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# Effects of Temperature Stress on Pre-imaginal Development and Adult Ptero-fitness of the Vector Mosquito, *Culex quinquefasciatus* (Diptera: Culicidae)

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**Abstract** Day-old first instar larvae of *Culex quinquefasciatus* mosquitoes were cultured to imago eclosion, at constant temperatures of 30.00, 32.00, 34.00 ℃ and ambient water temperature (28.00±0.02 ℃, Control). The duration and survivorship of larval and pupal life stages were monitored daily. The wings of adult mosquitoes were measured for length and fluctuating asymmetry. The results indicated significant ($p<0.05$) effects of water temperature on developmental indices investigated. The duration of larval and pupal stage was significantly shortened from 10.82±1.02 days (28.00±0.02 ℃) to 7.65±2.15 days (34.00 ℃) and 2.04±0.70 (28.00±0.02 ℃) to 1.19±0.27 days (34.00 ℃), respectively. Survivorship of immature stages showed inverse relationship with increasing water temperature; with survivorship of the pupae significantly higher than those of the larvae at all the temperatures tested. Wing length and fluctuating asymmetry were also affected by rise in temperature. The findings of this study indicate limited thermal adaptation of *Cx. quinquefasciatus* to relatively warm areas; this information should help in developing effective environmental mosquito-vector control against the species by the discouragement of ecological settings that may reduce micro-climatic temperatures around breeding habitats of the mosquito species.
**Keywords** Fluctuating Asymmetry; Larva; Breeding Temperature; pupa; Thermal Adaptation; Wing Length

## Introduction

*Culex* species are important mosquito vectors, responsible for the transmission of important human diseases including, West Nile virus, filariasis, encephalitis, etc, that pose serious threats to global public health (Curtis, 1996; Micheal and Bundy, 1997; Smith, 2006). According to World Health Organisation (2004), more than 1.3 billion people in 83 countries and territories; with 120 million of them dying yearly from secondary complications of the disease (Terranella et al., 2006a; 2006b; Sichangi et al., 2009) and approximately 18% of the world's population live in areas at risk of infection with filariasis (Carter Centre, 2008; Mukhopadhyay et al., 2008).

The breeding success of *Culex* Mosquito vectors of human diseases is largely influenced by prevailing ecological conditions in the larval habitats. Studies have shown that *Culex* species are principally cosmopolitan and breed preferentially in large water bodies such as natural swamps and man-made irrigation-related water receptacles, as well as, accessible septic tanks in urban slums (WHO, 1975; Wayne, 2010). Mean physico-chemical conditions in these varieties of active *Culex*-breeding sites differ considerably (Loetti et al., 2011).

Yet, water temperature is one of the most important physico-chemical factors that influence productivity of mosquito larval habitats (Clements, 1992). To this end, mosquitoes are conditioned to breeding preferentially in relatively narrow range of types of water bodies, especially, small sun-lit rain pools, characterized by warm water temperature for faster immature development (Secil et al., 2009; Loetti et al., 2011). Being ectothermic, mosquitoes are greatly influenced by environmental temperatures (Atkinson, 1994; Silby and Atkinson, 1994), and the rates of immature development are crucially dependent on water temperature of the larval habitats (Mpho et al., 2002a; Mourya et al., 2004; Carrington et al., 2013). Exposure of mosquito larval cohorts to widely different water temperatures, as obtainable in the diverse active habitats of *Culex* species, could impose significant stress on a population resulting in increased developmental and anatomical deficiencies, as well as, genetic instability (Mpho et al., 2001; 2002b). Therefore, effective management of *Culex* mosquito population development in its diverse larval habitats, most of which are unavoidably associated with anthropogenic communities and activities, demands a good understanding of the influence of water temperature on immature development of the mosquitoes. This information is presently scanty, and in order to bridge the research gap, this study was carried out to evaluate the effects of water temperature on survival rates and duration of development of immature life stages, as well as, adult body size and fitness, of *Culex quinquefasciatus* mosquitoes under laboratory conditions.

## 1 Materials and Methods

### 1.1 Source and exposure of larvae to different temperature regime

The *Culex quinquefasciatus* mosquitoes used in this study came from a colony raised in the Laboratory of the Department of Biological Sciences, Federal University of Technology, Minna, Nigeria. Twelfth Filial generation ($F_{12}$) were used in the study. The experimental set-up consisted of four water temperature treatments namely, 28.00±0.02°C (i.e., ambient room temperature, Mean ± Standard Deviation), 30.00, 32.00 and 34.00°C. The ambient temperature served as the control experiment. For each treatment, 100 approximately day-old first instar larvae of the mosquito were placed in thermal tanks (15 litres capacity), containing ten litres of bore-hole water (i.e., a density rate of 10 larvae per 1 000 ml). The temperatures of treatments 30.00 – 34.00°C were maintained constant with the aid of aquarium tube heaters regulated by digital thermostats (Model: 300W, LifeTech Aquarium®GB4706.67-2003). The ambient temperature treatment (i.e. Control) had no water heater but simply kept under the influence of room temperature. The larvae were fed with pulverized fish feed (Cuppens®), maintained generally according to the techniques of Olayemi et al. (2012). The mosquitoes were monitored for mortality, ecdysis and metamorphosis, during the hours of 0800 and 0900 daily. At pupations, the mosquitoes were transferred in plastic cups (350 ml) to adult-holding cages for eclosion. Survival rates and duration of development of the immature life stages were estimated as described by Ukubuiwe et al. (2013). The whole experiment was repeated three additional times at weekly intervals, resulting in the monitoring of 800 larvae per water temperature treatment.

### 1.2 Measurements of indices of wing quality

The adult Mosquitoes were sacrificed within 24 hours post-emergence, and had their wings carefully detached for analysis. Wing length was determined as the interval between the base of the Costa and distal extremity of the R3 vein, excluding the fringe setae (Loetti et al., 2011). Wing fluctuating asymmetry was determined as the difference between right and left wings of the mosquitoes (Ukubuiwe et al., 2016).

### 1.3 Data analysis

All data obtained from experimental replicates and repeats were processed as Mean±SD, and subsequently pooled for statistical analysis. Differences in mean values of immature life stage duration and survival rates, as well as adult wing lengths, among the water temperature treatments were compared for statistical significance using ANOVA at $p=0.05$.

## 2 Results

Table 1 highlights the influence of temperature on duration of immature stages of the *Culex* mosquitoes. Duration of aggregate immature stage reduced significantly ($p<0.05$) with increase in water temperature, ranging from 12.86±1.72 days in the Control Mosquitoes (i.e., 28.00±0.02°C), to 8.84±2.24 days among those raised at the highest temperature (34.00°C). However, duration of aggregate immature development was not significantly ($p>0.05$) affected by temperatures between 30.00 and 32.00°C. Generally, the influence of temperature on duration of development was more pronounced on the larval than pupal stage. While the Control temperature significantly extended duration of the pupal stage (2.04±0.70 days) compared to the treatments, subsequent increases in temperature resulted in insignificant reduction in duration of the stage (range = 1.40±0.20 days at 30°C to 1.19±0.27 days at 34.00°C). Duration of the larval stage ranged from 10.82±1.02 days at the Control temperature, to 7.65±2.15 days at 34.00°C.

Table 1 Immature developmental and survival rates of Culex quinquefasciatus mosquitoes exposed to different temperature regimens

| Water Temperature (℃) | Duration of Immature Life Stage (Days) | | | Survivorship of Immature Life Stage (%) | | |
|---|---|---|---|---|---|---|
| | Larval | Pupal | Aggregate | Larval | Pupal | Aggregate |
| 28.00±0.02 (Control) | 3.98±0.03b$^{c*}$ | 3.98±0.03b$^{c*}$ | 3.98±0.03b$^{c*}$ | 74.66±10.21 d $^{a**}$ | 91.05±2.80$^{d}$ $_{b}$ | 82.86±6.50$^{d}$ |
| 30.00 | 4.01±0.02$^{c}$ | 4.01±0.02$^{c}$ | 4.01±0.02$^{c}$ | 51.50±7.10 $^{c}$ $_{a}$ | 63.38±12.50$^{c}$ $_{b}$ | 57.44±9.80$^{c}$ |
| 32.00 | 3.60±0.25$^{b}$ | 3.60±0.25$^{b}$ | 3.60±0.25$^{b}$ | 28.63±5.98 $^{b}$ $_{a}$ | 44.24±6.70$^{b}$ $_{b}$ | 36.44±6.34$^{b}$ |
| 34.00 | 2.70±0.16$^{a}$ | 2.70±0.16$^{a}$ | 2.70±0.16$^{a}$ | 2.89±2.30 $^{a}$ $_{a}$ | 44.24±6.70$^{b}$ $_{b}$ | 3.99±2.75$^{a}$ |

Note: *Values followed by same superscript alphabets, in a column, are not significantly different at p = 0.05; **Values followed by same subscript alphabets, in a row of larval and pupal survivorship, are not significantly different at p = 0.05

The survivorship of the immature mosquitoes in response to increasing temperatures is presented in Table 2. Much more than duration of development, temperature significantly ($p<0.05$) influenced survival rates of the immature mosquitoes. Survival rate of the aggregate immature stage showed significant decrease with every increase of about 2.00°C in water temperature (range=82.86±6.50% at 28.00±0.02°C, to 3.99±2.75% at 34.00°C). Similar trends of decrease in survival rates, with increasing temperature, were recorded for the larval and pupal stages with ranges of 74.66±10.21 to 2.89±2.30% and 91.05±2.80 to 5.08±3.20%, respectively. The survival rates of the pupal stage were consistently higher than those of larvae, except amongst the mosquitoes cultured at the highest temperature, i.e., 34.00°C. While, survivorship of the larvae ranged from 74.66±10.21% at Control temperature, to 2.89±2.30% at 34.00°C, those of the pupae were 91.05±2.80 to 5.08±3.20% at respective similar temperatures.

Table 2 Effects of temperature on wing length of Culex quinquefasciatus mosquitoes exposed to different temperature regimens

| Water Temperature (°C) | Wing Length (mm) | | |
|---|---|---|---|
| | Left | Right | Mean |
| 28.00±0.02 (Control) | 3.98±0.03$^{bc*}$ | 3.98±0.03$^{bc*}$ | 3.98±0.03$^{bc*}$ |
| 30.00 | 4.01±0.02$^{c}$ | 3.98±0.03$^{bc*}$ | 3.98±0.03$^{bc*}$ |
| 32.00 | 3.60±0.25$^{b}$ | 3.98±0.03$^{bc*}$ | 3.60±0.25$^{b}$ |
| 34.00 | 2.70±0.16$^{a}$ | 2.70±0.16$^{a}$ | 2.70±0.16$^{a}$ |

Note: *Values followed by same superscript alphabets, in a column, are not significantly different at P = 0.05

The effects of water temperature on wing fluctuating asymmetry of *Cx. quinquefasciatus* is shown in Figure 1. The responses of length of wings of the emergent mosquitoes to temperature variation were less pronounced than those of survivorship and developmental rates, as wing length (WL) was not significantly different ($p>0.05$) between mosquitoes raised at the Control and 30.00°C temperatures. However, mean wing length of the mosquitoes significantly ($p<0.05$) reduced with increase in water temperature above 30.00°C. The fluctuating asymmetry (FA) of the wings of the mosquitoes equally increased with increase in breeding temperature; becoming more-or-less exponential at temperatures above 32.00°C (Figure 1).

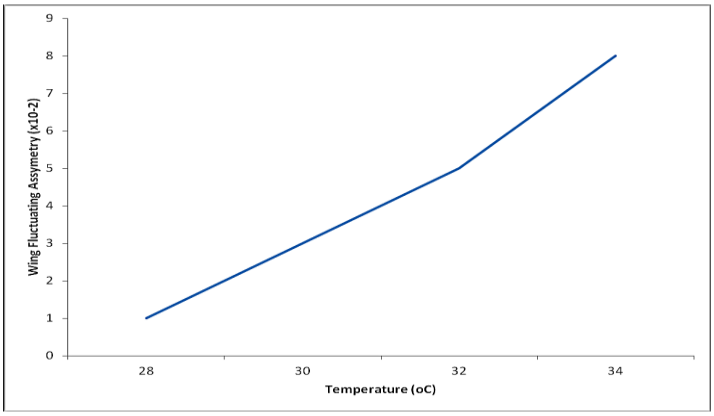


Figure 1 Effects of temperature on wing fluctuating asymmetry of *Culex quinquefasciatus* mosquitoes exposed to different temperatures

## 3 Discussion

Water temperature significantly affected the development of the immature life stages of the *Culex quinquefasciatus* mosquitoes; with reduction in life stage duration been inversely temperature-dependent. Similar temperature-immature development relationships have been reported for other mosquito species (Rueda et al., 1990; Ribeiro et al., 2004), and were attributed to enhanced relatively higher temperature within the optimum range requirement for growth and/or development mediating enzymatic activities. Insect size has been correlated with temperature (Lyimo et al., 1992; Atkinson, 1994; Angilleta et al., 2010; Fischer et al., 2011), as higher temperatures tend to produce smaller adults. Loetti et al. (2008) observed a positive linear relationship between water-breeding temperature and developmental rates of immature *Culex hepperi* mosquitoes within the thermal tolerance range. The duration of larval development was not significantly affected at water temperatures between 30.00 and 32.00$^{o}$C and, hence may be regarded as the optimum developmental temperature range for the species.

Though, the duration responses of the pupae to increasing water temperature were also inversely related, the influence of temperature on this immature life stage was less pronounce compared with the larvae, and higher temperatures above the Control (i.e., 28.00±0.02$^{o}$C) had no significant effect on duration of development of the pupae. This finding on pupal development was rather surprising, as been a metabolically active stage (as a result of the drastic anatomical and physiological re-organization of an aquatic pupa to a terrestrial imago that occurs during this stage), enhanced temperature especially within tolerance/optimum range of an insect's immature life stage ordinarily should quicken developmental rates, i.e., shorten duration of development (Pfadt, 1978; Dodson et al., 2012). Therefore, it may mean that the optimum/ critical temperature for development of pupae of *Cx. quinquefasciatus* is close to that provided by the Control experiment (i.e., 28.00±0.02$^{o}$C**)** and of course lower than that of the larvae (30.00-32.00$^{o}$C) as revealed by the results of this study. The variations observed in the sensitivity of larval and pupal stages of the mosquito species to water temperature in this study may be due to the differential dominant biological or developmental activities (i.e., growth, preceded by cell division and re-organization of tissues, occasioned by hormonal secretions, respectively associated with the two immature stages).

The results of this study showed that water temperature was much more impacting on survivorship of the immature stages than duration of their development, with every 2.00$^{o}$C increase in temperature resulting in significant decrease in survival rate. Survival rates of the aggregate immature stage was critically low (< 4.00%) at the highest temperature, i.e., 34.00$^{o}$C, tested in this study; and such survivorship may not be able to sustain occurrence of the species in an area. According to Clements (1963), enzymatic activities are seriously impaired at temperatures above the optimum and, thus, explain the near 100% mortality recorded among the immature mosquitoes exposed to 34.00 $^{o}$C in this study.

Consistently, the pupal stage exhibited higher tolerance to increasing temperatures than the larvae, till 32.00$^{o}$C after which they both succumbed statistically equally to 34.00$^{o}$C water temperature. The significantly higher adaptability of the pupal stage to higher temperatures may be due to the fact that, unlike larvae, pupae possess much tougher integument (Hoskins, 1932; Davis, 1932), and do not ecdyse into pupal instars, during which an immature stage is surrounded by a thin, vulnerable cuticle that may be easily detrimentally impacted by high temperatures (Davis, 1932). Interestingly, there is considerable disparity between the optimal temperatures for duration of development and survivorship of the immature mosquitoes, thus, suggesting that other factors, probably endogenous, play more important roles in the development of immature mosquitoes.

The results of this study revealed that beyond 30.00$^{o}$C, wing length (proxy for adult body size) of the emergent adult mosquitoes reduced significantly with increasing breeding water temperature. Since metabolic rate (i.e., histogenesis) is a limiting factor and temperature-dependent particularly in poikilotherms such as insects (Oda et al., 2002), then mosquito larvae raised in low temperatures should give rise to large adults with longer wings than their counterparts cultured in higher temperature. This fact, probably, explain the significantly smaller adult mosquitoes from breeding water media maintained at the relatively higher temperatures of 32.00 and 34.00$^{o}$C in mosquitoes, reduced adult body size (i.e., wing length) is associated with impaired ecological adaptability, low fecundity and, hence, reduced vectorial potential (Briegel 1990a; 1990b). Ptero-deficiency also manifested in the fluctuating asymmetry of the wings with increasing temperature, thus, further confirming the vectorial fitness liability incurred by immature mosquitoes raised in relatively high temperatures.

## 4 Conclusions

The findings of this study have provided further evidence of the limiting-potentials of breeding water temperature against population development, ecological adaptability and vectorial fitness of *Culex quinquefasciatus* mosquitoes. Though, increasing water temperature enhanced the rate of immature development, by significantly shortening the duration of larval and pupal stages, this metabolic gain was effectively neutralized by the set-back manifested as critical reduction in survivorship, production of relatively smaller mosquitoes with its consequent ecological liabilities, and pronounced vectorial ptero-misfitness. It, therefore, seems that *Cx. quinquefasciatus* may be poorly adapted to relatively warm ecological zones, and this information should help in developing effective environmental mosquito-vector control against the species by discouraging ecological settings (such as adjourning vegetation, increased wind flow, etc) that may reduce micro-climatic temperatures around breeding habitats of the mosquito species.

### Authors' Contributions

Conceived and designed the experiment: **OIK** and **UAC.** Analysed the data: **OIK.** Wrote the first draft of the manuscript: **OIK** and **VO.** Contributed to the writing of the manuscript: **UAC,** and **JAI.** Agree with manuscript results and conclusion: **OIK, VO, UAC,** and **JAI.** Jointly developed the structure and arguments for the paper: **OIK, VO, UAC,** and **JAI**. Made critical revisions and approved final version: all authors. All authors reviewed and approved of the final manuscript.

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