INFLUENCE OF PHOTOPERIOD ON LARVAL GROWTH INDICES AND ENERGY BUDGET FOR METAMORPHOSIS IN *Culex quinquefasciatus* MOSQUITO (DIPTERA: CULICIDAE); ITS IMPLICATION IN INTEGRATED VECTOR MANAGEMENT

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Abstract

This study was designed to elucidate, for the first time, the effects of photoperiod on mosquito larval growth indices and energy reserve utilised for metamorphosis, using *Culex quinquefasciatus* as a model vector. Approximately Day-old larvae of the mosquitoes were exposed to different photoperiodic regimens, ranging from zero to 24-hours of Light (hL) and reared using standard protocols. Analyses revealed significant effect of photoperiod on mosquito larval growth rate. This decreased as the duration of photophase increased, with range of values of 0.0412±0.0023 to 0.1044±0.0021 mg/day. It was also discovered that, the effects of photoperiod on Total Larval Body Size (TLBS) were not significant at the first larval instar, L1 (range=0.84±0.05 to 0.88±0.02 mm). However, as the mosquitoes progressed from L2 through L4, there were significant reductions in TLBS, with respective values at 24 and 0 hL, ranging from 2.29±0.14 to 2.73±0.10 mm, 2.55±0.64 to 3.18±0.08 mm, and 3.86±0.46 to 4.53±0.10 mm, respectively, for L2, L3 and L4. Total Teneral Reserve Component utilised for the processes of pupation and eclosion, were significantly affected by photoperiod. As there was increased utilisation of teneral reserves as the duration of photoperiod increased from 0 to 24 hours, with range = 7.07±1.31 to 11.59±2.04 μg nutrient/ mosquito and 5.78±2.19 to 12.28±3.33 μg nutrient/ mosquito, respectively, for pupation and eclosion. This study, thus, revealed critical information on these important aspects of bio-ecophysiology of mosquitoes, which will be invaluable in the development of a robust, cost-effective, and eco-friendly integrated mosquito management protocols.

Key word; Total Larval Body Size, Pupation, Eclosion, Teneral Reserve

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INTRODUCTION

Photoperiod, also known as photophase, is the amount of light available within a 24-hour clock (Gillot, 2005; Shi *et al.,* 2017). The effects of light (natural or artificial) on organisms have been reported and well documented (Bradshaw and Holzapfel, 1975; Carmine and Ronald, 1993; Chocorosqui and Panizzi, 2003; Kollberg *et al.,* 2013). Apart from its widely known diapause phenomenon, Photoperiod, expressed in numbers of hour of Light versus darkness, L: D, or in hours of Light (hL) or darkness (hD), has a great influence on insects physiology (Adkisson, 1964; Saunders, 2012).

Photoperiod is, perhaps, the most important abiotic factor regulating most physiological processes in insects through its effects on ommatidial pigments and photoreceptors in brains and synthesis of growth hormones (Lopatina *et al.,* 2011; Kollberg *et al.,* 2013), generally, and mosquitoes (Mathias *et al.,* 2006), in particular. According to Bowen *et al.* (1984), the insect brain (cerebral lobe in particular) is not only capable of detecting, receiving, and measuring the relative amounts of light and dark (i.e., acting as a circadian clock) but it can also be the source of a hormonal effector(s) that triggers the appropriate physiological and behavioural responses elicited by the ambient photoperiod. Photoperiod influence processes such as growth (Leimar, 1996; MacRae, 2010), diapause (MacRae, 2005), survival (Urbaneja *et al.,* 2001), longevity (Chocorosqui and Panizzi, 2003), development of ovarian follicles (Oda and Nuorteva, 1987; Reznik and Vaghina, 2011), life span (Lanciani and Anderson, 1993), and vectorial morphometric indices (Vinogradova and Karpova, 2006; Benoit and Denlinger, 2007).

Mosquitoes are important in the spread of important public health diseases such as malaria, yellow fever, elephantiasis and Zika virus disease (ZVD) (Black and Kondratieff, 2005). Major control protocols, (such as use of insecticides, window and door screens and long-lasting insecticide treated nets) have focused on the adult life stage (Collins *et al.,* 1995; Curtis, 1996; CDC, 2014), with little success story (Fillinger *et al.,* 2004). This has necessitated radical approaches targeted against the weakest and most vulnerable life stage, larvae, through environmental manipulation (Bond *et al.,* 2004). Moreso, it has been agreed that a sound knowledge of the influence of micro-environmental factors, such as photoperiod, on the bio-eco-physiology of mosquitoes is critical for the development of such alternative control protocols.

Although less studied, duration of photophase greatly affect aspects of mosquito physiology relating to growth and development (Śniegula *et al.,* 2012), and there have been no systematic evaluation aimed at bringing into clearer perspective, the effects of this factor on larval growth indices and teneral reserve accumulation for metamorphosis, hence, this study.

MATERIALS AND METHODS

Source and Handling of Mosquitoes

Freshly laid egg raft of *Culex quinquefasciatus* Mosquito were collected from an established colony in the

Entomology Unit of the Department of Biological Sciences, Federal University of Technology, Minna. These eggs were incubated, and hatched larvae subjected to the various photoperiodic regimen. The larvae were reared as described by Ukubuiwe *et al.* (2013). At every larval instar, 10 larvae were selected randomly from each regimen to measure growth parameters and teneral reserve accumulated.

Simulation of Photoperiodic Regimen

This was done as described by Lanciani and Anderson (1993), with slight modification. Five different photoperiodic regimens of 24 hours light, 6, 12, 18 and 24 hours Darkness were simulated in the laboratory using 24-hour clock and light sources, while the prevalent photoperiod of 13 hours of light, was taken as the 'Control'. The mosquitoes were exposed to the varying duration of source of light equivalent to the photoperiodic treatment.

Determination of Growth Rates and Total body Length

Growth rate of individual mosquitoes was estimated as weight at emergence divided by the age at pupation, and thus indicated the average increase of weight per day throughout the larval period (Lyimo *et al.,* 1992). Total body length was determined by adding the total lengths of the head, thorax and abdomen, as determined under binocular microscope mounted with an ocular micrometer at X10 Magnification (Timmermann and Briegel, 1998).

Determination of Energy Budget for the processes of Pupation and Eclosion

The teneral reserves (lipid, protein, glycogen and glucose) of the mosquitoes were determined according to the methods of Bradford (1976), and Van-Handel and Day (1988). The energy reserves utilised for the processes of pupation and eclosion were obtained, respectively, by adding up the differences between each teneral reserve component accumulated at fourth instar and pupa, and at pupa and adult (Kaufmann and Brown, 2008).

Data Analyses

A goodness-of-fit was used to test the data before analyses. Data for larval growth rates, total body length, and rates of teneral reserve accumulation were normally distributed, and analysed using one-way ANOVA. All values were expressed as mean ± standard deviation and decisions on statistical comparison of means were taken at $p<0.05$ level of significance. The means were separated using Duncan Multiple Range Test (DMRT).

RESULTS

Effects of Photoperiod on Larval Growth Indices of *Culex quinquefasciatus* Mosquitoes

The influence of photoperiod on larval growth rates (LGR) and total larval body length (TLBL) is shown in Table 1. Analyses revealed a significant ($p<0.05$) effect of photoperiod on these parameters. Generally, as the photophase increased from 0 to 24 hours of light (hL), larval growth rate reduced significantly ($p<0.05$) from 0.0412 ± 0.0023 to 0.1044 ± 0.0021 mg/day. A different trend was, however, observed on its influence on TLBL of the species, as stage-specific response to photoperiod was elicited. The first larval instars (L1), were not significantly ($P>0.05$) affected by photoperiod, and had TLBL ranging from 0.86±0.03 (at 24 hL) to 0.88±0.02 (at 0 hL). However, as the life stages progressed from L2 through to L4, photophase significantly ($p<0.05$) affected the TLBL; with values ranging from 2.29±0.14 to 2.73±0.10 mm, 2.55±0.64 to 3.18±0.08 mm, and 3.86±0.46 to 4.53±0.10 mm, for L2, L3 and L4 at 24 and 0 hL, respectively (Table 1).

Effect of Photoperiod on Metabolic Reserve for Metamorphosis in *Culex quinquefasciatus* Mosquitoes

The effects of photoperiod on total teneral reserve utilised for the processes of pupation and eclosion is shown in Table 2. There was significant ($p<0.05$) variation in the metabolic reserves utilised for these processes as the duration of photophase increased from 0 to 24 hL. The total reserve utilised for the process of pupation ranged from 7.07±1.31 (at 6 hL) to 13.83±3.77 µg nutrient/ mosquito (at 18 hL), while that utilised for eclosion ranged from 5.78±2.19 (at 12 hL) to 12.28±3.33 (at 24 hL) µg nutrient/ mosquito (Table 2).

Table 1: Effects of Photoperiod on Larval Growth Indices of *Culex quinquefasciatus* Mosquitoes

Photoperiodic levels (Light: Dark Hours)	Larval Growth Rate (mg/day)	Total Larval Body Size (mm)			
		L1	L2	L3	L4
0:24	0.1044 ± 0.0021^{e}	0.88 ± 0.02^{a}	2.73 ± 0.10^{c}	3.18 ± 0.08^{d}	4.53 ± 0.10^{c}
6:18	0.0869 ± 0.0041^{d}	0.85 ± 0.04^{a}	2.64 ± 0.16^{bc}	3.09 ± 0.11^{c}	4.43 ± 0.07^{c}
12:12	0.0700 ± 0.0050^{c}	0.84 ± 0.05^{a}	2.51 ± 0.16^{b}	2.93 ± 0.42^{bc}	4.28 ± 0.19^{ab}
13:11 (Control)	$0.0697\pm0.0028^{c*}$	0.87 ± 0.03^{a}	2.51 ± 0.18^{b}	2.92 ± 0.11^{bc}	4.32 ± 0.04^{c}
18:6	0.0517 ± 0.0021^{b}	0.85 ± 0.04^{a}	2.45 ± 0.27^{ab}	2.76 ± 0.29^{a}	4.03 ± 0.45^{b}
24:0	0.0412 ± 0.0023^{a}	0.86 ± 0.03^{a}	2.29 ± 0.14^{a}	2.55 ± 0.64^{b}	3.86 ± 0.46^{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
L1-L4, Larval Stages 1 to 4.

Table 2: Effect of Photoperiod on Metabolic Reserve for Pupation and Eclosion of *Culex quinquefasciatus* Mosquitoes

Photoperiodic levels (Light: Dark Hours)	Total Teneral Reserve Component (µg nutrient/ mosquito) for pupation	Total Teneral Reserve Component (µg nutrient/ mosquito) for emergence
0:24	7.22 ± 1.04^{a}	6.87 ± 2.08^{ab}
6:18	7.07 ± 1.31^{a}	8.77 ± 1.32^{b}
12:12	9.30 ± 1.95^{b}	5.78 ± 2.19^{a}
13:11 (Control)	7.28 ± 2.10^{a}	9.31 ± 0.76^{b}
18:6	13.83 ± 3.77^{c}	11.62 ± 2.61^{c}
24:0	11.59 ± 2.04^{bc}	12.28 ± 3.33^{c}

*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

DISCUSSION

This study demonstrated that *Culex quinquefasciatus* mosquito larvae reared in shorter photophase (0 - 12 hL), had higher larval growth rates than their siblings reared under longer photoperiods (18 – 24 hL). According to Lyimo *et al.* (1992), growth rate is a measure of the average increase of weight per day throughout the larval period. It, thus, imply that mosquitoes raised under short day-length had a higher increase of weight per day as larvae than siblings raised under long day-length, and would be bigger and better-fit for disease transmission (Briegel, 1990a;b). This reduction in growth rates as period of light increased (i.e. longer day length) could suggest a negative impact of light on be metabolism, and respiration, which regulates tissue synthesis and growth of insects (Carmine and Ronald, 1993) or the decreased production of growth hormone (Tauber and Kyriacou, 2001). It could also be due to altered feeding behaviour (Poteat and Buchwalter, 2014). Similar observations have been made in other insects by Lanciani (1992; 1993), Carmine and Ronald (1993), and Vinogradova and Karpova (2006), who reported decreased morphometrics in insects exposed to longer duration of light. However, increased growth rate has been reported for Tasmanian Lacewing, *Micromus tasmaniae* (Yadav *et al.,* 2008).

An attempt was also made in the present study, for the first time, to appreciate the influence of photoperiod, on the morphology of the mosquito species across the life instars and stages. Interestingly, day-length did not statistically affect the morphometrics of first instar larvae. This could suggest non-maturation of the photoreceptors and neural components for photoperiodic response, as maturation of these have been positively correlated with stage of development of photoreceptors in insects (Shintani *et al.,* 2009). However, as the mosquitoes progressed to second through to fourth larval instar, the effect of photoperiod became apparent (indication of increased photo detection and reception); with larvae reared at zero and shorter day-lengths relatively bigger/longer than their longer day-length siblings. The shortest/smallest individuals were obtained from those reared under the longest photoperiodic (24L: 0D) regimen.

In the present study, there was disparity in the quantity and component of teneral reserve utilized by the mosquitoes in the reorganisation processes at pupation and for eclosion. The mosquitoes reared under longer photophases utilised the highest quantities of teneral components for these processes, while mosquitoes from shorter day-lengths and control used significantly lower quantities of reserves. These has huge consequences on the quantity of teneral components that would be available for adult-life activities, and hence quality of adult life mosquitoes (Akoh *et al.,* 1992). For greater teneral reserve availability at adult life stage has been correlated with greater fecundity (Blackmore and Lord, 2000), and increased life span (McCay *et al.,* 1989).

Although, further studies are advocated, the results from this study can be incorporated in the development of alternative cost-effective control strategies, by targeting larval habitats with little or no exposure to light during the day, or, illuminating these dark habitats at night-time. The second option is actually feasible, as most mosquito habitats (septic tanks, Drainages, rain pools, and drains) are not suitable breeding grounds for economically important animals and livestock.

CONCLUSION

The present study has elucidated a significant influence of photoperiod on growth rate indices and energy reserves for pupation and eclosion in *Culex quinquefasciatus* Mosquitoes. This has increased the scientific knowledge on the bio-ecophysiology of the species and mosquitoes, in general. This information is valuable in making well-informed decision in developing cost-effective and environmental friendly control protocol.

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