



BREEDING SITES UTILIZED BY ANOPHELES MOSQUITOES IN KATSINA STATE, NIGERIA

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ABSTRACT

The transmission of malaria and the development of the *Anopheles* vector are directly linked to the breeding sites. Consequently, the breeding sites of *Anopheles* in Katsina State were determined during dry and wet seasons. To locate larval habitats, each sampling location was screened systematically within a 1Km radius and all water collections containing larvae were tagged, and subsequently drawn on a map using Arcicad software. *An. gambiae* were the most preponderant in pools with 332 (20.13%) and least abundance were 120 (7.28%) in overhead tanks. There was no significant difference ($p > 0.05$, $F=9.28$) between pool and pond nor between concrete reservoir and overhead tank in terms of collected larvae. *Anopheles* larvae were found in water bodies that ranged in size between 12 cm for e.g. rain pools to 50 m for earth dams. *Anopheles* were found breeding in association with vegetation in the water bodies, e.g. *Typha* species (*Typha angustifolia*) and water lilies (*Nymphaea* spp.) and rotten vegetation. Some of the observed ponds were permanent because they contain water throughout the year while others were temporary because they dried off during dry season between March and April of every study year from 2009 to 2014. The observed water bodies that were clean include rain pools while other pools were contaminated with oil, faeces, waste water, sewage etc. Others were polluted with organic matter, algal growth, decaying plant, animal waste etc. Habitats like earth dams and broken plastic reservoirs were completely exposed to sunshine however domestic water containers like buckets were only partially light and partial shaded.

Keywords: Arcicad software, larval habitats, breeding sites, population structure, concrete and overhead water reservoirs.

INTRODUCTION

The transmission of malaria and the development of the *Anopheles* vector are directly linked to the breeding sites. There are about four different types of mosquito habitats, e.g. Running Water, Transient Water, Permanent Water and Container habitats (www.rci.rutgers.edu, 2013). Mosquitoes prefer to lay their eggs in breeding sites such as shallow, stagnant water such as ponds, marshes, swamps, floodwater, ditches and woodland pools (www.rci.rutgers.edu, 2013). However, they have been living successfully in many different environments; they live even in environments that are not normally theirs. Mosquitoes also grow well in hot, humid environments especially in tropical areas. Some species live in very cold countries, like those in the Arctic Circle (www.rci.rutgers.edu, 2013).

Mosquito larvae are usually found in different habitats including water bodies that contain vegetation which larvae attach to so as to avoid being swept away by water currents. The *Anopheles*, *Culex*, *Culiseta*, *Coquillettidia* and *Uranotaenia* species breed in permanent bodies of water and can survive in polluted water as well as freshwater, acid water and brackish water swamps.

Other mosquito larvae may be present in container water sources such as puddles, upon leaves and stagnant water within small pools (www.rci.rutgers.edu, 2013).

Habitat and climate determine which mosquito species will be present in an area. Larval requirements can be quite specific and vary a lot. Mosquito larvae can be found in numerous habitats. Each habitat produces and shows a seasonal progression of mosquito species (www.rci.rutgers.edu, 2013).

To control diseases that are vectored by mosquitoes e.g. filariasis, malaria and others in Katsina State, we must carry out studies to determine the types of breeding sites utilised by mosquitoes. Consequently, the current study identified breeding sites used by mosquitoes in Katsina State. This is timely and important now more than ever before.

Study Area

Katsina State is 1,696 feet above sea level, it is located between 12°15' N - 7°30' E and 12° 25'N - 7.5° 0'E (Fig 1). It has an area of 24,194 km², with a population of 3,878,344 million and a

population density of 160.3/km² (KTHSB, 2004). The climate of Katsina State is characterized by two well marked seasons, the rainy season, extending from May to September and the dry season from October to April. The dry season is characterized by harmattan dust between November and February. The maximum amount of rainfall occurs between August and September.

Collection of *Anopheles* Larvae

Anopheles breeding sites were identified according to Onyabe & Conn (2001), 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. Sample size per water body was not determined and samples were mixed (Onyabe and Conn, 2001). In water bodies (e.g. pools, reservoirs, puddles etc.) mosquito larvae were collected using standard dipping techniques with a 350ml dipper and 3 cm wide tea sieve (Service, 1993). At each sampling location, anopheline and culicine mosquitoes that were collected were differentiated based on the floating habit of the mosquito larvae (Rozendaal, 1997).

Pictures of all *Anopheles* breeding sites within sampling areas across all the three ecological zones within Katsina State were taken using an ES15 Samsung digital camera and was transferred to a computer laptop.

Rearing of Larvae to Adults in the Laboratory

The larvae collected from the field were transferred into white plastic containers filled to two-thirds of their volume with non-chlorinated distilled water and reared in the laboratory according to the methods of Service (1993).

Characterization of Potential Mosquito Breeding Sites

Characterization of potential mosquito breeding sites was carried out by recording the characteristics of the different categories of breeding sites present within the sampling areas according to Sattler *et al.* (2005).

Mosquito breeding sites were geo-referenced, using hand-held global positioning system (GPS) receivers (eTrex and Garmin 12X1, Garmin International Inc.; Olathe, U.S.A.). GPS was used to determine the exact coordinates of each breeding site. Maps of some of the study areas were obtained from google map and some were created in version 9.0 ESRI ArcMap (Redlands, CA, U.S.A.).

Mosquito Identification

Mosquitoes analyzed were from two sources: (i) indoor resting adult catches and (ii) adults reared from field collected larvae (series). The adult mosquitoes were first identified using morphological characteristics according to Gillies and Coetzee (1987), Hopkins (1952) and Proft *et al.* (1999). Members of the *Anopheles gambiae* s.l complex were identified into *An. gambiae* s.s and *An. arabiensis* using species specific multiplex polymerase chain reaction (PCR). After morphological identification, specimens were stored in vials containing 70% ethanol for PCR.

RESULTS

Morphological Identification

During the period of study (2009 – 2011) a total of 3,027 mosquitoes were collected across the three ecological zones and all were morphologically identified as anopheline mosquitoes. There was no significant difference between *An. gambiae* and *An. funestus* larvae collected from Zone A, Zone B and Zone C (χ^2 , $p > 0.05$, $F = 5.05$) during the period 2009 - 2011.

Of the 1649 adults reared from larval collections, *An. gambiae* s.l was the highest with 1027 (62.28%) followed by *An. funestus* with 580 (35.17%) and the least was *An. maculipennis* with 16 (0.97%) (Table 1). Chi-Square have shown no significant difference between *Anopheles* species. However Pearson Correlation between the *Anopheles* species is significant at 0.05 level.

Of the 1378 adult mosquitoes from indoor adult collections, the preponderant species was *An. gambiae*, with 926 (67.2%), followed by *An. funestus*, 431 (31.3%) while the least abundant was *An. maculipennis* with 9 (0.65%) (Table 2). *An. maculipennis* and *An. quadrimaculatus* were collected from Zone A only, (Funtua and Dandume towns) (Table 2; Fig 2). Of the mosquitoes collected indoors, *An. maculipennis* was the least abundant with 16 (0.9%).

Variability was observed in the spatial distribution of *An. gambiae* s.s and *An. funestus* larval populations. Zone A recorded the highest preponderance of larvae at Dandume, 555 (33.7%) and at Funtua with 298 (18.1%) while least abundance was 148 (9.0%) in Zone C at Katsina (Fig 2). Similarly, adults were more preponderant in Zone A at Dandume with 301 (21.8%) and Funtua 299 (21.7%) and were not abundant in Zone C at Daura with 98 (7.1%) (Table 2; Fig 2). There is a significant correlation between the species ($p < 0.05$)

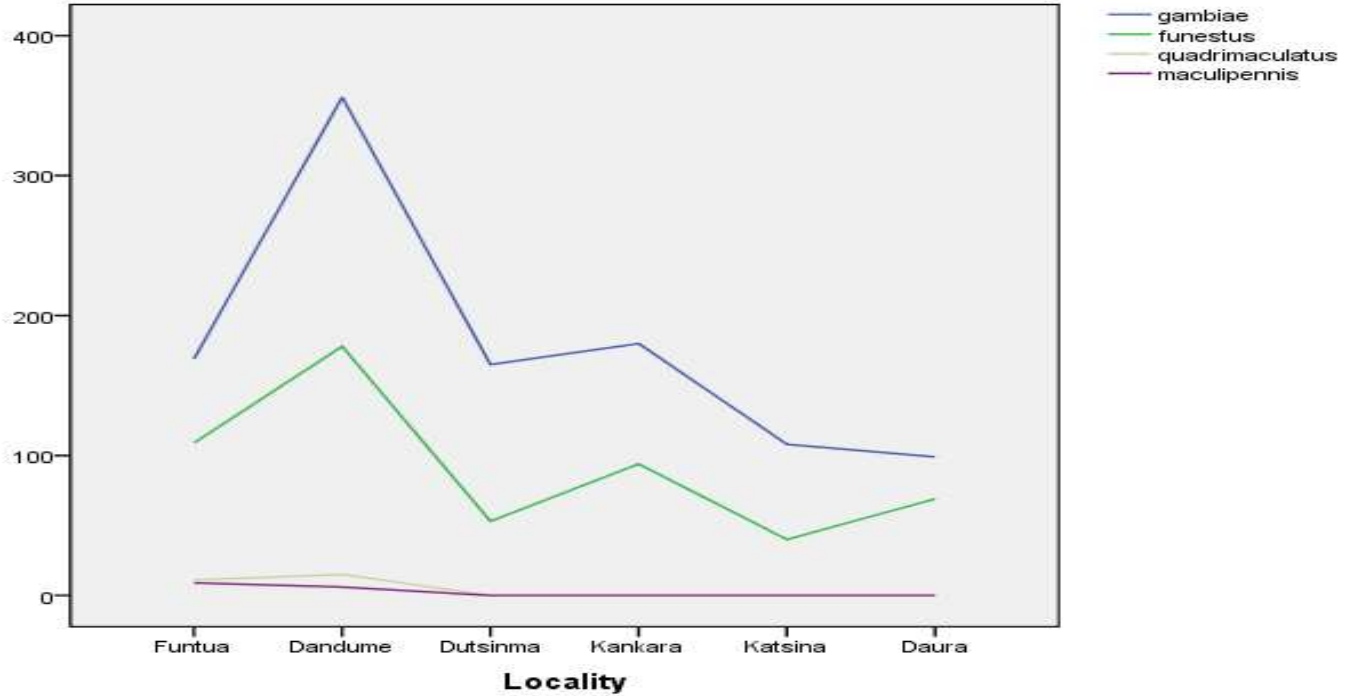


Fig 1: Sequential distribution and abundance of *Anopheles* species based on Locality

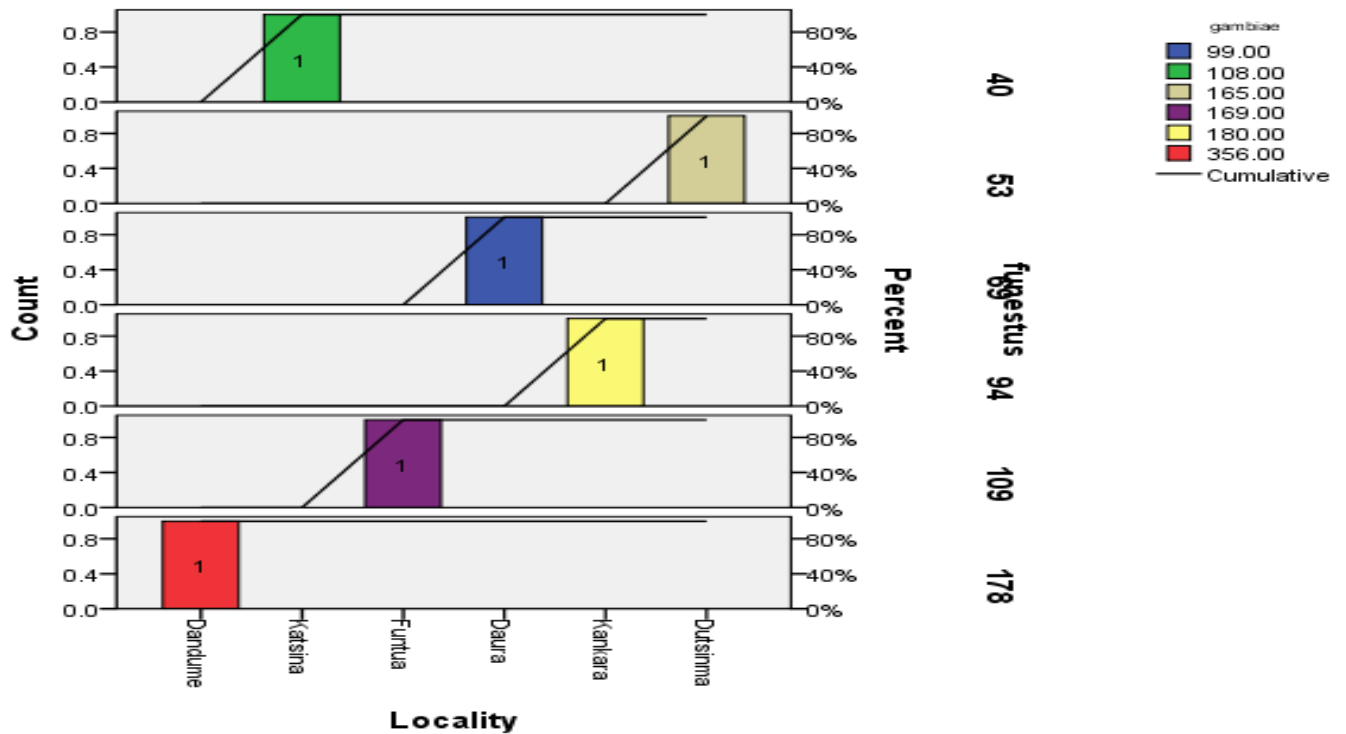


Fig 2: Pareto charts on distribution and abundance of *Anopheles* species based on Locality

The pareto chart above suggest that the highest impact of *Anopheles* mosquito control in Katsina State will be felt by targeting Dandume (356.00), Kankara (180.00) and Funtua (169.00).

Larvae

The highest abundance of *An. gambiae* larvae collected in a pond was 17 in Zone B at Dutsinma in September, 2009. 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). Pools and rice farm breeding sites were only active during wet months while ponds and reservoirs were active during both wet and dry months. Fewer number of *An. gambiae* larvae were collected during the dry months of October to May, 2010. However, the *An. funestus* collections increased in number during the dry months but reduced during the wet months (Fig 1 & 2).

Distribution of Mosquitoes across Breeding Sites

An. gambiae was the most proponderant species collected with 332 larvae from pools and the least abundant were 120 from

overhead tanks. On the other hand, the highest number of *An. funestus* larvae were recovered from cemented reservoirs (Fig 2). Chi-Square have shown no significant difference between *Anopheles* species. However Pearson Correlation between the *Anopheles* species is significant at 0.01 level. There was no significant difference in number of *An. gambiae* and *An. funestus* collected from pools and ponds ($p>0.05$, $F=9.28$), from cemented reservoirs and overhead tanks ($p>0.05$, $F=9.28$) and those from rice fields and ponds ($p>0.05$, $F=9.28$). *An. maculipennis* and *An. quadrimaculatus* were found breeding in rice farms only (Fig 3).

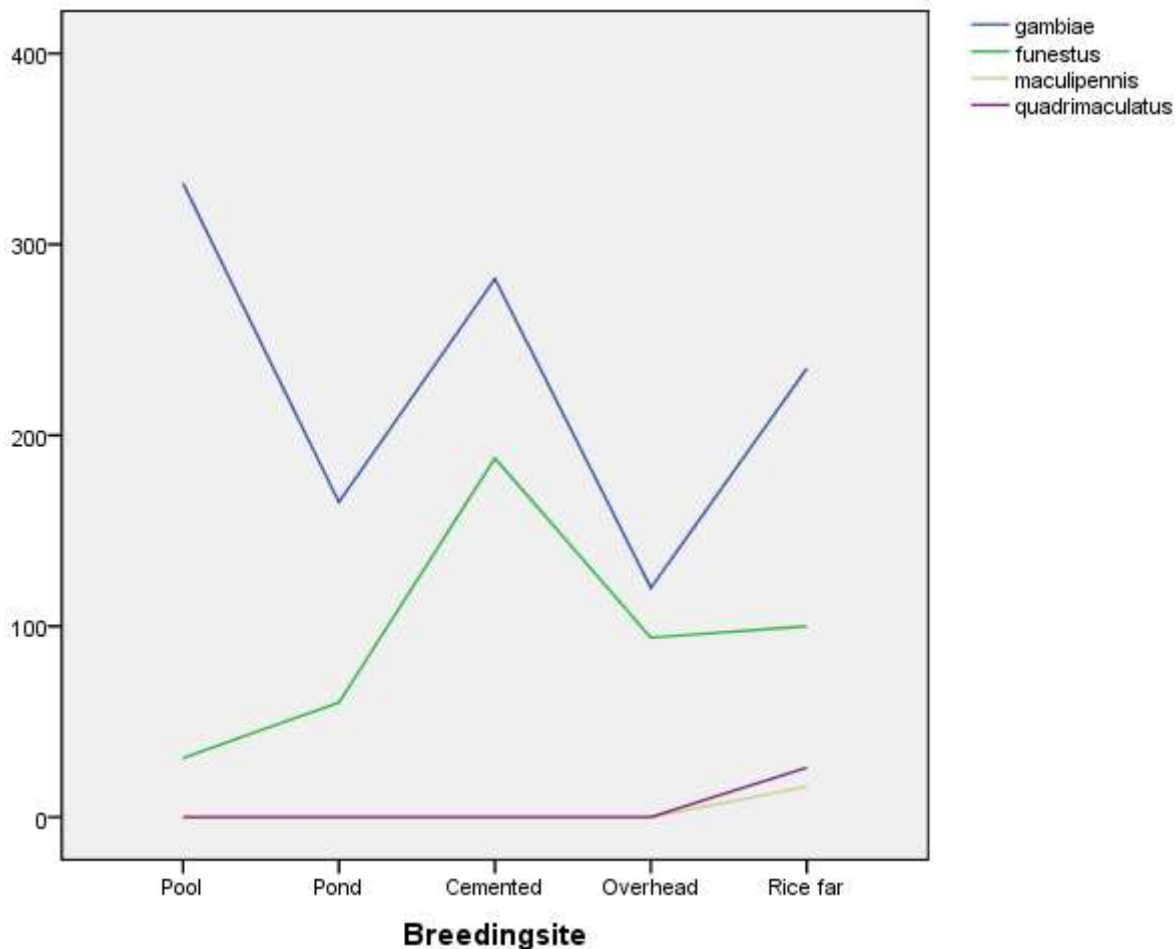


Fig 34: Sequential distribution and abundance of *Anopheles* species based on Locality

ANOVA shows no significant difference between the species. Correlation is significant at the 0.01 level (2-tailed). A binomial test presented and exact significance between *An. maculipennis* and *An. quadrimaculatus*.

Characteristics of the Breeding Sites

Anopheles mosquitoes were collected from five different breeding sites within the three ecological zones. Some were collected from pools, ponds and cemented reservoirs while

others were collected from overhead tanks and rice farms. Pools, ponds, cemented ground and overhead reservoirs were present in all the three ecological zones, however rice farms were fewer in Zones B and C but abundant in Zone A. The information on

physical characteristics of the breeding sites observed during the study were: size of water body, type of vegetation, habitat permanence, water condition and exposure to sunlight are detailed below.

Size of water body

Anopheles larvae were found in water bodies which ranged in size between 12 cm for e.g. rain pools to 50 m for earth dams.

Type of vegetation

Anopheles were found breeding in association with vegetation in the water bodies, e.g. *Typha* species (*Typha angustifolia*) and water lilies (*Nymphaea* spp.) and rotten vegetation.

Habitat permanence

Some of the observed ponds were permanent because they contain water throughout the year while others were temporary because they dried off during dry season between March and April of every study year from 2009 to 2011.

Water condition

The observed water bodies that were clean include rain pools while other pools were contaminated with oil, faeces, waste water, sewage etc. Others were polluted with organic matter, algal growth, decaying plant, animal waste etc. Some water

bodies are turbid because they contain mud and other organic debris.

Exposure to sunlight

Habitats like earth dams and broken plastic reservoirs were completely exposed to sunshine however domestic water containers like buckets were only partially light and partial shaded.

The following pictures though not showing all the breeding points sampled, are however, a good representation of the different types of breeding sites peculiar to the ecological zones and sampled towns.

1. Pools: Some were brought about by water leaking from pipes (Plates I & II) mostly shallow and clear with little vegetation. However pools located in houses were polluted, deeper and contained more volume of water (below). Pools located in gutters were more turbid, more polluted and smell. They contain a lot of debris from vegetation, waste, sewage among others. Some pools were marshy while others were made by animal hoof prints and tyre marks and they contained plant debris. Pools were also observed at water fetching points. They were formed by seepage water from pipes and wells. There were also pools made on river beds during the dry season when there was little water. These pools ranged in size from 5 cm to 100 cm and contained rotten vegetation and other debris.



Plate I: Pools within a house containing *Anopheles* larvae (arrow) at Funtua.



Plate II: Pools made by stagnant water at a washing point containing *Anopheles* larvae in a house at Daura



Plate III: Pools of water in a narrow alley containing *Anopheles* larvae at Katsina.

2. Rice farms: they contained numerous pools, some small (5 cm long) others were big (1 m long). They contained little vegetation at initial planting (Plate IV) but became filled with rice seedlings and rotten vegetation later in the farming season. Some became muddy especially during transplanting and other farm activities (Plate V).

3. Concrete reservoir

They were open reservoirs about 12 cm deep, with rotten vegetation and other debris. Some had clear water (Plates VII,

VIII) while others have turbid water (Plate IX). Some of them were located in block making industries, some were abandoned fish ponds, others were reservoirs for boreholes etc.

Overhead water tanks

These were made of plastic. They contained from 1000 to 3000 litres water and abundant algal (*spirogyra*) growth. Majority

were clear and clean but some were turbid because of the presence of heavy algal growth (Plates X, XI & XII).

water lilies (Plates XIII, XIV & XV) while others had vegetation only at their perimeter. Majority were turbid and contained rotten vegetation. Most were temporary because they dried up during the dry season.

5. Ponds

These were big water bodies ranging from 2m to 20 m wide. Some contained aquatic plants all over the water surface e.g.



Plate IV: Pools made from irrigation canals containing *Anopheles* larvae at Dutsinma



Plate V: Puddles in a rice field where *Anopheles* larvae were collected at Dutsinma



Plate VI: Concrete water reservoirs containing *Anopheles* larvae in a water reservoir at Katsina.



Plate VII: *Anopheles* larvae in plastic reservoirs at Katsina



Plate IX: *Anopheles* larvae in temporary earth ponds during dry season at Daura

DISCUSSION

Anopheles funestus occurred at high densities in large non-moving water bodies like ponds, cemented reservoirs and overhead tanks especially to survive and proliferate during the dry season so as to remain closely associated with groups of houses. This is consistent with the reports of Charlwood *et al.* (1997) and Lamidi (2009).

In all the sampled towns, the water supply system was erratic and this explains the use of numerous water storage containers to provide water for domestic chores, irrigation, car wash, block making and other construction purposes. These turned out to be conducive breeding sites for mosquitoes within and near human habitations. During all the three study years, it was observed that water storage in cemented and plastic tanks was largely responsible for the abundance of *Anopheles* mosquitoes in all the zones, especially in the dry season. Similar findings were made by Sharma (2008) in the semi-arid districts of Rajasthan, India.

Zone A had a preponderance of mosquito breeding sites, which may be linked to the abundance of rainfall in that zone which is above that found in the other two zones. This may explain why *Anopheles* mosquitoes were highly prolific and thus highly abundant in the zone.

The presence of large water reservoirs (used for irrigation purposes) located close to some towns e.g. Sabke dam near Daura, Ajiwa dam near Kasina and Zobe dam at Dutsinma, were also responsible for the high preponderance of *Anopheles* species, which easily invaded nearby houses. According to Keiser *et al.* (2005), 87.9% of the world malaria cases occurred in 9.4 million people who live near dams and irrigation schemes in sub-Saharan Africa. Many authors have reported that mosquitoes can travel up to 5 kilometers from their breeding points to invade human habitations (Takken *et al.*, 2007). Besides that, extensive irrigation within the three zones has altered soil characteristics which has impacted negatively on the desert physiography, vector abundance, distribution and vectorial capacity, thus triggering off changes in *Plasmodium falciparum*-dominated malaria.

The change in crop pattern, retention of high surface moisture, excessive use of irrigation canals and poor handling of irrigation water have attracted several anophelines to the three zones. The distribution of *Anopheles* and malaria observed in the three zones in Katsina State is more or less directly related to the distribution of irrigation water used for agriculture. 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 a similar vein, intense rains, farming activities undertaken through irrigation of enormous rice fields and other crops were also responsible for the very high population of *Anopheles* species recorded. In the irrigated areas and rice fields, mosquitoes and malaria transmission was very high. This is 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 rice harvest, were highly suitable for mosquito breeding, causing large numbers of anophelines to emerge. By implication, the malaria transmission season underwent profound alterations when compared to areas where irrigation was not practiced (Takken *et al.*, 2007).

Anopheles gambiae and *An. funestus* were the dominant species in rice farmlands, more than a third of *Anopheles* larval habitats were observed in farmlands, thus confirming farmlands as major contributors to mosquito and malaria prevalence in the three zones. This is consistent with the report of Himeidan and Kweka (2012) that farmlands constituted 40% of anopheline larval habitats.

Anopheles maculipennis and *An. quadrimaculatus* were identified morphologically during this study. They were collected only in zone A, from rice farms only and were absent from all other breeding points sampled, e.g. pools, ponds and reservoirs. Their presence was probably facilitated by the numerous breeding sites as a result of high rains and also the low temperatures prevailing in Zone A. This is consistent with Briet (2009) who reported that During the main season cropping period, both rice-cultivated and uncultivated inland valley surfaces have a positive correlation with the *An. gambiae* biting density, in the savannah villages of Côte d'Ivoire, that was directly related to rice cultivation in the inland valleys in a 2-km radius around each village and that the onset of the rainy season was accompanied by a rise in mosquito biting density.

Numerous *Anopheles* breeding sites were found exposed to direct sunlight and in turbid water during the current study; this is consistent with the findings of Mutuku *et al.* (2006). According to Minakawa (1999), female *Anopheles* use open habitats, which are directly under the sun for oviposition because they are warmer and the warmth reduces the larva and pupal development time. In addition, there is less predation and more algae which served as food for larvae. Some of the breeding sites were very turbid, yet numerous *Anopheles* were observed from such sites. Consequently, the presence of *Anopheles* in turbid and polluted water is an indication that some physical qualities of a water body may not play a role in their proliferation. This is consistent with the report of Gimning *et al.* (2001).

Similarly, during the current study, contrary to conventional thinking that *A. gambiae s.l.* only breeds in clean and clear water, its larvae were found in habitats polluted by vegetation, human

faeces, muddy water and even oil. This is contrary to the findings of Muirhead-Thompson (1951) but consistent with the recent findings of Sattler *et al.* (2005). These sites were the drain of mechanic workshops, polluted swamp used as garbage dump and one sewage pond with organic pollution from human faeces. It was also observed that breeding sites that were less than one meter in diameter e.g. buckets, small plastic tanks etc. were more likely to contain *Anopheles* larvae, as long as they contained some debris. Although *Anopheles* larvae were present in large drains, swamps and puddles, these sites contained much lesser densities of *Anopheles* larvae.

Aquatic plants like *Typha* species (*Typha angustifolia*) and water lilies (*Nymphaea* spp.) were present in some of the sampled breeding sites. These water plants facilitated *Anopheles* breeding by slowing down water current, blocking water flow and providing shade and breeding points for laying eggs. This is consistent with the report of Asaeda *et al.* (2005).

Results of the current study showed few *Anopheles* larvae were observed in swamps. This finding agrees with previous reports that *An. gambiae s.l.* prefers temporary breeding sites. Though *Anopheles* species are less likely to inhabit swamps if other habitats more preferable to *Anopheles* mosquitoes are present, the importance of swamp habitats to mosquito proliferation should not be underestimated, because of their great size and their role in supplying water for irrigation ditches, rice farms and various other agricultural activities. Consequently swamps should be targeted by appropriate control measures.

Similarly, the high preponderance of *An. funestus* recorded during this study could be explained by the clearing of natural wooded savannah and increases in cultivated areas which modified soil surface characteristics. In addition, building of temporary ponds like dams and enhanced water run-off. This is also in agreement with the reports of Gillies and De Meillon (1968) and Gillies and Coetzee (1987).

By the end of the current study, seasonal variations in the population of *Anopheles* species collected across the three ecological zones of Katsina State and across the seasons were noted. *An. gambiae* was the most synanthropic species and the most ubiquitous of all the anophelines collected within the three zones. The significantly higher *Anopheles* mosquitoes collected was as a result of a lot of breeding sites created by the abundant rainfall experienced, which is similar to the situation in the northern guinea savannah. In addition is high level of heterogeneity in anopheline mosquito species composition at macro-geographic scale. Mbogo *et al.* (2003) reported differences in the relationship between mosquito population and rainfall in different districts of Kenya and narrowed that to environmental heterogeneity.

Similarly, the high preponderance of *Anopheles* in the wet seasons was because the range and relative abundance of *An. gambiae* and *An. arabiensis* are determined by the amount of annual rain, annual and wet season temperatures and high vegetation (Molineux, 1988; Le Sueur and Sharp, 1991), which are all adequate in Zone A. In addition, the oviposition of mosquito eggs by gravid females and their development to larvae and subsequently to adults depends on the presence of suitable breeding sites and is therefore dependent on rainfall (Le Sueur and Sharp, 1991; Molineux, 1988). This is also consistent with the findings of Lindsay *et al.* (1998), Shililu *et al.* (1998) and Gimnig *et al.* (2001). Numerous reasons were advanced by different authors to explain the afore-mentioned observations, for example; McMichael *et al.* (2003) reported that rainfall provides the medium for the aquatic stages of the mosquito life cycle and also increases the relative humidity and thus the life span of the adult mosquito.

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 of a particular geographic region and depends on local habits of mosquitoes. 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 *et al.*, 1996).

The data obtained shows that the population density of *Anopheles* larvae and adults increase as one moves from the arid towns of Zone C (Katsina and Daura) towards the less arid towns of Zone B (Dutsinma and Kankara) and culminates in a dramatic increase in population density of *Anopheles* in Zone A (Funtua and Dandume) because of abundant rain, which lead to the formation of water bodies like pools, ponds etc and the low temperatures experienced, which collectively provide conducive conditions suitable for *Anopheles* proliferation.

A review of literature indicated that they are secondary vectors of malaria (Hopkins, 1987). The two species do not play a considerable role in malaria transmission in those towns where they are present. However control measures should also be directed towards them if the complete elimination of mosquitoes and malaria were to be accomplished.

An. gambiae was more preponderant in moist environments, and *An. arabiensis* is more common in arid areas (Lindsay *et al.*, 1998). Thus, other biotic or abiotic factors are responsible for species composition variation at micro-geographic scale.

An. gambiae s.l. breeds in polluted water in Nairobi and 95% of the larvae were *An. arabiensis*, which were also anthropophilic thus showing ecological flexibility within the species.

Similarly, Godwin *et al.* (2005) identified 17 vector species belonging to three genera (*Anopheles*, *Culex* and *Aedes*) as the

vectors of four human diseases prevalent in the areas surveyed. A total of 736 mosquito larvae were encountered in artificial sources and 568 larvae were harvested from natural sources. Pools, plastics and metal cans were the dominant artificial sources of mosquito larvae.

CONCLUSION

Anopheles mosquitoes breeds predominantly in pools (formed by rain), ponds, cemented reservoirs, overhead tanks, rice farms and numerous water bodies created by rain in addition to small water storage containers utilized by people for household chores. *Anopheles maculipennis* and *An. quadrimaculatus* were identified morphologically during this study only in rice farms.

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