

An investigation of physicochemical parameters of *Anopheles* and *Culex* breeding habitats in Port Harcourt Metropolis, Rivers State, Nigeria

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ABSTRACT: Monitoring and understanding the bionomics of mosquitoes is a key to devising ecologically friendly, alternative vector control strategies. Malaria and lymphatic filariasis are among the major mosquito-borne diseases primarily transmitted by female *Anopheles* and *Culex* mosquitoes, respectively, in Sub-Saharan Africa. Malaria mortality remains alarmingly high in Africa, particularly in Nigeria, which accounts for 26% of all global malaria deaths. This study was carried out to characterise the physicochemical parameters of breeding sites utilised by mosquito vector populations from June 2022 through October 2023. The physicochemical properties of *Anopheles* and *Culex* mosquito breeding habitats in three communities, each of the two Local Government Areas (Obio/Akpor and Port Harcourt), Rivers State, Nigeria, were analysed. Water samples from different study breeding habitats for physico-chemical investigation were collected concurrently with larvae and pupae in dark specimen bottles of 500 ml capacity to guarantee accurate representation. A total of 12 water quality parameters were measured from samples collected at six sites (three from each of the two LGAs). The parameters measured included alkalinity (mg/L), chloride (Cl⁻) (mg/L), electrical conductivity (EC) (μS/cm), nitrite (NO₂) (mg/L), sulphate, phosphate, hardness, dissolved oxygen (DO), total hydrocarbon content (THC), temperature, pH, total dissolved solids (TDS), and total hydrocarbon content (THC) using standard methods. Mosquito larvae and pupae (*Anopheles* spp and *Culex* spp) were sampled and collected from these habitats, reared to adulthood under controlled conditions, and morphologically identified. Results from the study showed significantly higher levels of TDS, conductivity, alkalinity, DO, hardness, nitrates, sulphates, phosphates, and THC compared to the reference breeding habitats, with THC reaching 384 mg/l and 350 mg/l in *Anopheles gambiae* sensu lato (sl) and *Culex species* breeding habitats, respectively. This study demonstrates that *Anopheles* and *Culex* mosquitoes can thrive in highly contaminated habitats marked by elevated organic pollution indicators, such as high THC levels. The findings highlight significant public health implications, including increased nuisance biting and a heightened risk of disease transmission in polluted environments. These results underscore the need for improved environmental management to mitigate the spread of mosquito-borne diseases.

Keywords: *Anopheles*, breeding sites, *Culex*, physicochemical parameters, Port Harcourt Metropolis, urban pollution, vector ecology.

INTRODUCTION

Mosquitoes are among the most important medical and public health vectors transmitting diseases worldwide, and vector control has been identified as the preferred method

for effective malaria control (Yina *et al.*, 2023). According to Powell (2018), several diseases are transmitted by mosquito species, eventually causing severe disease

burdens in human and animal populations. One of the major mosquito-borne diseases is malaria, which has been known to be a major public health problem in Africa (Yina *et al.*, 2023), and Nigeria continues to bear the largest proportion of this disease globally (WHO, 2024).

Sub-Saharan Africa continues to carry the heaviest burden of the disease, accounting for an estimated 94% of malaria cases worldwide in 2023. Nigeria contributes the heaviest estimated burden of malaria cases in 2023 (26%) as well as the highest contributor to the global malaria deaths (23%), underscoring the need to scale up and sustain current control efforts (WHO, 2024). Lymphatic filariasis is one of the Neglected Tropical Diseases (NTDs) that has plagued Africa and affects and damages the lymphatic system of human beings (Boateng *et al.*, 2025). This disease is caused by a microscopic thread-like worm called *Wuchereria bancrofti*, which is responsible for about 90% of cases (WebMD, 2022). The parasites are transmitted through mosquito bites, which serve as vectors (WebMD 2022). *Anopheles* mosquitoes have been found to be the major vectors in tropical Africa, while *Culex quinquefasciatus* are the common vectors in America. *Aedes* and *Mansonia* species are the vectors in the Asia Pacific. (CDC 2018).

Determination of malaria transmission intensity and efficacy of vector control in the fight against malaria will always require accurate information on the abundance of the vectors – *Anopheles* mosquitoes. On the other hand, *Culex pipiens* complex mosquitoes have a global distribution and are primary vectors of pathogens with public health significance, including West Nile disease, St. Louis encephalitis, Sindbis, Rift Valley fever viruses, and periodic filariasis and encephalitis (Liu *et al.*, 2019).

Vector control programmes in Nigeria have always consistently focused on the utilisation of synthetic insecticides in the form of bed nets and indoor residual spraying (IRS), with little effort invested in understanding the bionomics of the immature stages ecology, which is important for control using larvicides. Most baseline studies on vector identification and insecticide susceptibility status are often conducted to guide malaria vector control programmes, and obviously, in most cases, less emphasis and attention are placed on non-malaria vector species such as *Aedes* and *Culex* species (Oduola *et al.*, 2016). In most proven cases, the often-neglected species are responsible for the transmission of other life-threatening human diseases, which include yellow fever, lymphatic filariasis (LF) and dengue fever (Oduola *et al.*, 2016). Research has shown that the water quality of the breeding habitats of mosquitoes significantly influences mosquito presence and abundance, highlighting the need for effective water management strategies to control mosquito populations (Avramov *et al.*, 2024). In the tropics, it is no news that mean temperatures are suitable throughout the year; however, in the rainy season, the convergence of weather conditions, slightly reduced temperatures, and, as a result, build-up of humidity offers

peak conditions for mosquito survival and density (Beck-Johnson *et al.*, 2013). The physicochemical factors of aquatic habitats affect the survival, development, and fecundity of *Anopheles* and *Culex*; consequently, they can play a key role in malaria and lymphatic filariasis outbreaks (Fazeli-Dinan *et al.*, 2022).

MATERIALS AND METHODS

Study area

The study was conducted in two Local Government Areas (LGAs) of Port Harcourt Metropolis, Port Harcourt Local Government and Obio/Akpor Local Government (Figure 1). Port Harcourt (4.8156°N, 7.0498°E), the capital of Rivers State, Nigeria, lies along the Bonny estuary in the south-south region of the country, also referred to as the Niger-Delta. It is situated in the tropical mangrove, with rain extending from February to December (Muhammad *et al.*, 2021). These areas are mainly characterised by industries and pockets of farms, markets and living settlements. Obio-Akpor, Port Harcourt, and Eleme LGAs make up the Port Harcourt metropolis, which is on firm ground and about 66km from the Atlantic Ocean (Kura *et al.*, 2024). It is one of the major centres of economic activities in Nigeria, and a major city in the Niger Delta, said to be the richest LGA in Rivers State. This has brought about rapid urbanisation and the rising industrial and commercial growth of the city of Port Harcourt (Obianuju *et al.*, 2017). It is evergreen and ever raining because only the months of January and December can be truly called dry season; as such, malaria is perennial in the region due to the availability of *Anopheles* breeding sites year-round (Muhammad *et al.*, 2021). The heaviest rains in Port Harcourt occur in September, with an average of 367 mm, while December is the driest month of the year with an average rainfall of 20 mm (Muhammad *et al.*, 2021). The topography is flat, and pockets of forest, stream and freshwater bodies are found. The climate is characterised by two distinct seasons, the wet and dry seasons, with the former taking place from April to October and the latter between November and March (Ebere *et al.*, 2019).

Selection of study sites

The two sites (LGAs) were chosen because of the relatively divergent human activities in them, as well as for comparing their insecticide resistance profiles. Obio/Akpor and Port Harcourt are two of the three cities that make up the Port Harcourt metropolitan area, located in Rivers State. These study sites were chosen due to their locations in the urban areas and their involvement with lots of activities going on in the metropolis, like the generation of domestic waste due to improper disposal of waste, oil spills from automobile mechanic shops, and small factories like

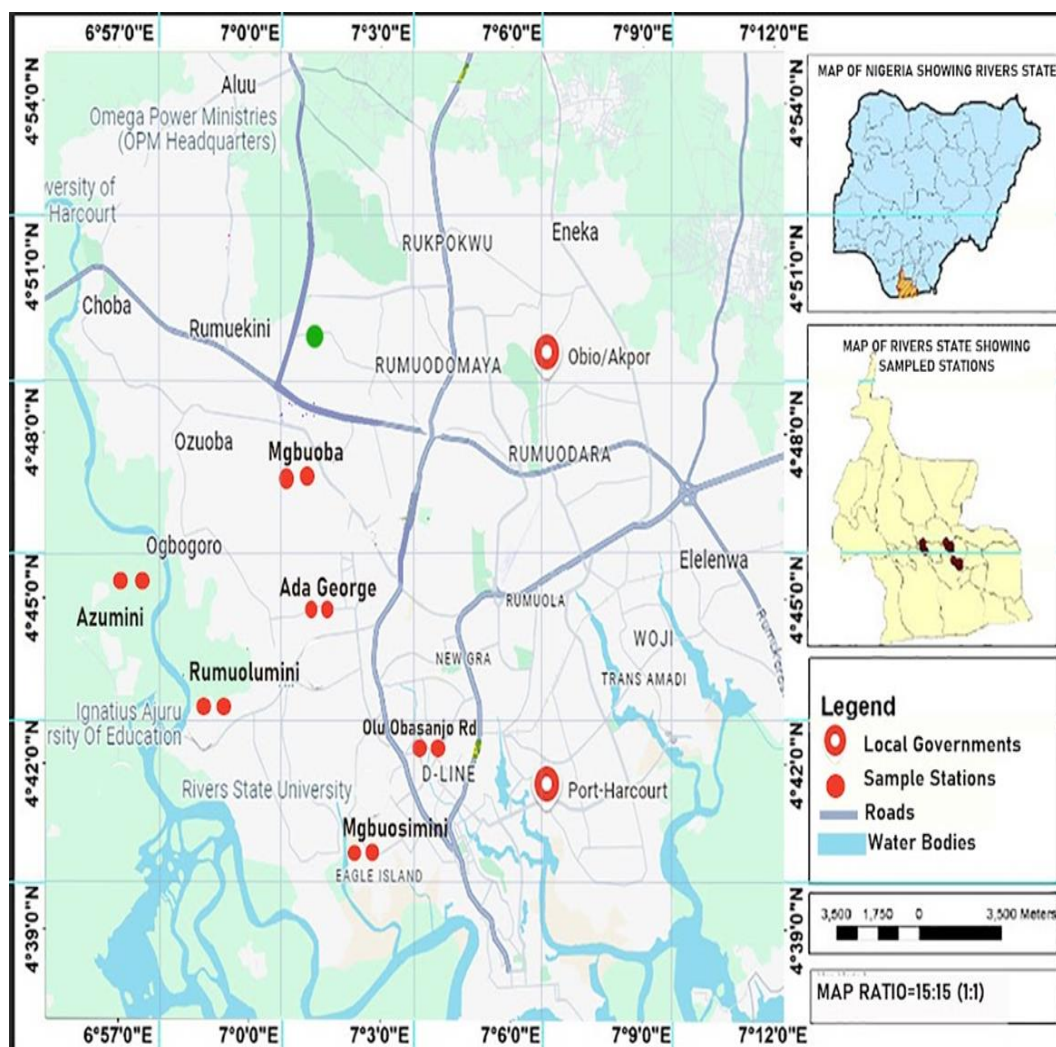


Figure 1. Map of Rivers State showing the study area.

paint factories. Mosquito larvae were randomly collected from six communities in the two LGAs. The mosquito larvae collected were transported to the insectary at the Rivers State University for sorting and rearing into adult stages for the susceptibility bioassay test. Advocacy visits were carried out to the LGA secretariats and the Community heads to explain to them the purpose of the work and to obtain permission and support for the study in their locality.

Sampling of mosquito larvae

Anopheles and *Culex* mosquito larvae were randomly collected from breeding within the metropolis drainage systems, ground cisterns, roadside ditches, low-lying pools in farm fields, and gutters randomly selected from each site (Plates 1 and 2), respectively. Sampling was done within the months of June 2022 to October 2023. The larvae were identified on the basis of their resting positions

on the surface of the water. A 350-ml dipper attached to the end of an approximately 1.2 m long handle was used to sample mosquito larvae by dipping from the breeding water bodies. 20 dips were taken per breeding habitat and emptied into a large container (Williams and Pinto 2012). The container was then inspected for the presence of *Anopheles* and *Culex* larvae, and pipetted for rearing (Plate 3).

All samples collected from a particular breeding habitat were stored in separate containers based on larval Genus (i.e., *Anopheles* or *Culex* spp.) and labelled according to site and location. This process was continued throughout the sampling period until a large number of larvae were collected. Larval collection for susceptibility tests was intensively carried out around the communities in Obio Akpor and Port Harcourt Local Government Areas in Port Harcourt metropolis, which were Obio/Akpor LGA coordinate: 4.8729 N, 7.0011E and Port Harcourt / LGA coordinate 4.7952N 7.0283E. The locations under Obio



Plate 1. Mosquito breeding sites in Port Harcourt.

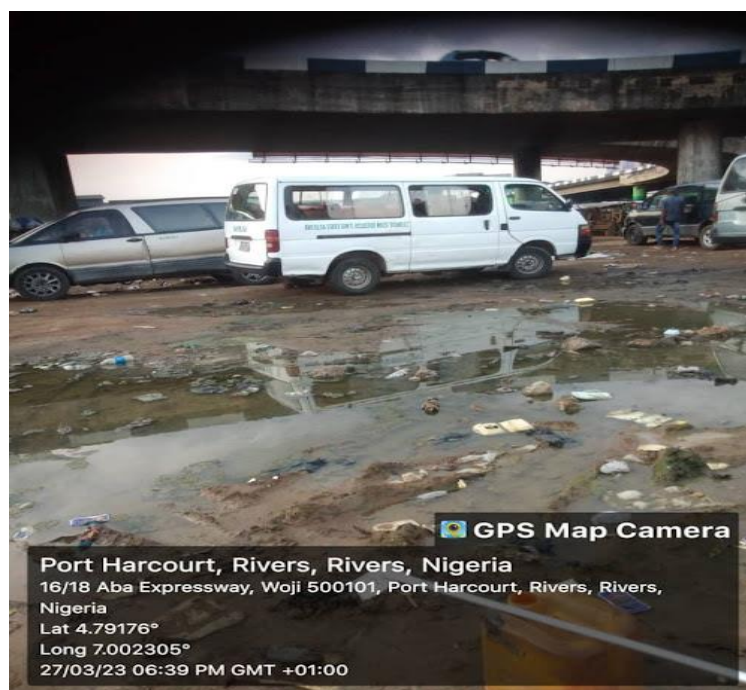


Plate 2. Mosquito breeding sites in Port Harcourt.

Akpor LGA where the study samples were collected from were Azumini (4.804116N, 6.94332E; 4.81454N, 6.94929E), Mgbuoba (4.81452N, 6.94855E; 4.80838N, 6.93081E), Rumuolumeni (4.30175E, 6.94165E; 4.84210N, 6.97211E; 4.83969N, 6.97236E) and the

locations under Port Harcourt LGA sampled were: Ada George (4.805406N, 6.993755E; 4.798942N, 6.991895E), Olu Obasanjo road (4.79176N, 7.002305E), Mgbuosimini (4.80804N, 6.98249E; 4.805406N, 6.993755E; 4.80804N, 6.98249E; 4.80958N, 6.97716E; 4.80960N, 6.97725E).

Reference site Latitude 4.79734 and longitude 6.97935.

It is most important to know the preferred breeding sites of the mosquitoes in the area. To identify the preferred breeding sites, it was essential to be systematic, and all possible breeding places were checked, even those that are hard to reach in some cases, like the smelly gutters. Other places where samples were carried out include small rain pools, drains and ditches, brackish water (where fresh water and salt water mix), streams, which should be searched at the edges where there is vegetation and the water moves slowly, ponds, swamps and marshes where larvae usually occur in vegetation around the edges. When collecting, care was taken to always approach the breeding place cautiously, facing the sun so as not to disturb larvae by shadows and movement, so they would not swim downwards and disappear from view. The collections were then carried out using a standard dipper (350 ml) and a white dipper with an adjustable ladle, where 20 dips were made per breeding site. The reference sites were areas where the habitats were not contaminated.

Set-up of the laboratory insectary for mosquito rearing

Mosquito larvae were sampled from the breeding habitat types of the study areas using a 350 ml capacity dipper. The mosquitoes sampled were sorted into the *Anopheles* and *Culex* genera, and the larvae were reared in the laboratory in different larval holding cages (LHC) that were labelled according to collection areas. Each LHC was 25.70cm tall, with a 27.30cm upper diameter covered in 0.05 mesh size netting material and a 16.40cm lower base. The larvae were fed with biscuit solution and monitored until they were pupated, under controlled conditions of 25–28°C and 70–80% relative humidity (Plate 2). The pupae were collected with a rubber dropper and placed in each transparent rubber basin, each measuring 5.50m high with an upper diameter of 11.50 cm and a lower base of 7.9 cm, along with a small amount of larval rearing water.

Each of the rubber basins with its pupae was then put inside a separate dry adult holding cage (DAHC) labelled accordingly. Each DAHC was 30.40cm high with an upper diameter of 25.40cm filled with netting material (0.05 inch diameter) and a lower base of 21.50cm in diameter with a circular hand entrance of 10.00cm in diameter attached to a long tubing net of 0.05 inches in diameter that was closed with a zip to prevent emerged adult mosquitoes from escaping the cage. The mosquitoes in a modified dry adult holding cage (DAHC) were monitored until they matured into adults. The newly emerged adults were fed with a 10% sugar solution soaked in cotton wool and placed on the netting cover of the cage for further investigation. Morphological identification was conducted using the Coetzee (2020). The larvae were reared to adulthood and identified as *An. gambiae* complex. The *Culex* larvae were identified as belonging to *Culex* genera using the morphological keys of Gillies and Coetzee (1987). All the

female adults (*Anopheles* and *Culex*) that emerged were identified morphologically (Muhammad *et al.*, 2021).

Collection of water samples and analysis

Field collection methods

Water samples were collected from all the breeding habitats sampled within the two LGAs in the metropolis. The water samples were kept in clean, dry cans prior to analysis. Each water sample was analysed for physicochemical constituents. In each site, the pH, temperature and the Total Dissolved Solids (TDS) were determined on-site (in situ) using the TRI-METER pH/TDS & TEMP-986 (pH meter model 29 1 MK2) portable device according to the manufacturer's manual (Plate 3).

Laboratory investigation of physicochemical parameters

Water samples from different study breeding habitats for physico-chemical investigation were collected concurrently with larvae and pupae in dark specimen bottles of 500 ml capacity to guarantee accurate representation. The water sample was fixed using 2 ml manganous sulphate and added to 2 ml potassium hydroxide, and 2 ml potassium iodide was added to the mixture. The fixed samples were taken to the laboratory for additional investigation; one litre of each water sample was investigated for the following physico-chemical parameters in the laboratory. A total of 12 water quality parameters were measured from samples collected at six sites (three from each of the two LGAs). Standard methods outlined by the American Public Health Association (APHA, 2005) were followed. The parameters measured included alkalinity (mg/L), chloride (Cl⁻) (mg/L), electrical conductivity (EC) (µS/cm), nitrite (NO₂) (mg/L), sulphate, phosphate, hardness, dissolved oxygen, and total hydrocarbon content (THC). Alkalinity and chloride concentrations were measured using titration techniques, electrical conductivity was determined using a conductivity/total dissolved solids (TDS) meter, and nitrite was detected using a spectrophotometer. All analyses were conducted following the procedures outlined by the American Public Health Association (APHA, 2005). Both *in situ* and *ex-situ* measurements were employed during the study. *Ex-situ* analyses were conducted at the Water Analysis Laboratory (Lab. Analytica) in Port Harcourt, Rivers State.

Hydrogen ion concentration (pH)

The effluent was collected in a clean glass bottle. Measurement of pH was carried out as described by APHA (2005). pH of the water samples was measured *in situ* in



Plate 3. Taking the readings *in situ* using TRI-METER pH/TDS & TEMP-986 portable device.

the sample site, using TRI-METER pH/TDS & TEMP-986 portable device according to the manufacturer's manual (pH meter model 29 1 MK2). pH meters were calibrated using standard buffer solutions of pH 7, 4, and 10. This was done by pouring a small amount of the buffer, pH 7, into a clean beaker, and a magnetic stirrer bar dropped into it, and placing the beaker on a magnetic stirrer to get a homogenous mixture. The pH meter electrode was lowered into the beaker so that the tip became immersed in the buffer solution, and the magnetic stirrer started. The meter was adjusted to take readings of the buffer. The electrode was removed, washed using distilled water then dried. The same process was repeated using pH 4 and 10. After calibration of the meter, the pH of the sample was analysed using the same procedure as stated above, but *in situ* and the meter results were recorded for the samples (APHA, 2005).

Determination of temperature

The temperature of the water samples was determined *in situ* using the TRI-METER pH/TDS & TEMP-986 portable device according to the manufacturer's manual. The measurements were obtained by immersing the probe of the TRI-METER *in situ* at the mosquito breeding sites, and the reading for temperature were recorded (APHA, 2005).

Total Dissolved Solids (TDS)

Total dissolved solids (TDS) measurement was carried out

in situ using the TRI-METER pH/TDS & TEMP-986 portable device according to the manufacturer's manual. This was done by immersing the electrode of the meter into about 50 ml of the test water in the beaker. Sufficient time was allowed for the meter to attain a constant reading. The readings were recorded in mg/L for the samples.

Electrical Conductivity (EC)

To check the conductivity result, a standard solution of potassium chloride of known conductivity cell was used (0.01 M KCl, 745.6mg in 1.0L de-ionised water = 1413umhos/cm). Thus, the conductivity cell (electrode) was washed three times in the 0.01M KCl solution, and the conductivity of the solution was measured. The conductivity cell was finally immersed in the sample, and the conductivity was recorded (APHA, 2005).

Chloride (Argentometric Method)

One (1) ml of potassium chromate indicator solution was added to 100 ml of the sample. This was titrated with standard silver nitrate titrant (0.0141N) to a pinkish yellow endpoint. Silver nitrate was standardised using a standard sodium chloride solution (0.0141N), 25 ml of standard sodium solution was introduced into a 150ml Erlenmeyer flask, and 6 drops of potassium chromate indicator were added to it. The solution was titrated with silver nitrate solution until a red precipitate appeared. The flask was stoppered and shaken vigorously to break the curds of

silver chloride. Titration was continued to the endpoint, and the volume of silver nitrate utilised, recorded. A blank prepared with distilled water was also titrated with silver nitrate to the endpoint, and the volume of the titrant was recorded. Chloride concentration was calculated thus:

$$CT \text{ (mg/L)} = (A + B) \times N \times \frac{35450}{ml} \dots\dots\dots \text{eq. 1}$$

Where: A = titration volume for sample (ml), B = titration volume for blank (ml), N = normality of silver nitrate

$$NaCl = CT \times 1.65 \text{ (mg/L)} \text{ (APHA, 2005)} \dots\dots\dots \text{eq. 2}$$

Alkalinity

Alkalinity was determined volumetrically using the customary method of the Bureau of Indian Standards (BIS). Volumetric flask, Burette, Pipette, Burette Stand, Glass rod, Conical flask, Beakers, Chemical balance with weight box were used. Standard alkalinity-resistant grade chemicals were used for solution preparation. 13.25 g anhydrous Na_2CO_3 , accurately weighed and (previously dried at $140^\circ C$ for 2 hours), was suspended in CO_2 free diluted water, diluted to 250 ml in a volumetric flask. 0.1N HCl solution was prepared by diluting 8.3 ml of concentrated HCl to 1000ml. It was standardised against Na_2CO_3 . Phenol 0.5 g powder was dissolved in 500 mL of 95% ethyl alcohol, and 500 mL of distilled water was added. Action for determination of alkalinity was followed in two parts: Phenolphthalein alkalinity and Methyl Orange alkalinity. Using HCl as a standard solution for titration, decolourisation of phenolphthalein indicator at pH 8.3 would mean complete neutralisation of hydroxides, while at pH 4.3, a change from yellow to orange for methyl orange indicator would mean total alkalinity.

Hardness

About 50ml of properly mixed buffer solution (16.9 g of ammonium chloride and 1.25 g of magnesium salt of EDTA and 1.0g of $CaCO_3$; and HCl) was pipetted into a conical flask, and 2-3 drops of Eriochrome black-T indicator added. The mixture was titrated against standard 0.01M EDTA until the wine colour changed from red to pale blue.

$$\text{Total hardness (mg/L)} = T \frac{1000}{V} \dots\dots\dots \text{eq. 3}$$

Where: T = Volume of titrant, V = Volume of sample (APHA, 2005).

Dissolved Oxygen (DO)

This is the concentration of oxygen in the sample. A fresh sample was collected in 300 ml biochemical oxygen demand (BOD) bottles, completely filled with the sample. Entrapped atmospheric oxygen was avoided. The bottles

were carefully stoppered and water sealed. The sample was examined immediately upon arrival at the laboratory. The dissolved oxygen (DO) in the sample was determined using a DO meter calibrated against a sample of known DO, as determined by the 10-iodometric method. The water seal was decanted from BOD bottles containing the sample. The ground glass stopper was then removed, and the electrode system/probe of the DO meter was immersed in the sample in the BOD bottle. The DO meter reading was carefully observed and recorded.

Determination of Nitrates

This was measured using an atomic absorption Spectrophotometer (Model: SPEC 720 VIS). Principle: A yellow colored nitrate derivative in alkaline solution is obtained when Nitrates react with phenol disulphonic acid. The colour produced is directly proportional to the amount of nitrates available in the sample. About 50ml of the effluent was collected in clean glass bottles, pipetted into a ceramic dish and evaporated to dryness on a hot water bath. Constant stirring with a bent rod was done to enable the residue to dissolve when 2 ml of phenol disulphonic acid was introduced.

Furthermore, ammonium hydroxide and distilled water were introduced into the mixture to make it alkaline. The mixture was filtered into a Nessler's tube and made up to 50ml with distilled water. Taking into consideration the colour development in the tube, the absorbance was read at 410nm using a spectrophotometer (Model: SPEC 720 VIS). The calculation was obtained by plotting a graph of the concentration against absorbance, and nitrate values were obtained by comparing the absorbance of effluents with the standard curve (mg/L).

$$\text{Nitrates (mg/L)} = \frac{\text{Absorbance of sample} \times \text{Conc. of standard solution (std)}}{\text{Absorbance of std} \times \text{Sample taken}} \times 1000 \text{ (APHA, 2005)} \dots\dots\dots \text{eq. 4}$$

Determination of Phosphates

Phosphates were determined using the Spectrophotometric method. Two milliliter (2 ml) of the water sample was transferred into 25 ml volumetric flask and one drop of phenolphthalein indicator followed by 2 ml of ammonium molybdate and 1 ml of freshly diluted stannous chloride solution was added to the water in the volumetric flask. Distilled water was added to make a volume of 25ml and mixed vigorously. The absorbance (colour intensity) was determined at a wavelength of 660nm in a Spectrophotometer (model SPEC 720 VIS) after 5 to 6 minutes (APHA, 2005).

Determination of Total Hydrocarbon Content (THC)

Hundred millilitres (100 ml) of the sample was measured

into a graduated glass bottle, acidified with H_2SO_4 to a pH of 2 or less at the time of sample collection. Thereafter, 4 ml of the organic solvent (Xylene) was added and shaken vigorously for 2 minutes. The contents of the bottle were emptied into a separating funnel. The bottle was rinsed with solvent and emptied into a separating funnel. The remaining solvent was then added to the separating funnel and shaken vigorously, intermittently releasing the stopper of the funnel to release pressure buildup. The contents in the separating funnel were allowed to settle. The bottom layer was then transferred into a clean bottle through a glass funnel in which cotton wool and about 1 g of anhydrous sodium sulphate had been stuffed at the aperture of the glass funnel to absorb water. The Total Hydrocarbon Content (THC) value was measured at 420 nm absorbance using a Spectrophotometer.

Statistical analysis

Statistical analysis was done using GraphPad Prism 8.0.2, and analysis of Variance (ANOVA) was used to compare the mean values of the physicochemical parameters of *Culex* and *Anopheles* breeding habitats. Tukey Post hoc test was also done to know which of the means were significantly different from the other.

RESULTS

Determination of the physicochemical properties of the breeding sites

Three rounds of sampling and physicochemical analyses were conducted, and the results showed significantly high levels of alkalinity, hardness, nitrate and total hydrocarbon contents in the study sites of *Culex* species and *Anopheles* species in the 3 localities in Obio/Akpor LGA (Table 1). The consistently low chloride levels (0.01 mg/l) were observed in both *Anopheles* and *Culex* breeding habitats. The alkalinity levels recorded (10.33 mg/l for *Anopheles* and 10.50 mg/l for *Culex*). The low DO levels (2.57 mg/l for *Anopheles* and 3.02 mg/l for *Culex*) as found in this study are consistent. The result also shows the insufficient dissolved oxygen, which can severely impact larval survival, posing a critical challenge for both *Anopheles* and *Culex* populations in these breeding habitats. Low nitrate (0.60 mg/l for *Culex*) and phosphate (0.02 mg/l) levels were also recorded. Total hardness levels reported (12.81 mg/L for *Culex*) are lower than those in other studies; the extreme hardness levels observed could pose challenges for larval survival. Similarly, high total dissolved solids (TDS) levels may negatively impact mosquito larvae.

All study areas showed similar low chloride levels (0.01 mg/l), well below WHO guidelines (>250 mg/l), indicating no significant pollution from chloride sources. Alkalinity varied significantly, with Obio-Akpor having the highest mean (10.50 mg/l) compared to the other sites (PHALGA:

8.12 mg/l, reference area: 1.52 mg/l). All values exceed the WHO guideline (>30 mg/l), suggesting suitable conditions for mosquito breeding. Hardness was highest in Obio-Akpor (12.81 mg/l) and significantly lower in the reference area (1.91 mg/l). Dissolved oxygen levels were critically low, especially in the PHALGA site (2.54 mg/l), well below the WHO threshold (<5 mg/l). THC was significantly higher in both Obio-Akpor and PHALGA compared to the reference area, where the habitats were not contaminated. The means (213.56 and 214.67 mg/l) exceed the WHO limit (<1 mg/l), indicating potential water quality issues. Temperatures were generally warm, with the highest in Obio-Akpor (29.05°C).

In Table 2, the P-value of 0.00 indicates critically low DO levels in both Obio-Akpor and PHALGA. Low DO is detrimental to larval survival, suggesting that the oxygen-poor conditions may hinder the development of *Anopheles* larvae, potentially leading to population declines. A P-value of 0.01 reveals significant differences in total hardness, with extremely high levels observed in Obio-Akpor. These elevated hardness levels can create an unsuitable environment for larvae, affecting their growth and survival rates. The significant difference noted (P-value 0.01) indicates mildly elevated temperatures, which can enhance breeding rates. However, if temperatures exceed optimal levels, they may also increase mortality risks for larvae, presenting a dual challenge for *Anopheles* populations. A P-value of 0.03 suggests significant differences in pH levels; however, the levels remain within the range suitable for breeding. This indicates that while pH varies across sites, it does not pose a significant barrier to larval development.

Chloride levels were very low across all sites (0.01–0.02 mg/l), significantly below WHO guidelines (>250 mg/l), indicating minimal chloride pollution. Alkalinity values were higher in Obio-Akpor (10.33 mg/l) compared to PHALGA (6.25 mg/l) and the reference area (1.18 mg/l). All measurements are below the WHO guideline (>30 mg/l), suggesting that alkalinity is not a limiting factor for larval development. Hardness was highest in Obio-Akpor (12.60 mg/l) and lower in the reference area (1.44 mg/l). All values fall below the WHO threshold (<200 mg/l), indicating suitable conditions for larval survival. DO levels were low, particularly in Obio-Akpor (2.57 mg/l) and PHALGA (2.50 mg/l), which are significantly below the WHO guideline (<5 mg/l). This low oxygen concentration may adversely affect larval survival. Nitrate levels were low across all sites (0.54–0.66 mg/l), well below WHO guidelines (>10 mg/l). This suggests limited nutrient enrichment, which may affect food availability for larvae. Sulphate levels were low and consistent (0.04–0.06 mg/l), remaining under the WHO guidelines (>250 mg/l), indicating minimal environmental impact from sulphates. Phosphate levels were also low (0.01–0.02 mg/l) and below WHO guidelines (>0.1 mg/l), suggesting limited potential for algal blooms that could provide food for larvae. THC was substantially higher in Obio-Akpor (226.89 mg/l) compared to the reference area (14.00 mg/l). The high THC values indicate

Table 1. The mean values and standard deviation of the physicochemical Parameters of *Culex* mosquitoes breeding sites water samples.

Parameters	Study area			P-value	WHO Guidelines
	Obio-Akpor (Mean $\pm$ SD)	PHALGA (Mean $\pm$ SD)	Reference area (Mean $\pm$ SD)		
Chloride (mg/L)	0.01 $\pm$ 0.00 ^a	0.01 $\pm$ 0.00 ^a	0.01 $\pm$ 0.00 ^a	0.58	>250
Alkalinity (mg/L)	10.50 $\pm$ 29.19 ^a	8.12 $\pm$ 8.62 ^b	1.52 $\pm$ 0.00 ^a	0.05	>30
Hardness (mg/L)	12.81 $\pm$ 43.44 ^a	9.90 $\pm$ 12.90 ^b	1.91 $\pm$ 0.00 ^a	0.05	<200
DO (mg/L)	3.02 $\pm$ 0.01 ^a	2.54 $\pm$ 0.35 ^b	6.80.80 $\pm$ 0.00 ^b	0.00	<5
Nitrate (mg/L)	0.60 $\pm$ 0.49 ^a	0.55 $\pm$ 0.65 ^a	0.00 $\pm$ 0.00 ^a	0.47	>10
Sulphate (mg/L)	0.06 $\pm$ 0.00 ^a	0.06 $\pm$ 0.00 ^a	0.02 $\pm$ 0.00 ^a	0.21	>250
Phosphate (mg/L)	0.02 $\pm$ 0.00 ^a	0.07 $\pm$ 0.00 ^a	0.02 $\pm$ 0.00 ^a	0.47	>0.1
THC(mg/L)	213.56 $\pm$ 613.93 ^a	214.67 $\pm$ 4133.33 ^b	86.00 $\pm$ 0.00 ^b	0.01	<1
Temp 0C	29.05 $\pm$ 1.67 ^a	28.06 $\pm$ 0.17 ^b	26.00 $\pm$ 0.00 ^b	0.01	>30
PH	7.92 $\pm$ 0.10 ^a	7.87 $\pm$ 0.00 ^b	7.40 $\pm$ 0.00 ^b	0.03	<6.5
TDS (mg/L)	307.78 $\pm$ 722.70 ^a	264.67 $\pm$ 2483.11 ^b	33.00 $\pm$ 0.00 ^b	0.00	>500
(EC)(μ S/cm)	318.78 $\pm$ 8334.04 ^a	313.67 $\pm$ 736.33 ^b	150.00 $\pm$ 0.00 ^b	0.02	400

Parameter with same alphabet (a) = Statistically significant; Parameter with two alphabets (a&b) = Not statistically significant. SD = Standard Deviation, DO = Dissolved Oxygen; THC = Total Hydrocarbons; TDS = Total dissolved solids; EC = Electrical Conductivity, Ph= Potential of Hydrogen.

Table 2. The mean values and standard deviation of the physicochemical Parameters of *Anopheles* mosquitoes breeding sites water samples.

Parameters	Study area			P-value	WHO Guidelines
	Obio-Akpor (Mean $\pm$ SD)	PHALGA (Mean $\pm$ SD)	Reference area (Mean $\pm$ SD)		
Chloride (mg/L)	0.01 $\pm$ 0.00 ^a	0.01 $\pm$ 0.00 ^b	0.02 $\pm$ 0.00 ^b	0.00	>250
Alkalinity (mg/L)	10.33 $\pm$ 45.41 ^a	6.25 $\pm$ 5.75 ^a	1.18 $\pm$ 0.00 ^a	0.09	>30
Hardness (mg/L)	12.60 $\pm$ 67.60 ^a	8.13 $\pm$ 4.29 ^a	1.44 $\pm$ 0.00 ^a	0.08	<200
D O (mg/L)	2.57 $\pm$ 0.23 ^a	2.50 $\pm$ 1.02 ^b	6.50 $\pm$ 0.00 ^b	0.00	<5
Nitrate (mg/L)	0.66 $\pm$ 0.52 ^a	0.54 $\pm$ 0.52 ^a	0.06 $\pm$ 0.00 ^a	0.46	>10
Sulphate (mg/L)	0.06 $\pm$ 0.00 ^a	0.04 $\pm$ 0.00 ^a	0.02 $\pm$ 0.00 ^a	0.36	>250
Phosphate (mg/L)	0.02 $\pm$ 0.00 ^a	0.02 $\pm$ 0.00 ^a	0.01 $\pm$ 0.00 ^a	0.14	>0.1
THC (mg/L)	226.89 $\pm$ 2663.26 ^a	160.29 $\pm$ 14285.14 ^b	14.00 $\pm$ 0.00 ^a	0.03	<1
Temp 0C	28.62 $\pm$ 0.92 ^a	27.64 $\pm$ 0.06 ^b	25.00 $\pm$ 0.00 ^b	0.00	>30
PH	7.84 $\pm$ 0.00 ^a	7.86 $\pm$ 0.01 ^b	7.30 $\pm$ 0.00 ^b	0.00	<6.5
TDS (mg/L)	261.90 $\pm$ 11827.15 ^a	308.22 $\pm$ 3307.26 ^b	29.00 $\pm$ 0.00 ^b	0.01	>500
EC (μ S/cm)	289.33 $\pm$ 7321.00 ^a	279.90 $\pm$ 14506.81 ^a	120.00 $\pm$ 0.00 ^a	0.09	400

Parameter with same alphabet (a) = Statistically significant; Parameter with two alphabets (a&b) = Not statistically significant. SD = Standard Deviation, DO = Dissolved Oxygen; THC = Total Hydrocarbons; TDS = Total dissolved solids; EC = Electrical Conductivity, Ph= Potential of Hydrogen.

potential water quality concerns, exceeding the WHO limit (<1 mg/l). The temperatures recorded were warm, with Obio-Akpor being the highest (28.62°C) compared to the reference area (25.00°C). All values exceed the optimal range (>30°C), which may favour *Anopheles* breeding. pH values were within a suitable range for mosquito breeding, with Obio-Akpor (7.84) and PHALGA (7.86) higher than the reference area (7.30). All values exceed the WHO threshold (<6.5). TDS levels were higher in both Obio-Akpor (261.90 mg/l) and PHALGA (308.22 mg/l) compared to the reference area (29.00 mg/l), but all remain below the WHO guideline (>500 mg/l). EC values were similar across sites, with all measurements below the WHO standard

(400 μ S/cm), indicating acceptable ionic concentration levels.

DISCUSSION

The results indicated that the physicochemical parameters were generally higher in the study areas compared to control sites, aligning with findings by Ononamadu *et al.* (2020). *Anopheles* and *Culex* mosquitoes inhabit a variety of aquatic environments, with *Culex* species often thriving in more contaminated, stagnant waters, while *Anopheles* species are typically associated with cleaner, oxygen-rich

habitats (Huzortey *et al.*, 2022). Monitoring parameters such as water temperature is crucial for understanding the suitability of breeding habitats and enhancing vector management strategies. The recorded temperatures (28.62°C for *Anopheles* and 29.05°C for *Culex*) fall within the range reported in other tropical studies (Seal and Chatterjee 2023). Temperature has been found to play a key role in the development of the Culicidae larvae. Thus, knowledge about the temperature of oviposition sites could be helpful for designing appropriate vector control methods (Rueda *et al.* 1990). Temperature fluctuates widely during the day and night, which can affect the survival and reproduction of mosquito species (Azari-Hamidian and Azarihamidian, 2020). Therefore, this parameter varies widely in such investigations.

Concentrations of dissolved ions can significantly impact water chemistry and larval survival. This is corroborated by research in Akure North, which found a strong correlation between electrical conductivity and the abundance of *Anopheles gambiae* and *Anopheles funestus* (Akeju *et al.*, 2022). Low chloride levels (0.01 mg/L) observed in both mosquito habitats correspond with findings from tropical regions, particularly Southeast Asia, where low salinity is linked to favourable breeding conditions (Caminade *et al.*, 2025). This suggests that such environments may support higher larval densities.

The recorded alkalinity levels (10.33 mg/L for *Anopheles* and 10.50 mg/L for *Culex*) are significantly lower than those reported in regions like India, where alkalinity often exceeds 30 mg/L (Giesen *et al.*, 2020). The influence of water chemistry on mosquito populations is critical for understanding their reproductive success. Higher alkalinity typically enhances larval survival, indicating that the low alkalinity observed in this study may hinder successful breeding. All sites are below WHO guidelines (<200 mg/l), indicating that water hardness should not limit mosquito survival.

Variations in physicochemical properties also significantly affect oviposition and development rates in mosquitoes. Research by Seal and Chatterjee (2023) demonstrated that the physicochemical characteristics of breeding habitats can influence the reproductive success of *Anopheles subpictus*. Furthermore, breeding water quality is a crucial determinant of oviposition and larval development, as highlighted by Kenawy *et al.* (2013).

Low dissolved oxygen (DO) levels (2.57 mg/L for *Anopheles* and 3.02 mg/L for *Culex*) found in this study are consistent with urban breeding sites in Africa, where organic pollution often leads to reduced oxygen availability (Giesen *et al.*, 2020). Insufficient DO significantly threatens larval survival, as mosquito larvae rely on both atmospheric oxygen and DO, especially when access to the surface is limited (Huzortey *et al.*, 2022). Reduced DO levels can lead to lower survival rates and prolonged development times, which is supported by the findings of Huzortey *et al.* (2022). This suggests potential challenges for larval survival due to low oxygen availability. Nitrate

levels were low across all sites, with no significant differences. All measurements (around 0.60 mg/l) are below the WHO guideline (>10 mg/l), indicating low nutrient enrichment. Sulphate levels were consistent across sites, all well under WHO guidelines (>250 mg/l), indicating minimal environmental impact from sulphate sources. Phosphate levels were low and not significantly different among sites. All values are below WHO guidelines (>0.1 mg/l), suggesting limited algal growth potential.

For *Anopheles* mosquitoes, preferences for breeding sites with higher DO levels have been documented, as seen in studies by Seal and Chatterjee (2023) and Silberbush *et al.* (2015). However, the low DO levels observed in this study highlight a potential risk to *Anopheles* larvae, emphasising the need for further investigation into species-specific responses to varying oxygen levels.

The study also found low nitrate (0.60 mg/L for *Culex*) and phosphate (0.02 mg/L) concentrations, which are typically favourable for mosquito breeding. Elevated nutrient levels can lead to increased competition and predation, thus reducing mosquito populations, as noted by Giesen *et al.* (2020).

The measured temperatures (28.62°C for *Anopheles* and 29.05°C for *Culex*) fall within ranges reported in tropical studies (Giesen *et al.*, 2020). While temperatures above 30°C can accelerate development, they may also increase mortality rates if conditions become extreme. The current temperatures suggest a conducive breeding environment but also raise concerns about potential heat stress on larvae.

Total hardness levels (12.81 mg/L for *Culex*) were lower than those found in other studies, where higher hardness is often associated with reduced mosquito populations (Caminade *et al.*, 2025). Similarly, high total dissolved solids (TDS) levels may negatively impact larval survival, underscoring the importance of maintaining optimal water chemistry for mosquito management.

Overall, this study reveals both favourable and challenging conditions for *Anopheles* and *Culex* mosquito breeding. While low chloride and nutrient levels suggest suitable environments, low DO and alkalinity present significant obstacles. This comparative analysis highlights the necessity for targeted interventions to improve mosquito habitat conditions, drawing on insights from similar ecological contexts.

The slightly lower pH levels in *Anopheles* breeding sites compared to *Culex* habitats in the Obio/Akpor area suggest that *Anopheles* may have a higher tolerance for more acidic conditions. The similarity in pH levels between the two species in Port Harcourt indicates that this factor may not significantly influence their breeding preferences in that location. All values exceed the optimal threshold (>30°C), which may favour mosquito breeding. pH values were within a suitable range for mosquito breeding, with Obio-Akpor (7.92) slightly higher than the reference area

(7.40). All values exceed the WHO limit (<6.5). TDS was significantly higher in the Obio-Akpor site (307.78 mg/l) compared to the reference area (33.00 mg/l), but all values remain under the WHO guideline (>500 mg/l). EC values were significantly higher in both Obio-Akpor and PHALGA compared to the reference area, indicating higher ion concentrations, but still below WHO standards (400 μ S/cm). In a previous study conducted in Hormozgan province, Iran, *Cx. quinquefasciatus* was identified at a pH of 6.9, which was the lowest one, while the total hardness was at the highest level (353.8 mg/L) (Hanafi-Bojd *et al.* 2017). These results indicated that there could be a negative association between the water pH and the abundance of mosquito larvae in aquatic habitats. The presence of predators in the larval habitats may have affected the abundance of mosquito larvae.

Both mosquito species demonstrate tolerance to varying pollution levels in their breeding environments (Abo *et al.*, 2024). The total petroleum hydrocarbon levels reached 384 mg/L, which likely influenced mosquito larval abundance (Mbanzulu *et al.*, 2022). The presence of larvae indicates their ability to thrive even in highly contaminated conditions, supporting findings by Amawulu *et al.* (2020).

Consistent organic pollution and deteriorating water quality, particularly after the rainy season, may create more favourable breeding conditions for mosquitoes, thereby increasing transmission rates of filariasis and malaria in urban areas. This aligns with the work of Lawal *et al.* (2022), who also reported *Culex* and *Anopheles* breeding in polluted water bodies, although they did not focus on vector density. The ability of both mosquito genera to tolerate elevated physicochemical parameters is corroborated by the work of Emidi *et al.* (2017).

Anopheles coluzzii has been shown to tolerate more contaminated breeding sites than other species (Osse *et al.*, 2019). However, certain *Anopheles* breeding sites exhibited significant electrical conductivity and TDS levels, with p-values < 0.0001, indicating a potential correlation between these parameters and mosquito abundance. The *Culex* breeding sites also showed significant levels of electrical conductivity and TDS (p<0.0001).

Environmental stressors, such as varying water quality, may indirectly contribute to resistance development. High TDS or conductivity in breeding habitats could expose local mosquito populations to environmental toxins or pesticides, influencing their selection pressure. This exposure may compel mosquitoes to adapt physiologically, potentially enhancing their resistance to insecticides. These findings are consistent with research conducted in Lagos, where Awolola *et al.* (2007) reported that the *Anopheles gambiae* complex, primarily *An. gambiae* s.s. and *An. arabiensis*, is adapting to various polluted water bodies in urban settings, contributing to rising malaria incidence. More recently, Osse *et al.* (2019) reported similar observations for *An. coluzzii* in Cotonou, highlighting the need for targeted vector management strategies in light of these ecological changes.

Conclusion

Findings in this study reveal that many mosquito species, particularly *Anopheles gambiae* s.l., demonstrate a remarkable tolerance to varying degrees of contamination in their habitats. This data is crucial for establishing a database on mosquito vectors and their ecological preferences, aiding in the strategic planning and implementation of effective control measures in the region.

The increased tolerance of *Anopheles gambiae* s.l. to pollutants due to its adaptation to urban environments raises significant epidemiological concerns regarding the transmission of malaria and other vector-borne diseases. While elevated levels of dissolved solids and other physicochemical parameters may not directly cause resistance, they create a chemical environment that can diminish the effectiveness of mosquito control methods, potentially fostering resistance over time. Such conditions may also promote higher mosquito populations, thereby increasing the need for pesticide application and the likelihood of resistance development. It is recommended that larval habitats with high THC or TDS should be targeted. There is a need to promote environmental sanitation in the communities to reduce mosquito larval abundance. Integrating supplemental interventions like larval source management (LSM) with community education will enhance mosquito vector control.

This study provides a comprehensive analysis of the physicochemical parameters in mosquito breeding sites, highlighting the urgent need for targeted interventions and community engagement to improve water quality and mitigate health risks associated with mosquito breeding in contaminated environments.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Supplementary Materials

Contact the corresponding author at vivian.woke@ust.edu.ng for the supplementary materials