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Full Length Research Paper

Physico-chemical characteristics of *Anopheles* breeding sites: Impact on fecundity and progeny development

I.O. Oyewole[1]*, O.O. Momoh[1], G.N. Anyasor[1], A.A. Ogunnowo [1], C.A. Ibidapo[2], O.A. Oduola[3], J.B.Obansa[3] and T.S. Awolola[4]

[1]Babcock University Ilisan Remo, Nigeria.
[2]Lagos State University, Lagos, Nigeria.
[3]University of Lagos, Nigeria.
[4]The Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria.

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Mosquitoes exploit almost all types of aquatic habitats for breeding. Prevailing physicochemical parameters in these habitats are important factors for survival and development of mosquito. Here, six water samples from Atlantic Ocean, River, well, distilled (control), rain and borehole water were used to culture *Anopheline* eggs collected from pure bred of Kisimu species. The development of eggs to 1st, 2nd, 3rd and 4th instars larval, pupal and adult stages were observed from day 1 to 6 using six replicates of each water sample. The number of eggs laid (fecundity) by the emerged adults were estimated using counting microscope. Level of development in emerged adults was determined using the wing size. The analysis of the physicochemical parameters of the water samples was carried out in the Nigerian Institute of Marine and Oceanography. The physicochemical characteristics were compared with the rates of development and fecundity of the *Anopheline* species. Statistical analysis using ANOVA indicates no significant difference (P > 0.05) in the hatchability of the eggs. However, the rates of larval development to pupal stage and subsequent adult emergence showed a level of significant difference (P < 0.05). Also, wing length for determining adult size showed no significant difference (P > 0.05). This study provides information on mosquito ecology in relation to breeding habitat which may have bearing on vector population and distribution as well as malaria transmission in a particular area.

Key words: Malaria, *Anopheles* mosquitoes, breeding habitat, physico-chemical properties.

INTRODUCTION

Mosquitoes exploit almost all types of lentic aquatic habitats for breeding. Larvae of *Anopheles* mosquitoes have been found to thrive in aquatic bodies such as fresh or salt water marshes, mangrove swamps, rice fields, grassy ditches, the edges of streams and rivers and small, temporary rain pools. Many species prefer habitats with vegetation while some breed in open, sunlit pools. A few species breed in tree holes or the leaf axils of some plants (CDC, 2004). *Anopheline* species are known to be ground pool breeders, although large numbers have been observed in gutters, periodomestic runoff and domestic containers (Mafiana et al., 1998; Aigbodion and Odiachi, 2003). *Anopheles* mosquito has been found to breed in clear water of suitable pH, temperature and nutrient composition (Okorie et al., 1978). However, high water current and flooding have been reported to lead to *Anopheles* species larval deaths due to reduction in oxygen tension causing physical harm to the larvae (Okogun, 2005). Water of a near neutral pH 6.8 - 7.2 was found most optimal for the weakening of the egg shells for the first instar larvae stage to emerge (Okogun et al., 2003). Various chemical properties of the larval habitat related to vegetation, ranging from pH, optimum temperature, concentration of ammonia, nitrate and sulphate have been found to affect larval development and survival (Mutero et al., 2004). The present study exa-mined under laboratory conditions some of the physico-chemical parameters germane to the survival, growth, develop-

*Corresponding author. E-mail: oyewoleio@gmail.com.

ment and fecundity in *Anopheles gambiae*.

MATERIALS AND METHODS

Water sample collection and analysis

Water samples were collected from the following sites: Majidun river in Majidun, Ikorodu Local Government Area of Lagos State, well located about 3 km from Majidun River, borehole water located in Abule Egba, Agege Local Government Area of Lagos State, Atlantic Ocean at Victoria Island in Lagos, Rain water from Nigerian Institute of Medical Research (NIMR) compound at Yaba, Lagos and distilled water from the insectary unit in NIMR. The water samples were kept in clean, dry 5-litre jerry cans prior use.

One litre of each water sample was analyzed for physico-chemical constituents from the Nigerian Institute of Oceanography and Marine Research (NIOMR). Conductivity and salinity were measured using YSI moulting meter, salinometric method for salinity and conductance for conductivity. Nitrate, sulphate, and phosphate were measured using spectrophotometer HACHDR 2000 while iron, silica, copper, zinc, calcium, magnesium, cadmium and lead were measured using Flame Atomic Absorption Spectrometry (FAAS). Surface water temperature and dissolved oxygen were determined using thermometric method; pH using potentiometric method, turbidity**,** total dissolved solids, total suspended solids and colour were determined using spectrophotometric technique while alkalinity and total hardness were obtained via titration.

Progeny development and fecundity

Water samples of different sources were put into 6 different plastic containers, each representing 6 replicates while distilled water was used as control. One hundred and twenty eggs of pure culture *A. gambiae* from Kisimu in Kenya were bred in each of the water samples. The mosquitoes were culture from egg to the adult stage. The first instar larvae emerged on day 2 and this was monitored in all the water samples. All the instar larvae were fed with mashed biscuit of low fat (12.30 g) plus yeast capsules (7.59 g). Pupae began to emerge after day 8 followed by adult emergence on day 3 post pupal emergence. The plastic bowls were covered with nets to prevent adults from flying out. Aspirator was used to collect the emerged mosquito from each water sample into rearing cages. These were provided with 10% glucose solution (w/v) in cotton wool placed in plastic tubes while 3 - 4 day old female mosquito was fed with blood meals from the forearm of a human volunteer twice a day to aid egg production. Filter papers sprinkled with distilled water were placed in each cage for egg collection. The experiment was maintained under room temperature. Number of eggs collected was estimated with the aid of counting microscope (stereomicroscope).

Determination of wing length

The wings of 10 mosquitoes from each water sample were removed gently with forceps and mounted on microscope glass slide. Wings were measured using dissecting microscope to the nearest 0.1 mm with an ocular micrometer from the distal end of the alula to tip, excluding the fringe scales.

Statistical analysis

Data collected were analyzed using SPSS software (version 10 for windows, SPSS inc. Chicago, IL) and Analysis of variance (ANOVA) was used as test statistics.

RESULTS

The results of the physicochemical analysis of various water samples showed that rain water constitutes the highest pH while Atlantic Ocean recorded the highest conductivity. Zero salinity was recorded in the borehole, distilled and rain water samples, however, highest levels of salinity and sulphate were recorded in Atlantic Ocean. Turbidity was highest in River but zero turbidity was recorded in distilled and rain water. Copper and zinc were absent in all the water samples (Table 1).

The proportion of emerged larvae and pupae from day 1 to 6 and the mean of the six replicates from different water samples are summarized in Tables 2 and 3. The emerged larvae were highest in distilled and river water respectively while borehole and rain water produced the least. However, statistical analysis showed that there was no significant difference ($P > 0.05$) in larvae emergence from mosquito eggs exposed to all the water samples. On the day 1($F_{(5,30)} = 1.331$, $P = 0.28$), day 2 ($F_{(5,30)} = 0.956$, $P = 0.46$), day 3 ($F_{(5,30)} = = 0.795$, $P = 0.56$), day 4 ($F_{(5,30)} = 54.715$, $P = 0.23$), day 5 ($F_{(5,30)} = 24.493$, $P = 0.13$) and day 6 ($F_{(5,30)} = 24.017$, $P = 0.100$).

Pupal emergence showed a level of significant difference ($P < 0.05$) on day 1($F_{(3,20)} = 3.11$, $P = 0.049$) and day 3($F_{(3,20)} = 5.20$, $P = 0.008$) for larvae reared in River water, Atlantic Ocean, distilled and well water samples. However, there was no significant difference on day 2 ($F_{(3,20)} = 1.027$, $P = 0.402$) and day 4($F_{(3,20)} = 1.824$, $P = 0.175$) of pupae emergence in these water samples.

Table 4 shows the emergence of adult mosquitoes of which the least was recorded in well water with highest mortality rate. Fecundity rate of the survived mosquitoes from different water samples are shown in Table 5.

Table 6 showed the mean length of wings measured from 10 mosquitoes collected from the six water samples. There was no statistical difference ($F_{(3,36)} = 0.073$, $P = 0.974$) in the length of wings of the adult mosquito from all the water samples.

DISCUSSION

Knowledge of the local epidemiology and ecology of malaria/vector is germane to control and reduction in malaria transmission. One of the major reasons for the study of mosquito ecology is to glean information on factors that may determine oviposition, survival, the spatial and temporal distribution of this important disease vector. Water is an important component of ecosystem and its quality in the breeding site is an important determinant of whether or not the female mosquitoes will lay their eggs and the resulting immature stages will successfully complete their development to the adult stage (Piyaratne et al., 2005).

In the present study, *A. gambiae* seems to prefer river and ocean as breeding habitat since these water bodies contain physico-chemical properties such as calcium,

Table 1. Physicochemical parameters in the six water samples.

Physicochemical parameters	Well water	River water	Borehole water	Atlantic ocean	Distilled water	Rain water
Temperature(^{0}C)	30.10	30.40	30.50	27.70	29.40	29
Dissolved oxygen (mg/L)	9.77	9.73	9.82	9.94	9.27	9.20
pH	6.15	6.51	4.74	7.11	7.16	7.54
Conductivity (µS/cm)	375	308.20	71	30800	20.30	6
Total Dissolved solids (mg/L)	187.50	154	36	15400	10.15	3
Salinity (%)	0.30	0.10	0	18	0	0
Turbidity (FTU)	10	30	1	15	0	0
Colour (Pt-Co)	43	353	0	37	0	0
Total Suspended Solids (mg/L)	1	12	0	5	0	0
Sulphate (mg/L)	51	0	0	3400	0	0
Nitrate (mg/L)	1.30	2.60	2.20	4.8	1.50	1.80
Iron (mg/L)	0.12	0.11	0.16	0.14	0.12	0.09
Silica (mg/L)	4.10	10.10	4.50	0.40	2.20	1.30
Phosphate (mg/L)	0	0.17	0	0.79	0.25	0
Alkalinity	204	40	20	120	20	4
Total Hardness (mg/L)	230	60	80	9000	120	20
Lead (mg/L)	0	0	0	0.016	0	0
Copper (mg/L)	0	0	0	0	0	0
Zinc (mg/L)	0	0	0	0	0	0
Calcium (mg/L)	140	110	45	5000	15	6
Magnesium (mg/L)	50	40	15	2300	15	2
Cadmium (mg/L)	0	0	0	0.0028	0	0

magnesium sulphate, nitrate, phosphate and dissolved solids in high proportion as nutrient composition. Other factors such as optimum temperature, pH and dissolved oxygen might have provided conducive environment for survival and breeding activity of the *Anopheline* species. Geller et al. (2000) reported that under laboratory conditions, *A. gambiae* carries out normal development when pH varies as much as from 4.0 to 7.8 as long as there is sufficient phytoplankton and zooplankton for it to consume. Also cool, still and clear water with suitable pH, temperature and nutrient composition has been found to encourage breeding in *Anopheles* species (Okorie et al., 1978; Okogun, 2005). However, high water current and flooding are detrimental to *Anopheles* larval survival due to reduction in oxygen tension and invariably physical harm to the larvae. These attributes may not be uncommon in oceanic water, hence, naturally it is not expected to support growth of the *Anopheline* mosquito. The growth and survival of *Anopheline* species in oceanic water as observed in the present study may be attributed to the laboratory conditions under which the experiment was carried out and the presence of other factors brackish water in the coastal areas with low water current, less flooding and as little saline content as 18‰ necessary for growth in the water sample. However, as found in oceanic water in this study could support breeding activity in *Anopheline* mosquito. Meanwhile, there was no significant difference in hatching of eggs in all the six water samples, but the rate of oviposition was significantly high in well, river, Atlantic Ocean and distilled water respectively, this may indicate that *Anopheline* mosquito discriminately lays its eggs in preferred water body. However, there was a discriminate growth of both the larvae and pupae in all the water samples while growth was significantly high in specimen cultured in both river and ocean water samples. Various chemical properties of the larval habitat in relation to vegetation, optimum pH and temperature, concentration of ammonia, nitrate and sulphate have been reported to affect larval development and survival (Pal, 1945; Mutero et al., 2004). High mortality rate recorded in the larvae cultured in well water could be due to the high alkaline content of this aquatic habitat. Previous reports have shown that the mosquito larvae can survive well in neutral or slightly alkaline water habitat (MacGregor, 1929; Abdullah and Merden, 1995; Pelizza et al., 2007. However, MacGregor (1929) reported that tree-hole mosquitoes (*Finlaya geniculata*) bred in water of a pH value of 4.4 developed and survived better than those from the local streams with a pH value of 8.2 - 8.4 which succumbed or remained stunted. Other factors associated with growth and survival of mosquito larvae includes temperature, pH and salinity of the breeding habitat. The present study showed that the growth rate of the emerged adults is independent of the water source as indicated in the sizes of their wings. The implication of this is that breeding

Table 2. Larval emergence from the six water samples.

Water Samples	Days of Emergence	Mean ± S.D
River water	1	14.50 ± 2.26
	2	16.83 ± 2.23
	3	17.17 ± 2.14
	4	16.67 ± 2.73
	5	16.50 ± 2.51
	6	16.67 ± 2.50
Borehole water	1	12.0 ± 1.67
	2	15.5 ± 2.95
	3	15.67± 2.94
	4	1.83 ± 1.72
	5	1.00 ± 1.26
	6	0.50 ± 0.55
Atlantic ocean	1	13.83 ± 2.23
	2	14.67 ± 1.97
	3	15.33 ± 1.80
	4	14.83 ± 2.14
	5	13.83 ± 2.99
	6	10.33 ± 4.13
Rain water	1	14.33 ± 3.39
	2	15.83 ± 2.56
	3	16.33 ± 3.14
	4	1.83 ± 2.79
	5	1.83 ± 2.99
	6	0.00 ± 0.00
Distilled water	1	15.50 ± 1.97
	2	17.00 ± 1.55
	3	17.5 ± 1.22
	4	15.83 ± 2.32
	5	10.67 ± 4.50
	6	9.50 ± 4.42
Well water	1	14.67 ± 3.08
	2	16.50 ± 1.87
	3	16.50 ± 1.87
	4	14.33 ± 2.16
	5	12.33 ± 4.03
	6	11.33 ± 4.59

Note: No significant difference recorded. Six replicates were used per day for each water sample

blood meal volume of the adult mosquitoes which are factors of body size (Lyimo et al., 1992). The present study highlights some of the factors resident in different bodies of water and the impact of such factors on survi-

Table 3. Pupal emergence from the six water samples.

Water Samples	Days of Emergence	Mean ± S.D
River water	1*	0.83±0.15
	2	5.00±3.29
	3[a]	6.67±2.88
	4	0.17±0.41
Borehole water	1*	NIL
	2	NIL
	3[a]	NIL
	4	NIL
Atlantic ocean	1*	0.17±0.41
	2	2.50±1.87
	3[a]	1.00±0.63
	4	1.50±1.38
Rain water	1*	NIL
	2	NIL
	3[a]	NIL
	4	NIL
Well water	1*	0.33±0.52
	2	4.83±2.48
	3[a]	2.83±4.45
	4	0.67±1.21
Distilled water(control)	1*	0.00±0.00
	2	4.33±3.20
	3[a]	1.50±1.38
	4	0.5±0.84

*, indicates a significant difference; a, indicates a significant difference.
Note: Six replicates were used per day for each water sample.

Table 4. Emergence and Mortality rate of adult mosquitoes.

Water samples	Mosquito emergence	Number of death	Number survived	Mortality rate (%)
Rain water	34	27	7	79.4
Borehole water	28	23	5	82.1
River water	73	52	21	71.2
Distilled water	42	31	11	73.8
Well water	58	53	5	91.4
Atlantic ocean	29	20	9	69.0

val, growth, development and breeding activity of *A. gambiae*. Important of this study cannot be overemphasized in formulating control strategies against this important malaria vector through proper environmental planning and management that will help in reducing breeding habitat.

Table 5. Fecundity rate of the survived mosquitoes from different water samples.

Water sample	Number of survived adult mosquitoes	Total number of eggs laid
Borehole water	15	213
Distilled water	31	367
Atlantic Ocean	21	711
Rain Water	18	174
River water	52	732
Well water	53	1802

Table 6. Mean length of wings of 10 mosquitoes from each water sample.

Water samples	Number of mosquitoes	Mean length of wings (mm)	Standard deviation
Distilled water	10	3.00	0. 33
Ocean water	10	3.30	0.36
Well water	10	3.27	0.38
Borehole water	10	3.28	0.41
River water	10	3.27	0.35
Rain water	10	3.34	0.44

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