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Original Article

Influence of Physico-chemical Conditions of Mosquito Breeding Habitats on Bacterial Communities in Minna, Nigeria

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ABSTRACT

Physico-chemical characteristics are key determinants of the occurrence, abundance, and distribution of bacterial communities in mosquito habitats, varying across habitat types and locations. This study was designed to evaluate the bacterial communities associated with conventional mosquito larval breeding habitats in Minna, Niger State, Nigeria. Water samples were collected from four habitat types (septic tanks, rain pools, rice fields, and drainages) within the city. Bacterial species were isolated from the samples, and the physico-chemical conditions of the habitats were analyzed using standard methods. Eight bacterial species were identified: *Bacillus subtilis*, *Lactobacillus bulgaricus*, *Bacillus licheniformis*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Priestia megaterium*, *Neobacillus niacini*, and *Bacillus lentus*. Apart from temperature (29.06±2.21 °C to 29.88±1.01 °C), all other physico-chemical parameters varied significantly across the habitats, including pH, dissolved oxygen, chemical and biochemical oxygen demand, phosphate and nitrate contents, total alkalinity and hardness, and electrical conductivity. Pearson's correlation analyses revealed several interrelationships among the variables, while focused principal component analysis (FPCA) showed distinct interactions between environmental variables and bacterial species. These findings provide insight into environmental influences on bacterial ecology in mosquito habitats and are relevant for the development and deployment of bacterial larvicides.

Keywords: Bacteria, Focused Principal Component Analyses, Interaction, Habitats, Physico-chemical

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INTRODUCTION

Mosquitoes play a major role in the transmission of human diseases such as malaria, dengue fever, yellow fever, and lymphatic filariasis [1]. Mosquitoes spend most of their immature periods in almost any available water-holding environment [2], and their development is influenced by the prevailing physico-chemical conditions of these habitats. These conditions regulate nutrient availability [3], and the occurrence, abundance, distribution and composition of bacterial communities [4, 8]. Bacterial communities in mosquito habitats, on the other hand, play a major role in larval development by serving as a food source [5] and influencing important adult traits such as longevity and insecticide resistance [6]. Members of these bacterial communities are often toxic to insects and have been used as biological control agents for several mosquito species [7].

The physico-chemical conditions of mosquito habitats are in a continuous state of flux, varying with time, weather condition, habitat types [9],locations [10] and agrochemical inputs [11]). It is therefore understandable that mosquito species and bacterial community profile will also vary with these fluctuations [12, 13]. Several studies have examined how physico-chemical conditions influence mosquito populations [14, 17] and bacterial community composition [15, 16]. However, there is a dearth of information on this subject in Minna, Niger state.

The influence of physico-chemical conditions on the occurrence of bacterial species in the study area is crucial for deploying mosquitocidal agents in larval source management and for developing indigenous bacterial consortia for mosquito control in the region. The present study is therefore designed to elucidate the key determinants of bacterial species abundance, and their species-specific interactions with the physico-chemical parameters in conventional mosquito breeding habitats in Minna, Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out in Minna, the capital of Niger State, North Central Nigeria. Located within longitude 6^0 33' E and latitude 9^0 27 ' N, covering a land area of 88 km^2, it has an estimated human population of 1.2 million. The area has a tropical climate, with mean annual temperature, relative humidity and rainfall of 30.20 ^{0}C, 61.00 % and 1334.00 mm, respectively. The climate presents two distinct seasons: a rainy season between May and October and a dry season (November - April). The vegetation in the area is typically grass dominated savannah with scattered trees [18].

Selection of Mosquito Breeding Habitats

Four (4) breeding habitat types were selected for the study, namely, Rain pools, Rice fields, Drainages, and Septic tanks. Water and bacterial samples were collected from ten representative sites of each habitat type.

Collection of water samples for isolation of bacteria from conventional mosquito breeding habitats

Water samples were collected from the selected mosquito habitats within the study area. Ten (10) replicates of water samples were collected from the randomly selected habitats for isolation of micro-organisms. These were put in 4 L water receptacles, labelled based on date, time, habitat-type and location of collection. The samples were transported to the laboratory of the Department of Microbiology, Federal University of Technology, Minna, for immediate isolation of the bacteria.

Isolation of bacteria from conventional mosquito breeding habitats

Water sample (0.1 mL) from each breeding habitat-type (immature breeding environment) were collected and spread separately on 20 cm diameter plates containing NA, MSA, and EMB agar media separately. Plates were incubated at 30 °C for 1–2 days and examined for bacterial growth and identification, following the methods of Wu et al. [7] and Abalaka et al. [19]. Inoculation of samples were carried out using the streak plate method. A loopful of each sample is taken and streaked onto prepared nutrient agar plates. The agar plates were incubated at 37°C for 24 hours. The plates were then screened for the presence of discrete colonies after incubation.

Isolation of pure bacterial cultures

Characteristically distinct colonies obtained after incubation were sub-cultured onto fresh Maconkey agar plates repeatedly to obtain pure cultures. Pure bacterial isolates obtained were stored on NA slant for identification and further analysis [19].

Phenotypic characterization of the bacterial Isolates

Phenotypic characterization was performed and compared to known reference data as described by Krieg *et al.* [20], including standard Gram staining procedures [19].

Biochemical characterization of the bacterial isolates

Gram staining, Citrate utilization test, Catalase test, Coagulase test, Sugar fermentation test (glucose, lactose and sucrose), Starch hydrolysis test, Indole test, Methyl red test (MR), Voges-Proskaeur's test (VP), Oxidase test, Urease test, and Motility test were employed in the identification system following standard procedures [21].

Collection of water samples from conventional mosquito breeding habitats for physico-chemical analyses

Water samples were collected for determination of the physico-chemical properties of the mosquito breeding habitats. Collection of this sample were done simultaneously as collection of water samples for isolation of micro-organisms and mosquito species. Water samples were labelled based on date, time, location, and habitat type. Collected samples were transported to the laboratory of the Department of Water, Aquaculture and Fisheries Technology (WAFT), Federal University of Technology, Minna, for analyses.

Surface water from mosquito larval breeding sites were collected using 250 mL bottles (for Biological Oxygen Demand (BOD), Dissolved Oxygen (DO) and 1 Litre-capacity rubber containers (for other chemical analyses). The water samples collected for analyses of DO were fixed at the site using Winkler's Method, by adding Reagents A and B. However, water samples for analyses of other physico-chemical parameters were not preserved. Other chemical parameters were determined according to procedures outlined in standard procedures for the examination of water and wastewater [22].

Physico-chemical parameters of the habitats

The physico-chemical parameters were determined following standard procedures of APHA [22]. The water temperature and water pH were measured *in situ* across the habitats in the sampling locations using a Mercury-in-glass Thermometer and a pH meter (Model: Jenway 3305). Other parameters such as Electrical conductivity (μS/cm), Total hardness (mg/L), Dissolved oxygen (mg/L), Total Alkalinity (mg/L), Nitrate and Phosphate Contents (mg/L), Biological (BOD) and Chemical Oxygen Demand (COD) (mg/L) were measured in the laboratory.

Data Analysis

All data from the physico-chemical analyses were curated and cleaned in Microsoft Excel, Version 2019 (Microsoft®, Redmond, WA, USA) and the R statistical environment software Version 4.3.1 [23]. Descriptive statistics (mean, standard deviation, and range) of the physico-chemical variables were computed using the *BiodiversityR* package [24]. The Shapiro-Wilk test was deployed to test for the normality of the data obtained for the physico-chemical variables. For testing homogeneity of variance, Bartlett's test (for normally distributed data) or Fligner-Killeen Test (for non-normally distributed data) was done in the *stats* package. Following these, differences among means of physico-chemical variables among the habitats were analysed using the Kruskal-Wallis test for non-normally distributed data, and analyses of variance (ANOVA) for normally distributed data, as appropriate. The differences among the means were separated statistically using post hoc tests (Ferroni test for non-normally distributed data and Dunn-Bonferroni for normally distributed data). Decisions on the statistical comparison of means were taken at the *p*-value of 0.05 level of significance. Focused principal component analysis (FPCA) in the *psy* package was applied to determine the influence of physico-chemical variables on the abundance of the bacteria species in the habitats [25].

RESULTS

Isolation, characterization, and identification of bacteria species associated with mosquito larvae and conventional breeding habitats

Biochemical screening of the bacterial isolates from mosquito larval samples and conventional mosquito habitats in Minna is shown in Table 1. Analyses of the isolates revealed the presence of eight bacterial isolates. These comprised six Gram-positive and two Gram-negative organisms, and all the isolates were rod-shaped. Biochemical tests further revealed the presence of eight suspected organisms, namely, *Bacillus subtilis, Lactobacillus bulgaricus, B. licheniformis, Klebsiella pneumoniae, Proteus mirabilis, Priestia megaterium, Neobacillus niacini,* and *B. lentus* (Table 1).

Physico-chemical conditions of conventional mosquito breeding habitats

The physico-chemical conditions of the selected mosquito breeding habitats in Minna during the study period are shown in Table 2. All parameters investigated showed significant ($p < 0.05$) differences, except for water temperature, with the mean temperature (29.47±1.65 °C; $p = 0.532$) of the habitats in Minna ranging from 29.06±2.21 °C (in septic tanks) to 29.88±1.01 °C (in drainages) (Table 2).

The mean water pH was 7.54±0.54 and ranged from 7.35 to 7.7 in drainages and rain pools, respectively (Table 2). The water pH of the habitats varied significantly ($p < 0.05$, df = 59) across the locations: drainages ($p = 0.01$), rain pools ($p = 0.012$), septic tanks ($p = 0.017$), and rice fields ($p = 0.012$). The dissolved oxygen (DO) levels (mean = 4.95±2.33 mg/L; $p = 0.00014$) were lowest in drainages (3.37±0.68 mg/L) and highest in septic tanks (6.55±0.15 mg/L) (Table 2).

The levels of chemical oxygen demand (COD) varied significantly ($p = 0.01261$) among the habitats, with a mean value of 94.30±191.71 mg/L; ranging from 44.80±6.69 mg/L in septic tanks to 231.70±90.79 mg/L in drainages (Table 2). Similarly, biochemical oxygen demand (BOD) also differed significantly across habitat types (mean = 44.48 ± 84.65 mg/L; range = 22.51–104.05 mg/L), with significant variation detected in drainages ($p = 0.0091$), rice fields (p = 0.0087), rain pools (p = 0.012), and septic tanks ($p = 0.012$) (Table 2).Nutrient levels, specifically nitrate and phosphate, showed significant differences (p < 0.05) among the habitat types. Phosphate concentrations were highest in septic tanks (1.55 ± 1.34 mg/L) and lowest in rice fields (0.43 ± 0.29 mg/L), with a mean value of 1.08 ± 0.95 mg/L ($p = 0.0037$). Nitrate levels also varied significantly ($p = 0.002$), ranging from 16.64 ± 14.49 mg/L in rain pools to 41.68 ± 23.08 mg/L in drainages, with a mean of 32.42 ± 20.79 mg/L (Table 2). Further analysis using the Kruskal-Wallis test confirmed significant differences in nitrate levels among habitat types: drainages (p = 0.0091), rice fields (p = 0.020), rain pools (p = 0.011), and septic tanks (p = 0.0091).Total alkalinity also varied significantly between habitats (p = 0.014), with values ranging from 205.90 to 280.10 mg/L and a mean of 228.30 ± 74.58 mg/L. Total hardness ranged from 141.40 mg/L in rain pools to 227.10 mg/L in drainages, with a mean value of 190.73 ± 60.55 mg/L, and showed highly significant variation ($p < 0.0001$). Electrical conductivity also differed significantly among habitats ($p < 0.0001$), with a mean of 530.78 ± 222.65 µS/cm, ranging from 373.90 to 756.20 µS/cm (Table 2). Correlation among the indices of physico-chemical characters in mosquito breeding habitats in Minna is shown in Figure 1. Pearson's correlation analyses showed that total hardness level was significantly positively correlated with electrical conductivity ($p < 0.001$; r = 0.746), alkalinity ($p < 0.01$; r = 0.620), nitrate ($p < 0.01$; r = 0.634), BOD ($p < 0.05$; r = 0.458), and phosphate level ($p < 0.05$; r = 0.490) (Figure 1). Additionally, there was a significant positive correlation between BOD and COD ($p < 0.001$; r = 0.998). However, there was a significant negative correlation between DO and COD ($p < 0.05$; r = - 0.557) and between DO and BOD ($p < 0.05$; r = - 0.558) (Figure 1).

Table 1: Biochemical Screening of the Bacterial Isolates from larval samples and conventional mosquito breeding habitats in Minna

Isolates	Gram Stain	Shape	Coagulase	Catalase	Indole	Citrate	H_2S	Voges-Proskauer	Methyl Red	Urease	Lactose	Glucose	Sucrose	Starch hydrolysis	Oxidase	Suspected organism
1	+	rods	-	+	-	+	-	+	-	-	-	+	+	+	+	*Bacillus subtilis*
2	+	rods	-	+	-	+	-	+	-	-	+	+	+	+	-	*Lactobacillus bulgaricus*
3	+	rods	+	+	-	+	-	+	-	+	+	+	+	+	-	*Bacillus licheniformis*
4	-	rods	-	+	-	+	-	+	-	+	+	+	+	+	-	*Klebsiella pneumoniae*
5	-	rods	+	+	-	+	-	-	+	+	-	+	+	+	-	*Proteus mirabilis*
6	+	rods	-	+	-	+	-	-	-	-	+	+	+	+	+	*Bacillus megaterium*
7	+	rods	-	+	-	+	-	-	+	+	+	-	+	+	-	*Bacillus lentus*
8	+	rods	-	+	-	-	-	-	-	+	+	-	+	+	+	*Neobacillus niacini*

Table 2: Physico-Chemical Conditions of selected Mosquito Breeding Habitats in Minna during the Study Period

Parameter	Mean	Range		Degree of Freedom (*df*)	*p*-Value
Water Temperature (°C)	29.47 95 % CI (29.04, 29.89)	29.06 (Septic Tanks)	29.88 (Drainages)		0.53263
pH	7.54 95 % CI (7.40, 7.68)	7.35 (Drainages)	7.7 (Rain Pools)		0.15491
Dissolved Oxygen (mg/L)	4.95 95 % CI (4.35, 5.55)	3.37 (Drainages)	6.55 (Septic Tanks)		0.00014
Chemical Oxygen Demand (mg/L)	94.30 95 % CI (44.78, 143.82)	44.80 (Septic Tanks)	231.70 (Drainages)		0.01261
Biochemical Oxygen Demand (mg/L)	44.48 95 % CI (22.61, 66.35)	22.51 (Rice Fields)	104.05 (Drainages)		0.01526
Phosphate (mg/L)	1.08 95 % CI (0.83, 1.33)	0.43 (Rice Fields)	1.55 (Septic Tanks)	59	0.00368
Nitrate (mg/L)	32.42 95 % CI (27.05, 37.79)	16.64 (Rain Pools)	(41.68) Drainages		0.00184
Total Alkalinity (mg/L)	228.30 95 % CI (209.03, 247.57)	205.90 (Septic Tanks)	280.10 (Drainages)		0.01424
Total Hardness (mg/L)	190.73 95 % CI (175.08, 206.37)	141.40 (Rain Pools)	227.10 (Drainages)		7E – 05
Electrical Conductivity (µS/cm)	530.78 95 % CI (473.26, 588.29)	373.90 (Rain Pools)	756.20 (Drainages)		1.6E – 06

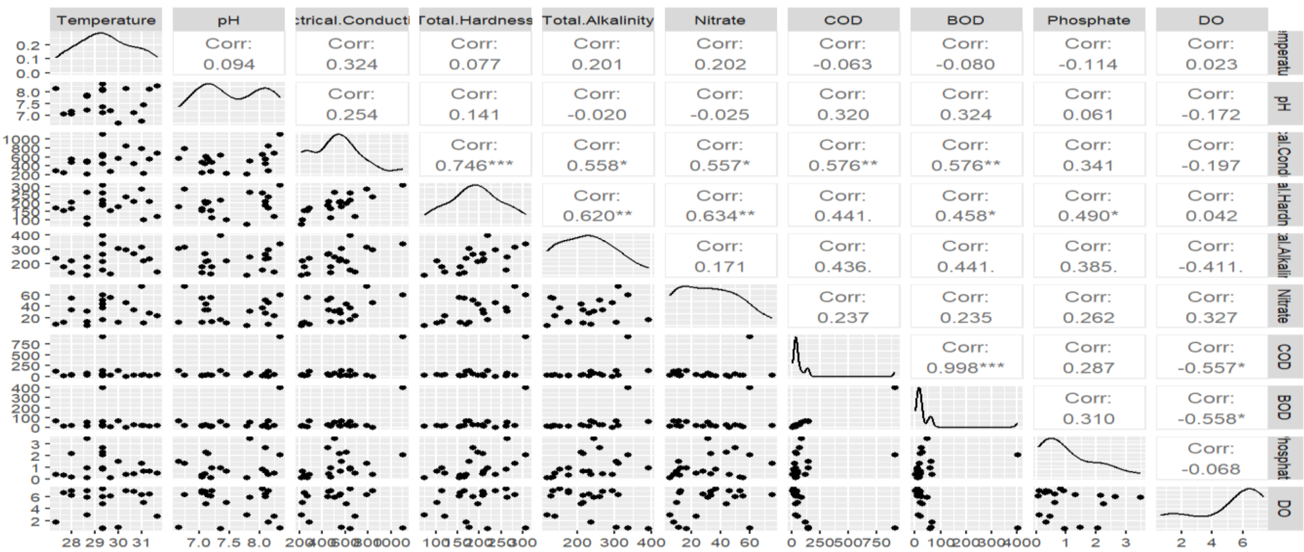

Figure 1: Correlation among the indices of Physico-Chemical characters in mosquito breeding habitats in Minna. COD – Chemical Oxygen Demand, BOD – Biological Oxygen Demand, DO – Dissolved Oxygen. * = 0.05, ** = 0.01, * = 0.001.**

Influence of physico-chemical conditions of conventional mosquito breeding habitats on bacterial communities

The influence of physico-chemical conditions of conventional mosquito breeding habitats on bacterial communities is shown in Figures 2 to 9. Focused Principal Component Analyses (FPCA) showed that most physico-chemical variables showed a positive correlation (green dots) with the abundance of *B. subtilis* in the habitatsThe species' abundance, on the other hand, had a negative correlation (yellow dots) with the nitrate, DO, and pH levels (Figure 2). Electrical conductivity, BOD, COD, and temperature all had a negative (yellow dot) impact on abundance of *L. bulgaricus*, whereas DO, pH, nutrients (nitrate and phosphate), and total hardness all had positive impact on the species' abundance in the habitats (Figure 3).

Temperature, water pH (r = 0.6), and DO all had a significant positive impact on the abundance of *B. licheniformis* (r = 0.8). However, other (yellow dots) variables investigated, particularly COD and BOD, had a negative impact on the species' abundance (Figure 4). The abundance of *K. pneumoniae* in the habitats was positively impacted by most of the physico-chemical factors (green dots), but weakly by temperature, water pH, and total alkalinity (Figure 5).

The abundance of *P. mirabilis* was positively impacted by nitrate levels, DO, and water pH, but negatively and significantly by phosphate levels in the habitats (Figure 6). Except for water pH and DO, the levels of the majority of the physico-chemical variables under investigation had a positive impact on the abundance of *P. megaterium*. Nitrate level and electrical conductivity, however, had an impact on the species' abundance ($p < 0.05$) (Figure 7).

While DO, phosphate level, and total hardness showed a weakly positive correlation with the abundance of the species, total alkalinity, water temperature, and pH had a negative impact on the abundance of *N. niacini* (Figure 8). Water temperature and DO had a negative and weak impact on *Bacillus lentus* abundance, but water pH levels had a strong negative correlation with it. The abundance of the species was positively correlated with every other physico-chemical variable that was examined (Figure 9).

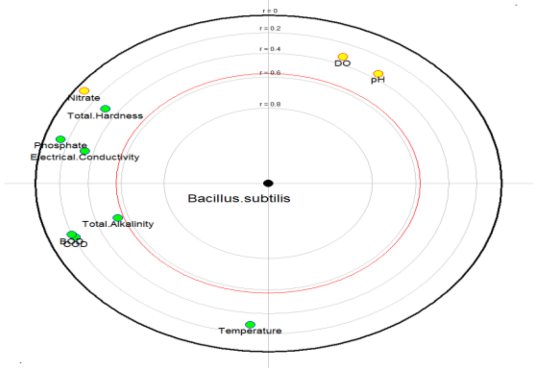

Figure 2: Interaction of *B. subtilis* and physico-chemical conditions of mosquito habitats. COD – Chemical Oxygen Demand, BOD – Biological Oxygen Demand, DO – Dissolved Oxygen

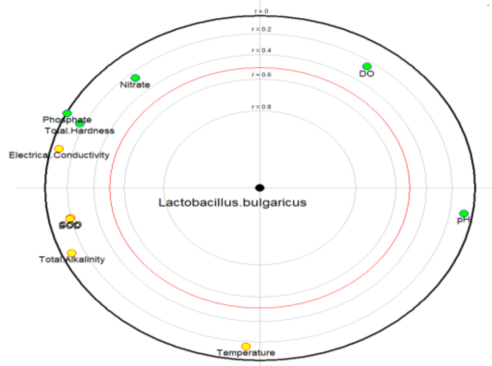

Figure 3: Interaction of *L. bulgaricus* and physico-chemical conditions of mosquito habitats. COD – Chemical Oxygen Demand, BOD – Biological Oxygen Demand, DO – Dissolved Oxygen

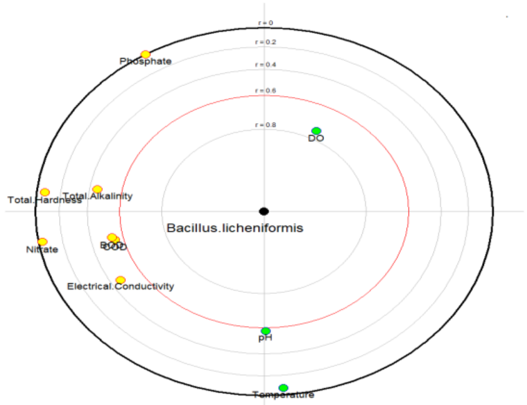

Figure 4: Interaction of *B. licheniformis* and physico-chemical conditions of mosquito habitats. COD – Chemical Oxygen Demand, BOD – Biological Oxygen Demand, DO – Dissolved Oxygen

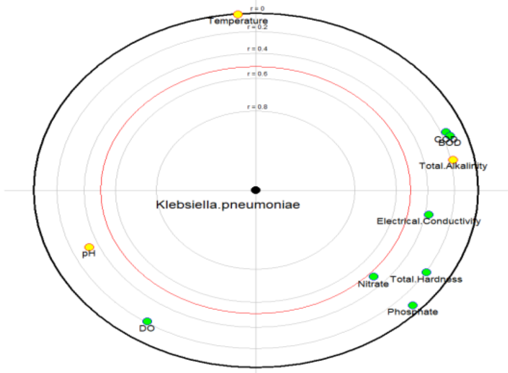

Figure 5: Interaction of *K. pneumoniae* and physico-chemical conditions of mosquito habitats. COD – Chemical Oxygen Demand, BOD – Biological Oxygen Demand, DO – Dissolved Oxygen

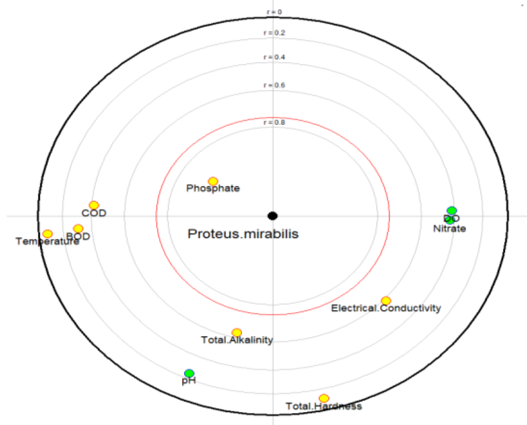

Figure 6: Interaction of *P. mirabilis* and physico-chemical conditions of mosquito habitats. COD – Chemical Oxygen Demand, BOD – Biological Oxygen Demand, DO – Dissolved Oxygen

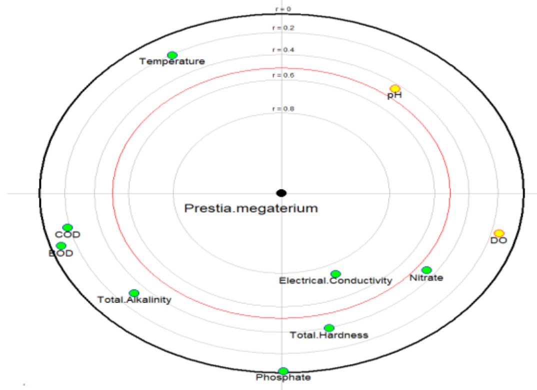

Figure 7: Interaction of *P. megaterium* and physico-chemical conditions of mosquito habitats. COD – Chemical Oxygen Demand, BOD – Biological Oxygen Demand, DO – Dissolved Oxygen

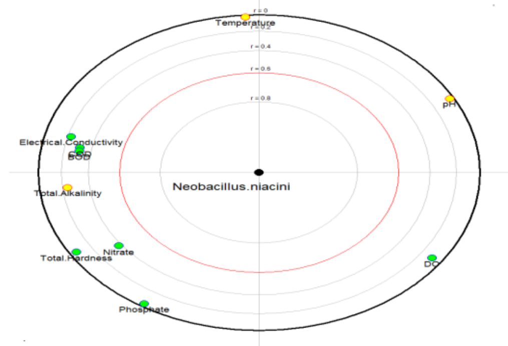

Figure 8: Interaction of *N. niacini* and physico-chemical conditions of mosquito habitats. COD – Chemical Oxygen Demand, BOD – Biological Oxygen Demand, DO – Dissolved Oxygen

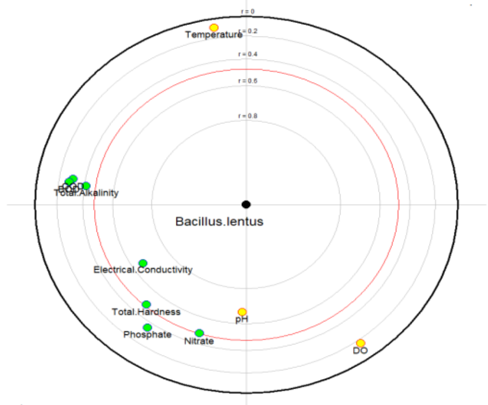

Figure 9: Interaction of *B. lentus* and physico-chemical conditions of mosquito habitats. COD – Chemical Oxygen Demand

Demand, BOD – Biological Oxygen Demand, DO – Dissolved Oxygen

DISCUSSION

Bacterial species composition, and distribution in mosquito larvae and conventional mosquito breeding sites in Minna

Eight bacterial isolates from six genera, four families, three orders, and two classes were found in the current study: *B. subtilis, L. bulgaricus, B. licheniformis, K. pneumoniae, P. mirabilis, Pr. megaterium, N. niacini,* and *B. lentus*. These were lower than those found in a study conducted in Puerto Rico [8] on the bacterial profiles of *Aedes aegypti* mosquitoes (seven classes and 23 families). Additionally, fewer classes of bacteria were isolated in this study than those reported by da Silva *et al.* [16]. These authors identified three different classes of bacteria: Gammaproteobacteria, Firmicutes, and Bacteriales in São Paulo State, Brazil. In the present study, however, Bacillota was the most common phylum.

Physico-chemical conditions of conventional Mosquito Breeding Habitats

The productivity of mosquito habitats in terms of larval mosquito presence and abundance [6] and availability of bacteria food sources [13] determined the quality of any mosquito breeding site [26]. In the present study, analyses revealed that apart from temperature, there were significant differences in the physico-chemical conditions of the mosquito habitats sampled both within habitat types and across the sampling locations.

As a poikilotherm, temperature plays a direct role in the development of immature life stages of mosquitoes [27]. According to Onchuru *et al.* [15], the growth of bacterial species is directly proportional to ambient temperatures. It therefore connotes that temperature may also have an indirect effect on the development of mosquitoes through their effects on bacterial growth; a major source of food for mosquito larvae [5]. These bacteria also produce volatile compounds that influence the oviposition dynamics in mosquitoes [40], and by implication, the productivity of the habitat [28]. In the present study, there were no significant differences in the temperature of the sampled habitats.

The mean temperature of all the habitats in Minna was 29.47 ^{o}C. Further, irrespective of sampling locations, the temperature was lowest in septic tanks (29.06 ^{o}C) and highest in drainages (29.88 ^{o}C). These temperature differences may account for the productivity of these habitats. Further, the temperature ranges recorded in this study are similar to those reported for the development of mosquitoes both in the laboratory [27] and in field studies [32]. These temperature ranges have been reported to support bacterial growth [29]. It therefore means that the conventional mosquito habitats in Minna are excellent for mosquito proliferation. Hence, there is a strong need to control the productivity of these habitats to avert the outbreak of disease in the study area.

Water pH varied significantly across the sampled habitats being lowest in drainages (7.35) and highest in rain pools (7.54). For Minna, the water pH of conventional mosquito breeding habitat had a mean value of 7.54. The pH conditions reported in this study are similar to those earlier reported by Ukubuiwe *et al.* [1] in the study area. According to Afolabi *et al.* [9] and [30], these pH values are within the range of pH values ideal for the growth and survival of mosquitoes in the field and laboratory, respectively.

Nambunga *et al.* [31] reported a strong correlation between the level of hydrogen ions and the availability of nutrients in mosquito breeding habitats; affecting their appeal to gravid female mosquitoes. Bacteria growth is also affected by the prevailing pH level, depending on the species and environment [8]. Earlier, Okoh *et al.* [14] reported a pH range of 7.33 to 8.60 in three Local Government Areas (LGAs) of Ekiti State, Nigeria, and related the absence of mosquitoes in some habitats to the high pH levels. Similarly, Auta *et al.* [2] reported a pH range of 6.00 to 8.00 in three LGAs in Kaduna State, Nigeria.

The Dissolved Oxygen (DO) concentration is the mass of oxygen gas present, in milligrams per litre of water [22], and its values indicate the amount of oxygen readily available for aquatic organisms. According to Kenawy *et al.* [26], most aquatic life may not be readily sustained in water bodies with DO levels below the threshold of 5 mg/L. Apart from anaerobic bacterial species, DO levels also affect the bacterial community structure of mosquito habitats [8]. Mosquitoes, however, rely more on oxygen from the atmosphere than DO as a source of oxygen [32]. In the present study, DO levels differed significantly among habitats and across sampling locations, with a mean DO level of 4.95 mg/L, ranging from 3.37 mg/L in drainages to 6.55 mg/L in septic tanks. The DO concentrations recorded during the study were higher than the minimal (3 mg/L) recommended threshold for aquatic life's sustenance [22]. The study also shows that septic tanks have a high level of DO to support organic food sources of mosquitoes including bacteria. The DO levels reported in this study are higher than that reported by Okoh *et al.* [14] in Ekiti State, Nigeria for mosquito breeding habitats (range = 0.73 to 1.62 mg/L) but lower than that reported in Akure, Ondo State, Nigeria (range = 7.67 – 8.56 mg/L) [32] .

Chemical Oxygen Demand is a measure of the oxygen required to chemically stabilize the organic matter in a water body [11]. In the present study, the COD levels differed significantly among the habitats and across the habitats with a mean value of 94.30 mg/L. The values were lowest in septic tanks (44.80 mg/L) and highest in drainages (231.70 mg/L). These COD values obtained in the present study were higher than those reported in Northwest Nigeria (6.50 to 3.30 mg/L) [33] and for the study area (2.23 to 14.85 mg/L) in an earlier study [1]. The higher values obtained in this study could be due to the difference in sampling location and season of sample collection.

Biological oxygen demand is defined as the amount of oxygen required by bacteria while stabilizing decomposable organic matter under aerobic conditions. It is the amount of oxygen required by aerobic bacteria to decompose organic matter at a standard temperature of 20^{o}C for 5 days [22]. In the present study, the mosquito habitats in Minna had a mean BOD value of 44.48 mg/L, with as low a value as 22.51 mg/L and as high as 104.05 mg/L. Analyses also revealed significant differences in the BOD levels of the habitats sampled.

The study also revealed a strong negative correlation between DO and COD, on the one hand, and between DO and BOD, on the other hand. The BOD and COD levels were, however, reported to be significantly positively correlated. Higher BOD values connote higher levels of organic content of a water body [34]. Spatial variations in organic contents could have accounted for the difference in values recorded across the sampled habitats and locations in this study. A lower BOD level (29.60 to 40.20 mg/L) in Batagarawa town in Northwest Nigeria [33] while a higher BOD level (140 mg/L) in Khoshk River in Shiraz, Fars Province, Iran [35]. Ukubuiwe *et al.* [1] earlier reported a BOD level range of 4.29 to 8.23 mg/L in the study area.

Nitrate is a nutrient needed by all aquatic plants and animals to build protein. It is released into the ecosystem through the decomposition of dead plants and animals, excretions of living animals, fertilizers, and agricultural run-offs [12]. Excess nitrate levels increase plant growth and decay, promote bacterial decomposition, and therefore, decrease the amount of oxygen available in the water [36]. According to APHA [22], the natural levels of nitrate are usually less than 1 mg/L. In the present study, nitrate levels were significantly different among the habitats, and across the sampling locations, being lowest in rain pools (16.64 mg/L) and highest in drainages (41.68 mg/L). The high value in drainages may be a deposit of organic wastes through human actions or rainfalls. The nitrate level reported in this study is higher than that reported by El Ela *et al.* [3] in El-Fayoum Governorate, Egypt but lower than that reported by Ukubuiwe *et al.* [1] in the study area.

Phosphate is a nutrient needed for plant and animal growth and is also a fundamental element in metabolic reactions. High levels of this nutrient can lead to overgrowth of plants, increased bacterial activity, and decreased dissolved oxygen levels. Phosphate comes from several sources including human and animal waste, industrial pollution, and agricultural run-offs [17]. In the present study, phosphate levels were significantly different among the habitats across the locations, being highest in septic tanks (1.55 mg/L) and lowest in rice fields (0.43 mg/L). The phosphate levels obtained in this study are lower than those reported earlier for the study area by Ukubuiwe *et al.* [1] (with range = 13.98 to 75.84 mg/L). These higher values could be due to the difference in time of study.

The alkalinity or the buffering capacity of a water body refers to how well it can neutralize acidic pollution and resist changes in pH [22]. It measures the quantity of alkaline compounds such as carbonates, bicarbonates and hydroxides in the water. In the present study, total alkalinity levels were significantly different among the habitats and across the sampling stations, with a mean value of 228.30 mg/L (range = 205.90 to 280 mg/L). The alkalinity level reported in this study is higher than that reported in an industrial site of the Hooghly River in India (156.07 mg/L) [29] but lower than that reported in Chamran School in Shiraz, Iran [35].

Total hardness is an indication of the presence of some mineral contents like ions of calcium, magnesium, chloride, sulphate, carbonate, and hydrogen carbonates. These minerals are important in the normal physiology of aquatic organisms including mosquitoes. Therefore, the level of hardness of water can determine the productivity of a habitat and thus mosquito abundance [29]. The present study also revealed a significant positive relationship between total hardness level and electrical conductivity, alkalinity, nitrate, BOD, and phosphate levels. This implies that as total hardness increases, the levels of EC, alkalinity, nitrate, BOD and phosphate also increase. These could be due to the increased levels of ions present in the water.

In the present study, the total hardness level across the habitats was lowest in rain pools (141.40 mg/L) and highest in drainages (227.10 mg/L). The mean hardness level in the drainages sampled in Minna was 190.73 mg/L. Analyses also revealed significant differences in the total hardness levels of all the habitats sampled. The hardness level reported in this study is higher (range = 71.37 to 114.62 mg/L) than that reported in West Bengal [29] but lower than that reported in Chamran, Fars Province (700 mg/L) [35].

Electrical conductivity (EC) is a measure of the ability of water to carry an electrical current, and it depends mainly on the presence of anions and cations in water and depends on mobility, valence of ions and temperature. It also signifies the amount of total dissolved salts, which regulate the flow of water in and out of organisms' cells and are building blocks of the molecules necessary for life [4]. In the present study, EC levels were significantly different among the habitats and across the sampling stations with a mean value of 530.78 µS/cm. The EC level reported in this study is higher than that reported mosquito habitats in Bohicon and Parakou, Benin (308.71 µS/cm) [4] and in Niger Delta University (203.67 µS/cm) [10] but lower than that reported in El-Fayoum Governorate, Egypt (8,400 µS/cm) [3].

Influence of physico-chemical conditions of conventional mosquito breeding habitats on relative abundance of bacterial species

The relative abundance of bacterial species as food materials and as oviposition attractants plays a major role in the productivity of mosquito habitats [4]. Further, the relative abundance and distribution of microorganisms are affected by the prevailing physico-chemical conditions of the surrounding media. The bacterial density in Lancang River China have been positively correlated with the levels of EC and TDS [37]. The present study revealed that the relative abundance of the isolated bacterial species was dependent on different physico-chemical parameters.

In the present study, the abundance of *Bacillus subtilis,* for example, was affected negatively by nitrate and DO levels in the mosquito habitats. Similarly, the abundance of *L. bulgaricus* and *P. mirabilis* were negatively affected by phosphate levels. The abundance of *B. licheniformis* and *K. pneumoniae* was negatively affected by dissolved oxygen, while high pH and total alkalinity levels reduced the abundance of both species. Temperature was a significant positive predictor of the abundance of *K. pneumoniae, P. mirabilis,* and *P. megaterium,* while negatively affecting the abundance of *N. niacini*. Nutrient (phosphate and nitrate) levels were positive predictors of the abundance of *P. mirabilis* and *B. lentus.*

The relative abundance of *Streptococcus* and *Escherichia coli* were influenced by dissolved oxygen and total dissolved solids [29]. The levels of pH, water temperature, and inorganic nutrient were strong determinants of the abundance of the most prevalent bacterial species in the Vaal River, South Africa [38]. In the Benin Republic, a strong correlation was noted between bacterial coliform and mosquito abundance [4]. Likewise, in China, the abundance of Alphaproteobacteria, Actinobacteria, and Flavobacteria in a pond water was strongly affected by the nitrate and phosphate levels [39].

Conclusion

This study, thus, revealed different forms of interactions between the physico-chemical conditions of mosquito habitat and bacterial species. Future studies are recommended to investigate the relationship between these species and mosquito larval abundance .

Declarations

Authors Contribution

UAC, AME, ANU, and OIK conceived and designed the experiments. UAC, OIF, UCC, and LS performed the experiments. ACU, and OIF analysed the data. UCC, and LS wrote the first draft of the manuscript. AME, ANU and OIK corrected the draft copy. All AUTHORS agreed to the final state of the manuscript

Disclosure of Conflict of Interests

The authors declare that they have no competing interests. This study was funded by a grant from TETFund (Institutional Based Research). However, the funder has not influenced the results or decision to publish.

Ethics Approval and Informed Consent

This study did not use human or animal subjects. Therefore, ethical consideration was not applicable.

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REFERENCES

1. Ukubuiwe, A. C., Abioye, J. O. K., Olayemi, I. K., Arimoro, F. O., Abolarinwa, S. O., Ukubuiwe, C. C., Mustapha, O. M., Sule, B. U., Aina, A. O., & Bala, E. (2024). Occurrence and Distribution of *Culex* Mosquito Larvae in relation to Breeding Habitat Characteristics in Four Communities in Minna, North-Central, Nigeria. *International Journal of Tropical Insect Science,* **44(2)**,. https://doi.org/10.1007/s42690-024-01399-y.

2. Auta, K. I., Oguche, O. D., Ibrahim, B., Yayock, H. C., & Dadi-Mamud, N. J. (2020). The physico-chemical Parameters associated with Mosquito Abundance in Breeding sites of Selected Locations within Three Local Government Area of Kaduna State, Nigeria. *Journal of Natural and Applied Sciences*, **9(1),** 8 – 20.

3. El Ela, A. A. A., Mostafa, A., Ahmed, E. A., Khalil, A., Ghonaim, M. & Ahmed, A. M. (2023). Mosquito abundance and physicochemical characteristics of their breeding water in El-Fayoum Governorate, Egypt. *Journal of King Saud University – Science*, **36**, 103040. https://doi.org/10.1016/j.jksus.2023.103040

4. Hessou-Djossou, D., Djegbe, I., Boukari, M. K. Y. G., Nonfodji, O., Tchigossou, G., Djouaka, R., Cornelie, S., Akogbeto, M., Djogbenou, L., & Chandre, F. (2024). Influence of physico-chemical and microbiological parameters of mosquito larval habitats on the species composition and insecticide resistance of *Anopheles gambiae sensu lacto* in the cities of Bohicon and Parakou in Benin. *International Journal of Biological and Chemical Sciences,* **18(3)**, 737-753

5. Merritt, R.W., Dadd, R. H. & Walker, E. D. (1992) Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual Review of Entomology* **37**, 349–374.

6. Kahamba, N. F., Limwagu1, A. J., Mapua, S. A., Msugupakulya, B. J., Msaky, D. S., Kaindoa, E. W., Ngowo, H. S. & Okumu, F. O. (2020). Habitat characteristics and insecticide susceptibility of *Aedes aegypti* in the Ifakara area, south-eastern

Tanzania. *Parasites and Vectors,* **13(53)** .https://doi.org/10.1186/s13071-020-3920-y

7. Wu, J.,Wei, L., He, J., Fu, K., Li, X., Jia, L.,Wang, R., & Zhang,W. (2021). Characterization of a Novel *Bacillus thuringiensis* Toxin Active against *Aedes aegypti* Larvae. *Acta Tropica*, **223**, 106088.

8. Caragata, E.P., Otero, L. M., Tikhe, C. V. , Barrera, R., & Dimopoulos, G. (2022). Microbial Diversity of Adult *Aedes aegypti* and water collected from different Mosquito Aquatic Habitats in Puerto Rico. *Microbial Ecology,* **83(1)**, 182 - 201, https://doi.org/10.1007/s00248-021-01743-6

9. Afolabi, O. J., Ndams, I. S., Mbah, C. E. & Kogi, E. (2010). The effects of alteration of pH on the breeding characteristics of mosquitoes in phytotelmata in Ahmadu Bello Zaria, Nigeria. *International Journal of Bioscience*, **5(1)**, 32-36.

10. Amawulu, E., Commander, T., & Amaebi, A. (2020). Effect of physicochemical parameters on mosquito larva population in the Niger Delta University Campuses, Bayelsa State, Nigeria. *International Journal of Zoological Research*, **16(2)**, 63-68.

11. Salihu, I. M., Olayemi, I. K., Ukubuiwe, A. C., Garba, Y., Gusau, A. Y., Nma-Etsu, M. and Usman, M. D. (2017). Influence of Agro-chemical Inputs on Suitability of Physicochemical Conditions of Rice-Fields for Mosquito Breeding in Minna, Nigeria. *Journal of Mosquito Research,* **7(17)**, 134 -141.

12. Kaur, M., Li, J., Zhang, P., Yang, H., Wang, L. & Xu, M. (2022) Agricultural soil physico-chemical parameters and microbial abundance and diversity under long-run farming practices: A greenhouse study. *Frontiers in Ecology and Evolution.* **10**, 1026771. doi: 10.3389/fevo.2022.1026771

13. Medeiros, M., Seabourn, P. S., Rollins, R. L., & Yoneishi, N. M. (2022). Mosquito microbiome diversity varies along a landscape-scale moisture gradient. *Microbial Ecology*, **84**, 893–900

14. Okoh, H. I., Adeogun, A. O., Omotayo, A. I., Olawumi, H. B., Afolayan, C. B., Mogaji, H. O., Olakiigbe, A. K., Oyeniyi, T. A. & Awe, O. B. (2022). Physicochemical Parameters of Anopheles Mosquitoes Breeding and Non-Breeding Habitats in Ekiti State, Nigeria. *Pan African Journal of Life Sciences*, **6(1)**, 412 – 418.

15. Onchuru, T. O., Ajamma, Y. U., Burugu, M., Kaltenpoth, M., Masiga, D., & Villinger, J. (2016). Chemical parameters and bacterial communities associated with larval habitats of *Anopheles*, *Culex* and *Aedes* mosquitoes (Diptera: Culicidae) in western Kenya *International Journal of Tropical Insect Science* **36**, 3, 146–160

16. da Silva, H., Oliveira, T.M.P., and Sallum, M.A.M. (2022). Bacterial Community Diversity and Bacterial Interaction Network in Eight Mosquito Species. *Genes*, **13**, 2052. https://doi.org/10.3390/ genes1311205

17. Olayemi, I. K., Omalu, I. C. J., Famotele, O. I., Shegna, S. P., & Idris, B. (2010). Distribution of mosquito larvae in relation to physico-chemical characteristics of breeding habitats

in Minna, North Central Nigeria. *Reviews in Infection*, **1(1)**, 49-53.

18. Ukubuiwe, A. C., Olayemi, I. K., Omalu, I. C. J., Jibrin, A. I., & Oyibo-Usman, K. A (2013). Molecular Bases of reproductive and Vectorial Fitness of *Culex pipiens pipiens* (Diptera: Culicidae) Mosquito populations, for the transmission of Filariasis in North Central Nigeria. *Journal of Medical Sciences,* pp 1-7. JMS (ISSN 1682-4474)/DOI:10.3923/jms.2013

19. Abalaka, M. E., Daniyan, S.Y, Adeyemo, S. O., & Abdulsalam, R. (2013). Comparative Studies on Microbiological and Nutritional Qualities of Juice Produced from Pineapple. *Journal of Biology and today's world*, **2(1)**, 10-20

20. Krieg, N. R. Staley, J. T., Brown. D. r., hedlund, B. P., Paster, B. J., Ward, N. L., Ludwig, W., & Whitman, W. B. (2010). *Bergey's Manual of systematic Bacteriology*. 2nd Ed. Vol. Four, Springer New York Dordrecht Hiedelberg London. ISBN: 978-0-387-95042-6. Pages 1- 926.

21. Varghese, N. & Joy, P. P. (2014). Microbiology Laboratory Manual. *Kerala Agricultural Research University Press.* Page 1 – 78.

22. American Public Health Association, APHA, (2017). *Standard Methods for the Examination of Water and Waste water* (23rd Ed.). Washington, D.C.

23. R Core Team (2023). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. *https://www.R-project.org/* accessed 12.05.23

24. Kindt, R. & Coe, R. (2005). *Tree Diversity Analysis. A manual and software for common Statistical Methods and Biodiversity Studies. World Agroforestry Centre (ICRAF): Nairobi*

25. Falissard, B. (2022). *psy: Various Procedures used in Psychometrics R package version 1.2.* http://cran.r-project.org/package=psy accessed 12.05.23

26. Kenawy, M. A., Ammar, S. E., & Abdel-Rahman, H. A. (2013). Physicochemical characteristics of the mosquito breeding water in two urban areas of Cairo Governorate, Egypt. *Journal of Entomological and Acarological Research,* **45**, 96.

27. Ukubuiwe, A. C., Olayemi I. K., Omalu, I. C. J., Arimoro, F. O., Baba, B. M., Ukubuiwe, C. C., Odeyemi, M. O., & Adeniyi, K. A. (2018). Influence of Rearing-water Temperature on Life Stages' Vector Attributes, Distribution and Utilization of Metabolic Reserves in *Culex quinquefasciatus* (Diptera: Culicidae): Implications for Disease Transmission and Vector Control. *The Journal of Basic and Applied Zoology,* **79(32)**, 1-15. https://doi:10.1186/s41936-018-0045-3

28. Ponnusamy, L., Wesson, D. M., Arellano, C., Schal, C. & Apperson, C. S. (2010). Species composition of bacterial communities influences attraction of mosquitoes to experimental plant infusions. *Microbial Ecology* **59**, 158–173. doi: 10.1007/s00248-009-9565-1.

29. Basu, S., Banerjee, T., Manna, P., Bhattacharyya, B., & Guha, B. (2013). Influence of Physicochemical Parameters on the

Abundance of Coliform Bacteria in an Industrial Site of the Hooghly River, India. *Proceedings of the Zoological Society,* **66(1)**, 20–26 DOI 10.1007/s12595-012-0054-z

30. Ukubuiwe, A. C., Ojianwuna, C. C., Olayemi, I. K, Arimoro, F. O., & Ukubuiwe, C. C. (2020). Quantifying the roles of Water pH and Hardness Levels in Development and Biological Fitness in *Culex quinquefasciatus* (Diptera: Culicidae). *The Journal of Basic and Applied Zoology,* **81**, 5. Doi.org/10.1186/s41936-020-0139-6.

31. Nambunga, I. H., Ngowo, H. S., Mapua, S. A., Hape, E. E., Msugupakulya, B. J., Msaky, D. S., Mhumbira, N. T., Mchwembo, K. R., Tamayamali, G. Z., Mlembe, S. V., Njalambaha, R. M., Lwetoijera, D. W., Finda, M. F., Govella, N. J., MatokeMuhia, D., Kaindoa, E. W., & Okumu, F. O. (2020). Aquatic habitats of the malaria vector, *Anopheles funestus* in rural southeastern Tanzania. *Malaria Journal,* **19(219)**. https://doi.org/10.1 186/s12936-020-03295-5

32. Akeju, A. V., Olusi, T. A. & Simon-Oke, I. A. (2022). Effect of physicochemical parameters on *Anopheles* mosquitoes larval composition in Akure North Local Government area of Ondo State, Nigeria. *The Journal of Basic and Applied Zoology,* **83**,84. https://doi.org/10.1186/s41936-022-00298-3.

33. Lawal, N., Idoko, A. S., Abdullahi, H., Jibiya, S. A., Ibrahim, N., Osibemhe, M., & Imam, A. A. (2022) Assessment of physico-chemical characteristics of mosquito breeding sites in Northwest Nigeria. *International Journal of Mosquito Research,* **9(1)**, 132-138

34. Ayawei, N. & Bennetta, V. (2022). Investigation of Biochemical Oxygen Demand (BOD) in Ntanwoba Creek using Dilution Method. *Research and Reviews: Journal of Chemistry,* **11(5)**, 1 – 5.

35. Soltan-Alinejad, P., Bahrami, S., Keshavarzi, D., Shahriari-Namadi, M., Hosseinpour, A., & Soltani, A. (2023). Physicochemical characteristics of larval habitats and biodiversity of mosquitoes in one of the most important metropolises of southern Iran. *Heliyon,* 9, e22754. doi.org/10.1016/j.heliyon.2023.e22754

36. Abuelhaded, K., El Howeity, M. A., Nofal, A. M. & Zanoun, G. H (2022). Optimization of the Growth Condition and Molecular Identification of Two Bacterial Isolates from Egyptian Agricultural Wastes. *Acta Scientific Microbiology,* **5(9)**, 73-79.

37. Luo, X., Xiang, X., Huang, G., Song, X., Wang, P., & Fu, K. (2019). Bacterial Abundance and Physicochemical Characteristics of Water and Sediment Associated with Hydroelectric Dam on the Lancang River China. *International Journal of Environmental Research and Public Health*, **16,** 2031; doi:10.3390/ijerph16112031

38. Jordaan, K. & Bezuidenhout, C. C. (2013). The impact of physico-chemical water quality parameters on bacterial diversity in the Vaal River, South Africa. *A paper was originally presented at the 2012 Water Institute of Southern Africa (WISA) Biennial Conference, Cape Town.* **39(3)**, 385 – 396. doi.org/10.4314/wsa.v39i3.7

39. Sun, F., Wang, C. & Yang, H. (2021). Physicochemical Factors Drive Bacterial Communities in an Aquaculture Environment. *Frontiers in Environmental Science,* **9**, 709541. doi: 10.3389/fenvs.2021.709541

40. Sumba, L. A., Guda, T. O., Deng, A. L., Hassanali, A., Beier, J. C. & Knols, B. G. J. (2004). Mediation of oviposition site selection in the African malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) by semiochemicals of microbial origin. *International Journal of Tropical Insect Science,* **24**, 260–265. doi: 10.1079/IJT200433.