Public Health Research 2013, 3(3): 50-53
DOI: 10.5923/j.phr.20130303.04

Breeding Sites of *Anopheles gambiae* s. l. (Gilles) in Rural Lowland Rainforest, Rivers State, Nigeria

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Abstract Studies were undertaken in the rainy season, May-September, 2009, to determine the breeding sites of the major malaria vectors in four villages (Omanwa, Ipo, Omademe, Ubima) in the Ikwerre Local Governement Area (LGA), Rivers State, Nigeria. A 100ml-ladle and Pasteur pipette were used for collections in large volume of water and Phytotelmata respectively. Standard keys were used for identification. A total of 979 immatures (larvae and pupae) were collected from 3 general breeding site- types: "Pools" (open-lit drainage, Puddle, Depression caused by tyre marks), Containers (plastic and metal) and Phytotelmata (Leaf Axils and depressions on felled trees). The 3 general site-types ("Pools", Containers, Phytotelmata) yielded 674, 280 and 25 immatures respectively. Numbers of immatures per site, in the three categories were "Pools" (19.47/site), Containers (36.77/site) and Phytotelmata (0.60/site); these differences were significant ($F=3.58$; $df=1,12$; $p<0.05$). Percent frequencies of immature occurrence in relation to numbers examined in the general site-types were, "Pools" (73.68%), Containers (32.25%) and Phytotelmata (21.43%). Frequency differences were significant ($F=3.04$; df: $1,2=3.02$, $p<0.05$). These results are compared to those from other studies and discussed within the context of the behavioural plasticity of *An. gambiae* s.l. *An. gambiae s.l.* predominantly utilized the classical breeding sites, but atypical sites were also used.

Keywords *Anopheles Gambiae* s.l., Breeding Sites, Malaria, Rural Rainforest, Nigeria

1. Introduction

A recent review concluded that about 1 million deaths (range 744,000-1,300,000) from the direct effects of malaria occur yearly in Africa, more than 75% of them in children[1]. Malaria accounts for 30% of all childhood deaths and 11% of maternal deaths in Nigeria[2]. Significant variations in vector biology within and between countries have been reported in malaria epidemiological studies[3]. It is now generally agreed that a clear understanding of the detailed epidemiology of the disease is a pre-requisite to effective malaria control in the African sub-region[4].

A number of factors have contributed to the remarkable success in the reduction of the malaria burden in four countries: Brazil, Eritrea, India and Vietnam[5]. These include, a targeted technical approach using a package of effective tools and data-driven decision-making. An Integrated approach to Vector Management (IVM) has been recommended by the World Bank[5]: useofInsecticide-Impregnated Bed Nets, Indoor Residual Spray (IRS) and Larviciding. An important component of larviciding is an accurate knowledge of the breeding sites of major malaria vectors. Studies to identify the breeding sites of *Anopheles gambiae* s.l. were therefore undertaken, April-September, 2009 in five villages (Ipo, Omanwa, Omademe, Ubima, Ozuaha) in the Ikwerre Local Government Area (LGA), Rivers State. This was to complement the Indoor Residual Spray (IRS), being undertaken simultaneously.

2. Materials and Methods

2.1. Study Area

The four villages are located in the lowland rainforest: Ipo (06º57.5´N, 05º02.3´E), Omanwa (06º53.5´N, 05º03.8´E), Omademe (06º575N, 05º05.1´E), Ozuaha (06º55.3´N, 05º03.5´E) and Ubima (06º54.2´N, 05º07.4´E). There are two overlapping seasons: April-September (rainy) and October-March (dry). The main occupation of the local people is farming, but some are involved in wildlife hunting and bushmeat trade.

2.2. Methods

Randomly selected grids (100m x 100m) were surveyed in each village. All possible ground level larval habitats and phytotelmata were identified in each grid and the type of habitat noted. Larvae were collected in the mornings on

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Published online at http://journal.sapub.org/phr

weekdays during the period, April-September, 2009. A 100ml-laddle and Pasteur pipette were the main sampling tools. Immatures were transported daily to the laboratory for identification, using standard keys[7-8]. During the preliminary study, collected immatures were reared to adults and subsequently identified by several keys[6-8] to confirm that they were those of *An gambiae* s.l.

3. Results

3.1. General Breeding Site-types

A total of 979 immatures (larvae and pupae) of *An. gambiae* s.l. were collected. These were collected from 3 general site-types: "Pools" (Open-lit drainage, Puddle, Depression caused by tyre marks); containers (Plastic, Metallic) and Phytotelmata (Leaf Axils, Depressions on standing, cut or felled trees). Sizes of containers varied, 4.0-20.0L. The 3 general site-types: "Pools", Containers, Phytotelmata) yielded 674, 280 and 25 immatures respectively. These differences were significant (F= 15.46; df 2,3=9,55; P<0.05) (Table 1). The numbers of immatures per site in each general site-type were: "Pools" (19.47/site), Containers (36.77/site) and Phytotelmata (0.60/site); these differences were significant (F=3.58; df 1,12= ; P<0.05) (Table 1). Percent frequencies of occurrence of immatures in relation to numbers of sites examined in each general site-type were: "Pools" (73.68%), Containers (32.25%) and Phytotelmata 21.43%) (Table2).

Table 1. Numbers of *Anopheles gambiae* s.l. immatures collected from different site-types

General site-type	Specific site-types	No of sites examined	Nber of immatures collected	No per site
"Pool"	Open-lit drainage	22	453	20.59
	Puddle	12	155	12.92
	Depressions caused by tyres	4	66	16.5
	Sub-total	38	674	
Containers	Plastic	52	262	5.04
	Metal	10	18	1.08
	Sub total	62	280	
Phytotelmata	Leaf Axils	34	08	0.24
	Depressions on standing, cut or felled trees	08	17	2.13
	Sub-total	42	25	

Table 2. Frequencies of Occurrence of *Anopheles gambiae* s.l. immatures at site-types

General site-type	Specific site-types	Number of sites examined	Number containing *Anopheles*	Percent frequency of occurrence
"Pool"	Open-lit drainage	22	18	81.82%
	Puddle	12	06	50%
	Depressions caused by tyres	04	04	100%
Sub-total		38	28	73%
Containers	Plastic	52	17	32.69%
	Metal	10	3	30.0%
Sub total		62	20	32.25%
Phytotelmata	Leaf Axils	34	04	11.77%
	Depressions on standing, cut or felled trees	08	05	62.50%
Sub-total		42	09	21.43%

Table 3. Numbers of *Anopheles gambiae* s.l. immatures collected from the 4 villages

VILLAGES	BREEDING SITE-TYPES			
	"POOLS	CONTAINERS	PHYTOTELMATA	TOTAL
UBIMA	121(95.28%)*	0 (0%)	6 (4.72%)	127
IPO	133 (49.08%)	127 (46.86%)	11(4.06%)	271
OMADAME	263 (64.15%)	145 (35.37%)	2 (0.49%)	410
OZUAHA	157 (90.75%)	8 (4.62%)	6 (3.46%)	173

(%)* Percent of total larvae in each village

3.2. Distribution within "Pools" Category

In the "Pools" category the distribution of immatures was: open-lit drainage (453), Puddle (155) and depression caused by tyres (66); these differences were significant (F=3.04; df 1,2=3.02; P<0.05). The numbers of immatures per site in these sub-categories were: open-lit drainage, 20.59/site; puddle, 12.92/site and depressions caused by tyres, 16.5/site (Table1).

3.3. Distribution within Container Category

More immatures were collected from plastic (262) than metal (18) containers. The numbers per site were 5.04/site (plastic) and 1.08/site (metal); these differences were significant (F=1.85; df 1,2= 1.20; P<0.05).

3.4. Distribution within Phytotelmata

Immatures were distributed almost equally between Leaf Axils (04) and Depressions on standing, cut or felled trees (05); the frequencies of occurrence were quite disparate: Leaf Axils (11.77%) and Depressions on standing, cut or felled trees (62.50%).

3.5. Variation among Communities

Numbers of immatures collected from "pools" at Ubima and Ozuaha were more than 90% of total collection in each village, but these declined to 64.15% and 49.08% at Omademe and Ipo respectively. The numbers from containers rose from 4.62% at Ozuaha to 35.37% and 46.86% at Omademe and Ipo respectively (Table 3).

4. Discussion

Parental capacity to enhance offspring survivorship and fecundity contributes, by definition, to parental fitness. In organisms with no parental care or juvenile dispersal, offspring survival and growth may depend strongly on the quality of the habitat in which they are deposited. Thus, when potential habitats vary in their suitability for juveniles, females are expected to choose habitats that maximise their fitness[9]. In mosquitoes, such oviposition habitat selection has been demonstrated in response to physical and chemical suitability for larval development[10], habitat size and resource availability[11-12], the presence and density of conspecific competitors[13] and the presence of predators [14]. The absence of *An. gambiae* s.l. immature from the randomly selected grid at Omanwa, highlighted the permanence of the colonisation of rural areas by *Cx quinquefasciatus*.

Earlier workers described the typical breeding habitats of freshwater *An. gambiae* s.l. as shallow sun-lit pools, such as burrow pits, drains, brick pits, car tracks, ruts, hoof prints around ponds and wells. Nearly 70% of larvae from this study were collected from these classical breeding sites. According to[9] Kitflawi *et al.*, these sites maximize the fitness of *An. gambiae* s.l.

However, other authors observed *An. gambiae* s.l. breeding in vegetative pools, irrigation canals, swamp edges and permanents wells[6]. Although Okorie[15] observed that *An. gambiae* s.l. was least attracted to containers, approximately 30% of all immatures were collected from containers and at Omademe and Ipo, the percents from containers rose to 35% and 47% respectively. These results indicate the behavioural plasticity of *An. gambiae* s.l. in the selection of breeding site. Plasticity in *An. gambiae* s.l. had been discussed extensively by White[16]. The low number of immatures in phytotelmata is not surprising because they had never featured as preferred breeding sites of *An. gambiae* s.l., probably because of limited size and resource availability[11-12]. Although the frequencies of occurrence of immatures in metal and plastic containers were almost identical, the higher numbers of immatures and numbers per sites in plastic containers were probably associated with variation in physical and chemical suitability of media for larval development[10].

5. Conclusions

Based on the total numbers and frequency of occurrence of immature, *Anopheles gambiae s.l.* predominantly utilized the classical breeding sites (Open-lit drainage, puddles, depressions caused by tyre marks); atypical breeding sites (plastic and metal containers, phytotelmata) were also used. These observations highlighted the plasticity of *An. gambiae s.l.*

ACKNOWLEDGEMENTS

This study was undertaken during a World Bank-funded project on the Indoor Residual Spray (IRS)-Integrated vector Management (IVM) for malaria control. The beneficiary was the Rivers State Ministry of Health, Port Harcourt, Nigeria. We are indebted to village chiefs, who granted us access to the study sites and the youths that served as guides in each of the villages. The commitment of Messrs Pasiya Otufu, Stephen Ozule and Ibrahim Sow Kamara during field work is appreciated. The assistance on statistical analyses by Mr Bukola Amoo Oyebade of the Forestry Biometrics and Measurement Unit, Department of Forestry and Wildlife Management, Faculty of Agriculture, University of Port Harcourt is gratefully acknowledged.

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