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Research Article

The Influence of Teneral Reserves Mobilization on Wing Development for Vectoral Fitness in *Anopheles Gambiae* and *Culex Quinquefasciatus* Mosquitoes

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Abstract

The mobilization of teneral reserve components across mosquito life stages (eggs-pupae) is vital because these teneral components are required for disease transmission. This study was conducted to determine the influence of teneral reserve accumulation on wing development and vectoral fitness in Anopheles gambiae and Culex quinquefasciatus mosquitoes. The first live instar (L1) of the selected mosquitoes was sampled using a 350 ml dipper from larval habitats that included gutters, swamps, and large water bodies of the selected sites in Minna metropolis, reared to the fourth live instar (L4), pupae, and adults under laboratory conditions of temperature and relative humidity (28^{oC} and 73%, respectively) in separate modified larval and dried adult holding cages (LHC and DAHC). At the adult stage, the wings of mosquitoes were removed and measured under a microscope, and nutritional reserves and vectorial fitness were determined using measured wings as an index. Differences in the nutritional composition of the fourth instar An. gambiae were as follows: sugar (3.66±0.20 microgram/larvae), glycogen (2.09±0.26 µg/lar), lipid (8.50±1.0 µg/lar) and proteins (22.39±0.63 µg/lar). Cx. quinquefasciatus had teneral reserves as follows (4.14±0.30, 2.07±0.28, 10.00±0.68 and 22.56±0.46 µg/Lar) of sugar, glycogen, lipid, and protein respectively. The dissimilarity in wing length among species populations emerged only within a narrow range. The relatively larger wings were visible in the average wing length of Cx quinquefasciatus (3.77±0.12 millimetre). The proportion of wings of both left and right wings were

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0.03±0.01 mm among the two different species populations. In addition, significant positive to highly positive correlations were observed between nutritional components and wing length, sugar (r= 0.489), protein (r=0.991**), and wing length in An. gambiae, whereas a negative correlation was observed for all teneral content in Cx. quiquefasciatus (r= -0.112 to -0.830). These findings were evaluated for the suitability of disease spread and are expected to contribute to the development of anti-larval control strategies through the manipulation of larval habitat nutrient contents.

Keywords

Teneral Reserves, Larval Instar, Wing Length, Vectoral Fitness, Nutritional Composition

1. Introduction

The dietary environment encountered by larvae has a substantial influence on female fitness-related features, such as body growth, fecundity, flight, creation of new tissues and organs, blood meal intake, and insect utilisation [1, 2]. The accumulation of resources during the larval stages of mosquitoes determines the energy reserves of the adults. Energy reserves can be considered a life-history trait because they are linked to mosquito durability and fecundity. Mosquito body size (such as pupal weight, adult weight, and wing length) is also controlled by larval food resources; hence, larval habitat food resources play an important role in mosquito life cycle and abundance [3, 4].

Most features of adult life, such as survival, flight, vitellogenesis, and longevity, are dependent on the accumulation, distribution, and use of teneral reserves (lipids, glycogen, glucose, and protein) during juvenile development [1, 5]. The mobilisation of teneral reserve components throughout mosquito life stages (first, second, third, and fourth larval stages, pupae, and adults) is important because these teneral components are required for a variety of immature and mature life activities [5, 6]. Lack of adequate nutrition during mosquito larval development may result in delayed or failed development, as well as the output of adults with inadequate nutritional stores, making pathogen transmission impossible [7].

The higher the protein, glycogen, sugar, and lipid reserves during emergence for a specific mosquito species, the greater the fertility and life span, and hence, adds to the health of the insect. Because small, starving mosquito larvae have lower energy stores than larger ones, smaller female mosquitoes must consume more blood during the adult stage to mature their eggs [3]. Therefore, pathogen transmission is influenced by healthy mosquitoes. Food availability is particularly crucial in host-pathogen interactions because it affects both partners. In different species of culicines, for example, larger bodies increase the likelihood of survival and success in obtaining a blood meal, as well as parasite infectivity, parity, and vectoral potential (VP) in some species [8].

Some modifications in survival and dispersal can be explained by variations in adult size, which is simply a representation of larval habitat quality (as well as other important components of vectoral ability, such as fecundity and blood-feeding behaviour) [3]. Thus, energy reserves are important markers of larval efforts to acquire resources and assimilate them into adult body size, as well as determinants of survival and fecundity, as evidenced by the pattern of transmission of mosquito-borne diseases such as malaria, filariasis, Japanese encephalitis, and dengue [9]. Autogenous mosquitoes do not require blood to mature their first egg hatching, and egg production is largely dependent on the presence of a female nutrient reserve [10].

As a result, both larval-derived teneral reserves and blood meal function as yolk precursors and stimuli for the hormonal control of egg maturation in autogenous females [9]. Although genetic and environmental factors influence mosquito physiology, including those that affect teneral accumulation and energy availability at each life stage through effects on feeding and teneral reserve distribution, the quality of the teneral reserve and the degree of accumulation during the mosquito's immature life stages determine the quality of adults [11]. As a result, the energy reserves of mosquitoes are health predictors that connect larval resource acquisition efforts to adult survival and fecundity. Carbohydrates, which are made and stored as glycogen, are used as energy sources in *Aedes aegypti*, whereas proteins are stored as lipids for development.

Proteins aid larval development through enzymatic reactions and oogenesis [12] and act as energy sources during starvation. Increased dietary protein and shorter larval developmental times have been linked to increased larval size and protein storage [13, 14]. Fourth-instar larvae usually require five times (5×) the amount of food consumed during the first instar. The ability of an adult mosquito to fly is primarily determined by its nutritional reserves, which are determined by wing length, with the relationship between wing length and body mass varying among mosquito species. Mosquito wing length has been linked to fertility, adult life duration, blood meal success, and vectoral competence [13]. The physiological significance of the strong association between major larval teneral reserves and wing symmetry has been created as a proxy for adult mosquito vectoral fitness [7].

There is scant knowledge of these critical drivers of mosquito vectoral ability, making it difficult for researchers to

understand the role of teneral components in the epidemiology of mosquito-borne diseases, which is necessary for the creation of long-term vector control strategies. Studies have found significant regional variations in mosquito vectoral capability [15], which could be attributed to similar changes in fitness and reproductive capacity, as well as differential vector requirements in different areas. This study was conducted to fill these information gaps and clarify the implications of larval nutritional reserves on the late larval stages of *Anopheles gambiae* s.l. Giles and *Culex quinquefasciatus*, such as Fatigan mosquito populations, on wing symmetry for vectoral fitness, and to provide baseline data for the development of sustainable locality-sensitive mosquito vector control strategies.

2. Materials and Methods

2.1. Study Area

The study was conducted in Niger State in the Middle Belt region of Nigeria (6° 33E and Latitude 9° 37N), covering 88 km and representing 9.30% of the total land area in Nigeria. The state consists of 85% arable land with a current population of approximately 5.2 million citizens. The State has a tropical climate with mean annual rainfall, temperature, and relative humidity of 1334 mm, 30.2℃, and 61%, respectively. Niger State is bordered by several states: Kaduna (Northeast), Federal Capital Territory Abuja, FCTA (Southeast), Zamfara (North), Kebbi (West), Kogi (South), Kwara (Southwest), and the Republic of Benin along Agwara LGA borders (Northwest). Niger State experiences two distinct climates: the rainy season (May – October) and dry season (November – April). Additionally, the vegetation in the area is typically grass-dominated savannah with scattered trees [16-18]. Within the state capital (Minna), four sampling sites {A, B, C, and D) with Gutters, Swamps and Large water bodies (i.e. 12 different breeding habitats) were selected in a wide range from one another [18, 19]. Figure 1.

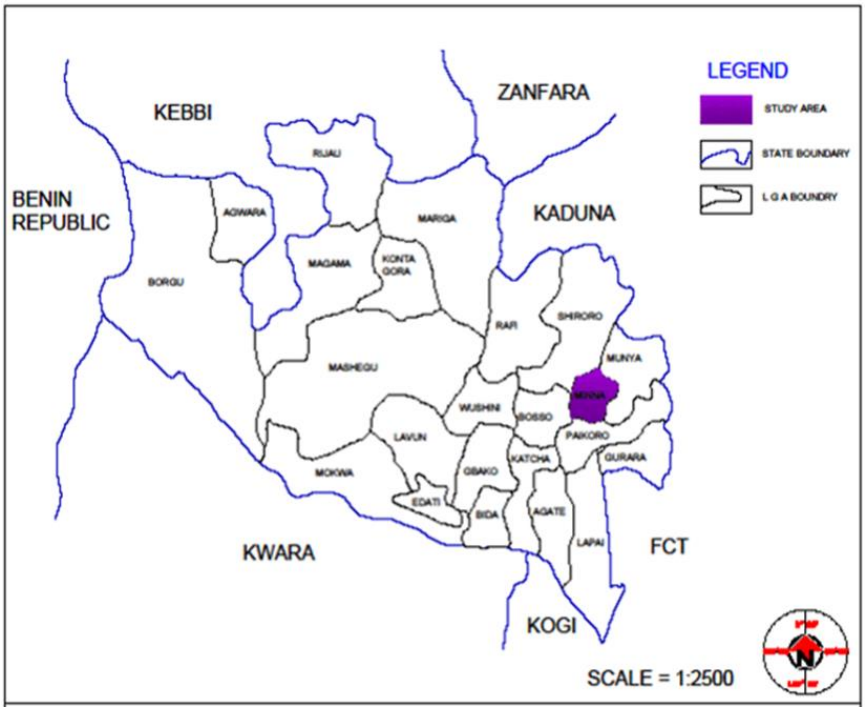

Figure 1. *Map of Niger State showing the study area.*

2.2. Selection and Identification of Mosquitoes for Teneral Nutrients

Of the three mosquito genera (*Aedes, Anopheles,* and *Culex*) commonly found in Niger State, two (*Anopheles* and *Culex*) were considered for the teneral test. This was because these two mosquitoes were considered to be more prevalent with diseases of epidemiological importance, although *Aedes* was among the most important; however, its effect was mild [5, 9, 16, 20, 23]. The sampled immature mosquitoes were initially identified macroscopically based on the observation of larval breathing tubes in the water and guided by a voucher key [21]. Larvae with long breathing tubes, narrow and inclined at a specific angle to the surface of the water, were *Culex and Anopheles* mosquito larvae that floated horizontally [20]. Immature mosquitoes were then separated into morph groups in the laboratory and identified

to the genus level based on visible characteristics such as (e.g., presence or absence of siphons, position of hair tufts, length of the siphon, arrangement of comb scales, and several others) [22]. Additionally, using a dissection microscope and morphological keys, the length of the larvae of each genus was used to distinguish life stages. Based on the genus abbreviation, names for the morphs-groups were assigned (e.g., *Anopheline- An.,* and *Culex-Cx.*) [21].

2.3. Larval Rearing in the Laboratory Insectary for Teneral Reserves

The first live-in stars (L1) of the selected mosquito vectors sampled in larval breeding habitats, including Gutters, Swamps, and Large water bodies, at the study sites (i.e. A, B, C, and D) were collected using 350 ml capacity dipper. The larvae were sorted into the following genera: *Anopheles* and *Culex*. Larvae belonging to each genus were reared under laboratory conditions of temperature and relative humidity (28$^{\circ C}$ and 73%, respectively) set in the laboratory using temperature and relative humidity reading devices. The larvae were held inside a separate modified Larval Holding Cage (LHC), measuring 25.70 cm long transparent container with an upper diameter of 27.30 cm covered with a netting material (0.05 mish size) and a lower base 16.40 cm in diameter [21].

Larval Holding Cages (LHC) were placed on a flat laboratory bench and monitored until they transformed into fourth instar (L4) and pupae. Fourth-instar larvae were separately identified to the species level as *Anopheles gambiae* and *Culex quinquefasciatus* by microscopy, guided by a voucher key, and used for the fourth-instar teneral reserve test as previously described [5]. A total of 120 L4 mosquitoes (five in each *An. gambiae* and *Cx. quinquefasciatus*) in rearing containers labelled Gutter, Swamp, and Large water bodies at each site (i.e. A, B, C, and D) within the Minna metropolitan area were investigated for mineral components (sugar, glycogen, lipid, and protein). Teneral reserve mobilisation was carried out following standard procedures at the insectary of the Department of Biology, Federal University of Technology, Minna, Niger State [22].

2.4. Rearing of Pupae to Adults in the Insectary for Wing Length Measurement

A quantity of live pupae of each species was collected with a rubber dropper from their rearing containers labelled as Gutter, Swamp, and Large water bodies at each study site (i.e. A, B, C, and D) within the Minna metropolitan area and transferred together with a small quantity of larval breeding water (that is, Gutters, Swamps, and large water bodies) into four different transparent rubber basins labelled at each study site (A, B, C, and D). Each basin measured 5.50m long, with an upper diameter of 11.50 cm and a lower base of 7.90 cm in diameter. The rubber basins with pupae were placed inside four separate dry adult holding cages (DAHC) labelled according to the study site (A, B, C, and D). Each DAHC measured 30.40 cm long transparent container with an upper diameter of 25.40 cm covered with a netting material (0.05-inch diameter) and a lower base 21.50 cm in diameter with a circular hand entrance of 10.00 cm in diameter attached to a long tubing net of 0.05 inch in diameter tight to disallowed emerged adult mosquitoes escaping from the cage as described by [5]. The emerged adults were identified under a microscope as *An. gambiae* and *Cx. quinquefaciatus*. They were fed a 10% sucrose solution soaked in cotton wool, placed on the netting cover of the cages [23], and used to measure right and left wing lengths.

2.5. Measurement of Wing Length of Mosquitoes in the Laboratory Insectary

A total of 360 reared and emerged adult mosquitoes were selected from two species (*An. gambiae* and *Cx. quinquefascius*) from the twelve (12) selected breeding habitats (Gutters, Swamps and Large water bodies) of the study site (A, B, C, and D) within the Minna metropolis were killed, and their left and right wings were removed gently and used for wing measurements to test the vectoral fitness of mosquitoes. Thirty left and right wings (15 *An. gambiae* and *Cx. quinquefasciatus*) were carefully mounted on a slide and labelled as left-wing (LW) and right-wing (RW). The wings were measured under a ×4 magnification ocular microscope with an ocular graticule mounted on the lens. Using a stage graticule, the acquired wing length data were computed and converted to the nearest millimetre [5].

2.6. Preparation of Standard Values for Sugar, Glycogen, Lipid, and Protein

2.6.1. Standard Values for Sugar and Glycogen

Anhydrous glucose (100 mg) was dissolved in 100 mL of distilled water, and 25, 50, 100, 150, and 200µl of glucose solution was added to glass tubes in triplicate. The anthrone reagent [95-98 per cent sulphuric acid (385 ml) + distilled water (150 ml) + anthrone (750 mg)] kept at 4$^{\circ C}$ was added to the 5 ml solution and mixed. The mixture was boiled for 17 min at 100$^{\circ C}$ and then allowed to cool. Optical density (OD) was measured at 625 nm using a spectrophotometer, and a graph of glucose against OD was calculated [9, 24].

2.6.2. Standard Values for Lipid

In triplicate, 100 mg of soybean oil was combined with 100 ml chloroform, and 50, 100, 200, and 400µl of the solution was added to glass tubes and placed on a heating block (at 100$^{\circ C}$) to evaporate the solvent. Sulphuric acid (0.2 mL) was added and the mixture was heated at 100$^{\circ C}$ for 10 min. The vanillin-phosphoric acid reagent [(vanillin (600 mg) + hot distilled water (100 ml) + 85 per cent phosphoric acid (400 ml)] was added to a 5 ml solution and stirred. This was heated and al-

lowed to cool until it turned a crimson colour. The optical density (OD) was read at 625 nm using a spectrophotometer, and lipids were plotted against the OD [5].

2.6.3. Standard Values for Protein

In the test tubes, 50 microlitres (50µl) of successive concentrations of 10, 20, 40, 80, and 100 g of bovine serum albumin were pipetted. Phosphate buffer was used to adjust the volume in the test tube to 1 ml (0.1 M, pH 6.6). The contents of the test tube were combined with five millimetres of protein reagent [Coomassie Brilliant Blue G-250 (100 mg) + 95 per cent ethanol (50 ml)], and the OD was read at 595 nm using a spectrophotometer [5].

2.7. Quantitative Extraction of Sugar, Glycogen, and Lipid from Mosquitoes

Five individuals at each L4 instar stage of *An. gambiae* and *Cx. quinquefasciatus* of the 12 breeding habitats (Gutters, Swamps and Large water bodies) of sites A, B, C, and D were emptied into each centrifuge tube, and sodium sulphate solution (0.2 ml, 2%) was added and homogenised until there were no discernible parts left. The glass rod used to homogenise the larvae (L4) was washed with equal volumes of chloroform/methanol solution (0.8 ml) in a centrifuge tube and centrifuged at 3000 rpm for 1 min. After transferring the supernatant to a clean centrifuge tube, 0.6 mL deionized water was added and mixed carefully before spinning at 3000 rpm for 1 min. The top fraction (water/methanol) was separated from the bottom fraction (chloroform) for sugar analysis, the bottom portion for lipid analysis, and the pellet for glycogen analysis [5].

2.7.1. Sugar Analysis

The sugar analysis part was placed in a tube with a 5 ml mark, which was then placed in a heating block at 90-110^{OC} (DB-2A hot plate), and the solvent was evaporated to 0.1-0.2 a level. Anthrone reagent was added to a 5 ml volume, properly mixed, and heated at 90-110^{oC} for 17 min. The optical density at 625nm was then calculated using a spectrum photometer [5, 24].

The glycogen analysis portion was poured into a tube with a 5 ml marking, which was then placed in a heating block at 90-110OC (DB-2A hot plate) and the solvent evaporated down to 0.1, 0.2 level. Anthrone reagent was added to a 5 ml level, properly mixed, and set aside to cool. A spectrophotometer was used to determine the optical density at 625 nm in 17 min. The optical density at 625 nm was calculated using a spectrum photometer [9, 24].

2.7.2. Lipid Analysis

The portion for lipid analysis was placed in a tube (with a marking at the 5 ml level) and heated (at 100$^{°C}$) to evaporate the solvent. Sulphuric acid (0.2 ml) was then added and the mixture was heated for 10 min at 100 °C. Vanillin reagent was added to a 5 ml solution and mixed. This was removed from the heating block, allowed to cool to allow the development of a reddish colour, and was stable for up to 30 min. OD was determined at 625 nm using a spectrophotometer [5, 9, 24].

2.7.3. Protein Analysis

Ice-cold saline buffer (0.9% NaCl) was added to the mosquitoes placed in a centrifuge tube, homogenised, and centrifuged at 4000 rpm. (20 min, 4°C). The supernatants were stored at -20$^{°C}$ until use. Phosphoric acid (85%, 100 ml) was then added to the protein reagent. The resulting solution was diluted with distilled water to obtain a final volume of 1 litre, by diluting with distilled water. The sample solution (50 µl) was pipetted into test tubes and adjusted to 1 ml with phosphate buffer (0.1 M, pH 6.6). OD at 595 nm was measured after 2 min and 1 h against a blank prepared from 1 ml of phosphate buffer and 5 ml protein reagent [5].

2.8. Statistical Analysis of the Data

Using Microsoft Office Excel and SPSS version 23 packages, the data generated were transformed into means and standard errors of the mean. One-way analysis of variance (ANOVA) was performed at a significance level of 0.05, and the statistical results obtained were converted to charts. Significant differences between means across the teneral components, their association with mosquito wing length, and the correlation between mosquito wing length and teneral reserve were determined as needed.

3. Results

3.1. Teneral Mobilization of the Fourth Instar of *Anopheles Gambiae* Mosquitoes

The teneral reserve components (that is. Sugar, Glycogen, Lipids and, Protein) accumulated by the fourth instar A. *gambiae* mosquitoes varied significantly at all sampling sites (A, B, C, and D) of Minna (Table 1). The highest sugar content was obtained for C (4.14±0.50µg/lar). This highest value was not significantly different (P>0.05) from the recorded value of B (4.06±0.35µg/lar) but significantly differed (P<0.05) from the values recorded for D and A sites with sugar reserve values of 2.82±0.17µg/lar and 3.60±0.23µg/lar, respectively. There was a significant difference (P<0.05) in the glycogen reserve between site A and the remaining sampling sites, with the highest value (3.66±0.22µg/lar). The lowest glycogen reserve was recorded in B, with a value of (1.21±0.11 µg/lar). The lowest glycogen content was insignificant (P>0.05) from glycogen containing D, but differed significantly (P<0.05) from the glycogen content of C, with the latter having the highest value (2.15±0.54 µg/lar).

Sampling site A showed a significant difference (P<0.05) from the remaining sites for lipid reserve (14.66±1.33µg/lar).

There was no significant difference (P>0.05) in the lipid reserves between C and D. Furthermore, there was no significant difference in the protein reserves of the fourth instars of *An. gambiae* in A, B, and D, except for fourth instars in C. The highest protein reserve was obtained from A with value (24.49±0.93 µg/lar) and the least value of 19.46±0.60 µg/lar of *An. gambiae* was obtained in the C site**.** The highest mean of the teneral reserves was recorded for protein (22.39±0.63 µg/lar) and the lowest mean was recorded for glycogen (2.09±0.26 µg/lar).

Table 1. *Teneral reserves of fourth-instar larvae of Anopheles gambiae mosquitoes from selected sites.*

Food Nutrients				
Sites	**Sugar (µg/Lar)**	**Glycogen (µg/Lar)**	**Lipid (µg/Lar)**	**Protein (µg/Lar)**
A	3.60±0.23^{ab}	3.66±0.22^{c}	14.66±1.33^{c}	24.49±0.93^{b}
B	4.06±0.35^{b}	1.21±0.11^{a}	8.67±0.82^{a}	24.03±0.61^{b}
C	4.14±0.50^{b}	2.15±0.54^{b}	5.33±1.33^{a}	19.46±0.60^{a}
D	2.82±0.17^{a}	1.35±0.14^{a}	5.33±0.82^{a}	21.56±1.40^{a}
Mean	3.66±0.20	2.09±0.26	8.50±1.01	22.39±0.63

Values are represented in the Mean± SE, of their replicates, values followed by the same superscript alone in the column are not significantly different at P>0.05, Lar= Larvae

The teneral reserves of fourth-instar *Culex quinquefasciatus* mosquitoes from different larval habitats are presented in (Table 2). The results revealed that the highest values of all teneral reserves were in the fourth instar of *Cx. quinquefasciatus* of A with values of 5.70±0.60 µg/lar, 3.97±0.34 µg/lar, 11.34±2.26 µg/lar, and 24.34±0.68 µg/lar for Sugar, glycogen lipids, and proteins, respectively. The lowest value of Sugar, glycogen, and proteins was recorded in the fourth instars of D (2.88±0.13 µg/lar, 1.10±0.04 µg/lar and 21.1±0.92 µg/lar for sugar glycogen and protein, respectively), while the least lipid reserve was obtained from C with a value of 8.67±0.82 µg/lar. There was no significant difference (P>0.05) in the teneral reserves of the fourth instars of C and D. Additionally, there was no significant difference (P>0.05) in the lipid content between A and B. The highest mean teneral reserves of the *Cx. quinquefasciatus* was recorded for protein (22.56±0.46 µg/lar) and the lowest mean was recorded for glycogen (2.07±0.28 µg/lar).

Table 2. *Teneral reserves of fourth instar larvae of Culex quinquefasciatus mosquitoes from selected sites.*

Food Nutrients				
Sites	**Sugar (µg/Lar)**	**Glycogen (µg/Lar)**	**Lipid (µg/Lar)**	**Protein (µg/Lar)**
A	5.70±0.60^{c}	3.97±0.34^{b}	11.34±2.26^{a}	24.34±0.68^{b}
B	4.60±0.36^{b}	1.56±0.24^{a}	10.00±1.05^{a}	23.13±0.83^{ab}
C	3.38±0.16^{a}	1.63±0.19^{a}	8.67±0.82^{a}	21.68±0.67^{a}
D	2.88±0.13^{a}	1.10±0.04^{a}	10.00±1.05^{a}	21.11±0.92^{a}
Mean	4.14±0.30	2.07±0.28	10.00±0.68	22.56±0.46

Values are represented in the Mean± SE, of their replicates, values followed by the same superscript alone in the column are not significantly different at P>0.05, Lar= Larvae

3.2. The Main Teneral Reserve Mobilization of the Fourth Instar of *An. Gambaie* and *Cx. Quinquefasciatus* Mosquito Population

The mean teneral reserves accumulated in the fourth instar of *An. gambiae* and *Cx. quinquefasciatus* larvae are shown in (Figure 2). The results revealed a significant variation in the mean reserves accumulated by the fourth instar of different mosquitoes. The highest means of sugar, lipid and protein reserves were recorded for *Cx quinquefasciatus* with values of 4.14±0.30 µg/lar, 10.00±0.68 µg/lar, and 22.56±0.46 µg/lar, respectively. The highest mean glycogen reserve was obtained in *An. gambiae* with a value of 2.09±0.26 µg/lar. The lowest sugar, lipid, and protein reserves were observed in *An. gambiae* with values of 3.06±0.20, 8.50±1.01 µg/lar and 22.39±0.63 µg/lar, respectively, while the least value of glycogen reserve was recorded for *Cx. quinquefasciatus,* with a value of 2.07±0.28 µg/l. No significant differences were observed among the components.

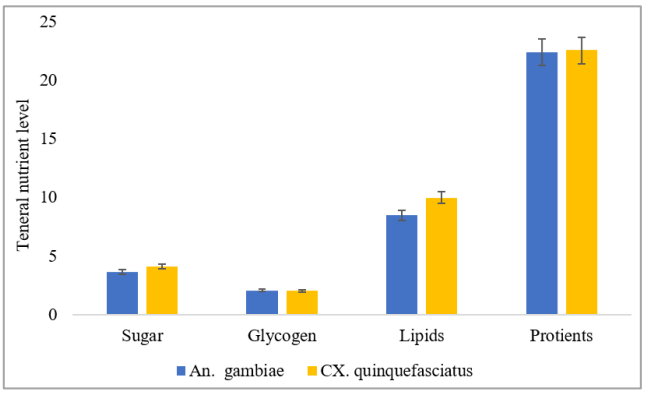

Figure 2. *Mean distribution of teneral nutrients (µg/mosquito) on the fourth instar of An. gambaie and Cx. quinquefasciatus mosquito population.*

3.3. The Wing Length and Fluctuating Asymmetry of *Anopheles Gambiae* Mosquitoes

The wing lengths and fluctuating asymmetry of *Anopheles gambiae* mosquitoes are shown in (Figure 3). The results showed significant disparities between wing lengths and fluctuating asymmetry of mosquitoes obtained from different sampling sites. The highest right-wing, left-wing, and mean wing lengths were recorded for *An. gambiae* obtained from D sampling sites (2.83±0.06, 2.81±0.06 mm), respectively. The highest wing length values were not significantly different (P >0.05) from those of *An. gambiae* wing lengths obtained from sampling sites B and A The lowest RWL, LWL, and MWL of the same species were recorded from the C sampling site with values of 2.65±0.05 mm, 2.64±0.05 mm, and 2.65±0.05 mm, respectively.

In contrast, the highest fluctuating asymmetry of the same species was recorded in B (0.05±0.01 mm), and the lowest FA was recorded in both D and C (0.01±0.01 mm). The highest FA differed significantly (P <0.05) among all sampling sites. However, the fluctuating asymmetry of *An. gambiae* from group A differed significantly (p<0.05) from those from groups C and D, with a value of 0.03±0.01 mm. Overall, the highest total mean wing length of *An. gambiae* was recorded in RWL and MWL (2.80±0.03mm). The highest value was not significantly different (p>0.05) from that of the LWR of the same species.

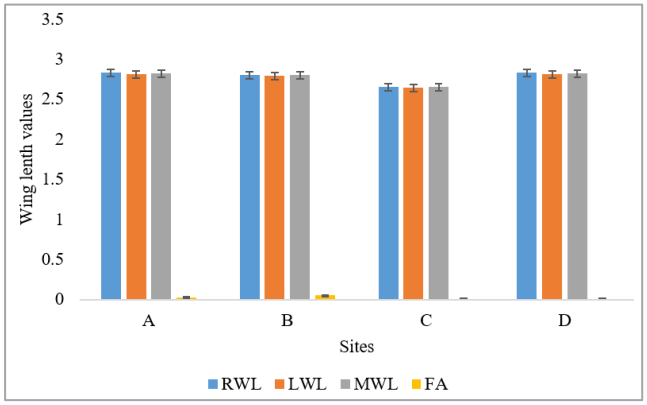

Figure 3. *Wing lengths and fluctuating asymmetry of An. gambiae from different studies sites.*

3.4. The Wing Length and Fluctuating Asymmetry of *Culex Quinquefasciatus* Mosquitoes

The wing length and fluctuating asymmetry of *Culex quinquefasciatus* are shown in (Figure 4). The results revealed significant differences among wing lengths of the same species obtained from different sampling sites. The highest right-wing length, left-wing length and mean wing length of mosquitoes were recorded from the D sampling site with values of 4.07±0.36 mm, 4.09±0.35 mm, and 4.10±0.36 mm, respectively. The highest values were significantly different (P<0.05) from the wing lengths of the same species obtained from the other sites. The lowest wing lengths were recorded for mosquitoes from site A; these lowest values were not significantly different (p>0.05) from those of the same species recorded from site B.

The highest fluctuating asymmetry of the same mosquitoes was recorded at site B (0.05±0.02 mm). The highest fluctuating asymmetry differed significantly (P <0.05) from the records of the remaining sampling sites. The lowest values were recorded in A, C, and D. Overall, the highest total length of mosquitoes was recorded in MWL (3.77±0.12 mm); this highest value was not significantly different (p>0.05) from RWL and LWL of the same species.

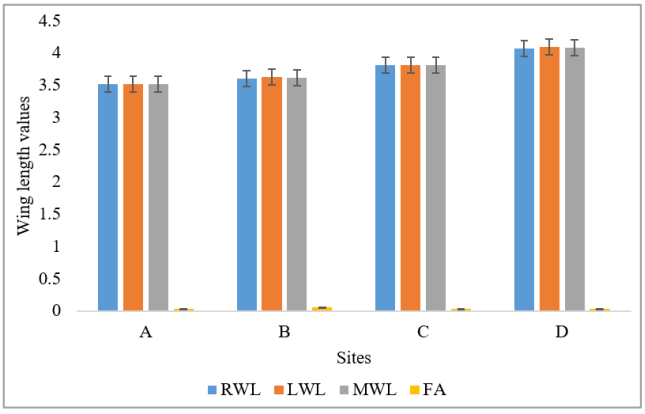

Figure 4. Wing lengths and fluctuating asymmetry of Cx. quinquefasciatus from selected studies sites.

3.5. The Mean Wing Length and Asymmetry of Adults *An. Gambiae* and *Cx. Quinquefasciatus*

Wing length and asymmetry in adult *An. gambiae* and *Cx. quinquefasciatus* are shown in (Figure 5). The mean wing length and fluctuating asymmetry of *An. gambiae* and *Cx. quinquefasciatus* mosquitoes revealed significant differences among the wing lengths and fluctuating asymmetry of mosquitoes obtained from the studied species. The highest overall mean length of the *An. gambiae* and *Cx quinquefasciatus* mosquitoes were recorded for RWL, MWL (2.80±0.03mm) and MWL (3.77±0.12mm). Both mosquito species recorded the lowest mean length in FA (0.03±0.01 mm).

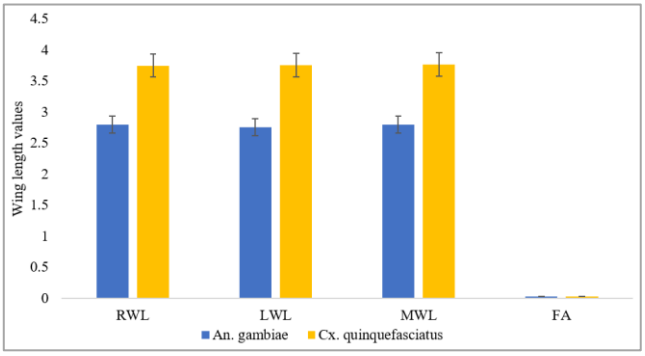

Figure 5. Mean wing length (mm) and asymmetry of adults An. gambiae and Cx. quinquefasciatus population.

3.6. Relationship Between Teneral Reserves and the Wing Length of *Anopheles Gambiae* Mosquitoes

The relationship between wing length and the teneral reserve parameters of *Anopheles gambiae* mosquitoes is detailed in (Table 3). The results revealed a significant positive correlation between the wing length and teneral reserves. There was a positive correlation between Right Wing Length, Left Wing Length, and Mean Wing Length of *Anopheles gambiae* mosquitoes and protein reserves, with values of r=0.974*, 0.978*, and 0.991**, respectively. A positive and reasonable association was observed with FA. The sugars, lipids, and proteins in *An. gambiae* (r=0.884, 0.746, and 0.600, respectively), and there were moderate positive correlations between sugar content and RWL, LWL, MWL, and Fluctuating Asymmetry (range r= 0.423 to 0.884), while a moderate positive correlation was found between FA and lipids (r= 0.746). On the other hand, there were negative correlations between wing parameters (except for FA) and all teneral reserves.

Table 3. Correlation coefficients between wing lengths and teneral reserved of adult An. Gambiae.

	RWL (mm)	LWL (mm)	MWL (mm)	FA (mm)
Sugar	0.407	0.423	0.489	0.884
Glycogen	-0.071	-0.119	-0.070	-0.242
Lipid	-0.230	-0.200	-0.136	0.746
Protein	0.974*	0.978*	0.991**	0.600

*Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed) RWL= Right wing length, LWL = Left wing length, MWL = Mean wing length, FA = Fluctuating Asymmetric

3.7. Relationship Between Teneral Reserves and the Wing Length of *Culex Quinquefasciatus* Mosquitoes

The relationship between wing length and the teneral reserve parameters of *Culex quinquefasciatus* mosquitoes is detailed in (Table 4). Significant positive correlation occurred between sugar, lipids, glycogen, and FA, with values (r= 0.959*, 0.228 and 0.012, respectively). Parameters such as RWL, LWL, and MWL were negatively correlated with sugar, glycogen, and lipid contents.

Table 4. Correlation coefficients between wing lengths and teneral reserved of adult Cx quinquefasciatus.

	RWL (mm)	LWL (mm)	MWL (mm)	FA (mm)
Sugar	-0.804	-0.797	-0.830	0.959*
Glycogen	-0.648	-0.650	-0.658	0.012
Lipid	-0.746	-0.753	-0.701	0.228

	RWL (mm)	LWL (mm)	MWL (mm)	FA (mm)
Protein	-0.103	-0.107	-0.112	-0.525

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed) RWL= Right wing length, LWL = Left wing length, MWL = Mean wing length, FA = Fluctuating Asymmetric.

4. Discussion

Regardless of the epidemiological significance of different life stages, poor or good teneral reserve mobilisation has a major impact on biological fitness [25-27]. The quality of a mosquito's teneral reserves and the degree to which they accumulate throughout its immature life stage dictate the quality of its adult existence [7]. The findings of the current study demonstrated that larvae of *Anopheles gambiae* and *Culex* significantly accumulated teneral reserve components that had an impact on mosquito disease transmission, including Sugar, Glycogen, Lipid, and Protein, obtained from larval breeding habitats that include Gutters, Swamps, and Large water bodies at all sampling sites (A, B, C, and D). The values of these teneral components investigated were within the ranged values of 7.66-13.62 sugar/glycogen, 12.05-23.95 for lipid, and 20.19-38.30, for protein. This is consistent with the findings of [28], who found comparable ranges of teneral reserves of fresh crude protein and fat in mosquitoes. However, this contrasts with the findings of [9] in stem borer research, who found greater ranging levels of food teneral reserves. The inconsistency in the results could be explained by variances in insect species, as well as the fact that the sampled stem borers weighed more than the mosquito larvae. Protein and fat levels in the fourth instars of the two species were substantially greater than sugar and glycogen levels at all sampling sites in the present study.

This could explain why nutrient flow in breeding habitats may be rich in proteins and lipids compared with sugar flow in breeding habitats. In addition, water channels from residential areas that contain organic waste and have a link with breeding habitats may end up dumping this organic waste, thus increasing the nutrients (i.e. Proteins and Lipids) in larval breeding habitats. This contradicts the findings of [5], who discovered that glycogen was considerably greater than protein in mosquitoes across all sample sites; however, the same study revealed substantially higher lipid reserve levels. As seen in our current study, the peak concentrations of sugar and glycogen in the fourth instar of the two mosquito species were found across all sample sites, indicating a high quality of carbohydrate meal in the breeding habitats that include Gutters, Swamps and Large water bodies where they occurred.

Nutritional reserves, particularly glycogen, influence the ability of larvae to pupate and play a regulatory role in insect development. [2, 10] reported that during the larval stages, energy reserves are generated and stored for use during metamorphosis, and supply reserves (lipids, sugar, and glycogen) for the adult stage. Therefore, the amount of nutrients retained in larvae has a significant effect on the lifespan of adult mosquitoes. Therefore, teneral reserves, which are a product of larval biosynthesis [29], ensure a restricted flight potential and short survival duration, which is maintained by the mobilisation of the majority of glycogen and lipid reserves available. The low sugar and glycogen contents of the fourth larval instar of the various mosquitoes found in the current study could be related to the low quality of the nutrients and competition between mosquitoes and non-mosquito invertebrates in such breeding settings. This will put mosquitoes in a nutritional bond, causing them to die or become incapable of serving as disease vectors.

It has been claimed that a few malnourished mosquito larvae have fewer energy stores than larger ones, requiring smaller female mosquitoes to consume more blood meals for egg maturation during the adult stage [4], and during pre-immature growth, teneral reserve build-up, distribution, and consumption determine an adult mosquito's physiological fitness [11, 30]. The lipid reserve distributions in the two mosquito species investigated differed significantly among the mosquito populations at all sample sites, as observed in the current study with the fourth instars of *An. gambiae* (14.66 ±1.33 µg/Lar) *and Cx. quinquefasciatus* (11.34±2.26 µg/Lar) was the highest lipid reserves in the A site and had the highest lipid reserves. These findings suggest that lipid production and storage are critical for the dietary physiology of mosquito species. [6] documented the role of nutrient reserves, especially in insect larval habitats, and particularly in lipids in yolk formation, as well as their energy value. Thus, lipid reserves built up during the larval stage play a significant role in the reproductive potential of female mosquitoes.

Nonetheless, the findings of the present study revealed low to moderate lipid reserves in the two mosquito species tested, with lipid reserves of 5.33±0.82 µg/Lar. *gambiae* from C and D, while moderate lipid reserve (8.67±0.82µg/Lar). *gambiae* and *Cx. quinquefasciatus* in Groups D and C. This finding is consistent with the lipid findings of [28], who discovered high- and low-range values of lipid reserves in the fresh mass of *Cx. pipiens pipiens* mosquito population in Minna. This discovery poses a substantial threat to vitellogenesis, fecundity, and eventually, the population density of these mosquito species in the low-teneral conditions of the study sites. However, sugar meals are frequently consumed by female mosquitoes to compensate for low-teneral lipids, which then enter lipogenic pathways, usually before blood meals are consumed [2].

For example, the *Aedes vexan*, a successful large-bodied mosquito with low teneral protein and lipids, must consume sugar meals within the first week of adult emergence or die within a few days. *An. albimanus*, a species of small size, uses the first blood meal instead of yolk to synthesise maternal lipids and protein stores, compensating for its poor teneral

reserve [4]. Thus, it appears that. *gambiae* and *Cx. quinquefasciatus* have evolved mechanisms to cope with the low-teneral lipid reserves in these low-teneral reserve sampling sites. Physiological research to explicate such tactics, on the other hand, is necessary to better understand the reproductive biology and ecological adaptability of species.

The protein reserve distribution in the mosquitoes varied significantly among the populations, whereas the mosquito fourth instar of *An. gambiae* (24.49±0.93 µg/Lar). *quinquefasciatus* (24.34±0.68µg/Lar) in A, site had the highest protein reserve. These findings indicate that proteins in mosquitoes are vital for growth and development and can speed up the development of fertilised eggs and hatching to ensure population dynamics of species which are typically influenced by the nutritional quality of larval breeding habitats. For example, carbohydrates generated and stored as glycogen are used as energy sources in *Aedes aegypti*, whereas proteins are used as lipids for growth. Proteins aid in larval development and oogenesis through enzymatic processes [12] and act as energy sources, especially during famine. *Anopheles* increase larval size and protein storage, which are linked to higher dietary protein intake and shorter larval developmental time [13].

Normally, fourth-instar larvae need five times (5×) the amount of food used in the first instar. Consequently, the nutritional quality of the larval breeding sites in this study fully supported the development of these studied mosquitoes which fully reflected the late fourth instar in both *An. gambiae* and *Cx. quinquefasciatus,* which has been investigated for teneral reserves in the current study since 80-90% of larval growth and teneral biosynthesis occur during this instar. Variances in larval nutrient elements, such as nitrogen, phosphate, calcium, sulphur, and potassium, could explain the significant differences in crude protein composition among populations in the study area. In this study, the fourth instar accounts for 80-90% of larval growth and teneral biosynthesis; thus, the nutritional quality of larval breeding sites sufficiently reflects the nutritional quality of the larvae.

The two mosquito populations showed considerable differences in wing length (WL), a surrogate for adult body size. This finding supports the idea that larval teneral reserves, especially proteins and lipids, drive adult body size, and that body size/WL is a good indicator of teneral resource conditions. This viewpoint was like that of [6], who claimed that total protein consists primarily of structural components, whereas lipids, on the other hand, can be linearly associated with body size in the same way as protein or display steeply inclined exponential correlations. The wing lengths were measured (in mm) in the current study for *An. gambiae* and *Cx. quinquefasciatus* were above 2.5 mm and 3.5 mm respectively, indicating that the sampling sites frequently consisted of large individuals. This is consistent with the findings of [28, 32], who found the wing length of the *Culex pipiens pipiens* mosquito to be greater than 3 mm. In *Anopheline* and *Culicine* mosquitoes, wing lengths of 2 and 3 mm are commonly considered threshold requirements for significant vectoral ability.

This observation has major epidemiological significance for the vectoral capacity of the mosquitoes currently under study. Mosquitoes that live longer and deposit more eggs than those that are smaller make them a formidable vector of *Anopheles* and *Culex-borne* diseases in the area. The largest adult size of *An. gambiae* (2.83±0.04 mm) and *Cx. quinquefasciatus* (4.09±0.35) mm in the current study were collected from sampling sites A and D. This shows that the larval breeding habitats where mosquitoes are of high nutritional quality and adult mosquito body size are determined by larval feeding circumstances [8], which is vital for the development of strategic mosquito control interventions, bearing in mind the crucial role played by teneral reserves (i.e. Sugar, Glycogen, Lipids and Proteins) in the bio-ecophysiology of mosquitoes.

The large adult body size of mosquito species among the sample sites could indicate that the species in the research locations had different disease transmission potentials. Except for *Cx. quinquefasciatus* at sites B and D, where left-wing lengths were slightly greater than right-wing lengths, in the remaining sampling sites the right-wing length slightly increased than or almost the same as the left-wing length in the *mosquito* populations investigated. This observation could be attributed to physiological processes that take place in the bodies of some members of these investigated mosquitoes, and could also be due to endogenous variables, such as the species' genetics, rather than the influence of environmental conditions or teneral nutrients in the larval breeding habitats.

However, a consistently longer left-wing than the right wing was found in some members of the *Cx. quinquefasciatus* populations in the research area may harm the species' flying activity by limiting manoeuvrability, lowering blood meals, mating success, and predator escape. Thus, further research on the physiology and genetics of disproportionate wing length in *Cx. quinquefasciatus* populations as well as their effects on flying habits. The degree of right-wing and left-wing asymmetry varied slightly between the two mosquito populations studied, indicating that mosquitoes are subjected to the same external and endogenous stress and hence are equally suited as vectors of mosquito-borne human diseases [31].

5. Conclusion

In the present study, the dietary components of mosquito larvae were significantly related to wing length and symmetry. These findings add to a growing body of information that suggests a link between mosquito larval diet and adult vectoral success. The physiological implications of a substantial correlation between primary larval teneral reserves (protein and lipids) and wing symmetry are used as proxies for adult mosquito vector fitness. Mosquito vector control programs that incorporate tactics aimed at interrupting food obtainability and larval feeding success in their breeding habitats, in

addition to the application of larviciding chemicals, may go a long way toward lessening vectorial fitness and, hence, emerging adult mosquito vectorial potential.

Abbreviations

FCTA	Federal Capital Territory Abuja
LGA	Local Government Area
An.	Anopheline
Cx	Culex
L1	Larval Stage 1
L2	Larval Stage 2
L3	Larval Stage 3
L4	Larval Stage 4
LHC	Larval Holding Cages
DAHC	Dry Adult Holding Cages
OD	Optical Density
RWL1	Right Wing Length
LWL1	Left Wing Length
MWL	Wing Length
FA1	Fluctuating Asymmetry

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Declarations

We declared that the manuscript is an original research and have under gone a thorough checking for its relevance and not been submitted else were apart from Nigeria Journal of Parasitology.

Author Contributions

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Ethical Approval and Informed Consent

The protocol for the study was approved by the Universiti Sains Malaysia ethics committees in partnership with the Niger State Ministry of Health and Ministry of Education. Before authorization was given, community leaders and local government executives were briefed on the study's goals. Members of selected households of selected local government areas were given a thorough explanation of the study and given their consent before being included as participants. Throughout the study, privacy and secrecy were preserved.

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Conflicts of Interest

The authors declare no conflicts of interest.

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