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Effect of Different Constant Temperature on the Life Cycle of a Fly of Forensic Importance *Lucilia cuprina*

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Abstract

Lucilia cuprina is one of the forensically important Calliphoridae fly. *L. cuprina* is a helpful resource at the crime scenes as well as a nuisance to sheep. It is known to be one of the first flies to occupy a corpse upon its death. Due to this, it has great importance in forensic field to find out post-mortem interval. In this study, the development of *L. cuprina* is studied in an incubator at different constant temperatures. Larvae of the *L. cuprina* were reared in an incubator at 20°C, 25°C, 30°C, 35°C, and 40°C. During study the developmental data, temperature and relative humidity of the rearing room as well as weight, length of the larvae were recorded from the time the larvae were collected until the adult flies emerge out.

Results obtained show that development of the *L. cuprina* was slow at lower temperature. At the high temperature, developmental rate was fast. At low temperature *L. cuprina* attained greatest body weight whereas at high temperature there was a decrease in the weight. When flies were reared at 40°C the development of the fly was much more rapid but slight mortality was observed. Above study shows that temperature plays a very important role in the development of *L. cuprina*. For the correct estimation of post-mortem interval this study is very useful, because each dipteran fly has its own developmental period with respect to temperature and region.

Keywords: Forensic; Incubator; Calliphoridae; Post-mortem; *Lucilia cuprina*; Temperature

Introduction

Forensic entomology is an extensive discipline where arthropod science and judicial system interact [1]. Information gained from medico legal entomology typically is used to determine time of death, place of death and other issues of medical or legal importance. The sue of insect development in medico criminal investigations has increased as it is the most accurate measurement of post-mortem interval (PMI) [2,3].

There are over 60 insect families which play an important role in carrion ecology. However, only the families (Calliphoridae, Sarcophagidae, Staphylinidae, Cleridae and Dermestidae) of Coleoptera (beetles) are the most important to be used in forensic entomology [4,5].

Out of all the insects visiting a dead body, the maggots of blowflies (Calliphoridae) and flesh flies (Sarcophagidae) are responsible for the maximum consumption of terrestrial carrion [6-11].

In India Calliphoridae is represented by 119 species belonging to 30 genera under 9 subfamilies [12,13].

Temperature and access to a body are the two most important factors affecting insect succession. Temperature is the most important variable influencing the rate of maggot development. High temperatures generally reduce the development time of the flies. Large aggregations of dipteran larvae (maggot masses) develop heat due to their frenetic activity and fast metabolism, thus raising the micro environmental temperature [14]. The relationship between insect development and temperature has been well-established [15-18].

Lucilia (=Phaenicia) *cuprina*, the Australian sheep blow fly is an insect of medical and veterinary importance in many parts of the world. It causes Myiasis in humans and particularly cutaneous Myiasis (fly strike) in sheep. Therefore, fly species is responsible for considerable amounts of damage and can have an economic impact on sheep industry [18-22]. More recently, this fly has proven to be of forensic importance since specimens of this species have been found associated with human corpses in various regions [23-26].

The succession or arthropods development is mostly affected and influenced by temperature and humidity [27,28]. In warmer temperature and high moisture condition, insects have also been known to grow faster. The opposite conditions have also been noted to retard insect growth significantly.

L. cuprina is closely related to *L. sericata*, another forensically important species with which it may be confused due to very similar morphology [29]. The main aim of this study is to provide data on development for *L. cuprina* at various constant temperatures for more accurate estimation of PMI in medico legal forensic investigations.

Development rate of the flies are frequently used to estimate PMI in homicide investigations in the first few weeks after death. Since development of immature insects is temperature dependent, PMI is normally calculated by the accumulated degree day/hour (ADD/ADH) model (measure of thermal time taken to reach each developmental event, K) which is associated with basal temperature called the lower temperature threshold or the developmental zero (temperature below which development ceases) value [30,31].

The rate of larval growth depends on its body temperature, which is affected by environmental conditions as ambient temperature and the heat generated by maggot aggregations [32]. In addition, an important detail for PMI determination is that each species has its own temperature dependent growth rate. Therefore, for correct estimation of PMI it is very important to know the developmental rate of each forensic fly at particular temperature.

In forensic entomology, species identifications and development of baseline data for all insect species found in human and/or at death scenes are crucial pieces of information that are required to conduct accurate future forensic analyses.

The variation of developmental times between different populations emphasizes on specific characterization of regional developmental times of each forensic species.

The aim of this study is to provide data on the development of *L. cuprina* at various constant temperatures for more accurate estimation of PMImin in medico legal forensic investigations.

In this study developmental rate of *L. cuprina* is studied in an incubator at constant temperature. This study can provide important control strategies of the fly *L. cuprina*. Due to an increase of forensic entomology and its applications, it is very important to understand the rate of development of all flies of forensic importance. This developmental study may be in accordance with varied temperature, humidity ranges.

Materials and Methods

Osmanabad district is the main research area to carry out this study.

Osmanabad is one of the district of Marathwada region of Maharashtra. It is situated in the Southern part of the state abutting Andhra Pradesh on South and lies between north latitudes 17˚37' and 18˚42' and east longitude 75˚1 6' and 76˚47' and falls in parts of survey of India degree sheets 47 N, 47 O, 58 B and 56 C.

The climate of the district is characterized by a hot summer and general dryness throughout the year except during monsoon season, i.e., June to September. The mean minimum temperature is 8.5˚C and mean maximum temperature is 42.5˚C (1607/DBR/2009).

Taking this into consideration present study includes 40˚C as the highest temperature. No study has been carried out in this region on forensic flies so present study may provide accurate PMImin to the flies found in this region.

The collection of the carrion flies on the rotten liver and flesh of the dog carcass was done from various regions of Osmanabd district. Collection was done from the Urban, rural areas of the Osmanabad district. Collected flies were maintained in a cage under laboratory condition for rearing and identification purpose.

Maintenance of the flies in the laboratory

After the collection of the samples, pure cultures for *L. cuprina* was obtained by separating eggs or larvae of one female and cultured them for further experiment. Adult flies were identified morphologically using various taxonomic keys.

Fresh liver was used as oviposition or larviposition site for females. Maggot culture was provided with fresh liver as a food till the prepupae stage. The collected flies were cultured under the laboratory conditions and their identification was done at each stage of development.

As soon as *L. cuprina* laid eggs those eggs were transferred from room temperature to an incubator set at constant temperature. All the larvae were incubated inside an incubator at different constant temperatures (20˚C, 25˚C, 30˚C, 35˚C, and 40˚C). Two incubators were used to carry out study. At each level of temperature, eggs, larvae, pupae and adult flies were observed daily. The length of the larvae and pupae was also measured. Life duration of the flies studied under five different ranges of constant temperature.

The instar stages of the larvae were recorded by determining their posterior spiracle by dissecting them. Length of the larvae was measured by using 1.0 mm × 1.0 mm graph paper. Ohaus milligram sensitive balance was used to weigh the larva (Ohaus Corporation, USA, Item PAG 213, readability: 0.001 g). The developmental data and relative humidity of the rearing room were recorded from the time larvae were collected until the emergence of the adult flies.

Hygro-thermometer (Mextech TM-1, humidity Temperature clock) was used to record temperature and humidity. Same study was repeated thrice in the same incubator with former generations of the flies.

Result

At 20˚C, the lowest temperature was tested, the life cycle of the *L. cuprina* was completed in 627 h (26 days). When temperature increased to 25˚C, *L. cuprina* completed its life cycle in 531 h 22 days. At lower temperature larvae attained greatest body weight. Further increase in the temperature by 30˚C, 35˚C and 40˚C leads to the completion of life cycle of *L. cuprina* in 333 h (14 days), 287 h (11 days) and 267 (11 days) simultaneously. At lowest temperature maggots spent 627 h to complete their life cycle and 410 h they were in the pupa form. As temperature increased to 25˚C development of the fly was completed in 531 h. Observation table 1 shows the all recorded parameters during study. When temperature was 20˚C and 25˚C flies have attained 43 mg and 44.6 mg maximum weight respectively. AS temperature increased there is decrease in weight of the fly. Lowest weight was recorded at 40˚C.

At 35˚C temperature the development of *L. cuprina* was completed much rapidly in only 12 days. There is slight decrease in the weight of the maggots. At 40˚C *L. cuprina* shows slight mortality. Flies are unable to tolerate high temperature that's why mortality was observed when larvae reared at high temperature. Somehow, *L. cuprina* completed its life cycle in 11 days. There is not much difference in the development of the fly at 35˚C and 40˚C.

Date	Stage	Weight in mg.	Length in mm.	Duration in hours (h)	Temperature	Humidity
29.08.15	Egg	0.002	1.1	1 pm	20 ± 1°C	84
30.08.15	I Instar	16	3	22 h	20 ± 1°C	86
31.08.15	II Instar	30	6.1	23 h	20 ± 1°C	79
01.09.15	III Instar	38	8.6	23 h	20 ± 1°C	80
02.09.15 to 06.09.15	Prepupa	42	9.5	149 h	20 ± 1°C	77
07.09.15 to 22.09.15	Pupa	43	10	410 h	20 ± 1°C	66
23.09.15	Adult	---	---	627 (26 days)	20 ± 1°C	50
29.08.15	Egg	0.002	1.1	3 pm	25 ± 1°C	67
30.08.15	I Instar	15.9	3.1	18 h	25 ± 1°C	62
31.08.15	II Instar	30.6	6.4	26 h	25 ± 1°C	64
01.09.15	III Instar	37.8	8.3	23 h	25 ± 1°C	60
02.09.15 to 05.09.15	Prepupa	42.6	9.6	128 h	25 ± 1°C	56
06.09.15 to 18.09.15	Pupa	44.5	12	336 h	25 ± 1°C	70
19.09.15	Adult	--	--	531 (22 days)	25 ± 1°C	72
19.09.15	Egg	0.002	1	4.30 pm	30 ± 1°C	79
20.09.15	I Instar	13.9	2.9	16h 30 min	30 ± 1°C	60
21.09.15	II Instar	20.6	4.8	23 h	30 ± 1°C	63
22.09.15	III Instar	35.8	7.6	21 h 30 min	30 ± 1°C	68
23.09.15 to 25.09.15	Prepupa	42	11.5	83 h 30 min	30 ± 1°C	61
26.09.15 to 02.10.15	Pupa	40	10.7	193 h	30 ± 1°C	64
03.10.15	Adult	--	--	333(14) days	30 ± 1 °C	69
24.09.15	Egg	0.002	1.1	5.30 pm	35 ± 1°C	58
25.09.15	I Instar	15.9	3.1	14 h	35 ± 1°C	61
26.09.15	II Instar	30.6	6.4	34 h	35 ± 1°C	64
27.09.15	III Instar	37.8	8.3	14 h 30 min	35 ± 1°C	59
28.09.15 to 29.10.15	Prepupa	43.9	10.4	81 h 30 min	35 ± 1°C	63
30.10.15	Pupa	43.2	9.5	144 h	35 ± 1°C	60
04.11.15	Adult	--	--	287 h	35 ± 1°C	65
03.10.15	Egg	0.002	1.1	3.30 pm	40 ± 1°C	69
04.10.15	I Instar	12.9	3	16 h 30 min	40 ± 1°C	65
05.10.15	II Instar	28.6	4.8	33 h 30 min	40 ± 1°C	70
06.10.15	III Instar	30.8	7.3	14 h	40 ± 1°C	71
07.10.15 to 08.10.15	Prepupa	34.9	8.5	71 h 30 min	40 ± 1°C	70

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09.10.13	Pupa	30.2	9.5	122 h	40 ± 1°C	63
13.10.13	Adult	--	--	267(11 days)	40 ± 1°C	60

Table 1: Effect of various constant temperature on the life cycle of *L. Cuprina*.

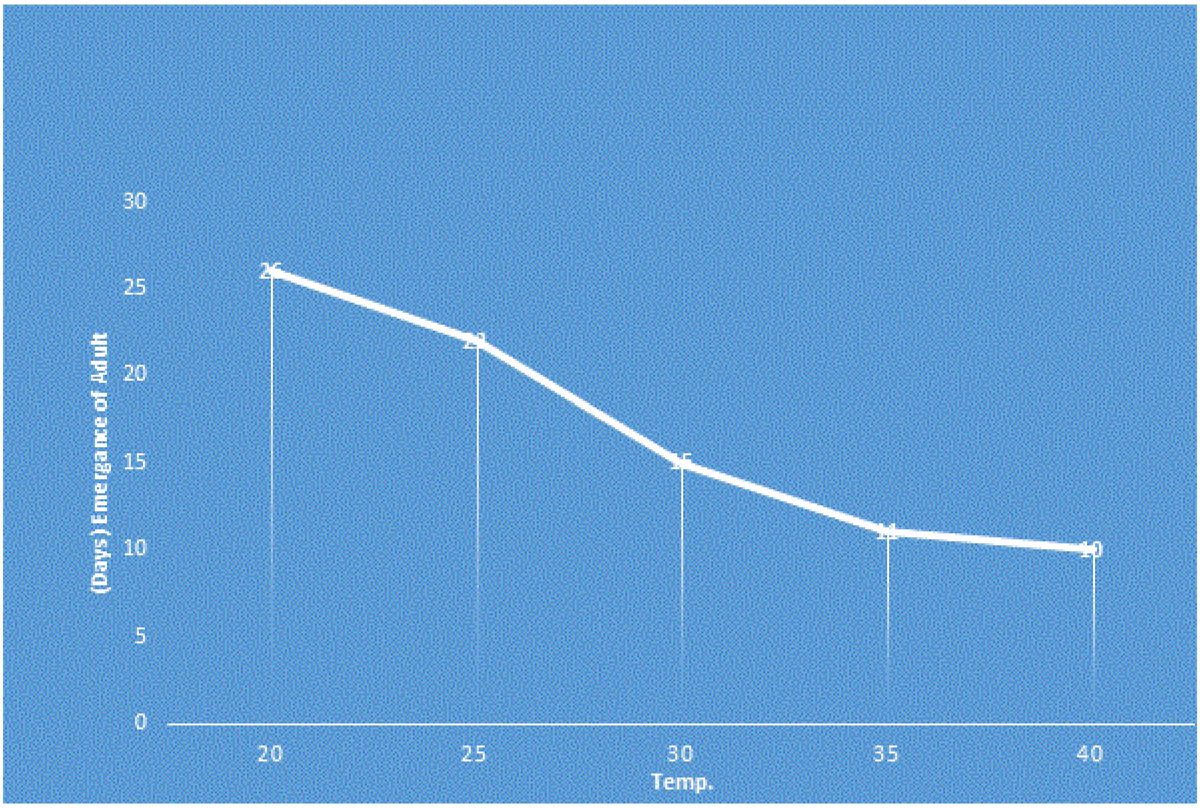

Figure 1: Graphical representation of the effect of constant temperature on development of *L. cuprina*.

Discussion

The succession of arthropods development is mostly affected and influenced by temperature and humidity [27,28]. In warmer temperature and high moisture condition, insects have been known to grow faster. The opposite conditions have been noted to retard insect growth significantly. Apart from the effect of surrounding conditions, studies have also noted that heat created by normal putrefaction processes of the body and larva mass has been known to influence the overall rate of insect development [2]. Based on this, it always has been suggested that when taking larva samples from decomposed body, the history of temperature and humidity should be noted and taken into consideration when determining the estimate time of death [33].

Abd Algalil and Zambare [34] have studied the effects of temperature on the development of Calliphorid fly of forensic importance *Chrysomya* at constant temperature. He observed that low temperature not only delays the duration of life cycle but also have an impact on the morphological parameters like length, width, and weight. At normal room temperature in the rainy season the length, width and weight of the second instar were 8.4 ± 0.16 mm, 1.8 ± 0.66 mm, 23.2 ± 0.37 mg, respectively. While at low temperature 10 ± 0.08 mm and 18.5 ± 0.67 mg. Thus in the rainy season, the duration required for laying eggs to reaching the second instar was 77 h (3021 days), but at the constant low temperature same period was 153 h. (6.38 days). In rainy season, the total larval duration was 143 h (5.96 days) at room temperature, while at low temperature it was 343 h (14.29 days). The pupal stage remained for 122 h (5.08 days) at room temperature in rainy season while at low temperature (10°C) it was 266 h (11.08 days).

Abd Algalil and Zambare [34] have studied the effect of temperature on the life cycle of *Chrysomya ruffiacies* in a different season.

Constant temperature increases the duration to complete the life cycle, reduce the activity and causes mortality [34].

Davidson [35] has also studied effects of temperature on the developmental time of blow fly life cycle and has defined the relationship between temperature and rate of development of insects at constant temperature.

In warmer temperature and high moisture condition, insects have been known to grow faster. The opposite conditions have also been noted to retard insect growth significantly.

Kotze, Villet and Weldon [36] have also studied the effect of temperature on development of the blow fly L. cuprina. They have studied the life cycle of *L. cuprina* at six constant temperatures from 18°C to 33°C to calibrate a thermal accumulation model of development for forensic applications. Their study shows *L. cuprina* has completed it life cycle in 53.9 days at 19°C, 13.5 days at 27°C and 10.4 days at 35°C. Higher temperature accelerates the larval growth whereas lower temperatures extend the period of larval development.

At low temperatures, the metabolic rate may be markedly reduced and this could result in greater bodyweight and a tendency to burrow deeper in order to escape at low temperatures. [27].

Dallwitz [37] reported that pupal survivorship for *L. cuprina* did not drop below 75 % for temperatures below 30°C. In his study *L. cuprina* completed its life cycle well at 30°C. Larvae of *L. cuprina* survived best at 24°C and mean larval length peaked at 27°C. At lower temperatures, larval development was slower and survivorship was compromised, especially at 18°C, with many larvae dying as they reached the wandering phase. As temperatures rose from 24°C, growth was increasingly compromised, and the physiological stress of developing at more extreme temperatures was evident in decreased survivorship [36].

Temperature has a significant effect on the body weight of *L. cuprina* larvae. Body weight of the larvae decreased as temperature Increased and the greatest body weight was attained at lower temperature. Larvae attained greatest body weight below 20°C. In this study also *L. cuprina* has attained greatest body weight at 20°C.

The blowfly, *L. cuprina*, has a peak of 33% activity at 20°C. Another peak of 6% activity at 42°C, and is immobile at 5°C [38].

Nideregger et al. [39] have compared the development of larvae of forensically important flies under constant, low, medium and high temperatures as well as under daily fluctuating temperatures in climatic chambers.

Conclusion

It is observed that temperature affects the life cycle of the flies. Low temperature increases the duration to complete the life cycle whereas high temperature decreases the duration to complete the life cycle. Variation in temperature and humidity influence growth and indirectly influence the estimation of time since death. Thus to ensure a more accurate estimation, history of surrounding temperature and humidity in the location where body was found must be taken into consideration.

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