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Larval habitats of anopheline vectors of malaria on the highlands of Mambilla Plateau Taraba State North East Nigeria

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Abstract
Identification of larval habitats on the highlands of Mambilla Plateau and mosquito tolerance range to physico-chemical parameters may be helpful to control insurgent of the vector mosquitoes and to put in place mechanisms to prevent epidemics. This study was conducted to determine and characterize the larval habitats of anopheline vectors of malaria in five altitudinal locations on the highlands of Mambilla Plateau. Larval density for each study location was determined from the breeding index using the formula: BI= TLP/ND×BP, where BI= breeding index, TLP= total number of larvae and pupae sampled, ND= number of dips and BP= number of breeding places/sampling sites. Logistic regression analysis was used to determine relationship between larval abundance and altitude as well as mean physicochemical parameters. Larval mosquito abundance per study location was recorded and compared. In all, 60 larval habitats were sampled only six were productive for anopheline larvae these were from three altitudinal locations. About 179 larvae were collected from breeding habitats and reared to adulthood they comprised of four species; *Anopheles gambiae*, *An. coustani*, *An. funestus* and *An. pharoensis*. Mosquitoes thrived in still, clear sunlit temporary pools and swamps with wide range of Physico-chemical parameters. There was no significant relationship between larval abundance and physicochemical parameters as well as altitude $p<0.05$. Altitude and physicochemical parameters of the larval habitats should be considered in planning and implementing targeted larval mosquito control programmes in the highlands.

Keywords: larval habitat, mambilla plateau, nigeria, *Anopheles*

1. Introduction

One of the efforts to control malaria is reduction in the mosquito vector-humans contacts [1].Mosquito larval habitats are places where mosquitoes breed, from the point of egg laying to completion of the life cycle, to emergence as adults [2]. The larval habitat is therefore very vital for mosquito population dynamics because the development and fitness of the adult mosquitoes are impacted by the status of their aquatic larval habitats [3]. Larval habitats are made up of diverse water bodies ranging from temporary to permanent, natural to artificial, small to large fresh water to salt water, shaded or sunny [4,5]. A greater number of anopheline species have been observed to breed in a broad range of habitats [6] but each species shows preference for various breeding site for oviposition [7].

Larvae of *An. gambiae s.l.* can be found in both temporary and permanent habitats [9] in large bodies of water such as flood plains [10] and in pools along lake shores [11]. Sibling species of the *An. gambiae* complex differ in their preference for breeding habitats, *An. melas* and *An. merus*, are most productive around the coastal areas where they exhibit great tolerance for salinity, surviving in relatively high degrees of salinity sometimes reaching or even exceeding that of sea water [8].Quality of the breeding habitat and physicochemical parameters among others are factors that impact on larval development and growth [12]. The understanding of each species threshold to environmental variables and habitat adaptation across altitudinal gradients is one potentially significant point in malaria vector control in the highlands this may provide useful insight into mosquito congregation[25].

Until now, the larval habitats and the physicochemical parameters that influence anophelines mosquitoes in their breeding habitats on the Mambilla Plateau are unknown therefore knowledge acquired from the current study could help to develop an effective measure to control malaria through vector management and to put in place mechanism to prevent epidemics.

2. Materials and Methods

2.1 Study area

Mambilla Plateau is located at longitude 6.8212̊ N, 11.5345̊ E and latitude 7.3523̊ N, 10.7723̊ E in Taraba state North-Eastern Nigeria with an area of about 3765sq km while the adjoining lowland covers about 1,250sqkm. It has boundaries with Gashaka Local Government Area (LGA) in the north-east, Kurmi Local Government Area in the north-west and Republic of Cameroun by an international boundary in the south. It is located in a savannah landscape with a peculiar topography and climate. The topography of Mambilla Plateau comprises undulating lowland, low hills and irregular plains, ridges, hills, and escarpment. Climate is semi-temperate with mean annual temperature of 16̊ C. Mambilla Plateau has an average altitude of 1600m above sea level. Mean annual rainfall is 1800mm; the rainy season extends from early March to October while a short dry season occurs between November and February.

Mambilla Plateau has a population of 224,357 people [13]. The people engage in agriculture and stock herding. Crops grown on the Mambilla Plateau include; maize, millet, sorghum, sweet potatoes and Irish potatoes. Tea, coffee, cocoa Avocadoes and pears are also grown on the Mambilla Plateau. The study was conducted in five communities namely; Nguroje (N06˚57'50.1''E0 11˚07'00.7''; elevation 1885m above sea level), Yelwa ((N07˚05'10.5'' E011˚05'27.9''; elevation 1674m above sea level), Gembu ((N06˚42.640 E011˚15.486''; elevation; 1584m above sea level), Kakara ((N06˚49'23.8''E011˚07'26.7''; elevation 1496m above sea level) which are located on the highlands of Mambilla Plateau while Mayo-selbe ((N07˚16'54.8''E011˚81'14.5'' elevation; 484m above sea level) located at the foot of the mountain.

2.2 Sampling Design

Various anophelines larval habitats were sampled in all the five altitudinal locations.The habitats include ponds, wells, swamps, dug pits, rivers and streams among others. These were visited once every month from November 2016 to October 2017 geographical positions and elevations of each habitat were recorded using Global Positioning System (GPS Garmin Oregon 550).

2.3 Characterization of larval habitats

Characterization of larval habitats was carried out by observing and recording the features of the different breeding sites present within the sampling locations, for example habitat permanence, the presence or absence of vegetation, water condition and exposure to sunlight. Light intensity as either shady or light, area of the larval habitat covered by shade was visually estimated as percentage canopy cover. Water current was determined by visual scrutiny as still or slow-flowing. Other parameters were measured using hand-held digital meters, temperature with thermometer, pH with Wagtech pH meter, electrical conductivity and total dissolved solids with Wagtech conductivity/TDS meter, turbidity with Wagtech turbidity meter, salinity and dissolved oxygen with Horiba Dip Electrode.

Water samples were collected from the various habitats in 1litre plastic bottles tightly closed and labeled according to altitude, location, coordinates and date of collection. The water samples were transported to Taraba state ministry of water resources and analyzed for other parameters such as; alkalinity, hardness, dissolved oxygen, sulphate, iron, lead, arsenic and nitrate with Wagtech photometer.

2.4 Larval collection and rearing

Larval collections were done from various aquatic habitats inall the communitiesusing the standard dipping method [14] from between 0.8am to 10.00am. In all the locations where larvae were sighted larvae of all available instar were collected anopheline larvae were distinguished from culicines based on their resting habits in water and the siphon. The edge of the dipper was submerged, dipped at about 45 degrees about an inch below the surface of the water quickly but gently, the dipper was moved along a straight line in the water. The stroke was ended just before the dipper was filled to avoid overflowing. The dipper was then gently raised out of the water without spilling the water and the larvae.Depending on the size of each larval habitat, about 10-30 dips were taken at intervals along the edge for about 30minutes at each larval habitat [15] and transferred into plastic containers along with the breeding sites water and taken to the laboratory to be reared.

To rear the mosquitoes, larvae from the field were transferred into small white transparent plastic buckets filled to two-thirds of their volume with the breeding site water. The mouths of the plastic buckets were covered with mosquito net a small hole was made at the centre of the net and plugged with a dry cotton wool until adults emerged [14]. Larvae were fed with baker's yeast.

2.5 Data analysis

Larval density for each study location was determined from the breeding index according to the formula of Belkin: BI= TLP/ND×BP, where BI= breeding index, TLP= total number of larvae and pupae sampled, ND= number of dips and BP= number of breeding places/sampling sites. Logistic regression analysis was used to determine relationship between larval abundance and altitude as well as mean physicochemical parameters. The significance level was considered at $p<0.05$.

3. Results and Discussion

A total of 60 larval habitats were sampled these include swamps15 (25.00%), old tyres 9(15.00%), streams and wells7 (11.67%) each, dug pits6 (10.00%), rivers 5(8.33%), building sites 4(6.67%), ponds and rock pools 3(5.00%) and tree trunk 1(1.66 %). The highest number of habitats 17(28.33%) were observed in Yelwa, Gembu and Mayo-selbe had 12(20.00%) each, Nguroje 11(18.33%) while Kakara had the least 8(13.33%).This is indicated in Table 1.

About 179 larvae were bred to adulthood they comprised of four species *An. gambiae*113 (63.13%), *An. coustani* 33(18.44%), *An. funestus* 20(11.17%) and *An. pharoensis* 13(7.26%). Gembu recorded the highest number of larvae 101(56.43%), Nguroje 57(31.84%) and Kakara 21(11.73%).

Larvae were sampled from only three altitudinal locations, Gembu, Nguroje and Kakara.Gembu recorded the highest larval presence 101(56.43%), Nguroje 57(31.84%) and Kakara 21(11.73%). Only six of the larval habitats were positive for anophelines larvae breeding index was in the range of 0.0 to 20.2 (Table 2). There was variability in the spatial distribution of anopheline larval populations. Larvae were associated with temporary habitats such as pits that were dug for blocks moulding and in clear, sunlit stream edges and swamps. They also had preference for habitats with wide range of Physico-chemical parameters and thrived in habitats with temperature range of 21.39±2.93-25.98±3.04, pH 6.52±.87-7.11±.56, Electrical conductivity (EC) 43.35±34.31-102±53.44, Total Dissolve Solutes (TDS)21.85±17.11-50.45±26.30, Turbidity 3.53±1.19-22.88±41.41, Hardness 27.57±13.01-41.00±8.20, Salinity 14.36±7.71-26.33±19.44, Alkalinity 15.14±7.51-21.66±10.22, Dissolve oxygen**(**DO**)** 5.57±1.13-5.66±1.66, Nitrates(NO_3^-)15.37±14.35-29.68±25.75, Sulphates (SO_4^{2-}**)**5.54±3.18-14.71±9.03, Iron (Fe).06±.06-.12±.10, Lead (Pb)00±.00-.01±.00, Arsenic (As).00±.00. This is represented in Table 3.There was no significant relationship between larval abundance and physicochemical parameters and altitude at $p<0.05$.

Table 1: Larval habitats of anopheline vectors of malaria along altitudinal locations on the highlands of Mambilla Plateau

	Larval habitat										
Location	**Dug pit**	**Pond**	**River**	**Stream**	**Swamp**	**Well**	**Tree trunk**	**Old tyres**	**Rock pools**	**building sites**	**Total**
Nguroje	2	1	0	1	4	3	0	0	0	0	11
Yelwa	2	0	0	3	2	1	0	9	0	0	17
Gembu	0	1	0	2	6	3	0	0	0	0	12
Kakara	2	1	0	1	3	0	1	0	0	0	8
Mayo-selbe	0	0	5	0	0	0	0	0	3	4	12
Total	6	3	5	7	15	7	1	9	3	4	60

Table 2: Larval abundance, species composition and breeding index of anophelines along altitudinal locations on the highlands of Mambilla Plateau

	Species/Number of larvae					
Study locations	***An. gambiae***	***An. coustani***	***An. funestus***	***An. pharoensis***	**Total (%)**	**Breeding index**
Nguroje	47	7	1	2	57((31.84)	11.4
Yelwa	-	-	-	-	0(0.00)	0.0
Gembu	52	21	17	11	101(56.43)	20.2
Kakara	14	5	2	-	21(11.73)	4.2
Mayo-selbe	-	-	-	-	0(0.00)	0.0
Total	113	33	20	13	179(100)	35.8

There was a relatively low occurrence of positive breeding sites along the altitudinal locations only six out of the 60 breeding sites were positive for anopheline larvae. This could be due to their temporary nature and variability. In the dry season the anophelines larvae survived and proliferated by breeding in temporary stream edges and man-made habitats such as pits that were dug for moulding blocks in the rainy season however these were replaced by swamps that had formed from the rain pools. Most of the streams were overgrown with tall grasses and the temporary pits, filled up with refuse. This finding concurred with Aditya, *et al*., [25] who observed that the mosquito habitats in the hill town of Darjeeling were temporary in nature and existed only for a brief period of time. In the dry season, larvae were collected only in Nguroje and Kakara but in the rainy season they were collected only in Gembu and none in other altitudinal locations especially Mayo-selbe which is a lowland and recorded highest number of adult mosquitoes. This finding contrasts with the impression that flat areas provide more breeding sites than steep slopes [21].

The non-availability of larvae in Mayo-selbe both in the dry and rainy seasons could be attributed to elevated temperatures which resulted in shorter development time of larvae therefore more adult presence. Immature stages and adults of mosquitoes are sensitive to temperature changes in their aquatic environment. Water temperature of above 34ºC generally has a negative impact on the survival of vectors [22, 23]. It could also be due to the fast flowing nature of the rivers in Mayo-selbe which may not be conducive for the adult female *Anopheles* mosquitoes to breed.

Larvae were found breeding in only one site in the rainy season as compared with five during the dry season the larvae might have been washed away by the heavy rainfall as mosquito larvae were observed to prefer still, clear and sunlit temporary habitats. In Kakara most of the breeding sites were observed close to the forest with banana and tea plantations about 50m away from houses and therefore beyond flight distance of the adult mosquitoes [18] these temporary breeding sites were cultivated during the rainy season in Nguroje they were located just within 7m of residential houses and were also cultivated into vegetable gardens this could account for their absence during the rainy season. In rural Gambia, a significant decline in the number of mosquitoes with distance to nearest breeding habitats was observed in agreement with the present study [19]. It is suggested that for female mosquitoes seeking blood meals orientation towards a host accounts only for short range movement of over 30m or less[19] and mean flight range of female *An. gambiae* was estimated at 0.64miles [20].

Table 3: Average Physicochemical Parameters of larval habitats on the highlands of mambilla plateau

	Elevation	**Temp**	**pH**	**EC**	**TDS**	**Turbidity**	**Hardness**	**Salinity**	**Alkalinity**	**DO**	**NO_3^-**	**SO_4^{2-}**	**Fe**	**Pb**	**As**
Nguroje	1748 ±12.18	25.98 ±3.04	6.52 ±.87	102 ±53.44	50.45 ±26.30	3.53 ±1.19	39.90 ±13.23	14.36 ±7.71	17.63 ±8.84	5.63 ±1.28	15 ±14.35	5.54 ±3.18	.12 ±.10	.01 ±.00	.00 ±.00
Yelwa	1641 ±25.23	25.33 ±3.40	6.82 ±.62	44.56 ±31.28	22.50 ±15.47	4.31 ±2.49	30.37 ±11.74	11.62 ±4.80	15.62 ±6.30	7.00 ±2.97	34.17 ±22.75	6.75 ±4.55	.03 ±.02	.02 ±.00	.00 ±.00
Gembu	1548 ±6.02	21.39 ±2.93	7.11 ±.56	83.89 ±67.46	42.16 ±33.52	22.88 ±41.41	41.00 ±8.20	26.33 ±19.44	21.66 ±10.22	5.66 ±1.66	26.50 ±28.42	9.75 ±5.47	.12 ±.10	.00 ±.00	.00 ±.00

Kakara	1468 ±6.21	23.75 ±2.48	6.69 ±.61	43.35 ±34	21.85 ±17.11	14.54 ±29	27.57 ±13.01	16.28 ±11.94	15.14 ±7.51	5.57 ±1.13	29.68 ±25	14.71 ±9.03	.06 ±.06	.01 ±.00	.00 ±.00
Mayo Selbe	465 ±5.27	31.92 ±1.70	5.88 ±.22	87.02 ±46.	43.80 ±22.86	5.36 ±1.32	51.80 ±28.02	13.60 ±2.70	16.00 ±3.16	8.60 ±1.81	45.60 ±8.2	9.60 ±2.30	.02 ±.01	.00 ±.00	.00 ±.00

In the current study temperature of water in the breeding habitats at the time of sampling ranged from 21.39°C ±2.93to 31.90°C±1.70 and pH of the breeding habitats varied from 5.88± .22to 7.11±.46. Earlier studies from other parts of Nigeria and Africa had reported temperatures and pH within the same range as the present study. Adebote, *et al*., [24] reported temperature range of 14 to 40°C and pH range of 5.86 to 9.85, Afolabi, *et al*., [12] recorded temperatures of 26.5 to 29.3°C and pH 7.1 to 7.3, Dida, *et al*., [25] recorded a pH range of 6.7 to 8.4 along the Mara River in Kenya.
The anopheline larvae were associated with temperature range of 20.7 to 28.0°C and pH 5.8(slightly acidic) to 7.8(slightly alkaline) this is similar to study by Hanafi-Bojd, *et al*.,[8] who found malaria vectors breeding in habitats of between 20°C - 30°C. Larvae thrived in habitats with 6 mg/L dissolved oxygen this was the amount of DO in all the habitats in the three altitudinal locations from where larvae were sampled that is Nguroje, Gembu and Kakara this concurred with 6.4mg/L recorded by [25]in Kenya. Electrical conductivity in the range of 14.5mg to 159mg/L, total dissolved solids 8 to 87mg/L and turbidity was 1.4 to 138mg/L.

4. Conclusion
There was a relatively low occurrence of positive breeding sites along the altitudinal locations and with wide range of physico-chemical parameters. This study has provided baseline information on larval habitats and physico-chemical parameters of the larval habitats on the Mambilla Plateau that would be helpful for the sustainable management of vector mosquitoes and to take precautionary measures against malaria epidemics. Altitude and physicochemical parameters of the larval habitats should be considered in planning and implementing targeted larval mosquito control programmes in the highlands.

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