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Studies on Biology of the Blowfly *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) Under Fluctuating Temperature in Three Different Seasons

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Abstract: *Precise developmental data for forensic indicator blowfly by species are essential for accuracy in the estimate of the post-mortem interval (PMI) and medical science. Laboratory reared both male and female blowflies at Gorakhpur were used for studying their biology under fluctuating temperature and humidity in three different seasons. The range of temperature and humidity in the local atmosphere during the period when three sets of experiment conducted were 19°C - 24°C/ 85% - 96%, 24°C - 28°C/ 82% - 94% and 28°C - 32°C / 78% - 84% for A, B and C sets respectively. Incubation period recorded for eggs laid by three sets of individuals i.e. (A), (B) and (C) which were reared at fluctuating temperature and humidity at three different seasons were 17.36 ± 2.86 hrs, 14.52 ± 2.39 hrs and 12.65 ± 2.24 hrs, respectively. It was observed that at higher temperature and lesser humidity the nymphs emerged at more quickly than at lower temperature and high humidity; this might be due to increased metabolic activities in the individual at higher temperatures. The total stadial period observed for male in sets (A), (B) and (C) were 114.16 ± 3.43 hrs, 108.83 ± 5.01 hrs and 106.00 ± 2.64 hrs respectively, whereas for female these readings were decreased gradually when surrounding temperature increased similar to male but shorter than male. Adult longevity of for males was slowly increased then gradually decreased when the temperature was raising high but this data was not significant statically. For example in males, the reading recorded were 66.50 ± 4.50 hrs, 71. 00 ± 5.88 hrs and 67.83 ± 4.63 hrs for sets (A), (B) and (C) respectively. This experiment was performed in order to understand the biology of the blowfly C. megacephala which is most abundant species in Gorakhpur district and its greater importance in forensic science. In this study, the effect of fluctuating temperature was investigated as it is one of the most helpful aspects of the environmental variables in this process.*

Keywords: *Biology, Blowfly, Chrysomya megacephala, Fluctuating Temperatures, Gorakhpur Condition.*

1. INTRODUCTION

The family *Calliphoridae* has non-metallic and metallic flies (Ambrose 2007). Worldwide the blowfly family includes over 1000 species and 150 genera (Rognes 1991). Most species of blowflies have wide geographical distributions and they are habitually linked to the human activities. Some species are typically urban and others have veterinary importance (Rognes 1991). Blowflies are used in medical surgery for removal of dead tissue and are also used by forensic experts in calculation of time of death interval in humans and other animals (Vincent *et al*. 1985, Smith 1986). The Indian blowfly larvae, *Chrysomya megacephala* was associated with carcasses throughout the year in the northern part of India (Bharti and Singh 2003, Bharti et. al. 2007) and these flies can with stand extreme temperature fluctuations and thus can help to calculate the post mortem interval (PMI) in all the seasons of the year. Greenberg (1991), Byrd and Allen (2001), and Clarkson et al. (2004) found that fluctuating temperatures would impact larval development which could be helpful in many ways in forensic investigation and medical treatment compared with constant temperature rearing. Variety of insects including blow flies, rates of development are different when raised under fluctuating temperatures compared to a corresponding constant temperature (Ratte 1984). Moreover, any difference between development rates determined in the lab (constant temperatures) and those at an actual crime scene (fluctuating temperatures) could potentially lead to inaccurate time of calculations. Bharti et. al. (2007) attempted the age grading of the immature stages of *C. megacephala* at different constant temperature at Patiala, Punjab and suggested the need of this type of studies at various part of India for better utilisation of this Science to society. However specimens in the nature are subjected to fluctuating, not constant temperature (Nabity et. al 2006). By realising this importance, an attempted

was made at Gorakhpur to study the biology of locally collected blowfly *C. megacephala* under fluctuating temperature in three different seasons.

2. MATERIAL AND METHOD

Six female fertilized blow flies (*Chrysomya megacephala*) were collected from the surroundings of a butcher's shop by simple hand picking method at Gorakhpur (26° 13'N and 27° 29'N). These individuals were brought in the laboratory, identified and segregated into six different round plastic containers of size 16cm diameter and 7cm depth. These containers were covered with a fine muslin cloth in order to facilitate fresh air to the individuals. 100 grams of chicken flesh was offered to the individuals for laying the eggs. Some refuge and sugar syrup soaked cotton was kept in each container for feeding of individual and maintaining the humidity (50%- 70%) and temperature (25°C - 26°C) of the container at room. Soil was also provided for pupa development and adult emergence. After adults emergence again eighteen female and male flies were selected from these laboratory reared individuals for further experiments. In three groups (A), (B) and (C) were created, each group having six male and female in pairs and were allowed to mate, in order to study their biology. The container used for studying their biology was of same measurement as mentioned above and were placed outdoors on an apartment balcony in Bashratpur, Gorakhpur. They were protected from rain and direct sunlight by a roof, but they were still exposed to natural temperature fluctuations. These individuals were also feed with sugar syrup and 100g of flesh was provided for oviposition. After emergence of 3rd instar, soil was transferred in another container so that larvae can burrow underneath and carry out their development into adult. Every half an hour containers were checked for oviposition and development rate of individuals. This experiment was repeated in three different situations when temperature and humidity fluctuation were accordingly happened as 19°C - 24°C / 85% - 96% (A), 24°C - 28°C / 82% - 94% (B) and 28°C - 32°C / 78% - 84% (C).

3. RESULT AND DISCUSSION

The oviposition of blowflies occurs shortly after death of the organism; yet, various circumstances such as diural verus nocturnal oviposition pattern, access of body, or cold temperature may delay oviposition (Nabity et. al. 2006). The eggs of *C. megacephala* easily hatched into small long whitish larvae on the chicken flesh conditions were favourable for them (plate1c). Incubation period recorded for eggs laid by individual of *C. megacephala* were 17.36 ± 2.86 hrs, 14.52 ± 2.39 hrs and 12.65 ± 2.24 hrs for (A), (B) and (C) categories of fluctuating temperatures and humidity at three different seasons respectively (Table1). Similar observation was also made by Clarkson et al (2005) and stated that incubation period of another blowfly *Protophormia terraenovae* was progressively decreased with increasing temperatures. It was observed that, at higher temperature and lesser humidity the nymphs emerged at more quickly than at lower temperature and high humidity. It was also observed that time taken for development was more quick in males when compared with the females (Fig 1a). This temperature bound female biased quicker development was reported by other workers (Claver et al 2010, 2011). When the temperature fluctuated to increase as the humidity drops the stadial period was also decreased, which allowed adult to attain adult hood quickly (Nabity et. al. 2006). An increase in the temperature will increase the rate of metabolism (Gomes et. al. 2009). An earlier study done on *P. terraenovae* by Davies and Ratcliffe (1994) found that fluctuating temperature actually led to significant faster rates of development, similar to what we have found in our study. The stadial period observed for 1st instar, was higher (ie., 27.16 ± 2.40 hrs, 24.00 ± 2.58 hrs and 21.16 ± 1.77 hrs for A, B, C categories, respectively) when compared to 2nd and 3 rd instars. But in contrary Nabity et al. (2006) and Bharti et al. (2007) recorded that 2nd and 3rd instars took prolong time for development as compared to 1st instars; this may be due to different method of studies. Moreover, they performed the observations at constant temperature whereas in our observation the temperature was fluctuating (Table1). Greenberg (1991), Byrd and Allen (2001), and Clarkson et.al (2004) also reported in other blowflies that fluctuating temperatures would have provided more stress to the 1st instar larva than to the rest of the stages; which was noticed less in constant temperature situations. Pupa was dark brown to red in colour with marking on them (Plate1 d).Time period taken for the formation of pupa was observed 57.66 ± 3.54 hrs, 55.16 ± 3.71 hrs and 51.83 ± 2.85 hrs for sets (A), (B) and (C) respectively. Time period required for pupa formation declined with the increase in temperature and decreasing humidity in other blowflies (*Chrysomya albiceps* and *Lucilla cuprina*) too Gomes et al. (2009). Since less time is required to pupate at higher temperature. Pupas were formed buried in soil provided so that they can escape from the lower temperature during adult formation (Grassberger and

Reiter 2003). Time period required for adult emergence was also found in similar trend as previous data, i.e. 93.33 ± 2.49 hrs, 89.66 ± 2.86 hrs, 86.66 ± 2.98 hrs for set (A), (B) and (C) respectively. This Indian blowfly species spent minimum development times that were similar to, but somewhat faster than, those recorded by Grassberger and Reiter (2002) and Marchenko (2001).

The emerged metallic dark greenish blue adult *C. megacephala* also responded with the fluctuating temperature and humidity (Plate1 a). Egg laid by the three sets of flies (A), (B) and (C) was observed 118.16 ± 8.78, 148.00 ± 17.58 and 153.33 ± 19.71 respectively. At higher temperature and low humidity females laid more eggs on rotten flesh provided to them (Byrd and Butter 1996). Eggs laid by *C. megacephala* were slender, long, white and narrow at the ends (Plate1b). Female individuals mostly preferred to lay the eggs on the decomposing flesh but it was also observed that some female individuals laid the eggs on the walls of containers (Bharti et. al. 2007). Hatchability percentage was also greater for eggs those faced higher temperature and low humidity conditions then the eggs heads to low temperature and high humidity. Davies and Ratcliffe (1994) reported that cold temperature would not really provide enough strength for the insects to break the chorion in order to eclose. Temperature had significant effect on nymphal mortality percentage; greater percentage og mortality was recorded at two extreme fluctuating temperature ranges i.e. 19°C- 24°C and 28°C- 32°C, whereas it was observed nearly half for middle range i.e. 24°C- 28°C (Table1). Although the fluctuating temperature has not showed much effect on the survival of individuals reared on all three sets, it made slightly higher effect on the individuals of 'B' set over other two sets (Table1). Insect development rate increases linearly, but only between the temperature extremes; developmental rate becomes curvilinear at both high and low extremes with increases or decreases in temperature (Nabity et.al. 2006). Preoviposition period for all three sets of individuals were observed as 30.66 ± 3.14 hrs, 28.66 ± 3.85 hrs and 26.50 ± 3.45 hrs respectively. These data clearly suggest that if temperature was lower and humidity was increased, egg laying time period was delayed. In the case of post oviposition period female individuals of set (B) were laid eggs for longer time period than the individual of other two sets (A) and (B) (Table 1). In set (B) post oviposition was prolong (34.83 ± 1.45 hrs) as these individuals were kept at optimum temperature for egg laying. Adult longevity period was decreased as the temperature increased along with declining humidity percentage (Fig.1b). The total lifespan for males and females were not uniformly increased as the temperature was increased (Fig.1c) but, it increased initially then slightly decreased due to rate summation effect and it alters the interpretation of data near thresholds when generated under fluctuating temperatures (Kaufmann 1932).

Table1. *Biological parameters of selected Blowfly on sugar syrup (n =6; X ± S.D)*

Biological parameters	Temperature/ Humidity wise Categories		
	A (19-24°C/85-96%)	B (24-28°C/ 82-94%)	C (28-32°C/ 78-84%)
Incubation period (Hrs)	17.36 ± 2.86	14.52 ± 2.39	12.65 ± 2.24
Stadial period (Hrs)			
1stInstar	27.16 ± 2.40	24.00 ± 2.58	21.16 ± 1.77
2nd Instar	18.66 ± 1.97	15.83 ± 1.40	14.00 ± 2.23
3rd Instar	15.66 ± 2.68	9.16 ± 1.35	7.83 ± 1.67
Pupa formation (Hrs)	57.66 ± 3.54	55.16 ± 3.71	51.83 ± 2.85
Adult emergence (Hrs)	93.33 ± 2.49	89.66 ± 2.86	86.66 ± 2.98
Total stadial period (Hrs)	111.08 ± 3.98	108.41 ± 3.32	104.41 ± 1.99
Fecundity (no.)	118.16 ± 08.78	148.00 ± 17.58	153.33 ± 19.71
Hatchability (%)	72.21%	76.12%	77.17%
Nymphal Mortality (%)	17.78%	6.8%	17.04%
Survival rate (%)	82.22%	93.20%	82.96%
Preoviposition period (Hrs)	30.66 ± 3.14	28.66 ± 3.85	26.50 ± 3.45
Postoviposition period (Hrs)	30.83 ± 1.76	34.83 ± 1.45	31.6 ± 1.49
Adult longevity (Hrs)	70.66 ± 4.06	74. 58 ± 4.92	68.99 ± 4.42
Total longevity (Hrs) (1st instar to adult)	203.75 ± 8.58	214.41 ± 10.26	207.49 ± 9.40

Plate1. *(a) Adult blow fly (b) Eggs (c) Larvae (d) Cocoon*

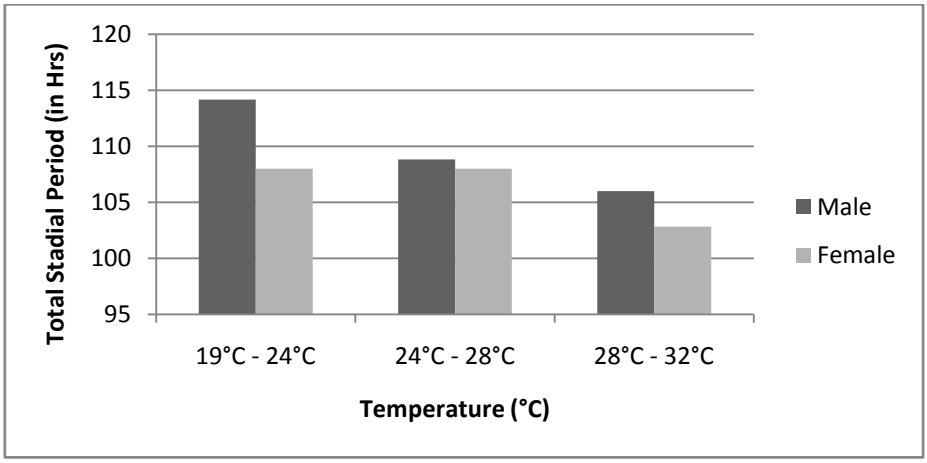

Fig1a. *Graphical representation of Total Stadial perod for Male and Female.*

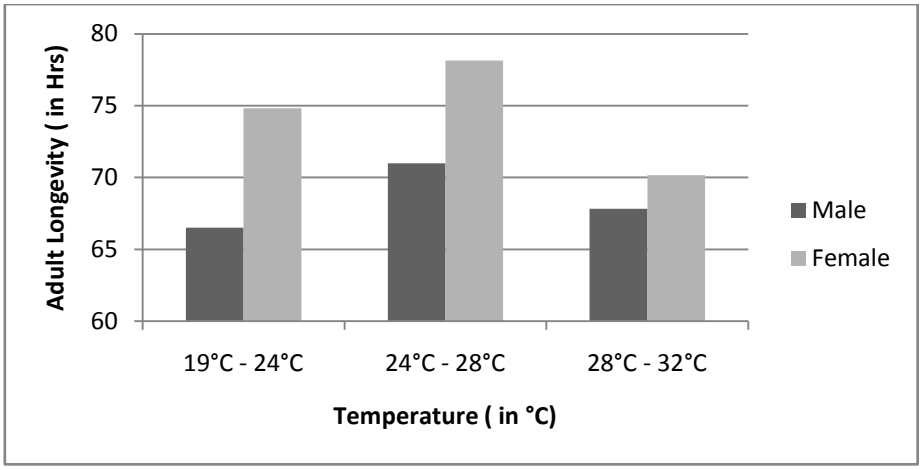

Fig1b. *Graphical representation of Adult Longevity for Male and Female.*

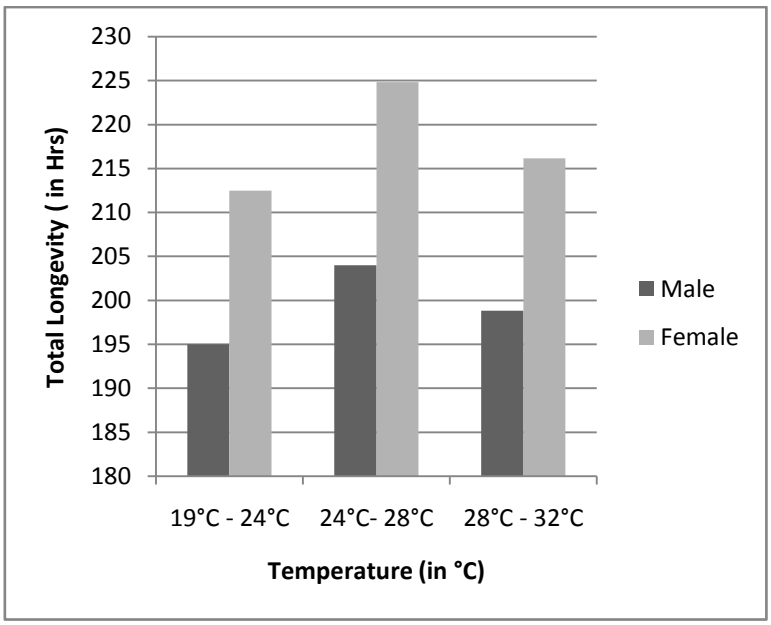

Fig1c. *Graphical representation of Total Longevity for Male and Female.*

4. CONCLUSION

We recorded the data for fluctuating temperature in the biology of *C. megacephala* which has more medical importance because specimens in nature are subjected to fluctuating temperature, not constant temperature. Experiment performed at three different varying temperatures and humidity on the life history of individuals gave a clear idea that as the temperature increases it causes a direct impact on the life history of insects. This data will provide valuable information in the forensic study of dead bodies in Gorakhpur region, where this species of blow fly is more common. Here result shows that number of eggs laid, hatchability, maggot duration and adult emergence all these phenomenon were affected by changed temperature and humidity.

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