**Influence of habitat quality on *Aedes*, *Anopheles* and *Culex* larval abundance in Edo state, Nigeria**

# Introduction

As one of the most important insect vectors of illness, mosquitoes can spread a variety of pathogens that affect both human and animal health. Many mosquito species that transmit illnesses like malaria, yellow fever, Zika, dengue, chikungunya, West Nile virus, and lymphatic filariasis are found in the genera *Aedes*, *Anopheles*, and *Culex* (Jupp, 2005; Dodson & Rasgon, 2017; Nebbak et al, 2022). Due to their extensive geographic spread and their broad range of breeding environments, they are threatening to public health, especially in tropical, subtropical, and temperate regions of Africa and Asia (Awolola et al., 2007; Chua et al., 2004; David et al., 2021; Muturi et al., 2007).

Mosquitoes propagate by female adults ovipositing in suitable breeding sites. Her choice of egg-laying is reprimanded by environmental and physiological factors (Chua et al., 2004; Muturi et al., 2007). 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 prefer temporary, stagnant water bodies with no predators and high organic content that offer nutrition for filter-feeding larvae (Benelli, 2015).

The qualities of water in breeding sites play a crucial role in both the laying of eggs and the growth of mosquitoes. Habitat quality for a mosquito species may be shaped by many factors including physicochemical properties, competing species and habitat structure ( Benelli, 2015; Chua et al., 2004; David et al., 2021; Medeiros-Sousa et al., 2020; Mwangangi et al., 2009). Physicochemical factors such as temperature, turbidity, acidity, and the concentrations of various substances, including ammonia, nitrite, nitrate, sulfate, phosphate, chloride, calcium, and hardness of the water are critical for egg hatching and larval development success (Awolola et al., 2007; Medeiros-Sousa et al., 2020; Nikookar et al., 2017). Furthermore, artificial habitats such as tyres, containers, puddles, tyre tracks and gutters may have differential potentiality for mosquito oviposition and larval development (Awolola et al., 2007; Chua et al., 2004; Medeiros-Sousa et al., 2020; Nikookar et al., 2017). Understanding how different habitat qualities govern the abundance and richness of mosquito vector species is crucial for disease prevention and mosquito control efforts.

Nigeria has a high prevalence of malaria, lymphatic filariasis, dengue fever and many other diseases transmitted by mosquito vectors. A better understanding of breeding behavioural patterns among mosquito populations is one of the key elements for reaching the goal of vector-borne disease elimination and eradication (Chua et al., 2004). In recent times, control of these vectors by targeting their larva stages, and manipulating their growing condition has been effective. While many studies focus on single populations, there is a paucity of studies showing the multiple effects of physicochemical properties on multiple mosquito populations simultaneously. Physicochemical properties of the habitat may not equally affect every mosquito larva due to species' physiology differences and environmental growth requirements (Silberbush & Blaustein, 2008; Mwangangi et al., 2009). Not much data currently exists regarding the physiochemical characteristics of mosquito larval habitats for multiple species. Many studies in Nigeria only compare a few physicochemical properties of mosquito abundance, which may be an oversimplification of the multivariable affecting mosquito abundance. Also, very few studies have included species co-occurrence as a factor for the population size of this vector larva. However, understanding the variables of topmost importance would be effective for environmental manipulation and target treatment of the vector’s larval stage (Benelli, 2015).

We conducted a field study to estimate the multivariate effects of physicochemical properties of water and species co-occurrence on *Aedes*, *Anopheles* and *Culex* larva abundance. We also investigated the difference in abundance across habitat types in three ecological zones. Furthermore, we aimed to identify the most important physicochemical properties that affect larval occurrence.

# Materials and methods

## Data analysis

Statistical analyses were conducted using R version 4.1.3. Artificial habitat types were categorized into five categories: container, gutter, puddles, tyres, and tyre tracks. Only sites that were positive for at least one mosquito sample were included in the analysis. 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. Prior to model construction, predictor variables underwent z-score transformation, resulting in a significant enhancement of the model's performance. Predictor variables demonstrating statistical significance (p < 0.05) were included as fixed effects, while ecozones 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 using the anova function. GLMMs were implemented using the lme4 package and the glmer function.

Separate negative binomial models were fitted to assess the impact of habitat type on *Aedes*, *Anopheles*, and *Culex* mosquito abundance. Negative binomial models were chosen over Poisson models due to observed over-dispersion in the data. The model was built with a log (x + 1) transformation was applied to the mosquito abundance variables prior to constructing the negative binomial model. 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.

Canonical analysis plots were employed to ordinate mosquito species associated with artificial habitat types. Before this analysis, a significant difference (p < 0.05) in the distribution of mosquito species across habitats was confirmed using the chisq.test function. Following this, a correspondence analysis biplot was generated using CA for analysis and fviz\_ca\_biplot for visualization.

Principal component analysis (PCA) of physicochemical parameters was conducted using the FactoMineR package. Graphical representations were created using ggplot from the ggplot2 package, while PCA biplots were generated using fviz\_ca\_biplot from the FactoExtra package.

# RESULT

Overall, 32 habitats were positive for at least one of Aedes, Anopheles and Culex larvae. In total, 642 larva mosquitoes were collected across all sites and habitats. This included 91 *Anopheles*, 200 *Culex* and 351 *Aedes* species. Overall, most mosquitoes were collected from used tyres (320), puddles (210) and containers (43) (see Tabe 1).

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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Habitat (n) | Anopheles (%) | Culex (%) | Aedes (%) | Mean ± SD | Total (%) |
| Containers (6) | 0 (0) | 4 | 39 | 7.17±11.29 | 43 (100) |
| Gutters (3) | 21 | 12 | 2 | 11.67±17.62 | 35 (100) |
| Puddles (5) | 25 | 25 | 160 | 42.00±70.03 | 210 (100) |
| Tyre track (5) | 34 | 0 | 0 | 6.80±6.49 | 34 (100) |
| Used tyres (13) | 11 | 159 | 150 | 24.61±20.78 | 320 (100) |

*n= number of samples; SD= Standard Deviation*

Culex larvae were more prevalent in used tyres compared to other environments, with a density of 12.23 ± 15.38. Aedes larvae showed significantly higher densities in both used tyres and puddles compared to other habitats, having mean densities of 11.54±15.34 and 32.00±68.79, respectively.

Used tyres harbored the highest density of Culex larvae (12.23±15.38), significantly differing from other habitats (P<0.05), while Culex larval abundance was highest in used tyres and absent in Tyre tracks.

For Anopheles, larval mosquito abundance did not significantly (P>0.05) differ between gutters (7.00±10.39), puddles (5.00±9.51), and tyre tracks (6.80±6.49). However. Containers had no presence of Anopheles larvae and did not significantly differ from used tyres which had a density of 0.84±2.15.

Culex and Aedes larvae were not observed in tyre tracks, while Aedes larvae were absent in containers.

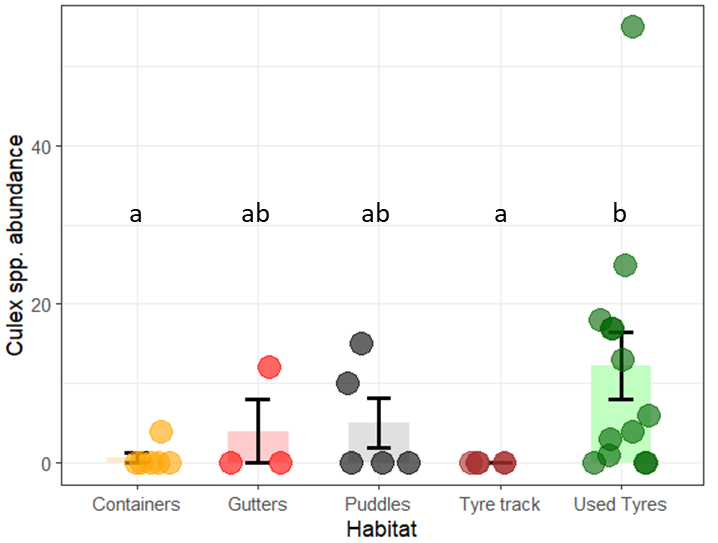


Figure 1: Abundance of *Culex* spp. larva at the artificial habitat.

There was no Culex larva in tyre tracks. However, used tyres, puddles and gutters had mean count of 12.23+15.38, 5.0+7.07 and 4.0+6.93 respectively, which were not statistically different from each other. Furthermore, an average of 0.67+1.63 culex larva was found in containers, but was not statistically different from the culex status in used tyres (0).

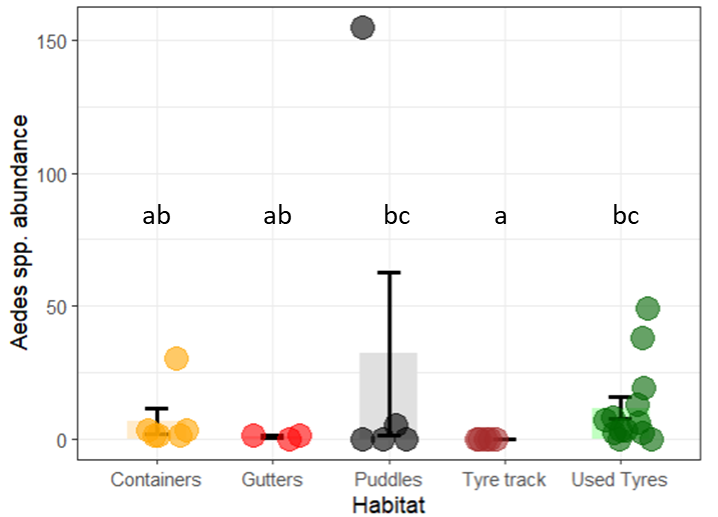


Figure 2: Abundance of Aedes spp. sampled at the artificial habitat

Similar to the occurrence among the sampled culex spp larvae, tyre tracks had no occurrence of Aedes spp. larvae from all samples. Here, puddles had the highest count (32+67.79) of Aedes larvae, though not statistically differing from that in used tyres (11.54+15.33). Furthermore, containers and puddles habitats did not differ significantly from tyre tracks (which had no *Aedes* larvae)

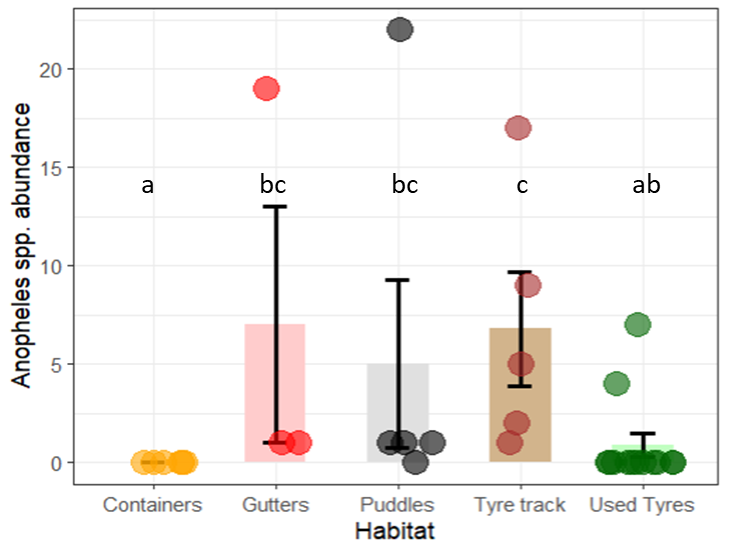


Figure 3: Abundance of Anopheles spp. larva sampled at the artificial habitats.

Unlike Aedes and Culex samples, Containers showed no presence of Anopheles, which was comparable to the absence found in used tires (P>0.05). Gutters, tire tracks, and puddles exhibited average Anopheles abundances of 7+10.39, 6.8+6.50, and 5.0+9.5, respectively, with no significant statistical variance observed among them (P>0.05).

Correspondence analysis (CA) biplot showed that *Aedes* was most associated with containers and puddles, Culex with used tyres, and anopheles with Tyre tracks (Figure 5). This supported evidence shown in the descriptive statistics. For example, Aedes had high prevalence in puddles (mean +SE), and Anopheles was mostly found in tyre tracks (mean + SE).

Descriptive statistics of the physicochemical properties are summarized in Table 2. There were variations between habitats and this were simplified using the PCA plot (Figure According to the Principal component analysis (PCA), the first two axes explained 55.5% of total variation. The first axis accounted for 41.2% of data variance, with total solid, suspended solid, colour, magnesium, sulphate, hardness, chloride, turbidity and Nitrate as the variables that most contributed to explaining the dataset variation. All physicochemical parameters were positively correlated with the first dimension, except for DO. TDS, Conductivity, Alkalinity, pH, phosphate and turbidity were the main variables explaining the second component which account for 14.3% of the variance (Figure 7).

PCA of the habitats and ecozones is represented in Figure 4. Puddles and tyre tracks had the most heterogeneous clusters. Containers and used tyres were the most homogenous, with great overlap in their clustering. Furthermore, the PCA ordination showed disparity in the homogeneity physicochemical characteristics of each ecozones. From the ordination plot, the lowland rainforest appeared more homogenous, relative to the large heterogeneity in derived savanna and freshwater swamps.

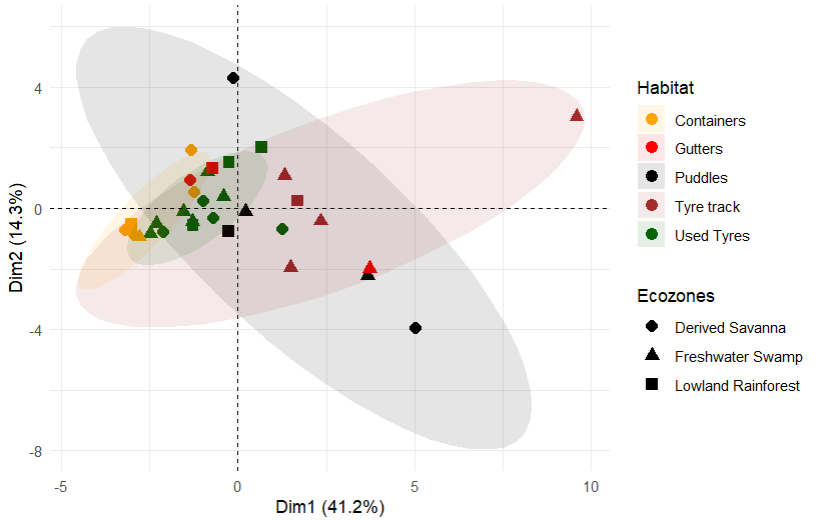


Figure 4: PCA of habitat and their ecozones. Ellipse was set to a 95% confidence interval (CI). However, CI could not be calculated for “Gutters” due to too few data points.

Table 2: Physicochemical properties of mosquito larva habitats, represented as mean ± standard deviation

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Habitat | Container | Gutters | Puddles | Tyre tracks | Used tyres |
| pH | 6.15±0.35 | 7.03±1.21 | 6.94±0.67 | 7.3±1.01 | 6.6±0.88 |
| Colour | 81.16±36.82 | 1878±2877.07 | 3139.6±2781.72 | 4153.4±31 | 608.15±797.83 |
| Turbidity | 12.333±7.94 | 436.33±661.42 | 817.4±842.95 | 855.8±484.35 | 77.92±89.82 |
| TDS | 57.42±67.32 | 81.27±46.31 | 108.12±104.37 | 106±64.04 | 70.94±30.94 |
| Suspended Solid | 8.33±5.98 | 250.67±378.77 | 374±324.79 | 776±687.10 | 129.92±278.72 |
| Total Solid | 65.75±67.47 | 331.93±370.20 | 482.12±287.72 | 882±730.19 | 200.86±273.88 |
| Conductivity | 108.33±127.03 | 153.33±87.37 | 204±196.93 | 200±120.83 | 133.85±58.39 |
| Chloride | 14.12±6.31 | 32.94±4.07 | 50.832±40.06 | 39.536±22.66 | 22.81±16.34 |
| Alkalinity | 29.33±21.75 | 86±72.58 | 57.6±15.71 | 87.2±66.19 | 53.85±35.11 |
| Hardness as CaCO3 | 25.67±29.59 | 53.33±41.05 | 83.6±66.31 | 116.4±68.31 | 54.15±36.28 |
| Phosphate | 0.49±0.40 | 3.67±2.49 | 36.082±74.12 | 5.046±3.51 | 1.13±0.99 |
| Sulphate | 17.5±18.98 | 88.67±101.93 | 35±26.63 | 119.8±128.26 | 29.46±14.40 |
| Nitrate | 5.94±7.43 | 28.73±37.18 | 28.538±25.68 | 27.14±17.71 | 10.75±11.95 |
| DO | 7.38±1.58 | 4.8±4.42 | 2.62±1.64 | 3.82±2.49 | 4.08±1.82 |
| BOD | 2.56±1.42 | 16.03±20.67 | 10.282±6.69 | 12.54±17.42 | 9.09±14.66 |
| Calcium | 7.61±11.61 | 18.95±15.45 | 26.934±21.08 | 28.70±19.31 | 16.28±13.40 |
| Magnesium | 1.38±1.08 | 1.62±1.13 | 3.988±3.64 | 10.79±9.70 | 2.62±1.65 |

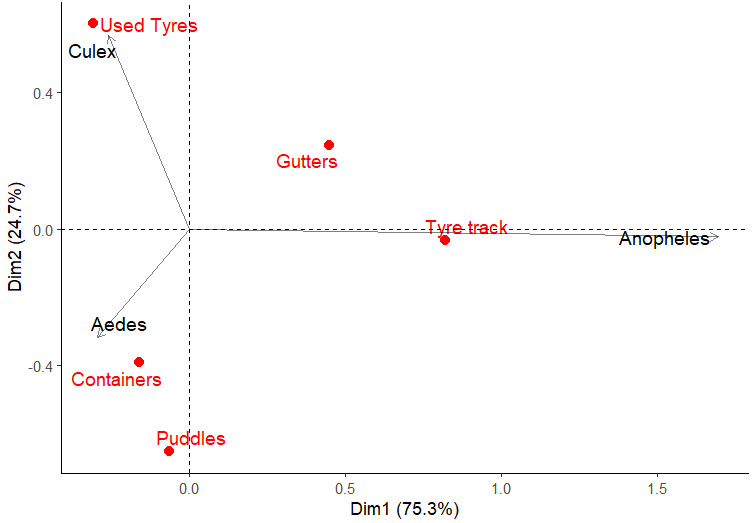


Figure 5: CA biplot representing the relationship between mosquito larvae and the Habitats.

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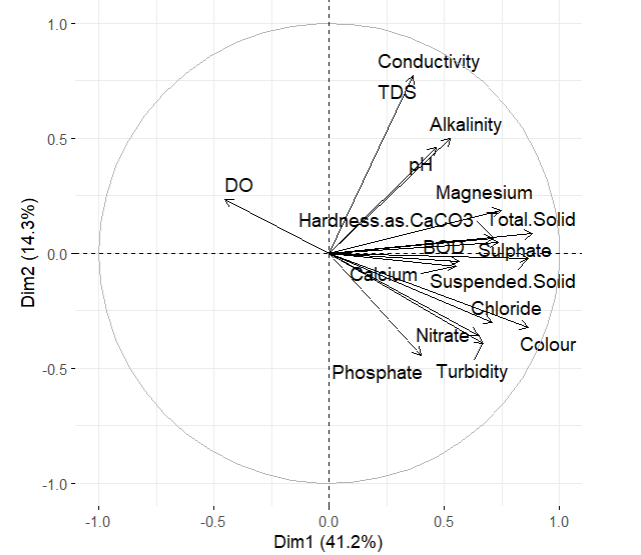


Figure 6: Principal component analysis biplot showing physicochemical parameters of sampling sites in a two-dimensional space.

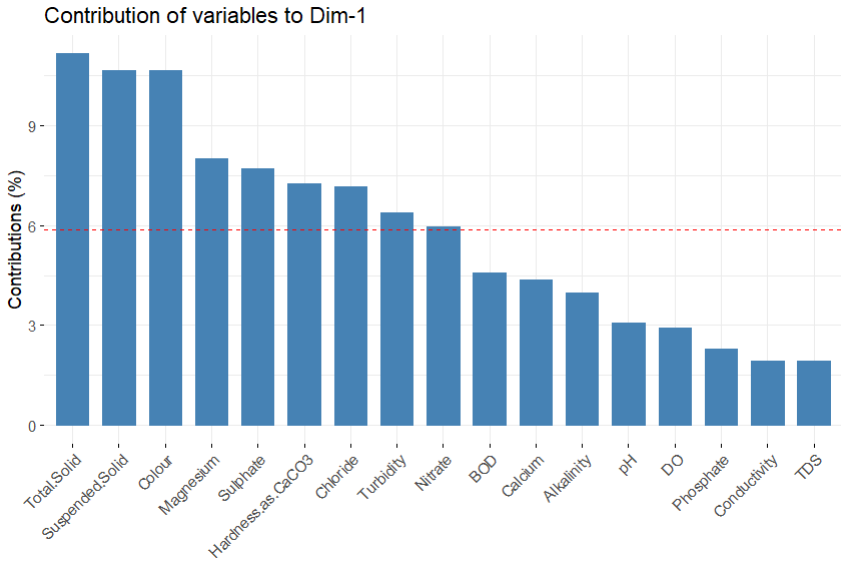


Figure 7: Contribution of physicochemical characteristics surveyed to the first principal component dimension.

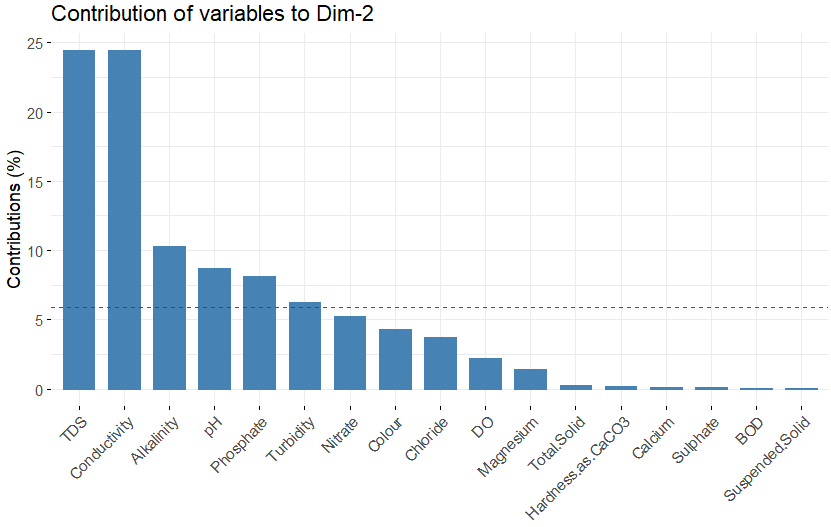


Figure 8: Contribution of physicochemical characteristics surveyed to the second principal component dimension.

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** |
| Anopheles Count | Fixed | Intercept | -0.1065 | 0.9494 | -0.112 | >0.05 |
|  |  | Turbidity | -0.8887 | 0.2863 | -3.104 | <0.01 |
|  |  | DO | -2.6321 | 0.4059 | -6.485 | <0.001 |
|  |  | *Culex* count | -0.5686 | 0.2636 | -2.157 | <0.05 |
|  |  | *Aedes* count | -1.0627 | 0.2632 | -4.037 | <0.001 |
|  |  | Depth | 1.3394 | 0.3687 | 3.633 | <0.001 |
|  |  | Magnesium | -0.6989 | 0.1834 | -3.810 | <0.001 |
|  | Interaction | Turbidity \* DO | 1.2109 | 0.2756 | 4.394 | <0.001 |

Turbidity, DO, Depth, Magnesium, Culex and Aedes count influenced the Anopheles abundance. There was an interactive effect between turbidity and DO which had a positive relationship with Anopheles density. Increased depth was positively associated with increased Anopheles abundance, while DO, magnesium, turbidity, Aedes and Culex count were negatively related to their abundance.

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** |
| Aedes Count | Fixed |  | -0.7078 | 1.6976 | -0.417 | P >0.05 |
|  |  | Anopheles Count | -0.9053 | 0.2375 | -3.812 | P<0.001 |
|  |  | Suspended Solid | 2.1395 | 0.6143 | 3.483 | P<0.001 |
|  |  | TDS | -1.9979 | 0.1691 | -11.813 | P<0.001 |
|  |  | Chloride | 0.2304 | 0.1486 | 1.550 | P >0.05 |
|  |  | Colour | -3.7158 | 0.3981 | -9.334 | P<0.001 |
|  |  | BOD | -1.9126 | 0.4001 | -4.781 | P<0.001 |

The GLMM model was utilized to predict the prevalence of Aedes larvae. It was constructed with six independent factors (Anopheles population, suspended solids, total dissolved solids (TDS), chloride levels, color, and biological oxygen demand (BOD)), all of which except Chloride showed notable impact on Aedes abundance. t

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** |
| Culex Count | Fixed | Intercept | -0.6317 | 1.1381 | -0.555 | P>0.05 |
|  |  | Turbidity | -4.7911 | 0.6119 | -7.830 | P<0.001 |
|  |  | pH | -0.4662 | 0.1768 | -2.636 | P<0.01 |
|  |  | Nitrate | 1.6897 | 0.2405 | 7.025 | P<0.001 |
|  |  | BOD | 0.3048 | 0.1457 | 2.092 | P<0.01 |
|  |  | DO | -0.7392 | 0.2333 | -3.169 | P<0.01 |
|  |  | TDS | -0.8355 | 0.2097 | -3.985 | P<0.001 |
|  | Interaction | DO\*TDS | 1.2212 | 0.4028 | 3.032 | P<0.01 |

Culex density was most affected by turbidity, pH, Nitrate, BOD, DO and TDS. GLMM showed that turbidity, pH, DO, and TDS had negative relationships with culex density. On the other hand, the model shows that BOD and Nitrate increased *Culex* abundance.

**DISCUSSION**

We surveyed 32 sites for the abundance of *Aedes* and Culex sp. mosquitoes and physicochemical parameters of these sites were recorded.

We observed a high correlation between conductivity and total dissolved solids, which were not surprising. The connection between electrical conductivity (EC) and total dissolved solids (TDS) has been thoroughly examined, consistently revealing a strong correlation between them.

***Culex* abundance**

Firstly, we find that Culex species larva were most associated with used tyres, which was not sprising, given reports of culex in containers by Joseph et al. (2013), who found that *Cu. andersoni*, and *Cx. fatigans* in high abundance in containers in Akure City, Nigeria. But notably, containers had relatively lower pH than other artificial habitats, from which, our study has shown high relationship of culex with areas of lower pH. Though, (Joseph et al., n.d.) had found high abundance of Culex in containers with pH ranged 7.1 to 7.3, which is slightly higher than average pH observed in this study. However, we found relatively high number of Culex larvae in gutters and puddles, which had pH more similar to Joseph et al (2013)’s pH reported. Nonetheless, the pH recorded across all samples was within optimal pH levels for Culex larval development, as suported by Ukubuiwe et al., (2020)’s study.

We observed that lower turbidity was water in containers supported Culex larvae abundance. Muturi, (2007) reported *Cx. quinquefasciatus* was positively associated with turbid water, but, Cx. *annulioris* larvae have been more associated with clear water (Muturi, 2007). We reported low turbidity in containers, which may result from low organic matter in the water (Muturi, 2007).

Research conducted by Kenawy et al. (2013) and Ibrahim et al. (2011) demonstrated a direct relationship between Nitrate levels and the density of Culex larvae. The progression of larvae through multiple generations has been associated with a decline in water turbidity. This decline boosts the activity of nitrifying bacteria, consequently elevating the concentrations of nitrate and nitrite ions (Darriet and Corbel, 2008). We found that Culex abundance was positively associated with Nitrate level in water. This was not surprising sine increased nitrogen level encourages microbial growth, which is likely favourable for mosquito larval growth, since they serve as diet.

Emidi et al. (2017) reported a negative (though not statistically significant) association of conductivity with (which in our study has a strong positive relationship with TDS) *Culex* larvae abundance.

Nikookar et al. (2017) studies on many Culex species in Iran showed that Cx. pipiens showed a significant positive correlation with conductivity, alkalinity, total hardness and chloride.

Our findings on Culex association to low level of DO aligns with (Muturi et al., 2007) who found negative association of DO with *Cx. quinquefasciatus*.

**Anopheles abundance**

We observed that *Anopheles* mosquito larvae were more abundant in tyres, puddles and gutters. These habitats have been seen to have high levels of *Anopheles* larvae in them. Studies

David et al. (2021) reported a positive association of conductivity with Anopheles larva abundance.

From our study, *Anopheles* larvae seemed to prefer sites with lower magnesium, DO and turbidity. Also, they were negatively associated with *Aedes* and *Culex* larvae abundance. The preference of Anopheles larvae for site with lower turbidity was not surprising. Clear waters with associating vegetation were majorly observed in the breeding habitats in Ojianwuna et al. (2021)’s study, in Delta state, Nigeria.

Awolola et al. (2007) observed higher Anopheles larvae abundance with low dissolved oxygen, high conductivity and turbidity. A similar observation by (Muturi et al., 2007) in a rice field showed that Anopheles arabiensis were associated with low levels of DO.

**Aedes Abundance**

We found that Aedes abundance was positively associated with chloride and suspended solid level, but negatively with BOD, Colour, TDS and population size of Anopheles spp. larvae.

David et al. (2021) was reported positive association between dissolved organic carbon concentration and number of immature Aedes.

Aedes abounded more in puddles and used tyres where anopheles were seen in very low numbers. This may be due to the physicochemical properties associated with both habitats. This may be due to high differences in the physiological requirements for their development and survival.

Limitations of study

We did not record the temperature, which we are sure highly influences mosquito abundance. Just like (Ojianwuna et al., 2021) found high abundance of Anopheles larvae with increasing temperature. Therefore, some of the surprising results may be due to this unaccounted factor in the survey.

**CONCLUSION**

The presence of *Aedes*, *Anopheles*, and *Culex* larvae indicates the likelihood of diseases such as malaria, yellow fever, dengue fever, and filariasis in the region. Vigorous vector control management should be implemented and education on concerning human behaviour that fosters mosquito breeding.

Research on ways that water's physical and chemical characteristics influence mosquito composition in different breeding sites can shed light on the complex ecosystem interactions that determine the suitability of habitats for different species. The data obtained in the present study expand our understanding of the ecology and interaction of the investigated species with abiotic factors in the aquatic environment, providing useful data for future studies that seek to elucidate the underlying mechanisms in the selection process and colonization of breeding sites by mosquitoes of epidemiological importance.

The physicochemical properties of the habitats may be altered by several anthropogenic or natural factors. Also, we could not determine how many generations of mosquito were at the habitat. There has been reports to show increased nitrate levels with longer microbial settlements in water. Microbial processes, such as nitrification, can lead to higher nitrate levels and turbidity. Bacteria present in the water can convert organic nitrogen compounds into nitrate during nitrification, while also releasing particles and organic material into the water, thus increasing turbidity.

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