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SPECIES COMPOSITION, DISTRIBUTION, ABUNDANCE AND VECTORIAL CAPACITY OF *AN. FUNESTUS* IN AN AFROTROPICAL ENVIRONMENT OF KATSINA STATE, NIGERIA.

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ABSTRACT

The *Anopheles funestus* group is one of the major malaria vectors in tropical Africa; however, there is scanty data about the dynamics of members of this group. Consequently, a study was undertaken from 2009 to 2013 to establish the species composition, distribution and abundance and vectorial capacity of *An. funestus* in parts of Katsina State, Nigeria. Mosquitoes collected were identified morphologically using mosquito morphological keys of Hopkins (1952) and Gillies and Coetzee (1987). Authentication of the members of the *Anopheles funestus* group was done according to Cohuet *et al.* (2003) using the cocktail PCR method based on the ITS2 region developed by Koekemoer *et al.* (2002). The cocktail PCR-assay samples were 518 (51.24%) *An. funestus* s.s., 67 (6.624%) *An. brucei* and 35 (3.46%) *An. rivoluru.* Zone A recorded the highest preponderance of larvae at Dandume, 178 (32.8%) and the least abundance was 40 (7.4%) in Zone C at Katsina. Similarly, adults were more preponderant in Zone A at Funtua 88 (22.8 %) and were least abundant in Zone C at Daura with 28 (7.1%). The highest isolations for *P. falciparum* and *P. malaria* were 120 and 193 respectively in *An. funestus s.s.* and the lowest were 11 and 24 of *P. falciparum* and *P. malaria* respectively isolated from *An. rivulorum*. Pools and Rice farms breeding sites were only active during wet months, however ponds and reservoirs were active during both wet and dry months. There is significant Pearson Correlations between species (0.05 level: 1-tailed) and within *An, funestus* larvae caught across sampled towns.

***Keywords**: Species composition, distribution, abundance, vectorial capacity, Afrotropical.*

INTRODUCTION

An. funestus group is one of the major malaria vectors in Africa, however, little is known about the dynamics of members of this group across the continent (Mulamba *et al*., 2014). Consequently, it is important to establish the species composition, distribution and abundance, vectorial capacity, ecological and behavioral differences of *Anopheles* species in Katsina State.

Diseases transmitted by mosquitoes have been responsible for killing more people than all the wars in history (Beerntsen, 2000). Worldwide, mosquitoes transmit diseases to more than 700 million people annually (Fradin, 1998) out of which about 300 - 500 million clinical malaria cases are transmitted by the *An. funestus* complex. Malaria is responsible for the deaths of 1 child every 30 seconds (Shell, 1997; WHO, 2008). It is responsible for about 1 million deaths (range 744,000 – 1,300,000) in Africa every year; with about 75% of cases occurring in children that are 5 years and below (Snow *et al.*, 1999).

Malaria killed 401 people in the last four weeks of September (2011) in Katsina state, according to local health officials (WHO, 2011). Up to 50,311 malaria cases were recorded in Katsina State in 2011, and was attributed to the unusually heavy rainfall recorded that year in Daura, Funtua, Ingawa, Kurfi and Bindawa towns of Katsina State (WHO, 2011).

An. funestus increases malaria transmission and is also preponderant during the dry season when the population of *An. gambiae* and *An. arabiensis* are low (Gillies and De Meillon, 1968). There is a need therefore for more research focusing on improving our understanding of *An. funestus, a* malaria vector population ecology on a local scale to enable the formulation of effective and vector-specific control strategies, aimed at reducing human vector contact and disease transmission. Reduction of mosquito population has been used world over to control malaria (Killeen *et al.*, 2003). The vector remains the key link in the transmission of malaria, and hence, warrants research and control efforts.

The control of malaria through mosquitoes should also be aimed at the specific biology of *An. funestus* and other non popular malaria vectors (Coetzee and Fontenille, 2004). It is not known how *An. funestus* breed and sustain themselves within the different ecological zones and also within the wet and dry seasons of Katsina State. Therefore this study aims at determining the species composition, distribution, abundance and vectorial capacity of *An. funestus* in an Afrotropical environment of Katsina State.

MATERIALS AND METHODS

Zone B (Sudan Savannah, yellow area)**:** The zone is represented by Dutsinma (12º 27' 18" N: 7º 29" E, Alt 604m above sea level (asl) and Kankara (11º 93" N : 7º 41" E) as sampled towns (KTSEPA, 2013).

SAMPLING ZONES AND SITES

Six towns were selected using cluster sampling method, out of which two towns were selected randomly from each of the three ecological zones (Sahel, Sudan and Guinea savannah) of Katsina State.

Zone A (Guinea Savannah, marked red in Fig 1**):** Funtua (11º 32" N; 7º 30" E) (Fig 1) and Dandume (11º 25" N ; 7º 12" E) (KTSEPA, 2013) were selected as representative towns of this zone.

Zone C (Sahel Savannah, green area)**:** This zone is represented by Katsina (12º 59" N: 7º 36" E, and Daura (13º 2' 11" N: 8º 9' 4" E (KTSEPA, 2013).

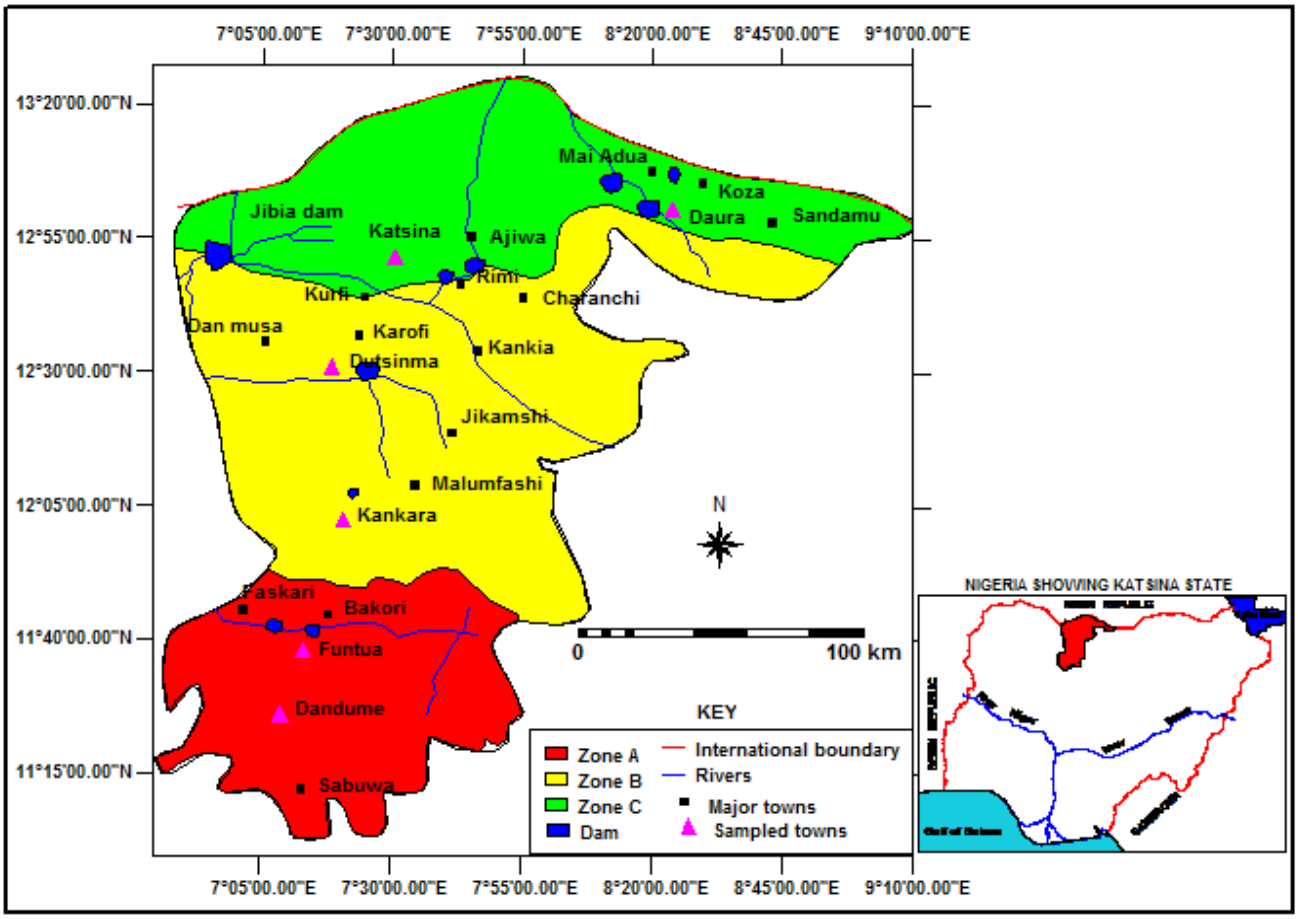

FIGURE 1 : KATSINA STATE SHOWING THE SAMPLED TOWNS AND VEGETATION ZONES

Source : Adapted and Modified from Katsina State Map, Ministry of Lands and Surveys

Collection of adult indoor resting mosquitoes

Indoor resting *Anopheles* were collected according to Faye *et al*. (1997), using Pyrethrum insecticide spray. In each town, 3 representative households were choosen and sprayed once bimonthly to determine the indoor resting density.

Collection of *Anopheles* larvae

Anopheles breeding sites were identified during the dry and wet seasons. In each locality, larvae of all available in stars were collected from all water bodies within a radius of 1 km according to Onyabe and Conn (2001) and larvae were reared according to Service (1993).

Mosquito identification

Mosquitoes analyzed were from two sources: 1) indoor resting adult catches and 2) adults reared from field collected larvae (series). The adult mosquitoes were first identified using morphological characteristics according to Hopkins (1952) and Gillies and Coetzee (1987).

PCR authentication for the members of the *Anopheles funestus* group was done according to Cohuet *et al*. (2003) using the cocktail PCR method based on the ITS2 region developed by Koekemoer *et al.* (2002).

RESULTS

Morphological identification

Five hundred and fourty three *An. funestus* species were identified (Table 1). Indoor adult collections were 394, *An. funestus* species (Table 2).

Variability was observed in the spatial distribution of *An. funestus* larval populations. Zone A recorded the highest preponderance of larvae was at Dandume, 178 (32.8%) and the least abundance was 40 (7.4%) in Zone C at Katsina (Table 1). Similarly, adults were more preponderant in Zone A at Funtua 88 (22.8 %) and were least abundant in Zone C at Daura with 28 (7.1%) (Table 2).

Table 1: Abundance and Distribution of *An. funestus* species larvae from different localities

Zone	Locality	*An. funestus*
Zone A	Funtua	109 (20.1%)
"	Dandume	178 (32.8%)
Zone B	Dutsinma	53 (9.8%)
"	Kankara	94 (17.3%)
Zone C	Katsina	40 (7.4%)
"	Daura	69 (12.7%)
	Total	543

Table 2: Abundance and Distribution of *An. funestus* species indoor adult collections from different localities

Zone	Town	*An. funestus*
Zone A	Funtua	88 (22.3%)
"	Dandume	75 (19.0%)
Zone B	Dutsinma	72 (18.3%)
"	Kankara	77 (19.5%)
Zone C	Katsina	54 (13.7%)
"	Daura	28 (7.1%)
	Total	394

Distribution of *Funestus* species across sampled towns

The highest preponderance of *An. funestus* adults was 88 in Zone A at Funtua and the lowest of 28 in Zone C was found at Daura. The highest number of 25 *An. funestus* larvae was recorded in Zone B in May in ponds at Dutsinma and Kankara and in a rice farm in June in Funtua (Zone A)(Table 2). Pools and rice farms breeding sites were only active during wet months while ponds and reservoirs were active during both wet and dry months. *An. funestus* collections increased in number during the dry months but reduced during the wet months.

Distribution of mosquitoes across breeding sites

The highest number of *An. funestus* larvae were collected from cemented reservoirs (Table 4).

Table 4: Breeding sites and *Anopheles* species identified morphologically

Breeding site	*An. funestus*
Pool	31 (6.6%)
Pond	60 (12.7%)
Cemented reservoir	188 (39.8%)
Overhead tank	94 (19.9%)
Rice farm	100 (21.1%)
Total	473

During this study the highest number of adult *Funestus* species identified were 88 at Funtua, 56 of which were *An. funestus s.s.* (Fig 3) and only 9 were *An. rivulorum* (Fig 4) and the lowest were 28 at Daura. The highest collection for larvae identified were 175 at Dandume, 99 of those were *An. funestus* s.s. and only 28 were *An. rivulorum*. The lowest was 36 at Daura (Table 4 & 5).

Table 5: Distribution of the members of the *An. funestus* complex with respect to locality

	Adults caught indoors					Larvae reared to adults				
Locality	Total collected	Total identified	Af	A r	Abr	Total collected	Total identified	Af	A r	Abr
Katsina	54	53 (98.2%)	29	9	15	40	36 (90%)	21	6	9
Daura	28	25 (89.3%)	16	2	10	69	69 (100%)	39	8	22
Dutsinma	72	68 (94.5%)	40	8	20	53	52 (98.1%)	25	11	16
Kankara	77	77 (100%)	42	8	27	94	94 (100%)	43	16	33
Funtua	88	88 (100%)	56	10	22	109	106 (97.3%)	58	19	29
Dandume	75	75 (100%)	50	7	19	178	175(98.3%)	99	28	48
Total	431	386	233	44	113	580	532	285	88	157

Af = An. funestus s.s. Ar = An. rivulorum Abr = An. brucei

Table 6: One-Sample Test For adults *Funestus* species caught indoors

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
An. Funestus s..s	6.568	5	.001	38.83333	23.6340	54.0326
An. rivulorum	6.404	5	.001	7.33333	4.3899	10.2767
An. brucei	7.892	5	.001	18.83333	12.6991	24.9675

One-Sample t-test indicates no significant diference between *Funestus* species caught indoors at 95% CI and 2-tailed (Table 6 and 7). However there is significant Pearson Correlations at the 0.05 level (1-tailed). Within *An, funestus* larvae caught across sampled towns, Pearson correlations also shows significant difference (Table 8).

Table 7: Pearson Correlations analysis for adult *Funestus* species caught indoors

		An. funestus s.s	*An. brucei*	*An. rivulorum*
An. funestus s.s.	Pearson Correlation	1	.740*	.763*
	Sig. (1-tailed)		.046	.039
	N	6	6	6
An. rivulorum	Pearson Correlation	.740*	1	.675
	Sig. (1-tailed)	.046		.071
	N	6	6	6
An. brucei	Pearson Correlation	.763*	.675	1
	Sig. (1-tailed)	.039	.071	
	N	6	6	6

*. Correlation is significant at the 0.05 level (1-tailed).

Table 8: Pearson Correlations analysis for *Funestus* larvae

		An. funestus s.s	*An. brucei*	*An. rivulorum*
An. funestus s.s.	Pearson Correlation	1	.944**	.939**
	Sig. (2-tailed)		.005	.005
	N	6	6	6
An rivulorum	Pearson Correlation	.944**	1	.940**
	Sig. (2-tailed)	.005		.005
	N	6	6	6
An. brucei	Pearson Correlation	.939**	.940**	1
	Sig. (2-tailed)	.005	.005	
	N	6	6	6

**. Correlation is significant at the 0.01 level (2-tailed).

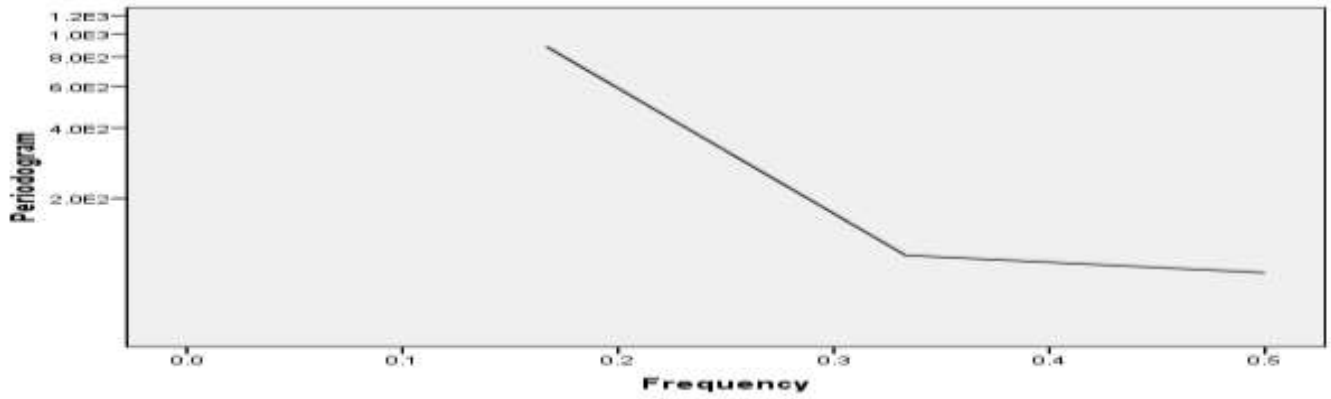

Fig 3 : Periodogram of *An. funestus* s.s. species by frequency

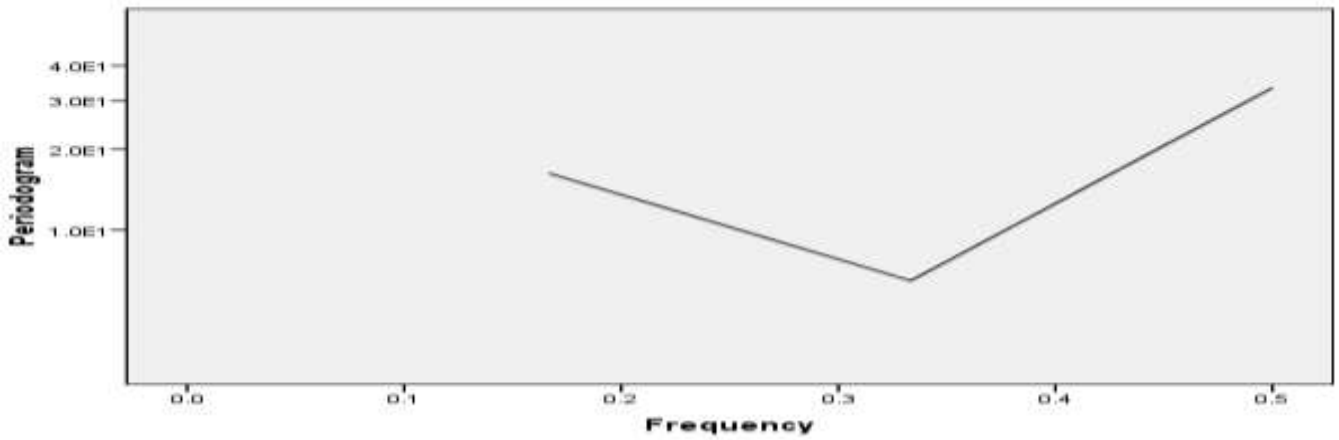

Fig 4: Periodogram of *An. rivulorum* species by frequency

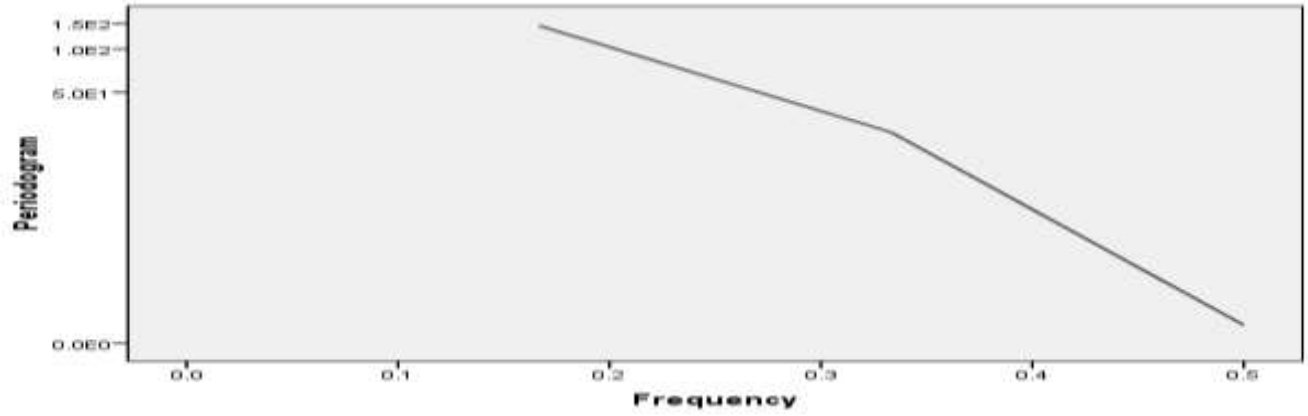

Fig 5: Periodogram of *An. brucei* species by frequency

Periodogram of *An. funestus* species by frequency indicates that: *An. funestus* s.s. population was high during the dry season but drops sharply as the rains set in (Fig 3); but population of *An. rivulorum* increased dramatically during the rainy season (Fig 4). However the population of *An. brucei* was low during both dry and wet seasons (Fig 5).

The highest number of collections and isolations for *P. falciparum* and the highest for *P. malaria* were 120 and 193 respectively isolated from *An. funestus*. The lowest were 11 and 24 of *P. falciparum* and *P. malaria* respectively isolated from *An. rivulorum* (Table 5).

Table 9: Prevalence of *Plasmodium* sporozoites isolated in the identified *Funestus* species.

Anopheles species	*Plasmodium* sporozoites identified by Vectest				Total
	P. fal.	*P. mal.*	*P. vivax (210)*	*P. vivax* (247)	
An. funestus s.s	120	193	0	0	313
An. brucei	30	51	0	0	81
An. rivulorum	11	24	0	0	35
Total	161	268	0	0	429

P. fal. = Plasmodium falciparum *P. mal. = P. malariae*

Pearson Correlation is significant between these *Funestus* species at the 0.01 level (2-tailed)(Table 10).

Table 10: Results of Pearson Correlation between *An. funestus s.s.* and *An. brucei*

		An. funestus s.s	*An. Brucei*	*An. rivulorum*
An. funestus s.s	Pearson Correlation	1	1.000**	1.000**
	Sig. (2-tailed)		.	.
	N	2	2	2
An. brucei	Pearson Correlation	1.000**	1	1.000**
	Sig. (2-tailed)	.		.
	N	2	2	2
An. rivulorum	Pearson Correlation	1.000**	1.000**	1
	Sig. (2-tailed)	.	.	
	N	2	2	2

****. Correlation is significant at the 0.01 level (2-tailed).**

DISCUSSION

This study identified three species of the *Funestus* complex in Katsina State, Nigeria. These are *An. funestus s.s., An. brucei and An. rivulorum.* This is consistent with previous reports that *An. funestus*, *An. leesoni*, *An. rivulorum* and *An. brucei* are found in West and Central Africa. However their biology and vectorial capacity are very different. Only *An. funestus s.s*, feeds on man, therefore the other members of the complex are mostly not malaria vectors. In addition, Cohuet *et al*. (2003) reported an *An. rivulorum*-like species from Burkina Faso and Cameroon, however it differs from the South African *An. rivulorum*, and does not seem to play any role in malaria transmission.

All the *Funestus* species identified during this study were found living in sympatry. This is consistent with studies in Senegal (West Africa), where 3 chromosomal populations exhibiting different anthropophilic activities were recognized, and were sometimes found in sympatry. However in Cameroon, a cline of inversion frequencies was reported from the humid forest in the South (with 'Folonzo'-like inverted populations) to the dry savannas in the North (with 'Kiribina' standard populations), with both forms displaying strong heterozygote deficiency when sympatric. All these data suggest restricted gene flow between chromosomal forms of *An. funestus*.

Excessive irrigation for agriculture within the three zones of Katsina State, Nigeria, has changed the features of the soil and it has dictated the abundance, distribution and vectorial capacity of *An. funestus*, thereby facilitating the establishment of malaria cases caused by *P. falciparum*. Therefore water reservoirs for irrigation should be equipped with motorised aerators that will constantly stir the water so that it will be uninhabitable to the early stages of mosquitoes and thus contribute immensely to reduction of mosquitoes and by implication malaria.

In the irrigated areas and rice fields of the study area, mosquitoe and malaria transmission was very high because o*ver 30% of An. funestus* larvae were collected in rice farms,

thus confirming farmlands as major contributors to *Funestus* proliferation and by implication, malaria prevalence in the three zones of Katsina State. This is similar to the report of Himeidan and Kweka (2012), that farmlands constituted 40% of anopheline larval habitats. This is also consistent with the reports of Marrama *et al*. (2004, 2009) who stated that malaria transmission was 150 times higher in irrigated areas than in the natural ecosystems and 90% of infections were caused by *An. funestus*.

This study had observed that lands left fallow after harvesting rice, were highly suitable for breeding of numerous *Funestus* species and by implication, the malaria transmission in areas having rice plantations underwent profound alterations when compared to areas where irrigation was not practiced. This finding is supported by Takken *et al*. (2007).

The study has also observed that *An. funestus* occurred at high densities in large non-moving water bodies like ponds, cemented reservoirs and overhead tanks; so as to survive and proliferate during the dry season near human habitations. This finding is consistent with the reports of Charlwood *et al*. (2000) and Lamidi (2009).

The high preponderance of *An. funestus* recorded during this study could also be explained by the clearing of trees, shrubs and grasses to pave way for farming activities, as a result of which soil surface characteristics were changed. In addition, building of temporary ponds like dams for irrigation purposes facilitated the increase in the population of *An. funestus* species. This findings are in agreement with the reports of Gillies and De Meillon (1968) and Gillies and Coetzee (1987).

Similarly, the significantly higher *Funestus* mosquitoes collected during this study was as a result of a lots of breeding sites created by rainfall, in addition to the high level of heterogeneity in the *Funestus* species composition throughout the area of study. This is similar to Mbogo *et al.* (2003), who reported on observed differences in the relationship between mosquito population and rainfall in different districts of Kenya and then narrowed that to environmental heterogeneity. Hence, the association of mosquitoes and malaria with rainfall is due not only to greater breeding activity of mosquitoes, but also to the rise in relative humidity and higher probability of survival of female *Anopheles* mosquitoes. This varies with the circumstances prevailing in a particular geographic region and also depends on local habits of mosquitoes. Consequently malaria transmission can only be sustained by about 80mm of rain for 5 months, however 60mm for as many months as well as 80mm for less than 5 months will be inadequate (Lindsay and Birley, 1996).

The above findings are important for mosquito control by determining the ecology and distribution of various *Funestus* species and is important in the determination of mosquito vector abundance and associated malaria prevalence. Thus the effective control of malaria through vector management requires information on distribution and abundance of *Funestus* species in an area like Katsina State. Besides, individual species within the species complex differ in host-biting preference, abundance and vector competence, consequently, identification of the mosquito vectors to species level and mapping species distribution in heterogeneous environments are necessary for a successful control programme (Coetzee *et al*., 2000; Minakawa *et al*., 2002 and Godwin *et al*., 2005).

More cases of malaria were recorded in the study area i.e. Katsina State, during July and August throughout the period of study. This is consistent with reports that low humidity levels force mosquitoes to feed more frequently in order to take care of dehydration. Under favourable humidity, mosquitoes tend to survive for a longer period, which push them to disperse farther and this permit them excel in malaria transmission cycles.

Where temperature is not a limiting factor, malaria transmission is highly seasonal, with its peak following the period of peak rainfall. In addition, *Anopheles* species can aestivate as eggs for up to 20 months and majority of the eggs are able to hatch as soon as the rains resume thereby increasing the population density of *An. funestus* (Charlwood *et al*., 2000 and Kasili *et al*., 2009).

During this study, it was observed that *An. funestus* was very preponderant at the end of the rainy season, and thus contributed immensely to sustain the endemicity of malaria in the sampled towns across the three zones of Katsina State. Similar findings were reported by Lamidi (2009). Consequently, *An.gambiae* s.s., *An. arabiensis*, and *An. funestus* s.s alternate in malaria transmission across the year (Ebenezer *et al*., 2014) especially during the rains as such all should be targeted in order to achieve effective malaria control.

All three identified *An. funestus* subspecies i.e. *An. funestus, An. rivolurum and An. brucei* were all found positive for circumsporozoite protein, this is a confirmation that the afore-mentioned *Anopheles* species were all active in malaria transmission. This finding agrees with Mulamba *et al*. (2014). Therefore for any malaria control programme to be successful in Katsina State, Nigeria, *An. funestus, An. rivolurum and An. brucei* must all be targeted simultaneously and wholistically.

This study discovered that heterogeneity in oviposition site preference between the *An. funestus, An. rivolurum and An. brucei* enabled vector densities to be high in both the wet and dry seasons, allowing for a year round transmission of malaria, especially during the dry season. Malaria transmission was maintained by these species which were anthropophilic as well as zoophilic, and this behaviour allowed for low level but persistent malaria transmission as reported by Manh *et al*. (2010). Zoophilic behaviour was observed in some houses that kept animals such as cattle, camel, sheep, goats and donkeys. Therefore insecticides should be sprayed on and around these animals instead of only indoor spraying of insecticides, so as to reduce *Anopheles* populations.

CONCLUSION

Three *Anopheles funestus* complex species were identified in Katsina State, Nigeria and *P. falciparum* and *P. Malariae* were isolated form these species.

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CONFLICT OF INTEREST

The authors hold no conflict of interest of any shape or form.

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