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Research Article

Influence of Fluctuating Temperatures on Morphometry of *Culex quinquefasciatus* (Diptera: Culicidae) Mosquito

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Abstract

Background and Objective: The thermal response of *Culex quinquefasciatus* to increase in temperature was investigated in this study, using techniques of morphometry. This was aimed at understanding the interplay between temperature and body size of all life stages of the mosquito species. **Materials and Methods:** First instar larvae of the species were reared at 28.00, 30.00, 32.00 and 34.00°C till adulthood. Body parts were measured at every life instar and stage. Volumes of fourth instar larvae and adult were also determined. **Results:** Generally, temperatures above 30.00°C significantly reduced the dimensions of the body parts, resulting in small-sized mosquitoes with reduced biological fitness indices. The body parts of the species responded differentially to the temperature changes. **Conclusion:** The study, thus, puts in clearer perspective, phenotypic plasticity of life stages of the species at different thermal conditions.

Key words: Morphometry, biological fitness, thermal condition, phenotypic plasticity, size, dimensions

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mosquitoes are considered one of the most epidemiologically important medical insects, capable of infecting man with various disease pathogens through their ferocious blood-feeding biting habits. The burdens of vectored diseases have continued to ravage man, hampering growth and development in most third world countries, especially in tropical climates. The efficiency of these vectors are enhanced by the clemency of Afrotropical weather conditions, especially, temperatures. Being an ectotherm, the basal temperatures of mosquitoes are directly dependent on the temperature of the surrounding micro-environment. In insect ecology, temperature plays critical role in the distribution and abundance of a species^{1,2} and for each species, there exists a range, within which, development is optimised and vectorial success enhanced. Temperature values above or below this range, adversely affect the physiology of the species³. The importance of temperature cannot be over-emphasised, as it determines most biological fitness attributes of mosquitoes, such as immature survivorship, duration of development, emergence success, teneral accumulation and mobilisation, susceptibility to viruses, adult longevity, survivorship and body size⁴⁻⁷.

Studies have shown that the temperature of immature mosquito breeding habitats plays critical role in the resultant body size of the adult insect, an index for most biological fitness attributes of mosquitoes^{8,9}. For example, the body size of adults determines mating success, blood-meal intake, fecundity, survival, longevity, flight range and ultimately, fitness^{9,10}. Although, there exist an avalanche of empirical studies on the influence of various temperature regimens on almost all aspect of bio-eco-physiology of mosquitoes, little or nothing exist on the influence on body parts of larval instars, pupal and adult life stages. Further, most conclusions of the influence of temperature on development and growth of mosquitoes are based on data obtained from adult wing measurements¹¹⁻¹⁶. These, though accurate, are not sufficient to elucidate the complex morphological transformation associated with various temperature regimens, especially during the immature life stages (i.e., larval instars I-IV and pupae). Such information is vital in epidemiological studies for making informed-decisions and developing sustainable integrated vector control strategies.

With recent interest in the techniques of morphometry¹⁷, the influence of environmental variables on growth and development of insects, especially, culicidae can be better understood¹⁸. Quantitative data from the measurement of body parts of mosquitoes during pre-imaginal development, will provide first-hand-evidence on ontogeny of the species. For example, in mosquitoes, the head (enclosed by the head

capsule) is specialised for sensory inputs and bears the mouthparts for food intake, its size determines feed-intake proficiency. Cube of thoracic width of the fourth larval instar serves as index for biomass accumulation¹⁹. The abdomen contains most digestive, respiratory and reproductive structures and its length contributes to total body length of a species. The sizes of the siphon and trumpet are critical for gaseous exchange during immature development, while the anal papillae are for ion regulation²⁰. More so, wing traits serve as proxy for adult body size, fluctuating asymmetry and volume of blood-meal²¹.

Therefore, quantitative data on influence of temperature on their sizes are critical in understanding the interplay between temperature and biological fitness of a mosquito species. Thus, using techniques of morphometry, the present study aims at elucidating morphologic plasticity of mosquitoes at various temperature regimens, using *Culex quinquefasciatus* as a model species.

MATERIALS AND METHODS

Duration of study: This study was carried out at the Entomology Unit of the Department of Animal Biology, Federal University of Technology, Minna, Nigeria. The duration of the study was from April, 2017-June, 2018.

Simulation and maintenance of temperature regimens:

Four temperature regimens (28.00, 30.00, 32.00 and 34.00°C) were simulated in the study as described by Olayemi *et al.*¹⁵. Three of these regimens were generated and maintained (in four replicates) using digital thermostats (Model: 300W, LifeTech Aquarium® GB4706.67-2003) placed in aquaria filled with water. To be double sure and monitor fluctuations in temperature (if any) during the course of the experiment, mercury-in-glass thermometer were inserted into each aquarium and reading taken twice daily.

Source, rearing and maintenance of experimental insects:

Freshly laid eggs were obtained from the Insectary of the Entomology Unit of the Department of Animal Biology, Federal University of Technology, Minna. The larvae were reared and adults maintained following the methods described earlier²². Briefly, the egg rafts were incubated for 24 h and hatched-out larvae reared at the standard rate of 1 larva per 4 mL in the temperature-controlled water media. The larvae were fed daily by gently spraying pulverised fish feed (Coppens®) until pupation. Pupae were placed in adult holding cages till emergence, while successfully enclosed adult mosquitos were fed with clean cotton pad soaked in 10% sucrose till collection for morphometric analyses.

Sample size of measured mosquitoes: To provide for adequate sample size, each temperature regimen was setup in four replicates. The whole study was repeated immediately after the first (therefore, resulting in two rounds of experimentation).

Immature life stages: At each immature life instar or stage, 20 mosquitoes were selected, randomly for measurement of body parts. Hence, within a temperature regimen, 400 immature mosquitoes were measured comprising of 20 individuals \times 5 life instars/stages (LI-LIV instars and pupa) \times 4 replicates. Therefore, for the 4 temperature regimens, 1,600 immature mosquitoes for the 4 temperature regimens. This resulted in the measurement of 3,200 immature mosquitoes during the study.

Adult life stage: For adult measurements, 40 adult mosquitoes (comprising of 20 individuals each of male and female mosquito) were randomly selected from each replicate of temperature regimen. Hence, within a temperature regimen, 160 adult mosquitoes [i.e., 20 individuals \times 2 sexes (male and female) \times 4 replicates] were measured and a total of 640 adult mosquitoes for all temperature regimens for each round of study. This resulted in the measurement of 1,280 adult mosquitoes during the study.

Measurement of morphometric parameters: Immature morphometrics: Measurement of larval body was done according to the methods of Bar and Andrew²³ with slight modification, as reported by Ukubuiwe *et al.*²⁴. All body measurements were carried out at 4X magnification with an ocular micrometer mounted on a calibrated binocular microscope (with conversion factor of 0.263). For larval body parts, lengths and widths of head, thorax, abdomen and siphon were measured. Others include lengths of antennae, anal papillae and total body length (lengths of head+thorax+abdomen). Pupal body parts measured include length of trumpet, cephalothorax, abdomen and total pupal length (lengths of cephalothorax+abdomen).

Adult morphometrics: Adult body parts measured include wing lengths, widths and surface area (a product of length and width of each wing), length and width of the abdomen²⁴.

Vectorial attribute parameters: The volume of 4th instar larvae (a proxy for biomass accumulation) was estimated as cube of the width of the thorax and volume of adult (an index for blood-meal ingestion) was determined as

cube of length of the wing. Fluctuating asymmetry, FA (i.e., difference between right and left wings of the mosquito and proxy for ontogenic stress) was also determined¹⁹.

Data analysis: Morphometric data generated from the study were processed into means and standard deviation using Microsoft Office Excel 2016. Differences in means of lengths and widths of body parts among temperature treatments were compared using one-way and two-ways Analysis of Variance (ANOVA) as appropriate. The means were separated using Duncan Multiple Range Test (DMRT) and decisions on statistical comparison of means were taken at $p = 0.05$ level of significance. Principal Components Analysis (PCA) was used to determine the influence of water temperature on adult morphometrics.

RESULTS

Effects of water temperature on length and width of head capsule of *C. quinquefasciatus*: Analyses showed significant ($p < 0.05$) effects of rearing water temperature on the length of the head capsule in all larval instars (Fig. 1a). The mosquitoes reared at 34°C, consistently had the shortest head capsule, while those reared at ambient ($28.66 \pm 0.32^\circ\text{C}$) and 30°C had the highest values (Fig. 1a, b). At the LIII and IV stages, temperatures below 34°C did not significantly ($p > 0.05$) affect the length of the head capsules of species. The width of LIV was widest at 30°C.

Effects of water temperature on thoracic length and width of *C. quinquefasciatus*: During the first (LI) and second (LII) instars, the longest thorax was recorded at 30°C. As the species progressed to LIII and IV, the longest thoracic length was observed at temperature below 32°C (i.e., $\leq 30^\circ\text{C}$) (Fig. 2a). Temperatures above 30°C produced the smallest lengths. The width of the species at LI and II instars were not significantly affected by temperature below 34°C. However, among the last larval instar (LIV), the cohorts reared at 30°C had the widest width (Fig. 2b).

Effects of water temperature on abdominal length and width of *C. quinquefasciatus*: At LI, there were no significant effects of water temperature on the length of abdomen of the species. However, at LII to IV, temperature increase significantly reduced abdominal length. Generally, cohorts reared at temperatures 34°C had the lowest lengths (Fig. 3a) and widths (Fig. 3b).

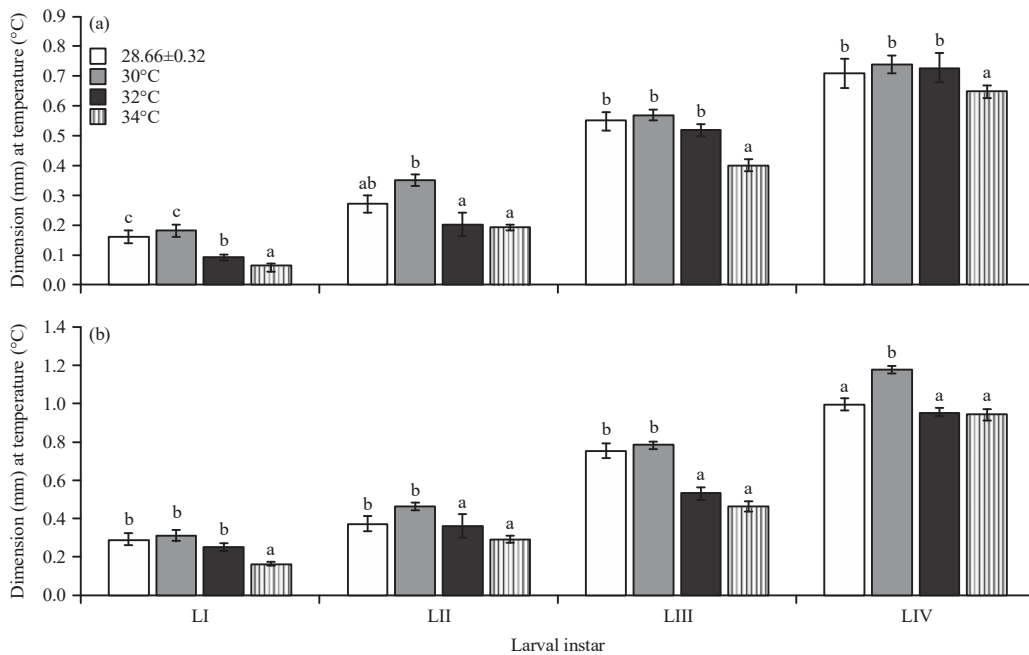


Fig. 1(a-b): Effects of water temperature on (a) Head capsular length and (b) Width of *C. quinquefasciatus* mosquito larvae. Bars with same letter are not significantly different at $p < 0.05$ according to analysis of variance. Values expressed as Mean \pm SD

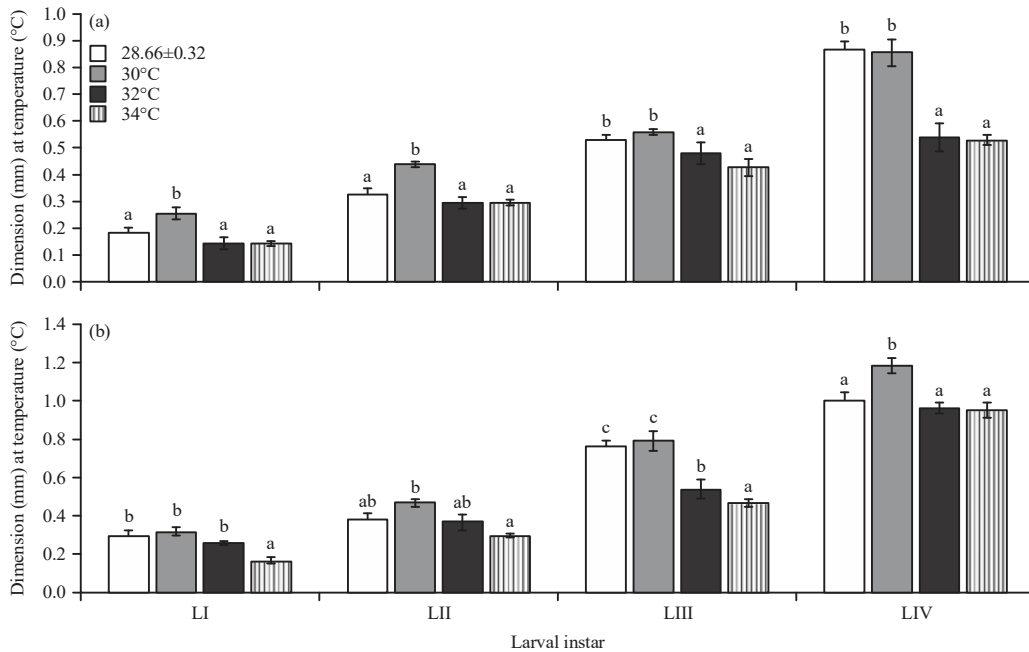


Fig. 2(a-b): Effects of water temperature on (a) Thoracic length and (b) Width of *C. quinquefasciatus* mosquito larvae. Bars with same letter are not significantly different at $p < 0.05$ according to analysis of variance. Values expressed as Mean \pm SD

Effects of water temperature on siphonal length and width of *C. quinquefasciatus*. The length and widths of the siphon of all larval instars were significantly reduced at temperatures above 32°C (Fig. 4a, b).

Effects of water temperature on antennal length of *C. quinquefasciatus*. The antennae of the species showed similar response to temperature increase as other body parts measured (Fig. 5).

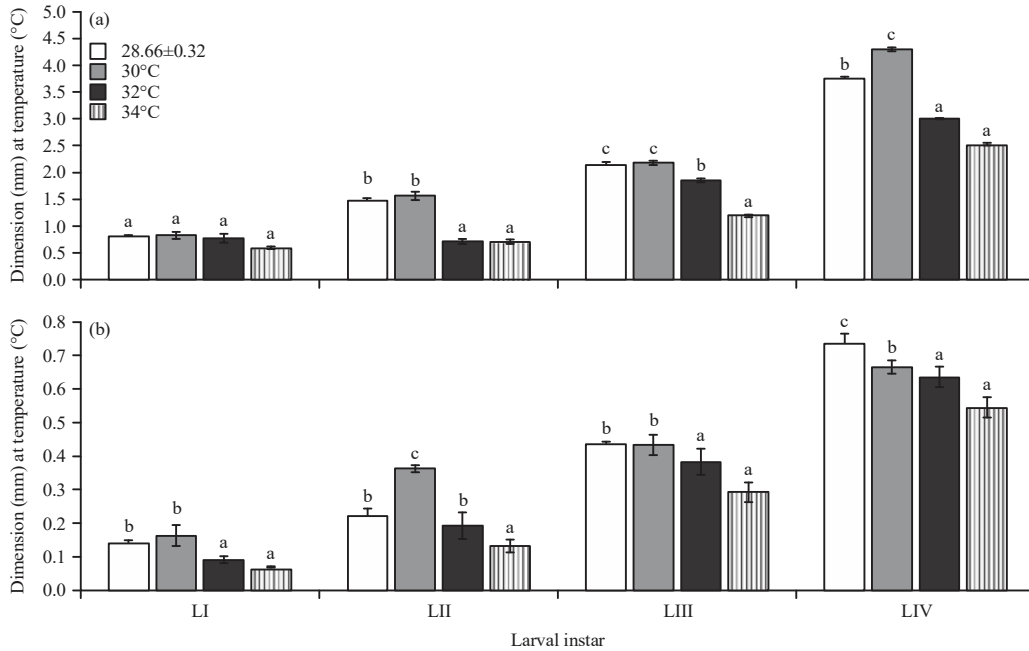


Fig. 3(a-b): Effects of water temperature on (a) abdominal length and (b) width of *C. quinquefasciatus* mosquito larvae. Bars with same letter are not significantly different at $p < 0.05$ according to analysis of variance. Values expressed as Mean \pm SD

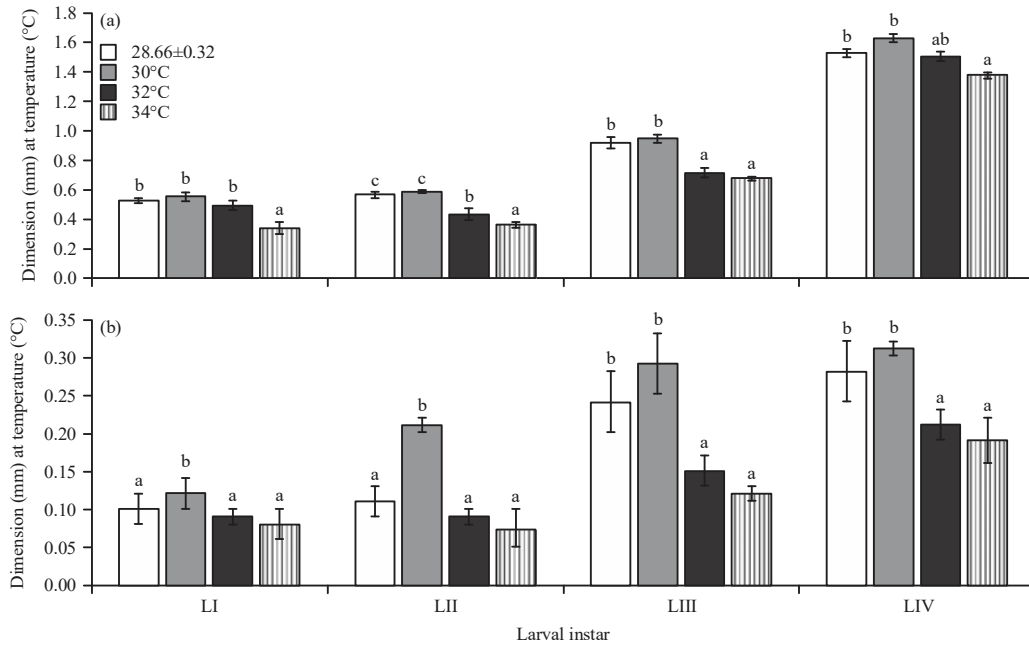


Fig. 4(a-b): Effects of water temperature on (a) Siphonal length and (b) Width of *C. quinquefasciatus* mosquito larvae. Bars with same letter are not significantly different at $p < 0.05$ according to analysis of variance. Values expressed as Mean \pm SD

Effects of water temperature on anal papillae of *C. quinquefasciatus*. The anal papillae of all instars responded, negatively, to the increase in temperature. For example, cohorts reared at 34°C, consistently, had the shortest anal papillae (Fig. 6).

Effects of water temperature on total body length (TBL) of larval instars of *Culex quinquefasciatus* mosquito: Analyses show that increasing water temperature had significant negative effects on the TBL of all larval instars. These negative effects were, however, more obvious at 34°C (Table 1).

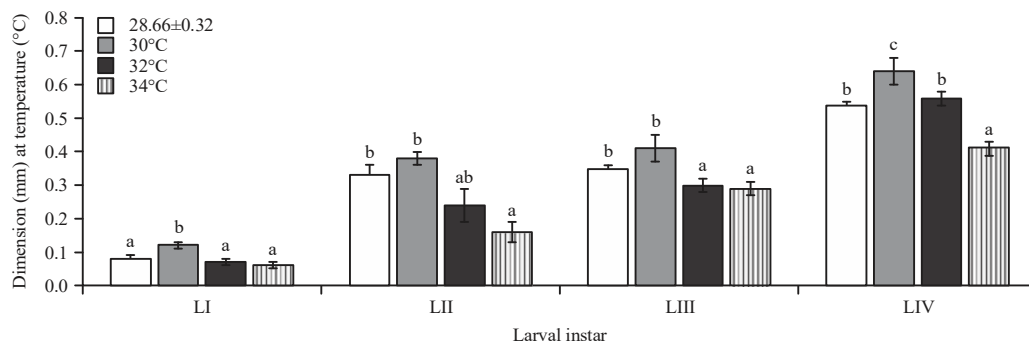


Fig. 5: Effect of water temperature on antennal length of *C. quinquefasciatus* mosquito larvae
 Bars with same letter are not significantly different at p<0.05 according to analysis of variance. Values expressed as Mean ±SD

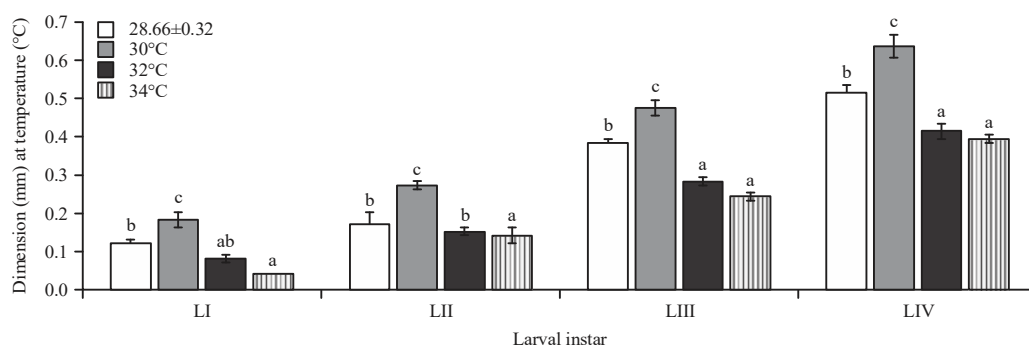


Fig. 6: Effects of water temperature on length of anal papilla of *C. quinquefasciatus* mosquito larvae
 Bars with same letter are not significantly different at p<0.05 according to analysis of variance. Values expressed as Mean ±SD

Table 1: Effects of water temperature on body lengths of larval stage of *Culex quinquefasciatus* mosquito

Larval stage	Dimension (mm) at temperature (°C)			
	28.66±0.32	30	32	34
I	1.14±0.11 ^{c*}	1.26±0.04 ^c	1.00±0.07 ^b	0.78±0.06 ^a
II	2.08±0.17 ^c	2.34±0.08 ^c	1.22±0.07 ^b	1.20±0.08 ^a
III	3.20±0.08 ^c	3.29±0.23 ^c	2.82±0.09 ^b	2.00±0.09 ^a
IV	5.30±0.32 ^c	5.84±0.18 ^c	3.76±0.20 ^b	3.47±1.05 ^a

*Values followed by same superscript alphabet in a row are not significantly different at p<0.05, values are expressed as Mean ±SD

Table 2: Effects of water temperature on morphometrics of pupal stage of *Culex quinquefasciatus* mosquito

Body parts	Dimension (mm) at temperature (°C)			
	28.66±0.32	30	32	34
Cephalothorax	1.72±0.35 ^{c*}	1.73±0.03 ^c	1.58±0.11 ^b	1.31±0.13 ^a
Trumpet	0.48±0.12 ^b	0.53±0.01 ^b	0.39±0.02 ^a	0.34±0.01 ^a
Abdomen	2.56±0.07 ^b	2.76±0.13 ^c	2.49±0.03 ^b	2.15±0.08 ^a

*Values followed by same superscript alphabet in a row are not significantly different at p<0.05. Values are expressed as Mean ±SD

Effects of water temperature on body parts of pupal stage of *Culex quinquefasciatus* mosquito: Analyses revealed significant (p<0.05) decrease in lengths of cephalothorax, trumpet and abdomen with increase in temperature (Table 2).

Influence of water temperature regimen on morphometrics of adult life *Culex quinquefasciatus* mosquito: The effects of rearing water-temperature on body parts and wing parameters of male and female *C. quinquefasciatus* mosquito are shown in Table 3 and Fig. 7. Mosquitoes reared at 30°C had the longest adult body parts. More so, temperatures above this, resulted in significant reduction (Table 3).

Effects of water temperature on wing length and fluctuating asymmetry of *Culex quinquefasciatus*: Analyses revealed an initial increase in adult Wing Length (WL) as temperature increased from 28.66±0.32 to 30°C, with subsequent decrease at higher temperatures. Meanwhile, fluctuating asymmetry (FA), increased, steadily, with temperature rise (Table 4). However, WLs and FA of female mosquitoes were, significantly, longer and lower, respectively, than those of their counterpart male mosquitoes (Table 4).

Effect of water temperature on volumes of 4th instar larvae and adult *Culex quinquefasciatus*: The influence of water temperature on the body volumes of fourth instar larvae and adult of *C. quinquefasciatus* is shown in Table 5.

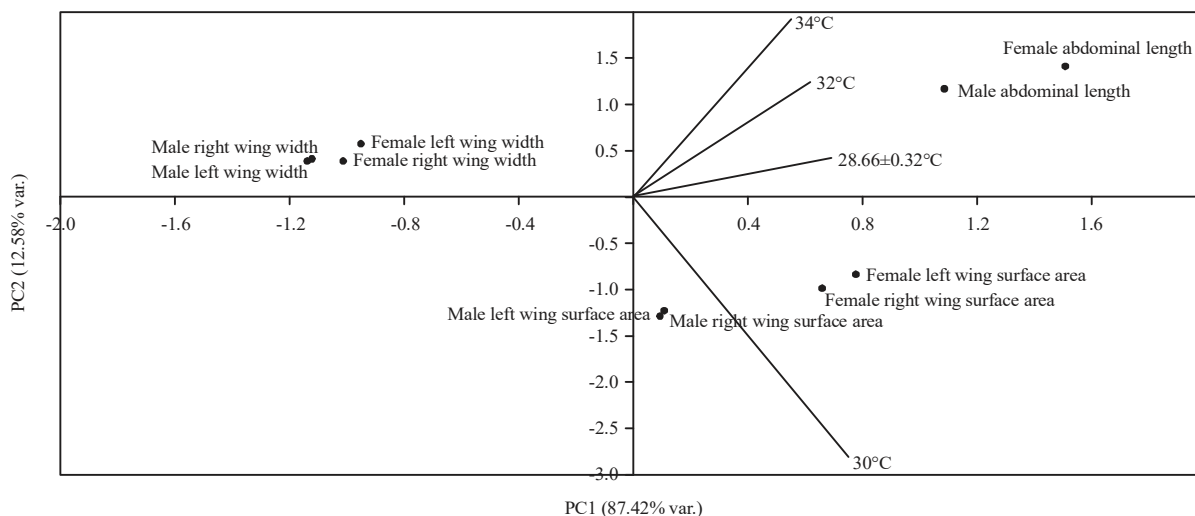


Fig. 7: Principal components analysis (PCA) biplots for influence of water temperature on adult morphometrics in *Culex quinquefasciatus* mosquito species (n = 1,280). For each axis, the amount of variation explained by each axis is shown. Arrows indicate the direction and relative importance of the water temperature regimens. Temperature regimens: 28.00, 30.00, 32.00 and 34.00 °C

Table 3: Effects of water temperature on morphometrics of adult life stage of *Culex quinquefasciatus* mosquito

Sex	Parameters	Dimension (mm) at temperature (°C)			
		28.66±0.32	30.00	32.00	34.00
Male	Abdominal length	2.55±0.02 ^{b*}	2.30±0.05 ^b	1.99±0.05 ^a	1.81±0.03 ^a
	Wing width	0.62±0.03 ^b	0.83±0.01 ^b	0.46±0.03 ^a	0.32±0.02 ^a
	Wing surface area	1.58±0.06 ^b	2.49±0.05 ^c	0.87±0.04 ^a	0.64±0.05 ^a
Female	Abdominal length	2.58±0.03 ^b	2.64±0.04 ^b	2.44±0.02 ^b	2.25±0.04 ^a
	Wing width	0.68±0.02 ^b	0.93±0.02 ^c	0.64±0.05 ^b	0.42±0.03 ^a
	Wing surface area	1.79±0.05 ^b	2.91±0.03 ^c	1.64±0.05 ^b	1.05±0.04 ^a

*Values followed by same superscript alphabet in a row are not significantly different at p<0.05. Values are expressed as Mean±SD

Table 4: Effects of water temperature on wing length and fluctuating asymmetry of *Culex quinquefasciatus* mosquito

Temperature regimen (°C)	Wing length (mm)		FA	
	Male	Female	Male	Female
28.66±0.32 (Ambient)	2.54±0.13 ^{c*}	2.53±0.07 ^b	0.00±0.01 ^a	0.01±0.01 ^a
30	3.01±0.11 ^d	3.13±0.14 ^d	0.01±0.01 ^a	0.01±0.02 ^a
32	2.13±0.03 ^b	2.68±0.05 ^c	0.03±0.02 ^b	0.02±0.02 ^b
34	1.72±0.19 ^a	2.10±0.06 ^a	0.05±0.02 ^b	0.03±0.03 ^c

FA: Fluctuating asymmetry. *Values followed by same superscript alphabet in a column are not significantly different at p<0.05. **Values followed by same subscript alphabet in a row are not significantly different at p<0.05

Table 5: Effects of water temperature on body volumes of 4th instar larvae and adult *Culex quinquefasciatus*

Temperature regimen (°C)	Larval size (DT ³) (mm ³)	Adult size (WL ³) (mm ³)
28.66±0.32 (Ambient)	0.99±0.20 ^{b*}	16.47±1.42 ^c
30	1.66±0.13 ^c	22.70±6.22 ^d
32	0.86±0.07 ^a	13.88±0.72 ^b
34	0.84±0.20 ^a	9.27±1.59 ^a

*Values followed by same superscript alphabet in a column are not significantly different at p<0.05. All values are expressed as Mean±SD of Mean. DT³: Cube of Diameter of fourth (L4) instar larvae. WL³: Cube of wing length of adult

Analyses revealed an initial increase in both entomological indices as temperature increased from ambient to 30°C, these decreased with further increase in temperature.

The Principal Components Analysis (PCA) biplots showed that temperature of 30°C had the most significant effects on body parts measured (i.e., length of abdomen, width of wings and surface areas of wings) (Fig. 7).

DISCUSSION

The present study investigated the influence of different temperature regimens on the body parts of *Culex quinquefasciatus* mosquito. The results revealed high phenotypic plasticity due to thermal stress at all life stages of the species. Generally, rearing temperatures above 30°C, significantly, reduced lengths and widths of most body parts. For example, smaller values of lengths and widths of immature measured body parts (i.e., head capsule, thorax, abdomen, siphon, juvenile antennae, anal papillae, cephalothorax, trumpet and abdomen) were observed for the species at higher temperatures of 32 and 34°C.

The head capsules of mosquito cohorts reared at temperatures above 30°C were smaller in lengths and widths; for example, mosquito larvae reared at 34°C had the least values for these parameters. This implied that these cohorts of mosquito larvae may possess small mouth parts and hence, reduced feeding capabilities. On the other hand, mosquitoes reared at 30°C had the longest and widest thoracic dimensions, hence, greater tendency of higher biomass accumulation.

Further, with significantly higher abdominal width and length of larvae (especially, in LIV), mosquito in cohorts reared at 30°C will have greater abdominal distension during feeding.

Siphonal dimensions were smallest in mosquitoes reared at temperature $\leq 32^\circ\text{C}$. A condition that could impair gaseous exchange processes, with resultant negative effect on development. The antennae and anal papillae of mosquitoes reared at 34°C were the smallest and perhaps, these structures may not be as efficient, functionally, as the larger forms observed in mosquitoes reared at 30°C. Further, mosquitoes reared at 30°C had the longest TBL. This was expected as mosquito cohorts at this temperature regimen had significantly longer abdominal lengths.

The reduced size of body parts observed at higher temperature in this study, could be attributed to decreased feeding rates associated with smaller feeding apparatus and/or thermal-stress conditions elicited by temperatures above optimum¹⁴. These conditions can affect mobilization of reserves⁷ and hence, growth, development and general morphology of insects²⁵. Le Sueur and Sharp¹¹ had earlier reported reduction in size of larval head capsule with increase in temperature.

The present study also showed that increase in water temperature significantly affected the wing length (WL) and fluctuating asymmetry (FA) of *C. quinquefasciatus* mosquito.

For example, adult mosquitoes (both male and female) reared at higher temperatures above 30°C had smaller WLs, while those reared at 30°C had the longest WL. Further, WL and FA have been described as proxies for body size and index for fitness of mosquito species, respectively. It, therefore, means the former temperatures produced smaller adults and may be less fit for disease transmission, while the latter produced bigger and better vector⁹. These observations were consistent with earlier thermal studies by Bochnanovits and De Jong⁴, Mourya *et al.*⁵, Atkinson⁸, Le Sueur and Sharp¹¹, Loetti *et al.*²⁶ and Phasomkusolsil *et al.*²⁷.

The findings of the present study on small body phenotypes produced by high temperatures ($>30^\circ\text{C}$) explain the general pattern proposed for the relationship between body sizes of organisms and temperatures, especially, ectotherms (i.e., smaller sizes of organisms result from higher temperatures, 'hotter is smaller')⁹. More so, optimal temperature has been associated with maximal performance of organisms: The study shows that 30°C is the optimal temperature for this species of mosquito. Meanwhile, wing FA of the species was elevated with temperature increase; indicating developmental instability, which must have been elicited by thermal stress conditions²⁸. Similar fluctuations due to environmental stress have been reported by Mpho *et al.*¹⁴. However, contrary to their submissions, the present study showed that both sexes exhibited higher FA with rise in temperature.

In the present study, water temperature and larval biomass accumulation index exhibited a direct relationship at temperatures $\leq 30^\circ\text{C}$, however, as temperature increased further, an inverse relationship ensued. The highest biomass accumulation for the species was at 30°C, while the least was at 30°C. It, therefore, means that mosquito cohorts reared at the former temperature will be better fit for adult activities, than those from the latter due to high general reserves.

In mosquito physiology, the cube value of the diameter of thorax of the fourth instar gives an indication to volume of the larvae and degree of biomass accumulation during the larval life stage²⁹. Meanwhile, biomass accumulation at fourth larval instar has a resultant effect on the overall size of the adult, and, subsequently, success of adult life history traits. For example, it has been correlated with greater ability to fly and locate mate³⁰, host location and selection³¹, greater blood meal intake³² and efficiency in transmitting pathogens³³. It, therefore, implies that mosquito raised at 30°C may be better at disease transmission, while those reared at 34°C may be less fit.

CONCLUSION

The results of the present study reveal high morphologic plasticity in *Culex quinquefasciatus* due to different thermal conditions. Mosquito cohorts reared at 30°C had significantly, higher values for body parts measured, while, those reared at temperatures above this (i.e., >30°C), had significant smaller body sizes. This trend was observed through all life stages (i.e., larval to adult stage). The study has, thus, put into clearer perspective, structural responses of the species to thermal stress conditions, which produce adult mosquitoes of different sizes.

SIGNIFICANCE STATEMENT

This study employed morphometry in discovering the influence of higher temperatures in producing smaller-sized mosquito. This information is beneficial in understanding the interplay between temperature and biological fitness in the species. This study will help researchers to uncover critical areas such as the use of morphometrics in determining interactions between ecological factors and life stages' development of *C. quinquefasciatus* mosquito.

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