EFFECT OF LIVESTOCK, HUMAN HOST AND AQUATIC HABITAT DISTRIBUTION ON THE ABUNDANCE OF *ANOPHELES GAMBIAE* COMPLEX IN BORNO STATE ARID ZONE OF NIGERIA

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Abstract. A study was conducted in Maiduguri, the capital city of Borno state of Nigeria to investigate the influence of livestock, human hosts and larval habitat distribution on the abundance of *Anopheles gambiae* complex in a home. Both larvae and adult of anopheles species were collected in the beginning and late rainy season 2008 and dry season 2009 using standard methods. The results showed that *Anopheles gambiae s.s* was the predominant species in both larvae and adult samples in all the sample periods. Statistical analysis detected significant difference between larvae and adults specimens collected (χ^2 = 23.53, df =1, P<0.05). Similar result was obtained between sample periods and between species population (P<0.05). Multiple regression analysis revealed that the ratio of distance to a house from a larval habitat to a distance to a livestock shed from larval habitat significantly and negatively correlated with the distribution and relative abundance of *Anopheles gambiae* larvae in all the sample periods (r = - 0.52, P < 0.05; r = 0.61, P < 0.05; and r = 0.84, P < 0.05 for beginning of rainy season, late rainy season and dry season respectively) but positively correlated to the ratio of human density to livestock density in a homestead. Distance from a house to the nearest larval habitat significantly and negatively correlated to *Anopheles gambiae* complex adults density in a house (r = - 0.46, P < 0.05). The result therefore showed that livestock and human host availability affect the distribution and relative abundance of anopheles larvae in aquatic habitat, but the distribution of anopheles adults in a house is determined by distance from a house to larval habitat.
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1. Introduction

Previously it was thought that the control of malaria would be easy, base on the assumption that the relationship between the parasite, the vector and human host was clearly understood (Smith, 1996, Gimning *et al.,* 2001). The effective therapeutic and chemotherapeutic agents were available and that insecticides held a great promise for vector control.

However, despite the tremendous progress made in the acquisition of knowledge of the malaria parasite, the human host and development of anti-malarial drugs, the disease has proven far harder to control (Dobson, 1999). Malarial still remains an insidious and ever present scourge that constitutes obstacles to development (WHO, 2000, Gallap and Sachs, 2001).

Chief among other factors attributed to the inability to control this disease is lack of adequate knowledge of vector ecology which varies from one geological zone to another (Lehman *et al,* 1997).

Anopheles gambiae which is the major African malaria vectors is a group of closely related and morphologically indistinguishable species of which two or more coexist in many areas (Coetzee *et al.,* 2009).

Previous studies have demonstrated high level of heterogeneity in Anopheles mosquito species composition at macro-geographic scale (Gimnig et al., 2001; Shililu *et al.,* 1998) where the range and relative abundance of *Anophele gambiae* and *Anopheles arabiensis* are defined by climatic factors (Lindsat *et al.,* 1998). However, climatic factors are not the only variables that affect the relative abundance of Anopheles mosquitoes geographically defined area as evident from previous studies that species composition and abundant vary significantly among nearby village where climate is very similar (Joshi *et al.,* 1975). Thus, other ecological factors such as host and habitat characteristics and distribution could be involved in causing species composition and distribution variation at micro-geographic scale.

In this study therefore, the influence of host and aquatic habitats availability on relative abundance of anopheles were examined in Maiduguri, Borno State, of Nigeria.

2. Materials and methods

2.1 Study area

Maiduguri the study area is located between latitude 13°N and 14°N and longitude 12°E and 13°E It lies between the Sudan and Sahel savanna zone which is characterized by a short rainy season from June to September and a prolonged dry period between Decembers to May. The mean annual temperature ranged 28 - 29°C and a maximum temperature of 48°C (Udoh, 1981). The major occupation and socio-economic activities of people living in Maiduguri comprise of farming (both crops and animals) and livestock business is highly practiced. Borno state which has Maiduguri as its capital city has been reported to be a major livestock producing area with an estimated livestock population put at 40 % of national ruminant population count (Boun *et al.,* 1994) and the greater percentage of the livestock business takes place in Maiduguri.

2.2 Mosquito larvae sampling

All aquatic habitats in the study area were sampled for anopheline larvae in the beginning of rainy period once a week for seven weeks. Aquatic habitats were first inspected for the presence of anpheline mosquito larvae. If anpheline larvae were present, 2-25 dips at each site, depending on habitat sizes, were taken using a standard mosquito dipper (350 ml) (Minikawa *et al.,* 1999). Mosquito Larvae were then immediately preserved in 95 % ethanol. This sampling method permitted only comparison of relative abundance of each species among the habitats. Absolute abundance could not be estimated because only a proportion of mosquito larvae was sampled from large habitats while a greater proportion were collected from small habitats (Minikawa *et al.,* 1999).

2.3 Adult sampling

Adult mosquitoes were collected randomly from 38 houses using pyrethrum indoor spray catch method at week interval between larval collection periods also for seven weeks. The coordinates of each house was recorded using hand-held GPS unit. The distance to the nearest larval habitats from each house was estimated with a tape measure when the distance was less than 200 meters. When it exceeds 200 meters, the distance was measured on a wheel. The number of resident was recorded for each house.

2.4 Distribution of Livestock Hosts

Livestock in the study area were primarily sheep and cattle. The locations of all livestock sheds in the study area were taken and the number of livestock in each shed was recorded. Livestock density around each larval habit was estimated by averaging the number of livestock in the five nearest livestock shed (Dale, 1999). Similarly, human density around a larval habitat was estimated by averaging the number of residents in the five nearest houses. Livestock density and human density were also estimated by averaging the number of livestock in the five nearest livestock shed and the number of residents in the five nearest houses around each house where a mosquito collection was made.

2.5 Morphological identification of larval and adult anopheles mosquitoes.

Adult and larval Anopheline mosquitoes were examined microscopically to distinguish Anopheles species from other mosquito species using the taxonomy key of Gillies and Meillon (1966) and Service, (1980). Briefly, the larvae lack siphon, with the result that when they are at the water surface they lie parallel to it and are not subtended at an angle as are the culicinae.

The respiratory trumpets of anopheline pupae are short and broad distally, thus appearing conically. The most reliable characteristics for identifying anopheline pupae is the presence of short peg-like spines situated laterally near the distal margins of abdominal segments.

2.6 PCR identification of Anpheline species complex

Genomic DNA was extracted from each Anopheline mosquito that was identified morphologically using the modified protocol of Collins *et al.,* (1987). Each mosquito was homogenized in 10 ul bender buffer in 1.5 ml eppendorf tube and incubated in a hot water bath at 65°C for 30 minutes and there after centrifuged at 14,000 rpm for 15 minutes. The supernatant was recovered into a fresh tube and incubated at -20°C for 15 minutes at 14,000 rpm and supernatant recovered into fresh tube again. Then 100 ul phenol-chloroform mixtures was added and mixed by inversion for 3 mins and centrifuged at 14,000 rpm for 5mins. The aqueous phase was transferred into a new tube and 200 ml of pre-chilled absolute ethanol was added mixed by inversion and kept for 15 mins at – 20°C and then centrifuged for 15 mins again at 14,000 rpm. The pellet was washed in 150 ul of 70 % ethanol and then dissolved in 20 ul Tris-EDTA + RNAse (50 mg/ml), and kept at 4°C overnight and therefore kept at -20°C until used for PCR

The method of Coetzea *et al.,* (2000) was used in the identification of species-specific for *An. gambiae* complex. This involved using universal primers forward primer in a cocktail reaction that contains reverse primers. GA, for *An gambiae* s.s., ME for *An.*

melas, AR for *An. arabiensis* and QD *An. Quadriannulatus*. The lengths of the sequences amplified between universal primer and each of the four species-specific primers are 153 bp for *An. Quadriannulatus,* 315 bp for *An. arabiensis,* 390 bp for *An gambiae* and 464 bp for *An melas*.

The reaction mix contained 1x buffer C (300 mM Tris-HCl, 75 mM $(NH4)_2S0_4$, 2.5 mM $mgcl_2$ (pH 8.5) and 0.25ml of 20 mM DNTP mix. For the species complex identification, 0.25 ul each of the primers were used. The PCR reaction conditions were set at 94°C for 30 sec., 48°C for 30 Sec., and at 72°C for 2 mins for 35 cycle using Hybaid PCR Express (Thermohybaid Ltd, U.K), for the thermal cycling. The final extension step was at 72°C for 10 mins. The quantity of tag was 0.625 unit/25 ul per reaction and 50 ng of the extracted DNA was used as template. The volume was made up to 25 ul with sterile double distilled water.

2.7 Statistical Analysis

Multiple regressions analysis was used to establish how human/livestock distribution affects the relative abundance of anopheline species. The relative abundance of each species s.s. was calculated as the no of each species of anopheles s.l. and was arcsine transformed in the analysis. Because there were multiple larval habitats in the study area, geographic distances among larval habitats were represented by a distance matrix (Noboru *et al,* 2002). All distance matrices were computed using Progicial R. Legendre and Vaudor (1991) statistical package. Distance matrix of the dependent variable among sites was computed using multiple regressions on distance matrices method (Legendre *et al.,* 1994).

3. Results

3.1 Anopheline larvae species composition and distribution in Maiduguri in the study periods.

Table 1 gives the distribution of species compositions of *Anopheline larvae* and adults during the study periods.

In the beginning of the rainy season (June/July 2008), 8 aquatic habitats were found in the study area. All habitats had mosquito larvae, 7 contained *Anophelin Larvae* and 1 site had other species. For the 7 anopheline-positive habitats, four were human made habitats including stagnant water, ditches, dams and water logged site.

A total of 261 anopheline larvae were collected from the 8 habitats and 93.9 % of the specimens were identified.

In the late rainy season (October/November, 2008), 40 aquatic habitats were sampled. 23 sites were human-made habitats. The remaining 7 sites were natural habitats. Of the 40 aquatic habitats, 30 habitats had Anpheline larvae, 6 contained mixed species while 2 contained only *Anopheline melas and Anopheline quadriannalatus* and the remaining 2 had no anopheles larvae but other unidentified species. A total of 1012 Anopheline larvae was collected, 89.1 % were identified to species by PCR.

In the dry season (March/April) 2009, only 5 aquatic habitats were found and all were man-made habitats consisted of stagnant water from domestic use. The entire 5 habitat contained only *Anophele gambiae* larvae and none contained other mosquito species. A total of 32 larvae were collected and all the 32 were successfully identified by PCR (see Table 1 for species compositions and relative abundance).

Overall, the relative abundance of Anopheline mosquito larvae varied significantly between sampling periods ($\chi^2 = 2.86$, df = 2, P = < 0.05).

3.2 Adult species composition and distribution

In the beginning of rainy season (June/July) a total of 114 adult anopheline mosquitoes were collected from 22 houses. 9 houses did not have any mosquitoes. (see Table 1 for species relative abundance). The average density of adult Anopheles mosquito per house was 5.18.

In the late rainy season (September/October), 882 Anopheline adults, including both males and females were collected in 32 houses. Six houses did not have mosquitoes.

In the dry season (March/April), 608 adult anopheles mosquitoes were collected from 39 houses. Eleven houses had no mosquitoes. There was significant difference in species composition between larvae and adult specimens ($\chi^2 = 231.53$, df = 1, P < 0.05). Also a significant difference was observed in anophelin mosquito species between the sample periods ($\chi^2 = 302.11$, df = 2, P < 0.05).

Table 1. The species composition and percentage distribution of Anophelin larvae and adults in early and late rainy season 2008 and dry season 2009.

Sampling date		*An gambiae*	*An arabiensis*	*An quadriannulatus*	*An melas*	*other* species
Early rainy	LV	82.45	11.02	0.81	4.49	1.23
season	AD	68.98	8.51	0.49	12.14	9.88
Late rainy	LV	51.00	19.73	8.31	13.33	7.43
Season	AD	68.31`	19.20	5.42	5.18	1.89
Dry season	LV	100	*	*	*	*
	AD	79.03	9.34	*	8.88	2.70

LV = Anopheles larvae AD = Anopheles adults * = not found

3.3 Relationship between relative abundance of Anopheline larvae and the measured variables.

Multiple Regression analysis detected two variables (the ratio of human density to livestock density in a homestead and the ratio of distance to a house from a larval habitat to distance to a livestock shed from a larval habitat) significantly and associated with the relative abundance of anopheles larvae for all the sample periods. Correlation coefficient ($r = -0.52$, $P < 0.05$; $r = 0.61$, $P < 0.05$ and $r = 0.84$, $P < 0.05$ for beginning of rainy season, late rainy season and dry season respectively), but positive association ($r = 0.38$, $P = 0.02$) was shown for the variable ratio of human density to livestock density in a homestead. The standard partial regression coefficients suggest that the distance ratio played a more important role than the ratio of human density to livestock density.

Table 2. Regression analysis result for association between relative abundance of Anopheline larvae and host availability in the study period.

Period of study	Variable	Standard partial regression coefficient	P. value
Early rainy season	Distance from larval habitats to house/ Distance from larval habitats to livestock shed	0.810	<0.01
	Human density/Livestock density	0.362	<0.01
	Distance matrix among larval habitats	-0.354	>0.05
Late rainy season	Distance from larval habitats to house/ Distance from larval habitats to livestock shed	0.562	<0.01
	Human density/Livestock density	0.410	>0.05
	Distance matrix among larval habitats	-0.281	>0.05
Dry season	Distance from larval habitats to house/ Distance from larval habitats to livestock shed	0.663	< 0.01
	Human density/Livestock density	0.394	>0.05
	Distance matrix among larval habitats	-0.359	>0.05

3.4 Association between Anopheline adult mosquito density and host availability in the study period.

For the adult mosquito samples collected, of the six independent variables analyzed, distance from a house to the nearest larval habitat was the only variable significantly associated with adult anopheline mosquito density (Table) 3.

There was negative association between Anopheline adult density in a house and distance from the house to its nearest larval habitat ($r = -0.46$, $P<0.05$).

Table 3. Regression analysis results for association between Anopheline mosquito density and host availability.

	B R S		L R S		D R S	
Variable	**sprc**	**p**	**sprc**	**p**	**sprc**	**p**
i. Average human density in 5 houses surrounding the sample house	0.121	>0.05	0.286	>0.05	0.412	>0.05
ii. Distance to the 5 nearest houses from the house where mosquito were sampled	-0.032	>0.05	0.258	>0.08	0.397	0.05
iii. Average livestock number in the 5 nearest livestock shed from the sample house	-0.016	>0.05	0.062	>0.05	0.513	>0.5
iv. Average livestock number in the 5 nearest livestock shed from the sample house	-0.0750	>0.05	0.042	>0.05	0.381	>0.05
v. Distance to the nearest breeding site from the sampled house	0.273	<0.05	0.322	<0.05	0.480	<0.05
vi. Distance matrix among larval habitats.	0.152	>0.05	-0.038	>0.05	-0.022	>0.05

BRS = Beginning of rainy season LRS = Late rainy season DRS = Dry rainy season sprc = spartial partial regression coefficient P = Probability value

4. Discussion

Studies somewhere have shown that the distribution of *Anopheles gambiae* in house within the same geographical zone varies significantly, but the ecological factors underlying the variation in species composition, distribution and abundance was unknown. This study have shown that ratio of human density to livestock in homestead and the ratio of distance between larval habitats and houses to the distance between larval habitats and livestock sheds were significantly associated with the relative abundance of anopheline larvae. More larvae would be found in a habitat closer to houses and farther away from livestock sheds.

For the adult mosquitoes, distance from house to larval habitat was the only variable significantly associated with anopheline adult density. *An. gambiae* in a house was not correlated with either human or live stock density in a homestead or with the distance from livestock shed from a house. More anopheline would be found in houses near larval habitats than in houses farther from. In fact, this study have shown that about 90% of *An. gambiae* adults were found in houses within 500 meters from the nearest larval habitat except for the late rainy season where a lot of multiple larval habitats were found. The distribution and relative abundance of *An. gambiae* significantly varied among the sampling periods ($P < 0.05$). More collections were recorded during the late rainy season. Comparison between larval and adult sample collection showed a significantly more adult samples than the larval samples in all the study period. Such difference may be attributed to difference in feeding and resting behavior of different species. For examples, *An. gambiae* prefers to rest indoor after a blood meal (Service, 1980; Hangstone *et al.,* 1979) while *An. Arabiansis* prefers to rest outdoor (White and Rosen, 1973; Githeko *et al.,* 1996).

This result showed that distance from houses to larval habitats was the only variable significantly associated with *An.gambiae* adult density in houses. Other variables such as human and livestock densities and distance of livestock shed from a house had no significant association with *An. gambiae* density. This finding is in agreement to the findings of Charewood and Edoh (1996) who reported that anopheline adults density is negatively correlated with distance to larval habitats from houses in Kenya and also Shidrawi (1972) who also found that no correlation between *An. gambia* and cattle density. It was observed that more than 90 % of anopheline adults were found in houses less than 300 meters from larval habitats in all the study periods. Suggesting that anopheline mosquitoes tend to inhabit houses around larval habitats (Manga *et al.,* 1993). The complete absence of other mosquito species during, dry season collection could be due to unsuitable water environment for their breeding. This is so because other Anopheline mosquito species for example *An. funestus* tend to breed in large permanent waters with aquatic vegetation (Gillies and Meillon, 1968). Unfortunately this kind of water habitats was not found in the study area. Maiduguri which is the study area is located in desert arid zone

of Northern Nigeria where large permanent water body is very hardly to be found.

The results of this finding have several interesting implications on malaria vector control in Maiduguri. First eradication of mosquito larval habitats in one home stead is important, but is not good enough for reducing mosquito densities in a community and therefore vector control should be community-based.

Secondly, zoo prophylaxis may not be effective control measure of *An. gambiae* population abundance. This is so because the finding of this study showed that anopheline adult mosquito density had no correlation with livestock density or distance to livestock shed. Perhaps environmental management through elimination of larval habitat and larval control using bioinsecticides may be a more effective approach for reducing adult mosquito densities. For now, these implications is more specific to arid zone ecology northern Nigeria, the validity of this results needs to be determined in different areas under different ecological conditions.

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