INTRODUCTION
Mosquitoes are tiny arthropods that are known to be vectors of many diseases like malaria, yellow fever, dengue fever and filaria (Wilkerson et al., 2021). Mosquito vectors transmit more than 17% of mosquito-borne diseases globally and kill more than 700,000 people annually (WHO, 2020).

*Aedes aegypti* is the primary vector for some arboviral diseases such as Yellow fever, dengue, Zika and Chikungunya. This species of invasive mosquito has gained attention globally because of their fast-spreading trend and successful adaptation. This study was designed to determine the larval abundance and physico-chemical parameters of *Aedes aegypti* from three breeding sites within Kano metropolis. Determination of larval abundance was performed using ovitraps according to the protocol described by Wan-Norafikah et al., 2012. Twenty ovitraps were placed BUK old site, BUK New site and SOT from April – September, 2023.

Physico-chemical parameters such as pH, Temperature, Electrical conductivity, Dissolved Oxygen, Total Dissolved Solids, Turbidity and Total Suspended Solids were measured for all breeding habitats. A total of 2959 *Aedes aegypti* larvae were collected in the study areas within Kano metropolis which comprised of BUK old site 31.80%, BUK New Site 32.75% and School of Technology (Kano Poly) 35.45%. The study revealed that temperature ranges from 29.98°C to 31.05°C, pH range of 6.37 – 6.74, TDS level of 109.80 – 305.09 mg/L and Electrical Conductivity ranges from 270 – 390 μS/cm. It can be concluded from this study that *Aedes aegypti* are abundant in the study area and also physico-chemical parameters of the breeding habitats showed significant variations among the three breeding habitats. This data may help in management of *Aedes aegypti* especially in eliminating stagnant water which serves as the breeding habitat.

Keywords: Aedes, Physicochemical, Temperature, Vector

A critical control strategy to combat major mosquito-borne diseases involves specifically targeting mosquito vectors in order to effectively disrupt disease transmission. (Asma’u et al., 2021). Indiscriminate and prolonged use of insecticides in agricultural practices alters the natural environment of mosquitoes, leading to the development of resistance (Alhassan et al., 2015). Understanding the ecology and spatial distribution of mosquito larvae is crucial for ensuring the effectiveness of vector control measures (Mereta et al., 2013). Breeding habitats can be classified as either artificial or natural, and further categorized based on their stability as temporary, permanent, or semi-permanent in nature (Adel et al., 2024). Places with stagnant water are ideal for mosquito reproduction and the growth of their larvae. Additionally, breeding sites typically consist of various types of vegetation, whether natural or cultivated, dense or sparse, and submerged
or emerged. Research has shown that mosquitoes tend to prefer habitats with shorter vegetation rather than those with taller vegetation (Sowilem et al., 2017).

The physicochemical parameters greatly affect the species, density, and development of mosquito larvae, and these parameters can vary across breeding sites. These parameters include temperature, pH, dissolved organic and inorganic materials, salinity, turbidity, and electrical conductivity (Gopalakrishnan et al., 2013). Changes in these parameters of the breeding sites can have both positive and negative effects on the vectors. For instance, when the temperature drops below 14°C or rises above 30°C, the larval growth rate in most vector species decreases. Additionally, mosquito larvae are typically found in environments with pH levels ranging from 3.3 to 10.5 (Liu et al., 2021). Monitoring and knowledge of environmental factors like physicochemical parameters are important in tackling insecticide resistance challenges (Ononamadu et al., 2020). Understanding the environmental characteristics of mosquito breeding sites as well as their prevalence has been demonstrated to be a successful strategy in reducing mosquito-borne diseases. Hence this study was therefore designed to determine the larval abundance and physico-chemical parameters of Aedes aegypti breeding sites in Kano metropolis.

MATERIALS AND METHODS

Study Area
The study was conducted in three breeding habitats within Kano metropolis. These include Bayero University Kano old site (11°58’46"N, 8°28’42"E), Bayero University, Kano New Site (11°58’15"N, 8°26’5”E), and School of Technology Kano State Polytechnic (11°59’29”N, 8°32’26"E) (Figure 1).

Identification of Mosquitoes to Species Level

Morphological Identification
A total of 210 (50 from BUK old site, 72 from BUK New Site and 88 from SOT) adults Aedes aegypti were identified using the morphological and pictorial keys (Rueda, 2004). They were identified using a 7X–45X, Trinocular XTL Stereo Zoom microscope.

Molecular Identification
Genomic DNA Extraction
DNA was extracted according to the protocol described by Livak, 1984. Livak grind buffer was first prepared using 1.6 ml 5mM NaCl, 5.48g sucrose, 1.57 g Tris, 10.16 ml 0.5M EDTA, 2.5 ml 20% SDS and sterile water to make the final volume to 100 ml. The solution was then sterilized, filtered and stored at -20°C until used.

Genomic DNA was extracted from 30 mosquitoes (10 mosquitoes from each location). The livak grind buffer was first pre heated in a water bath at 65°C until clear. A single adult mosquito was put in a 1.5ml effendorf tube and homogenized in 100 ul of pre-heated livak grind buffer with plastic pestle. The samples were incubated for 30 minutes at 65°C and microfuge briefly to collect condensation. Then 14
ul of 8M potassium acetate was added into each tube and mixed before incubation on ice for 30 minutes. The samples were then centrifuge at 13,000 rpm at 4°C for 20 minutes. The supernatant was transferred to a new eppendorf tube and 200 ul of 100% ethanol was added. The samples were centrifuged again at 13,000 rpm for 15 minutes at 4°C. The supernatant was then discarded and the DNA pellet was rinsed with 100 ul of 70% ethanol. The pellet was left on the bench to dry overnight and then re-suspended with 30 ul of nuclease free water and incubated at 65°C for 10 minutes. DNA concentration was measured using Nanodrop and stored at –20°C until use.

**Molecular Identification**

Molecular identification of *Aedes aegypti* to species level was done using polymerase chain reaction based on the method described by Beebe et al., (2007). The primers used are: forward primer ITS1A, 5'-CCTTTGTACACACCACCGTCG, and reverse primer ITS1B, 5'-ATGTGTCCTGCAAACTGAC. The final 12.5 ul of PCR mixture consist of 2 ul of genomic DNA, 6.25 master mix, 1 ul of each forward and reverse primer and 2.25 ul of nuclease free water. The PCR cycling conditions were 95°C 5 minutes and 35 cycles of 94°C for 30 seconds, 51°C for 40 seconds and 72°C for 45 seconds. Restriction analysis was carried out in a 0.5 ml microfuge tube containing 6.25 ul of PCR product and 0.5 ul of RsaI buffer. The mixture was incubated at 37°C for 2 h and then size separated on a 1.5% agarose gel stained with ethidium bromide at 100 V for 55 min. The gel was visualized on gel imaging system.

**Determination of Physico-chemical Parameters**

Water samples from each collection site were collected and analyzed using water testing kit (model no. ISO9001, 1575-02-1504) for the following physicochemical parameters: pH, Temperature, Electrical conductivity, Dissolved oxygen, Total Dissolved Solid, Turbidity and Total Suspended Solid.

**Data Analysis**

Data analysis was done using R Software version 4.3.1. Physico-chemical parameters were analyzed using one way ANOVA. Larval abundance was calculated as percentage using this formula as described by Olajide et al., 2019.

\[
\text{Larval abundance} = \frac{\text{Number of larvae per site}}{\text{Total number of larvae collected}} \times 100
\]

**RESULTS AND DISCUSSION**

**Larval Abundance**

A total of 2965 *Aedes aegypti* larvae were collected in the study areas within Kano State which comprised of BUK old site 31.80%, BUK New Site 32.75% and School of Technology (Kano Poly) 35.45%.

![Figure 2: Aedes aegypti larvae Collected from Kano state](image)

Table 1 presents the monthly distribution of *Aedes aegypti* larvae collected in Kano State for the months of July, August, and September. In July, the collected data indicates that there were 1144 Aedes mosquito larvae, the number of mosquito larvae decrease to 570 in August. In September, the data indicates an increase in the number of mosquito larvae compared to August. There were 1251 Aedes mosquito larvae. There were total of 2965 *Aedes aegypti* larvae collected across the three location in Kano State (Table 1).
Table 1: Monthly Distribution of Mosquito larvae collected in Kano State

<table>
<thead>
<tr>
<th>Month</th>
<th>Aedes</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>0</td>
</tr>
<tr>
<td>June</td>
<td>0</td>
</tr>
<tr>
<td>July</td>
<td>1144</td>
</tr>
<tr>
<td>August</td>
<td>570</td>
</tr>
<tr>
<td>September</td>
<td>1251</td>
</tr>
<tr>
<td>Total</td>
<td>2965</td>
</tr>
</tbody>
</table>

Morphological identification of *Aedes aegypti*

Morphological identification confirmed that all collected *Aedes* mosquitoes belonged to the *Aegypti* species. The head region displays white flat-scale patches on the clypeus and vertex, with only a few erect forked scales present on the occiput. The proboscis was entirely dark without any white band. The legs had a white stripe on the femoral knee-spot and the front part of the mid-femur, as well as on the thorax. The scutum was black with two white stripes running along the center.

Molecular identification of *Aedes aegypti*

After Polymerase Chain reaction for species identification, gel electrophoresis was carried out to view the results. A band of 750 bp PCR product was observed on 1.5% agarose gel confirming that these mosquitoes belong to genus *Aedes* (Fig. 2). The digestion of the PCR product using RsaI determined the species of the sample. The gel images obtained after incubation with the RsaI enzyme show clear bands indicating the correct base pair sizes for *Aedes aegypti* (bands at 120, 180 and 400 bp) (Figure 3).

![Figure 2: Gel electrophoresis picture showing bands observed after conducting PCR using internal prescribed spacer region 1 primer (ITS1) (Step one of a two-step PCR protocol) for species identification.](image-url)
Physico-chemical Parameters
The result for the physicochemical parameters of *Aedes aegypti* breeding sites from Kano State is presented in Table 2. For pH water sample from SOT (6.41 ± 0.39) has the highest pH level while water sample from BUK old (6.74 ± 0.4) site has the lowest pH level (Table 3). For Temperature, water sample from BUK New Site has the highest temperature (33.18 ± 1.64) and water sample from SOT recorded the lowest temperature (29.98 ± 1.58). For Electrical Conductivity and Dissolved Oxygen, water samples from BUK Old Site showed the highest levels than the water water samples with 390.0 ± 0.0 and 109.08 ± 11.0 respectively (Table 2). For Turbidity and Total, water samples from BUK New Site showed higher values than BUK Old site and SOT.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BUK old site</th>
<th>BUK New Site</th>
<th>SOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.74 ± 0.44a</td>
<td>6.37 ± 0.44a</td>
<td>6.41 ± 0.39a</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>31.05 ± 1.65a</td>
<td>33.18 ± 1.64b</td>
<td>29.98 ± 1.58a</td>
</tr>
<tr>
<td>EC (µS/cm)</td>
<td>390.0 ± 0.04a</td>
<td>270.0 ± 0.05a</td>
<td>320.0 ± 0.04b</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>5.63 ± 0.63a</td>
<td>4.65 ± 0.61b</td>
<td>4.96 ± 0.63b</td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>109.08 ± 11.0a</td>
<td>179.98 ± 11.27a</td>
<td>305.09 ± 10.92b</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>77.54 ± 11.98a</td>
<td>226.16 ± 11.99b</td>
<td>97.56 ± 11.86c</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>301.68 ± 11.77a</td>
<td>337.2 ± 12.14b</td>
<td>351.54 ± 11.94b</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM of Three Replicates. Values with the same Alphabet within the same row are not significantly different. BUK = Bayero University Kano, SOT = School of Technology (Kano Poly).

Discussions
This study was designed to determine the larval abundance, identification and physico-chemical parameters of *Aedes aegypti* breeding habitats in Kano metropolis, Kano State Nigeria. Morphological and molecular identification of the *Aedes* mosquitoes in this study confirms the presence of *Aedes aegypti*. This study confirms that ovitraps are suitable for the oviposition preferences of female *Aedes aegypti* mosquitoes, as they tend to lay their eggs in black containers like cans and discarded tires. This is in agreement with the findings of Rydzanic et al., (2003). This study reveals that larval abundance was found during rainy season (July – September) and no any larvae was found during the months of April – May (Table 1). This sheds light on the absence of *Aedes aegypti* during dry seasons which could be because there was no enough water to favor unlimited suitable habitats that leads to massive *Aedes aegypti* production, abundance and distribution. This is in conformity with the results of many reports which indicated that *Aedes aegypti* population density is strongly influenced by rainfall and temperature (Lourenço-de-Oliveira et al., 2004; Brown et al., 2014).

Table 1 showed the monthly larval abundance of *Aedes aegypti* in the study area. The month of September recorded the highest larval abundance followed by July. However, the lowest larval abundance was recorded during the month of August. This could be attributed to the increase in the frequencies of rain falls in August that might facilitate the washing away of the developing larvae from the ovitraps thereby leading to the decline in the population of mosquitoes. This is in agreement with the report of Barde et al., (2019).

The physicochemical properties showed significant variation amongst the three breeding sites of *Aedes aegypti* (Table 2). The results indicated variation in temperature from 29.98°C to 31.05°C, and there were no much variation in pH values ranging from 6.37 – 6.74. This is In agreement with other studies which report that a wide range of pH levels from 6.5 to 8.0 is suitable for *Aedes* mosquito survival and breeding (Waewwab et al., 2019). According to Umar and Donpedro (2008), the pH of the breeding habitats of mosquitoes interferes with osmoregulation and oxygen transport mechanisms. An increased in alkalinity, salinity and pH may lead to quicker mosquito growth larvae up to 9.0 pH. After pH reaches the substantial alkalinity range, pH inhibits the further
growth of *Aedes* larvae at the field level. So, at the household level, basic actions like common salt may be used to reduce *Aedes* breeding (Rasika et al., 2022).

Total dissolved solids (TDS) is a measure of the combined dissolved content of all inorganic and organic substances present in water - molecular, ionized, or micro-granular suspended form. Natural sources, wastewater, municipal and agriculture run-off and industrial effluents are all sources of TDS in bodies of water (Bernard and Ayeni, 2012).

Oscoregaard et al. (2017) reports that immature *Aedes aegypti* infestation was positively associated with total dissolved solids but negatively associated with dissolved oxygen. This is in conformity with the current research as can be seen in Table 2. The highest TDS level was observed in SOT and the same site has the highest percentage of *Aedes aegypti* larvae (Figure 1).

**CONCLUSION**

The findings from this study revealed the presence of *Aedes aegypti* in the studied area. It can also be concluded that physicochemical parameters of *Aedes* mosquito breeding habitats showed significant variations among the three breeding sites. Controlling *Aedes* mosquitoes in artificial breeding habitats such as cans and discarded tyres are critical for controlling diseases associated with *Aedes aegypti*. This study highlighted the importance of vector control interventions especially by eliminating stagnant water which serves as *Ae. Aegypti* breeding sites.

**REFERENCES**


