



## **Impact of Environmental Factors on Anopheline Larval Density**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors YDM and BMM designed the study and managed the analyses of the study. Author NEO performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author SCH managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aim:** This study aim is to determine the impact of environmental factors on mosquito larval density breeding within Lugbe and Gosa communities.

**Study Design:** Anopheline mosquito larval breeding sites were identified and characterized in both dry and wet seasons. Samplings were done in the morning (08:00-12:00 h) or afternoon (14:00-17:00 h) for about 45 mins at each larval habitat. Larval sampling was done using USAID approved techniques.

**Place and Duration of Study:** The Presidential Malarial initiative PMI/USAID - funded Insectary Laboratory at Nasarawa State University, Keffi/six (6) Months.

**Methodology:** Standard 350 mL dipper for large water bodies; ladle and plastic pipettes for small water bodies where dippers will not be effective, once forth nightly. Water collected were emptied into a white basin and checked for mosquito larvae. The Anopheline larvae were passed through a 100 mesh sieve and stored in labelled container. Environmental characteristics of each larval habitat were measured and recorded during larval collection. The Environmental data determined included habitat hydrological variables. Data on the amount of rainfall and humidity was gotten from Nigerian Meteorological Agency (NIMET). The temperature was determined in-situ using the mercury – in – glass thermometer.

**Results:** The study findings indicate that larval density was seen to be higher in Lugbe than in Gosa. This may not be unconnected to the fact that more habitats were found in Lugbe than in Gosa. Also, it is evident from the result that temperature, rainfall and relative humidity have various degrees of impact on larval density with temporary waters having higher larval density. In this study, Principal Component Analysis (PCA) extracted two significant Principal Components with eigenvalues > 1, explaining about 69.50% and 61.23% of the total variance in corresponding larvae density.

**Conclusion:** This study, has shown that targeting the Anopheline larvae can be an effective tool in the fight against Malaria. Moreover, temperature, relative humidity and rainfall have a great effect on the Anopheline larval densities in Lugbe and Gosa areas of the Federal Capital Territory (FCT).

**Keywords:** Malaria parasite; Anopheline mosquito larvae; breeding density.

## 1. INTRODUCTION

Environmental effects on human health have been a subject of debate in the science community, especially as it pertains to malaria transmission [1]. Any variation in the environment that affects relevant aspects of malaria vector biology could result in a change in transmission risk via effects on vectorial capacity [2]. A recent study shows that changes in temperature (both means and diurnal fluctuation) and rainfall events can have substantial effects on the transmission potential of malaria [3]. However, many studies examining the effect of environment on mosquito biology and aspects of vectorial capacity have focused directly on the adult mosquitoes. This is logical, as it is only the adult female mosquitoes that transmit malaria and the frontline interventions used for control primarily target the adult stage. Based on studies in other invertebrate systems, it is expected that variation in quality of larval habitats could feed through to impact adult life history, which in turn could affect transmission [4].

Malaria, is an entirely preventable, treatable but life-threatening mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans belonging to the genus *Plasmodium*. Only the female of the *Anopheles* mosquito can transmit malaria and only five species out of more than 100 are known to infect man [5,6]. The economic and social burden of malaria is huge with 40% of the world population estimated to be living in malaria-

endemic areas Sturchler et al. [7], 500 million episodes of clinical infection and 2.7 million deaths annually [8]. The most important factor that leads to the successful development of the parasite within the mosquito is humidity and ambient temperatures. Poor quality housing also facilitates malaria transmission as the populations are continually exposed to mosquito bites. Malaria, which is endemic in tropical countries where stagnant pools of water from rain and blocked drainage aid the breeding of mosquitoes, takes a heavy toll on life in Nigeria. This necessitated the use of Lugbe and Gosa villages as study areas. In the southern part of the country, transmission occurs all year round while in the north it is more seasonal [9]. The importance of detailed knowledge of local determinants of malaria is of primary importance in the development of area-specific control interventions that will effectively lead to the control of the disease. Larval control through Larval Source Management (LSM) is one of the malaria vector control measures advocated by the World Health Organisation, particularly in situations of high mosquito population density. Among the variables that affect the effectiveness of this approach of control partly is a habitat characteristic of the area [10]. Knowledge of the socio-economic characteristics of the community is indispensable in this regard.

WHO [8] identified seven endemic malaria regions in the world, each having a predominant *Anopheles* mosquito driving malaria

transmission. Sometimes there is diversity even in regional transmission. Secondary vectors exist to augment the transmission but cannot sustain the disease cycle in the absence of the principal vector. *Anopheles gambiae* and *Anopheles funestus* are the principal vectors in sub-saharan Africa [11]. Malaria cases and deaths are still being witnessed in large numbers in Nigeria and this is attributable to paucity of information on the main drivers of malaria transmission among other factors like injudicious use of antimalarial drugs, delay in seeking medical help and reliance on clinical judgment without seeking laboratory confirmation in most peripheral health facilities [2]. Also, lack of health care facilities close to peoples' home and wrong diagnoses all contributed to the huge disease burden being experienced [6,12]. Robust information on the determinants of local burden of the disease forms the basis of control strategy. Detailed knowledge of local determinants of malaria is of primary importance in the development of area-specific control interventions that will effectively lead to the control of the disease. Information on Anopheline habitat characteristics and environmental impact is basic for critical planning. There is presently no available literature on the impact of environmental factors on larval density and malaria transmission in these very important satellite towns of the Federal Capital Territory (FCT), Lugbe and Gosa.

In sub-Saharan Africa, the most important vectors of malaria, are of the *Anopheles gambiae* complex, consisting of about seven sibling species with different abilities to transmit malaria [13]. *Anopheles gambiae sensu stricto* Giles and *An. arabiensis* Patton, are the most broadly distributed and efficient vectors of malaria. Members of the *An. funestus* group Giles are also significant in terms of distribution and vectorial capacity in Africa [13]. The range and relative abundance of *An. arabiensis* and *An. gambiae* s.s. are partly governed by climatological factors, especially total annual precipitation. The Anopheline mosquito undergoes four stages of development: egg, larva, pupa and adult. During its life-cycle the mosquito undergoes two changes (metamorphosis), from larva to pupa and from pupa to adult. After a blood meal and subsequent development of her eggs, the female adult lays egg in unpolluted water (rain pools, ponds, riversides, lakes, etc.) in batches of 50–200 eggs. Length of time the eggs take to hatch into larvae largely depends on temperature: At 30°C

eggs hatch into larvae in about 2-3 days and at 16°C, it takes about 7-14 days.

The optimum temperature for the development of most mosquito species is around 25°C to 27°C, and their development can be completely arrested at 10°C or 40°C, when high mortality may occur [14]. Three of the four stages in the life cycle of the mosquitoes occur in water, thus the occurrence and abundance of mosquito is closely associated with the availability of suitable larval breeding habitats [15]. The larva has a well-developed head with "mouth brushes" used for feeding (filter- feeders). The larva feeds on micro-organisms (For example; algae, bacteria) and organic matter in the water where they breed. With no respiratory siphon, the larva lies parallel to the surface of water to breathe. *Anopheles* does not usually breed in swiftly moving streams or rivers since larvae are not adapted to withstand wave action. But breeding sites can be as diverse as swamps, marshes, rice fields, temporary pools (puddles), ditches, drains, gulleys, rock-pools, tree holes, water storage containers and empty tins. However, some anopheline species show preference to specific conditions. Even when many different studies have been devoted to study the factors that affect the survival of larvae and the mechanisms that control the emergence of adults, some complementary data are still very necessary to literature contribution. Rainfall, temperature, humidity and time of year are however known to influence larval survival and emergence of adults [16].

Tropical areas including Nigeria have the best combination of adequate rainfall, temperature and humidity allowing for breeding and survival of Anopheline mosquitoes. Malaria transmission in Nigeria takes place all year round in the south but is more seasonal in the northern regions [6]. Aigbodion and Odiachi [4], recorded higher larval densities in Benin City during the wet season coinciding with high amount of rainfall, with peak densities between June and August across various habitats. Mafiana et al. [17], also reported the highest larval density in May, also in the rainy season with increased aquatic vegetation but this time in Nigeria. However, Soleimani-Ahmadi et al. [18], reported higher larval densities during the dry season which correlated positively with filamentous algae, emergent plants and sun exposure. Presently, there is little information on these drivers of malaria transmission in Lugbe and Gosa thus the aim of this study is to determine the impact of

environmental factors on mosquito larval density breeding within these communities.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was conducted in different sites in Lugbe and Gosa, both satellite towns in AMAC Area Council of Federal Capital Territory (FCT). The FCT, in North Central Geopolitical zone, hosts Abuja the nation's capital. Lugbe, (named after a bird) was originally inhabited by the Gbagyis. It is one of the popular suburban settlements in Abuja, mainly residential and densely populated due to its proximity to the city centre. It is divided into five main districts Lugbe South, Lugbe North, Lugbe Central, Lugbe West and Lugbe East. It houses agencies like National Space Research Agency, National Biotechnological Agency, Voice of Nigeria and Federal Inland Revenue (FIRS). Apart from Lugbe village, several estates abound including FHA estate, Trademoore estate, Wisdom estate and so much more. Gosa, which is under the same chieftdom as Lugbe, houses the popular weekly Gosa market and an MDA, Nigeria Nuclear Regulatory Authority. It consists of several villages and lacks basic infrastructure.

The sites are designated 1,2,3,4 and 5. Site 1 is in Gosa Toge located at 8°56' N, 7°17' E coordinates. Site 2 is Kiyami I at 8°56" North and 7°17' East. Site 3 is Lugbe village 8°56' North and 7°19' East while Site 4 is behind AMAC Estate Sauka, Lugbe 8°57' North and 7°21' East while Site 5 is FHA Lugbe 8°58' North and 7°22' East. Both areas are still developing thus stagnant water are common features; including pot holes on the roads, gullies, construction sites, farmlands and many others. Various forms of vegetation also surround human habitation sometimes permanently in some places. These provide conducive breeding ground for mosquitoes. Water supply also remains a major problem; ponds, hand dug wells and bore holes are used as sources of water supply. Streams and other water bodies also exist in both communities.

### 2.2 Larval Collection

Anopheline mosquito larval breeding sites were identified and characterized in both dry and wet seasons. Samplings were done in the morning (08:00-12:00 h) or afternoon (14:00-17:00 h) for

about 45 mins at each larval habitat. Larval sampling was done using United States Agency for International Development (USAID) approved techniques [10]. Standard 350 ml dipper for large water bodies; ladle and plastic pipettes for small water bodies where dippers will not be effective, once forth nightly. Water collected were emptied into a white basin and checked for mosquito larvae. The Anopheline larvae were passed through a 100 mesh sieve and stored in labelled container. Larvae present were identified morphologically using keys developed by Gillies and Coetzee (1987) in the Presidential Malaria Initiative PMI/USAID - funded Insectary at Nasarawa State University, Keffi. In the laboratory, each larva was individually mounted in Berlese's medium on a microscope slide and identified to ascertain its true Anopheline identity by morphological characters.

#### 2.2.1 Sites of larval collection

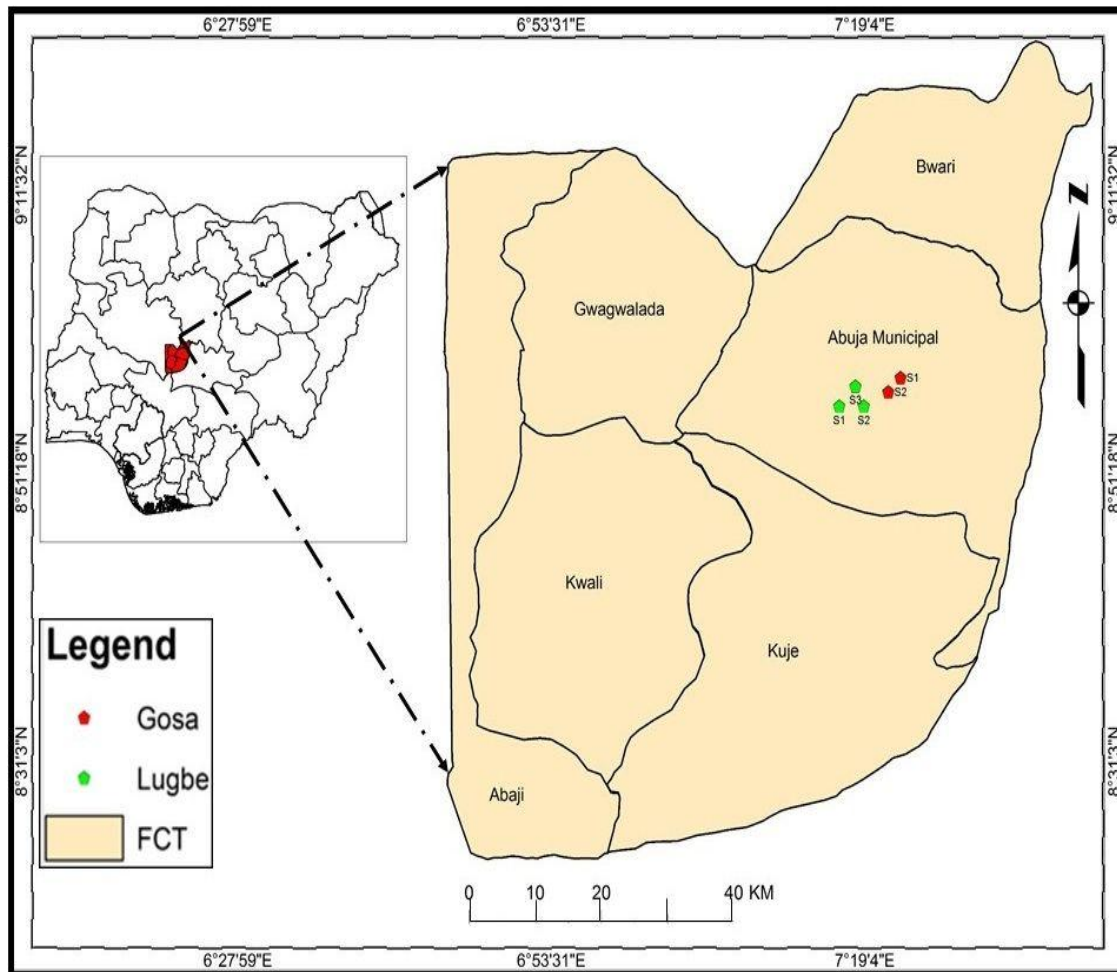
The various sites selected for larval collection are as presented in Table 1.

**Table 1. Variational sites and distances for larval collection**

Name	Distance From University of Abuja permanent site (KM)
Gosa Toge	1.2
Kiyami	1.9
Lugbe Village	2.3
Sauka Lugbe	1.9
Lugbe FHA	2.5

#### 2.2.2 Characterization of larval habitats

Environmental characteristics of each larval habitat were measured and recorded during larval collection. The Environmental data determined included habitat hydrological variables. The habitat hydrological variables including intensity of light, being natural or artificial, vegetation covering, the presence of algae, substrate type, and permanence of the habitat were recorded. Light intensity was visually categorized as full sunlight, partial sunlight and shade. Habitats were categorized as artificial or natural (Soleimani-Ahmadi et al, 2014). Data on the amount of rainfall and humidity was gotten from Nigerian Meteorological Agency (NIMET). Temperature was determined in-situ using the mercury – in – glass thermometer.



**Fig. 1. Map of Abuja (FCT) showing the site locations points at Lugbe and Gosa communities**

### 2.3 Calculation of Larval Density

The larval density was calculated using the Mosquito Breeding Index (BI) according to Belkin [15].

$$BI = TL \div ND \quad (1)$$

Where:

TL = Total number of larvae collected  
 ND = Total number of dips performed

### 2.4 Analysis of Environmental Factors Impact on Larvae Density Breeding

Principal Component Analysis (PCA) which is intended to change the original variables into new, uncorrelated variables (axis), known as essential components, which are direct consolidation of the original variables was used

in analyzing the impact of environmental factors on larvae density at the two sampled community. PCA gives data on the significant parameter that portray most of the information set, managing information diminishment with least loss of unique data. The obtained data were subjected to principal component analysis to estimate the larvae density variation in relation to the environmental factors measured at different months for Lugbe and Gosa community.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

This study was conducted for a period of six (6) months between March and August, 2016, from which 1678 Anopheline larvae were collected; 920 from Lugbe and 761 from Gosa respectively.

**Table 2. Environmental characteristics in relation to larvae density measured for Gosa community**

Month (2016)	Larvae density	Rainfall	Relative humidity	Temperature	Larvae	Larvae density
March	16	0.45	58.5	30	33	17
April	16	0.8	69.5	29.7	46	30
May	23	12.2	72.9	28	61	38
June	25	6.5	83.2	26.1	51	26
July	23	11.9	86.5	26.1	51	28
August	18	10.8	89.2	25	39	21

**Table 3. Environmental characteristics in relation to larvae density measured for Lugbe community**

Month	Larvae count	Rainfall	Relative humidity	Temperature	Total larvae count
March	17	0.45	58.5	30	33
April	30	0.8	69.5	29.7	46
May	38	12.2	72.9	28	61
June	26	6.5	83.2	26.1	51
July	28	11.9	86.5	26.1	51
August	21	10.8	89.2	25	39

Environmental features of an area have been used for decades in the fight against disease vectors and invariably, diseases. Results of characteristics factors as measured from Lugbe and Gosa community at varying months is as presented in Tables 2 and 3.

Larval density in Gosa was at its peak in the month of June, 2016 which had a short bout of rainy season, though the rains came in significant amount. As a result of persistent rise in humidity over the months, temperature dropped significantly from the previous month (Fig. 2).

In Lugbe, however, larval density was highest in May, 2016 which also recorded high amount of rainfall (Fig. 3). Temperature for this period was stable as relative humidity increased.

Table 4 summarises the breeding site for Anopheline mosquitoes in the two communities. Site 1(Gosa Toge) is characterized as a turbid, temporary habitat with no vegetative covering. It is an artificial seepage hole made close to the Toge River by the locals. Site 2 (Kiyami) is a natural temporary pool fed by the stream and rain. Site 3 (Lugbe village) is dependent on the river, a natural marshy area, that is sunlit and polluted. Site 4 is a gully dug and abandoned by a block making factory behind AMAC estate Sauka Lugbe. Site 5

(FHA Lugbe) is an artificial ditch with no vegetation.

Principal component loadings and scores for the various PCs at the two different communities are both displayed in Bi-plots as shown in Figs. 4 and 5.

### 3.2 Discussion

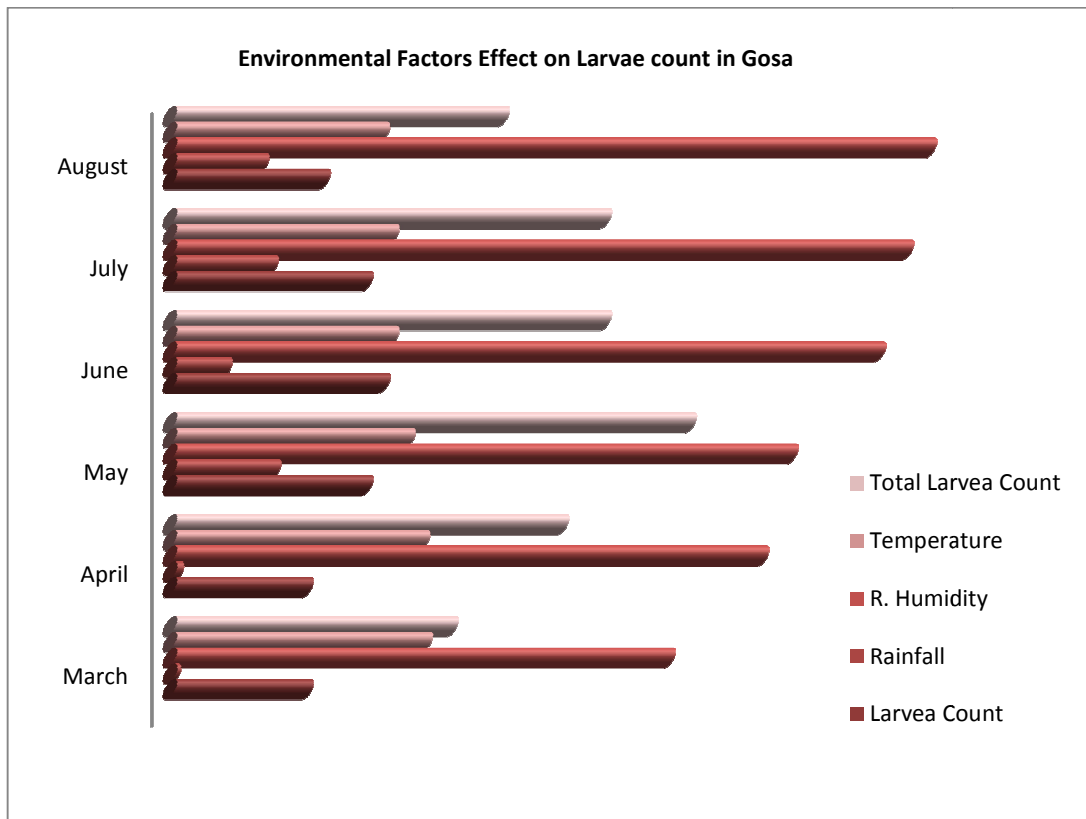
The findings from this study indicate that larval density was seen to be higher in Lugbe than in Gosa. This may not be unconnected to the fact that more habitats were found in Lugbe than in Gosa. However, for individual habitats; Anopheline larvae were more abundant in temporary than in permanent ones. This agrees with the report of Fontenille and Simard [16], that, permanent waters also tend to have more predators and competitors. This certainly would affect the choice of the mosquitoes for oviposition.

Also, shaded habitats had more larvae than sunlit ones. Polluted water was the least in terms of abundance, sometimes no larvae was seen in them. However, the reverse is the case for clear waters where the larvae thrived. This might be attributed to the fact that Anopheline larvae are surface feeders and will find it difficult to breathe in such waters, supporting the proposition of Soleiman-Ahmadi et al. [18] which noted that the

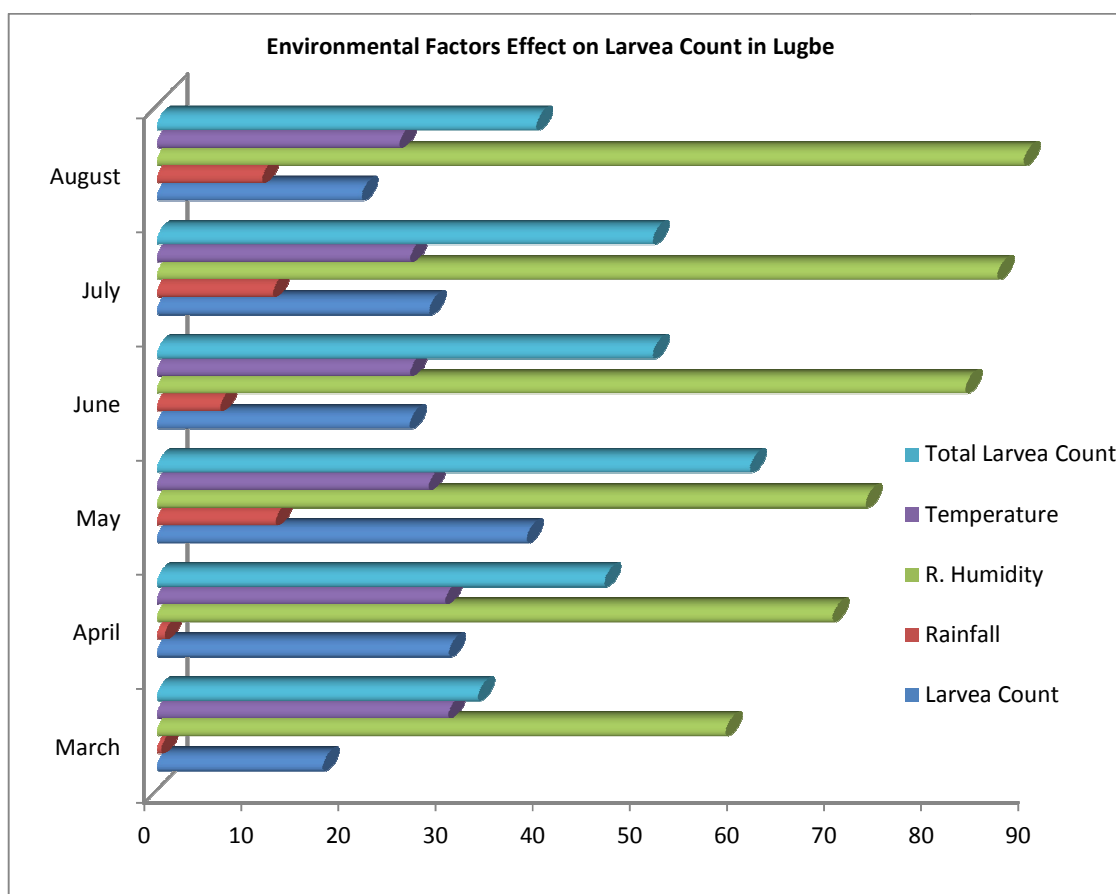
most productive breeding sites are natural and clear water bodies.

Water bodies that are more permanent in nature recorded low abundance of the larvae. The possible reason for this is the presence of predators. Also, the turbulence and constant motion of these water bodies might have contributed to the low abundance of larvae. Still waters provide favourable conditions in which larvae can stay close to the surface with their spiracle open to the air for breathing. This contradicts the postulations of Soleiman-Ahmadi et al. [18], who reported more larvae in permanent waters of Iran. The conflict might be due to the climatic condition of the first study in Iran, where there was relatively low precipitation and as such riverbeds were almost always dry, giving their permanent habitats same features with our temporary ones here in the tropics. Habitat with vegetation and especially those with algae recorded more number of larvae than those without. Anopheline larvae are known to feed on algae [17].

The increase in humidity positively affected larval density. Increased temperature also led to a decrease in larval density, corroborating the findings of Moller-Jacobs [2]. This is of importance to malaria transmission as Moller-Jacobs., [2] demonstrated that temperature during larval stages affect adult survivorship. Principal component analysis (PCA) was performed on normalized data sets (6 parameters × 6 months) for larva density determinant parameters within the environment of study to reduce the dimensions of the original data sets and to identify latent factors affecting larvae density for Lugbe and Gosa community. The number of significant principal components (PCs) was determined based on bi-plots graphs. In this study, PCA extracted two significant PCs with eigenvalues > 1, explaining about 69.50% and 61.23% of the total variance in corresponding larvae density. In Gosa sample analysis, F1 and F2 correspond to 91.36% variations. The most influential parameters in F1 were total larvae count, rainfall, relative humidity and mean larvae density. On the other hand, in



**Fig. 2. Number of anopheline larvae collected in Gosa and monthly rainfall, humidity and temperature**  
 Please change Larvea to larvae (Total larvae count and Larvae count)



**Fig. 3. Number of anopheline larvae collected in Lugbe and monthly rainfall, humidity and temperature**

*Please change Larvea to larvae (Total larvae count and Larvae count)*

F2, only temperature causes the variation. Also in Lugbe sample analysis, PCA extracted two principal components F1 and F2 accounting for 94.89%. The main parameters which affect the variation in larvae density are total larvae count, rainfall, relative humidity and mean larvae density.

The comparative measured data conformed with the results of the PCA analysis were temperature was directly in the opposite quadrangle as relative humidity, larvae density and rainfall, hence an indication that an increase in temperature leads to a decrease in larvae density. However, relative humidity and rainfall falls into same quadrangle adjacent to larvae density indicating a trend of similar characteristics at with stringent control measure. This result shows that rainfall and relative humidity can either influence the rate of larvae density count positively or negatively depending

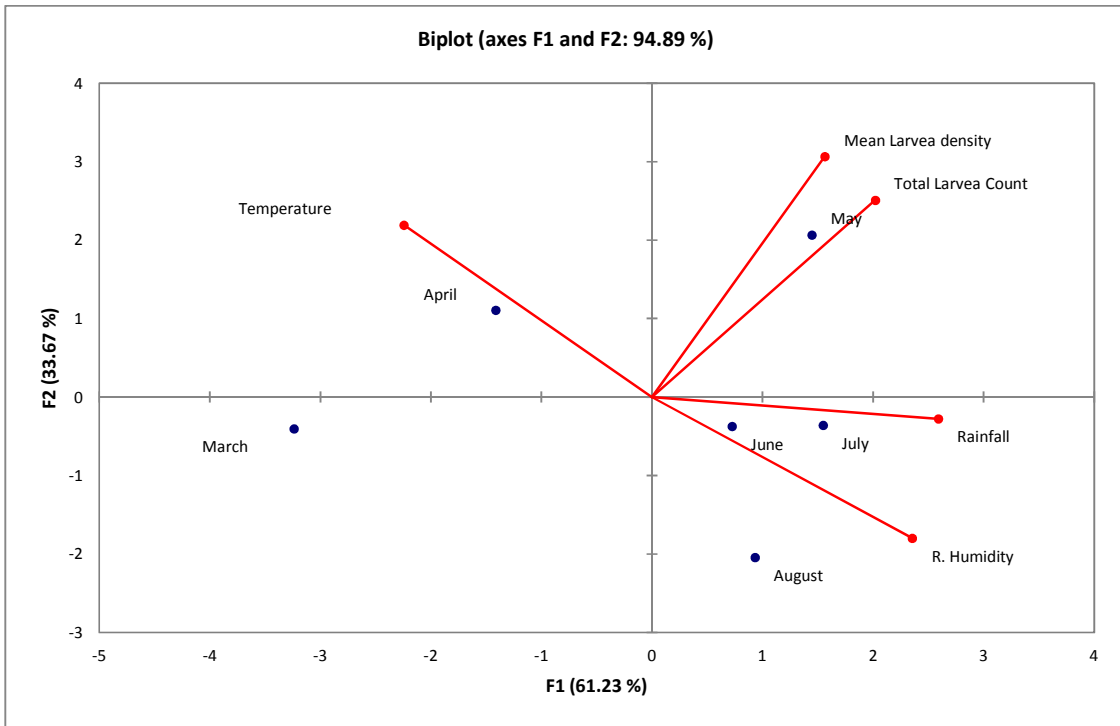
on the balanced shots of the immediate environment. More so, the months of June and May fell into the positive (++) quadrangle of Biplot for Lugbe, with month May recording the highest rate of larvae density count, as compared to that of Gosa where only month May (with the highest larvae density count) also fell into the positive (++).

Rainfall correlated negatively to larval density. This trend is seen especially in Gosa where larval density peaked in June with decreased humidity and rainfall. Rainfall tends to lead to flooding of the habitats, washing away the larvae present. The washing away of the larvae affects the density being recorded. Paaijimans et al. [14], submitted that high water current and flooding is detrimental to Anopheles larval survival as a result of the physical harm to the larvae and reduction in their oxygen tensions.

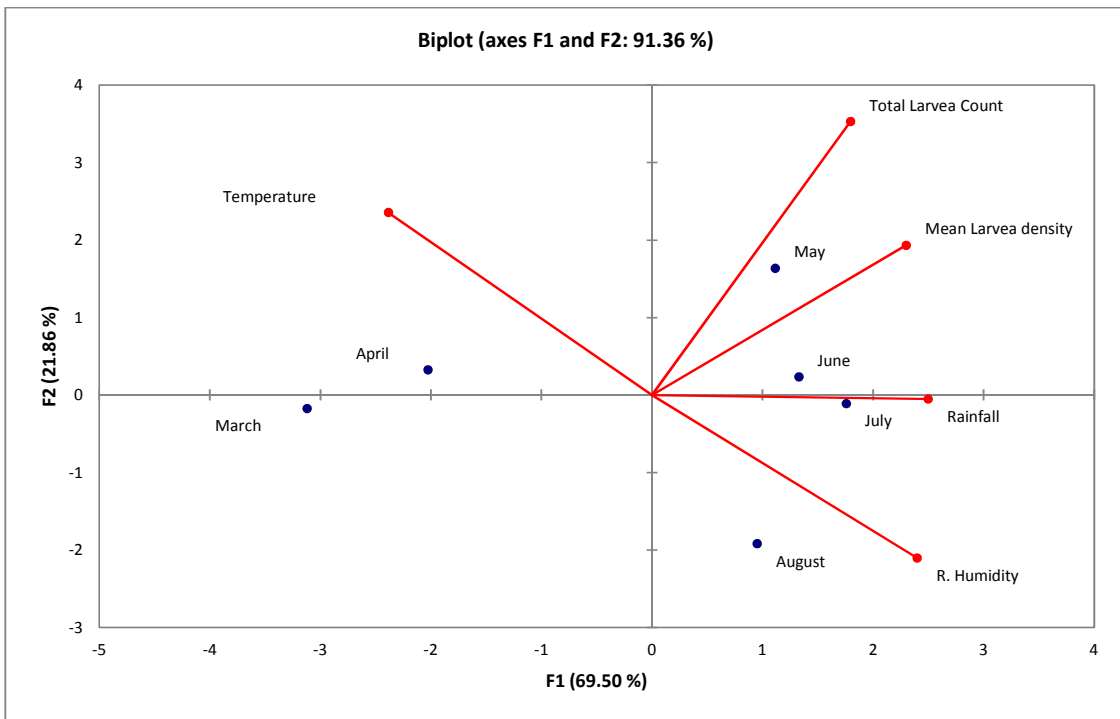


**Table 4. Characterisation of breeding sites**

<b>Parameters</b>	<b>Site 1 (Gosa Toge)</b>	<b>Site 2 (Kiyami)</b>	<b>Site 3 (Lugbe Village)</b>	<b>Site 4 (Sauka Lugbe)</b>	<b>Site 5 (FHA Lugbe)</b>
Type of Breeding Site	Temporary	Temporary	Permanent	Permanent	Temporary
Origin of the Water	River	Stream/Rain	River	Stream	River
Nature of Water collection	Seepage hole	Temporary Pool	Marshes	Gulley	Ditch
Exposure to Sunlight	Shaded	Shaded	Sunlit	Shaded	Sunlit
Presence of Vegetation	None	Floating	Emergent	Emergent	None
Characteristics of the Water	Turbid	Clear	Polluted	Clear	Turbid



**Fig. 4. Biplot of environmental characteristics measure in relation to Larvae density and month for Lugbe community**



**Fig. 5. Biplot of environmental characteristics measure in relation to larvae density and month for Gosa community**

#### 4. CONCLUSION

Despite the on-going argument for and against the relationship between larval density and environment and their impact on malaria incidences, the study, has shown that targeting the Anopheline larvae can be an effective tool in the fight against Malaria. From findings of this study, the following conclusions can be made. First is that the temperature, relative humidity and rainfall has affected the Anopheline larval densities in Lugbe and Gosa areas of the FCT. The implication is that the environmental effects have a lot to contribute to the eradication of malaria. A lot of these effects on the other stages of life of the vector are still not well understood, especially in this part of the world. This battle field cannot be left for the government alone since everyone has a role to play as has been shown in the study.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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