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SEASONAL ASSESSMENT OF PUPAL HABITAT PRODUCTIVITY OF MALARIA VECTOR: *Anopheles gambiae* s.l AS INFLUENCE BY PHYSICO-CHEMICAL CONDITIONS AT SELECTED BREEDING HABITATS IN NIGER, NIGERIA

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Abstract

Malaria is the most significant protozoan disease in Africa and the principal vector-borne disease (VBD) in Nigeria, which is influenced by the quality of breeding habitats that are reflected through the stage preceding adult. Control of *Anopheles gambiae* s.l. populations through source reduction is still considered the most effective way of prevention and control, although it has proven unsustainable in Niger State. Physico-chemical cues were investigated. Samples were taken inside and outside 1 m^2 cages weekly by dipping and emptying the cages from May 2019 to March 2020. The data subjected to analysis of variance (one and two-way Anova). The findings revealed the mean pupae abundance (MPA), were significantly higher in Large Water bodies (624.50±217.81), and followed by Gutters (436.00±184.2) and Swamps (285.50±125.06). The mean pupae productivity (MPP), followed the order of descending rate>GT (717.50±219.38)>LW (677.21±145.10)>SW (530.40±136.97). The result also showed that emptying technique (ET) was more sufficient and reliable than dipping technique (DT). The peak abundance and productivity of the pupal stage was June to August then declined in March,2020 both habitats. However, MPP differed significantly ($p<0.05$) from one another across the months in all the habitat types. The physical and chemical cues of the breeding sites, varied significantly, except in temperature, total hardness, biochemical oxygen demands, conductivity, and pH in all the habitats. This study revealed high utilization of physico-chemical properties and poses increased risk of malaria. Thus, emphasis on the vector management strategies should be given specially on gutters and large water bodies as breeding habitats of malaria vectors (MV), in Niger State.

Keywords: Pupae, density, Malaria, Niger, Nigeria, Vector borne diseases, Anopheles gambiae s.l, Physico-chemical, habitats, cages.

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1. Introduction

In public health, the term "vector" refers to any organisms that can communicate infectious diseases to mankind and other living organisms. In Africa, particularly Nigeria, malaria is the most dreadful disease and the primary vector that play vital role for its transmission to humans is *Anopheles gambiae* s.l. [1]. In African, female *An. gambiae* s.l is the most stubborn vector that harass all people with tremendous effects specifically in under-age children due to their low immune response, most recent, *An. stephensi* (An Asian malaria vector) was discovered in the northern part of Nigeria, also in Niger State, *An. rufipes* (Neocilia group) was discovered in 2019 [2] however, these two different malaria vectors are not widely distributed [2, 3]. In the physiological process, malaria vectors ingest the pathogen (i.e., *Plasmodium* species) that causes disease while feeding on the blood of a diseased person and later transmit it to others, due to the lack of effective vaccines or specific pharmacological drugs dead occurs. Globally, Malaria constitute 67 % deaths most especially on children under the age of five and [1], and reported in 2022, that there were an estimated 241 3 million malaria cases and 627,000 deaths in 2020 [4], thus, declared mosquitoes as "public enemy number one" [5].

In sub-Saharan Africa, this disease is the cause of morbidity and mortality and contributes to over 94 % of global cases [6], with Nigeria accounted for 25 % of the global malaria burden [7]. This curable, disease due to prompt diagnosis received treatment by only 22 % patients with symptoms within 24 hours which increases their risk of death. The accumulative number of cases and deaths due to malaria were recorded to be 19 % infection, 55 % infant childhood mortality, 60 % outpatients, and 30 % of hospitalizations across the country and Niger State [8]. As in many mosquitoes, malaria vectors (MV) have four life stages (egg, larva, pupa, and adult), the larval stage (comprising of four instars i.e., L1, L2, L3 and L4), and pupal (stage preceding adult), these stages have unbreakable link with water and have ability to adapt to condition of breeding environments [9]. Collectively, these stages take about 7 to 14 days to complete development however. depend on the species. Pupal productivity of most mosquito species varies with the availability and quality of water and nutrient resources for larvae.

Thus, the habitat type is an important parameter for successful breeding and subsequent emergence as adults. Estimation of the productivity of a given mosquito species and the larval breeding habitat involves the analysis of not only the stability of the adult populaces but also the cues such as (physical and chemical) influencing immature richness and distribution [10]. The factors that are said to impact on the malaria vectors and thus influence malaria transmission are changes in rainfall, temperature, humidity, and physico-chemical parameters (such as dissolved oxygen, carbon dioxide, carbonates, bicarbonates, nitrates, sodium chloride, chlorine, turbidity, conductivity, water depth, pH, etc.) These factors must be manipulated for successful control program for example, a lack of rainfall has been found to make larval and pupal stages of mosquitoes vulnerable to desiccation, except in areas where human-made breeding habitats were available providing opportunities for breeding [11].

Although malaria vectors are found to breed in specific breeding habitats such as swamps, rice fields, wells, ponds, puddles, hoof prints, and both permanent and temporary water collection habitats [12], however cohabitation occurs with different mosquitoes in different breeding habitats. According to [2], that *Anopheline* and *Culicin*e larvae and pupae coexisted and there seems to be some evidence of differential habitat utilization, irrespective of whether the breeding environments were clean, unclean, or polluted. Even habitats with extremely high *Culicine* immature stages proliferation had consistent numbers of *Anopheles*, implying that estimating pupal habitat productivity for the control of specific *Anopheles* habitats must consider all the breeding habitats that allow utilization and adaptability of immature stages of mosquito species that coexist in such habitats for mosquito disease control in endemic areas of Africa [13–15]. Pupae indices are the best estimators of malaria transmission risk, because pupa mortality is minimal compared with larvae mortality. Several pupae indices have been described, including: pupae per household; pupae per person; pupae per hectare; and even more specific ones, such as an index of sexual dimorphism focused on the female pupae [16].

From both epidemiological and health policy perspectives, it is therefore crucial to acquire a clear and comprehensive understanding of the location and distribution of productive breeding habitats, the extent of their pupae productivity, and how they may be affected by household ecological factors [17]. Research into mosquito pupal habitat characteristics and population growth patterns may provide useful information on mosquito population growth trends, species diversity, and breeding locations for the control strategies [18, 19]. According to [9], the ability of adult mosquitoes to transmit disease depends on the features of the individual's immature stages constituting the population. Physico-chemical cues serve as proxy markers to comment on the possible variations among individual mosquitoes, their breeding habitats and their disease transmission potential.

There is death of information on the estimation of pupal density of malaria vectors (MV) using 1 M^2 cage, and the knowledge on status of the breeding habitats as influence by physico-chemical conditions is scares or neglected in Niger State. Since these factors have effect on the life history traits of the pupal stage such as their growth, development and survival, which affect habitat productivity and hence, the transmission of malaria. This research reports an analysis of pupae measurements in the rainy and dry seasons and examines the impact of pupal habitat productivity for trial intervention on *An. gambiae s.l* pupal production in both rainy and dry seasons.

The purpose of the study was to estimate habitat-specific pupal productivity, shades light on impact of cage sampling method and determine the extent to which habitat productivity for *An. gambiae* s.l can be predicted generally in a typical LGA where the study conducted in Niger North-central, Nigeria. The feasibility of a source reduction program could logically be assessed based on the knowledge acquired and further control strategies can be recommended.

2. Materials and Method

2. 1. Description of study areas

Nigeria is a country in West Africa that lies between the Equator and the Greenwich Meridian, roughly between 4° and 14° north latitude and 2° 2' and 14° 30' east longitude. It covers 923,768.5 km^2 and is characterized by undulating topographic relief patterned by valleys formed by its river systems. The southern coastal plains have a mean elevation of around 150 meters above sea level. The northern plains rise to around 600–700 meters, with the Jos Plateau (over 1,500 meters) in the geographic center of Nigeria and the Mambilla plateau (over 2,100 meters) among the mountains on the Cameroon border [20]. The temperature varies depending on the ecological zone, with tropical at the coast (in Humid Forest and Derived savannas) with extremely low and high temperatures of 10 °C and 37 °C, sub-tropical inland (in Derived and Guinea savannas), and semi-arid in the far north (in Sudan and Sahel savannas) with extremely low and high temperatures of 6 °C and 44 °C. Normal monthly temperatures in the Jos and Mambilla plateaus' mid-altitude region range from 21 °C to 25 °C. In the north, annual rainfall varies from 500 mm to 750 mm, while in the south, rainfall ranges from 1,200 mm to over 4000 mm. Nigeria has population of about 200 million people residing in different regions of 36 states including Federal Capital Territory (FGT-Abuja).

Niger State is one of Nigeria's Middle Belt states, located between Longitude 60'33E and Latitude 90'37 N on a land area of 88 km^2 (representing 9.30 % of the country total and area with 85 % arable land), with a population of 3950.249 people as of the 2006 census, however, by 2012, a projected population of around 4.2 million people was recorded, and in 2019, the projected population of Niger State people by National Bureau of Statistics (NBS) may reached 6 million, and by 2023 the projected population will reach 6,783,300 People and distributed among twenty-five local governments.

The state has a mean annual rainfall temperature of 61 %, 30.20 mm, and relative humidity of 1334mm, the region has a tropical climate. Kaduna State and the Federal Capital Territory to its north-east and South East borders, respectively; Zamfara State borders the north, Kebbi State borders the West, Kogi State borders the South, and Kwara State borders the South West; and the Republic of Benin, along Agwara LGA, its North-west border [21]. The climate is divided into two seasons: a wet season from May to October and a dry season from November to April, with annual rainfall varying from 1,100 mm in the north to 1,600 mm in the south. The vegetation in this area is typically grassy savannah with a few scattered trees (**Fig. 1, 2**) [9].

In the sampling breeding habitats of the four local government areas, all aquatic ecosystems were mapped and Bosso, Katcha, Lapai, and Shiroro were among the local government areas. Bosso is located 20 km^2 from the State capital; it has coverage area of 1,592 km^2 and estimated population of 180,094, and host sampling sites of Shango, Bosso dam, and Maikunkele. Katcha is 143 km^2 from the State capital with a land cover area of 1,681 km^2 with estimated population of 149,317 people, and host Tako kure, Efukyadya, and Tkogbako sampling sites, Lapai is about 71 km^2 from the state capital, it has an area of 3,051 km^2 with an estimated population of 134,591 people, and host sampling site includes Batagi, Akuvera, and Bakajeba while, Shiroro is 69 km^2 from the state capital. It covers an area of 5,015 km^2 with estimated population of 287,698 people it has Jindau, Mutumdaya, and Shieyi sampling sites as describe by [22, 23].

Twelve sampling points within the selected larval habitats (i.e., Gutters, Swamps, and Large water bodies) were pre-sampled and selected based on the indication of the presence of mosquito larvae by dipping technique. Selection was done based on the visual presence of larvae and pupae using a 350 ml dipper [24, 25].

The pre-sampling and selection were made between March and April 2019 before the inundation of the breeding habitats with rainwater at the beginning of the rainy season and was focused on water quality monitoring for mosquito breeding and vector disease distribution in the areas **(Fig. 3)**.

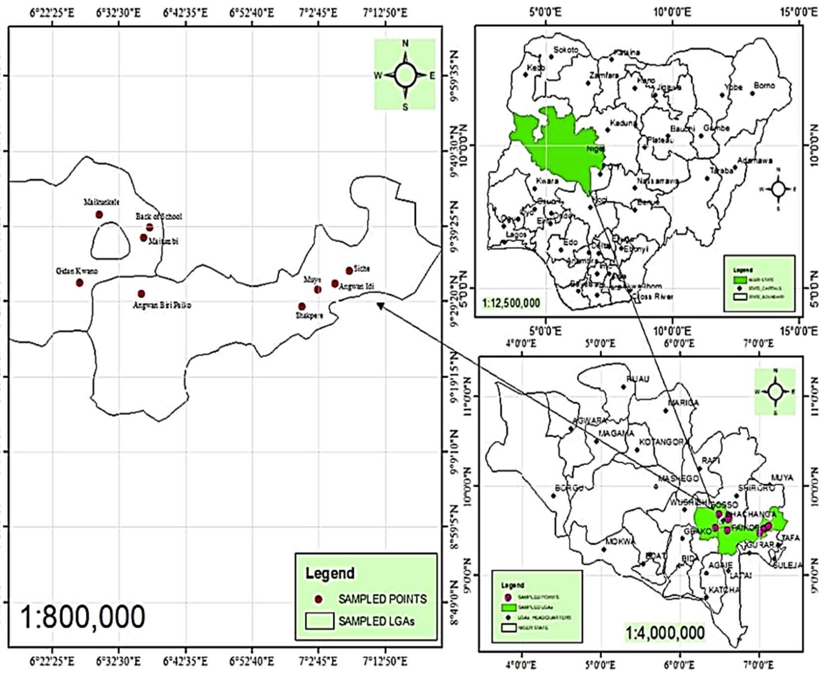

Fig. 1. The map of Nigeria showing geographical position of Niger State

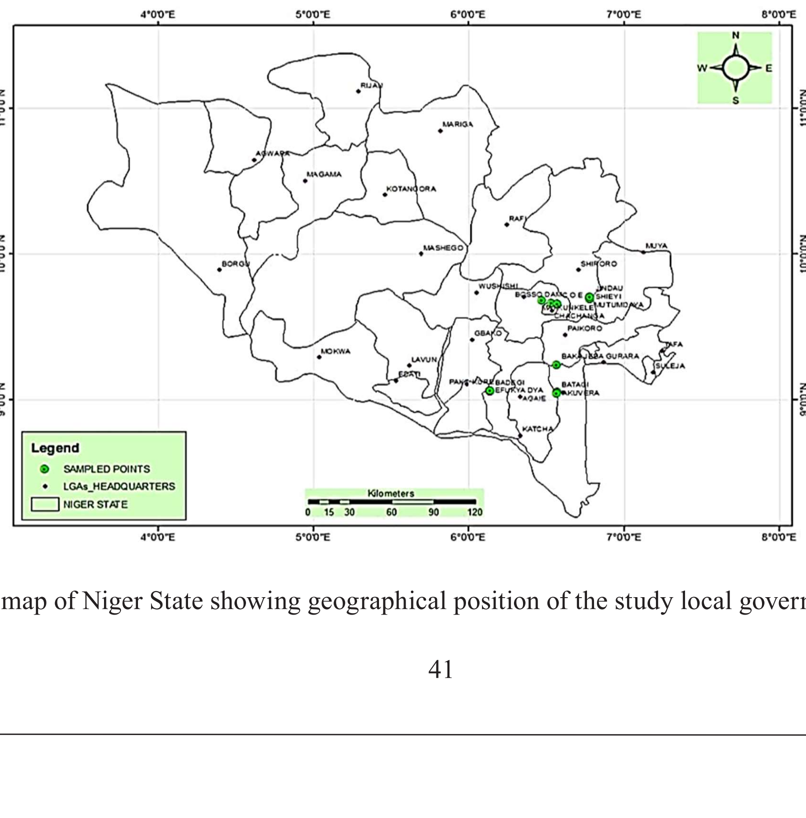

Fig. 2. The map of Niger State showing geographical position of the study local government areas

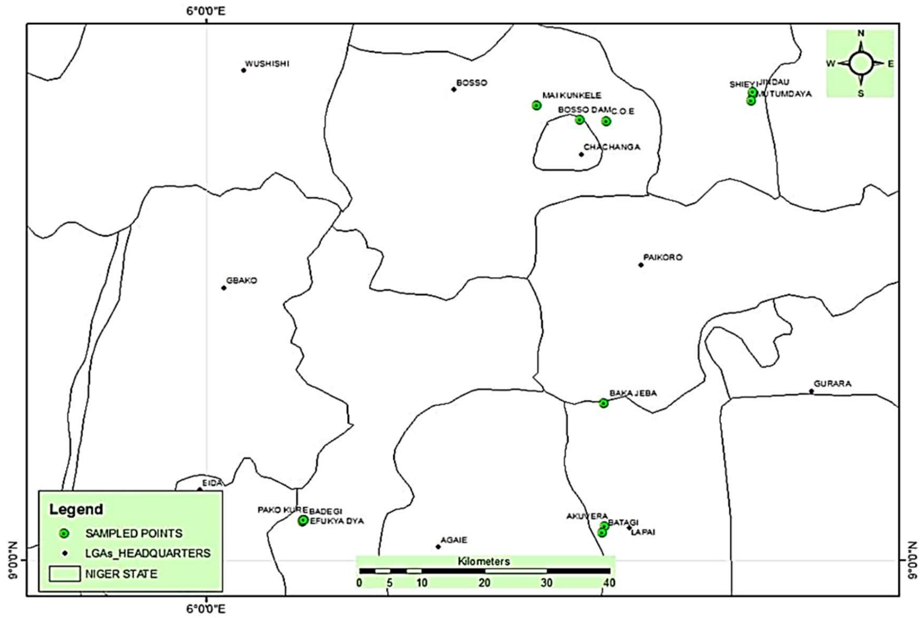

Fig. 3. Map of sampling sites showing the position of the breeding habitats of the studied LGAs

2. 2. Characterization and selection of breeding habitats

This research work had two types of classes of breeding habitats: temporary and semi-permanent breeding habitats. The habitats were characterized based on the nature of their existence and availability. Therefore, temporary larval habitats are those that hold water during the rainy season and dry up around 2–3 weeks after the rainy season ends, while semi-permanent larval habitats may or may not dry up 1 month after the rainy season ends as described by [26]. Gutters and Swamps are temporary larval while large water bodies are the semi-permanent breeding habitats in this study.

2. 3. Experimental design

The study involves field and laboratory works, L1-L4 larvae and pupae were sampled together with larval water both outside and inside the breeding cages by dipping (i.e., outside cages) and by emptying the cages (i.e., inside cages) from the three types of conventional mosquito breeding habitats (i.e., Gutters, Swamps, and Large water bodies) of the study areas. The collected samples of the immature stages of mosquitoes were subjected to laboratory investigations following standard procedures.

2. 4. Field collection of larvae and pupae of mosquitoes

For the outside sampling, dipping process was done at random using a standard pint 350 ml dipper at a rate of 20 per sampling site in the breeding habitat's surface areas where the concentration of larvae and pupae was observed. While, for the inside cages, it was a modified method from [27], and utilized for the fact that it was the only method involving a square meter instrument, and done between 2006–2007, only in Kenya, West Africa. 72 experimental cages each measuring 1×1 (1 m^2) were deployed and distributed among the larval breeding habitat types. Each larval breeding habitat had 6 cages to serve as replicates for the study (i.e., R1, R2, and R3 R4, R5, and R6, respectively), these cages serve to reduce the estimation errors. These cages were constructed from strong woods, covered with a fine untreated netting material (0.05 inch in diameter), and tucked in the soil substrate covering, only a small fraction of the main aquatic habitats material tucked in the soil to ensure that there is no movement of the mosquito larvae in and out of the cages [28]. The distribution of the sampling cages in some of the selected study sites and total number of cages used in all the breeding habitats were presented in **(Table 1, Fig. 4).**

Each pressed sampling cage in each breeding habitat was unzipped to allow mosquitoes from the main habitat to enter for breeding. Every day, the sampling cages were checked for live mosquito larvae and pupae, for 6 days, and on the seventh day, the cages were gently raised and removed. The gathered mosquito larvae and pupae of each sampling cage of the habitats were collected in a large water bath containing clean water from the breeding sites and a standard dipper 350 ml capacity was used to collect the samples as described by [29]. The live larvae and pupae were sieved and collected with a white plastic spoon. The cages were then re-used in their original placements, and the procedure was repeated at each breeding habitat for the duration of the study. All the sampling activities were carried out during the periods of the seasons starting from May to November 2019 to March, 2020. These immature stages of different mosquitoes were kept in the laboratory for further studies.

Fig. 4. Distribution of experimental cages in gutter, large water and swamp breeding habitats

Table 1

The distribution of 1m^2 cages in the different breeding habitats

	Replicates				
Habitats	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**No of cage**
Gutters	BGT	KGT	LGT	SGT	24
Swamp	BSW	KSW	LSW	SSW	24
Larger water	BLW	KLW	LLW	SLW	24
Total	–	–	–	–	72

Note: B – Bosso, K – Katcha, L – Lapai, S – Shiroro, GT – Gutters, SW – Swamps, LW – large waters

2. 5. The laboratory rearing and identification of immature stages of *Anopheles gambiae* s.l

Certain members of these larval stages (L1-L4) of sampled mosquitoes were preserved into 10 % formalin and categorized macroscopically based on the observation of the placement of the position of the respiratory tube by the larvae in the water. They were most certainly *Culex* if the respiratory tube was narrow, long, and angled at a certain angle to the water surface. Mosquito larvae that drifted horizontally to the water's surface were most likely *Anopheles*. The mosquitoes were then sorted into morph groups in the laboratory and identified to genera level based on visible characteristics such as (e.g., presence or absence of siphon, the position of hair tufts, length of the siphon, arrangement of comb scales and several others) also, life stages were differentiated by the length of the larva of each genus, with the aid of a dissection microscope and guided by morphological keys [30]. In this study, only genus *Anopheles* was considered, and members of the live identified *An. gambiae* s.l larvae strains were reared and fed with 0.32 ml yeast solution separately under laboratory conditions of temperature 24.2 °C and relative humidity 64 % until they emerge to pupae. Male and female pupae were recognized macroscopically based on the observation of their size (usually, the female pupa is larger than the male pupa) [31]. The identified pupae were transferred to formalin sample bottles containing 10 % formalin for preservation and kept in laboratory of Federal University of Technology, Minna.

2. 6. Counting of pupae of *Anopheles gambiae* s.l

On each week of the collection on both methods (i.e., dipping and emptying the breeding cages), pupae were accounted, the mean average was calculated, and the larval habitats productivity rate (i.e., sampled per dipper and per $1M^2$) was estimated in each breeding every month throughout the period of study.

2. 7. Collection of water samples for physico-chemical parameters investigations

Water samples from different study breeding habitats for physico-chemical investigation were collected concurrently with larvae and pupae in dark specimen bottles of 500 ml capacity to guarantee accurate representation. The water sample was fixed using 2 ml manganous sulphate and added into 2 ml potassium hydroxide, and 2 ml potassium iodide was added to the mixture. The fixed samples were taken to the laboratory for additional investigation; however, a mercury thermometer, pH, and conductivity meters were used to determine the water temperature, pH, and conductivity at the larval collection site. One liter of each water sample was investigated for the following physico-chemical parameters in the laboratory; Total dissolved solute, dissolved oxygen, conductivity, nitrate concentration, phosphate, sulphate, carbonate, chlorine, alkalinity, and biochemical oxygen demand as described by [32].

2. 8. Statistical analysis of the data

The cumulative estimate of the productivity rate of pupae in each breeding habitat and each month was finalized at the end of the study periods. SPSS software was used to carry out the statistical analysis (version 23 for windows, SPSS Inc. Chicago II). Different breeding habitats' pupal productivity was analyzed using one-way and two-way analysis of variance (ANOVA). Duncan Multiple Range (DMR) was used to distinguish the means and $p<0.05$ was used to search for a meaningful difference, and the analyzed pupae were converted to plot graphs. For the physico-chemical parameters, One-way analysis of variance was used in the statistical analysis at ($p=0.05$)

3. Result

3. 1. Mosquito pupal occurrence of selected breeding habitats

The monthly pupal occurrence (Pupal per season – P/S) of *Anopheles gambiae* s.l in the three breeding habitats is detailed in **(Fig. 5)**. The highest pupal occurrence in the gutters was recorded in June (2nd) (152.00±93.30 P/S) and the lowest was in December (8th) with the value of 0.00±0.00 P/S. These highest and lowest values differed substantially ($p<0.05$) from one another and the abundance of all other months. However, there was no substantial difference ($p>0.05$) between September (5th) and October (6th) records, but there was significant difference ($p<0.05$) between the values of August (4th), July (3rd) and November (7th) with the later having the least value (14.75±4.40 P/S).

In the swamps, the highest pupal occurrence was recorded in June (2nd) (97.25±68.37 P/S) and the lowest was recorded in March (11th) with the value of 00.00±0.00 P/S. This highest value significantly differed ($p<0.05$) from all other months while the lowest value had no significant difference ($p>0.05$) from December (8th), January (9th) and February (10th) months. The months of May (1st), September (5th) and October 6th) had significant different ($p<0.05$) from one another while, August (4th), and July (3rd) did not differed significantly ($p>0.05$) from one another.

A similar trend was observed in large water bodies, with the highest pupal occurrence recorded in June (2nd) (194.50±98.18 P/S) and the least was recorded in March (11th) (6.75±0.01 P/S). These highest and Lowest values significantly differed ($p<0.05$) from one another and the abundance of all other months. However, May (1st), July (3rd), August (4th), September (5th) and December (8th) significantly differed ($p<0.05$) from one another with later having the least value (21.00±1.12), while October (6th), November (7th), January (9th), and February (10th) had no significant difference (p>0.05) in the abundance.

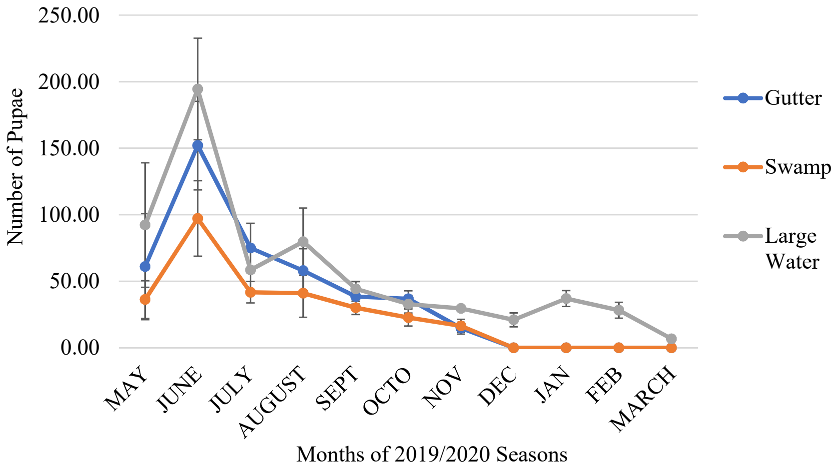

Fig. 5. The pupal abundance of the three mosquito genera of the selected breeding habitats

3. 2 Mosquito pupal productivity of the selected breeding habitats

The monthly pupal productivity (Pupal meter square per season PM^2/S) of *Anopheles gambiae* s.l in the three breeding habitats is detailed in **(Fig. 6**). The results indicated highest pupal production in gutters was obtained in June (2nd) (236.50±46.52 PM^2/S), and the least was recorded in December (8th) (0.00±0.00 P/M^2/S). These values were significantly different (p<0.05) from one another and the production of all other months. The month of May and October had no significant difference (p>0.05) with the latter having a higher record (118.25±66.11 PM^2/S). There was no significant difference (p>0.05) between the records of July (3rd), and August (4th), however, significantly differed (p<0.05) from the month of September (5th). For the swamps, the highest pupae were recorded in June (2nd) with the value of 161.30±48.15 PM^2/S, while the least was recorded in December (8th) (0.00±0.00 P/M^2/S). The highest value significantly differed (p<0.05) from the production of the remaining months.

The months of October (7th) and May (1st) had no significant difference (p>0.05) from one another with the latter having the higher record of 44.15±8.76 PM^2/S. The Months of July (3rd) and September (5th) had no significant difference (p>0.05) from one another but significantly differed (p<0.05) from August (4th) with later having the higher record (106.25±20.36 PM^2/S). In large water bodies, the significant highest pupal production was recorded in June (2nd) (212.00±48.47 PM^2/S) and the lowest was recorded in Marchs (11th) (0.00±0.00 PM^2/S). However, there was a significant difference (p<0.05) in the production of October (6th), May (1st), July (3rd), September (5th), and August (4th) respectively.

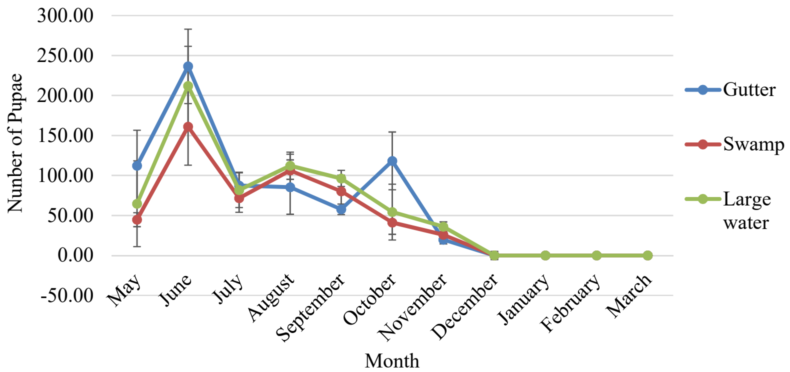

Fig. 6. The Pupal productivity of the three mosquito genera of selected breeding habitats

3. 3. Variation in water physico-chemical characteristics of the selected breeding habitats

The water physical properties investigated among the mosquito breeding habitats are presented in **(Table 2).** The result revealed no significant difference between temperature, total hardness, biochemical oxygen demands, conductivity, pH and all the breeding habitats. However, the dissolve oxygen record in large water bodies significantly differed (p<0.05) from the remaining habitats with the highest value (8.83±0.43 mg/l). The highest temperature (27.24±0.25 °C), total hardness (234.00±52.76 mg/l), and conductivity (284.25±95.73 µS/cm) were recorded in gutters while pH had the highest value (7.91±0.52) recorded in swamps. The least temperature and total hardness were obtained in swamps with the value of 26.98±0.30 °C and 143.00±13.38 mg/l respectively. The lowest conductivity (196.00±66.15 µS/cm) was recorded in Swamps while the lower pH (7.10±0.24) was recorded in gutters. Biochemical oxygen demands were highest (5.13±0.70 mg/l) in large water bodies and lowest in swamps with the value of 3.65±0.47 mg/l.

For water chemical parameters, the result showed that parameters such as nitrate, phosphate, calcium, sulphur, potassium, chlorine, and alkalinity showed no significant difference (P>0.05) between swamps and large water bodies but significantly differed (P<0.005) from gutters, however, gutters had the highest concentration in each of the parameters with values (4.57±0.85, 3.26±0.73, 88.19±12.79, 87.52±20.8, 28.23±2.45, 10.63±0.79, 187.00±0.19 mg/l) respectively. The lowest nitrate (0.21±0.02 mg/l), sulphur (12.07±1.68 mg/l), and potassium (4.18±0.66 mg/l) were recorded in swamps. The lowest phosphate, calcium, and chlorine were recorded in large water bodies with values (0.38±0.06, 23.19±4.79, and 27.91±11.79 mg/l) respectively.

Table 2

Variation in water physico-chemical characteristics of the selected mosquito breeding habitats

	Habitats		
Parameters	**Gutters**	**Swamps**	**Large water bodies**
Temperature (°C)	27.24±0.25^a	26.98±0.30^a	27.01±0.01^a
Total H (mg/l)	234.00±52.76^a	143.00±13.38^a	180.00±36.29^a
Cond (µS/cm)	284.25±95.73^a	196.00±66.15^a	211.75±32.01^a
pH	7.10±0.24^a	7.91±0.52^a	7.56±0.21^a
DO (mg/l)	5.73±0.45^a	6.25± 0.59^a	8.83±0.43^b
BOD (mg/l)	4.68±0.19^a	3.65±0.47^a	5.13±0.70^a
Nitrate (mg/l)	4.57±0.85^b	0.21±0.02^a	0.44±0.10^a
Phosphate (mg/l)	3.26±0.73^b	0.52±0.13^a	0.38±0.06^a
Calcium (mg/l)	88.19±12.79^b	32.93±14.74^a	23.19±4.79^a
Sulphur (mg/l)	28.23±2.45^b	12.07±1.68^a	14.17±0.91^a
Potassium (mg/l)	10.63±0.76^b	4.18±0.66^a	5.03±0.62^a
Chlorine (mg/l)	87.52±20.89^b	46.17±18.43^a	27.91±11.47^a
Alkalinity (mg/l)	187.00±26.39^b	73.00±30.42^a	53.00±9.95^a

Note: values are Means ± Standard Errors of Mean replicates. Values followed by the same superscript(s) along the row are not substantially different (p>0.05)

4. Discussion

Pupal stage represents the final step in metamorphosis of mosquitoes and is the transition from the aquatic, larval stage to the terrestrial, adult form. Estimates of pupal density are therefore, the best proxy measure of adult productivity from natural habitats [33]. Pupal stage of the principal malaria vector; *An. gambiae* s.l., in the north central of Niger State, Nigeria, emerged, from all habitat types investigated although significant variations in their productivity was recorded in which large water bodies had the most production (406.50±0.00 pupae) followed by gutters (388.50±0.00 pupae) and the least was swamps with value of 258.25±0.00 pupae. Existence of such variations in the occurrence and productivity of malaria vectors from habitats greatly emphasizes the need to develop tools that can identify those habitats, which could then be targeted during an LSM operation to make it more cost-effective [34].

To the best of our knowledge, this is the first systematic study of the productivity of pupae of *An. gambiae* s.l. mosquitoes reported in the study locations of Niger State. In this research, malaria density using pupae index was utilized and estimated in both P/S and P/M^2 and the technique involved is comparable to that of [27].

It appears that this productivity may be very specific to certain environmental conditions and hence it cannot be generalized. Therefore, in every target area all habitat types for *Anopheles* pupae would need to be characterized and field team researchers trained on how to identify the habitat types and their individual productivity established for local malaria vectors. Availability and stability of aquatic habitats are very crucial in determining seasonal productivity of malaria vectors. The quality of the larval habitats determines the pupal productivity and adult features of *Anopheles* mosquitoes which in turn are linked to the disease transmission potential [35].

In this study it is equally observed that pupae of *An. gambiae* s.l that occurred in all the habitat types contradict the generalization of [36], that in West Africa *An. gambiae* s.l immature stages develop in freshwater habitats that are small, temporary, clean and sun-exposed is correct, but our results suggest it might mislead those interested in working on larval control of African malaria vectors. [36], himself emphasized, 'unfortunately *An. gambiae* does not lend itself to generalization. This is supported by [37], observation that '*An. gambiae* does not follow the textbook in its habitat selection and it can be found in a wide variety of water bodies' independent of size'. He furthermore highlighted 'that it is difficult to attribute a definite type of breeding place *to An. gambiae* and that this *Anopheline* is likely to breed in almost any water that happens to be available; admittedly, it can be said to show a slight preference for small sunlit pools, but it does not follow that these represent the characteristic breeding place of this mosquito'. Furthermore, work in larger urban settings has indicated that *An. gambiae* can adapt to new habitat types over a relatively short number of years, thus, the flexibility of this species complex and worthy of known [38].

The study conducted by [19], has further confirmed that most of the *Anopheline* breeding habitats have been originated from anthropogenic influence such as irrigation activities and urbanization. This is very possible the fact that, communities of these study locations are mostly farmers at the same times fishermen while, other members engage on local industrial activities. These anthrongenic activities may lead the expansion suitable breeding ground of mosquitoes.

Additionally, [2], reported that the predominant determinant influencing species of mosquito to develop in each aquatic breeding habitat whether it is flowing or not, clean or contaminated, shaded or bright, permanent or temporary is the quantity and quality of such breeding habitats. Vectors of mosquito are common in tropical areas like in Niger State part of Nigeria for the reason that, their richness in the species diversity and potential as well as the abundance of ideal breeding habitats facilitates speedy instar development and high survival rates [39]. There is evidence that larval habitats influence the abundance and density of various vector species, including juveniles, and at the same time diversify and distribute adult mosquito species [40]. Thus, the prevalence of *An. gambiae* s.l pupae in the three aquatic environments corresponded to the spread of malaria in the study locations.

The study conducted by [41], noted the high prevalence of *An. gambiae* in all the dirty habitats such as gutters and domestic runoffs this could be due to the female species' preference for these settings for oviposition. This suggests that the same aquatic environments that are used to control *Culicne* immature stage could also be used to manage *Anopheline* immature stages. According to the earlier study, different mosquitos' immature stages are likely to cohabit in the same environments [42], likewise*, Anopheles* early and late instars were highly correlated at the three habitats.

In agreement with this finding [42] reported that mosquitoes exploited almost all types of aquatic habitats for breeding, and possessed larger adaptability. According to [14], the quality of larval habitats and adaptive features possessed by mosquitoes appear to have an impact on mosquito occurrence and growth, indicating sufficiency for immature stages. This is possible since ecological factors (abiotic and biotic factors) play a role in influencing the population dynamics of larvae and pupae.

For instance, *Anopheline* adapted to polluted habitats such as gutters by changing their behaviour likewise *Culicine* adapt to freshwater larval habitats similarly [41]. However, it is plausible that these habitats have always been able to host larvae and pupae at the same frequency as "cleaner" places. Although in West Africa that *Anophelines* such as *An. gambiae* s.l larvae and pupae develop in freshwater habitats however, in this current study despite there was no comparison with findings in some part of African countries apart from west African countries, focusing alone on fresh water habitats for malaria vectors may not produce the desire results in term of management and control strategies of malaria disease.

Unfortunately, *Anophelines* do not depend on habitat specification, can be found in a wide range of water bodies, regardless of size [43]. The significant highest occurrence of pupae mosquitoes in gutters and large water bodies could be attributed to the modification in the aquatic environment of the habitats due to changes in the habitat's ecosystem and the quality of the breeding habitats that they are found couple with the adaptability of these mosquitoes. Another possible reason was that pupae mosquitoes in both habitats in this current study were able to utilize the breeding habitats because of the adaptive qualities possess in them. According to [42], it has been previously stated that the most common contributing factor to the emergence and spread of infectious disease agents has to do with the changes of habitats such as irrigation systems, wetland, deforestation, road building, and natural resources, flow of water bodies rich in nutrients as well as the ability to with stand any chance that may occur in a given habitat, as far as such habitat can support the development and abundance of mosquitoes [44, 45]. Equally every habitat possesses specific ecological properties that are important to the fate of anti-larval biological control strategies aimed at reducing juvenile mosquito populations they support [46].

In comparison to large water habitats, smaller habitats are more likely to contain fewer mosquito species, sustain smaller populations, and have higher rates of extinction this could be so most especially where predators of immature stages are high, additionally nutrient competition between mosquitoes and non- mosquito immature stages can result in scanty population of these mosquitoes in each breeding habitats. Supporting the claim large water habitats were preferred by all pupae encountered in this study over gutters and swamp habitats. Likewise, the high productivity of large water bodies could be credited to the high amount of organic matter present in the system due to influx from various sources [47], reported that large breeding habitats absorb the organic waste as well as surface water from precipitation, street washing, car washing, grass watering, and other sources.

The findings also, revealed the overall highest peaks of *An. gambiae* s.l pupae (P/S) occurrence in seasons in the studied breeding habitats to be months of June (i.e., rainy season) for all the breeding habitats. [48], also, found that mosquito development was at peaks during rainy periods most especially the month of June. In support of this study, [41], reported higher densities of for example *Anopheline* species in the rainy season however in the month of July. The finding contrasts [49], which found no strong association between rainfall and immature *Anopheles* and *Culicines* counts, implying that mosquitoes thrive in semi-permanent or permanent water bodies during periods of low rainfall.

The smallest peaks of the pupae densities in the three breeding habitats were recorded in November, and these smallest pupae were because the rainy reason was terminated, and immature stages of mosquitoes cannot survive in dried breeding habitats. During May and November, there were very few mosquitos in the present study, and this least occurrence could be due to the onset of the dry season and early onset of the rainy season leading to scarcity of rainwater in the breeding habitats [50], indicated that very rare larval habitats are found during lack of water. Lack of rainfall and the fact that the month of May is the start of the rainy season, while November is normally dry season, mosquito eggs will not hatch and survive in the soil of the breeding habitats during this period.

In terms of seasonal pupal productivity (PM^2/S), the result revealed similar trend to pupal occurrence as the, June, being the most productive months of the *An. gambiae* s.l in temporary (i.e., Gutters and Swamps), and semi-permanent (i.e., Large water bodies) habitats. These observations were based on the nature of the breeding habitats, distribution of the quality and the quantity of the breeding factors (i.e., biotic, and abiotic factors), and nature of distribution of rainfall in the season, the fact that monthly rainfall (i.e., rainy season) remain same (do not change) in North Central part of Nigeria, but the distribution of rainfall may vary from location to location. In the three breeding habitats, the smallest peaks of the pupae densities were seen in November while in the months December, January February and March recorded zero population densities particularly in large water bodies. This is because in North Central part of Nigeria, November is generally considered to be end of rainy season while preceding months are the beginning of dry season and in these months the level of water reduces leading to dead of immature stages of mosquitoes.

As it occurred in terms of pupae occurrence similar trend in pupal productivity in this current study, the density of *An. gambiae* s.l pupae was found to be closely linked with rainfall. In support of this current study [51], also recorded larger populations of mosquito species, for example, *Anopheline* species in but in July (i.e., the peak of rainy season) thus, immature stages productivity depends on rainfall and subsequent changes in the water table and river levels. There were very little pupae during dry season most especially in swamps and large water bodies in this current study. The low species density during these periods could be due to a lack of rainfall; these conditions would prevent mosquito eggs from hatching and surviving in the soil of breeding habitats.

This cohorts the research work of [16], that recorded the overall number of *An, gambiae* s.l pupae found in the large water was higher during the rainy season than during the dry season, it is also in line with findings reported from Morelos in Mexico and from Thailand. Therefore, it requires that, responsible authorities are required to target the most productive breeding habitat management to undertake efficient measures for vector control [17]. The occurrence and productivity of pupae breeding habitats is governed by the quality of water in habitats and consequently fluctuates the adult densities capable of malaria transmission. The current study in addition, investigated the effect of eight physico-chemical parameters, namely water temperature, DO, pH, conductivity, salinity, TDS, turbidity, and hardness on *An. gambiae* s.l densities. In line with the observed seasonal variations in the physcico-chemical parameters of the pupae breeding habitats, there was a corresponding variation in the mosquito pupae occurrence and productivity in the various habitats [52], indicated that water is needed for the hatching of eggs because it dissolves the nutrients and physico-chemical properties into solutions and allows the eggs to float in larval breeding habitats; without water, such eggs will not hatch. Physico-chemical and nutrients have effects on egg hatchability and transformation into larval, pupal, and adult growth and development and life-history traits [53, 54].

Lack of adequate nutrition during mosquito egg hatching and larval development can result in delayed or failed development, as well as the production of adults lacking adequate nutritional stores [54]. [55], showed that chemical variables at mosquito breeding habitats had a specific impact on the growth and development of mosquito juvenile stages. *An. gambiae* s.l pupae found in the research habitats were linked to water quality and suitability to each breeding habitat type. The findings agree with the statement of [42], that mosquitos breed in practically all types of aquatic ecosystems and have greater adaptability. The water quality of the breeding sites is a significant component for female oviposition because female mosquitos prefer oviposition sites based on a mix of visual and chemical cues [56]. Also, in confirmation with these findings, it has earlier been stated that the survival of mosquitoes in a given habitat, is reliant on specific physico-chemical characteristics which strongly affect the larval density of individual mosquito species [57].

The findings of various physico-chemical parameters levels have some influence on *Anopheline* oviposition, larval instar that resulted on pupal densities, and survival in the selected conventional breeding habitats. This could be because mosquitoes require specific amounts of certain characteristics to live in each habitat; this statement is consistent with the findings of [57, 58], who discovered that certain physico-chemical factors appear to have a substantial impact on the larval and pupal density of individual mosquito species. The pH, temperature, calcium, alkalinity, chlorine, potassium, nitrate, and sulphate concentrations in the larval habitats have an impact on larval development and survival [10]. This result correlated with the earlier studies have also confirmed that some of the physico-chemical parameters such as temperature, DO, conductivity and pH associated strongly with Anopheline larval and pupae abundance [19].

The temperature at which an insect develops is critical in determining most immature and adult life traits, more so, for optimal growth and development, a temperature range is required. The temperatures obtained in the current study (range=26.98±0.30 to 27.24±0.25 °C) and this range temperatures within the optimal range of 16 °C to 34 °C that allows larvae and pupae of *Anopheles* most especially *An. gambiae* s.s. in which it is included in *An. gambiae* s.l to survive and develop into adults. According to reports by [59], low water temperatures reduce thermal pollution in aquatic environments and encourage invertebrates to thrive. However, higher temperatures create conditions more favourable for the survival of *An. gambiae* larvae [34].

The pH of neutrality (7.0) supported highest performance of the mosquito species (in terms of duration of embryogeny, hatchability rates, WL and FA) and may be the average tolerable pH condition for the species The pH range of 7.1 to 7.9 was recorded during the current study period in all the breeding habitats found to be supportive to *An. gambiae* s.l pupae and could be attributed to the required range values by the mosquitoes. This claim is in agreement with [60], who found that *Anopheles, Aedes,* and *Culex* immature live in acidic as well as alkaline mediums. However, alkaline pH with range (8–8.5) is considered to be favourable pH for the majority of *Anopheles*, pH range (6–8) for *Ae.* and a broader range of pH (6–10) for *Culex* [55]. Mosquitoes like a neutral pH range of 6.8 to 7.2 for breeding. Thus, outside of this range, mosquito eggs, larvae, and pupae growth are underdeveloped, and mortality occurs at pH levels below 4.5 or over 10 [53].

The amount of dissolved oxygen (DO_2) present in mosquito breeding habitats ranged from 5.73 mg/l to 8.83 mg/l. In gutter and large water habitats, different larval instars and pupae of *Anopheles* mosquitoes were able to thrive. This is the first time during our study period that DO_2 levels in gutters have been recorded at moderate levels, and this could be since dissolved oxygen levels in the water decrease with depth; at the surface, there is a high level of dissolved oxygen, and larvae are positioned in such a way that they can use the available DO_2 for survival. The depths of almost temporary breeding habitats (Gutters) are not as that of semi-permanent breeding habitats (Large water bodies) in this current study and most larvae prefers to breed at the banks of water bodies whose depths are shallow. The higher dissolve oxygen in swamps and large water and biochemical oxygen demands in large water bodies may be due to the high density of phytoplankton, which is the principal producers of oxygen, a by-product of photosynthesis, in aquatic habitats [52]. [59] reported that oxygen gas present in water is vital to the existence of most aquatic organisms; the diversity and abundance of organisms are greatest at higher dissolve oxygen concentrations. This is in line with the findings of [42], who found that low dissolve oxygen concentrations (<3 mg/l) made it difficult for many aquatic creatures to survive, but that the key value for optimal concentration and tolerance limits differed by species. This contradict [19], who said that, the DO_2 level of the water bodies which contained below 3 mg/l was categorized as third class although can support *Anopheline* mosquitoes, including for example An. *culicifacies*, can breed in such kind of wastewater bodies with low levels (<3 mg/l) of DO concentration.

The results of our study indicated that chemical nutrients such as K^+, S^-, Ca^{2+} Cl^-, PO and NO_3 at various concentrations play a vital role by increasing the number of surviving and successfully pupating mosquito larvae in the breeding habitat types. These findings are consistent with those of [61], who discovered that factors like PO_4 concentration had a favourable impact on mosquito larvae in the late aquatic stages. This could be due to the faster decomposition of leaf litter, which is linked to considerably higher phosphorus elements due to anthropogenic activities like farming and fishing, which need the use of pesticides containing these elements.

According to [62], microbial activity rises in tandem with quicker breakdown rates, affecting the availability of organic matter as a food source for mosquito larvae and, of course, the development of pupae for most *Culex* species in each habitat. Mosquito growth is also aided by calcium and organic carbon from outside sources, which also, play an important role in mosquito growth [63].

The number of pupae produced by *Anopheles* differed significantly across three habitat types, with swamps and large water bodies producing more *Anopheles* pupae than the gutters. This could be related to the fact that their nutrition requirements and breeding habitat utilization differ in this current study.

Previous research has found that adding nutrients to water accelerates mosquito larval growth and development, enhances immature survival, and increases the percent of mosquito larvae that pupate [53, 64]. Therefore, a lack of larval nutrition can lengthen development time, reduce pupation and emergence rates, and lead to reduced adult female body sizes [65, 66]. Nitrogen, for example, is essential to produce chitin and is extremely restricting for larval growth and development. Therefore, combining NO_3 and PO_4 nutrients, or NH_4 and PO_4 nutrients, promotes mosquito development and reduces pupation time.

In this current study, *An. gambiae* s.l pupa was seen actively breeding in gutters even though, their densities were not much as compare to other habitats studied, demonstrating that they can withstand varying degrees of these conditions. As a result, their abundance was positively related to these physico-chemical factors. According to [67, 68], the distribution of mosquitoes in different habitats is the result of more intricate relations of distinct habitat ranges of physico-chemical variables. Organic pollution is indicated by high levels of total hardness, nitrate, phosphate, calcium, chlorine, sodium, potassium, alkalinity, and conductivity in gutter habitats. This could be owing to the flood of residential and agricultural wastes caused by human activities, as well as residents' bad sanitary habits. This finding conforms with the earlier report of [45, 69–73], who concluded that ecological changes caused by human activities, in combination with a lack of proper sanitary practice.

5. Research limitation

This research work limited to malaria pupae of *Anopheles gambiae* s.l it does not included with the pupae of the other available genera (i.e., *Aedex* and *Culex*) commonly found in the study areas and equally limited to only four local government areas (LGAs) out of twenty-five LGAs of the State.

6. Conclusions

In conclusion, the findings from this study and from published work show that the productivity of pupae of *An. gambiae* s.l (malaria vectors) from different habitat types was highly varied. In this research, all habitat types produced pupae of malaria vectors. The overall productivity of *An. gambiae* s.l. pupae draw attention for on the dangers pose by all immature stages of malaria vectors (MV), therefore, we recommend sophisticated control strategies most especially larvicides in all aquatic habitats and should be continued within the north central of Nigeria lying emphases on Niger State. This study provides baseline data for further studies on mosquito and other aquatic insect fauna for effective vector management.

This study prospect for further research in the areas of the following:

1. Determination of quantity/classification of the immature stages of non-mosquito invertebrates.
2. Expansion of the vector management tactics that will include other mosquito genera.
3. Extension for the assessment of mosquito production and survivorship from more different habitats at a wide geographical scale in feature.
4. Phyto and zooplanktons associated with breeding habitats should also be investigated and their contributions to the development of mosquitoes.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper**.**

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Data availability

Manuscript has no associated data.

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