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Correlation between physicochemical parameters and larval abundance of Culex quinquefasciatus Say in Lagos State, Nigeria

Hajara Amao^{1*}, Emmanuel Taiwo Idowu² and Olubunmi Adetoro Otubanjo³

¹Parasitology Unit, Department of Zoology, University of Lagos, Nigeria. E-mail: hajaraamao@yahoo.com ²Parasitology Unit, Department of Zoology, University of Lagos, Nigeria. E-mail: etidowu@yahoo.com ³Parasitology Unit, Department of Zoology, University of Lagos, Nigeria. E-mail: adetoro2001@yahoo.com

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Abstract

Mosquito breeding habitats play an important role in determining adult mosquitoes' abundance and distribution. This study assessed the impact of physicochemical parameters of breeding water on Culex quinquefasciatus larval abundance. A larval survey was conducted in Epe, Eti-Osa, Kosofe, and Ojo local government areas (LGAs) of Lagos state. Emerged adults of Culex mosquitoes were identified. Members of the Cx. pipiens complex were identified into subspecies using polymerase chain reaction. Physicochemical parameters of water from larval sampling sites were analyzed using standard techniques. Six Culex species; Cx. quinquefasciatus, Cx. decens, Cx. nebulosus, Cx. cinereus, Cx. tigripes and Cx. duttoni were encountered. Cx. pipiens quinquefasciatus was the only member of the pipiens complex. Breeding habitat in Kosofe recorded the highest electrical conductivity, total dissolved solids (TDS), and nutrients followed by Ojo, Eti-Osa and then Epe. Larval densities were found to have significant relationship with ammonia (r = 0.507, P = 0.027) and sulfate (r = -0.539, p = 0.017) contents of the breeding habitats. The study revealed that Cx. p. quinquefasciatus is the most dominant and only member of the Cx. pipiens complex. Ammonia and sulfate have a significant impact on the abundance of Cx. quinquefasciatus immature stages. Manipulation of these two parameters could make Culex breeding water less suitable in the study areas.

Keywords: Culex quinquefasciatus, Larvae, Mosquito breeding habitats, Physicochemical parameters, Lagos State

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1. Introduction

Mosquitoes of the *Culex pipiens* complex are found in all temperate, subtropical and tropical regions of the world, where they are responsible for the high burden of nuisance, especially in urban and semi-urban areas (Nchoutpouen *et al.*, 2019). They are vectors of many arboviruses (Japanese encephalitis, St Louis encephalitis, Rift valley fever and West Nile fever viruses) and nematodes (*Wuchereria bancrofti, Brugia* and *Dirofilaria* species), which cause chronic debilitations and deaths (Bhattacharya and Basu, 2016).

Mosquitoes of the *Cx. pipiens* complex require a wide range of habitats with varying breeding water parameters to complete their lifecycles. The physicochemical parameters of mosquito breeding habitats are

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^{*} Corresponding author: Hajara Amao, Parasitology Unit, Department of Zoology, University of Lagos. Nigeria. E-mail: hajaraamao@yahoo.com

determined by the underlying rocks and microclimate (Amawulu et al., 2020). Urbanization, industrialization, and human activities can also change water systems, resulting in varied physicochemical characteristics of water ecosystems (Amawulu et al., 2020). These mosquito breeding water parameters include pH, temperature, alkalinity, dissolved oxygen (DO), total dissolved solids (TDS), biological oxygen demand (BOD) and total water hardness. They provide chemical cues for habitat selection and oviposition, help the females detect larval competitors and predators, and avoid such waters as oviposition sites (Paaijmans et al., 2008). These water features have also been reported to stimulate egg hatching, affect the development and survival of immature stages, adult fitness and distribution which determine the geographical pattern of mosquito disease transmission (Briegel, 2003; Kabula et al., 2011; and Garba and Olayemi, 2015).

Temperature, the amount of heat stored in a water body, affects the development of mosquito aquatic stages. Temperatures higher than 30°C reduce the rate of development of many mosquito species (Nikookar et al., 2017), while all mosquito species died at a temperature of 52°C (Paaijmans et al., 2008). The pH of a water body regulates the availability of soluble chemicals to aquatic organisms. An optimal pH value of 6.8-7.2 weakens mosquito eggshells to allow for the emergence of first instar larvae (Oyewole et al., 2009). There was reduced survival and adult emergence of *Cx. quinquefasciatus* with an increase in pH above 9.4, and larvae could not survive when pH level was more than 11.0 (Amarasing and Dalpapoda, 2014). Dissolved oxygen is important in respiration for aquatic organisms. *Cx. quinquefasciatus* tolerate DO levels of less than 4 mg/L, while the same mosquito immature stages were absent in habitats where the oxygen level was above 12 mg/L (Amarasing and Dalpapoda, 2014). Nutrients such as ammonia (NH₃), nitrite (NO₂), nitrate (NO₃), sulfate (SO₄) and phosphate (PO₄) promote the growth of aquatic fauna and flora, which serve as food for mosquito larvae (Nikookar et al., 2017).

There is little information on the physicochemical parameters of non-malaria mosquitoes breeding waters in Lagos State. Previous investigations elsewhere in Nigeria (Afolabi *et al.*, 2019; Owolabi and Bagbe, 2019; and Oniya *et al.*, 2019) only examined water features such as temperature, pH, electric conductivity, DO, biological oxygen demand and TDS. The most comprehensive work on physicochemical parameters of mosquito breeding sites in Lagos was conducted for *Anopheles* eleven years ago (Oyewole *et al.*, 2009). Ukonze *et al.* (2017) carried out a more recent work in Anambra State, Southeast Nigeria. Therefore, this study was undertaken to determine the association between the physicochemical parameters of *Cx. quinquefasciatus* breeding water and the abundance of their larvae in the study areas, which is necessary for effective larval control measures.

2. Materials and methods

2.1. Study areas

Lagos state is located in the southwestern part of Nigeria, between latitudes N6°22′, N6°42′ and longitudes E2° 42′, E4°15′. The state has an average elevation of 15 meters above sea level and occupies 3,577 square kilometers. Lagos state has a population of 9,013,534 people (NPC, 2006). The state has a tropical climate with two wet seasons from April- July and September-October and two dry seasons from November to March and in August. It has a mean average temperature of 27.5°C and a total annual rainfall of 1,824 mm (BNRCC, 2012). The state vegetation consists of swamp forests in the coastal areas and dry lowland rain forests. The economic activities in the State include fishing, lumbering, farming, commerce and manufacturing. Four Local Government Areas (LGAs), Eti-Osa, Epe, Ojo and Kosofe, were chosen (Table 1).

2.2. Study design

A *Culex* mosquito breeding habitat, which is a permanent drainage channel, was randomly selected from each LGA for larval sampling. *Culex* mosquito larvae were collected monthly for 12 months from May 2015-April 2016. The larval collection was done using the dipping method with the standard 350 ml WHO dipper (BioQuip Product, Inc. California, USA). 20 dips were taken per habitat at different locations. Mosquito immature habitats were mapped using a handheld global positioning system receiver (etrex Garmin International Inc. USA). Data on the total monthly rainfall for the study duration from 2015 to 2016 were gathered from the meteorological station at Murtala Mohammed International Airport Ikeja, Lagos.

Study site	Area (km²)	Population (NPC, 2006)	Geographic coordinates
Epe	965.0	181,499	N6°37′43.620″ E3°54′33.700″
Eti-Osa	129.5	287,785	N6 [°] 26′54.175″ E3 [°] 24′22.186″
Kosofe	178.9	682,772	N6°31′35.988″ E3°23′13.116″
Ojo	180.0	507,693	N6°29′42.054″ E3°11′56.169″

2.3. Mosquito rearing and morphological identification

Culex mosquito larvae were reared at the Nigerian Institute of Medical Research, Lagos. The emerged adults were identified into species level with the aid of dissecting microscope and the morphological keys of Edwards (1941) and Harbach (1988). Morphologically identified members of the Culex pipiens complex were preserved on silica gel in Eppendorf tubes for sibling identification using multiplex Polymerase Chain Reaction (PCR).

2.4. Molecular identification of Culex pipiens sibling species

One hundred and twenty (120) morphologically identified mosquitoes of *Culex pipiens* complex were randomly selected and subjected to PCR analysis to determine their sibling species. Genomic DNAs were extracted from whole mosquitoes using the Jena Bioscience DNA Mini-Prep Kit (Germany), following the manufacturer's instructions. The PCR was performed by amplifying the acetylcholinesterase-2 (*ace-2*) gene on the Techne TC 4000 thermal cycler using the two forward primers ACEpip (5'-GGAAACAACGACGTATGTACT-3'), ACEquin (5'CCTTCTTGAATGGCTGTGGCA-3') and the reverse primer B1246s (TGGAGCCTCCTCTTCACGG-3') following the method of Smith and Fonseca (2004).

2.5. Physicochemical analysis of mosquito breeding water

Five hundred milliliter (500 ml) of water samples from each mosquito breeding habitat were collected in plastic bottles on larval collection days and taken to the Chemistry Department, University of Lagos, for physicochemical analysis. Nutrients such as ammonia (NH $_3$), nitrite (NO $_2$), nitrate (NO $_3$), phosphate (PO $_4$) and sulfate (SO $_4$) were analyzed based on mg/L using the HACH DR2000 Spectrophotometer. Magnesium (Mg) and calcium (Ca) were determined by the atomic absorption spectrophotometry method using the Perkin Elmer 200 Atomic Absorption Spectrometer. Chloride (CI), biological oxygen demand and alkalinity were tested using the acid titration method. Physical characteristics of larval habitats, including temperature (°C), electrical conductivity (μ S/cm) and TDS, were measured with a Com-1000 conductivity meter. Acidity (pH) of breeding sites were determined using a portable pH Orion 4-star meter, while DO readings were taken with the potable Orion-3 DO meter. All analyses were conducted following the standard methods of the American Public Health Association (APHA, 1998).

2.6. Data analysis

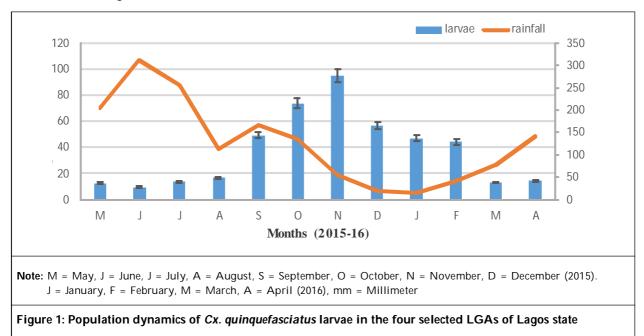
Culex larvae abundance was calculated in percentages while Analysis of Variance (ANOVA) was used to determine significant differences in abundance, and p-values of less than 0.05 were considered significant. The mean values of water parameters were calculated using descriptive analysis on SPSS software version 22. The physicochemical parameters of the breeding sites were compared using the Kruskal-Wallis analysis. The association between Culex larval abundance and physicochemical variables in breeding habitats was examined by Pearson correlation analysis.

3. Results

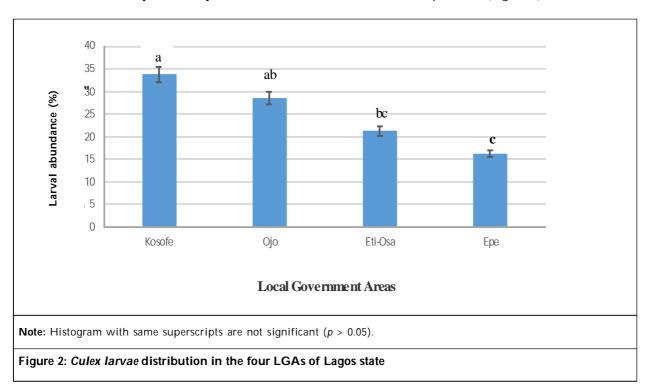
3.1. Culex larvae abundance, distribution and species identification

A total of 8,248 *Culex* larvae were collected. The larvae were more abundant from September to February, with the peak population recorded in November. *Culex* larval populations started decreasing from March, reaching

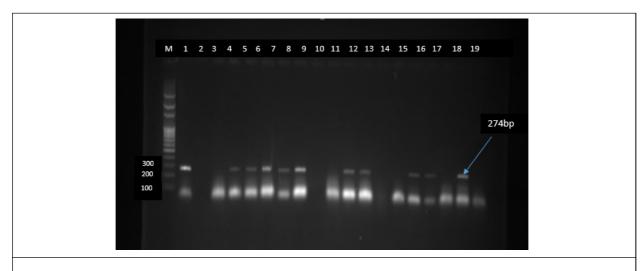
their lowest density in June. Larval abundance varied significantly (F = 6.49, p = 0.00, p < 0.01) with the months in all the LGAs (Figure 1).



Kosofe LGA recorded the highest number of *Culex* larvae (33.9%), followed by Ojo LGA (28.6%) and Eti-Osa LGA (21.3%), while Epe LGA (16.2%) recorded the least mosquitoes. There was a significant difference in larval population in all the study areas (F = 6.12, p = 0.001, p < 0.01). The larval abundance differed significantly between Kosofe and Epe LGAs and Kosofe and Eti-Osa LGAs. There was also a significant difference between the number of larvae collected in Ojo and Epe LGAs. However, the larval population did not vary statistically between Kosofe and Ojo LGAs, Ojo and Eti-Osa LGAs and Eti-Osa and Epe LGAs (Figure 2).



The emerged adults were morphologically identified as *Cx. quinquefasciatus* (61.9%) of the *Cx. pipiens* complex, while other species observed included *Cx. decens* (10.0%), *Cx. nebulosus* (6.9%), *Cx. cinereus* (12.0%), *Cx. tigripes* (3.9%) and *Cx. duttoni* (5.3%). The results of PCR analysis of members *Cx. pipiens* complex revealed *Cx. quinquefasciatus* as the only sibling species (Figure 3).



Note: M = Molecular marker, Lane 1 = Positive control, Lane 2 = Negative control, Lanes 4, 5, 6, 7, 8, 9, 11, 12, 16 and 18 = Positive samples.

Figure 3: Electrophoresis of PCR products of members of *Culex pipiens* complex showing the presence of *Cx. quinquefasciatus* with a 274 DNA base pair

Culex mosquito species occurrence differed with LGAs. Cx. quinquefasciatus had the highest and least occurrence in Kosofe and Epe LGAs, respectively. All the other species had their highest densities in Epe LGA and least in Kosofe LGA except for Cx. decens which had its most occurrence in Eti-Osa LGA (Figure 4).

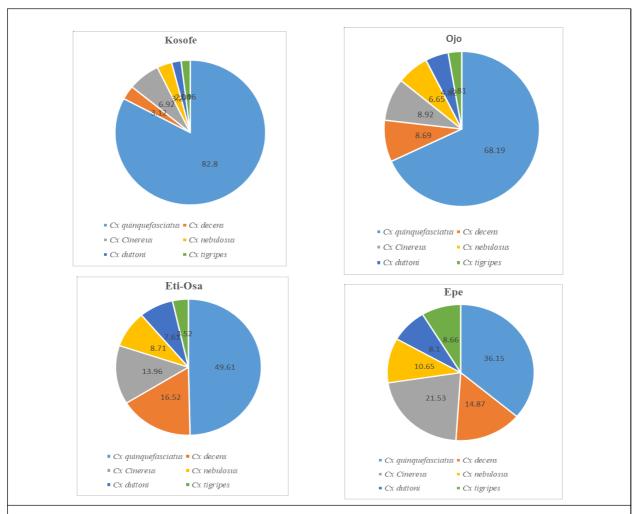


Figure 4: Culex species composition in the four selected Local Government Areas of Lagos state

3.2. Physicochemical parameters of Culex breeding habitats

A summary of the descriptive statistics of the physicochemical parameters of *Culex* larvae habitats in all four LGAs is shown in Table 2. A total of 14 water parameters were measured from water samples collected from the *Culex* mosquito breeding sites. The water temperature values of the breeding habitats ranged from 27.18 - 27.18°C and the pH readings varied from 6.64-7.75.

Culex habitats in Kosofe, Ojo and Eti-Osa LGAs recorded relatively higher electrical conductivity and TDS than Epe LGA. The total water hardness (amount of magnesium and calcium) of the breeding sites in Kosofe and Ojo LGAs were higher than the other two localities. High chlorine content was detected in Eti-Osa, Epe and Kosofe LGAs. Mosquito breeding sites in Kosofe LGA had the highest nutrients (NH $_3$ NO $_3$, SO $_4$ and PO $_4$) compared with the remaining three LGAs. The alkalinity and the amount of DO were lowest in Kosofe and highest in Epe LGA.

The relationship between the various water parameters was determined using the Pearson correlation analysis and represented in Table 3. The electric conductivity showed a positive significant correlation with total dissolved solids (1.000) and phosphate (PO_4) (0.994), while it showed a negative correlation with pH (0.998) at the 0.01 level. TDS was also significantly positively associated with phosphate (PO_4) (0.990) at 0.01 level. There was a significant negative correlation between pH, Total dissolved solids (0.996) and phosphate (PO_4) (0.998) at the 0.01 level. DO had a negative relationship with calcium (0.998) at a 0.01 significant level. Ammonia showed a positive correlation with nitrate (PO_3) (0.974) and a negative one with dissolved oxygen (BOD) (0.964) at 0.05 significant level. Calcium was positively significantly correlated with biological oxygen demand (BOD) (0.960), while sulfate (PO_4) had a positive association with magnesium (PO_4) (0.971) at the same 95% level of significance. No significant correlation was recorded between the rest of the breeding sites parameters.

Table 2: Physicochemical	Local	Government	Areas			
Physicochemical parameters (Mean ± SE)	Epe	Eti-Osa	Kosofe	Ojo		
Temperature (pC)	27.97 ± 0.272 ^a	27.84 ± 0.236 ^a	27.42 ± 0.353 ^a	27.18 ± 1.666b		
Acidity (pH)	6.64 ± 0.060^{a}	6.90 ± 0.043 ^a	6.96 ± 0.084 ^a	7.755 ± 0.082b		
Conductivity (μS/cm)	895.9 ± 4.824 ^a	866.0 ± 3.858 ^a	792.1 ± 2.201 ^a	126.2 ± 2.669b		
TDS (ppm)	453.33 ± 2.519 ^a	445.5 ± 1.751 ^a	394.72 ± 2.021b	64.32 ± 1.635°		
DO (mg/L)	2.056 ± 0.088 ^a	3.005 ± 0.110 ^b	4.01 ± 0.130°	4.128 ± 0.104°		
BOD (mg/L)	38.17 ± 0.422 ^a	35.51 ± 0.416 ^b	15.81 ± 0.124	18.21 ± 0.416 ^d		
Alkalinity (mg/L)	10.68 ± 0.394 ^a	6.54 ± 0.2824 ^b	6.873 ± 0.233 ^b	17.98 ± 0.480 ^d		
Magnesium (mg/L)	20.33 ± 0.386 ^a	161.33 ± 1.051 ^b	6.777 ± 0.571°	4.667 ± 0.431°		
Chloride (mg/L)	103.75 ± 5.734°	84.66 ± 0.611 ^b	120.7 ± 3.145 ^a	51.98 ± 0.899°		
Phosphate (mg/L)	33.97 ± 1.705 ^a	31.14 ± 0.339 ^a	31.90 ± 0.521 ^a	10.28 ± 0.348 ^b		
Calcium (mg/L)	184.08 ± 1.513 ^a	108.61 ± 1.183 ^b	6.016 ± 1.350	4.072 ± 0.805°		
Ammonia (mg/L)	1.229 ± 0.0264 ^a	0.528 ± 0.050b	0.307 ± 0.032b	0.183 ± 0.058°		
Nitrate (mg/L)	30.18 ± 0.7908 ^a	28.90 ± 0.286 ^a	26.13 ± 0.731 ^a	21.85 ± 0.1287b		
Sulfate (mg/L)	183.89 ± 4.O33 ^a	124.36 ± 0.681 ^b	85.93 ± 0.494°	71.99 ± 0.945°		

Note: Parameters with the same superscripts across columns did not vary significantly at 0.05 level. TDS = Total Dissolved Solid, DO = Dissolved Oxygen, BOD = Biochemical Oxygen Demand, μ S/cm = Siemens per centimeter, mg/L = Milligram per liter.

Ca

NH,

NO,

SO

0.929

0.884

0.954

0.612

-0.634 0.643

-0.742 0.732

-0.858 0.856

-0.538 0.589

	Table 3: Pearson correlation coefficients of physicochemical parameters of <i>Culex quinquefasciatus</i> breeding habitats							ing						
	Temp	PH	EC	TDS	DO	BOD	ALK	Mg	СІ	PO ₄	Ca	NH ₃	NO ₃	SO ₄
Temp	1													
РН	-0.831	1												
EC	0.849	-0.998*	1											
TDS	0.859	-0.996*	1.000**	1										
DO	-0.925	0.656	-0.661	-0.670	1									
BOD	0.933	-0.577	0.603	0.620	-0.941	1								
ALK	-0.604	0.890	-0.900	-0.899	0.294	-0.309	1							
Mg	0.552	-0.358	0.416	0.440	-0.290	0.571	-0.520	1						
СІ	-0.699	0.338	-0.393	-0.419	0.559	-0.805	0.333	-0.924	1					
PO ₄	0.802	-0.998*	0.994**	0.990**	-0.627	0.534	-0.893	0.317	-0.287	1				

Note: * Significant at 0.05 level; and ** Significant at 0.01 level. Temp = Temperature, EC = Electric Conductivity, TDS = Total Dissolved Solids, DO = Dissolved Oxygen, BOD = Biological Oxygen Demand, ALK = Alkalinity, Mg = Magnesium, CI = Chloride, PO₄ = Phosphate, Ca = Calcium, NH₃ = Ammonia, NO₃ = Nitrate, SO₄ = Sulfate.

-0.283

-0.363

-0.551

-0.709

0.339

0.106

0.280

 0.971°

-0.608

-0.350

-0.460

-0.850

0.602

0.726

0.841

0.505

1

0.945

0.932

0.343

1

0.974

0.174

1

0.372

3.3. Effect of physicochemical parameters on Cx. quinquefasciatus larval abundance

0.654 -0.998

 -0.964°

-0.947

-0.306

0.733

0.860

0.608

0.960

0.826

0.851

0.544

The relationship between the various water parameter and *Culex* mosquito larvae abundance was determined using the Pearson correlation analysis and represented in Table 4. *Culex* mosquito larval densities in the study locations were found to have a significant positive relationship with NH_3 (r = 0.507, p = 0.027) and SO_4 (r = 0.539, p = 0.017). Other parameters did not significantly affect *Culex* larvae population densities. Parameters such as temperature, conductivity, TDS, magnesium, chloride and calcium have positive non-significant associations with larval densities. Other characteristics, including pH, DO, alkalinity, nitrate, sulfate and phosphate, were negatively and insignificantly correlated with the larval population (Table 4).

Table 4: Pearson correlation showing the association between the physicochemical parameters of breeding water and larvae abundance				
Physicochemical parameters	Correlation	p-Value		
Temperature (°C)	0.001	0.99		
Acidity (pH)	-0.156	0.53		
Electric conductivity (µS/cm)	0.010	0.97		
Total dissolve solids (ppm)	0.167	0.49		
Dissolved Oxygen (mg/L)	-0.308	0.19		

Table 4 (Cont.)					
Physicochemical parameters	Correlation	p-Value			
BOD (mg/L)	-0.188	0.44			
Alkalinity (mg/L)	-0.196	0.42			
Magnesium (mg/l)	0.206	0.39			
Chloride (mg/L)	0.061	0.80			
Phosphate (mg/L)	-0.134	0.58			
Calcium (mg/L)	0.288	0.23			
Ammonia (mg/L)	0.507	0.03			
Nitrate (mg/L)	-0.295	0.22			
Sulfate (mg/L)	-0.537	0.02			

Note: BOD = Biochemical Oxygen Demand, mg/L = Milligram per liter, ppm = Parts per million, and μ S/cm = Siemens per centimeter.

4. Discussion

The study's objective was to determine the relationship between *Cx. quinquefasciatus* abundance and the physicochemical parameters of their breeding habitat. We observed a negative association between rainfall and the abundance of *Cx. quinquefasciatus* larvae. This finding concurs with previous studies by Mgbemena and Ebe (2012); Manyi *et al.* (2014); Akpan and Nwabueze (2015) in Nigeria, Muturi *et al.* (2007) in Kenya and Soh and Aik (2021) in Singapore. The heavy precipitation that characterizes the rainy season leads to flooding, which destroys mosquito breeding sites and washes away eggs and immature stages. Heavy rains also reduce the *Culex* mosquitoes' flight activities and infective rates (Cotar *et al.*, 2016).

Culex larvae abundance differed significantly between the four study locations. The variation in mosquito larval occurrence can be explained in differences in the LGAs human population densities and anthropogenic activities, which are essential for the proliferation of Culex habitats (O'Meara, 1997; and Mgbemena and Ebe, 2012). The urban and highly populated Kosofe LGA had more larvae than the rural and low populated Epe LGA. Urbanization and human activities significantly influence the biodiversity of an area (Townroe and Callaghan, 2014). Low biodiversity in Kosofe LGA indicates a high risk of disease transmission and infection (Keesing et al., 2010). Rural areas have diverse predators that prey on aquatic mosquito larvae reducing their survivorship. On the other hand, urbanization removes the favorable conditions required for the development of predators (Carlson et al., 2004).

Culex quinquefasciatus was the most common and abundant species in all locations examined. These findings are consistent with previous studies conducted in Nigeria and Benin republic (Amao et al., 2018; and Nchoutpouen et al., 2019). Culex quinquefasciatus is less susceptible to insecticides and other pollutants in its breeding habitat than most Culex mosquitoes (Subra, 1980). The mosquito has developed methods to adapt to a polluted environment, such as developing an effective detoxification mechanism, cuticle resistance in the larva, and dry resistant eggs (Nchoutpouen et al., 2019). Culex tigripes, on the other hand, was the least encountered Culex species. This finding is similar to those reported by Muturi et al. (2007), Odo et al. (2015) and Amao et al. (2018) in Kenya and Nigeria, respectively. The small number may be because Cx. tigripes are not usually associated with drainage systems (Harbach, 1988).

The abundance of immature mosquito stages depends, to a certain extent, on the physical and chemical characteristics of their breeding sites. Physicochemical parameters such as temperature, electrical conductivity, TDS, total water hardness, chloride and ammonia contributed positively by increasing the larval abundance. These findings were similar to the observations reported by Dejenie *et al.* (2011) in Ethiopia. Ammonia was the only parameter with a significant positive correlation with *Cx. quinquefasciatus* abundance in this study. This

finding, however, contradicts the results of Bashar *et al.* (2016), who reported a lack of association between larval abundance and ammonia in Bangladesh. Ammonia acts as a nutrient that favors the growth of aquatic fauna and flora such as bacteria, protozoa, fungi, algae and yeast, which serve as food for the larvae (Opoku *et al.*, 2007).

Mosquito breeding water parameters such as pH, DO, biochemical oxygen demand, alkalinity and phosphate, on the other hand, were found to be indirectly associated with the *Culex* larval densities. Lower alkalinity, biological oxygen demand, and DO have also been shown to increase the *Culex* population (Bashar *et al.*, 2016). In this study, there was a significant negative association between *Culex* abundance and the sulfate contents of the breeding waters. An excessive rise in sulfate can adversely affect some aquatic organisms, thereby reducing larval food sources. Excess sulfate gives water a shiny effect which reflects the sun's rays, thereby lowering the water temperature and reducing mosquito metabolism and reproduction (Nikookar *et al.*, 2017). However, results from India suggested that water temperature, DO, ammonia and nitrite were the best predictor parameters associated with mosquito immature abundance (Sunesh and Reuben, 2002). Physicochemical parameters with strong correlation could be helpful in mosquito larvae control through the manipulation of such parameters (Rao *et al.*, 2011).

5. Conclusion

Culex quinquefasciatus larval abundance could be influenced by the physicochemical features of their breeding habitats. The breeding water parameters varied with the rural-urban status and human activities of the four LGAs. Ammonia (NH_3) and sulfate (SO_4) significantly impact the occurrence and abundance of Cx. quinquefasciatus immature stages. It is suggested that more studies should be conducted to validate such associations in other parts of the state if mosquito larvae control interventions are to be implemented through the manipulation of mosquito habitats in the state.

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