academicJournals

Vol. 7(5), pp. 94-100, June 2015
DOI: 10.5897/JPVB2015.0193
Article No. 0205D9852843
ISSN 2141-2510

http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Full Length Research Paper

Culicid forms distribution and breeding sites in Nsukka ecological zone of South Eastern Nigeria

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Received 23 February, 2015; Accepted 15 May,2015

The culicid forms distribution and breeding sites in Nsukka ecological zone of south eastern Nigeria were investigated in four hundred and fifty eight (458) oviposition sites between October and December, 2010. Soup ladle dipper method was used in larvae/pupae collection while the environmental parameters were determined *in situ* using field meter. Anova were used to investigate the association between the physicochemical variables, the relative abundance and distribution of the mosquito larvae. Of the 458 containers sampled, automobile tyre had container index% of 59.62 while the least was 20.95%, recorded in clay pots. Four thousand, four hundred and twenty five species of culicid distributed in four genera (*Aedes, Anopheles, Culex* and *Toxorhynchites*) were identified. *Aedes aegypti* recorded 562 (12.70%) of the total collection in the automobile tyre. Also in the discarded cans, *A. aegypti* ranked second highest. The *Culex quinquefasciatus* had a prevalence of 302 (6.82%) in the automobile tyre while *Aedes albopictus and Toxorhynchites* 248 (5.60) and 242 (5.47) were recorded in the clay pots respectively. Also, Table 3 shows that a total of 458 (40.17) containers were sampled out of which 184 (38.14%) contained mosquito larvae. A total of 204 (20.95%) clay containers were sampled during the course of the studies. Water drums recorded 36 (30.55%) and a total of 104 (59.62) were observed in automobile tyres. Out of the 184 (40.17%) containers that held mosquito larvae, the highest container type that held mosquito larvae was automobile tyre with a total of 62 (59.62%). Among the oviposition sites, clay pots recorded highest dept of 18.50 cm and this was closely followed by buckets with 17.28cm while cans was the least with 5.2cm. The more important vectors of mosquito-borne diseases are those which show a close association with man and prefer him to other animals as source of food. These include *A. aegypti, A. albopictus, Culex fatigans* and *Agkistrodon bilineatus taylori.*

Key words: Culicid, breeding sites, ecological zone, Nigeria.

INTRODUCTION

The disease transmission and biting nuisance problems arising from the occurrence and interaction of mosquitoes with humans appear to have defied several scientific advances and health services instituted to combat them. Part of the problems militating against effective and sustained control of mosquitoes and the diseases

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transmitted by them is the overt advantages available to mosquitoes to breed in diverse aquatic media that are naturally occurring or the creation of human activities (Adebote et al., 2008). *Aedes aegypti* species is known to be the most thoroughly domesticated of any of the mosquitoes and apparently greatly prefers the blood of man to that of other animals (King et al., 1944).

Mosquitoes prefer an environment with certain resources (food, shelter, breeding sites; favourable temperature and suitable humidity) in sufficient amount at an appropriate time for survival and development (Adeleke et al., 2008). The policy, in the 1970s, of clearing forest in Enugu region of Nigeria for planting of the valuable timber trees, teaks and gmelina, increased the people's exposure to the bites of forest mosquitoes. They also noted that the situation was aggravated as a result of rot holes which developed in the tree stumps left behind after clearing operations which later became filled with water and formed ideal larval habitat for *Aedes africanus* – a sylvatic yellow fever vector (Onyido et al., 2009). According to Anosike et al. (2007), in their aquatic stages different species of mosquitoes may occupy the same habitat, and also form part of a single guild. The recent increase in ecological and environmental modification due to agricultural activities and urbanization has been observed to contribute to the breeding of various mosquito species (Amusan, 2005; Anizoba and Obudulu, 2006).

Mosquitoes develop in a wide range of aquatic larval habitats and in all climates from the Arctic to the Tropics (Edington and Gilles, 1978). There have been no known information on the culicid forms distribution and breeding sites in Nsukka ecological zone of south eastern Nigeria hence this study is concerned with exploring how different types of containers may act on the mosquito proliferation and how the knowledge on these mechanisms may help in establishing better policies to control the dengue vector. Accordingly, this study focused on surveying several containers considering their type of use, volume, factors, and materials as potential factors that could facilitate culicid forms distribution.

MATERIALS AND METHODS

Sample collection

Outdoor water holding containers within 270 dwellings (sites) in these ecological zones were examined. The containers holding water found in these sites were sampled. Five scoops in each of the water holding containers were obtained with a plastic soup ladle dipper for containers holding water of more than 250 ml while in containers holding below 250 ml of water was poured directly into plastic beakers. Specimens in recipients like barrels, drums and tanks were collected with the aid of plastic bowls and buckets of known volumes. The water collected was then poured into a white plastic bowl and carefully observed for the presence of mosquitos' larvae. Culicine larvae collected were concentrated in a sieve and carefully picked with dropping pipette into labeled small cups and covered with mosquito net. Specimens in recipients like reservoir and fish pond were collected by the sweep net method, as proposed by Tun-lin et al., 1995). These were transported to the post graduate research laboratory of the department of Zoology University of Nigeria where they were reared with a diet of baker yeast until they emerged to adults. Other invertebrates collected were preserved in labeled collecting bottles containing 70% alcohol.

Species identification

Mosquitoes collected were identified to species level and counted with the aid of binocular microscope in the Entomological laboratory of the Department of Zoology, University of Nigeria, Nsukka using pictorial keys of Gillett (1972) for the culicines.

Determination of physicochemical parameters of water in the container

Depths of water in containers were obtained by lowering a metre rule to the bottom of the container at three locations and the mean depths recorded. The surface areas of water in the containers were determined from length and width measurements with a metre rule. The pH, electrical conductivity, total dissolved solids and temperature of water in each container was determined by means of a HANNA HI 991300 pH/EC/TDS/Temp meter. Relative humidity, temperature and rainfall data covering the study area during the course of the research was obtained from Centre for Basic Space Science in the University of Nigeria, Nsukka.

Experimental design

The breeding sites were classified according to the guidelines of Medronho et al. (2009).

1. Volume.
2. Construction material.

Concerning volume, the recipients were classified as:

1. Very small-under 250 ml.
2. Small from 250 to 1,000 ml.
3. Medium-from 1,000 to 25,000 ml.
4. Large-from 25,000 to 1,000,000 ml
5. Very large-above 1,000,000 ml.

According to material, the recipients were classified as:

1. Automobile tyre.
2. Clay pots.
3. Water drums.
4. Buckets.
5. Bottles.
6. Cans.

Statistical analysis

Simpson's index was used in determining the relative abundance of each species of mosquito encountered in each site and during the entire course of the study using the formula given:

$$D = \frac{\sum n\,(n-1)}{N\,(N-1)}$$

n = the total number of organisms of a particular species
N= the total number of organisms of all species.
D = the diversity of species.

Table 1. Range (mean±SE) of physic chemical parameters of water in oviposition sites supportive of mosquito species around Nsukka, Nigeria.

	Physical parameters		Chemical parameters		
Oviposition site type	**Depth (cm)**	**Temperature (°C)**	**pH**	**Total dissolved solids (ppm)**	**Electrical conductivity**
Automobile tyre	4.50-17 (10.30±1.30)	25.8-29.4	6.86-8.74	0-193	0-376
Clay pots	4.20-30 (18.50±60)	20.2-25.2	6.23-7.53	10-120	26-170
Water drums	15-20 (17.28±2.01)	21.4-24.0	6.30-6.85	10-180	22-230
Buckets	9-18 (17.28±1.15)	24.2-29.2	5.82-7.25	15-115	25-165
Bottles	4.50-15 (6.2±1.42)	25.6-30.0	6.54-7.80	10-120	22-230
Cans	0.5-10 (5.2)	25.0-30.3	5.87-8.10	10-184	28-165

Table 2. Monthly relative abundance of larval mosquito species from oviposition sites around Nsukka area (September-December 2010).

Season/Month	*A. aegypti* No. (%)	*A.albopictus* No. (%)	*A. gambiae* No. (%)	*A. funestus* No. (%)	*Culer nebulosus* No. (%)	*C. Tigripes* No. (%)	*C.quinquefascitus* No. (%)	*Toxorhynchites viridibasis* No. (%)	Total No. (%)
Wet season									
September	535 (43.3)	324 (44.5)	372 (49.3)	103 (53.6)	46 (41.8)	66 (35.9)	167 (45.8)	82 (39.0)	1695 (44.9)
October	700 (56.7)	404 (55.5)	383 (50.7)	89 (46.4)	64 (58.2)	118 (64.1)	198 (54.2)	128 (61.0)	2084 (55.1)
Sub total	1235 (32.7)	738 (19.3)	755 (20.0)	192 (5.1)	110 (2.9)	184 (4.9)	365 (9.6)	210 (5.5)	3779
Dry season									
November	148 (75.5)	81 (58.3)	89 (88.1)	24 (75.0)	12 (92.3)	15 (55.6)	43 (58.1)	37 (57.8)	449 (69.5)
December	48 (24.5)	58 (41.7)	12 (11.9)	8 (25.0)	1 (7.7)	12 (44.4)	31 (41.9)	27 (42.2)	197 (30.5)
Sub total	196 (30.3)	139 (21.5)	101 (15.6)	32 (5.0)	13 (2.0)	27 (4.2)	74 (11.5)	64 (9.9)	646
Total	1431 (32.3)	867 (19.6)	856 (19.3)	224 (5.1)	123 (2.8)	211 (4.8)	439 (9.9)	274 (6.2)	4425

Simpson's index of diversity = 1- D.

Statistical package for social science (SPSS) was used to check the correlation amongst the physicochemical parameters of water in containers, and abundance of species of mosquito breeding therein. GENSTAT was used to test for One-way analysis of variance (ANOVA) test for significant differences in the relative abundance of mosquitoes amongst containers; using least significant difference to separate means that differ significantly. While a two way analysis of variance was used to test the interaction of mosquito species and containers.

RESULTS

Table 1 shows ranges of physic chemical parameters of water in oviposition sites supportive of mosquito species around Nsukka, Nigeria. Among the oviposition sites, clay pots recorded highest dept of 18.50 cm and this was closely followed by buckets with 17.28cm while cans was the least with 5.2cm.Temperature recorded highest in cans with 30.3°C as against 24.0°C that was observed in water drums. pH was highest in automobile tyre with 8.74 while buckets recorded the lowest of 7.25. Also, Total dissolved solids (TDS) was highest in automobile tyre with 193 ppm as against the lowest value recorded in buckets with 115ppm. The electrical conductivity was highest in automobile tyre with 376 while buckets registered the lowest value of 165.

Table 2 shows monthly relative abundance of larval mosquito species from oviposition sites around Nsukka area between September to December, 2010. Among the total number (4425) of larval mosquito species recorded, the *A. aegypti* was most abundant with (143) 32.7% in wet season when compare with (196) 30.3% abundance recorded in dry season. The *C. tigipes* larval species was least abundant among the mosquito species observed with 184 (4.9%) recorded in wet season as against 27(4.2) available in the dry season. Also, *Aedes albopictus* was second abundant mosquito species with 738 (19,3%) in the wet season as against 139 (21.5%) in the dry season. *Toxohynchies viridibas* recorded 210 (5.5%) in wet season as against 64(9.9%) that was observed in dry season.

Table 3. Artificial oviposition site preferences of mosquito species around Nsukka area, Nigeria (September to December, 2010).

Oviposition site type	No. examined	No. positive for larvae	Container index %	Total larvae collected (%)	Mosquito species	Species occurrences No. (%)
Automobile tyres	104	62	59.62	1252 (28.30)	*A. Aegypti*	562 (12.70)
					A. albopictus	329 (7.44)
					C. nebulosus	59 (1.33)
					C. quimquefasciatus	302 (6.82)
Clay pots	204	44	20.95	864 (19.53)	*A. albopictus*	248 (5.60)
					A. Gambiae	242 (5.47)
					A. Funestus	132 (2.98)
					Toxorhynchites viridibasis	242 (5.47)
Discarded buckets	60	20	46.66	709 (16.02)	*A. Aegypti*	234 (5.29)
					A. Albbopictus	156 (3.53)
					C. tigripes	211 (4.77)
					C. quinquefasciatus	108 (2.44)
Discarded cans	40	32	80.00	720 (16.27)	*A. Aegypti*	333 (7.53)
					A. albopictus	134 (3.03)
					A. Gambiae	224 (5.06)
					C. Quinquefasciatus	29 (0.66)
Water drums	36	11	30.55	513 (11.59)	*A. aegypti*	174 (3.93)
					A. Funestus	92 (2.08)
					A. Gambiae	247 (5.58)
Broken bottles	8	7	87.50	367 (8.29)	*A. aegypti*	128 (2.89)
					A. Gambiae	143 (3.23)
					C. Nebulous	64 (1.45)
					Toxorhynchites viridibasis	32 (0.72)
Total	458	184	40.17	4425	-	4425

Table 3 displayed artificial oviposition site preferences of mosquito species around Nsukka area , Nigeria (September to December 2010). A total of 4,425 mosquito species were collected and *A. aegypti* recorded 562 (12.70%) of the total collection in the automobile tyre. Also in the discarded cans, *A. aegypti* ranked second highest. The *Culex quimquefasciatus* had a prevalence of 302 (6.82) % in the automobile tyre while *A. albopictus and T. virdibasis* 248 (5.60) and 242 (5.47) were recorded in the clay pots respectively. Also, Table 3 shows that a total of 458 (40.17) containers were sampled out of which 184 (38.14%) contained mosquito larvae. A total of 204 (20.95%) clay containers were sampled during the course of the studies. Water drums recorded 36 (30.55%) and a total of 104 (59.62) were observed in automobile tyres. Out of the 184 (40.17%) containers that held mosquito larvae, the highest container type that held mosquito larvae was automobile tyre with a total of 62 (59.62%).

DISCUSSION

The species of mosquitoes encountered in this study compares favorably with the five species recorded in Nsukka (Agwu, 2005) and also with those recorded in Benin (Wagbatsoma and Ogbeide, 1995). The species also compares favorably with other works carried out in the south-eastern Nigeria. Which include the five species encountered by Onyido et al. (2009) in Awka and that of Anosike et al, (2007) in Imo

State. In the South- west, these *spp* encountered here were also compares favorably with the nine *spp* recorded in Abeokuta (Adeleke and Adeyi, 2008). This study is also not far from the report in Zaria (Adebote et al., 2008). The species recorded do not also differ much from what was recorded in Santo Domingo city (Carlos et al., 2003). The slight difference might be due to different microhabitat sampled and the distribution of the species and also due to the fact that different species of mosquitoes show individual ecological preference for breeding habitat (Service, 2004).

Agwu (2005), reported that temperature and relative humidity are factors that determine the preference of many mosquito species, and that low relative humidity is known to cause death of mosquitoes through desiccation and also at very low temperatures, mosquito development is slow, while at high temperatures, mosquito development is rapid but producing sterile adults. Death also occurs at temperature above 35°C. Adebote et al. (2008) also stressed on aquatic microhabitat drying out due to cessation of rainfall. This may be the reason why the number of mosquitoes encountered in this study decreased as one moves from the rainy to the dry season (that is from October to December). Water storage mode, the level of sanitation, frequent use of water from containers may determine the availability of containers holding water around dwellings thus this could be the reason why there was difference in the level of sites being positive in a given micro-ecological habitat. In October, *A. aegypti* was relatively more abundance (0.75) than all the other mosquitoes *species* encountered in Nsukka, and this is in agreement with the findings of Agwu (2005). The least encountered was *Toxoryhnchites sp and* this might be due to its prolonged life cycle. *Toxoryhnchites sp* spends longer time in developing from egg to adult than the other mosquito species. In November, *C. fatigans* was relatively more abundant than the other species encountered. *C. fatigans* are known to be most widely distributed mosquito in the world.

It is found on every continent except in Antarctica. This species of mosquito is known to breed in foul water like in septic tanks, soakage pits, cess-pit etc in urban environment. This mosquito is generally known to be more opportunistic in the selection of oviposition sites, and have an ubiquitous distribution of immature in various geographical areas and water body types. This may be the reason why this mosquito was more abundant, and also the period in which the study was carried out coincided with the dry season and according to Russell et al. (2001) the eggs of *A. aegypti* when embryonated, the eggs can survive up to one year until they are flooded and hatched and the completion of the immature stages depend on continued presence of water in the container and on the water temperature. This may account for the decrease in the prevalence of *A. aegypti* in the dry season since water tend to dry out of container as it was reported by Carlos et al. (2003) that mosquitoes species decrease due to seasonal changes. *A. albopictus,* native to the tropical and sub tropical area of South-east Asia was reported to have entered the USA in shipments of scrap tyres from Northern Asia.

According to Anosike et al. (2007), the route of importation of this mosquito into Nigeria is not clear but Agwu (2005), reported that the importation of used tyres from Asia might be the possible route. The impact of this mosquito is very unhealthy, is a fast colonizer, catholic in its feeding habit and is capable of breeding in both domestic and forest environments (Anosike et al., 2007). The relative abundance of this mosquito species might be affected due to the fact that its most preferred habitat (vulcanized rubber) did not hold water during the dry season and thus didn’t support its’ productivity as reported by Anosike et al. (2007). *A. Taylori,* reported by Gillett (1972), as a tree breeder was found in containers during this study, This might be due to ecological changes which include deforestation, land excavation and landscaping (Amusan, 2005; Anizoba and Obudulu, 2006; Onyido, 2009). This may also be the reason why it was the least encountered since it may be trying to adapt to container life. In Nsukka ecological zone, among the seven physicochemical parameters monitored, only total dissolve solid (ppm) and electrical conductivity correlated significantly ($p<0.01$) with the prevalence of *A. aegypti*. The correlation of *A. aegypti* with high total dissolve solids shows that it has preference for such habitat. The abundance of *A. albopictus* correlated significantly ($p<0.01$) with volume of water, and this might be that this species of mosquito has preference for containers having high volume of water. Though depth, water temperature, pH, total dissolve solid (ppm) and electrical conductivity did not correlate with its abundance.

Studies on physicochemical parameters carried out by Umar and Don-pedro (2008) reported that the survival of *A. aegypti* occurred mainly between pH 6.5 and 8.0, and that high mortality resulted above this level. Clark et al. (2004) reported that pH value of 4.0 has no significant effect on the growth and development of *A. aegypti*. Carpenter (1982), reported that larvae mortality was not significant at pH 6 to 8 and that pH 4.5, 10.0, and 10.5 had no significant difference on *C. fatigans.* According to Dario and Nicolas (2002), preimaginal stages of mosquito develop in artificial containers of small volume, such as flask, bottles and flower vases. This might be the reason why volume of water had no effect on most of the mosquito species except *e. albopictus* in Nsukka ecological zone. The fact that only a few *species* abundance was affected by some physicochemical parameters is in line with the report of Adebote et al. (2008) in which no physicochemical parameter correlated with larvae abundance in two of the sites he sampled in Zaria and while other species abundance correlated significantly with the physicochemical parameters in the third site. Mosquitoes utilize a great variety of water sources for breeding. These include ground pools, water in artificial containers, water holding tree, leaf axils, rock pools, gutters and septic tanks etc (Kittayapong and Strickman, 1993; Wagbatsoma and Ogbeide, 1995; Dario and Nicolas, 2002; Amusan, 2005; Adebote et al., 2008; Adeleke et al., 2008; Onyido et al., 2009).

Mosquitoes are reported to share habitat with each other or other invertebrate. Seng and Jute (1994) reported that the larvae of *A. aegypti* shared habitat with *A. albopictus* in urban houses while Adebote et al. (2008a), reported the sharing of habitat by different mosquitoes species. This was also reported by Agwu (2005). In this study there were also cases of different mosquito *species* co- existing together and also with other invertebrate which inhabit containers. Such included unidentified dragon fly nymph, unidentified chironomid larvae, unidentified earth worm, unidentified snail and unidentified water (pond) skater. The containers in which these organisms existed were examined and the level of container not holding mosquito larva might be due to the predatory nature of some of the organism that exist in the container thus feeding on the larvae of mosquitoes. According to Hammon (1984), the larvae of *Toxorhynchites sp* are predaceous to all instars of mosquito larvae. The report that this *species* of mosquito being low in abundance from its record against most mosquitoes as reported by Hammon (1984), Agwu (2005) and Anosike et al. (2007) was also encountered in this study. Though it is a mosquito *spp*, it got its name from the nature of its proboscis which means arrow (toxo) and snout (rhynch). According to Wikipedia (2010), it is known as mosquito hawk or mosquito eater. It is the largest known *species* of mosquitoes, but in spite of their alarming appearance, they are among the few kinds of mosquitoes that do not suck blood. Rather, the adults subsist on whatever carbohydrate- rich materials might be available. The larvae prey on the larvae of other mosquitoes and similar nektonic prey. In order for this mosquito *spp* to be use as an effective mosquito predator, then its limitations (low prevalence and long pupa phase) have to be improved on.

Among the mosquitoes *species* found only *Toxorhynchites spp* has not been incriminated to transmit disease since the adults don't feed on blood. This poses a serious public health implication. Also, in case of possible out breaks of infectious diseases which these mosquitoes are known to transmit like yellow fever, dengue, Chikungiya *Wuchereria bancrofti,* filarial worm etc, like the case of outbreak of yellow fever in Uganda after forty years which claimed 45 lives in December, 2010 was as a result of the prevalence of the vector in such area. The fact that these mosquitoes are collected in artificial containers around living houses is a sign that if, eventually, any infected person is around the area when this mosquitoes are abundant then there might be a sporadic man- to- man transmission as many will be exposed to it. The more important vectors of mosquito-borne diseases are those which show a close association with man and prefer him to other animals as source of food. These include *A. aegypti, A. albopictus, C. fatigans, C. trigripes* and *A. taylori.*

Conflicts of interest

Authors have none to declare.

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