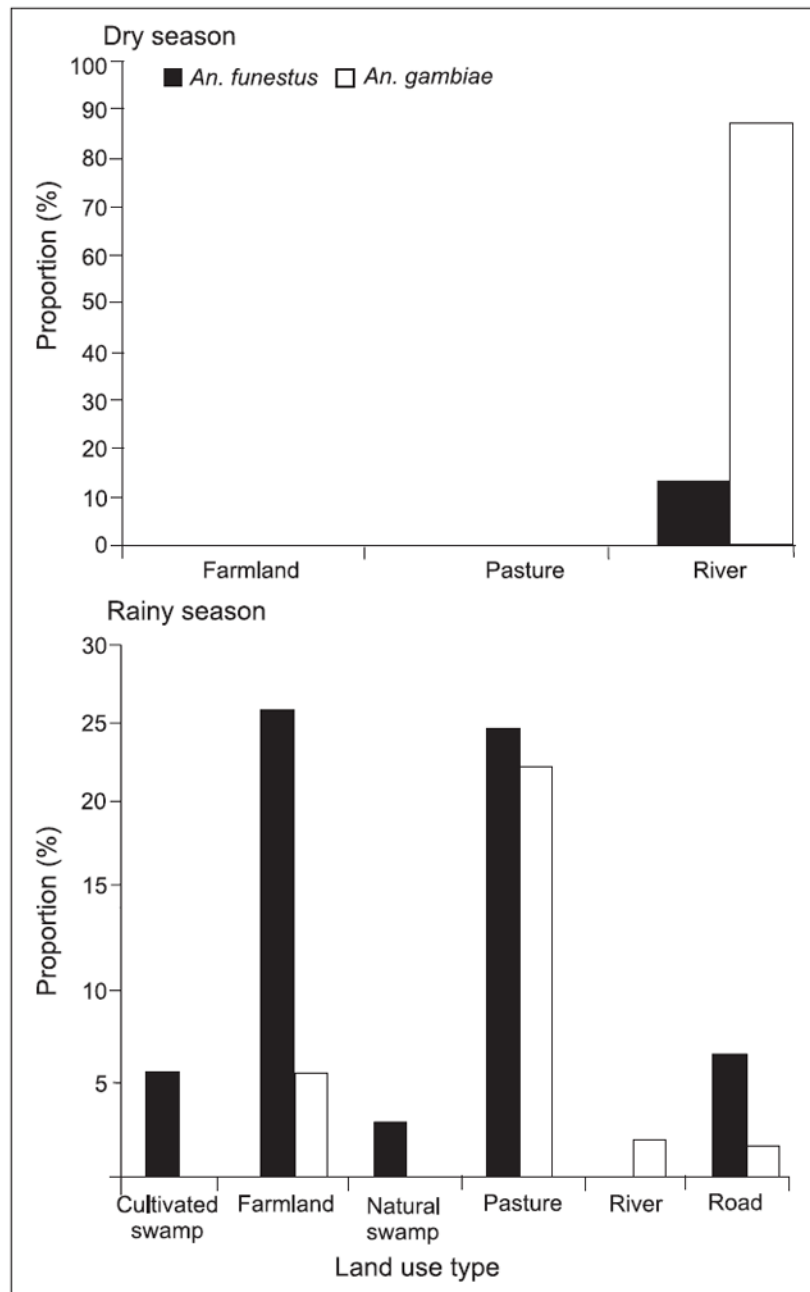


Fig. 3. Proportion of malaria vector species in different land-use types at Kombewa during rainy season.

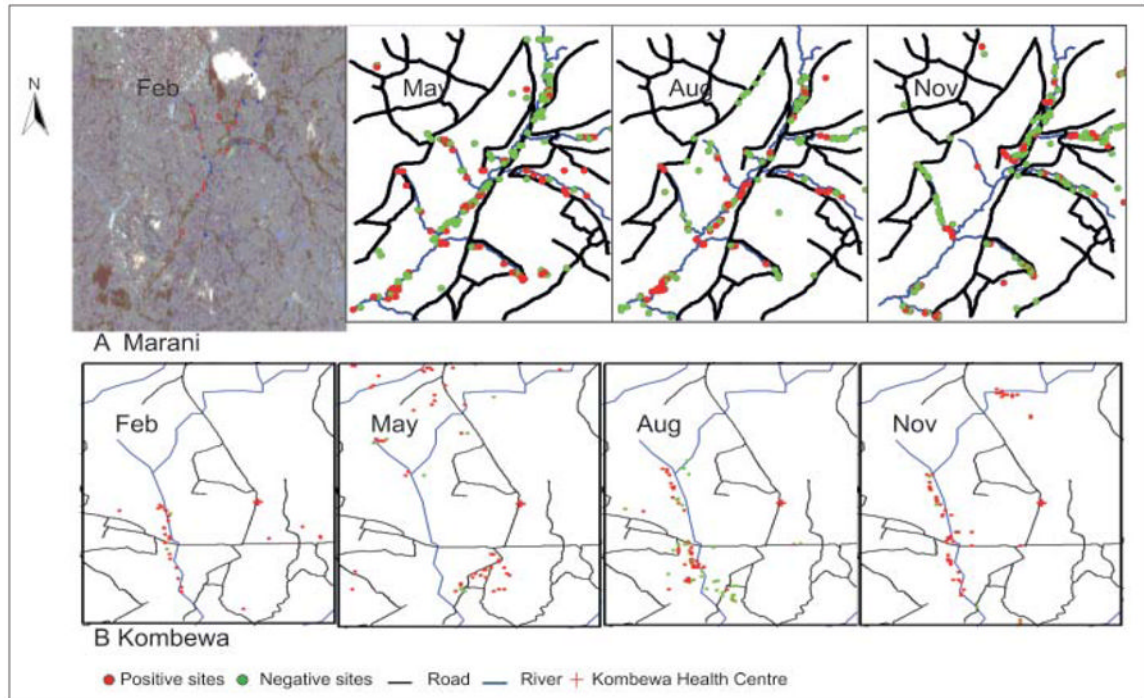


Fig. 4. Distribution of anopheline breeding sites at Marani and Kombewa in 2004.

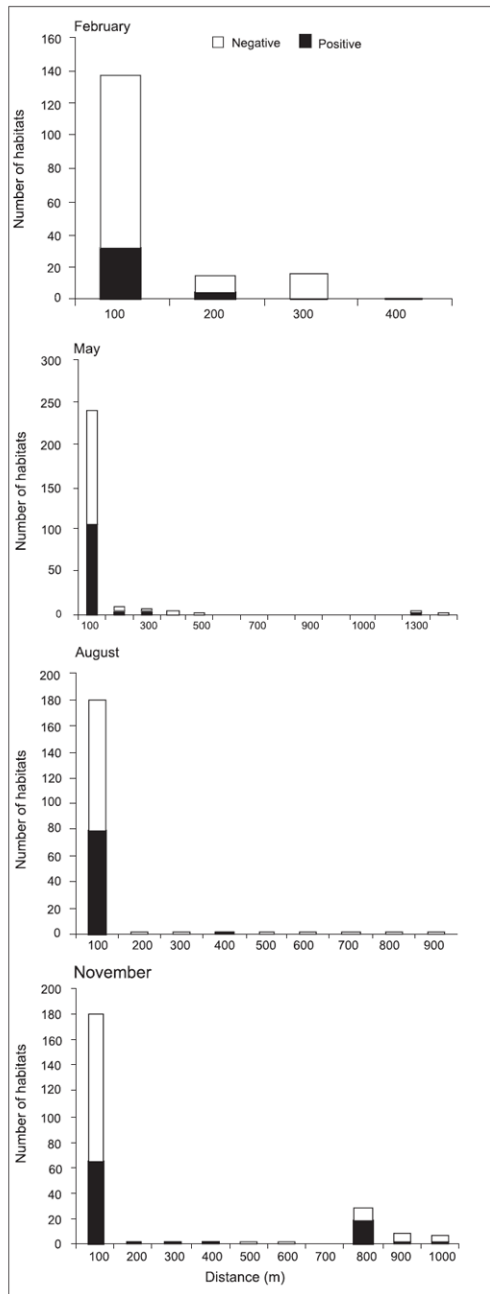


Fig. 5. Distribution of larval habitats with reference to distance from the nearest stream at Marani.

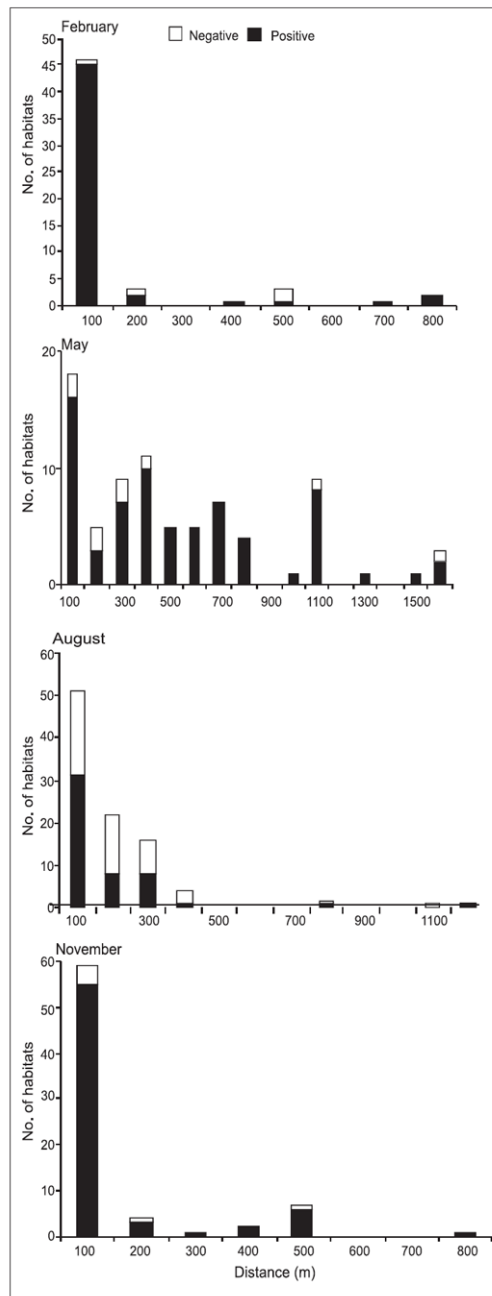


Fig. 6. Distribution of larval habitats with reference to distance from the nearest stream at Kombewa.

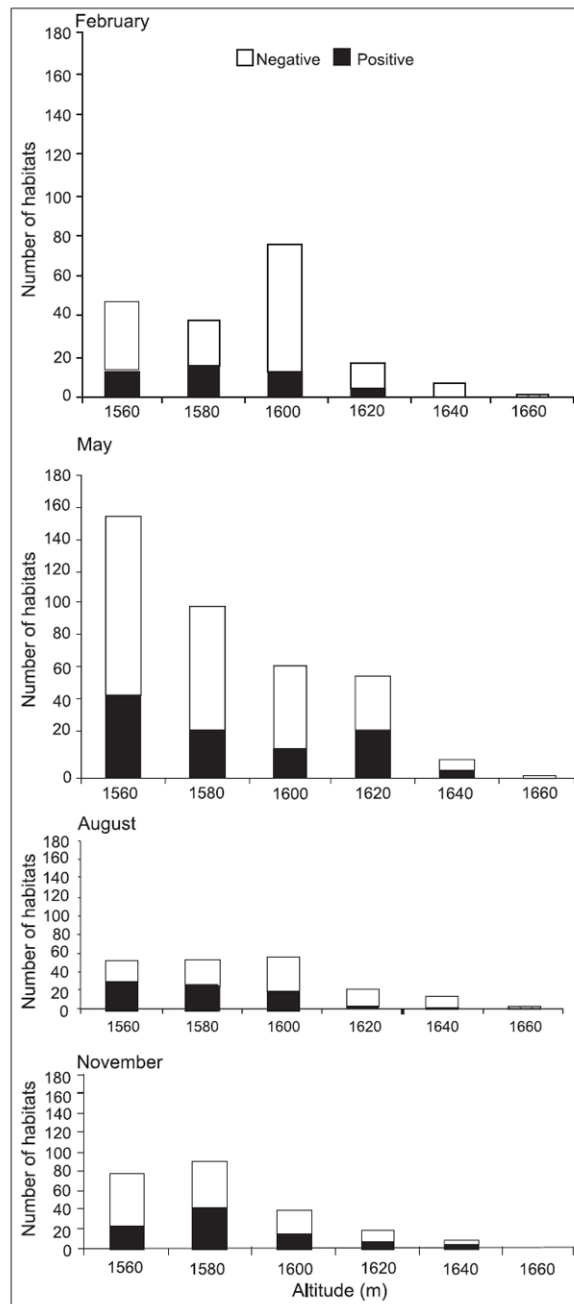


Fig. 7. Distribution of breeding sites against height above sea level at Marani in 2004.

Table 1

Occurrence of breeding sites in different land use/land cover types in February, May, August and November 2004 at Marani and Kombewa

Month	Habitats	Cultivated swamp				Marani				Kombewa			
		Farm land	Forest	Natural swamp	Road	Pasture	River	Road	Farm land	Natural swamp	Pasture	River	Road
February	No. of sites	196	5	22	4	39	70	4	2	0	1	25	0
	(+)ve habitats	116(59.2) ^b	1 (20)	6 (27.3)	0(0)	8 (20.5)	21 (30) ^{ab}	0(0)	2 (100)	0 (0)	1 (100)	23 (92)	0 (0)
May	No. of sites	187	4	53	14	57	20	14	58	4	72	8	8
	(+)ve habitats	53 (28.3) ^b	0 (0)	8 (15.1)	2 (14.3)	14 (24.6) ^{ab}	1 (5)	6(75)	50 (86)	4 (100)	66 (92)	6 (75)	8 (100)
August	No. of sites	128	13	66	28	54	51	8	13	0	22	47	8
	(+)ve habitats	64 (50) ^a	5 (38.5)	20 (30.3) ^b	1 (3.6)	11 (20.4) ^b	13 (25.5) ^b	5(63)	8(62)	0 (0)	5(23)	30 (64)	3 (37)
November	No. of sites	116	36	59	0	36	11	0	3	14	57	0	0
	(+)ve habitats	69 (45.7) ^a	10 (27.8) ^{ab}	25 (42.4) ^a	0 (0)	10 (27.8)	2 (18.2)	0 (0)	3 (100)	12 (86)	54 (95)	0 (0)	0 (0)

* Letters following positive proportions show results of Turkey multiple comparison tests; Value with different letters were significantly different at $p=0.05$ level; Figures in parentheses indicate percentages.

Table 2
Occurrence of breeding sites in different habitat types in Marani for February, May, August and November 2004

Month	Status	Drainage ditch	Foot prints	Manmade pool	Natural pool	Pond	Rock pool	Spring
February	No. of sites	95	12	26	34	0	6	9
	(+) ve habitats	30 (31.6) ^a	1 (8.3)	2 (7.7)	7 (20.6)	0 (0)	0 (0)	5 (55.6)
May	No. of sites	92	16	42	116	5	0	1
	(+) ve habitats	46 (50) ^a	7 (43.8)	21 (50) ^a	39 (33.6) ^a	3 (60)	0 (0)	1 (100)
August	No. of sites	62	8	57	38	0	4	0
	(+) ve habitats	29 (46.8) ^a	3 (37.5)	33 (57.9) ^a	14 (36.8) ^a	0 (0)	0 (0)	0 (0)
November	No. of sites	72	31	33	54	14	7	23
	(+) ve habitats	30 (41.7) ^a	7 (22.6)	10 (30.3)	28 (51.9) ^a	6 (42.9)	1 (14.3)	9 (39.1)

* Letters following positive proportions show results of Tukey multiple comparison tests; Values with different letters were significantly different at $p = 0.05$ level; Figures in parentheses indicate percentages.

Average elevation, distance from nearest stream, surface area, habitat number (%) and t-test results for association of variables to anopheline occurrence at Marani and Kombewa in 2004

Table 3

Month	Marani				Kombewa						
	Presence	Absence	t-value	df	p-value	Presence	Absence	t-value	df	p-value	
February	Elevation (m)	1571.2 ± 3.7	1581.2 ± 2.1	2.3	79	< 0.05	1197.4 ± 4.7	1178 ± 9.6	1.8	5	> 0.05
	Distance (m)	50.7 ± 10	70.5 ± 6.7	1.2	155	> 0.05	103.5 ± 23.3	254.1 ± 104.1	1.4	3	> 0.05
	Surface area (m ²)	3.4 ± 0.5	6.1 ± 1.3	2	169	< 0.05	12 ± 1.4	5.8 ± 1.9	2.9	7	< 0.05
	Habitats	53 (29.1)	129 (70.9)				26 (92.9)	2 (7.1)			
May	Elevation (m)	1572 ± 2.6	1567 ± 1.7	1.7	209	< 0.05	1191.3 ± 3.7	1205 ± 8.9	1.4	11	> 0.05
	Distance (m)	53.7 ± 11.7	112.2 ± 21.6	2.4	233	< 0.05	489.1 ± 48	427.6 ± 175	0.4	9	> 0.05
	Surface area (m ²)	25.8 ± 17.2	8.7 ± 2.9	1	128	> 0.05	27.4 ± 6.9	9.5 ± 3.4	2.1	71	< 0.05
	Habitats	132 (48.5)	140 (51.5)				70 (88.6)	9 (11.4)			
August	Elevation (m)	1571.4 ± 2.6	1585 ± 2.5	3.7	188	< 0.05	1200.8 ± 2.7	1192 ± 2.4	2.3	94	< 0.05
	Distance (m)	81.1 ± 46.9	108 ± 17.7	0.9	123	> 0.05	150.9 ± 26.3	170.4 ± 27.3	0.8	94	> 0.05
	Surface area (m ²)	16.1 ± 3.5	27.2 ± 17.7	0.6	124	> 0.05	11.5 ± 1.9	10 ± 3.2	0.4	77	> 0.05
	Habitats	84 (42.2)	115 (57.8)				54 (1000)	0 (0)			
November	Elevation (m)	1573.3 ± 2.4	1569.9 ± 2	1.1	196	> 0.05	1200.7 ± 4.6	1177.8 ± 11.7	4.1	69	< 0.05
	Distance (m)	232.4 ± 34.7	162 ± 23.4	1.7	169	< 0.05	110.4 ± 17	128.46 ± 59	0.3	6	> 0.05
	Surface area (m ²)	10.5 ± 1.3	6.4 ± 0.7	2.7	139	< 0.05	17.3 ± 2	12.1 ± 9	0.6	4	> 0.05
	Habitats	91 (38.9)	143 (61.1)				69 (93.2)	5 (6.8)			

Figures in parentheses indicate percentages.