**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; Eneanya et al., 2018; 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. This habitat quality 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; Medeiros-Sousa et al., 2020; Mwangangi et al., 2009; 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 faces a high prevalence of mosquito-borne diseases such as malaria, lymphatic filariasis, and dengue fever ( Eneanya et al., 2018; Awosolu et al., 2021). A better understanding of mosquito breeding behaviours is crucial for achieving vector-borne disease elimination and eradication (Chua et al., 2004). Recent control efforts have targeted the larval stages of mosquitoes by manipulating their growing conditions (Zoh et al., 2022). However, most studies focus on single populations, and there is a lack of research on the multivariate effects of physicochemical properties on multiple mosquito populations simultaneously (Silberbush & Blaustein, 2008; Mwangangi et al., 2009). This gap in data necessitates further study on the physicochemical characteristics of mosquito larval habitats.

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.

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.

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.

# 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.

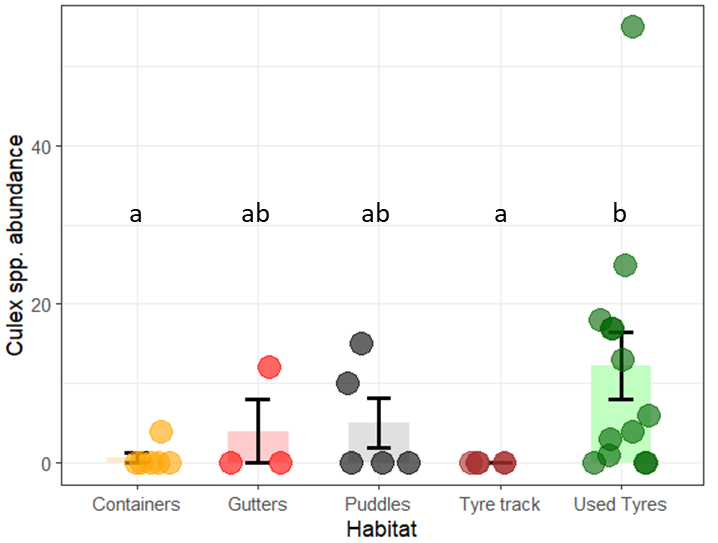


Figure 1: Abundance of *Culex* spp. larva at the artificial habitat.

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).

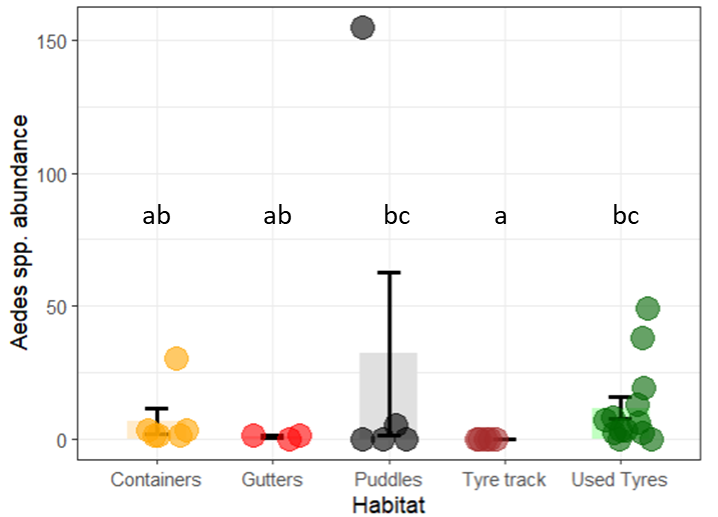


Figure 2: Abundance of Aedes spp. sampled at the artificial habitat

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)

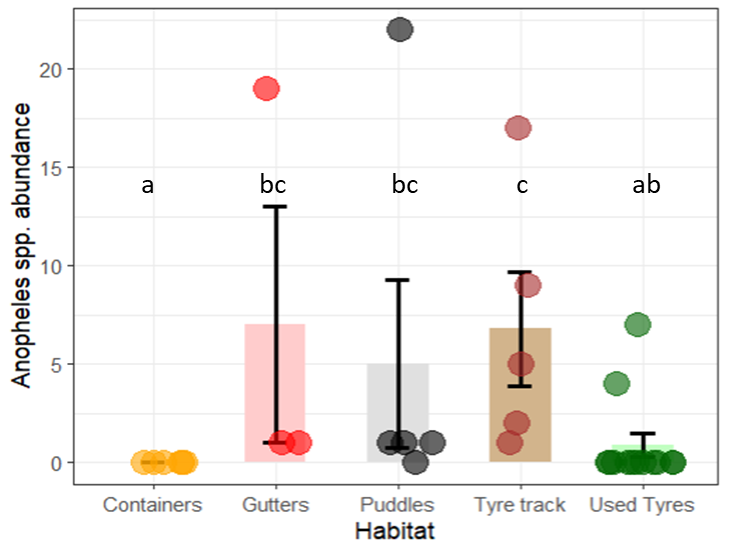


Figure 3: Abundance of Anopheles spp. larva sampled at the artificial habitats.

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.

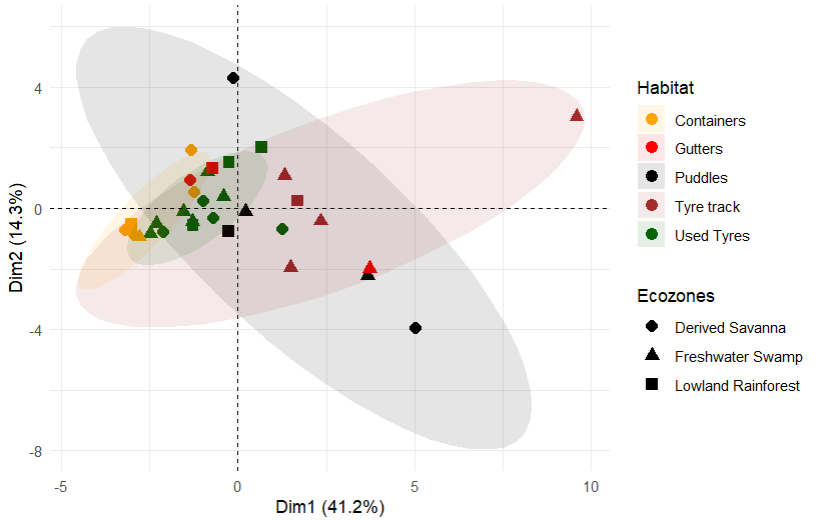


Figure 4: PCA of habitat and their ecozones. Ellipse was set to a 95% confidence interval (CI). However, CI could not be calculated for “Gutters” due to too few data points.

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 |

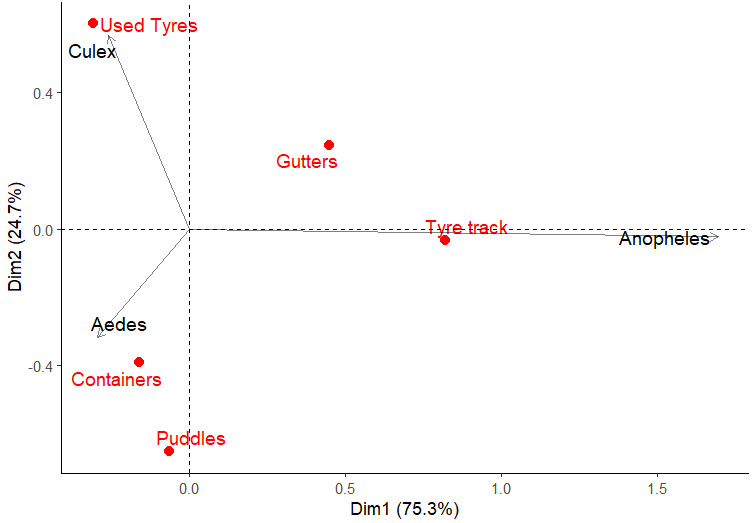


Figure 5: CA biplot representing the relationship between mosquito larvae and the Habitats.

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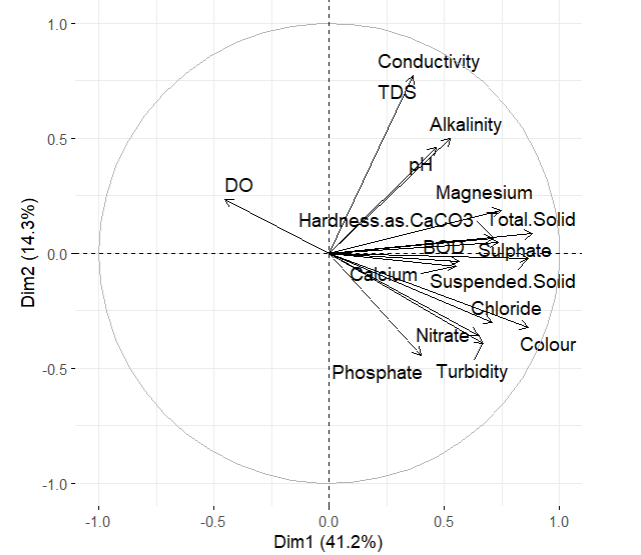


Figure 6: Principal component analysis biplot showing physicochemical parameters of sampling sites in a two-dimensional space.

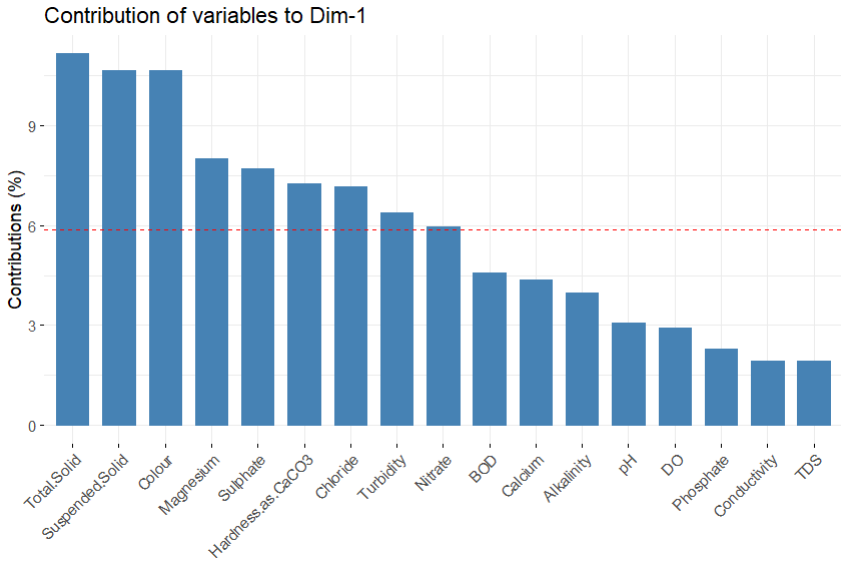


Figure 7: Contribution of physicochemical characteristics surveyed to the first principal component dimension.

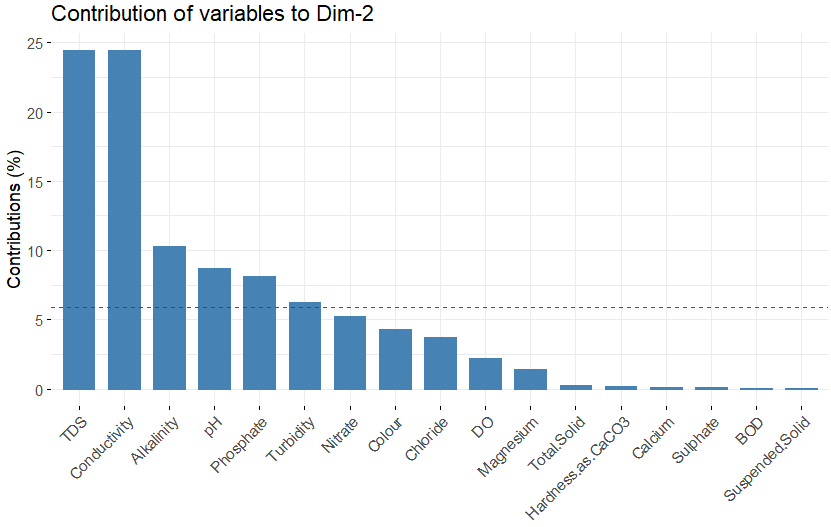


Figure 8: Contribution of physicochemical characteristics surveyed to the second principal component dimension.

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 | 0.1942 | 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 |

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 |  | -0.5580 | 1.4129 | -0.395 | P >0.05 |
|  |  | *Anopheles* 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 |

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.

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 | -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 |

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

**DISCUSSION**

We surveyed 32 sites for the abundance of *Aedes* and Culex sp. mosquitoes and physicochemical parameters of these sites were recorded. Our aim was to identify important physicochemical variables that significantly impacted the mosquito larvae abundance, and further understand how the habitat type affect their preference.

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.

1. Physicochemical properties associated with abundance of each mosquito

***Culex* abundance**

Culex was negatively associated with pH, turbidity and TDS, and was positively associated with nitrates.

Firstly, we found that Culex species larva were most associated with used tyres, which was not surprising. A study in some villages in Rivers state, Nigeria by Okiwelu & Noutcha (2012) showed that 80% of sampled *Cx. quinquefasciatus* in container-type breeding sites, which includes tyres, etals, plastic containers and ‘calabashes’. Obi et al., (2020)’s study also supports a high occurrence of mosquito larvae in used tures compared to other breeding sites like rock poles and electric poles.

We observed that lower turbidity was water in containers supported Culex larvae abundance. Contrastingly, Muturi, (2007) reported *Cx. quinquefasciatus* was positively associated with turbid water, but also reported that Cx. *annulioris* larvae have been more associated with clear water. We reported low turbidity in containers, which may result from low organic matter in the water (Muturi, 2007). Here, *Culex* was most associated with used tyres, and this habitat was less turbid compared to puddles, gutters, and tyre tracks.

Soltan-Alinejad et al. (2023) reported 8.3 as the optimum pH level for *Cx. quinquefasciatus* and *Cx. laticinctus*, but our study shows that used tyres had pH of 6.6±0.88, yet had the most abundant in *Culex* compared to other habitats. We were not surprised our model predicted negaative association with pH, considering that other habitats had relatively higher pH (except for containers).

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. Also, Nikookar et al. (2017) studies on many *Culex* species in Iran showed that *Cx. pipiens* showed a significant positive correlation with conductivity and chloride, which are indication of dissolved solids. We had a perfect correlation between TDS and conductivity in our study.

**Anopheles abundance**

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

From our study, *Anopheles* larvae seemed to prefer sites with lower magnesium, turbidity and pH. 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. The association of Anopheles larva with clearer water may be due to their low tolerance for pollution. This is so because clear water likely has higher oxygen levels, which was also true from the outcome of our study. Experiments under controlled conditions have shown that *Anopheles* larvae exhibit higher survival rates, faster development, and better overall fitness in clean water compared to polluted or turbid water. They are known to have lower tolerance to polluted water compared to Aedes and Culex mosquitoes. Clean water tends to have a more neutral pH, while polluted water can be acidic or alkaline, or often contains harmful chemicals, heavy metals, and organic pollutants which can be detrimental to larval physiology and development. In this case, the Anopheles larva preferred lower pH level, which supports the idea of less turbidity since turbid waters tend to have compounds like ammonia that increases pH.

However, our observations on pH relationship was in contrast to (Emidi et al., 2017) whose study showed anopheles larvae density was associated with increased pH, after having recorded pH between 8.0 – 8.8 in their study.

It is also ntable that these sites (tyre tracks, puddles and gutters) have high variability in their physichochemical properties compared to containers and used tyres where Anopheles were barely found.

**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.

Reji et al., 2013 had similar observation to ours in their study on effect of physicochemical characteristics on abundance of container-breeding *Aedes* mosquitoes. They found a negative correlation between the abundance of the mosquito larvae and TDS.

(Ref: Physicochemical characteristics of habitats in relation to the density of container-breeding mosquitoes in Asom, India. J Vector Borne Dis 50, September 2013, pp. 215–219))

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.

Our findings contradict that of Mahata et al. (2022), who found a moderately positive correlation of Aedes larvae abundance with BOD and TDS. According to the authors, A. aegypti prefers clean water found in different domestic containers inside or near human dwellings, whereas A. *albopictus* is more likely to be present in natural containers or outdoor man-made habitats possessing a greater amount of organic debris.

We found that Aedes larvae abundance was did not increase with increase in Anopheles larva abundance. This may be due to differences in the physicochemical properties of the habitat for which they are found. For Anopheles, we saw that they were more abundant in tyre tracks, gutters, and puddles, while *Aedes* were more abundant in puddles and used tyres. Used tyre habitat had lower turbidity compared to gutters, tyre tracks and peddles. Similar occurrence was seen for magnesium, except that its level in gutters was lower than in used tyres.

1. Physicochemical properties of each habitat

The physicochemical properties off the habitat indeed differed, and this would affect the type of mosquito larvae and the choice of oviposition by gravid female mosquitoes. It seemed that containers and used tyres have more closely related homogenous properties, which may account for why *Aedes* and *Culex* larvae were particularly more abundant in them. Puddles were more heterogeneous in physicochemical properties, and seemed to have a fair number of each species of Aedes, Culex and Anopheles. Tyre tracks seemed heterogeneous, but afar off from the relatively homogenous physicochemical properties of containers. This may possibly account for why Anopheles were more appreciative of tyre tracks, and absent from containers. Furthermore, the result of our GLMMs seems to support Anopheles larvae would rather co-occupy habitats of Culex than that of Aedes.

Our study findings indicate that puddles and tire tracks exhibit considerable heterogeneity, which is evident from the diverse range of physicochemical properties observed within them. Specifically, key physicochemical properties identified through principal component analysis (PCA), including turbidity, color, suspended solids, total solid, chloride, and magnesium, demonstrate substantial variability in puddles. Notably, among the thirty-two sites studied, only five were identified as positive puddle sites. Similarly, tire tracks display significant variability in certain key variables identified by PCA, such as total solids, conductivity, chloride, nitrate, and magnesium. However, we believe that a larger sample size may better capture and account for the extensive variability observed in the dataset.

The high variability in overall physicochemical characteristics has implications for the tolerance and adaptation of mosquito vectors to diverse environments, complicating control strategies. Limited exchange with the surroundings results in stable physicochemical conditions and uniform properties in stagnant water environments like containers and used tyres. Over time, microbial communities in such confined water bodies may reach equilibrium, fostering consistent populations and metabolic processes that stabilizes the water's physicochemical characteristics. In contrast, puddles and gutters experience greater exposure to environmental fluctuations, including rainfall, runoff, and temperature changes. These dynamic inputs and outputs lead to heterogeneous physicochemical properties that vary across space and time. Puddles and gutters receive inputs from diverse sources such as organic matter, pollutants, and debris washed from the surroundings, further contributing to the variability in composition and properties of the water. The open nature of puddles and gutters facilitates greater microbial diversity and activity. Consequently, microbial communities in these environments exhibit spatial and temporal variations, resulting in dissimilarities in physicochemical properties driven by differences in metabolic activities and community composition.

Through this study, we can confirm that Anopheles and Culex larvae can co-occur in same habitat, despite the differences in their physiology.

**Limitations of study**

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

**Physicochemical properties of the ecozones**

Compared to habitat type, there was no clear distinction in the physicochemical properties of the mosquito sampling sites based on ecozones in Edo state, Nigeria. It may be obvious that the three ecozones (lowland rainforest, freshwater swamp and derived savannah) did not affect preference of the mosquitoes, as much as the habitat type.

Main focus of the discussion section

1. Physicochemical properties associated with abundance of each mosquito
2. Which mosquito is associated with which habitat
3. Mosquito species co-occurrence
4. Implication of physicochemical properties of habitat and ecozone on mosquito control

**CONCLUSION**

The presence of Aedes, Anopheles, and Culex larvae in the studied region underscores the potential risk of diseases such as malaria, yellow fever, dengue fever, and filariasis. To mitigate these risks, it is imperative to implement robust vector control measures and educate the community on behaviour that contributes to mosquito breeding. Furthermore, investigating how the physical and chemical characteristics of water influence mosquito composition across different breeding sites can provide valuable insights into the intricate ecosystem interactions governing habitat suitability for various mosquito species. This study enhances our understanding of the ecological dynamics and interactions between the investigated mosquito species and abiotic factors in aquatic environments, offering valuable insights for future research endeavours aimed at elucidating the underlying mechanisms driving the selection and colonization of breeding sites by epidemiologically significant mosquitoes.

It is important to note that the physicochemical properties of habitats may undergo alterations due to both anthropogenic and natural factors. Additionally, limitations in our study, such as the inability to determine the number of mosquito generations present at the habitat, warrant consideration. Reports have indicated that prolonged microbial settlements in water can lead to increased nitrate levels, with microbial processes such as nitrification contributing to higher nitrate levels and turbidity. Bacterial activity in water can result in the conversion of organic nitrogen compounds into nitrate during nitrification, accompanied by the release of particles and organic material, thus elevating turbidity levels.

Our findings reveal significant heterogeneity in puddles, tyre tracks, and gutters in terms of physicochemical properties, posing challenges for control efforts. This variability underscores the adaptive capacity of mosquitoes to thrive in diverse environmental conditions, highlighting the complexity of mosquito management strategies. Addressing these complexities requires a multifaceted approach that integrates scientific knowledge with effective control measures tailored to the specific characteristics of breeding sites.

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