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EFFECT OF TEMPERATURE ON THE POSTEMBRYONIC STAGES AND ADULTS OF THE BLOWFLY, *CHRYSOMYA CHLOROPYGA* (DIPTERA: CALLIPHORIDAE).

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ABSTRACT

Chrysomya chloropyga biology was studied under controlled temperature (15.6, 22.2, 28.8, 32.2, and 36.1 °C) for an incubation period of eight hours. Development and survival of incubated eggs, larval stages I, II and pupae to adult emergence were highest between 22.2 and 28.8 °C and resistant to temperatures below and above that range. Third larva stage showed a difference, with emergence higher at 15.6 than at 22.2 °C. No adult fly emerged at 36.1 °C. The male and female flies of ages 0, 5, 10, 15 and 20 days were divided into batches (n = 20). Batches of male and female flies was incubated separately for eight hours and further monitored for 72 hours in the laboratory for survival. Adult males and females survived within 15.6 – 28.8 °C than at higher temperatures irrespective of the ages; 36.1 °C had a lethal effect on the flies. There was no significant difference (P>0.05) in the survival of adult flies at incubation and after 72 hours of exposure. The results suggest that the effect of temperature depends upon the stage of development of *Chrysomya chloropyga.* Temperature is fundamental in the development of *C. chloropyga* and should be considered during PMI determination and in the formulation of control strategies.

KEYWORDS: Calliphoridae, *Chrysomya chloropyga,* thermal requirements, temperature-dependent development, developmental duration.

INTRODUCTION

Blowflies are part of the order Diptera and family Calliphoridae. The blowfly family includes a variety of carrion feeding species (e.g. *Calliphora*, *Lucilia*, and *Phormia*) that are important to forensic and medical entomologists (Sulakova & Bartak, 2013; Joseph *et al*., 2015). They are attracted to scenes where bleeding occurred, and are quick to arrive in large numbers when an animal dies. *Chrysomya* like other fly genera undergo complete metamorphosis and develop along four stages which are egg, larva, pupa and adult. *Chrysomya* species develop through three larval stages. Adult *C. chloropyga* lives for an average of 66 – 68 days under laboratory condition on mixed diet of ground rice and fish (Ajayi & Muse, 2015). Knowledge of the general biology of *Chrysomya* species is relevant in the studies of forensic science (Williams & Villet, 2006).

Time-temperature interaction has been reported as a determinant in insect survival (Terblanche *et al*., 2008). The minimal duration of development from oviposition to adult emergence in *Chrysomya* species was inversely related to temperature, and the developmental time from oviposition to adult eclosion might be different in various regions (Grassberger & Reiter, 2001; 2002; Claver & Yaqub, 2015; Bansode *et al*., 2016).

Metabolic rate of flies is known to be higher and lifespan shorter at elevated ambient temperature (Sohal *et al*., 1985). At lower temperature, the metabolic processes were delayed; developmental times, oviposition and egg eclosion were prolonged (Benkova & Volf, 2007). Mortality rate increased with increasing temperatures, with male longevities less than those of females in adult *Musca domestica*. Fecundity and longevity also decreased with increasing temperature (Fletcher *et al*., 1990; Lysyk, 1991). Development time differed significantly between strain and temperature (Tarone *et al*., 2011).

The developmental times of the eggs, larvae and pupae of the peach fruit fly *Bactrocera zonata* and the cucurbit fly *Dacus ciliatus* was significantly decreased with increasing temperature from 20 to 40 °C for the species (El-Sabah *et al*., 2012). There was a reduction in mortality of the larval stable fly *Stomoxys calcitrans* at warmer temperature (Lysyk & Selinger, 2012). Ricalde *et al*. (2012) reported that the duration and survival of the developmental stages of *Ceratitis capitata* varied with temperature, egg to adult development time was inversely proportional to temperature.

Since *C. chloropyga* feed on decomposing organisms and waste materials, the fly carries along with it germs from its filthy habitats, man could be in danger if his

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environment or food is contaminated. *C. chloropyga* also feeds and deposits their eggs in and around exposed wounds of ruminants causing myiasis (Bisdorf & Wall, 2008).

Health implications due to activities of *C. chloropyga* on humans and animals requires appropriate measures to control the population of *C. chloropyga.* In spite of the revealed control methods using synthetic insecticides, such as pyrethrin, aerosols, diazinon, flight traps baited with dimethyl trisulphide (Bates, 2004; Bisdorf & Wall, 2008); insect growth regulators such as diflubenzuron, cyromazine and dicyclanil (Wall & Lovatt, 2015); the species remain ubiquitous in our environments. Information on the effect of temperature variations on the biology of *C. chloropyga* in the tropics is scanty in spite of the ubiquity of the fly in our environment. It is against this background that the present study examines the possible effects of temperature variations (as population control strategy) on the developmental stages, viz. eggs, larvae, and pupae; and survival of adult *Chrysomya chloropyga*.

MATERIALS AND METHODS

Collection of *Chrysomya chloropyga*

Adults of *C. chloropyga* were collected with a sweep net from Obafemi Awolowo University incinerator, Ile-Ife, Nigeria. They were reared for five generations to breed a laboratory stock.

Rearing of *Chrysomya chloropyga*

A self-supporting colony of the fly species was raised in a climate room at temperature 25 °C and 60 ± 5% relative humidity and L13:D11 photoperiod. The adult male and female flies were kept in cages ($40\times30\times30cm^3$). Fine sawdust was laid at the bottom of the cages. The flies were provided continuously with sugar and mixture of ground dried fish and rice moistened with water in the ratio 1:1:1.5 w/v.

Eggs laid on culture medium were removed from the cage and transferred into another cage for hatching. The feed served not only as source of food-protein but also as breeding site for the fly larvae. After the eggs had been laid on the feed, it was removed from the cage and transferred into another cage where the eggs eventually hatched. The larvae fed voraciously to pass through the three larval stages. At the third and final stage, the larva migrated from the petri dish containing the feed and settled into the bedding sawdust in the cage where they eventually pupated. The effect of temperature variation was subsequently observed in four replicates on the development of egg, larva, pupa, adult eclosion and survival under controlled temperatures using SP X-II Biochemistry incubator in the laboratory.

Development of Eggs, Larvae and Pupae

Twenty (20) eggs, larvae and pupae were separately exposed to 15.5, 22.2, 28.8, 32.2 and 36.1 °C in the incubator for a period of eight hours. They were thereafter taken to the insectary for further development on mixture of ground dried fish and rice diet in a rearing chamber at 25 °C and 60% relative humidity, after which they were monitored for further developments and the number of those that eventually emerged to adults were counted and recorded.

Adult Survival

Twenty adult males and females each of ages 0, 5, 10, 15 and 20 days were exposed to various temperatures (15.5, 22.2, 28.8, 32.2 and 36.1°C). After eight hours of exposure, the number of dead flies were removed and recorded. The survived adults were further monitored for 72 hours on mixture of ground rice and fish diet in a rearing chamber at 25 °C and 60% relative humidity. Water and sugar were also provided.

Statistical Analysis

Data obtained were analysed by applying one way ANOVA using IBM SPSS® version 23 statistical package. Tukey's HSD Post Hoc test was used to resolve differences among means. A value of $P < 0.05$ was used to indicate significant difference among groups.

RESULTS

An array of constant temperatures between 15.6 and 36.1 °C were used for this study. Twenty eggs, larvae, pupae stages and adults of *C. chloropyga* were incubated for 8 hours, and the number of individuals that survived was recorded. The mean survival of the developmental stages was observed as shown in Table 1.

In sequence, eggs and pupae survival were highest at 22.2, 28.8, 15.6 and 32.2 °C. Larval instars I and II showed a similar survival trend. Survival was highest at 22.2 °C. Instar III survival was highest at 28.8 °C. All immature stages showed high mortality at 36.1 °C due to their inability to develop in hot temperatures. Analysis of variance revealed significant differences ($P < 0.05$) in the mean survival of developmental stages at various temperatures.

Table 1: Effect of temperature on the survival of pre-imago stages of *C. chloropyga* (N = 20).

Temperature (°C)	Egg	1st Instar Larva	2nd Instar Larva	3rd Instar Larva	Pupa
15.6	9.00 ± 0.9129[a]	7.75 ± 1.7500[a]	10.75 ± 0.4787[a]	11.25 ± 1.1087[ab]	13.00 ± 0.7071[c]
22.2	18.00 ± 0.4083[c]	17.25 ± 1.2500[b]	16.75 ± 0.8539[b]	9.75 ± 0.6292[a]	19.00 ± 0.4083[e]
28.8	12.50 ± 0.6455[b]	12.00 ± 0.8165[ab]	13.00 ± 0.7071[a]	14.25 ± 0.8539[b]	15.75 ± 0.8539[d]
32.2	8.50 ± 0.8660[a]	10.00 ± 1.4142[a]	11.00 ± 1.4720[a]	8.75 ± 0.4787[a]	8.25 ± 0.4787[b]
36.1	0.00	0.00	0.00	0.00	1.00 ± 0.4083[a]

Data are expressed as mean ± SE; Mean ± SE followed by the same alphabet, within a column, are not significantly different (P < 0.05) by Tukey HSD test.

The survival of incubated developmental stages were further monitored till adult emerged (Table 2). Growth rate for egg, larval instar I, larval instar II and pupa was highest at 22.2 °C and lowest at 36.1 °C. The emergence of adult from larval instar III was highest at 28.8 °C and lowest 36.1 °C. Males emerged quicker than females. The mean adult emergence from developmental stages showed significant difference with varying temperatures (P < 0.05).

Twenty each of adult male and female flies ages 0, 5, 10, 15 and 20 days were incubated for 8 hours; the number of individuals that survived were recorded immediately and further monitored for 72 hours to observe survival.

Table 2: Adult emergence from pre-imago stages of *C. chloropyga* pre-exposed to different temperatures.

Temperature (°C)	Adult Emergence				
	Egg	1st Instar Larva	2nd Instar Larva	3rd Instar Larva	Pupa
15.6	7.25 ± 0.6292[a]	6.00 ± 1.4720[a]	10.50 ± 0.6455[a]	9.75 ± 1.0308[a]	12.50 ± 0.6455[c]
22.2	17.00 ± 0.7071[c]	15.75 ± 2.0156[b]	16.50 ± 0.6455[b]	9.50 ± 0.6455[a]	17.75 ± 1.3150[d]
28.8	10.75 ± 0.6292[b]	10.25 ± 1.1087[ab]	10.50 ± 0.6455[a]	13.25 ± 0.7500[b]	13.75 ± 0.7500[c]
32.2	8.00 ± 0.4083[a]	9.50 ± 1.3229[a]	10.00 ± 0.8165[a]	8.25 ± 0.6292[a]	7.50 ± 0.8660[b]
36.1	0.00	0.00	0.00	0.00	1.00 ± 0.4083[a]

Data are expressed as mean ± SE; Mean ± SE followed by the same alphabet, within a column, are not significantly different (P < 0.05) by Tukey HSD test.

The mean survival of adult male and female *C. chloropyga* exposed to various temperatures are shown in Tables 3. Optimum survival was recorded at 22.2 °C among all the ages. 36.1 °C was lethal and did not support survival. Among the male flies, ages 0 and 10 days showed similar pattern of mean survival while ages 5, 15 and 20 days showed similar mean survival across the temperature gradients. There was significant difference (P < 0.05) for the mean survival of all ages of adult male flies.

Among the female flies, ages 0, 10, 15 and 20 days showed similar mean survival at 22.2, 28.8, 15.6, 32.2 and 36.1 °C while adult females at age 5 showed significant difference with mean survival at 15.6 than 28.8 °C. Analysis of variance showed significant differences (P < 0.05) in the mean survival of all ages of adult female flies.

Table 3: Survival of adult *C. chloropyga* exposed to different temperatures (N = 20).

Temperature (°C)	Age of adult (in days)				
	0	5	10	15	20
			Males		
15.6	12.00 ± 0.9129[b]	13.00 ± 0.7071[c]	11.50 ± 0.6455[b]	11.50 ± 1.0408[b]	12.50 ± 0.6455[b]
22.2	20.00 ± 0.0000[c]	20.00 ± 0.0000[d]	19.25 ± 0.4787[c]	19.00 ± 0.4083[c]	19.50 ± 0.2887[c]
28.8	13.25 ± 0.4787[b]	10.50 ± 0.6455[b]	12.25 ± 0.8539[b]	11.00 ± 1.0801[b]	10.75 ± 0.8539[b]
32.2	6.00 ± 0.4083[a]	5.00 ± 0.7071[a]	5.75 ± 0.2500[a]	5.50 ± 0.5000[a]	6.00 ± 0.4083[a]
36.1	0.00	0.00	0.00	0.00	0.00
			Females		
15.6	13.00 ± 0.9129[b]	14.25 ± 1.3150[b]	12.50 ± 1.4434[b]	13.50 ± 1.1902[b]	12.00 ± 0.8165[b]
22.2	20.00 ± 0.0000[d]	19.50 ± 0.2887[c]	20.00 ± 0.0000[c]	19.25 ± 0.2500[c]	20.00 ± 0.0000[c]
28.8	15.50 ± 0.6455[c]	12.50 ± 0.6455[b]	13.00 ± 0.4083[b]	13.75 ± 0.8539[b]	13.00 ± 0.9129[b]
32.2	7.00 ± 0.5774[a]	7.50 ± 0.6455[a]	8.25 ± 0.8539[a]	9.75 ± 0.4787[a]	8.50 ± 0.6455[a]
36.1	0.00	0.00	0.00	0.00	0.00

Data are expressed as mean ± SE; Mean ± SE of the same sex followed by the same alphabet, within a column, are not significantly different (P < 0.05) by Tukey HSD test.

Table 4 shows the mean survival of male and female *C. chloropyga* pre-exposed to the various temperatures and the monitored for a further 72 hours. The pattern of survival after 72 hours was similar across the board when compared with Table 3. Male and female flies showed the highest survival rate at 22.2 °C. Survival was critically low at 32.3 °C. Analysis of variance showed significant differences ($P < 0.05$) in the mean survival of all ages of adult female flies monitored for 72 hours.

Table 4: Survival of adult *C. chloropyga* pre-exposed to different temperatures further monitored for 72 hours.

Temperature (°C)	Age (in days)				
	0	5	10	15	20
			Males		
15.6	10.75 ± 0.7500^{b}	12.25 ± 0.8539^{b}	9.75 ± 0.2500^{b}	9.75 ± 0.8539^{b}	11.25± 0.4787^{b}
22.2	19.25 ± 0.2500^{c}	20.00 ± 0.0000^{c}	19.00± 0.5774^{c}	19.00± 0.4083^{c}	18.75± 0.4787^{c}
28.8	11.50 ± 0.2887^{b}	10.25 ± 0.8539^{b}	12.25± 0.8539^{b}	10.2 ± 1.2500^{b}	10.50± 0.6455^{b}
32.2	5.25 ± 0.7500^{a}	3.50 ± 0.8660^{a}	4.00 ± 0.9129^{a}	4.75 ± 0.6292^{a}	5.50 ± 0.6455^{a}
36.1	0.00	0.00	0.00	0.00	0.00
			Females		
15.6	11.75 ± 0.4787^{b}	14.00 ± 1.4720^{b}	12.00 ±1.2247^{b}	12.75 ±1.0308^{b}	11.25 ±0.4787^{b}
22.2	19.75 ± 0.2500^{c}	19.25 ± 0.2500^{c}	19.00 ±0.7071^{c}	17.75 ±0.7500^{c}	19.75 ±0.2500^{c}
28.8	13.00 ± 1.0801^{b}	12.50 ± 0.6455^{b}	12.50 ±0.5000^{b}	13.75 ±0.8539^{b}	13.00 ±0.9129^{b}
32.2	6.50 ± 0.2887^{a}	6.25 ± 0.6292^{a}	5.75 ± 1.3150^{a}	7.75 ± 0.8539^{a}	8.25 ± 0.4787^{a}
36.1	0.00	0.00	0.00	0.00	0.00

Data are expressed as mean ± SE; Mean ± SE of the same sex followed by the same alphabet, within a column, are not significantly different ($P < 0.05$) by Tukey HSD test.

DISCUSSION

Temperature is a major climatic factor that influences insect survival and development. Temperature affects various physiological and enzyme activities in insect (Trudgill *et al*., 2005; Terblanche *et al*., 2008). Insect development depends on thermal requirements (Honek & Kocourek, 1990; Haddad *et al*., 1999; Krebs, 2007). However, the thermal requirements of a species vary with developmental stage and geographic origin. Each insect species has an optimal temperature range for development limited by lower and upper thresholds. Below and above these temperature limits, development does not occur (Haddad *et al*., 1999; Ricalde *et al*., 2012).

The results from this study indicated that the optimum temperature for development and survival of *C. chloropyga* is between 22.2 and 28.8 °C. Frouz *et al*. (2002) reported that in *Chironomus crassicaudatus,* slowest development was observed at 15 °C, with developmental rate peaking between 25 and 27.5 °C. The percentage survival was similar between 15.6 and 28.8 °C but higher temperature around 36.1 °C had a lethal effect on the development of both immature stages and adults. This suggests that high temperatures have militating effect on the development and survival of *C. chloropyga*. This agrees with Bansode *et al*. (2016) who reported that developmental stages of *Chrysomya megacephala* grew normally up to 35 °C but at higher temperatures, there was mortality.

The immature stages tolerate wider temperature range, with the pupal stage showing higher tolerance to the non-optimal temperatures. Zuha *et al*. (2012) reported that *Megaselia scalaris* larva survival rate was almost equal at 27 and 30 °C but lower at 33 °C. Loetti *et al*. (2008) reported that for individuals of *Culex hepperi* reared from first instar larva stage to adult emergence, total development time was inversely related to temperature between 15 and 25 °C; no adults emerged at 33 °C and survival was highest at 20 °C.

In this study, the rate of development was slower at lower temperatures and faster at high temperatures, but immature stages that reached adult emergence after exposure to high temperatures lived shorter than those that were exposed to lower temperatures. According to Lysyk (1991), the longevity of *M. domestica* decreases with increasing temperature. Claver and Yaqub (2015) reported that larvae of laboratory reared *C. megacephala* under fluctuating temperatures emerged more quickly at higher temperatures than at lower temperatures. Xiaofei and Hui (2009) reported that the developmental time of the pre-imaginal stages in *Bactrocera correcta* significantly decreased with increasing temperature from 18 to 33 °C.

Males emerged more quickly than females. A pattern of progressive decrease in the number of emerged larval stages with increasing temperature was observed. In addition, males developed faster than the females. This study corroborates the findings of Frouz *et al*. (2002) who reported that males developed faster than females during adult emergence from immature stages of *Chironomus crassicaudatus* reared at nine constant temperatures from 12.5 to 32.5 °C.

The highest percentage survival for adult *C. chloropyga* was recorded at 22 °C with 100 % survival. Rate of survival was about 66% in males while females recorded 77% survival rate at 15.6 and 28.8 °C, and these values were significantly higher than the percentage survival recorded at 32.2 °C and 36.1 °C. Female *C. chloropyga* recorded higher survival than the male. There was no significant difference in the survival of adults with respect to their age after exposure to different temperatures. Fletcher *et al*. (1990) reported that mortality rates in houseflies increased with increasing

temperature, with male longevities less than those of females.

CONCLUSION

Temperature plays a significant role in the development and survival of both immature stages and adults of *C. chloropyga***.** The rearing temperature influences some characteristics of the adult which in turn may affect species fitness. Temperature effect was not dependent on the age of the fly, but has a marginal lethal effect on the males than the females. Although, under field conditions, *C. chloropyga* may develop at high temperatures in much reduced numbers. Since elevated temperatures have a negative effect on *C. chloropyga* species, then an increase of the ambient temperature could cause a displacement of its distribution towards higher latitudes. Therefore, the knowledge of the effect of temperature on the pre-imaginal development and adult survival of *C. chloropyga* is essential to understand the biology and ecology of the species. Information provided here can be used to calculate minimum PMIs and predict population growth rate of *C. chloropyga.*

RECOMMENDATION

Temperature is vital in the development of *C. chloropyga* and should be considered during post mortem interval determination. Moreover, temperature variation along with other measures can be used as an effective means of controlling the populations of both pre-imaginal and adult stages of *C. chloropyga.*

DISCLOSURE

The authors declare no conflict of interest.

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