

Research Report

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Influence of Variable Photoperiod on Life-stages Mobilization of Teneral Reserves in *Culex quinquefasciatus* (Diptera: Culicidae): Implication for Environmental Manipulation for Vector Control

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Abstract The influence of photoperiod on teneral reserve accumulation across life stages of *Culex quinquefasciatus* was investigated in this study. Day-old larvae of the mosquito were exposed to five simulated photoperiodic regimens, namely 0, 6, 12, 18 and 24 hours of Light (hL) and a Control (prevailing 13 hL). Rearing of immature stages and quantification of teneral components (lipid, glucose, protein and glycogen) at the life stages were done following standard protocols. Results revealed significant effects of photoperiod on teneral components in all life stages. Generally, there were significant increase in all teneral components as the larvae progressed from L1 to L4 instars, with subsequent reduction at pupal and adult stages. The quantities of teneral components also exhibited an inverse relationship with photo-duration, with glycogen and protein more distributed than lipid and glucose. Average immature component ranged from 12.05 ± 0.30 to 23.95 ± 0.15 , 7.66 ± 0.43 to 13.62 ± 0.29 , 38.06 ± 0.70 to 61.97 ± 0.44 , and 20.19 ± 0.58 to 38.30 ± 0.47 $\mu\text{g}/\text{mosquito}$, for lipid, glucose, glycogen and protein, respectively. While Adult values were, respectively, ranged from 10.47 ± 0.36 to 25.29 ± 0.99 , 6.46 ± 0.30 to 15.22 ± 0.90 , 33.54 ± 1.94 to 67.66 ± 0.49 and 17.29 ± 0.45 to 41.04 ± 0.24 $\mu\text{g}/\text{mosquito}$. The present study showed that photoperiod had significant influence on teneral reserves of *Cx. quinquefasciatus* mosquito.

Keywords Lipid; Protein; Photophase; Biological Fitness; Physiology

Background

The Common house mosquito, *Culex quinquefasciatus* (Say, 1826) is among the most well studied mosquito species (Ukubuiwe et al., 2012). They are catholic species, found practically in almost any habitat capable of water retention (Olayemi et al., 2014). The species are important vectors of lymphatic filariasis (Awolola et al., 2004), a disease that has ravaged the third world countries (WHO, 2004). These mosquitoes, have also been incriminated in the spread of other important zoonotic diseases such as St. Louis Encephalitis, West Nile encephalitis, Eastern equine encephalitis, Venezuelan equine encephalitis, Japanese encephalitis, Ross River encephalitis, Murray Valley encephalitis, and Rift valley fever (Gross, 2006). Due to their ubiquitous nature and hardiness (Olayemi et al., 2010), the species also double as suitable models for studying the biology and physiology of mosquitoes and the complex interaction of mosquito species with environmental factors (Olayemi et al., 2016).

Several environmental factors affect the physiology of growth and development of insects; some of which clearly affecting teneral accumulation and energy availability at each life stage, via the effects on feeding and teneral reserve distribution. One of such factors is photoperiod (day-length), defined as the amount of light available

within a 24-hour clock (Shi et al., 2017) and expressed in numbers of hour of light versus darkness, L: D, or in hours of Light (hL) or darkness (hD) (Saunders, 2012). Adequate understanding of interactions between this important abiotic factor (photoperiod) and regulation of molecular productivity of mosquitoes, vis-i-vis, energy availability through teneral reserve accumulation is paramount in developing seasonal-specific and habitat-specific control protocols.

Although, less studied than other environmental factors (e.g. temperature, rainfall, density, larval competition, and nutrition); photoperiodic conditions are capable of affecting mosquito ecology, life history traits and disease transmission potentials. Further, photoperiod can act as a cue to seasonal changes, and provide valuable information for initiation of physiological and behavioural changes to withstand impending unfavourable weather conditions (Gillot, 2005).

Previous studies have reported significant interactions of insects with photoperiod. For example, it affects vectorial traits such as duration of development (Yee et al., 2012), immature survivorship (Leimar, 1996), metabolic rates (Lanciani and Anderson, 1993) and larval growth indices (Leimar, 1996; Ukubuiwe et al., 2017) and total teneral budget for metamorphosis (Ukubuiwe et al., 2017). Others include, adult survivorship (Leisnham et al., 2011), adult size (Costanzo et al., 2015), fecundity (Carmin and Ronald, 1993), longevity (Lanciani and Anderson, 1993), gonotrophic cycles (Jordan and Bradshaw, 1978; Oda and Nuorteva, 1987) and diapause induction (Anderson, 1968; Lounibos et al., 2003). Despite these reports on life history and behavioural traits of insects, little or no work exist on the mobilisation of teneral reserve across life stages of mosquitoes, especially, *Cx. quinquefasciatus*.

This study was designed to determine, for the first time, if day-length affects mobilization of teneral reserve components across the life stages (first, second, third and fourth larval stadia, pupae and Adult) of *Cx. quinquefasciatus* mosquitoes. As these teneral components (i.e., lipid, protein, glucose and glycogen) are critical for various immature and post-immature life activities (Briegel, 2003). It focused on understanding if photoperiodic change will affect teneral components within a life stage and across the life stages. It is, therefore, expected that the result from this study will bring into clearer perspective this aspect of mosquito biology, update literature on the subject matter and assist in taking well-informed decisions in mosquito vectors' management.

1 Materials and Methods

1.1 Laboratory insectary set-up

The insectary was set up as described by Olayemi and Ande (2009) and mosquitoes reared as described earlier (Ukubuiwe et al., 2012). From the established cohorts of *Culex quinquefasciatus* colony, day-old larvae were collected and reared following standard techniques in plastic trays (30 cm x 25 cm x 5 cm), at the rate of 250 larvae/bowl in 1000 ml of distilled water. The mean laboratory temperature and relative humidity of insectary were recorded as $28.00 \pm 2.00^\circ\text{C}$ and $83.25 \pm 2.11\%$, respectively, while mean rearing water temperature was $28.00 \pm 0.20^\circ\text{C}$.

1.2 Simulation of photoperiodic regimen

Photo-phase regimens of 0, 6, 12, 18 and 24 hours of light (hL) were simulated by varying the duration of exposure of the mosquitoes to a source of light and regulated as described by Lanciani and Anderson (1993). The 'Control' was the prevailing photoperiod condition (13 hL). Test mosquitoes were exposed to these photoperiodic regimens from the larval stage through to adulthood.

1.3 Quantification of teneral reserves of life-stages of *Cx. quinquefasciatus*

At every life stage, ten (10) mosquitoes were selected, randomly, from each photoperiodic regimen and analysed for teneral components (i.e., lipid, glycogen, glucose and protein). Teneral reserve mobilization was carried out as described by Bradford (1976), Van-Handel (1985a; 1985b), Van-Handel and Day (1988) and Kaufmann and Brown (2008).

1.4 Preparation of standards for lipid, glucose and glycogen

For lipid:

Soya bean oil (100 mg) was mixed with chloroform (100 ml), and in triplicates, 50, 100, 200 and 400 µl of the solution was added to glass tubes and placed on a heating block (at 100°C) to evaporate the solvent. Sulphuric acid (0.2 ml) was added and heated for 10 minutes (at 100°C). Vanillin-phosphoric acid reagent [vanillin (600 mg) + hot Distilled water (100 ml) + 85% phosphoric acid (400 ml)], stored in a dark place) was added to 5 ml level and mixed. This was removed from the heating block and allowed to cool until reddish colour was formed. Optical density (OD) was determined at 625 nm and µg lipid plotted against OD (Van-Handel, 1985b).

For glucose and glycogen:

Anhydrous glucose (100 mg) was dissolved in distilled water (100 ml), and in triplicates, 25, 50, 100, 150 and 200 µl of glucose solution was added to glass tubes. Anthrone reagent [95-98% sulphuric acid (385 ml) + distilled water (150 ml) + anthrone (750 mg), stored at 4°C] was added to 5 ml level and mix. This was heated for 17 minutes (at 100°C), and allowed to cool. OD was read at 625 nm and a graph of µg glucose plotted against OD (Van-Handel, 1985a).

For protein:

Fifty micro-litres (50 µl) of serial concentrations containing 10, 20, 40, 80 and 100 µg bovine serum albumin were pipetted into test tubes. The volume in the test tube was adjusted to 1 ml with phosphate buffer (0.1 M, pH 6.6). Five millimetres of protein reagent [Coomassie Brilliant Blue G-250 (100 mg) + 95% ethanol (50 ml)] were added to the test tube and the contents mixed, and OD read at 595 nm (Bradford, 1976).

1.5 Quantitative extraction of lipid, glycogen and glucose fractions from mosquito

Sodium sulphate solution (2%, 0.2 ml) was added to the mosquito sample in a glass centrifuge tube and homogenized with glass rod until no identifiable parts remained. The glass rod was washed with equal volumes of chloroform/methanol solution (0.8 ml) into centrifuge tube and centrifuged at 3000 rpm for 1 minute. The supernatant was transferred to a clean centrifuge tube, while the pellets retained for glycogen analysis. Distilled water (0.6 ml) was added to the supernatant, mixed, and also centrifuged at 3000 rpm for 1 minute. The top fraction (containing water/methanol) was separated for sugar analysis, while the bottom portion (chloroform) was used for lipid analysis (Van-Handel and Day, 1988; Kaufmann and Brown, 2008).

Lipid analysis:

The portion for lipid analysis was placed in a tube (with a marking at the 5 ml level), and heated (at 100°C) to evaporate the solvent. Sulphuric acid (0.2 ml) was then added, and heated for 10 minutes at 100°C. Vanillin reagent was added to 5 ml level and mixed. This was removed from heating block and allowed to cool, for the development of reddish colour and was stable up to 30 minutes. OD was determined at 625 nm (Van-Handel and Day, 1988; Kaufmann and Brown, 2008).

Sugar analysis:

Portion for sugar analysis was placed in a tube (with a marking at the 5 mL level), and heated (at 100°C) to evaporate the solvent down to 0.15 ml. Anthrone reagent was then added to 5 ml level and mixed. This solution was heated (for 17 minutes at 100°C), removed to cool. OD was determined at 625 nm (Van-Handel and Day, 1988; Kaufmann and Brown, 2008).

Glycogen analysis:

Anthrone reagent was added (to 5 ml level) and mixed. The solution was heated (for 17 minutes at 100°C), removed and allowed to cool. OD was determined at 625 nm (Van-Handel and Day, 1988; Kaufmann and Brown, 2008).

1.6 Quantitative extraction of protein from mosquito

Total proteins were determined by the method of Bradford (1976). Briefly, Ice-cold saline buffer (0.9% NaCl) was added to the mosquitoes placed in a centrifuge tube, homogenized and centrifuged at 4000 r.p.m. (for 20 minutes at 4°C). Supernatants were stored at -20°C until used. Phosphoric acid (85%, 100 ml) was added to the protein reagent. The resulting solution made up to a final volume of 1 litre, by diluting with distilled water. Sample solution (50 µl) was pipetted into test tubes, and adjusted to 1 ml with phosphate buffer (0.1 M, pH 6.6). OD at 595 nm was measured after 2 minutes and before 1 hour against blank prepared from 1 ml of phosphate buffer and 5 ml protein reagent (Bradford, 1976; Bakr et al., 2010).

1.7 Data analysis

Data generated were processed into means and standard deviation using Microsoft Office Excel 2016. One-way and two-ways Analyses of variance (ANOVA) were used as appropriate to compare significant differences between means among the teneral components in a photoperiodic treatment and among photoperiodic treatments within a teneral component. All decisions on statistical comparison of means was taken at $p < 0.05$ level of significance. The means were separated using Duncan multiple range test (DMRT).

2 Results

2.1 Effects of photoperiod on lipid distribution among life stages of *Culex quinquefasciatus* mosquito

Analyses revealed a significant ($p < 0.05$) effects of photoperiod on the lipid accumulation and distribution in the species (Table 1). Generally, teneral component increased, significantly, as the larvae progressed from first instar (L1) to fourth (L4) instar, and reduced subsequently at pupal and adult stages. More so, increase in photo-duration (from 0 to 24 hL), significantly, decreased lipid composition in the life stages.

Table 1 Effects of photoperiod on lipid distribution (µg/mosquito) among life stages of *Culex quinquefasciatus* mosquito

Photoperiodic levels (hL)	Average larval composition	Pupal stage	Average immature composition	Adult stage
0	23.27±0.10 ^{d*}	26.66±0.49 ^d	23.95±0.15 ^c	25.29±0.99 ^c
6	22.08±0.33 ^d	24.99±0.11 ^d	22.67±0.25 ^c	24.03±0.06 ^c
12	19.05±0.24 ^c	22.90±0.08 ^c	19.82±0.21 ^b	21.25±1.01 ^b
13 (Control)	19.28±0.15 ^c	23.45±0.63 ^c	20.11±0.24 ^b	21.14±0.73 ^b
18	14.23±0.15 ^b	14.13±0.50 ^b	14.21±0.08 ^a	12.25±1.59 ^a
24	12.05±0.14 ^a	12.08±0.93 ^a	12.05±0.30 ^a	10.47±0.36 ^a

Note: *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

The range of values for the larval instars were, respectively, 10.02±0.13 to 15.87±0.43, 11.72±0.37 to 23.20±0.29, 12.21±0.30 to 25.45±0.73, and 14.24±0.72 to 28.56±0.51 µg/mosquito for L1, L2, L3, and L4, respectively at 24 and 0 hL (Figure 1). These translated into average larval composition (ALC), ranging from 12.05±0.14 to 23.27±0.10 µg/mosquito, also at 24 and 0 hL (Table 1). Composition of lipid at the pupal life stage also varied with photoperiod (range = 12.08±0.93 to 26.66±0.49 µg/mosquito). While average immature (range = 12.05±0.30 to 23.95±0.15 µg lipid/mosquito) and adult lipid composition (10.47±0.36 to 25.29±0.99 µg lipid/mosquito), also varied with photoperiodic lengths (Table 1).

2.2 Effects of photoperiod on glucose distribution among life stages of *Culex quinquefasciatus* Mosquito

There were significant ($p < 0.05$) effects of photoperiod on glucose distribution across the life stages of the mosquito species, with significant decrease in quantity as photo-duration increased from 0 to 24 hL (Table 2). Meanwhile, analyses revealed similar trend in composition pattern as lipid distribution, i.e., an initial increase in the teneral component as the larvae progressed from L1 to L4, with subsequent reduction at later life stages.

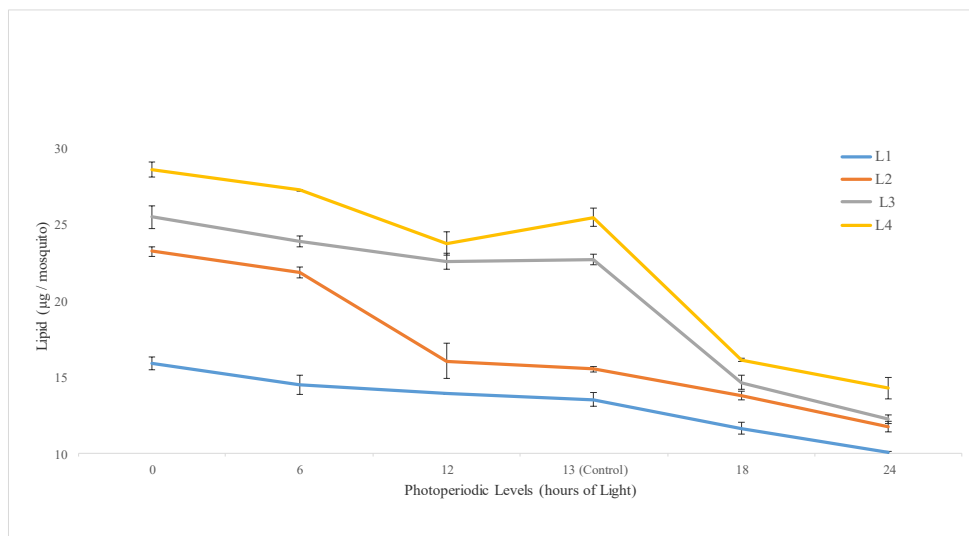


Figure 1 Influence of photoperiod on lipid mobilisation across the four larval instars (L1–4) of *Culex quinquefasciatus* mosquito

Table 2 Effects of photoperiod on glucose distribution (µg/mosquito) across life stages of *Culex quinquefasciatus* mosquito

Photoperiodic levels (hL)	Average larval composition	Pupal stage	Average immature composition	Adult stage
0	13.04±0.32 ^{e*}	15.97±0.17 ^e	13.62±0.29 ^c	15.22±0.90 ^d
6	11.63±0.59 ^d	14.03±0.13 ^d	12.11±0.50 ^d	11.46±0.72 ^c
12	9.86±0.33 ^c	11.87±0.21 ^c	10.26±0.30 ^c	9.23±0.72 ^b
13 (Control)	9.56±0.10 ^c	12.34±0.74 ^c	10.12±0.21 ^c	9.41±0.79 ^b
18	8.18±0.29 ^b	10.31±0.16 ^b	8.60±0.20 ^b	8.68±0.27 ^b
24	7.38±0.36 ^a	8.73±0.68 ^a	7.66±0.43 ^a	6.46±0.30 ^a

Note: *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

The values of glucose composition in L1, L2, L3, and L4 ranged from 5.32±0.27 to 8.23±0.19, 6.21±0.92 to 11.24±0.41, 8.09±0.05 to 15.48±0.55 and 9.89±0.31 to 17.20±0.20 µg/mosquito, respectively (Figure 2). Average larval glucose composition (range = 7.38±0.36 to 13.04±0.32 µg/mosquito) and pupal composition (8.73±0.68 to 15.97±0.17 µg/mosquito) reduced with increasing photoperiod. Average immature and adult glucose composition ranged, respectively, from 7.66±0.43 to 13.62±0.29 µg/mosquito and 6.46±0.30 to 15.22±0.90 µg/mosquito, respectively, at 24 and 0 hL (Table 2).

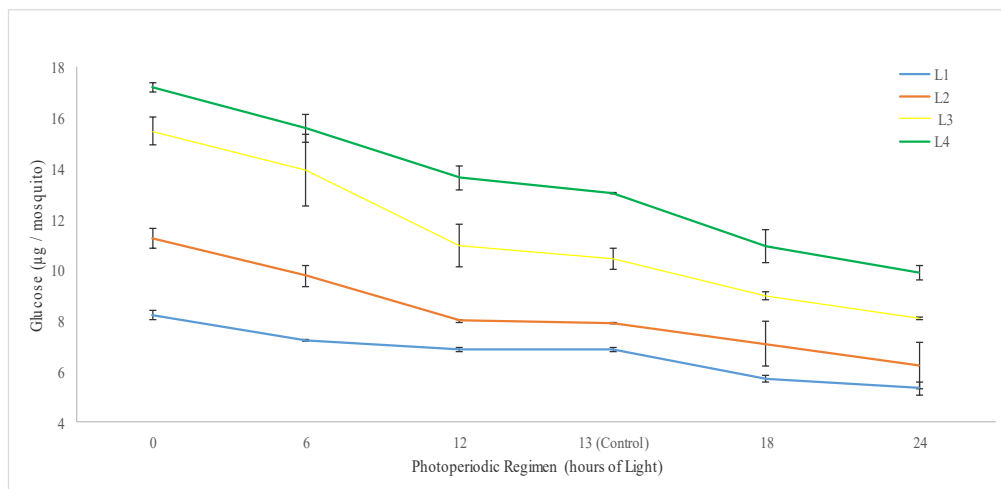


Figure 2 Influence of photoperiod on glucose mobilisation across the four larval instars (L1–4) of *Culex quinquefasciatus* mosquito

2.3 Effects of photoperiod on glycogen distribution among life stages of *Culex quinquefasciatus* mosquito

Analyses also revealed significant ($p < 0.05$) effects of photoperiod on the glycogen distribution across the life stages. These effects were similar to those observed on lipid and glucose distribution (i.e., significant decrease in quantity as photo-duration increased from 0 to 24 hL) (Figure 3 and Table 3).

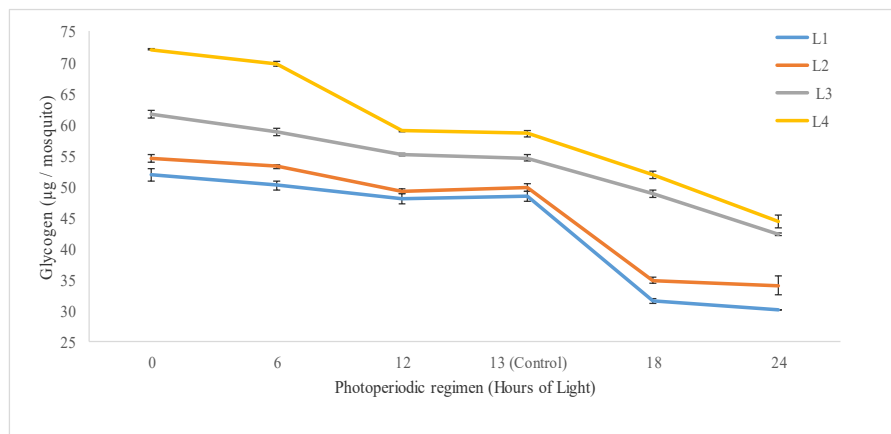


Figure 3 Influence of photoperiod on glycogen mobilisation across the four larval instars (L1–4) of *Culex quinquefasciatus* Mosquito

Table 3 Effects of photoperiod on glycogen distribution (µg/mosquito) among life stages of *Culex quinquefasciatus* mosquito

Photoperiodic levels (hL)	Average larval composition	Pupal stage	Average immature composition	Adult stage
0	59.79±0.53 ^{e*}	69.96±0.07 ^f	61.97±0.44 ^c	67.66±0.49 ^c
6	57.98±0.38 ^d	68.12±0.00 ^e	60.01±0.30 ^d	65.53±0.59 ^d
12	52.80±0.08 ^c	56.03±0.13 ^c	53.45±0.09 ^c	55.27±0.05 ^c
13 (Control)	52.81±0.19 ^c	57.64±0.47 ^d	53.77±0.23 ^c	55.04±0.84 ^c
18	41.75±0.33 ^b	45.58±0.68 ^b	42.52±0.34 ^b	41.70±0.54 ^b
24	37.67±0.72 ^a	39.63±0.66 ^a	38.06±0.70 ^a	33.54±1.94 ^a

Note: *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

The range of values for the glycogen accumulated during the L1, L2, L3, L4 and pupa life stages are, respectively, 30.12±0.01 to 51.76±1.03, 34.00±1.53 to 54.49±0.67, 42.25±0.24 to 61.58±0.55, 44.32±1.09 to 72.05±0.06, and 39.63±0.66 to 69.96±0.07 µg/mosquito (Figure 3). Average immature and adult glycogen composition ranged from 38.06±0.70 to 61.97±0.44 and 33.54±1.94 to 67.66±0.49 µg/mosquito, respectively (Table 3).

2.4 Effects of photoperiod on protein distribution among life stages of *Culex quinquefasciatus* mosquito

As in earlier teneral components analyzed (i.e., lipid, glucose and glycogen), increasing daylength from 0 to 24 hL significantly ($p < 0.05$) reduced protein accumulation, hence, distribution across the life stages (i.e., photoperiod exhibited an inverse relationship with protein content). Protein contents of the species increased significantly as the larvae progressed from L1 to L4, with significant ($p < 0.05$) reduction as it progressed to pupal and Adult life stages (Table 4).

Table 4 Effects of photoperiod on protein distribution (µg/mosquito) among life stages of *Culex quinquefasciatus* mosquito

Photoperiodic levels (hL)	Average larval composition	Pupal stage	Average immature composition	Adult stage
0	37.01±0.43 ^{e*}	43.49±0.67 ^e	38.30±0.47 ^c	41.04±0.24 ^c
6	34.94±0.19 ^d	42.41±0.49 ^d	36.43±0.25 ^d	39.76±0.58 ^d
12	31.24±0.28 ^c	32.61±0.67 ^c	31.52±0.33 ^c	31.89±0.32 ^c
13 (Control)	30.62±0.15 ^c	32.28±0.36 ^c	30.96±0.07 ^c	30.80±0.51 ^c
18	26.97±0.75 ^b	27.15±1.60 ^b	27.01±0.87 ^b	22.91±1.79 ^b
24	20.34±0.74 ^a	19.60±0.05 ^a	20.19±0.58 ^a	17.29±0.45 ^a

Note: *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

The range of values for accumulated protein in L1-4, pupa and adult were, respectively, 17.97±0.43 to 28.80±0.59, 19.73±0.54 to 34.41±0.79, 20.50±1.16 to 39.29±0.38, 23.18±0.96 to 45.50±0.67, 19.60±0.05 to 43.49±0.67, and 17.29±0.45 to 41.04±0.24 µg/mosquito, respectively (Table 4 and Figure 4). Meanwhile, Average Larval Composition (ALC), ranged from 20.34±0.74 to 30.62±0.15 µg/mosquito and Average Immature Composition ranged from 20.19±0.58 to 38.30±0.47 µg/mosquito (Table 4).

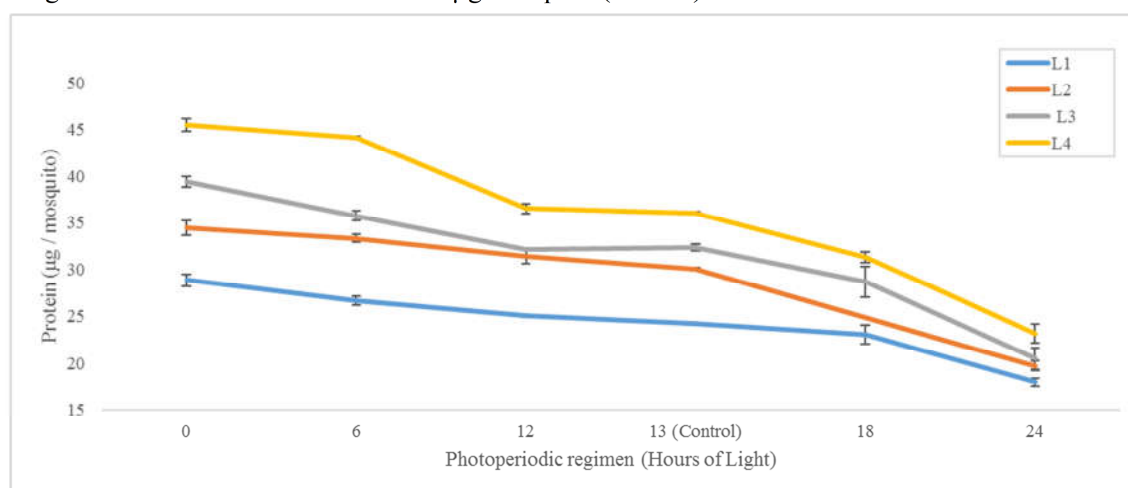


Figure 4 Influence of photoperiod on protein mobilisation across the four larval instars (L1–4) of *Culex quinquefasciatus* mosquito

3 Discussion

In the present study, analyses revealed significant effects of different photo-regimens on teneral reserve accumulation and mobilization across the different life stages of *Cx. quinquefasciatus* mosquito. The mosquito, developmentally, have three life stages, viz., larval stage (made up of four instars, L1 - L4), a pupal and an adult stage; and all life stages of mosquito are epidemiologically important in disease transmission. For example, the fourth is not only the last phago-stage (Timmerman and Briegel, 1998), but represents the pinnacle of biomass accumulation (Timmerman and Briegel, 1999). The Pupal stage, also, on the other hand, due to its low mortality rates and the proximity to the adult stage, is epidemiologically important, in determining species' success rate and adult population (Tun-Lin et al., 2009; Tran et al., 2013). While the Adult stage is important as it is solely responsible for transmission of important human disease (Olayemi et al., 2014).

Despite, the epidemiological significance of these life stages, poor or impaired mobilisation of teneral reserve (especially due to ontogenic stress) significantly affect biological fitness attributes (Timmerman and Briegel, 1993; Briegel, 2003). More so, the quantity of teneral reserve and degree of accumulation during the immature life stage of a mosquito determines the quality of adult life (Briegel, 1990).

In the present study, there were gradual increase in the quantities of all teneral components (lipid, protein, glycogen and glucose) as the mosquito progressed through the larval instars across all photoperiodic regimens. This is an indication that, irrespective of the photoperiodic regimen, growth and development was stimulated. Earlier, Timmerman and Briegel (1999) had correlated growth in mosquitoes with biomass accumulation. Suggestive that in nature, this mosquito species can grow and development at all range of photoperiod, ability that could have enhanced their vectorial capability in disease epidemiology.

More so, in this study, it was observed that irrespective of the photoperiodic regimen, the quantities of lipid and glycogen accumulated across all life stages were significantly higher than protein and glucose. This phenomenon was physiological, from the standpoint of the importance of the former two teneral components in mosquito's biology (Kerkut and Gilbert, 1985; Gäde, 2004). Similar trends in quantity of these tenerals were observed at the pupal and adult life stages of the mosquito at all regimen.

Among the photoperiodic regimens studied, short photophase (0 and 6 hL) favoured the accumulation of greater teneral contents, while longer photophase (18 and 24 hL) caused significant reduction. It, therefore, suggests that development under shorter photophases may encourage greater accumulation of teneral reserves. Earlier studies have correlated greater quantities of reserve components with higher body weights (Lanciani and Anderson, 1993); hence, it could be inferred that these individuals raised at zero and short day-lengths (6L) have greater body weights and could be better fit as vectors of disease. Previous work on *Anopheles quadrimaculatus* (Lanciani, 1992; 1993) and *Cx. pipiens* (Benoit and Denlinger, 2007), also reported higher body weights in individuals raised at short photoperiods.

These findings are important in mosquito-vector-management protocols, in the face of dwindling and/or scarce economic resources and poor government funding in vector control programmes in developing economies, especially, in the study area. Firstly, there are arrays of breeding habitats closer to human habitation, which are, either perpetually in darkness or receives less amount of light (e.g., damaged or abandoned septic tanks). These habitats are, unfortunately, usually productive for mosquitoes (Grech et al., 2013; Olayemi et al., 2014) and may be turning out mosquitoes that are physiologically fit for pathogen transmission. Unfortunately, such habitats are mostly ignored, either since they are not perceived as threat, epidemiologically, or due to the fact that, mosquitoes developing in them are usually unnoticed. Secondly, although, habitats with greater exposure to light (e.g., rice fields or water pools), which are productive for mosquitoes, and receive greater attention during vector control programs may be producing less fit mosquitoes. More so, unlike in dark habitats, the productivity of these exposed habitats are usually visible and, hence, attract greater attention and allocation of control resources. Therefore, scarce economic resources could be channeled in controlling mosquito vectors inhabiting dark habitations, while applying less expensive method like larval source management for the sunlit habitats.

Authors' contributions

Azubuikwe, Israel, Francis, and Innocent conceived and designed the experiment. Azubuikwe and Bulus performed the experiments. Azubuikwe, Israel and Chinenye analysed the data. Azubuikwe and Chinenye wrote the first draft of the manuscript. Israel, Francis and Innocent contributed to writing of the manuscript. All authors read and approved the final manuscript.

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