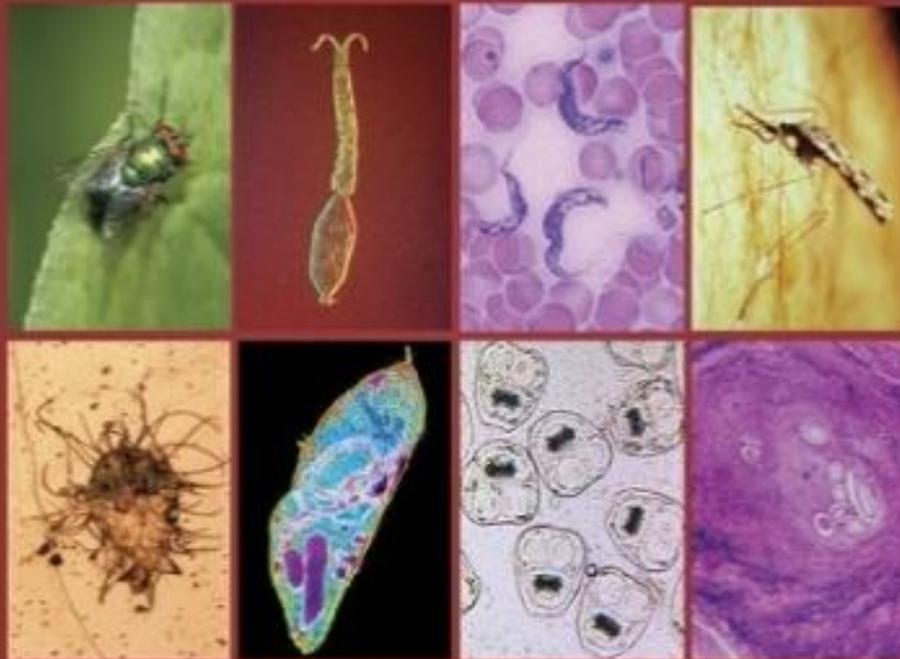




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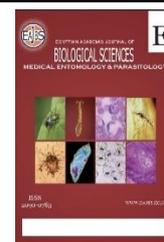
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Habitat Quality Influences *Aedes*, *Anopheles* and *Culex* sp. (Diptera; Culicidae) Larval Abundance and Co-occupancy in South-south Nigeria

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ABSTRACT

Several *Aedes*, *Anopheles*, and *Culex* mosquito species transmit diseases including malaria, yellow fever, Zika, dengue, and lymphatic filariasis. These mosquitoes thrive in diverse tropical and subtropical habitats, where physicochemical properties, habitat types, and biotic interactions influence oviposition and larval development. Understanding these extrinsic factors that influence mosquito breeding success, although commonly known, is crucial, particularly in regions lacking prior reports, and for periodic re-evaluation in other regions. Continuous assessment reports provide timely insights into mosquito activities in their habitats, which are now needed due to the realities of climate change. This understanding is essential for implementing effective and comprehensive control measures, even on a nationwide scale, in areas with similar climates. A survey was conducted across 16 localities in Edo State, southern Nigeria, covering 32 mosquito larval-positive breeding sites, comprising containers, gutters, puddles, used tyres, and tyre tracks. A total of 17 physicochemical properties were assessed at these sites. *Anopheles* larvae were abundant in tyre tracks, puddles, and gutters with high environmental variability, while *Aedes* favoured puddles and used tyres. *Aedes* abundance was positively linked to chloride but negatively associated with suspended solids, total dissolved solids (TDS), water colour, and *Anopheles* larvae. *Culex* were predominantly found in used tyres and were negatively associated with pH, turbidity, and TDS, but positively with nitrates. *Aedes* and *Culex* preferred containers and used tyres with stable physicochemical properties, while *Anopheles* were more common in puddles, gutters, and tyre tracks with higher heterogeneity. This variability highlights *Anopheles*' adaptability to diverse conditions, which complicates vector control.

INTRODUCTION

Mosquitoes play a crucial role as insect vectors, capable of transmitting a variety of pathogens that impact both human and animal health. Species responsible for spreading illnesses such as malaria, yellow fever, Zika, dengue, chikungunya, West Nile virus, and lymphatic filariasis are commonly found within the genera *Aedes*, *Anopheles*, and *Culex* (Jupp, 2005; Dodson and Rasgon, 2017; Eneanya *et al.*, 2018; Nebbak *et al.*, 2022). Due to their widespread distribution across various geographic regions and their ability to thrive in diverse breeding environments, mosquitoes pose a significant threat to public health, particularly in tropical and subtropical areas of Africa and Asia (Chua *et al.*, 2004; Awolola *et al.*, 2007; Muturi *et al.*, 2007a; David *et al.*, 2021).

Mosquitoes propagate by female adults ovipositing in suitable breeding sites. Their choice of egg-laying site is influenced by environmental and physiological factors (Chua *et al.*, 2004; Muturi *et al.*, 2007a). Gravid females use visual cues and olfactory chemosensors to detect and evaluate potential aquatic habitat quality (Turnipseed *et al.*, 2018). They are highly receptive to the volatile organic compounds released from stagnant water sources like containers, tyres, puddles, gutters, and natural pools (Chua *et al.*, 2004; Medeiros-Sousa *et al.*, 2020). These chemosignals provide information on the presence of microbial communities and nutrients that will support larval growth (Turnipseed *et al.*, 2018). Female mosquitoes also use non-chemical cues when choosing egg deposition sites. They generally prefer temporary, stagnant water bodies with no predators and high organic content that offer nutrition for filter-feeding larvae (Benelli, 2015).

The water quality in breeding sites significantly influences mosquito egg-laying and larval growth. Habitat quality can be influenced by various factors, including physicochemical properties, competing species, and habitat structure (Chua *et al.*, 2004; Mwangangi *et al.*, 2009; Benelli, 2015; Medeiros-Sousa *et al.*, 2020; David *et al.*, 2021). Physicochemical factors, such as temperature, turbidity, acidity, and the concentrations of various substances (including ammonia, nitrite, nitrate, sulphate, phosphate, chloride, calcium, and water hardness), play a critical role in the success of egg hatching and larval development (Awolola *et al.*, 2007; Nikookar *et al.*, 2017; Medeiros-Sousa *et al.*, 2020).

Furthermore, different habitat types, such as tyres, containers, puddles, tyre tracks, and gutters, may vary in their suitability for mosquito oviposition and larval development (Awolola *et al.*, 2007; Mwangangi *et al.*, 2009; Nikookar *et al.*, 2017; Medeiros-Sousa *et al.*, 2020).

Understanding how habitat quality influence mosquito vector abundance and species richness is crucial for disease prevention and effective control. Nigeria faces high rates of mosquito-borne diseases, including malaria, lymphatic filariasis, and dengue fever (Ebomwonyi *et al.*, 2015; Eneanya *et al.*, 2018; Ebomwonyi *et al.*, 2019; Awosolu *et al.*, 2021). Gaining insights into mosquito breeding behaviour and ecology is crucial for the effective eradication of vector-borne diseases (Chua *et al.*, 2004). This need has become even more pressing due to the accelerating rate of climate change, which has the potential to reorient both vectors and parasites. Climate change can significantly influence the distribution and behaviour of vectors (organisms that transmit pathogens) and parasites.

Variations in temperature, precipitation, and other climatic factors can alter their habitats and expand their ranges, potentially increasing disease transmission (de Souza and Weaver, 2024). Moreover, recent vector control efforts have focused on manipulating conditions affecting larval development (Zoh *et al.*, 2022). For better outcomes, it is imperative to enhance our understanding of the physicochemical nature of larval habitats in the wild. While numerous studies have explored the relationship between habitat quality and mosquito vectors in Nigeria, none has been conducted in Edo State. Even if similar studies exist in ecologically comparable regions, updated data are still needed. Moreover, it is common for multiple mosquito genera to breed within the same habitat type, although they may occupy distinct ecological niches (Gilbert *et al.*, 2008; Okwa *et al.*, 2009; Guo *et al.*, 2018; Omoregie *et al.*, 2019). This coexistence underscores the importance of understanding the multivariate effects of physicochemical properties on inter-genus mosquito larval populations, as previous reports have shown significant variation (Avramov *et al.*, 2024). This study

estimates the combined effects of water physicochemical properties and species co-occurrence on the larval abundance of *Aedes*, *Anopheles*, and *Culex* mosquitoes in Edo State, southern Nigeria, the influence of phytochemical parameters on mosquito genera has not been previously studied. Focusing on the genus level enabled us to gain broader insights that are crucial for establishing foundational understanding, particularly in this environment.

This approach was intended to allow us to gather comprehensive data applicable to multiple species within the genus, thereby providing a robust baseline for future studies. Such foundational knowledge is expected to guide more targeted research on specific species, ensuring that subsequent studies are built on a solid and broad understanding of the genus' responses to environmental factors. This is especially critical in a state with a high burden of mosquito-borne diseases, including malaria and yellow fever (Ebomwonyi *et al.*, 2015, 2019; Nwachukwu William *et al.*, 2022). Mosquito larval abundance were examined across various habitat types in three eco-vegetation zones in Edo State, Nigeria—freshwater swamp, lowland rainforest, and derived savannah. Our hypothesis posited that different habitat types would exhibit varying degrees of homogeneity in their physicochemical properties. In habitats with high homogeneity, the available resources will likely be relatively uniform, potentially favouring the larval development of mosquito taxa that are relatively more specialized to such environments.

MATERIALS AND METHODS

Study Area:

The research was conducted across all eco-vegetation zones within Edo State, including freshwater swamps in the south, lowland rainforests in the central and southern regions, and the derived savannah in the north (Fig. 1). Edo State is located between longitudes 06°04' E and 06°43' E, and latitudes 05°44' N and 07°34' N

(Segynola, 1993) in the south-southern part of Nigeria. It covers 19,794 km² of primarily low plains with extensive water networks (Olomukoro and Ezemonye 2005). The state has a tropical climate with four seasons: a long rainy season (March to June), and a short dry period (August break), a brief rainy season (peaking in late September), and a long dry season (late October to early March) (Asangwe 1993). Annual rainfall ranges from 1200 mm to 2200 mm, and temperatures vary between 25°C in the rainy season and 28°C in the dry season. The terrain consists of undulating plains prone to erosion.

Sampling Locations:

Mosquito larval samples were obtained from sixteen localities across the three eco-vegetation zones of Edo State over one year (April 2017 to March 2018). The localities included Uholor, Utaghan, Oghobaye-Ologbo, Rubber Factory-Ologbo, Ogheghe, Evboesi and Obanakhoro in the Freshwater swamp; Idunmwungha, Ugonoba and Ikhueni in the Lowland rainforest and Agenebode, Iviukwe, Iviarri, Sabongida-Ora, Iloje-Okpuje and Uzebba in the Derived savanna (Fig. 1). Sampled larval habitats included gutters, containers, puddles, used tyres and tyre tracks.

Mosquito Larval Sampling:

Mosquito larval samples were collected from randomly selected larval habitats using 148 mL plastic dippers, following WHO guidelines (WHO, 2003). Ten dips were made at each site and transferred into labelled containers with net covers, then taken to the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin for identification.

Water Sample Collection for Physicochemical Analysis

Immediately after mosquito sampling, water samples were collected for physicochemical analysis using standard methods. Clear and amber BOD and DO bottles were used, with the water in the BOD bottles fixed with 1 mL each of

Winkler's solutions A and B. Additional water samples for other physicochemical

parameters were collected in separate bottles.

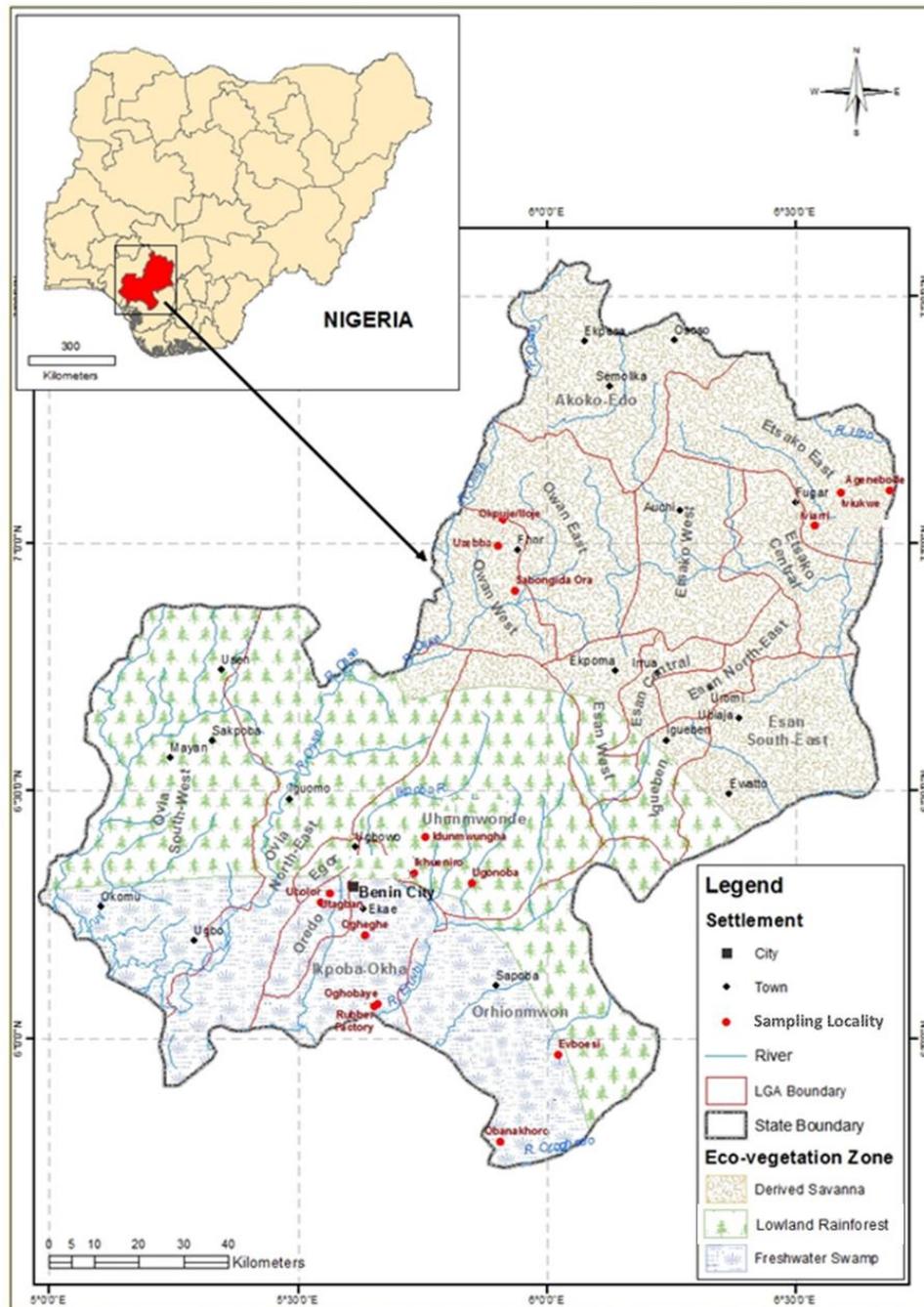


Fig. 1: Map of Nigeria showing Edo State, and the sampling localities.

Identification of Mosquito Larval Samples:

In the laboratory, visible larvae in the 2nd, 3rd, and 4th instars were identified to genus level using standard keys (Foster and Walker, 2002; Service, 2012) and subsequently discarded. Containers with field-collected larvae were kept at $27.53 \pm$

$1.59 \text{ } ^\circ\text{C}$ and $78.83 \pm 27.53 \%$ relative humidity for at least three days to allow smaller larvae to develop for final identification.

Physicochemical Analysis of Water Samples:

Physicochemical analyses of water samples were conducted using standard

methods at the Benin-Owena River Basin Development Authority/University of Benin Joint Analytical Research Laboratory, Benin City. Parameters measured included pH, colour, turbidity, total dissolved solids (TDS), suspended solid, total solid (TS), conductivity, chloride, alkalinity, hardness (as CaCO₃), phosphate, sulphate, nitrate, dissolved oxygen (DO), biological oxygen demand (BOD), calcium and magnesium.

Conductivity was measured with a Hanna 911 conductivity meter, and pH with a Hanna Hi-1922 pH meter. DO and BOD were evaluated using Winkler's method. Chloride was determined by titration with silver nitrate (AgNO₃). Alkalinity was assessed by titration with sulphuric acid, and total hardness by titration with ethylenediaminetetraacetic acid (EDTA). Calcium and magnesium were assessed using the complexometric titration (Ademoroti, 1996). Total solids (TS) were evaluated gravimetrically. Colour, suspended solids, turbidity, nitrate, sulphate, and phosphate were analysed with HACH colorimeter (Dr/890) (Hach, 2004).

Statistical Analysis:

Statistical analyses were conducted using R version 4.1.3. Habitat types were categorized into five groups: container, gutter, puddles, tyres, and tyre tracks. We used Principal component analysis (PCA) to show whether larval habitats had varying physicochemical properties according to habitat type and Eco-vegetation zone. This was conducted using the "FactoMineR" package. PCA biplots were generated using the "FactoExtra" package (Kassambara and Mundt, 2020).

A generalized linear mixed-effects model (GLMM) was developed using a stepwise regression approach with forward selection, employing a Poisson distribution and a log link function. Only most contributing variables (i.e., those that contribute more than the average contribution) from the PCA were included as predictors in the GLMM. Before model construction, predictor variables underwent

z-score transformation, significantly enhancing the model's performance. Predictor variables with statistical significance ($p < 0.05$) were included as fixed effects, while eco-vegetation zones and habitat types were incorporated as random effects due to their impact on observations. Model selection was guided by second-order Akaike's information criterion (AIC) scores and Bayesian Information Criterion (BIC), with a series of trial models compared. GLMMs were implemented using the "lme4" package and the 'glmer()' function.

Separate negative binomial models (NBM) were fitted to assess the differences in *Aedes*, *Anopheles*, and *Culex* mosquito abundance across all container type. The model was built with a 'x + 1' transformation applied to the mosquito abundance (x) variables prior to computing. To assess differences among each habitat, Tukey tests for multiple comparisons were conducted using the 'glht()' function from the "multcomp" package (Hothorn *et al.*, 2008), given the existence of five distinct habitat levels. Correspondence analysis (CA) plots were employed to ordinate mosquito species associated with habitat types. Before this analysis, a significant dependence ($p < 0.05$) in the abundance of the mosquito genus across habitat types was confirmed using the 'chisq.test()' function. Following this, a correspondence analysis was generated using 'CA()' function for analysis and 'fviz_ca_biplot()' function in the "FactoExtra" package, for biplot visualization.

RESULTS

A total of 642 mosquito larvae were collected from all sites combined, comprising 91 *Anopheles*, 200 *Culex* and 351 *Aedes* larvae. Overall, the highest number of mosquitoes were collected from used tyres (320), followed by puddles (210) and containers (43) (Table 1). Tyre track habitats contained no *Culex* or *Aedes* mosquitoes, whereas containers had no *Anopheles* mosquitoes.

Table 1: Mosquito larvae collected at the sampling locations and their abundance.

Habitat (n)	<i>Anopheles</i> (%)	<i>Culex</i> (%)	<i>Aedes</i> (%)	Mean \pm SD	Total
Containers (6)	0	4 (2.00)	39 (11.11)	7.17 \pm 11.29	43
Gutters (3)	21 (23.08)	12 (6.00)	2 (0.57)	11.67 \pm 17.62	35
Puddles (5)	25 (27.47)	25 (12.50)	160 (45.58)	42.00 \pm 70.03	210
Tyre track (5)	34 (37.36)	0	0	6.80 \pm 6.49	34
Used tyres (13)	11 (12.09)	159 (79.50)	150 (42.74)	24.61 \pm 20.78	320

n= number of samples; SD= Standard Deviation

* % relative to the total number of mosquitoes found in the respective genus.

No *Culex* larvae were found in tyre tracks. However, the mean abundance of *Culex* mosquitoes in other habitats including used tyres (12.23 \pm 4.27), puddles (5.0 \pm 3.16) and gutters (4.0 \pm 4.0), were not statistically different from each other ($p <$

0.05). Furthermore, an average of 0.67 \pm 0.67 *Culex* larvae per containers was observed, which was not statistically different from the *Culex* larvae in tyre tracks (0.0 \pm 0.0) (Fig. 2).

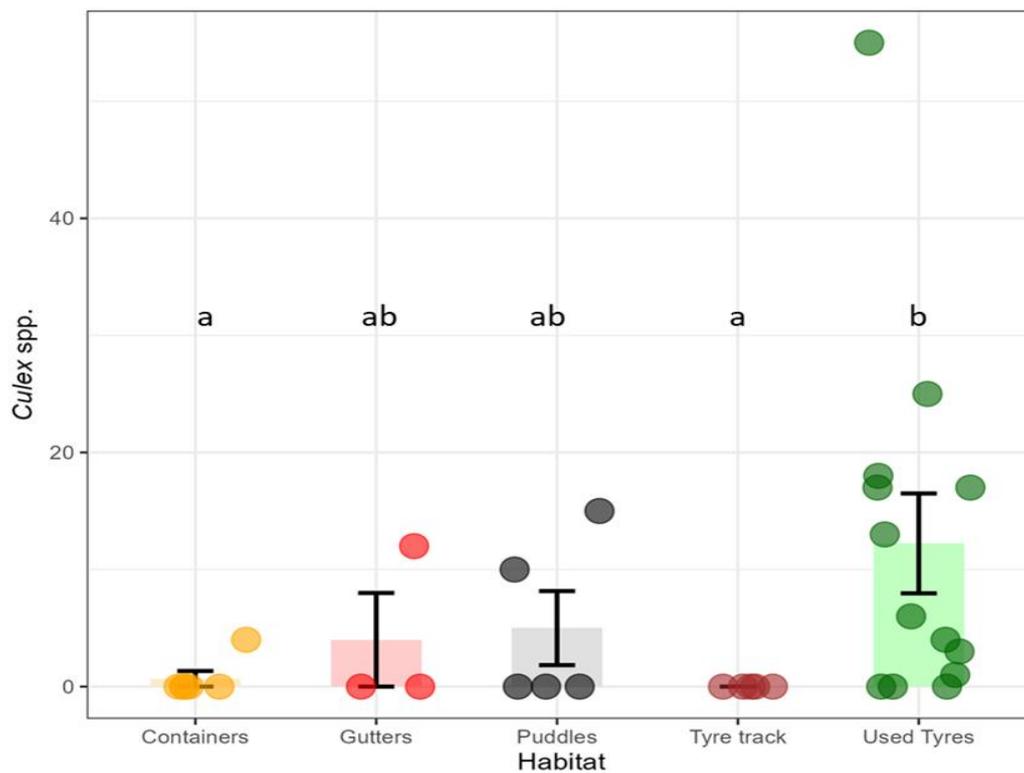


Fig. 2: Mean abundance of *Culex* spp larvae in artificial habitats. Error bars represent the standard error of the mean. Statistically significant differences are indicated by different letters.

As was the situation with *Culex*, no *Aedes* larvae were sampled from tyre tracks. The abundance of *Aedes* larvae was highest in puddles (32 \pm 30.77) and was also statistically similar with their abundance in

used tyres (11.54 \pm 4.25). Their abundance in containers and puddles were also not significantly different from tyre tracks (which had no larvae) (Fig. 3).

The mean *Anopheles* larval counts in gutters (7.0±6.0), tyre tracks (6.8±2.91) and puddles (5.0±4.25) did not differ

significantly ($p > 0.05$). Unlike *Aedes* and *Culex* samples, no *Anopheles* larvae were found in the containers (Fig. 4).

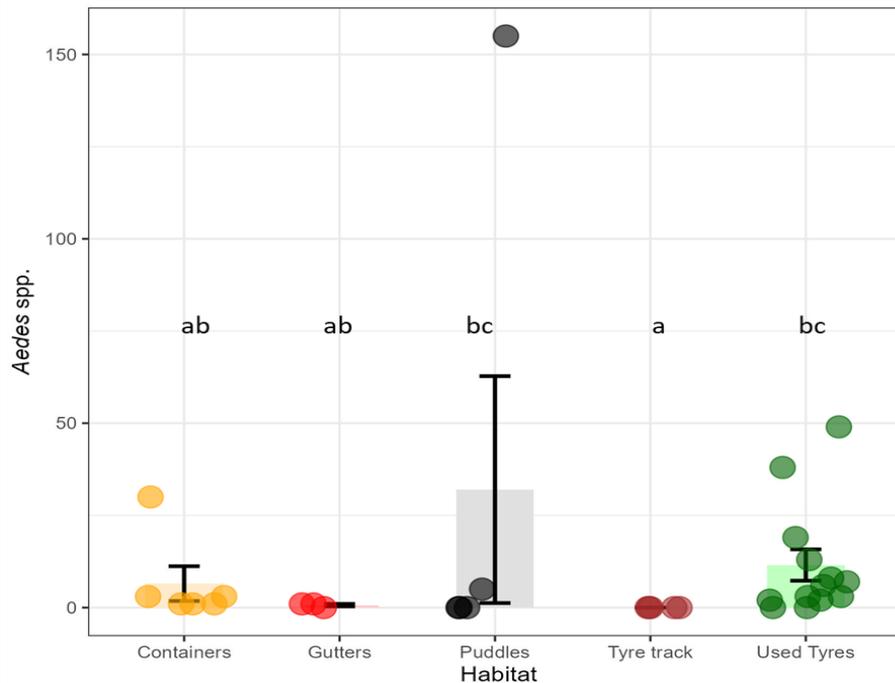


Fig. 3: Mean abundance of *Aedes* spp. larvae in artificial habitats. Error bars represent the standard error of the mean. Statistically significant differences are indicated by different letters

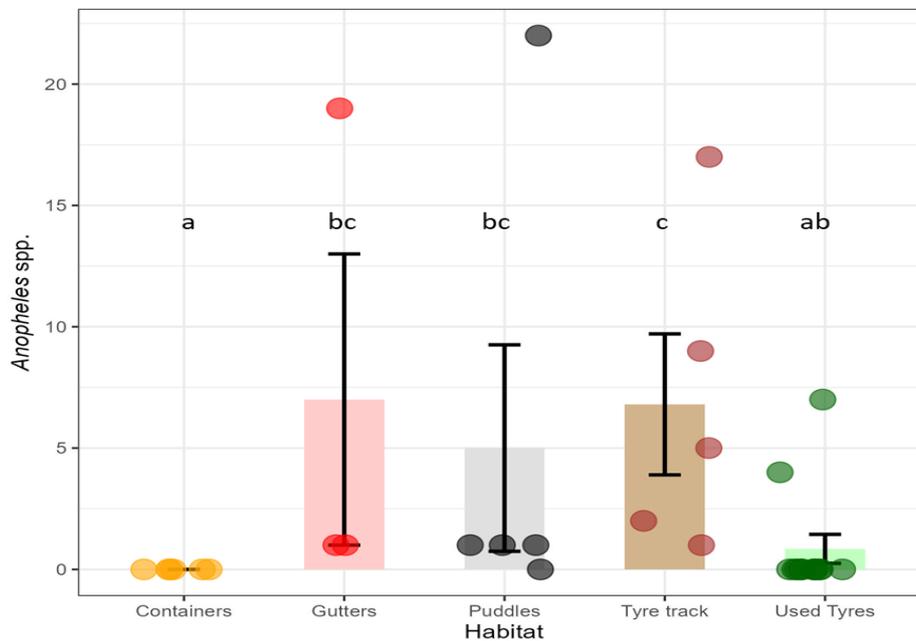


Fig. 4: Mean abundance of *Anopheles* spp. larvae in artificial habitats. Error bars represent the standard error of the mean. Statistically significant differences are indicated by different letters.

Descriptive statistics of the physicochemical properties as they differ by habitat type are summarized in Table 2.

The 17 physicochemical parameters assessed is presented in a two-dimension PCA plot (Fig. 8). The first two axes of the

PCA explained 55.5% of total variance in the data. Specifically, the first axis (principal component) accounted for 41.2% of data variance. Total solid, suspended solid, colour, magnesium, sulphate, hardness, chloride, turbidity and nitrate were the variables that most contributed to the principal component. Also, all physicochemical parameters were positively correlated with the first PCA dimension, except for DO (Fig. 8). The second principal component which explained 14.3 % of the variance was primarily associated with total dissolved solids (TDS), conductivity, alkalinity, pH, phosphate and turbidity (Fig. 8).

The PCA of habitats and eco-vegetation zones is presented in the biplot (Fig. 5). Puddles and tyre tracks had the most heterogeneous clusters. Containers and used tyres were the most homogenous, with some overlap in their clustering. Furthermore, the PCA ordination showed little disparity in the homogeneity of physicochemical characteristics of each eco-vegetation zone. From the PCA biplot, the sites in the lowland rainforest zone appeared more homogenous in physicochemical properties, relative to the derived savanna and freshwater swamps sites which were relatively heterogeneous (Fig. 6). The correspondence analysis (CA)

biplot showed that *Aedes* was most associated with containers and puddles, *Culex* with used tyres, and *Anopheles* with Tyre tracks (Fig. 7).

For predicting *Anopheles* abundance, the estimates and standard errors (SE) of significant most-contributing PCA variables according to the first and second dimension (Figs. 9 and 10) including *Culex* and *Aedes* abundance is presented in Table 3. The GLMM showed that Turbidity, Magnesium, and pH negatively ($P < 0.05$) influenced the abundance of *Anopheles* larvae. For predicting *Aedes* abundance, the prediction estimates and standard error of the most contributing variables in according to the PCA (Figs. 9 and 10) in addition to *Anopheles* and *Culex* abundance is shown in Table 4. The GLMM shows that Colour, TDS, suspended solid and *Anopheles* population were significant negative predictors of *Aedes* abundance, while Chloride was the only significant positive predictor (Table 4).

Culex density was most affected by turbidity, pH, nitrate, and TDS. GLMM showed that turbidity, pH, and TDS had negative relationships with *Culex* abundance. Conversely, the model shows that *Culex* abundance was associated with increased nitrate (Table 5).

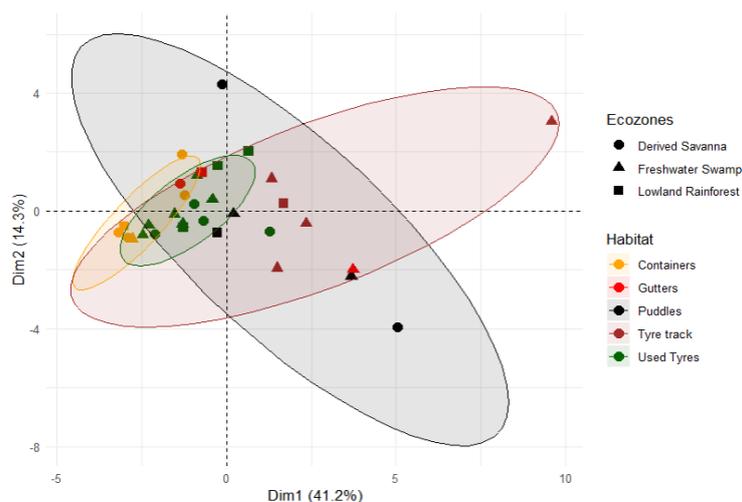


Fig. 5: PCA of sampling site represented by a point (N= 32). Sites are coloured by habitat. A larger eclipse would correspond to higher heterogeneity in the physicochemical properties of sites within a habitat type

*Eclipse could not be calculated for “Gutters” due to too few data points.

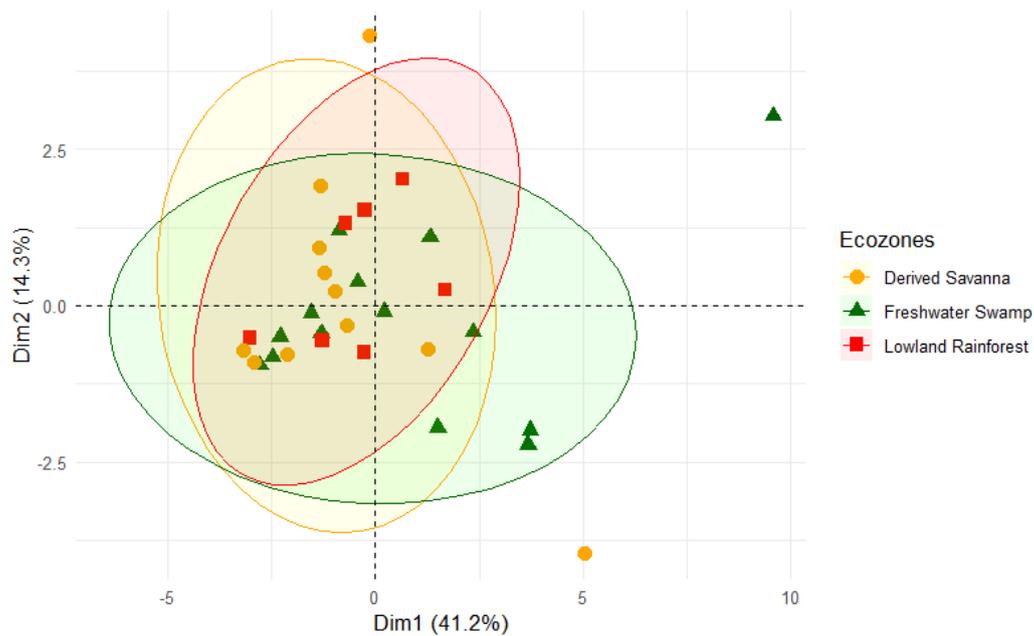


Fig. 6: PCA of sampling site represented by a point (N= 32). Sites are coloured by eco-vegetation zones. Larger eclipse would correspond to higher heterogeneity in physicochemical properties between sites in the same eco-vegetation zone.

Tab. 2: Physicochemical properties of mosquito larva habitats, represented as mean \pm standard deviation.

Physicochemical properties	Mosquito Larval Habitats				
	Container	Gutters	Puddles	Tyre tracks	Used tyres
pH	6.15 \pm 0.35	7.03 \pm 1.21	6.94 \pm 0.67	7.3 \pm 1.01	6.6 \pm 0.88
Colour (Pt. Co.)	81.16 \pm 36.82	1878 \pm 2877.07	3139.6 \pm 2781.72	4153.4 \pm 31	608.15 \pm 797.83
Turbidity (NTU)	12.333 \pm 7.94	436.33 \pm 661.42	817.4 \pm 842.95	855.8 \pm 484.35	77.92 \pm 89.82
TDS (mgL ⁻¹)	57.42 \pm 67.32	81.27 \pm 46.31	108.12 \pm 104.37	106 \pm 64.04	70.94 \pm 30.94
Suspended Solid (mgL ⁻¹)	8.33 \pm 5.98	250.67 \pm 378.77	374 \pm 324.79	776 \pm 687.10	129.92 \pm 278.72
Total Solid (mgL ⁻¹)	65.75 \pm 67.47	331.93 \pm 370.20	482.12 \pm 287.72	882 \pm 730.19	200.86 \pm 273.88
Conductivity (μ Scm ⁻¹)	108.33 \pm 127.03	153.33 \pm 87.37	204 \pm 196.93	200 \pm 120.83	133.85 \pm 58.39
Chloride (mgL ⁻¹)	14.12 \pm 6.31	32.94 \pm 4.07	50.832 \pm 40.06	39.536 \pm 22.66	22.81 \pm 16.34
Alkalinity (mgL ⁻¹)	29.33 \pm 21.75	86 \pm 72.58	57.6 \pm 15.71	87.2 \pm 66.19	53.85 \pm 35.11
Hardness as CaCO ₃ (mgL ⁻¹)	25.67 \pm 29.59	53.33 \pm 41.05	83.6 \pm 66.31	116.4 \pm 68.31	54.15 \pm 36.28
Phosphate (mgL ⁻¹)	0.49 \pm 0.40	3.67 \pm 2.49	36.082 \pm 74.12	5.046 \pm 3.51	1.13 \pm 0.99
Sulphate (mgL ⁻¹)	17.5 \pm 18.98	88.67 \pm 101.93	35 \pm 26.63	119.8 \pm 128.26	29.46 \pm 14.40
Nitrate (mgL ⁻¹)	5.94 \pm 7.43	28.73 \pm 37.18	28.538 \pm 25.68	27.14 \pm 17.71	10.75 \pm 11.95
DO (mgL ⁻¹)	7.38 \pm 1.58	4.8 \pm 4.42	2.62 \pm 1.64	3.82 \pm 2.49	4.08 \pm 1.82
BOD (mgL ⁻¹)	2.56 \pm 1.42	16.03 \pm 20.67	10.282 \pm 6.69	12.54 \pm 17.42	9.09 \pm 14.66
Calcium (mgL ⁻¹)	7.61 \pm 11.61	18.95 \pm 15.45	26.934 \pm 21.08	28.70 \pm 19.31	16.28 \pm 13.40
Magnesium (mgL ⁻¹)	1.38 \pm 1.08	1.62 \pm 1.13	3.988 \pm 3.64	10.79 \pm 9.70	2.62 \pm 1.65

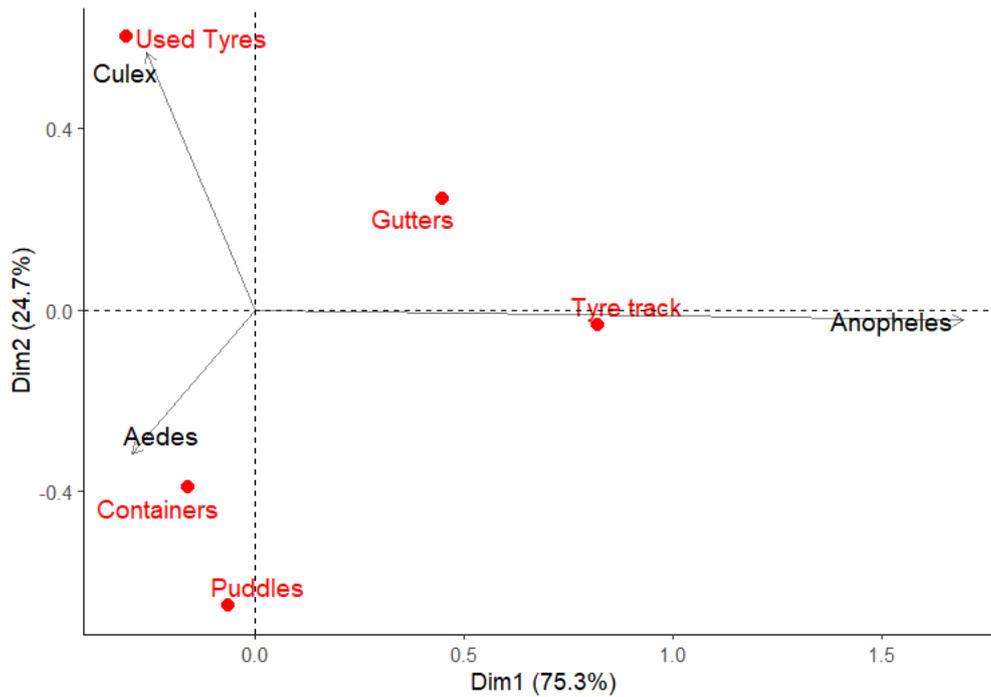


Fig. 7: Correspondence analysis (CA) biplot representing the relationship between mosquito larvae and the habitat type.

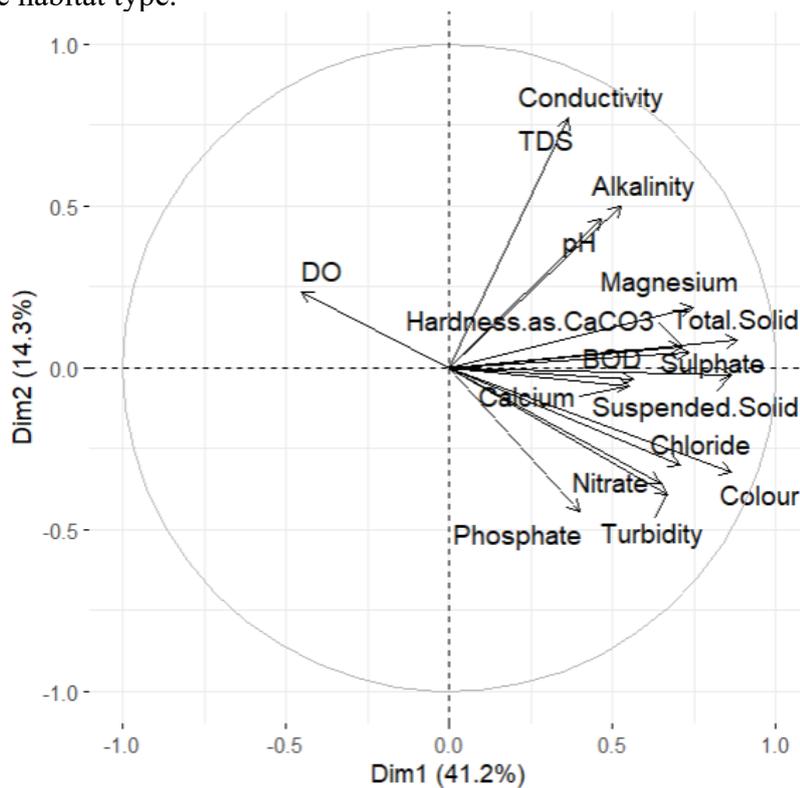


Fig. 8: Principal component analysis (PCA) biplot showing physicochemical parameters of sampling sites in a two-dimensional space.

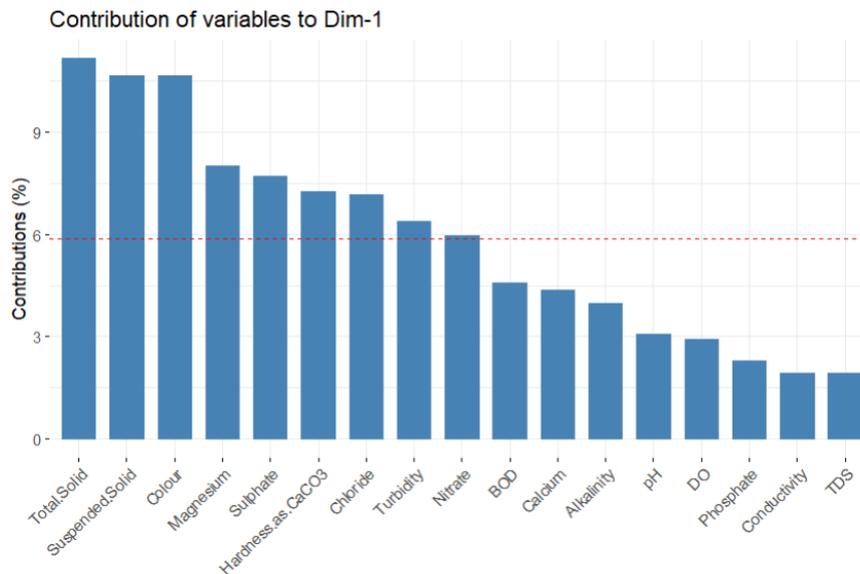


Fig. 9: Contribution of physicochemical characteristics surveyed to the first principal component dimension.
 *The red dashed line in the visualization represents the average contribution of the variables to the first principal component axis (Dim 1). Variables with bars above the dashed line contribute more than average to the axis (and vice-versa).

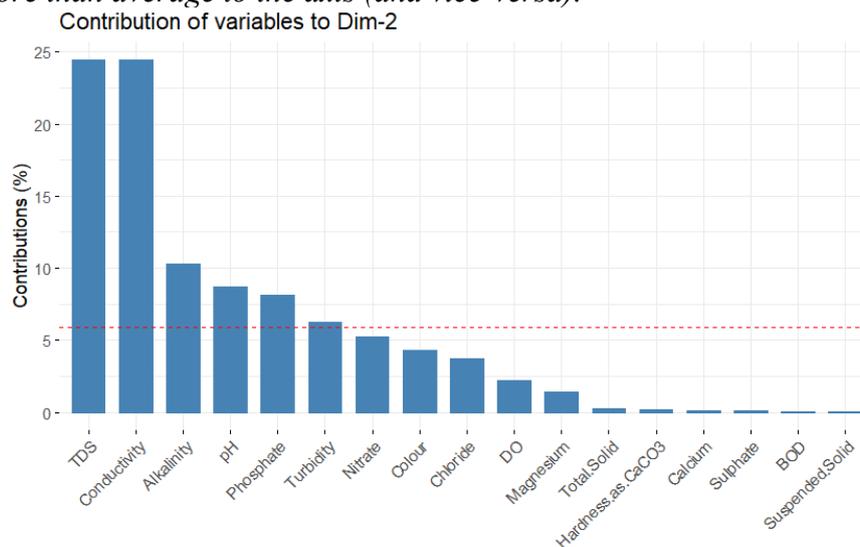


Fig. 10: Contribution of physicochemical characteristics surveyed to the second principal component dimension.
 * The red dashed line in the visualization represents the average contribution of the variables to the second principal component axis (Dim 2). Variables with bars beyond the dashed line contribute above average to the axis (and vice-versa).

Table 3: Results of the generalized linear mixed model (GLMM) of the number of immature *Anopheles* in larval habitats.

Dependent variable	Effect	Term	Estimate	SE	Z-Value	p-value
<i>Anopheles</i> abundance	Fixed	Intercept	0.1942	1.2795	0.152	>0.05
		Turbidity	-0.6205	0.1857	-3.341	<0.001
		Magnesium	-0.7247	0.1710	-4.238	<0.001
		pH	-0.5958	0.1520	-3.921	<0.001

Table 4: Results of the generalized linear mixed model (GLMM) of the number of immature *Aedes* in larval habitats.

Dependent variable	Effect	Term	Estimate	SE	Z-Value	p-value
<i>Aedes</i> abundance	Fixed	Intercept	-0.5580	1.4129	-0.395	P > 0.05
		<i>Anopheles</i> Count	-1.2163	0.2735	-4.448	P < 0.001
		Suspended Solid	-0.7419	0.1582	-4.688	P < 0.001
		TDS	-1.6200	0.1291	-12.544	P < 0.001
		Chloride	0.5479	0.1353	4.049	P < 0.001
		Colour	-2.7449	0.3086	-8.894	P < 0.001

Table 5: Results of the generalized linear mixed model (GLMM) of the number of immature Culicidae in larval habitats.

Dependent variable	Effect	Term	Estimate	SE	Z-Value	p-value
<i>Culex</i> abundance	Fixed	Intercept	-1.1405	1.2833	-0.889	P > 0.05
		Turbidity	-4.6823	0.6625	-7.068	P < 0.001
		pH	-0.5527	0.1095	-5.046	P < 0.001
		Nitrate	2.2995	0.3123	7.364	P < 0.001
		TDS	-1.4212	0.2795	-5.083	P < 0.001

DISCUSSION

This study investigated the relationship between the physicochemical properties of mosquito larval habitats and mosquito abundance. We examined (co-)occurrence patterns, hypothesizing that specific habitat types might favour certain mosquito genera due to their distinct physiological and nutritional needs. By surveying 32 breeding sites, we recorded the abundance of *Aedes*, *Anopheles* and *Culex* larvae along with 17 water physicochemical parameters. Our analysis identified variables significantly influencing larval abundance and highlighted disparities in habitat preferences among mosquito genera. We also assessed the consistency of physicochemical conditions across breeding sites and how this influenced larval habitat use by different mosquito genera.

Culex Abundance:

We found that *Culex* larvae were most commonly associated with used tyres. Okiwelu and Noutcha (2012) reported that 80% of sampled *Cx. quinquefasciatus* were found in container-type breeding sites, including tyres, plastic containers and

'calabashes'. Similarly, Obi *et al.* (2020) observed a high occurrence of mosquito larvae in used tyres compared to other sites like rock pools and electric poles. Among the 17 physicochemical properties assessed, *Culex* larvae were negatively associated with pH, turbidity and TDS, and positively associated with nitrates.

Lower water turbidity in containers supported *Culex* larvae abundance. Muturi (2007b) found *Cx. quinquefasciatus* positively associated with turbid water, while *Cx. annulioris* larvae preferred clear water, indicating intra-genus differences. Our study found *Culex* larvae most abundant in used tyres (12.23±4.26), with a pH of 6.6±0.88 and had lower turbidity compared to puddles, gutters, and tyre tracks. Lower turbidity may indicate cleaner water, reducing competition and predation, enhancing aeration, and providing more stable microenvironments. These conditions could improve *Culex* mosquito survival compared to more turbid environments. Our model predicted a negative association between *Culex* larvae and pH, likely because most habitats—except containers—had higher pH levels.

Interestingly, this contrasts with findings by Soltan-Alinejad *et al.* (2023), who reported an optimal pH of 8.3 for *Cx quinquefasciatus* and *Cx laticinctus*. This value is higher than the mean pH observed in used tyres and, in fact, higher than that of any habitat in our study. The success (i.e., high abundance) of *Culex* mosquitoes in used tyres is likely influenced by multiple factors beyond pH. Furthermore, used tyres may provide stable microhabitats with minimal pH fluctuation, potentially reducing environmental stress and promoting *Culex* larval survival.

Culex abundance was positively associated with nitrate levels in water. Increased nitrogen levels stimulate microbial growth, which in turn benefits mosquito larvae by enhancing their food supply (Kaufman and Walker, 2006). Research by Kenawy *et al.* (2013) and Ibrahim *et al.* (2011) demonstrated a direct relationship between nitrate levels and *Culex* larval density. Our study's PCA biplot showed a strong correlation between TDS and conductivity, but negatively associated with *Culex* abundance. Emidi *et al.* (2017) also reported a negative, though not statistically significant, association between conductivity and *Culex* larvae abundance. In contrast, Nikookar *et al.* (2017) observed a significant positive correlation between *Cx. pipiens* abundance and both conductivity and chloride levels, suggesting that environmental associations may vary by species or region.

***Aedes* Abundance:**

Aedes larvae thrived in puddles and used tyres. They showed positive associations with chloride and negative associations with suspended solids, colour, TDS, and *Anopheles* larvae. Gopalakrishnan *et al.* (2013) observed a negative correlation between TDS and container-breeding *Aedes* larvae, while Mahata *et al.* (2022) reported a positive correlation. *Aedes* mosquitoes show significant intra-genus variations in turbidity tolerance, with *Ae. aegypti*

preferring clean water and *Ae. albopictus* favouring habitats with organic debris.

In our study, *Aedes* abundance was negatively associated with suspended solids but positively correlated with chloride. Chatterjee *et al.* (2015) reported a significant positive association between per-dip *Ae. aegypti* larval density and TDS but found no significant relationship with chloride. Overgaard *et al.* (2017) also reported a positive association between *Ae. aegypti* immature infestation and TDS, but a negative association with dissolved oxygen. *Aedes* abundance did not increase alongside *Anopheles* abundance. The lack of co-occurrence between these two mosquito species may be attributed to differences in the physicochemical properties of their preferred habitats. Therefore, the presence and abundance of *Anopheles* serve as negative predictors of *Aedes* abundance.

***Anopheles* Abundance:**

Anopheles mosquito larvae were most abundant in tyre tracks, puddles, and gutters, whereas *Aedes* and *Culex* mosquitoes were predominantly found in containers and used tyres. This is supportive of Owolabi and Bagbe (2019)'s study which reported a higher percentage abundance of *Culex* sp. than *Anopheles* sp. in gutters, while *Anopheles* sp. showed greater abundance in roadside puddles compared to *Culex*. Additionally, Mwangangi *et al.* (2010) documented high densities of anopheline larvae in rice fields, canals, and marshes during their study on *Anopheles* larval abundance and diversity across three rice agro-village complexes in central Kenya. *Anopheles* larvae preferred habitats with lower magnesium, turbidity, and pH.

Anopheles were mainly associated with clearer waters, likely due to their low tolerance for pollution and higher oxygen levels. Studies have shown that *Anopheles* larvae thrive better in clean water compared to polluted water, which they tolerate less effectively than *Aedes* and *Culex* mosquitoes (Kudom, 2015; Jeanrenaud *et*

al., 2023). Our findings align with this, showing a preference for lower pH and less turbidity. However, this contrasts with Emidi *et al.* (2017), who found *Anopheles* larvae to be associated with higher pH levels (8.0 – 8.8). Tyre tracks, puddles, and gutters exhibit high variability in physicochemical properties compared to containers and used tyres, where *Anopheles* were rarely found. This suggests that *Anopheles* are better adapted to natural habitats with higher nutrient fluctuations and variations in physicochemical properties, giving them an advantage over *Culex* and *Aedes* mosquitoes, which appear more selective in their habitat preferences.

Mosquito Co-Occupancy and Physicochemical Properties of Habitat Types:

A strong positive relationship between conductivity and TDS was observed, consistent with previous studies (see, Thirumalini and Joseph, 2009; Rusydi, 2018). Our study shows that habitat physicochemical properties influenced mosquito larvae survival. Containers and used tyres, with more homogenous properties, had higher abundances of *Aedes* and *Culex* larvae. Puddles, with more heterogeneous properties, contained a mix of *Aedes*, *Culex*, and *Anopheles* species. Our PCA biplot indicated that tyre tracks, though heterogeneous, differed from the relatively homogenous properties of containers, explaining *Anopheles*' preference for tyre tracks and their absence from containers.

No clear distinction in physicochemical properties was found based on eco-vegetation zones (lowland rainforest, freshwater swamp, and derived savannah) in Edo State, Nigeria. Habitat type had a more significant impact on mosquito preferences than eco-vegetation zones.

High variability in physicochemical characteristics affects mosquito vectors' tolerance and adaptation, complicating control strategies. Stagnant water environments like containers and used tyres

results in stable conditions and uniform properties, while puddles and gutters experience environmental fluctuations, leading to heterogeneous properties. Temporal variability in physicochemical properties is a major determinant of mosquito abundance and species co-occupancy (Mwangangi *et al.*, 2010). These habitats accommodate variations due to nutrient inflow or outflow from rains, runoff, evaporation and other extrinsic factors (Owolabi and Bagbe, 2019).

Directions for Future Research;

Future studies should examine the dynamic nature of physicochemical properties in aquatic habitats, considering multiple mosquito generations. Capturing fluctuations in mosquito larvae breeding sites due to anthropogenic and natural factors and their effects on development should be explored. In addition, understanding nutrient inflow and outflow is necessary to determine their impact on mosquito abundance and species co-occurrence. Furthermore, investigating whether mosquitoes select or avoid breeding sites based on available alternatives could provide valuable insights into habitat preferences and potential adaptive behaviours, which may influence mosquito abundance and occupancy, as well as effectiveness of targeted vector control strategies.

CONCLUSION

This study enhances our understanding of the ecological dynamics between mosquito species and abiotic factors in aquatic environments. Our findings reveal that *Aedes* and *Culex* larvae predominantly inhabit containers and used tyres with stable physicochemical properties, while *Anopheles* larvae are more commonly found in puddles, gutters, and tyre tracks that exhibit higher environmental heterogeneity. This variability underscores *Anopheles*' remarkable adaptive capacity to diverse conditions, presenting significant challenges for vector control efforts. In this region, areas with high accumulations of

used tyres are likely to experience increased populations of *Aedes* and *Culex*, thereby elevating the risk of diseases such as yellow fever and elephantiasis. Conversely, regions with numerous puddles, gutters, and tyre tracks could face a heightened risk of malaria due to the prevalence of *Anopheles*. To mitigate these risks, it is imperative to implement robust vector control measures and to educate the community on reducing mosquito breeding sites. This study provides valuable insights into the selection and colonization of breeding sites by epidemiologically significant mosquitoes, thereby informing targeted interventions and policy decisions aimed at controlling mosquito-borne diseases.

Declarations:

Ethics Approval and Consent to Participate: This study was approved by the Ethics Committee of the Ministry of Health, Edo State, Nigeria (REF: HM.1208/199). It complied with ethical standards for both human and environmental research. Informed consent was obtained from all participants prior to their involvement in the study.

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