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Studies on Time Duration of Life Stages of *Chrysomya megacephala* and *Chrysomya rufifacies* (*Diptera:* Calliphoridae) during Different Seasons

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Abstract

Accurate estimation of post mortem interval (PMI) can be crucial to the successful investigation of suspicious death. The forensic indicator blowflies are essential for accuracy in estimation of PMI. The Calliphoridae species *Chrysomya megacephala* and *Chrysomya rufifacies* were reared in laboratory condition for studying their time duration of different stages under the fluctuating temperature in three different seasons (i.e. rainy season, winter season and summer season).

In laboratory, at the fluctuating temperature during rainy season, winter season and summer season, *Chrysomya megacephala* took 237 hours 47 minutes, 263 hours 51 minutes and 211 hours 13 minutes respectively. Whereas the *Chrysomya rufifacies* took 239 hours 14 minutes, 286 hours 02 minutes and 216 hours 26 minutes during rainy season, winter season and 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 to give a more accurate estimate of PMI.

Keywords: Forensic entomology; Postmortem interval; Seasonal life cycle duration; Fluctuating temperature

Introduction

The family Calliphoridae includes blow-flies (also frequently spelled green bottle, blue bottle or carrion 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 [1]. Worldwide there are 1100 species in the neotropics [2], but very numerous species in Africa and Southern Europe.

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 [3]. 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 arista on the last segment which is plumose or hairy throughout its length [4].

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 [5]. 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 [6]. 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 Phormia regina). 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 [7], but in one interesting exception currently documented literature, female from two species of *Chrysomya* (*C. rufifaces and C. albiceps*) are either arrhenogenic (laying only male offsprings) or thelygenic (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) [8]. 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 after 72 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 *Chrysomya megacephala* and *Chrysomya rufifaces* during different season so as to prepare the baseline data that will help the forensic experts to find correct PMI in Indian conditions.

Materials and Methods

The present research work was carried out at research laboratory. The species *Chrysomya megacephala* and *Chrysomya rufifacies* flies were used as the biomaterials and different appliances and tools were used.

Collecting and rearing of blowflies

The species *Chrysomya megacephala* and *Chrysomya rufifacies* flies were collected from carcass in Latur district, Maharashtra, India.

The fresh liver sample was purchased from the local slaughter house. Partially putrefied 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 rainy, winter and summer seasons at laboratory condition. The plastic jar was taken for rearing the instars of blowfly larvae. The liver meat was then placed into 6 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 50 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 instar 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 *Chrysomya megacephala* and *Chrysomya rufifacies* life cycle during different seasons was recorded. The temperature and humidity were recorded daily with the help of Hygro-thermometer clock. The experiment was repeated three times seasonally.

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 are of *Chrysomya megacephala* and *Chrysomya rufifacies* during different seasons are as follows

Chrysomya megacephala

Rainy season

The average temperature and humidity during the experiment was 24.1°C and 49.6% respectively. Table 1 showed the time duration of different stages of *Chrysomya megacephala* during rainy season. Result revealed that the eggs took 18 hours 37 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 25 hours 45 minutes and post mortem interval (PMI) duration persisted 44 hours 22 minutes since egg laid. The IInd instar larva took 26 hours 35 minutes to reach third instar larva and PMI duration was 70 hours 57 minutes. The IIIrd instar larva persisted 29 hours while PMI duration since egg laid upto IIIrd instar was 99 hours 57 minutes. The pre-pupal stage took 22 hours 40 minutes and PMI duration was 122 hours 37 minutes. The time taken by pupal stages upto adult fly emerged was 115 hours 10 minutes while the total duration took by *Chrysomya megacephala* in rainy season was 237 hours 47 minutes (Table 1).

Life cycle stages		Duration (H:MM)	PMI (H:MM)
Eggs		18:37	
Larva	Ist instar	25:45	44:22
	IInd instar	26:35	70:57
	IIIrd instar	29:00	99:57
Pre-pupa		22:40	122:37
Pupa		115:10	237:47
Total duration		237:47	

Table 1: Time duration of different stages of *Chrysomya megacephala* during rainy season.

Winter season

The average temperature and humidity during the experiment was 22.7°C and 35.8% repectively. Table 2 shows the time duration of different stages of *Chrysomya megacephala* during winter season. The result showed the eggs persisted 20 hours 36 minutes. After hatching eggs the Ist instar larva took 26 hours 30 minutes to become IInd instar larva stage. The PMI duration since egg laid was 47 hours 06 minutes. The IInd instar larva took 28 hours 30 minutes to reach third instar larva and the PMI duration was 75 hours 36 minutes. The IIIrd instar larva took 48 hours and PMI duration was 123 hours 36 minutes. The pre-pupal stage persisted 21 hours 50 minutes to reach pupal stage while PMI duration was 145 hours 26 minutes. The pupal stage took 118 hours 25 minutes to become adult fly emerged. The total duration of whole life cycle of *Chrysomya megacephala* during winter season was 263 hours 51 minutes (Table 2).

Life stages		Duration (H:MM)	PMI (H:MM)
Eggs		20:36	
Larva	Ist instar	26:30	47:00
	IInd instar	28:30	75:36
	IIIrd instar	48:00	123:36
Pre-pupa		21:50	145:26
Pupa		118:25	263:51
Total duration		263:51	

Table 2: Time duration of different stages of *Chrysomya megacephala* during winter season.

Summer season

The average temperature and humidity during the experiment was 32.5°C and 21.5% respectively. Table 3 shows the time duration of different stages of *Chrysomya megacephala* during summer season. The result showed the eggs persisted 18 hours 08 minutes. After hatching eggs the Ist instar larva took 26 hours 05 minutes to become

IInd instar larva stage. The PMI duration since egg laid was 44 hours 13 minutes. The IInd instar larva took 26 hours 45 minutes to reach third instar larva and the PMI duration was 70 hours 58 minutes. The IIIrd instar larva took 27 hours 05 minutes and PMI duration was 98 hours 03 minutes. The pre-pupal stage persisted 20 hours 10 minutes to reach pupal stage while PMI duration was 118 hours 13 minutes. The pupal stage took 93 hours to become adult fly emerged. The total duration of whole life cycle of *Chrysomya megacephala* during summer season was 211 hours 13 minutes (Table 3).

Life stages		Duration (H:MM)	PMI (H:MM)
Eggs		18:08	
Larva	Ist instar	26:05	44:13
	IInd instar	26:45	70:58
	IIIrd instar	27:05	98:03
Pre-pupa		20:10	118:13
Pupa		93:00	211:13
Total duration		211:13:00	

Table 3: Time duration of different stages of *Chrysomya megacephala* during summer season.

Chrysomya rufifacies

Rainy season

The average temperature and humidity during the experiment was 24°C and 42.1% respectively. Table 4 showed the time duration of different stages of *Chrysomya rufifacies* during rainy season. Result revealed that the eggs took 22 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 25 hours 06 minutes and post mortem interval (PMI) duration persisted 47 hours 44 minutes since egg laid.

Life stages		Duration (H:MM)	PMI (H:MM)
Eggs		22:38	
Larva	Ist instar	25:06	47:44
	IInd instar	27:35	75:19
	IIIrd instar	51:05	126:24
Pre-pupa		41:50	168:14
Pupa		71:00	239:14
Total duration		239:14	

Table 4: Time duration of different stages of *Chrysomya rufifacies* during rainy season.

The IInd instar larva took 27 hours 35 minutes to reach third instar larva and PMI duration was 75 hours 19 minutes. The IIIrd instar larva persisted 51 hours 05 minutes while PMI duration since egg laid upto IIIrd instar was 126 hours 24 minutes. The pre-pupal stage took 41 hours 50 minutes and PMI duration was 168 hours 14 minutes. The time taken by pupal stages upto adult fly emerged was 71 hours while the total duration took by *Chrysomya rufifacies* in rainy season was 239 hours 14 minutes (Table 4).

Winter season

The average temperature and humidity during the experiment was 25°C and 46.8% respectively. Table 5 shows the time duration of different stages of *Chrysomya rufifacies* during winter season. The result showed that the eggs persisted 19 hours 02 minutes. After hatching eggs the Ist instar larva took 46 hours 15 minutes to become IInd instar larva stage. The PMI duration since egg laid was 65 hours 17 minutes. The IInd instar larva took 28 hours 25 minutes to reach third instar larva and the PMI duration was 93 hours 45 minutes. The IIIrd instar larva took 50 hours 05 minutes and PMI duration was 143 hours 47 minutes. The pre-pupal stage persisted 24 hours 35 minutes to reach pupal stage while PMI duration was 168 hours 22 minutes. The pupal stage took 117 hours 40 minutes to emerge adult fly. The total duration of whole life cycle of *Chrysomya rufifacies* during winter season was 286 hours 02 minutes (Table 5).

Life stages		Duration (H:MM)	PMI (H:MM)
Eggs		19:02	
Larva	Ist instar	46:15	65:17
	IInd instar	28:25	93:45
	IIIrd instar	50:05	143:47
Pre-pupa		24:35	168:22
Pupa		117:40	286:02
Total duration		286:02	

Table 5: Time duration of different stages of *Chrysomya rufifacies* during winter season.

Summer season

The average temperature and humidity during the experiment was 32°C and 22.07% respectively. Table 6 shows the time duration of different stages of *Chrysomya rufifacie* during summer season. The result showed that the eggs persisted 19 hours 21 minutes.

Life stages		Duration (H:MM)	PMI (H:MM)
Eggs		19:21	
Larva	Ist instar	25:45	45:06
	IInd instar	28:00	73:06
	IIIrd instar	48:40	121:46
Pre-pupa		20:10	141:56
Pupa		74:30	216:26
Total duration		216:26	

Table 6: Time duration of different stages of *Chrysomya rufifacies* during summer season.

After hatching eggs the Ist instar larva took 25 hours 45 minutes to became IInd instar larva stage. The PMI duration since egg laid was 45 hours 06 minutes. The IInd instar larva took 28 hours to reach third instar larva and the PMI duration was 73 hours 06 minutes. The IIIrd instar larva took 48 hours 40 minutes and PMI duration was 121 hours 46 minutes. The pre-pupal stage persisted 20 hours 10 minutes to reach pupal stage while PMI duration was 141 hours 56 minutes. The pupal stage took 74 hours 30 minutes to become adult fly emerged. The total duration of whole life cycle of *Chrysomya rufifacies* during summer season was 216 hours 26 minutes (Table 6).

Discussion

Chrysomya megacephala and *Chrysomya rufifacies* were the two calliphorides, which were found in all the seasons of the year. The duration of the life cycle depended on climacteric conditions and reflected yearly temperature changes. Corpses in summer and rainy season decayed as much faster rate than those in winter. Warmer temperature in summer speeded up succession while low temperature in winter retarded succession by slowing down the development rate of larvae.

Large numbers of studies have been done on effect of temperature on insect life cycle. *P. sericata*. *P. regina*, *C. rufifacies* and *Cochliomyia macellaria* development was slightly longer at fluctuating temperature than constant temperature [8-10]. The effect of controlled temperature and humidity on the life cycle, rate of development of thirteen species of flies, representing nine genera within the family Calliphoridae was also investigated [11-14].

Forensic entomology in the Indian perspective, finds insects to be important forensic indicator [15]. They study the relationship between insects and corpse decomposition. Singh and Bharti enlisted the species of blowflies order *Diptera*, family Calliphoridae Species *C. megacephala*, *C. rufifacies*, *Calliphora vicina*, *L. sericata*, and *L. illustris* available in the state of Punjab which can be important from the forensic point of view. It is also studied on entomological evidences covering all the five seasons of the year that is summer, rainy, autumn, winter and spring [16].

The present research is similar with the developmental duration of *Chrysomya megacephala* (*Diptera:* Calliphoridae), in rainy season and low constant temperature of 10°C and humidity 19%, reported that the total life cycle duration in rainy season was completed in 265 h ± 2 h (11.04 days ± 0.08 days) when the temperature ranged between 26°C and 29°C and humidity ranged between 35% and 50%, while in the low constant temperature 10°C ± 0.5°C the life cycle was completed in 609 h ± 4 h (25.38 days ± 0.16 days) indicating a delay in the life cycle by 14.37 days ± 0.13 days [17]. Similarly our current results are in agreement with another study on the effect of different temperature and humidity on the life cycle duration and morphological parameters of *Chrysomya rufifacies* (*Diptera:* Calliphoridae) in different seasons, it was reported that the life cycle of *Chrysomya rufifacies* in summer was completed in 241 ± 2.17 h (10.04 days ± 0.12 days) when the temperature ranged between 30.1°C and 37.2°C, but in the rainy season it was completed in 275 h ± 2.27 h (11.46 days ± 0.45 days), when the temperature ranged between 26.2°C and 30.1°C, while in winter the life cycle was completed in 318 h ± 2.45 h (13.25 days ± 0.25 days) when the temperatures ranged between 26.4°C and 18.2°C respectively [18]. In present research work it is also observed that time duration of development of Ist instar larvae is lesser as compare to IInd and IIIrd instar larvae, it is similar with Nabity et al. [19] and Bharti et al. [20] 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.

Insect development is dependent on environmental temperature, where the warmer temperature rate of development is faster [21,22].

Conclusion

From the present research work reported here, it is concluded that the duration of total life cycle of *Chrysomya megacephala* and *Chrysomya rufifacies* species are different in different 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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