AFIT Journal of Science and Engineering Research, 2022 1(1): 1-14
ISSN: 2786-9873; e-ISSN: 2786-9811
Official Publication of Air Force Institute of Technology, Kaduna Nigeria.

Influence of Crowded Breeding Conditions on Morphometry and Biological Fitness Indices of *Culex quinquefasciatus* Mosquito (Diptera: Culicidae): Implication for Disease Transmission

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Abstract
The present study attempts to use morphometry to elucidate the influence of larval crowding on life stages' ontogenic progression of Culex quinquefasciatus. Four mosquito crowding conditions were adopted; namely; 1 larva in 1.25, 2.5, 5.0 and 10.0 cm^3*. Each density regimen was setup in four (4) replicates, with a repeat after the first experimentation. A total of 4,480 mosquitoes, comprising of 3,200 immature and 1,280 adult life stages were used in the study. Immature (larval and pupal) and adult body parts were measured at all life stages and density regimens. Lengths, widths and surface area of adult wings were determined. Volumes of fourth instar larvae and adult and wing fluctuating asymmetry were also determined. Analyses revealed crowding-dependent reduction in body size of all life stage and fitness indices with increase in crowding conditions. The information generated in this study is crucial in understanding the inter-play between density and biological fitness of Cx. quinquefasciatus.*
Keywords*: Ecological factor, Fitness, Morphology, Overcrowding.*

INTRODUCTION

In mosquito ecology, interactions between vectors and ecological factors are epidemiologically important in determining disease transmission potential of the vectors (Hinne *et al.* 2021). Such interactions often produce effects that alters the life history-traits of the vectors (Couret *et al.* 2014). Mosquito larval breeding conditions is one of the ecological factors that play critical role in determining the biological fitness of adult mosquitoes and the resultant capacity of such to act as vectors of disease pathogens (Dida *et al.*, 2018). Mosquito larval crowding conditions are crucial in determining the quality of adult mosquitoes (Muriu *et al.*, 2013). Studies have shown that such conditions result in various degrees of competition in insects, which affect physiologic and metabolic processes (Shaw *et al.*, 2019; Ukubuiwe *et al.*, 2019a; Cator *et al*, 2020).

Environmental growth conditions during preimaginal life stages of mosquitoes, especially, as larvae, affect physiological fitness. Body sizes of mosquito have been estimated from wing length, and its cube values serving as proxy for volume (Aminuwa *et al.*, 2018). Disparity in body sizes of mosquitoes have been correlated with several indicators of fitness such as survival rates and developmental rates (Ukubuiwe *et al.*, 2016). Others include host seeking capability and blood meal acquisition and utilization, teneral reserve accumulation, fecundity, infectivity rate and reproductive success (Brand *et al.*, 2016).

The technique of morphometry (i.e., measuring body parts) has been used to analyse the influence of various ecological (developmental) variable, especially, environmental and developmental stress on mosquitoes (Olayemi *et al.,* 2016; Aminuwa *et al.,* 2018). Measurement of body parts of immature and adult life stages have provided information on growth response of mosquitoes and their interactions with ecological variables (Ukubuiwe *et al.* 2018). Through morphometry, mosquito fitness indices such as volumes of adult (an indication of blood-meal acquisition propensity) and fourth larval stage (biomass accumulation indicator) have been estimated (Ukubuiwe *et al.*, 2019b); thereby opening gateway to understanding the physiology of these insects of public health concerns.

Although, laboratory studies have revealed variation in mosquito adult size due to larval crowding conditions, there is, however, dearth of information on morphometric evaluations of this interaction, especially, in *Cx. quinquefasciatus.* More so, there is little or no quantitative reports of morphometric evaluations of these phenotypic expressions across life stages progression in the species. This study was therefore, designed to elucidate the changes in the length of body parts of the life stages of *Cx. quinquefasciatus* under different larval crowding conditions.

MATERIALS AND METHODS

Duration and Location of the Study

The study was carried out during the months of May to October, 2017 and at the Insectary of the Entomological Unit of the Department of Animal Biology, Federal University of Technology, Minna, Nigeria.

Source and Rearing of Experimental Mosquitoes

Experimental mosquitoes were obtained from the insectary of the Entomological unit of the Department of Animal Biology, Federal University of Technology. Newly laid egg rafts of *Culex quinquefasciatus* were collected and incubated in transparent plastic hatching trays for 24 hours at ambient conditions of 27 ^oC, 78.24 % and 14:10 L: D, respectively, for mean temperature, relative humidity and photoperiod.

Hatched out larvae were reared at the different larval density simulated. The larvae were fed pulverised fish feed (Coppens®) at the rate of 0.32 mg/ larva, sprayed gently over the surface of the rearing trough until pupation. On pupation, the pupae were collected and placed in adult holding cages for emergence and maintained using 10% sucrose soaked in clean cotton pad (Ukubuiwe *et al.,* 2016).

Simulation of Larval Density Regimens

Four larval density conditions were adopted in the present study as reported earlier (Ukubuiwe *et al.,* 2019a). These conditions were obtained by varying the numbers of immature mosquitoes in fixed volume of distilled water. The larval conditions were 1 larva per 1.25, 2.50, 5.00 and 10.00 cm^3 of distilled, obtained by rearing 400, 200, 100 and 50 larvae in 500 cm^3 of water. Each larval density was in four (4) replicates. A second round of experimentation was carried out after the first to increase the population size.

Sample Size of Measured Mosquitoes

A total of 4,480 mosquitoes, comprising of 3,200 immature and 1280 adult life stages were measured in the study. The experiment was setup in four (4) replicates and the whole setup repeated immediately after the first study (i.e., duplicated). Therefore, for a density regimen, 1,120 mosquitoes were measures (i.e., 800 immature and 320 adult life stages). This is exemplified below:

A schematic Presentation of the Measured Mosquito Sample Size

Life Stage	Number measured (A)	Number of replicates (B)	Number of Treatments (C)	Round of Experimentation (D)	Number measured in a Life Stage/instar (AxBxCxD)	Total in the Study
			Immature Life Stage			
L1*	20	4	4	2	640	
L2	20	4	4	2	640	
L3	20	4	4	2	640	
L4	20	4	4	2	640	4,480
Pupae	20	4	4	2	640	
			Adult Life Stage			
Male	20	4	4	2	640	
Female	20	4	4	2	640	

***L1 = First Instar Larvae**

Morphometric Parameters

All body measurements were carried out at 4X magnification (with conversion factor of 0.263), with an ocular micrometer mounted on a calibrated binocular microscope (Model: Olympus XSZ-107BN). Total larval body length was determined as sum of lengths of head, thorax and abdomen. While total pupal body length was estimated as the sum of cephalothorax and abdominal lengths (Ukubuiwe *et al*., 2018). Larval morphometric data include lengths of antennae, head, thorax, abdomen, papillae, siphon and total body length. Widths of the head, thorax, abdomen, and siphon were also measured. Pupal Morphometric data include lengths of cephalothorax, trumpet, abdomen and total pupal length were determined. Adult Morphometric data include measurement of lengths, widths and surface area (a product of length and width of each wing) of wings, length and width of the abdomen (Ukubuiwe *et al*., 2018).

Other parameters measured include volumes of fourth instar larvae (a proxy for biomass accumulation), and adult (an index for blood-meal ingestion) were estimated as cube of the width of the thorax, and wing length. Fluctuating asymmetry (i.e., ptero-fitness) was determined as difference between the right and left wings (Olayemi *et al.*, 2016).

Data Analysis

Data obtained from measurements of body parts were processed and expressed as means and standard deviation using Statistical Package for Social Scientists (SPSS) version 21. One-way and two-ways Analysis of Variance (ANOVA) were used as appropriate to compare differences in means of lengths and widths of body parts among density regimen. Duncan Multiple Range Test (DMRT) was used to separate the means. All decisions on statistical comparison of means were taken at p=0.05 level of significance.

RESULTS

Effects of Larval Density on Morphometrics of Larval Stages of *Culex quinquefasciatus*

Lengths of Larval body parts:. Analyses revealed a significant (p = 0.002, df = 159) effect of larval density on the lengths of some body parts of first larval instar, L1. Apart from thoracic, antannal and anal papillal lengths, the lengths of the head capsule, abdomen and siphon of arvae reared at 1 larva/ 1.25 cm^3 were significantly (p = 0.032, df = 159) reduced (Figure 1a).

Similar trend as L1 was observed as the larvae progressed to L2. However, only, the lengths of the thorax and antennae were not significantly (p = 0.084, df = 159) affected; all other body parts were significantly reduced at 1 larva/ 1.25 cm^3. Second instar larve reared at 1 larva/ 10 cm^3 had, significantly, longer body parts (Figure 1b).

At L3, analyses also revealed significant reduction in lengths of body parts of this life stage as larval density increased from 1 larva/ 10 cm^3 to 1.25 cm^3. With larvae in the latter smallest,while those in the former longest. This was the case for all the body parts, except, the antennae (Figure 1c).

Interestingly, the effects of increasing larval density on lengths of body part of larvae were most evident at the fourth instar (Figure 1d). Analyses revealed significant (p = 0.023, df = 159) variations in all body parts measured; with significantly longer larval body parts in crowding condition of 1 larva/ 10 cm^3 and 5cm^3 (Figure 1d).

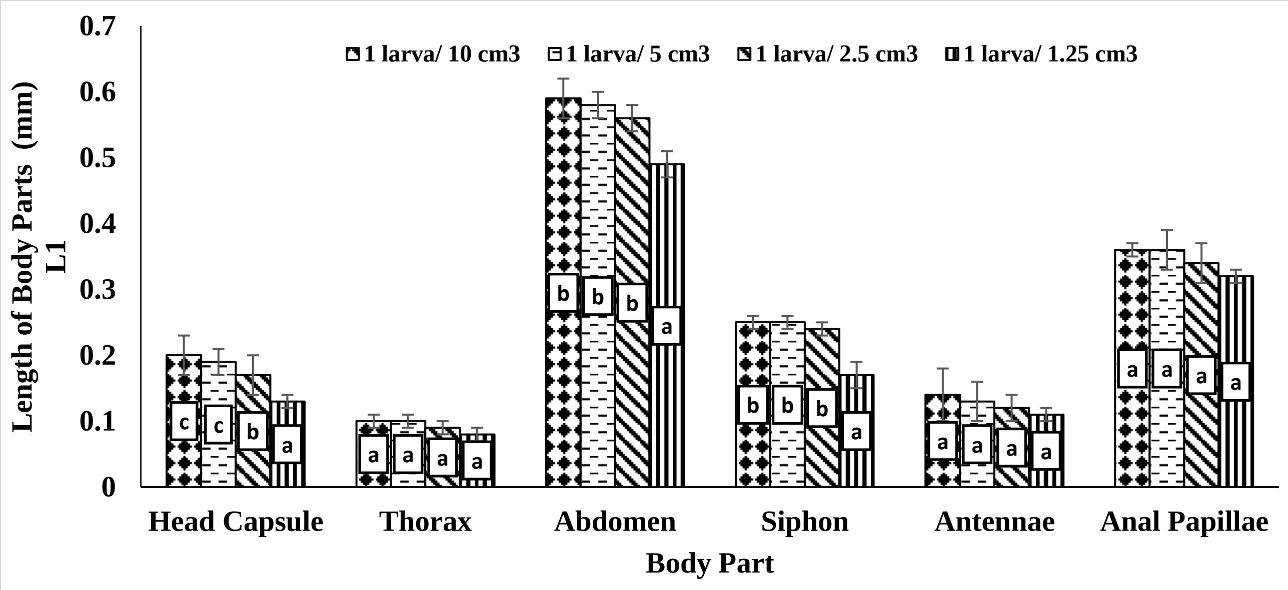

Fig. 1a: Effects of larval density on Length of Body Parts of First Larval Instar (L1) of *Cx. quinquefasciatus*. Bars with same letter are not significantly different at p<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA). Values expressed as Mean±SD

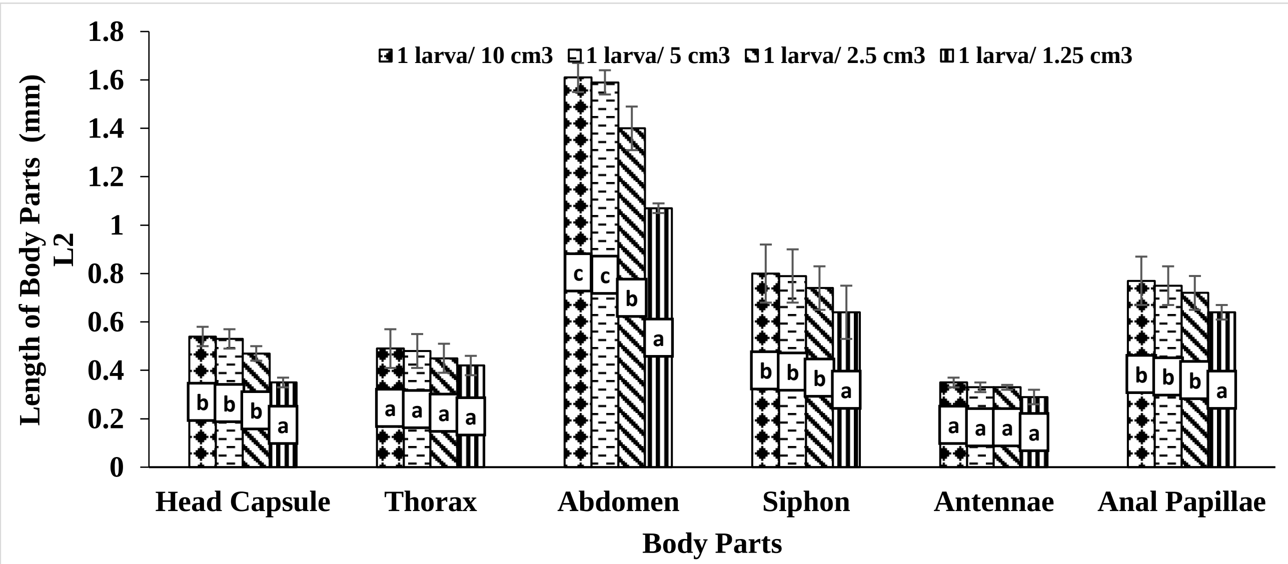

Fig. 1b: Effects of larval density on Length of Body Parts of Second Larval Instar (L2) of *Cx. quinquefasciatus*. Bars with same letter are not significantly different at p<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA). Values expressed as Mean±SD

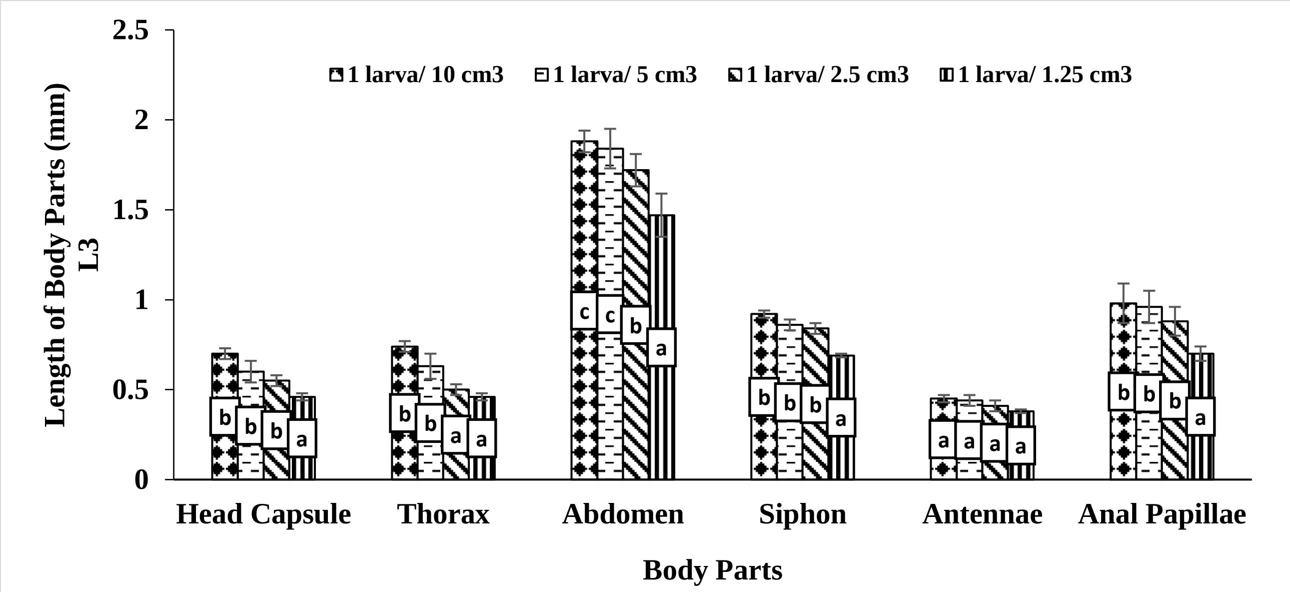

Fig. 1c: Effects of larval density on Length of Body Parts of Third Larval Instar (L3) of *Cx. quinquefasciatus*. Bars with same letter are not significantly different at p<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA). Values expressed as Mean±SD

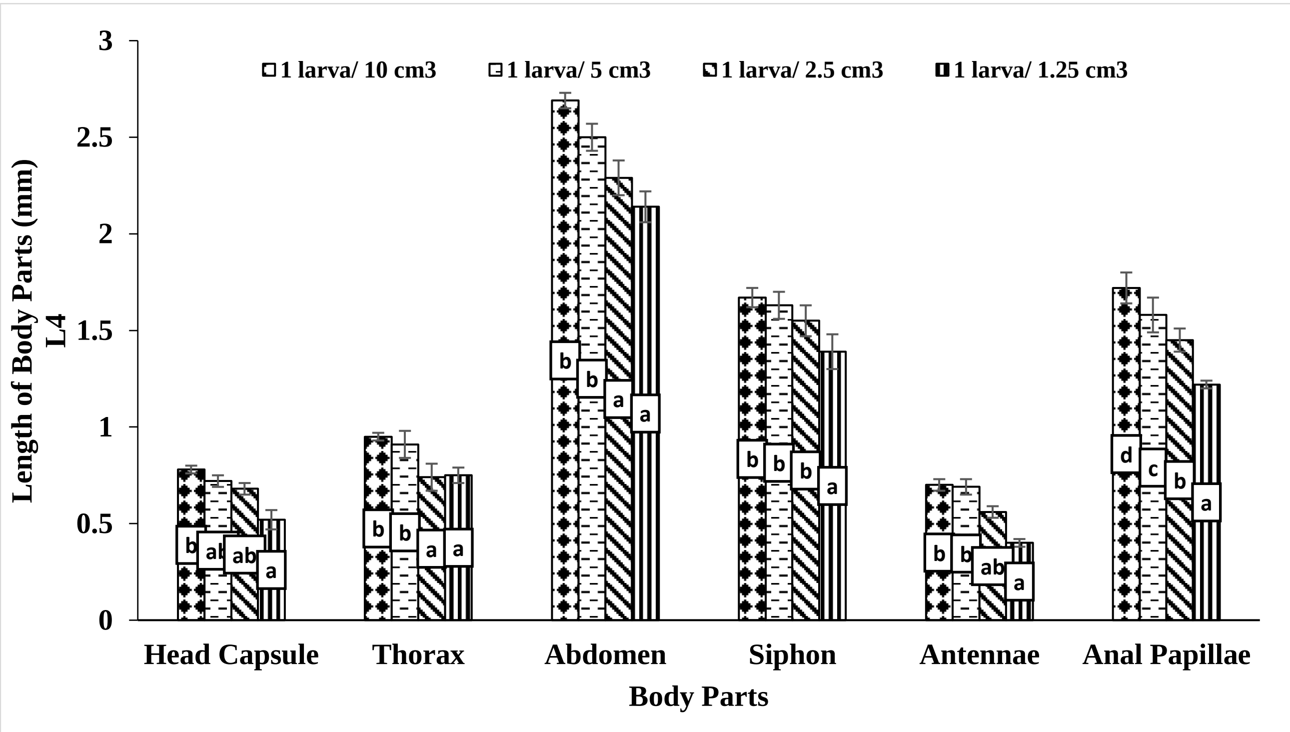

Fig. 1d: Effects of larval density on Length of Body Parts of Fourth Larval Instar (L4) of *Cx. quinquefasciatus*. Bars with same letter are not significantly different at p<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA). Values expressed as Mean±SD

Width of Larvae: At L1, analyses revealed no significant variation in the widths of the body parts of *Cx. quinquefasciatus*. However, at L2, significant changes were observed in the widths of the head capsule and abdomen; with the dimensions of mosquitoes from 1 larva/ 10 cm^3 widest and those from 1 larva/ 1.25 cm^3 narrowest (Figure 2b).

At L3, the changes were, remarkedly, visible (Figure 2c). Following similar trend as at L2, mosquitoes reared at 1 larva/ 5 cm^3 and 10 cm^3 had, significantly, wider body parts. At the last phagoperiod (i.e., L4), the effects of crowded conditions on the width of body parts were most evident; as mosquitoes reared at 1 larva/ 5 cm^3 and 10 cm^3 had the highest values.

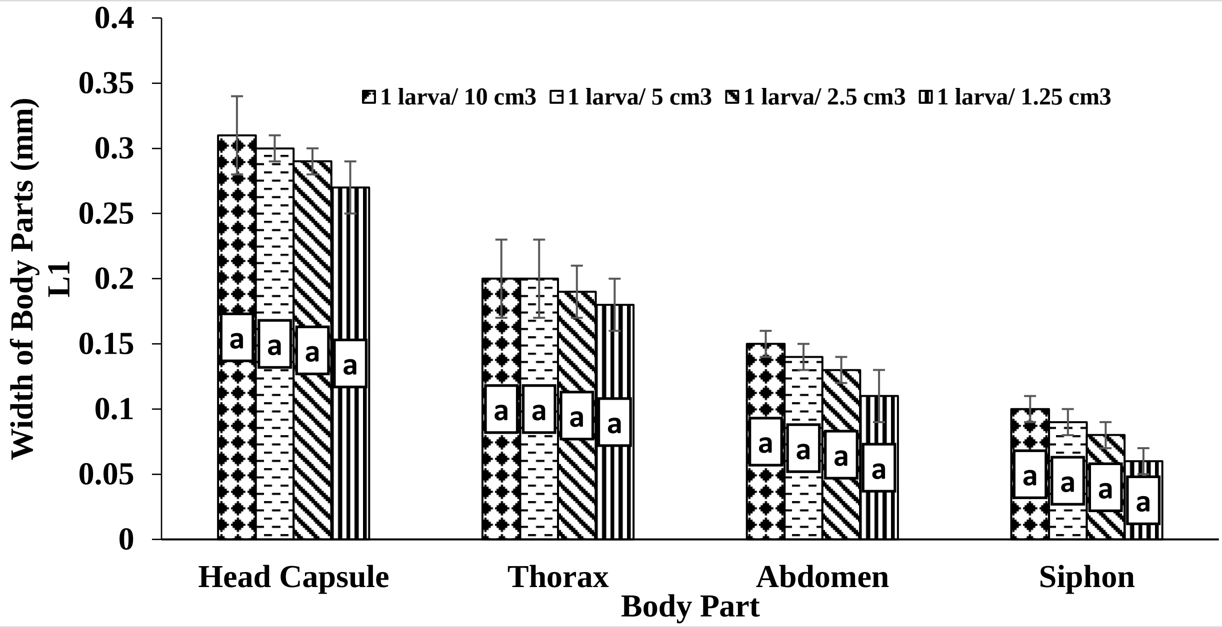

Fig. 2a: Effects of Larval Density on Width of Body Parts of First Larval Instar of *Cx. quinquefasciatus*. Bars with same letter are not significantly different at p<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA). Values expressed as Mean±SD

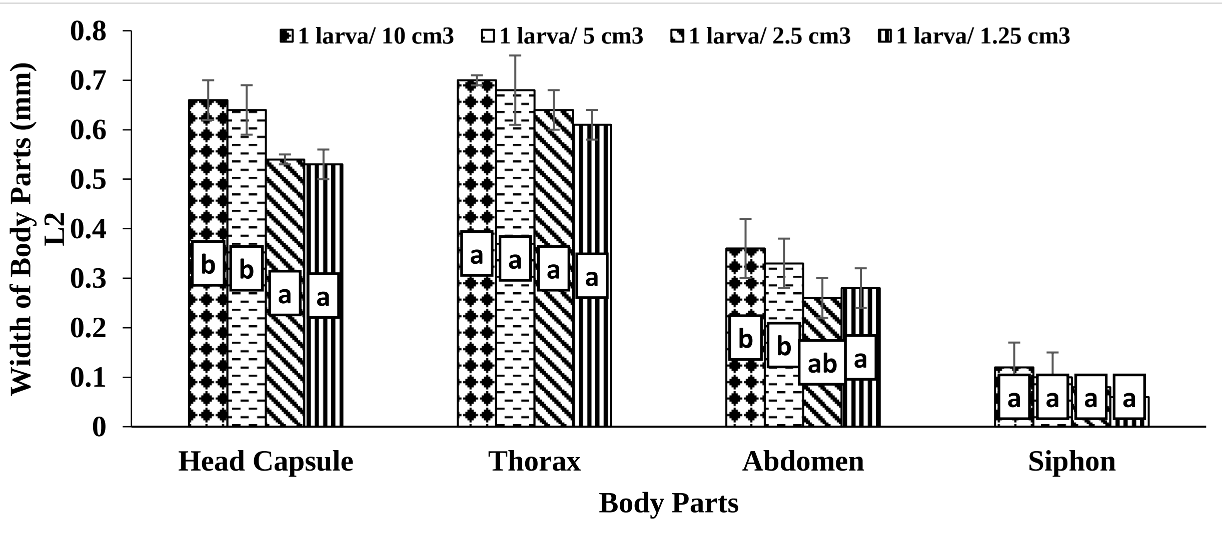

Fig. 2b: Effects of Larval Density on Width of Body Parts of Second Larval Instar of *Cx. quinquefasciatus*. Bars with same letter are not significantly different at p<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA). Values expressed as Mean±SD

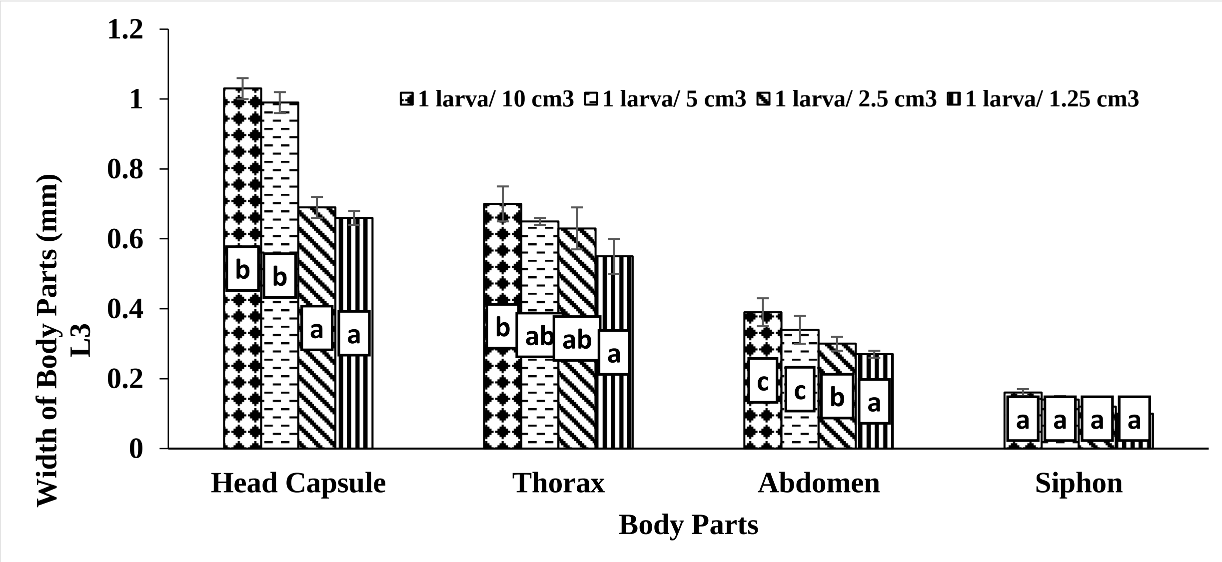

Fig. 2c: Effects of Larval Density on Width of Body Parts of Third Larval Instar of *Cx. quinquefasciatus*. Bars with same letter are not significantly different at p<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA). Values expressed as Mean±SD

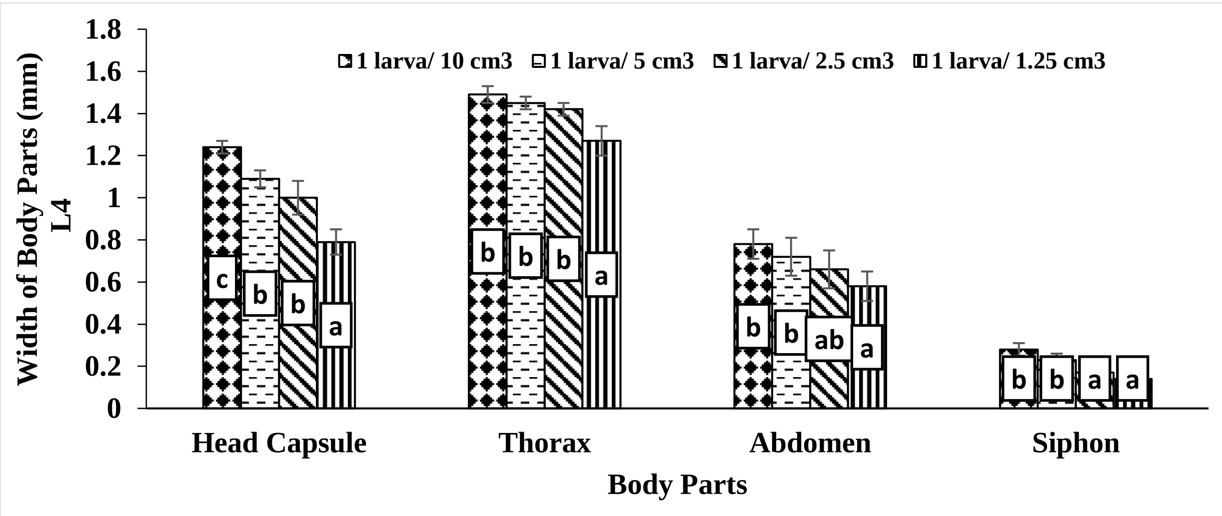

Fig. 2d: Effects of Larval Density on Width of Body Parts of Fourth Larval Instar of *Cx. quinquefasciatus*. Bars with same letter are not significantly different at p<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA). Values expressed as Mean±SD

Effects of Larval Density on Morphometrics of Pupal Stage of *Culex quinquefasciatus*

Analyses showed significant (p = 0.013, df = 159) variation in body lengths of pupae from the different larval density regimens. There were no significant differences in the length of cephalothorax and trumpet of pupae from 1 larva/ 5 cm^3 and 10 cm^3. However, the lengths of the abdomen varied, significantly, among the various density regimen. Generally, pupae from 1 larva/ 1.25 cm^3 rearing condition had the smallest body lengths (Figure 3).

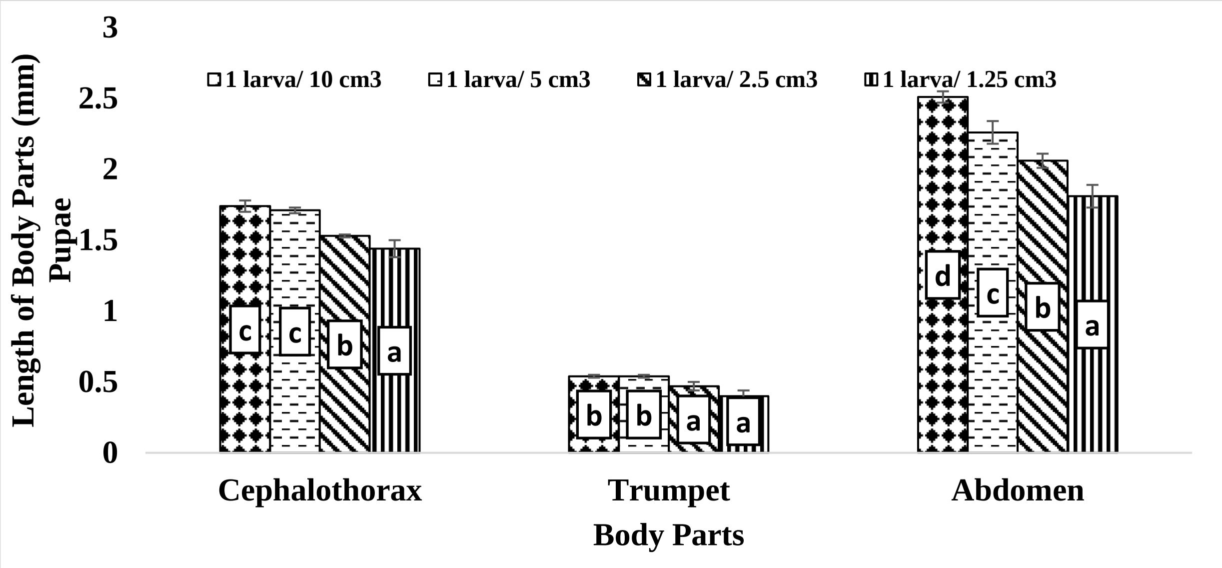

Fig. 3: Effects of Water Temperature on Length of Body Parts of Pupal Life Stage of *Cx. quinquefasciatus*. Bars with same letter are not significantly different at p<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA). Values expressed as Mean±SD

Effects of Larval Density on Total Body Lengths (TBL) of Immature Life Stage of *Culex quinquefasciatus*

Mosquitoes reared at 1 larva/ 1.25 cm^3 were the smallest. First instar larvae reared at 1 larva/ 1.25 cm^3 were the shortest (0.71±0.05 mm), while those reared at 1 larva/ 10 cm^3, the longest (0.89±0.04 mm). There were no significant difference ($p = 0.081$, df = 79) between the TBL of larvae reared at L2 and L3 at 1 larva/ 5 cm^3 and 10 cm^3.

At L4, analyses revealed significant ($p = 0.013$, df = 79) effects of larval density on the larvae; with the larvae reared at 1 larva/ 10 cm^3, the biggest (4.40±0.07 mm), while those at 1 larva/ 1.25 cm^3, the smallest (3.49±0.10 mm). Similarly, the total pupal body length followed the similar pattern as L4 (Table 1).

Table 1 Effects of Larval Density on Total Body Lengths of Immature Life Stages of *Culex quinquefasciatus*

Immature Stage		Dimension (mm) at Larval density			
		1 larva/ 10 cm^3	**1 larva/ 5 cm^3**	**1 larva/ 2.5 cm^3**	**1 larva/ 1.25 cm^3**
Larva	**I**	0.89±0.04^{c*}	0.86±0.02^{bc}	0.83±0.05^{b}	0.71±0.05^{a}
	II	2.64±0.10^{c}	2.60±0.05^{c}	2.26±0.06^{b}	1.98±0.08^{a}
	III	3.13±0.08^{c}	3.01±0.17^{c}	2.77±0.08^{b}	2.35±0.13^{a}
	IV	4.40±0.07^{d}	4.03±0.11^{c}	3.82±0.18^{b}	3.49±0.10^{a}
Pupae		4.79±0.09^{d}	4.51±0.11^{c}	4.20±0.05^{b}	3.72±0.18^{a}

*Values followed by same superscript alphabet in a row are not significantly different at P<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA); Values are expressed as Mean±SD

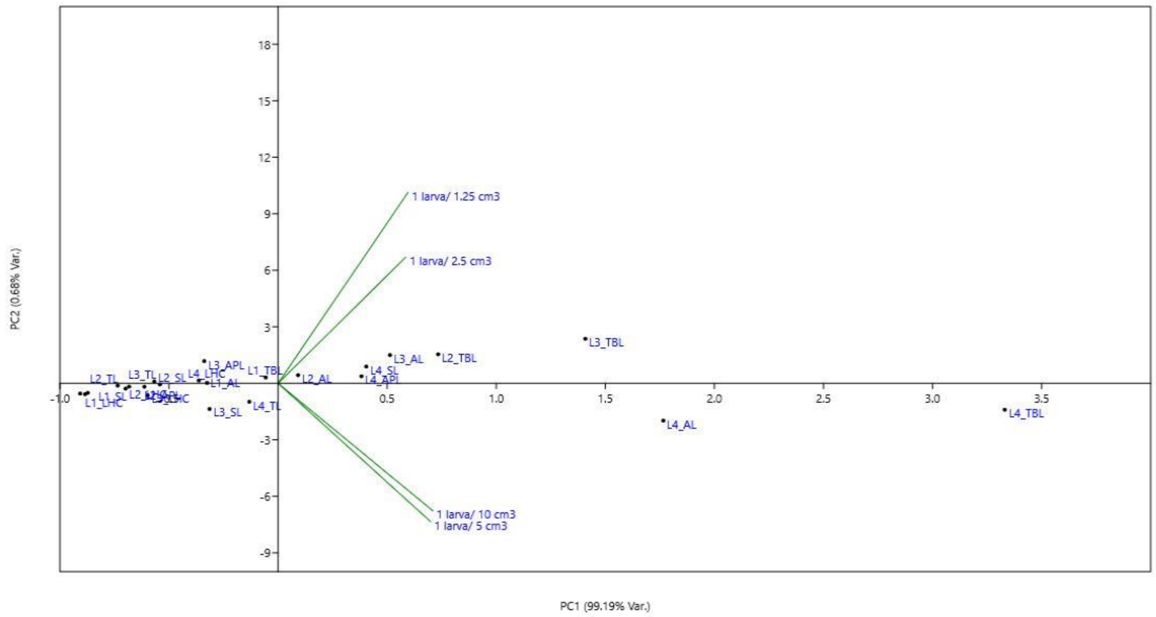

Figure 1a. Principal Components Analysis (PCA) biplots for influence of larval density on length of body parts of *Cx. quinquefasciatus* (n = 4,480). For each axis, the amount of variation explained by each axis is shown. Arrows indicate the direction and relative importance of larval density regimens. Density regimens: 1 larva in 1.25, 2.50, 5.00 and 10.00 cm^3 of distilled water. L1 to L4 (larval stages 1 to 4). Body Parts: HCL-Length of Head capsule, TL-thoracic length, AL-abdominal length, SL-siphonal length, APL-anal papillar length and TBL-total body length.

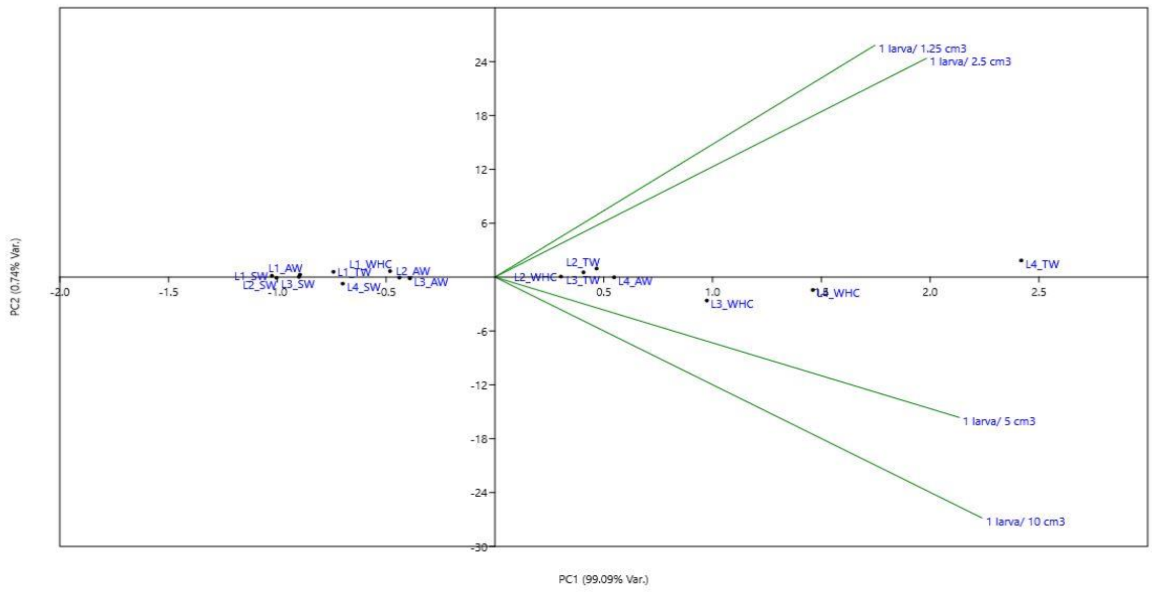

Figure 1b. PCA biplots for influence of larval density on widths of body parts of *Cx. quinquefasciatus* (n = 4,480). For each axis, the amount of variation explained by each axis is shown. Arrows indicate the direction and relative importance of the larval density regimens. Density regimens: 1 larva in 1.25, 2.50, 5.00 and 10.00 cm^3 of distilled water. L1 to L4 (larval stages 1 to 4). Body Parts: WHC-Width of Head capsule length, TW-thoracic width, AW-abdominal width, and SW-siphonal width.

Effects of Larval Density on Morphometrics and Fitness Attributes of Adult *Culex quinquefasciatus*

Adult mosquitoes from 1 larva/ 10 cm^3 were the biggest and had the lowest fluctuating assymetery (FA). The mosquitoes from this larval density had significantly (p = 0.01, df = 639) higher values of the body parts measured. Interestingly, mosquitoes reared at the highest density regimen had the lowest values for the body parts measured with the highest FA.

Table 2: Effects of Larval Density on Mophometrics and Vectorial Fitness Attributes of Adult *Culex quinquefasciatus*

Body Part	Parameter	Dimension (mm) at Larval density			
		1 larva/ 10 cm^3	1 larva/ 5 cm^3	1 larva/ 2.5 cm^3	1 larva/ 1.25 cm^3
Wing	Width (mm)	$0.78\pm0.02^{c*}$	0.76 ± 0.01^{b}	0.73 ± 0.03^{ab}	0.71 ± 0.03^{a}
	Length (mm)	3.16 ± 0.07^{c}	3.14 ± 0.04^{c}	3.05 ± 0.08^{b}	2.89 ± 0.11^{a}
	Surface Area (mm^3)	2.47 ± 0.12^{d}	2.37 ± 0.07^{c}	2.22 ± 0.11^{b}	2.09 ± 0.13^{a}
	FA**	0.00 ± 0.01^{a}	0.01 ± 0.01^{a}	0.02 ± 0.02^{b}	0.03 ± 0.01^{c}
Abdomen	Length (mm)	3.07 ± 0.10^{c}	3.04 ± 0.08^{c}	2.92 ± 0.10^{b}	2.79 ± 0.11^{a}

*Values followed by same superscript alphabet in a row are not significantly different at P<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA); Values are expressed as Mean±SD. **FA = Fluctuating Asymmetry (difference between right and left wings)

Effects of Larval Density on Volumes of Fourth Larval Instar and Adult *Culex quinquefasciatus*

Analyses revealed significant (p = 0.08) effects of density on volumes of L4 and adult life stage. The body volumes of L4 reduced with increasing larval density. Larvae reared at 1 larva/ 10 cm^3, also had the highest volume (3.31±0.30 mm^3), which was significantly higher than those reared at 1 larva/ 1.25 cm^3 (2.28±0.14 mm^3).

The volumes of the adult mosquitoes also varied significantly (p = 0.067) among the density regimens. Generally, mosquitoes reared at 1 larva/ 10 cm^3 had the highest volumes, closely followed by mosquitoes from 1 larva/ 5 cm^3, while those from 1 larva/ 1.25 cm^3 had the smallest volume. Further, the female mosquitoes were, significantly, bigger than their counterpart male mosquitoes (Table 3).

Table 3: Effects of Larval Density on Volumes of Fourth Larval Instar and Adult *Culex quinquefasciatus*

Density Regimen	Larval Volume (DT^3) (mm^3)	Adult Volume (WL^3) (mm^3)	
		Male	Female
1 larva/ 10 cm^3	$3.31\pm0.30^{c*}$	$25.69\pm2.42^{b}{}_{a**}$	$38.19\pm4.47^{b}{}_{b}$
1 larva/ 5 cm^3	3.06 ± 0.19^{b}	$24.66\pm2.03^{b}{}_{a}$	$36.19\pm4.47^{ab}{}_{b}$
1 larva/ 2.5 cm^3	2.77 ± 0.02^{ab}	$22.45\pm3.36^{ab}{}_{a}$	$35.52\pm3.75^{ab}{}_{b}$
1 larva/ 1.25 cm^3	2.58 ± 0.41^{a}	$19.31\pm1.45^{a}{}_{a}$	$30.05\pm5.96^{a}{}_{b}$

*Values followed by same superscript alphabet in a column are not significantly different at P<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA). **Values followed by same subscript alphabet in a row are not significantly different at P<0.05 according to Duncan Multiple Range Test (DMRT) following Analysis of Variance (ANOVA). All values are expressed as Mean±SD of Mean. DT^3= Cube of Diameter of fourth (L4) instar larvae. WL^3= Cube of Wing Length of Adult

Discussion

Effect of Larval Density on Morphometrics of Life Stages of *Culex quinquefasciatus*

The present study revealed progressive decrease in lengths of body parts and total body lengths of the life stages of *Cx. quinquefasciatus* mosquitoes, as density increased from 1 larva/ 10 cm^3 to 1.25 cm^3. Mosquitoes reared at 1 larva/ 10 cm^3 (i.e., 50 larvae/ 500 cm^3) were consistently bigger throughout the life stages, closely followed by those reared in 1 larva/ 5 cm^3 (100 larvae/ 500 cm^3).

The smallest mosquitoes were from cohorts reared at 1 larva/ 1.25 cm^3 (400 larvae/ 500 cm^3). Studies have revealed that overcrowded larvae often emerge as smaller adults, which have been associated with decreased survivorship, smaller teneral reserves, and reduced fecundities (Tsurim *et al.,* 2013). These conditions significantly affect vector competence in mosquitoes.

The effects of increasing larval density were minimal at early larval instars (L1 and L2), but evident at late larval instars (L3 and L4). This could be due to increased competition for limited resources (food and space) as the larvae increased in size or modified feeding rates and behaviour, especially, at these late larval instars.

Increase in larval age in an overcrowded environment elicit increased waste products excretion, semiochemicals production and secretion and/or release of other growth retardant factors (Tseng, 2004). These chemicals significantly affect growth and sizes of larval instars (Legros *et al.,* 2009). Density-dependent negative reduction in growth rate of has also been reported in *Aedes* mosquitoes (Bedhomme, *et al.,* 2003).

Effect of Larval Density on Wing Length and Fluctuating Asymmetry of *Culex quinquefasciatus*

The wing length (WL) of insects, especially, mosquitoes serves as a measure of body size. Its cubic values gives an estimate of the volume of the mosquitoes (Ukubuiwe *et al.,* 2019b). Wing fluctuating assymetry (FA), on the other hand, is a proxy for fitness of adult mosquitoes. Higher values in FA indicate the presence of environmental and/or genetic stress conditions during development (Mpho *et al.,* 2002).

The present study revealed a significant density-dependent decrease in WL in *Cx. quinquefasciatus* mosquito. There was a gradual decrease in mean WL as density increased from 1 larva/ 10 to 1.25 cm^3. Since WL is a proxy for adult body size, blood meal size uptake, and longevity (Briegel *et al.,* 2002), it therefore, implies that mosquitoes from 1 larva/ 10 cm^3 rearing condition are bigger, will take up more blood meal per time and will live longer; indication of efficiency as vector.

On the other hand, those reared at 1 larva/ 1.25 cm^3 will be less fit as vectors. Further studies is, however, advocated to confirm these submissions; although, in our earlier study (Ukubuiwe *et al.,* 2019a), mosquitoes in the former group (1 larva/ 10 cm^3) had significantly higher metabolic reserves (for life stages' activities) than the latter cohorts.

Based on wing measurements, the female mosquitoes were bigger (longer wing lengths) than male counterparts, even from the same tratment. Similar observations in sex-wise variation in wing lengths have been reported in *Aedes aegypti* (Macia´, 2009), and *Anopheles gambiae* (Muriu *et al.,* 2013), female *Ochlerotatus caspius* (Silberbush *et al.,* 2014).

However, unlike in the present study, Silberbush *et al.* (2014), reported that the size of adult male *O. caspius* were not affected by larval density. Furthermore, FA was relatively higher in mosquitoes reared at higher density regimen; an indicator of developmental stress in these cohorts of mosquitoes. This group may not be vector competent. Further studies are advocated to demonstrate vectoral competency in these cohorts of mosquitoes.

Effects of Larval Density on Volumes of Fourth Instar Larvae and Adult *Culex quinquefasciatus*

Analyses revealed greater volumes of fourth instar larvae and adult mosquitoes reared at 1 larva/ 10 cm^3 than those reared at 1 larva/ 1.25 cm^3. The former mosquito cohorts will have a higher biomass accumulation and blood-meal ingestion propensity, and hence, may be efficient vectors than the latter. This is epidemiologically important, considering the implications of these in disease pathogen transmission. Similar density-dependent observations have been reported in *Anopheles* mosquito species (Schneider *et al.,* 2000; Ye-Ebiyo *et al.,* 2003) and in other insects (Hooper *et al.,* 2003; Lazareviæ, 2004).

Body size is usually decreased in response to high density which could be associated with a decrease in development time or increased development time with supernumerary moults (Tammaru *et al*., 2000). Although, the latter is not possible in mosquitoes, as they can only afford four instars, however, the influence of high density on the mosquito larvae can be seen in the sizes of the fourth instar larvae, which is the last foraging stage, and an index for accumulation of biomass. As larvae in this study were fed *ad libitum*, the decreased body size associated with increased larval density could be due to stress conditions due to congestions which negatively affects digestive enzyme activities (Lazareviæ *et al*., 2004), hence, lowering degree of allocation of metabolic resources (Ukubuiwe *et al.,* 2019a). The reduction in body sizes of the fourth and adult could also be as a result of chemicals associated with high densities, like increased metabolic waste due to overcrowding and growth retarding factors. Increased density regiment have been reported to affect the accumulation of teneral reserves (Roberts and Kokkinn, 2010).

Conclusion

The present study has revealed the ontogenic progression of phenotypic plasticity due to larval crowding conditions. It also revealed that in *Culex quinquefasciatus*, the effects of over-crowding usually climaxed at fourth larval instar. Such information is crucial in understanding the inter-play between density and fitness of *Cx. quinquefasciatus* mosquito.

Acknowledgements

We wish to express our gratitude to Professor F. O. Arimoro for facilitating the grant from the United State Agency for International Development (USAID), USA, Higher Education Partnership/ University of Mississippi (UM).

Funding

This study was funded by a grant from United State Agency for International Development (USAID), Higher Education Partnership/ University of Mississippi (HEP/UM) (UM SUB-AWARD NO. 15-12-024).

Conflict of Interest

There is no conflict of interest.

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