Quantifying the Influence of Larval Density on Disease Transmission Indices in *Culex quinquefasciatus*, the Major African Vector of Filariasis

Azubuike Christian Ukubuiwe[1] [ID], Chioma Cynthia Ojianwuna[2],
Israel Kayode Olayemi[1], Francis Ofurum Arimoro[3],
Innocent Chukwuemeka James Omalu[4],
Chinenye Catherine Ukubuiwe[5] and Bulus Musa Baba[6]

[1]Applied Entomology Unit, Department of Animal Biology, Federal University of Technology, Minna, Nigeria. [2]Department of Animal and Environmental Biology, Delta State University, Abraka, Nigeria. [3]Applied Hydrobiology Unit, Department of Animal Biology, Federal University of Technology, Minna, Nigeria. [4]Applied Parasitology Unit, Department of Animal Biology, Federal University of Technology, Minna, Nigeria. [5]Department of Microbiology, Federal University of Technology, Minna, Nigeria. [6]Department of Water, Aquaculture and Fisheries Technology, Federal University of Technology, Minna, Nigeria.

ABSTRACT: Larval crowding is one of the abiotic factors affecting biological fitness in mosquitoes. This study aims at elucidating, quantitatively, the influence of more larval crowding on aspects of fitness in *Culex quinquefasciatus* mosquito. To this end, day-old larvae of the species were reared in 4 density regimens equivalent to 1 larva in 1.25, 2.5, 5, and 10 mL of distilled water. Developmental indices, adult fitness indices, and accumulation and utilisation of teneral reserves for metamorphosis were determined at these density regimens. The results revealed varying significant negative effects of larval density on all fitness indices measured for the species. The study also revealed high utilisation of teneral reserves for metamorphosis at high larval densities. The information generated will be useful in making informed-decisions in allocating scare resources for vector control, although field trials are advocated to establish these laboratory findings.

KEYWORDS: biological fitness, development, survivorship

Introduction

Lymphatic filariasis is a disease of the lymphatic system. The disease is caused by infection with thread-like nematode worms *transmitted* through bites from infected mosquitoes, principally, *Culex quinquefasciatus*.[1] Control of the disease remains elusive, despite the use of chemotherapy. Hence, the World Health Organization (WHO) recommended mosquito-vector control as a supplemental strategy for eliminating the disease.[2,3] However, effective mosquito-vector control must be predicated on an adequate knowledge of the ecophysiology of the vector species. Ecological interaction among immature mosquitoes is of particular importance as they affect egg hatching,[4] competition, and abundance.[5,6]

One of such ecological factors is larval crowding. Although there exist an avalanche of literature on the influence of larval crowding on the life cycle of insects,[7–19] this information is species-specific[20] and cannot be generalised to all mosquito species.[21] There is, therefore, need for detailed species-based ecological studies for understanding species-specific interactions for effective integration into control strategies.

In nature, the gravid female *C quinquefasciatus* are capable of exploiting most available water body for egg-laying, eg, drainages, rice fields, swamps, gutters, and rain pools for laying eggs.[22] These gravid females ensure optimal development and survivorship of the immatures by actively searching for and selecting nutrient-rich and productive habitats.[23] Presence of conspecifics,[24] semiochemicals from previously hatched-out larvae,[25] chemicals from decaying organic matters,[26,27] predators,[28] mineral salts,[29] water surface tension,[30] and quality and quantity of larval habitat[31] are some examples of cues, which the females depend on for oviposition site selection.

These conditions determine the attractiveness of larval habitats to the gravid females, and degree of egg rafts oviposition, and, hence, their relative productivity (ie, in terms of density of immature life stages). Female *C quinquefasciatus* mosquitoes lay an average of 30 to 350 eggs/raft; these eggs have high fertility, hatchability, and immature survival rates.[32,33] Therefore, with favourable oviposition attractants and immature breeding conditions, there is likelihood for increased larval density in the mosquito population and, often, competition for limited resources and space.[34]

Although there is the potential health implication of high mosquito larval density,[35–37] there is need to answer the question 'Does high mosquito population density constitute epidemiological threat?' This study, therefore, attempts to

quantitatively elucidate the impacts of such crowded conditions on developmental and adult fitness indices, and how these affect teneral reserve mobilisation and utilisation in *C quinquefasciatus* mosquito. Some adult fitness indices include duration of development (DD), immature survivorship, adult emergence success, daily survivorship, longevity, and teneral reserve. Teneral reserves, for example, determine the amount of energy available for most adult life traits such as body size, longevity, vitellogenesis, and flight.[38–40]

Therefore, this study aims at elucidating the influence of varying density regimen on these indices of fitness of the species. The information generated in this study will be important in developing efficient control protocols for cost-effective deployment of resources for control of the vector.

Materials and Methods

Duration of study

The study was carried out at the Entomology Unit of the Department of Animal Biology, Federal University of Technology, Minna, Nigeria. The study was carried out between May 2017 and April 2018.

Culture of experimental mosquito

Freshly laid egg rafts of *C quinquefasciatus* were collected from an established colony of mosquitoes in the Insectary Unit of the Department of Animal Biology, Federal University of Technology, Minna, Nigeria. These were placed in transparent plastic hatching trays and incubated for 24 hours at ambient room temperature. Hatched larvae were cultured at rates equivalent to simulated larval density regimens in plastic trays following standard techniques.[41] The larvae were fed with fish feed (Coppens®) at the rate of 0.32 mg/100 larvae which was sprinkled daily on the water surface. Excessive feed was removed using pipette to avoid mould formation. Water from the culture bowls were changed daily until pupation to prevent formation of scum. The pupae were collected in small bowls and placed in adult-holding cages for emergence. Emerged adults were maintained in the holding cages, and fed 10% sucrose solution soaked in clean cotton balls as described by Olayemi and Ande.[42] Prevailing laboratory conditions during experimentation were $28.00 \pm 2.00°C$, 86.00%, and 12:12 (light:dark hours), respectively, for temperature, relative humidity, and photoperiod.

Simulation of larval density regimens

Different mosquito larval densities were simulated according to the methods described by Takken et al,[13] though with slight modification. Briefly, 4 density regimens were adopted, namely, 50, 100, 200, and 400 larvae/500 mL of water to produce densities of 1 larva per 10.0, 5.0, 2.5, and 1.25 mL of distilled water, respectively. Each density regimen had 5 replicates. The entire experimental setup was repeated immediately after the first, resulting in 2 rounds of experimentation and 10 replicates.

Determination of entomological variables

Developmental indices. Duration (days) of development of larval instars (LI-IV) and immature life stages (average larval and pupal) were estimated as the time spent by the instar or life stage before entering the next. Survival rates of immature instars and life stages were determined as the percentage of mosquitoes at the beginning of a life instar or stage that successfully entered the next stage.[33] Larval growth rates (GR) were determined as average daily weight gain by the larvae during development. It was expressed as a ratio of weight at eclosion to age at pupation.[43] The weight of the mosquitoes was determined using electrical weighing balance (Model: JA303P) with sensitivity (of readability) of 0.001 g. Mean GRs were used for statistical analysis.

Adult success indices. Adult emergence success involved counting of the numbers of adult mosquitoes that successfully eclosed and expressing it as a percentage of the total larvae at start. The ratio of live adults to total adults on the preceding day was used to estimate daily adult survivorship, while post-emergence longevity was estimated as the number of days the adult mosquito spent post-emergence, while fed sugar solution.[33] Adult values are mean of male and female mosquito values.

Mosquito biochemical analyses. Mobilisation of teneral reserves (lipid, glycogen, glucose, and protein) at larval instars (LI-IV) and life stages (larval, pupal, and adult) were carried out as described by Van-Handel,[44,45] Van-Handel and Day,[46] and Kaufmann and Brown[47] and reported by Ukubuiwe et al.[48] Rates of teneral components accumulation were determined as the ratio of teneral components at terminal (fourth) larval instar and age at pupation and expressed in mg/component/day. All biochemical analyses were done in triplicates. Metabolic reserves for the processes of pupation and eclosion were estimated, respectively, as the difference between teneral reserve components at terminal larval instar and pupal stage, and between the pupal and adult stages.

Data analysis

All data generated in the study were processed into means and standard deviation using Microsoft Office Excel 2016 and IBM SPSS (Statistical Package for Social Science) Version 23. Differences in mean values of entomologic variables among density regimens were compared for significance difference using one-way analysis of variance (ANOVA) as appropriate. All decisions on statistical comparison of means were taken at $P = .05$ level of significance. The means were separated using Duncan Multiple Range Test (DMRT).
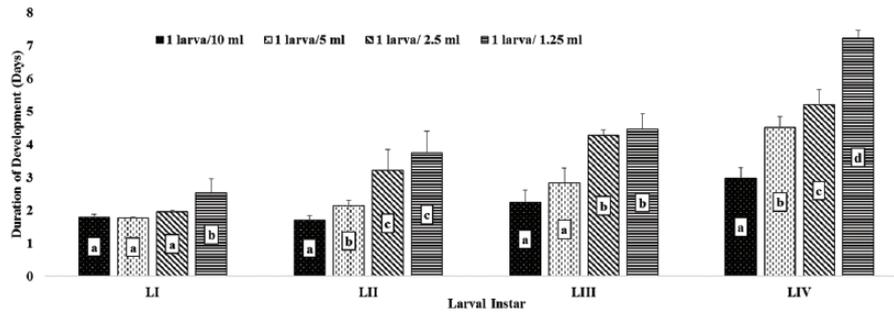
Figure 1. Effects of rearing density on duration of development of *Culex quinquefasciatus* mosquito larvae. Bars with same letter are not significantly different (*P* < .05) according to analysis of variance. Values are expressed as mean ± SD.

Table 1. Effects of larval density on duration (days) of development and larval growth rate (mg/day) of *Culex quinquefasciatus* mosquito.

DENSITY REGIMEN (LARVA: ML)	TOTAL LARVAL DURATION	PUPAL STAGE DURATION	TOTAL IMMATURE DURATION	GROWTH RATE (MG/DAY)
1: 1.25	17.99 ± 0.11[d]	1.39 ± 0.89[c]	19.38 ± 0.17[d]	0.0278 ± 0.0002[a]
1: 2.5	14.68 ± 0.15[c]	1.33 ± 0.14[c]	16.11 ± 0.21[c]	0.0341 ± 0.0004[b]
1: 5	11.27 ± 0.28[b]	1.28 ± 0.14[b]	12.55 ± 0.36[b]	0.0444 ± 0.0011[c]
1: 10	8.72 ± 0.50[a]	0.96 ± 0.31[a]	9.68 ± 0.69[a]	0.0575 ± 0.0035[d]

Values followed by same superscript alphabet in a column are not significantly (*P* < .05) different. All values are expressed as mean ± SD of mean.

Results

Effects of larval density on duration of immature development and larval GR of C quinquefasciatus mosquito

The effects of larval density on DD and GR of *C quinquefasciatus* are shown in Figure 1 and Table 1. Analyses revealed significant (*P* < .05) negative effects of increased density on these entomo variables.

There were variations in DD among the larval instars at different density, especially at the fourth larval instar (LIV) (Figure 1). Meanwhile, mosquito larvae reared at 1 larva/1.25 mL had the most delayed development time (17.99 ± 0.11 days), while those reared at 1 larva/10 mL developed fastest (8.72 ± 0.50 days) (Table 1).

Time taken till eclosion (ie, total immature DD) was significantly (*P* = .018) prolonged as rearing density increased, with mosquitoes reared at 1 larva/1.25 mL spending the longest time as immature (19.38 ± 0.17 days). GR significantly (*P* = .00) reduced with increasing density, exhibiting an inverse relationship (Table 1).

Effects of larval density on immature survivorship in C quinquefasciatus mosquito

The impacts of larval density on survivorship of immature life stages of *C quinquefasciatus* mosquitoes are shown in Figure 2 and Table 2. Generally, survivorship of immature life stages of the species exhibited a negative relationship with rearing density. Increasing density resulted in significant reduction in survivorship, with mosquitoes reared at 1 larva/1.25 mL having the lowest survivorship (<40% at LIV) (Figure 2).

There were no significant (*P* = .00) differences in immature (larval and pupal) survivorship of the species across the different density regimen. Furthermore, average immature survivorship was lowest at 1 larva/1.25 mL (65.18% ± 4.39%) and highest at 1 larva/10 mL (97.17% ± 0.43%) (Table 2).

Effects of larval density on emergence success, survivorship, and longevity of adult C quinquefasciatus

Adult emergence success, survivorship, and longevity of adult *C quinquefasciatus* are significantly affected by larval density (Table 3). Adult emergence success varied significantly (*df* = 3, *P* = .01) across the density regimens, ranging from 42.19% ± 2.58% to 76.50% ± 10.94% at 1 larva/1.25 mL and 1 larva/10 mL, respectively.

Mosquitoes reared at 1 larva/10 mL had the highest (75.00% ± 5.57%) daily adult survivorship, while those reared at 1 larva/1.25 mL had the lowest (62.08% ± 6.81%). Post-emergence longevity also followed a similar trend as daily adult survivorship (Table 3).

Effects of rearing density on teneral composition across life stages of C quinquefasciatus

Lipid composition. Analyses revealed significant effects of rearing density on mobilisation of lipid across the life stages of *C quinquefasciatus* mosquito (Table 4 and Figure 3). As
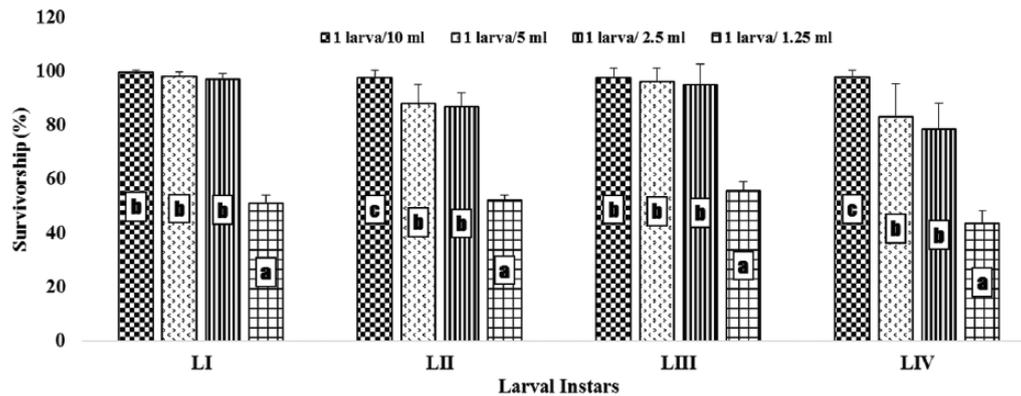
Figure 2. Effects of rearing density on survivorship of *Culex quinquefasciatus* mosquito larvae. Bars with same letter are not significantly different ($P < .05$) according to analysis of variance. Values are expressed as mean $\pm$ SD.

Table 2. Effects of larval density on survivorship (%) of immature stages of *Culex quinquefasciatus* mosquito.

DENSITY REGIMEN (LARVA: ML)	AVERAGE LARVAL SURVIVORSHIP	PUPAL STAGE SURVIVORSHIP	AVERAGE IMMATURE SURVIVORSHIP
1: 1.25	54.40 $\pm$ 5.40[a]	68.30 $\pm$ 9.05[a]	65.18 $\pm$ 4.39[a]
1: 2.5	76.73 $\pm$ 4.46[b]	81.16 $\pm$ 6.55[b]	87.62 $\pm$ 4.46[b]
1: 5	91.31 $\pm$ 3.92[c]	98.34 $\pm$ 2.09[c]	94.72 $\pm$ 2.92[c]
1: 10	96.88 $\pm$ 0.89[c]	98.36 $\pm$ 2.30[c]	97.17 $\pm$ 0.43[c]

Values followed by same superscript alphabet in a column are not significantly ($P < .05$) different. All values are expressed as mean $\pm$ SD of mean.

Table 3. Effects of larval density on emergence success, survivorship, and longevity of imaginal life stage of *Culex quinquefasciatus*.

DENSITY REGIMEN (LARVA: ML)	EMERGENCE SUCCESS OF ADULT (%)*	DAILY SURVIVORSHIP OF IMAGINES (%)*	POST-EMERGENCE LONGEVITY (DAYS)*
1: 1.25	42.19 $\pm$ 2.58[a]	62.08 $\pm$ 6.81[a]	3.63 $\pm$ 0.36[a]
1: 2.5	49.00 $\pm$ 12.94[b]	69.51 $\pm$ 3.50[b]	5.74 $\pm$ 0.58[b]
1: 5	70.50 $\pm$ 13.97[c]	74.01 $\pm$ 5.01[c]	11.25 $\pm$ 1.12[c]
1: 10	76.50 $\pm$ 10.94[d]	75.00 $\pm$ 5.57[c]	13.45 $\pm$ 0.30[d]

Values followed by same superscript alphabet in a column of a parameter are not significantly ($P < .05$) different. All values are expressed as mean $\pm$ SD of mean.
*Adult values are mean of male and female mosquito values.

larval density increased progressively, there was a significant ($P = .00$) decrease in the quantity of lipid across the life stages and within a life stage. Furthermore, irrespective of density regimen, there was a significant ($df = 4$, $P < .05$) progressive increase in larval stages' accumulation of lipid as the larvae progressed from LI through to LIV (Figure 3). However, the quantity accumulated was dependent on density regimen. Pupal values reduced significantly as it progressed to the adult stage (Table 4).

The rate of accumulation of lipid in the larval stages varied significantly among the mosquitoes, being lowest at 1 larva/1.25 mL ($0.87 \pm 0.03 \mu g$ lipid/mosquito/day) and highest at 1 larva/10 mL ($3.27 \pm 0.21 \mu g$ lipid/mosquito/day) (Table 4).

Glucose composition. The effects of larval density on glucose composition in *C quinquefasciatus* mosquito are shown in Figure 4 and Table 5. Analyses revealed a significant ($df = 4$, $P = .013$) negative effects of density on the glucose composition across all life stages; as larval density increased, the quantities accumulated by each life stage decreased significantly (Figure 4).

Generally, there was a significant ($df = 4$, $P < .05$) increase in glucose content as larvae progressed from LI to LIV. These values reduced significantly ($P < .05$) at later life stages (ie, pupae and adults). Furthermore, as density increased, larval rate of accumulation of glucose significantly ($P < .05$) reduced, ranging from $0.57 \pm 0.01 \mu g$ glucose/mosquito/day at 1 larva/1.25 mL to $3.27 \pm 0.21 \mu g$ glucose/mosquito/day at 1 larva/10 mL (Table 5).

Table 4. Effects of larval rearing density on life stages' composition and larval accumulation rates of lipid in *Culex quinquefasciatus* mosquito.

DENSITY REGIMEN (LARVA: ML)	PUPAE (μG LIPID/PUPA)	ADULT (μG LIPID/ADULT)*	LARVAL ACCUMULATION RATE (μG LIPID/LARVA/DAY)
1: 1.25	12.06 ± 0.07[a]	9.69 ± 0.33[a]	0.87 ± 0.03[a]
1: 2.5	16.05 ± 0.24[b]	12.39 ± 0.52[b]	1.44 ± 0.07[b]
1: 5	18.11 ± 0.31[c]	16.42 ± 2.01[c]	1.98 ± 0.05[c]
1: 10	25.20 ± 0.45[d]	22.18 ± 0.78[d]	3.27 ± 0.21[d]

Values followed by same superscript alphabet in a column are not significantly ($P < .05$) different. All values are expressed as mean ± SD of mean.
*Adult values are mean values of male and female lipid composition.
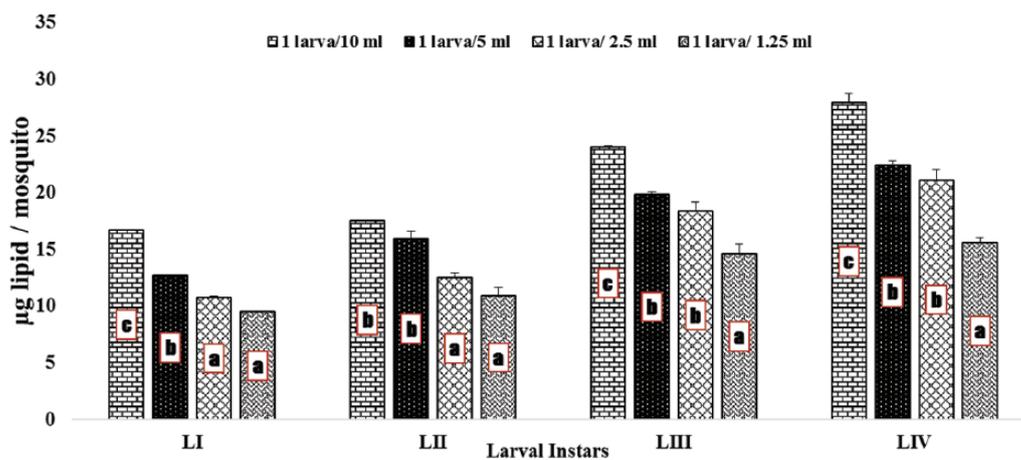
Figure 3. Effects of rearing density on lipid accumulation in larval instars of *Culex quinquefasciatus* mosquito. Bars with same letter are not significantly different ($P < .05$) according to analysis of variance. Values are expressed as mean ± SD.
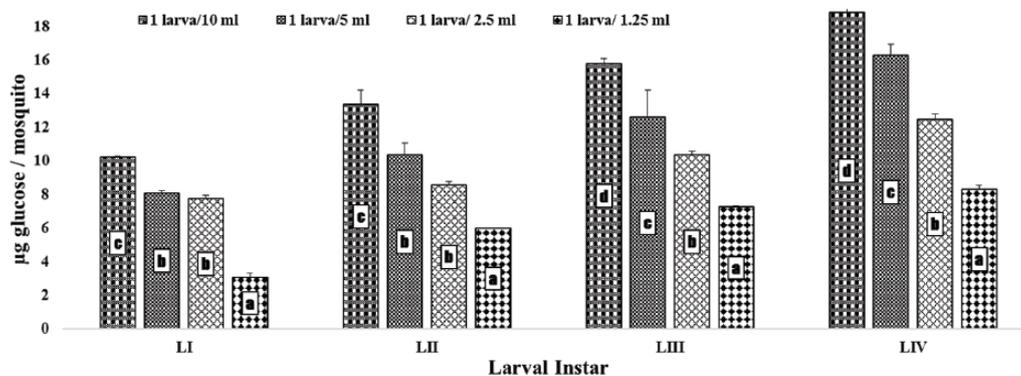
Figure 4. Effects of rearing density on glucose accumulation in larval instars of *C quinquefasciatus* mosquito. Bars with same letter are not significantly different ($P < .05$) according to analysis of variance. Values are expressed as mean ± SD.

Glycogen composition. Analyses revealed a similar trend in glucose composition as in lipid and glucose composition (Figure 5; Table 6). Progression through larval instars was associated with increasing glycogen content; this, however, reduced at pupal and adult stages. Pupae and adult mosquitoes from density treatment of 1 larva: 10 mL had the highest glycogen contents (44.72 ± 0.37 μg glycogen/pupa and 42.91 ± 2.30 μg glycogen/adult, respectively), with the highest larval daily accumulation rate (5.45 ± 0.37 μg glycogen/larva/day) (Table 6).

Protein composition. Increase in larval density significantly ($df = 4, P < .05$) reduced the quantity of protein accumulated at all life stages of *C quinquefasciatus* mosquito (Figure 6; Table 7). At all larval density treatment, earlier larval instars of the species had significantly lower protein contents than the succeeding instar (Figure 6), and adult life stage, consistently, had lower protein content than the pupal stage (Table 7).

The rate of larval accumulation in the species was highest (5.34 ± 0.33 μg protein/mosquito/day) at the lowest density

Table 5. Effects of larval rearing density on life stages' composition and larval accumulation rates of glucose in *Culex quinquefasciatus* mosquito.

DENSITY REGIMEN (LARVA: ML)	PUPAE (µG GLUCOSE/PUPA)	ADULT (µG GLUCOSE/ADULT)*	LARVAL ACCUMULATION RATE (µG GLUCOSE/LARVA/DAY)
1: 1.25	7.56 ± 0.48[a]	5.55 ± 0.39[a]	0.57 ± 0.01[a]
1: 2.5	9.85 ± 0.67[b]	7.91 ± 0.43[b]	0.85 ± 0.03[b]
1: 5	14.34 ± 0.73[c]	13.25 ± 0.25[c]	1.44 ± 0.07[c]
1: 10	17.27 ± 0.07[d]	15.44 ± 0.28[d]	2.22 ± 0.14[d]

Values followed by same superscript alphabet in a column are not significantly ($P < .05$) different. All values are expressed as mean ± SD of mean.
*Adult values are mean values of male and female lipid composition.
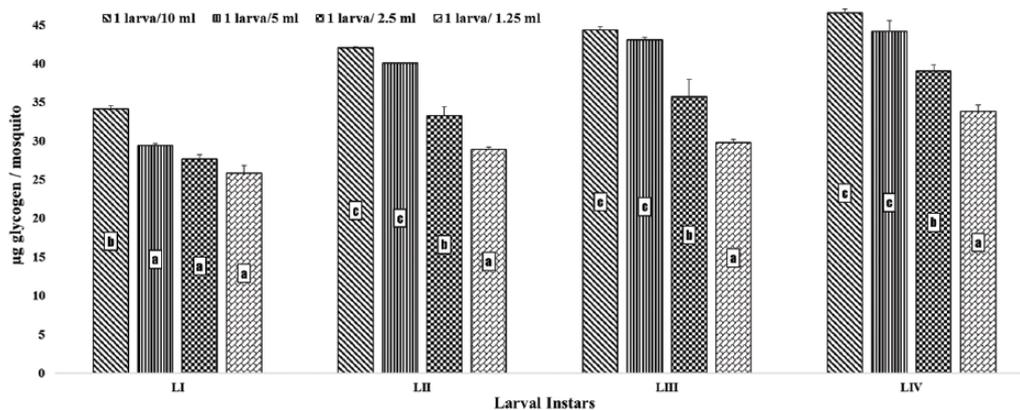
Figure 5. Effects of rearing density on glycogen accumulation in larval instars of *Culex quinquefasciatus* mosquito. Bars with same letter are not significantly different ($P < .05$) according to analysis of variance. Values are expressed as mean ± SD.

Table 6. Effects of larval rearing density on life stages' composition and larval accumulation rates of glycogen in *Culex quinquefasciatus* mosquito.

DENSITY REGIMEN (LARVA: ML)	PUPAE (µG GLYCOGEN/PUPA)	ADULT (µG GLYCOGEN/ADULT)*	LARVAL ACCUMULATION RATE (µG GLYCOGEN/LARVA/DAY)
1: 1.25	26.21 ± 0.12[a]	22.86 ± 1.72[a]	1.88 ± 0.92[a]
1: 2.5	31.22 ± 0.46[b]	27.97 ± 2.60[b]	2.66 ± 0.07[b]
1: 5	38.10 ± 2.42[c]	33.52 ± 0.55[c]	3.90 ± 0.16[c]
1: 10	44.72 ± 0.37[d]	42.91 ± 2.30[d]	5.45 ± 0.37[d]

Values followed by same superscript alphabet in a column are not significantly ($P < .05$) different. All values are expressed as mean ± SD of mean.
*Adult values are mean values of male and female lipid composition.

(ie, 1 larva/10 mL) and lowest (1.83 ± 0.03 µg protein/mosquito/day) at the highest density (ie, 1 larva/1.25 mL).

Effects of rearing density metabolic reserve for pupation and eclosion of C quinquefasciatus mosquitoes

The effects of larval density on metabolic reserve (lipid, protein, glycogen, and glucose) for pupation and eclosion in *C quinquefasciatus* are highlighted in Table 8. Generally, the mosquito species required more teneral components for pupation than for eclosion.

Higher larval densities utilised more protein, glycogen, and glucose contents for pupation. The total quantities and teneral components utilised for pupation varied significantly ($df = 4$, $P = .00$) among larvae from the different larval-density regimens. At the highest density (1 larva: 1.25 mL), the mosquito species expended more teneral components (13.56 ± 5.51 µg nutrient/mosquito) for eclosion than at lower density (1 larva: 10 mL; 7.22 ± 5.40 µg nutrient/mosquito) (Table 8).

Discussion

Effects of larval density on duration and larval GR of C quinquefasciatus mosquito

This study revealed significant negative impacts of increasing larval density on immature developmental times (ie, DD) and
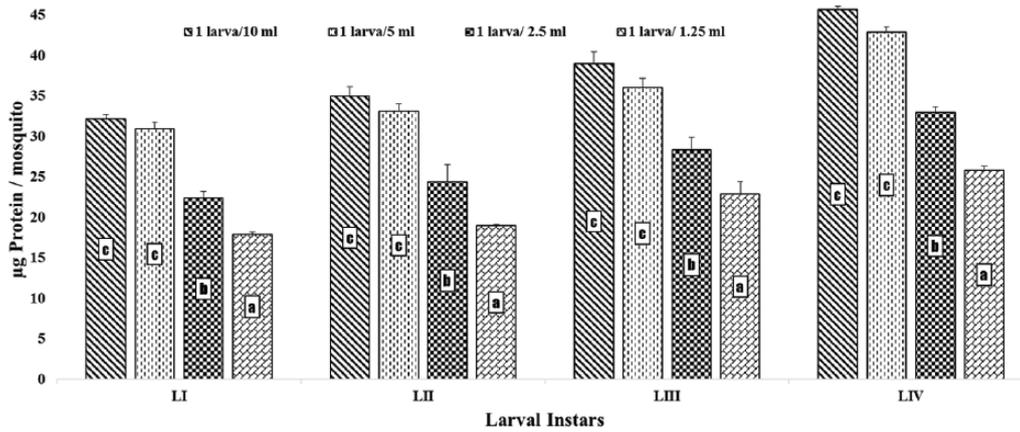
Figure 6. Effects of rearing density on protein accumulation in larval stages of *Culex quinquefasciatus* mosquito. Bars with same letter are not significantly different at $P < .05$ according to analysis of variance. Values are expressed as mean $\pm$ SD.

Table 7. Effects of larval rearing density on life stages' composition and larval accumulation rates of protein in *Culex quinquefasciatus* mosquito.

DENSITY REGIMEN (LARVA: ML)	PUPAE (µG PROTEIN/PUPA)	ADULT (µG PROTEIN/ADULT)*	LARVAL ACCUMULATION RATE (µG PROTEIN/LARVA/DAY)
1: 1.25	24.60 ± 0.37[a]	20.00 ± 2.14[a]	1.83 ± 0.03[a]
1: 2.5	29.58 ± 0.08[b]	24.76 ± 1.37[b]	2.79 ± 0.06[b]
1: 5	39.11 ± 0.76[c]	35.77 ± 0.60[c]	3.79 ± 0.08[c]
1: 10	42.58 ± 2.27[d]	41.28 ± 1.18[d]	5.34 ± 0.33[d]

Values followed by same superscript alphabet in a column are not significantly different ($P < .05$). All values are expressed as mean ± SD of mean.
*Adult values are mean values of male and female lipid composition.

larval daily weight gain (ie, GRs) of *C quinquefasciatus* mosquito. For example, as density increased, from 1 larva/1.25 mL to 1 larva/10 mL, there was general delay in DD in the larval instars and pupae.

In field studies, high relative density of immature life stages of the species within a habitat have been correlated with increased adult mosquito population within a specified time, with the implication of increased disease transmission tendency.[36,37] This study, although laboratory based, revealed that this increase in density within confined water space significantly delays the mosquito species' development time. A condition that may portend reduction in vector-human contacts, resulting from low adult emergence per unit time, and leading to decreased frequency of bites, hence, reduced transmission of pathogens. Field experimentations are, however, required to authenticate this observation.

Generally, in quantitative terms, mosquito cohorts reared at a density of 1 larva/1.25 mL spent about twice the time for those at 1 larva/10 mL to complete immature development. Among instars, LI mosquitoes reared at 1 larva/1.25 mL spent about 1.4 times the time spent by their counterparts at 1 larva/10 mL. LII, LIII, LIV, and pupae at the former density spent 2.2, 2, 2.4, and 1.5 times the time spent by the latter. These observations are very significant in epidemiology of disease vectored by this species (*C quinquefasciatus*), as

time spent in development determines vectorial efficiency of adult mosquito.[49]

The prolonged larval and pupal development times of *C quinquefasciatus* at higher *density* observed in this study may be attributed to, but not limited to, crowding-related stress conditions, such as general increase in body sizes with age, suppressed GRs (due to inadequate feed intake as a result of competition among the larvae), production of growth retardant chemicals, ie, growth retardant factor (GRF), and increased metabolic wastes production[50] which disrupt growth. More so, the delay could have been necessitated by the physiological need to attain threshold size for ecdysis and metamorphosis and accumulate teneral reserves for effective adult life.[51,52] The role of increased larval density in increasing DD has been reported in other *C* species: *C pipiens quinquefasciatus*,[6] *C quinquefasciatus*,[53] *C tarsalis*,[54] *C tritaeniorhynchus*,[55] and other mosquito genera.[15,56] Although, in other insects, overcrowding elicits shorter development times.[57–59]

Effects of larval density on survivorship of immature stages of *C quinquefasciatus* mosquito

In this study, survivorship of immature stages of *C quinquefasciatus* were, significantly, affected by density. Larvae and pupae mosquito cohorts reared at the lowest density regimen

Table 8. Effects of larval density on teneral reserve components (μg nutrient/mosquito) for pupation and eclosion in *Culex quinquefasciatus* mosquito.

DENSITY REGIMEN (LARVA: ML)	LIPID		PROTEIN		GLYCOGEN		GLUCOSE		AGGREGATE	
	PUPATION	ECLOSION	PUPATION	ECLOSION	PUPATION	ECLOSION	PUPATION	ECLOSION	PUPATION	ECLOSION
1:1.25	$3.59 \pm 0.42^{ab*}_{b**}$	$2.37 \pm 0.39^{b}_{b}$	$13.56 \pm 5.51^{c}_{d}$	$4.60 \pm 1.77^{c}_{c}$	$7.65 \pm 0.73^{c}_{c}$	$4.58 \pm 2.97^{c}_{c}$	$2.73 \pm 0.23^{b}_{a}$	$2.01 \pm 0.38^{c}_{a}$	27.53 ± 6.89^{b}	13.56 ± 5.51^{b}
1:2.5	$5.05 \pm 1.07^{c}_{b}$	$3.66 \pm 0.76^{d}_{b}$	$13.68 \pm 5.21^{c}_{d}$	$4.83 \pm 1.30^{d}_{c}$	$7.83 \pm 1.21^{c}_{c}$	$3.25 \pm 2.14^{b}_{b}$	$2.60 \pm 0.91^{b}_{a}$	$1.94 \pm 1.01^{b}_{a}$	29.16 ± 8.40^{b}	13.68 ± 5.21^{b}
1:5	$4.29 \pm 0.29^{b}_{a}$	$1.69 \pm 2.32^{a}_{b}$	$10.22 \pm 4.65^{b}_{d}$	$3.34 \pm 0.16^{b}_{d}$	$6.08 \pm 1.81^{b}_{c}$	$3.36 \pm 1.84^{b}_{b}$	$1.93 \pm 0.57^{ab}_{a}$	$1.83 \pm 0.33^{ab}_{a}$	22.52 ± 7.32^{b}	10.22 ± 4.65^{a}
1:10	$2.72 \pm 1.30^{a}_{b}$	3.02 ± 1.23^{c}	$7.22 \pm 5.40^{a}_{c}$	$1.30 \pm 1.09^{a}_{ab}$	$1.87 \pm 0.12^{a}_{a}$	$1.81 \pm 2.16^{a}_{a}$	$1.67 \pm 0.62^{a}_{a}$	$1.09 \pm 0.92^{a}_{a}$	13.48 ± 7.44^{a}	7.22 ± 5.40^{a}

Values followed by same superscript alphabet in a column are not significantly different at $P < .05$. Values followed by same subscript alphabet in a row (among teneral components of a process) are not significantly different at $P < .05$. All values are expressed as mean ± SD of mean.

(1 larva/10 mL) had the highest survivorship, while those at the highest density (1 larva/1.25 mL) had the lowest. There were, however, no difference in survivorship among larvae and pupae reared at 1 larva/10 mL and 1 larva/5 mL. Earlier studies by Suleman[53] on the species and Hooper et al[16] and Hirschberger[60] reported similar observations (ie, significant reduction in immature survivorship due to larval crowding) on other insect species.

The results from this study revealed evidence of developmental challenges by larvae of *C quinquefasciatus* in crowded environment (in terms of survivorship). Earlier field studies have revealed that gravid female of the mosquito[36,37] and other species in the genus, *C restuans*[31] and *C sitiens*[61] actively, search for habitats with conspecifics (a cue that suggests suitability for immature development); this tendency may result in overcrowded conditions (especially if the habitat is confined), which may result in reduced survivorship. Although there had been no report of 'avoidance' strategy by the species, Gimnig et al[15] reported that *Anopheles* mosquitoes avoid ovipositing in such confined crowded habitats, to avoid competition and predation.

Although the present data were generated from simulated crowded conditions, it suggests that *C quinquefasciatus* at high juvenile mortality is elicited at density greater than 1 larva/5 mL. This information could be deployed in developing cost-effective mosquito control protocols and in monitoring mosquito population outburst. Although subject to field trials, and information on larval density, habitat size, and volume of water, *C quinquefasciatus* mosquito in the wild from densely populated larval habitats may have lower survivorship, than in sparsely populated habitats.

Effects of larval density on emergence success, daily survivorship, and longevity of adult C quinquefasciatus mosquito

This study revealed significant negative effects of high larval density on number of emergent adults, daily survivorship, and post-emergence longevity of adult *C quinquefasciatus* mosquitoes. These entomological variables reduced, significantly, as density increased from 1 larva/10 mL to 1 larva/1.25 mL, and may have been elicited by the overcrowding experienced during immature life stage development of the species. Earlier, Roberts and Kokkinn[50] and Costanzo et al[62] opined that overcrowding in *C quinquefasciatus* and *C pipiens* is linked with competition. This results in modified foraging behaviour due to food and nutrient shortage, and exposure to and accumulation of waste products, which negatively affect adult survivorship.[38,50,61]

Although the biology of *Culex* species differs from those of other genera (*Anopheles* and *Aedes*), similar diminishing eclosion rates with increase in larval density have been reported.[10,63–65] Meanwhile, success at emergence has been positively correlated with increased vector-human contact,[66] while adult survivorship ensures, among others, foraging and mating successes,[67] and

longevity ensures development of pathogen within the mosquito and sucessess at pathogen transmission.[68]

This study provides further information on the influence of larval density on adult success traits of *C quinquefascatus* mosquito and, hence, capacity to transmit diseases. For example, the result revealed that at the highest density treatment, longevity was <3 days; these cohorts may not be, epidemiologically, important in disease transmission, as filarial parasites spend about 10 days within the mosquito to reach infective stage.[2,3,69,70] The reverse can be said for cohorts raised at lower density regimen, especially at 1 larva/10 mL. Further studies are advocated to verify the role of density regimens on vector–parasite interaction.

Effects of rearing density on teneral reserve accumulation and distribution among life stages of *C quinquefasciatus*

In mosquito ecology, overcrowding acting alone or coupled with limited food resources in *C quinquefasciatus* leads to either or both of exploitative (scramble) or interference competition.[52] This competition usually modifies the foraging behaviour, a situation which leads to variations in teneral reserve accumulation, especially during the phagoperiods.[61] These reserves (ie, lipid, glucose, glycogen, and protein) are chief determinants of quality of insect adult population and community dynamics.[51,59,60]

In this study, mosquitoes reared at very high density had significantly low teneral components. These low teneral values could be a direct effect of inefficient teneral mobilisation, which could have been elicited by altered food consumption rate and crowding-related stress conditions. Farrar et al[71] opined that food consumption rate in insects is a behavioural trait, which encompasses various physiological performances. Similar tendencies (ie, reduced food consumption due to crowding) have been reported in *C quinquefasciatus*,[50] *C sitiens*,[61] and lepidopterans.[72,73] In other insect species and orders, indifference in feeding rhythms[74] and increased food consumption[75,76] have been reported.

Effects of rearing density on utilisation of metabolic reserve for pupation and eclosion of *C quinquefasciatus* mosquitoes

Modified foraging behaviour and altered consumption rate can affect the total amount of energy intake and, hence, metabolic reserves.[77,78] These also affect the quantity of energy required and available for the metamorphic processes of pupation and eclosion. Pupation and eclosion, themselves, are energy consuming processes, which determines the energy available at adulthood and, hence, adult-life quality.

In this study, *C quinquefasciatus* mosquito utilised more teneral reserves for pupation than for eclosion. This was expected, as the process of pupation involves tissue re-organisation – a high-energy demanding process – that prepares the insect for life outside the aquatic environment.[79] This study also revealed disparity in the total amount of teneral reserve components available for pupation and eclosion across different regimens of mosquito densities. The mosquito cohorts reared at the lowest density regimen (1 larva/10 mL) required the least energy for pupation and eclosion, while those at the highest (1 larva/1.25 mL) required the highest.

The results from the study suggest that when subjected to different density regimen, *C quinquefasciatus* mosquitoes require greater amount of energy for pupation, than for eclosion. More so, as density regimen increased, the total amount of reserves and individual teneral component required for pupation greatly increased. Likewise, total amount of metabolic reserves and individual teneral component, available for eclosion (though lower than that required for pupation), was also density-dependent.

The above implies that *C quinquefasciatus* mosquito cohorts reared at higher density regimen may have depleted energy reserves for survival post-emergence. This condition may be responsible for the ephemeral post-emergence longevity observed in this study. More so, greater energy demand during metamorphosis by mosquitoes reared at higher density may signal impaired structural and developmental processes, resulting in greater demand of energy for repairs.

Conclusions

The results from this study are suggestive of reduction in biological fitness of *C quinquefasciatus* in overcrowded habitat conditions. Growth in the mosquito species from overcrowded treatment was impaired and manifested as delayed developmental times, reduced immature survivorship, reduced adult emergence success, daily survivorship, and short-lived. More so, these mosquitoes expended greater teneral reserves for metamophosis. Further studies are recommended to determine what (1) common levels of overcrowding are in the field, (2) whether overcrowding has comparable effects with those mentioned in this study, and (3) whether energy consumption for pupation and eclosion are comparable with field studies.

ORCID iD

Azubuike Christian Ukubuiwe https://orcid.org/0000-0002-7409-7568

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