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Studies on time duration, life cycle stages of family (Calliphoridae) species: *Calliphora vicina* and *Lucilia cuprina* during summer season

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Abstract
Exact detail estimation of post mortem interval (PMI) in the investigation of distrustful death, the forensic measurer blowflies are essential for accuracy in estimation of PMI. The family Calliphoridae species of *Calliphora vicina and Lucilia cuprina.* Were reared in laboratory condition for studying their time duration of different stages of life cycle under the fluctuating temperature in summer seasons. *Calliphora vicina* took 202 hours 45 minutes, where as the *Lucilia cuprina* took 226 hours 53 minutes, during summer season respectively. This study shows that forensic investigators will have to take each of these variables into consideration from the development of insects in order diptera to give clear or exact estimation of postmortem interval.

Keywords: forensic insect, PMI in summer, lifecycle duration, temp change

Introduction
The Poladpur taluka is a very remote and tremendous rainfall region due to this biotic fauna are available. Several various species are observed in family Calliphoridae but the actual time duration determining studies of the species of *Calliphora vicina and Lucilia cuprina* are studied. The family Calliphoridae includes blow-flies the well-known scavenger insects belonging to the order Diptera. Blowfly is usually the first insects to come in contact with dead body remains Worldwide. There are 1100 species in the biogeographic region of the world Developmental data for primary blow flies provide the most accurate means of estimating the PMI using arthropod insects information (Greenberg 1991) [1]. It is presumed that the first individuals that arrive at, and lay eggs in a corpse do so within hours after death (Catts and Goff 1992) [2]. Provided information the body is outside and there are no obvious barriers preventing egg-laying (such as environmental restrictions and whether the body is covered, buried or indoors). Therefore, time of death is assumed to be close to the time the first eggs are deposited.

In order for insect developmental analysis to yield an accurate PMI estimation, data must be available for the earliest colonizing species collected from the corpse at the time of discovery. Although there may be several early-arriving species, the oldest 12 individuals are the most relevant because they represent the first eggs deposited on the body. Because certain species can oviposit beginning a few hours following death and continuing for at least two weeks (smith K.G 1986) [3]. The typical habitat of blowflies are temperate to tropical areas that provide a layer of loose, damp soil and litter where larvae may thrive and pupate Johnson MD (1975) [4]. Adult blowflies are occasionally pollinator, being attracted to flowers and strong odour resembling rotting meat such as American pawpaw and dead horse arum. The larvae of Calliphoridae feeds on remains or other decaying matter. Most species of blowflies studied thus far are anautogenous; female requires a substantial amount of protein to develop mature eggs within her ovaries (about 800 µg per pair of ovaries in Phormiaregina). Both male and female adult Calliphoridae ranges from 6 to 14 mm in length. The adult size depends on species and food availability to the larval stages. The majority of these species are metallic in appearance with colour ranging from brilliant green or blue to bronze or shiny black (Ambrose DP (2007) [5]. In some species, a covering of fine hairs, powder or dust masks the bright metallic coloration of fly epicuticle. Adults are characterized by a three segmented antenna located between and anterior to the pair of compound eyes. This antenna has a hair or aristaon the last segment which is plumose or hairy throughout its length. The female visit remains for both protein and egg lay. Blowfly eggs are about 1.5 mm × 0.4 mm, white or yellowish, looks like rice balls when laid. While the female blowfly typically lays 150 to 200 eggs per batch, she is usually iteroparous, laying around 2000 eggs during her course of life. The sex ratio of blowfly eggs is usually 50:50. Khole V (1978) [6]. But in one interesting exception currently documented literature, female from two species of Chrysomya (C. rufifaces and C. albiceps) are eitherarrhenogenic (laying only male offsprings) or the lygenic (laying only female offsprings). Blowflies are usually the first insects to come in contact with carrion because they have an ability to smell dead animal matter from upto 10 miles (16 Km) Greenberg B (1991) [7]. Upon reaching carrion, female deposit eggs onto the body. Since development is highly predictable if the ambient temperature is known, blowfly are considered a valuable tools in forensic science to determine post mortem interval (PMI). Traditional estimation of time since death are generally unreliable after72 hours and often entomologist are the only officials capable of generating an actuating approximate time interval. This research work was taken up in order to study the time duration of different

stages of *Calliphora vicina and Lucilia cuprina* during summer season so as to prepare the baseline data that will help the forensic experts to find correct PMI in Indian conditions.

Materials and Methoods

The present research work was carried out at research laboratory. The species *Calliphora vicina and Lucilia cuprina* flies were used as the biomaterials and different appliances and tools were used.

Collecting and Rearing of blowflies

The species *Calliphora vicina and Lucilia cuprina* flies were collected from poladpur Taluka, district of Raigad, Maharashtra, India. For the collection of flies fresh liver sample was purchased from the local slaughter house. Partially putre fied liver and meat was exposed in the sampling site and within few minutes the flies were attracted. The flies' were collected by the insect net and after identification they were released in the insect rearing cages. Honey solution (water and honey) soaked in tissue paper was kept in petridish and fresh sliced liver meat of cattle was provided daily in separate petridishes in the rearing cages. After few days the mated adult female started laying eggs on sliced liver meat. The eggs were collected with the help of fine brush directly after laying. The blowflies laid eggs on the sliced liver meat which was later on reared in in laboratory condition at summer seasons. The plastic jar was taken for rearing the instars of blowfly larvae. The liver meat was then placed into 7 cm deeper jar covered with fine mesh to prevent the entry of parasitoids. The three experiments were conducted at the same time. Three groups of 60 larvae separately transferred into three plastic jars and fed them fresh liver meat daily till pupation. Observation was taken on hourly basis. The maggots were observed and collected with the help of forceps and preserved in small bottle throughout their developmental stages at different time duration. As the third instars larva finished feeding and reach wandering phase, they left the food and travel to the soil for pupation. After few days the adult fly emerged out from the pupa. The total time taken by each stages of *Calliphora vicina and Lucilia cuprina* life cycle during summer seasons was recorded. The temperature and humidity were recorded daily with the help of Hygrothermometer clock. The experiment was repeated three times.

Statistical Analysis

Statistical analysis was performed using the excel sheet, data were analyzed by using two way analysis of variances (ANOVA) and significance level at $P \leq 0.05$.

Observations and Results

In present research work it is observe that the blowflies' reaches from Ist instar larvae to IInd and then IIIrd instar larvae after their moulting completion. The time duration of different stages of Calliphora vicina *and Lucilia cuprina* during summer seasons are as follows.

Summer season

1. *Calliphora vicina*

The average temperature and humidity during the experiment was 36.8°C and 25.7% respectively. Table 1 shows the time duration of different stages of *Calliphora vicina during* summer season. The result showed the eggs persisted 18 hours. After hatching eggs the Ist instar larva took 25 hours 05 minutes to become IInd instar larva stage. The PMI duration since egg laid was 43 hours 05minutes. The IInd instar larva took 24 hours 30 minutes to reach third instar larva and the PMI duration was 67 hours 35 minutes. The IIIrd instar larva took 25hours 00 minutes and PMI duration was 92 hours35 minutes. The pre-pupal stage persisted 20 hours 05 minutes to reach pupal stage while PMI duration was 112 hours 40 minutes. The pupal stage took 90 hours 05 minutes to become adult fly emerged. The total duration ofwhole life cycle of *Calliphora vicina* during summer season was 202 hours 45 minutes (Table 1).

Table 1: Time duration of summer stages of *Calliphora vicina during* summer season.

Ife cycle stages		Duration (H:MM)	PMI (H:MM)	Temperature
Eggs		18:00		37°C
Larva	Ist instar	25:05	43:05	38°C
	IInd instar	24:30	67:35	38°C
	IIIrd instar	25:00	92:35	39°C
Pre-pupa		20:05	112:40	39°C
Pupa		90:05	177:45:00	40°C
Total duration		177:45:00		

2. *Lucilia Cuprina*

The average temperature and humidity during the experiment was26°C and 44.2% respectively. Table 2 showed the time duration of different stages of *Lucilia cuprina* during summer season. Result revealed that the eggs took 20 hours 38 minutes for incubation. After incubation there are three stages of larval instars (i.e. Ist instar, IInd instar and IIIrd instar). The duration of Ist instar larva took 24 hours 30minutes and post mortem interval (PMI) duration persisted 45 hours 08 minutes since egg laid. The IInd instar larva took 25 hours 15 minutes to reach third instar larva and PMI duration was 70 hours 23 minutes. The IIIrd instar larva persisted 49 hours 30 minutes while PMI duration since egg laid upto IIIrd instar was 119 hours 53 minutes. The pre-pupal stage took 38 hours30 minutes and PMI duration was 158 hours 23 minutes. The time taken by pupal stages upto adult fly emerged was 68 hours 30 minuits while the total duration took by *Lucilia cuprina* in summer season was 226hours 53 minutes (Table 2).

Table 2: Time duration of different stages of *Lucilia cuprina* during summer season.

Ife cycle stages		Duration (H:MM)	PMI (H:MM)	Temperature
Eggs		20:38		36°C
Larval stages	Ist instar	24:30	45:08	38°C
	IInd instar	25:15	70:23	36°C
	IIIrd instar	49:30	119:53	36°C
Pre-pupa		38:30	158:23	38°C
Pupa		68:30	224:53:00	39°C
Total duration		224:53:00		

Conclusion

From the present research work reported and concluded that the duration of total life cycle of *Calliphora vicina and Lucilia cuprina* species are different in summer seasons due to variation in temperature and humidity. Insect development is dependent on environmental temperature, where at the higher temperature, the development faster as compare to cooler temperature.

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