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Research Article

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Effect of Seasonal Temperature Variations on the Life Cycle Duration of Forensically Important Calliphorid Fly, *Chrysomya saffranea* (Bigot, 1877)

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Abstract

The present study deals with the effects of variation in temperature and humidity on the different life cycle stages of Calliphoridae fly *Chrysomya saffranea*. In India for first time, *Chrysomya saffranea* was recorded apart from Australia as a native place of this fly.

In this study different life cycle stages of *Chrysomya saffranea* in different seasons were observed. In summer season when the average temperature ranged between 30.5°C and 33.2°C and the average humidity ranged between 12% to 19%, life cycle duration was completed in 220 h (9.17 days) from the depositing of eggs up to the eclosion of adults. But in rainy season when the average temperature ranged between 25.6°C and 28.9°C, and the average humidity ranged between 50% to 65%, life cycle duration was completed in 259 h (10.79 days), while in winter season when the average temperature ranged between 17.8°C and 24.4°C, and the average humidity ranged between 17% to 28%, life cycle duration was completed in 341 h (14.21 days) from eggs up to emergence of the adults.

The temperature and humidity are the most important factors playing role in the larval development, decay and degradation of the cadavers. Therefore, the climatic fluctuations and environmental changes play an important role in the life cycle of *Chrysomya saffranea*. All these factors must be considered in the Post Mortem Interval determination.

Keywords: Forensic entomology; Postmortem interval; Calliphoridae fly; *Chrysomya saffranea*; Temperature; Humidity

Introduction

Forensic entomology is the use of insects and other arthropods as evidence in both civil and criminal investigations [1,2]. It deals with the insects associated with cadavers of human and animal remains to determine the Post-Mortem Interval (PMI) [3]. The wide range of this field can be divided into three subdivisions of urban, stored-product, and medico-legal forensic entomology [4]. Predominantly, blowfly larvae are the most common insects recovered from cadavers of humans; they can provide information on the conditions experienced by a body following death [5].

Chrysomya saffranea (Bigot, 1877), was recorded for first time in India [6], this fly also known as Steelblue fly or Australian blowfly [7]. In this study we used *Chrysomya saffranea* to study the effect of seasonal temperature on the life cycle duration and the time spent in the development of different stages of this fly and the impact of seasonal temperature on PMI determination. These blowflies of the genera *Chrysomya* (Diptera: Calliphoridae) are of considerable medical and sanitary importance since it is a myiasis producing agent in animals as well as humans. These flies are also important in forensic entomology since they can be used as biomarkers to estimate the Post Mortem Interval [8-10].

There are many factors which play an important role in the growth and development of arthropods, such as temperature, humidity, geographical location, seasons, decomposition time and habitat, which can affect the activity of the forensic flies and their life cycle [11-16]. Campobasso et al. [17] proved that the temperature and humidity are the most extrinsic factors playing an important role in the rate of postmortem decay and cadaver degradation.

Clark et al. [18] studied the insect activity at standard condition (21°C temperature and 30% humidity) and observed the insect activity within 96 h after death, while Campobasso et al. [17] reported that the oviposition can occur soon after death at favorable condition or at most within 2 days from the death.

Campobasso et al. [17] observed that the unfavorable temperature and humidity conditions can destroy the eggs easily, and found that in some cases the embryogenesis in eggs was slow down or stop showing a state of quiescence known as diapauses. However, the embryogenesis can resume normally once when the micro environmental condition become more favorable. Nielsen and Nielsen, [19] observed that the eggs of *Chrysomya vicina* (Diptera: Calliphoridae) did not hatch at temperature below 4°C while hatching of eggs and development of larvae occurred at 6°C to 7°C.

Considerations of critical environmental factors affecting the rate of decomposition are very important during the crime investigations. These factors include location of the body, temperature, general climate, time of year, insect activity, animal activity in the area, and the amount of rainfall [17,20,21].

To study the influence of fluctuating temperatures on insect development, one of five methods must be relied upon [22]. The first method involves transferring the insects from one temperature to another after a period of time has elapsed. The second is to record the temperatures of the natural environment by using a data logger or thermograph and the third method, similar to the first, is to transfer the insects to different temperatures according to a set pattern, The fourth method, which by no means emulates the natural environment, is to set up conditions where the insects develop at temperatures based on a gradient as a result of insect mobility. The fifth and final method is to set the desired conditions for the development and cycle the conditions every 24 h [22].

In this study we used the normal fluctuating temperatures at laboratory condition in different seasons and its effect on the life cycle duration, morphological parameters of different stages and the total life cycle duration from eggs depositing up to the adult eclosion.

Materials and Methods

Larvae of Calliphoridae species *Chrysomya saffranea* were collected from the dead body of cat at the garden of the Zoology Department at Dr Ambedkar Marathwada University Reserve (19° 522 483 N, 75° 192 123 E), Aurangabad City, Maharashtra State in India. Posteriorly, these larvae were reared in the laboratory by providing daily feed of buffalo`s fresh liver. Maggot culture was provided with fresh liver as a food till the prepupae stage. Prepupae were kept in 500 ml beakers containing dry soil which is required for the pupation. Adult flies which emerged out from pupae were reared in rearing boxes of size 22 × 12 × 10 inches in dimensions (length × width × depth respectively). Purity of culture was maintained by separating the eggs immediately after depositing from the female. Larvae and adult were dissected from the pure culture with the help of stereo-zoom microscope (ERMA Optical works, Tokyo, No. 44883) and light microscope (Magnus Trinocular Microscope MLX-DX, Olympus -India PVT. LTD. No. 4B525145) and identified according to the identification keys in [7,6,23].

Experimental Design

In these experiments eggs were collected with the help of fine brush directly after laying and reared in summer, and rainy winter seasons at laboratory condition. After maintaining the pure culture of *Chrysomya saffranea*, to insure the same environmental condition at laboratory three experiments were conducted at the same time. Three groups of 80 larvae separately transferred into three glass beakers 500 ml capacity; each group of larvae were daily fed on 50 gm of buffalo`s fresh liver till pupation. At the pupation time larvae were placed in small plastic tub 2 L capacity with 200 gm of dried soil which is necessary for the pupation. Life cycle duration of different stages and the morphological parameters were recorded daily. The temperature and the relative humidity were also recorded by using Hygro-thermometer clock. The experiment was repeated three times seasonably.

Statistical Analysis

Statistical analysis was performed using the excel sheet, data were analyzed statistically by using Two-ways analysis of variance (ANOVA) and significance level at P ≤ 0.05 was used.

Results and Discussion

Life cycle in summer season

The life cycle duration of *Chrysomya saffranea* has been studied when the average temperature ranged between 30.5°C and 33.2°C and the average humidity ranged between 12% to 19% (Table 1), PMI in h, different life cycle stages and morphological parameters were recorded as below:

Developmental stages	Summer season		Rainy season		Winter season	
	Average Temperature (°C)	Average Humidity (%)	Average Temperature (°C)	Average Humidity (%)	Average Temperature (°C)	Average Humidity (%)
Eggs	30.5	19	28.9	65	24.4	28
1st instar	31.3	19	28.1	65	23.5	27
2nd instar	31.6	17	27.8	59	21.6	25
3rd instar	32.3	16	27.4	55	20.6	23
Prepupae	32.7	16	27.1	55	20.6	21
pupae	32.5	13	26.3	50	19.4	21
Adult	33.2	12	25.6	51	17.8	17

Table 1: Temperature and humidity variations in different seasons during the different developmental stages.

Eggs: The length, width and weight of the eggs of *Chrysomya saffranea* were 1.34 mm, 0.4 mm, and 0.20 mg respectively. The egg development took 19 h after which they hatched into 1st instar larvae.

Larvae (Maggot): The 1st instar larva emerged from the eggs and took 20 h to develop. The average length, width and weight of the 1st instar larvae were 4.5 mm, 1.6 mm and 8.3 mg respectively. The 1st instar larvae molted into 2nd instar larvae which remained for 22 h from the 39th up to 61st hour of their life. The average length, width and weight of the 2nd instar larvae were 6.7 mm, 2 mm and 25.5 mg respectively. After 61 h of development the 2nd instar larvae molted into 3rd instar larvae which took up 24 h to develop. The average length, width and weight of 3rd instar larvae were 11.2 mm, 4 mm and 56.1 mg respectively.

Prepupae: Prepupae (post feeding stage) took 27 h, and the size of the prepupae reduced. The average length, width and weight of prepupae were 9.3 mm, 3.6 mm and 44.6 mg respectively.

Pupae: The pupal stage took up 108 h. The average length, width and weight of pupae were 8.5 mm, 3.4 mm and 33.7 mg respectively.

Adult: Adult flies emerged out from the pupae after 220 h of development. That means that the life cycle of *Chrysomya saffranea* in summer was completed in 220 h from the egg-laying stage up to the adult eclosion. The average length, width and weight of adult were 8 mm, 3 mm and 29.5 mg respectively. PMI in h and morphological parameters of the different stages in different seasons are shown in Tables 2-5.

Life cycle in rainy season

Life cycle duration of *Chrysomya saffranea* has been studied in rainy season when the average temperature and relative humidity were ranged between 25.6°C to 28.9°C, and 50% to 65% respectively (Table 1), different life cycle stages and morphological parameters were recorded as below:

Eggs: The average length, width and weight of the eggs were 1.46 mm, 0.4 mm and 0.21 mg respectively. The eggs hatched into 1st instar larvae after 22 h from the depositing.

Larvae (Maggots): The 1st instar larval development took 22 h, and the average length, width and weight of 1st instar was 5.5 mm, 1.9 mm and 12.6 mg respectively. The 1st instar larvae took 28 h to molt into 2nd instar larvae. At 72 hrs of development, the average length, width and weight of the 2nd instar larvae was 9.4 mm, 2.5 mm and 30.2 mg respectively. The 3rd instar larvae development started from 72 h after eggs depositing and continued up to 102 h, and the average length, width and weight of the 3rd instar larvae were 14.2 mm, 4.2 mm and 68.1 mg respectively.

Prepupae: The developmental duration of prepupae started from 102 h to 130 h. And the average length, width and weight were 10.5 mm, 4 mm and 47.5 mg respectively.

Pupae: The pupal stage development took 129 h. The average length, width and weight of the pupae were 9.4 mm, 3.6 mm and 38.2 mg respectively.

Adult: The adult emerged out from the pupae after 259 h of development. That means the life cycle duration of *Chrysomya saffranea* in rainy season was completed in 259 h, and the average length, width and weight of the adult were 9 mm; 3.3 mm and 32.3 mg respectively. PMI in h and morphological parameters of the different stages are shown in Tables 2-5.

Life cycle in winter season

Life cycle duration of *Chrysomya saffranea* has been studied in winter season when the average temperature ranged between 17.8°C and 24.4°C, and 17% to 28% average humidity (Table 1), different life cycle stages and morphological parameters were recorded as below:

Eggs: After depositing, the eggs were immediately collected; the average length width and weight of the eggs were 1.13 mm, 0.4 mm and 0.20 mg respectively. After 27 h of development the eggs hatched into 1st instar larvae.

Larvae (Maggot): The 1st instar larvae took 30 h to develop, the average length, width and weight of the 1st instar larvae were 4 mm, 1.3 mm and 7.6 mg respectively. The duration spent for 2nd instar to reach the 3rd instar was 30 h, and the average length, width and weight of the 2nd instar larvae were 6 mm, 1.8 mm and 20.1 mg respectively. The 3rd instar larvae took 42 h, and the average length, width and weight of 3rd instar larvae were 9.2 mm, 3.4 mm and 42.2 mg respectively.

Prepupae: This non-feeding stage took up 40 h to develop from 129 h up to 169 h. The average length, width and weight of prepupae were 8.7 mm, 3.2 mm and 32.2 mg respectively.

Pupae: The pupal stage took up 172 h to develop. The average length, width and weight of the pupae were 8 mm, 3 mm 30.4 mg respectively.

Adult: Adult emerged out from the pupae after 341 h from the depositing of eggs. That means life cycle of *Chrysomya saffranea* in winter was completed in 341 h. The average length, width and weight of adult were 7.4 mm, 2.7 mm and 24.3 mg respectively. PMI in hour and morphological parameters of the different stages in different seasons are shown in Tables 2-5.

The summaries of PMI duration in different seasons are shown in Figure 1.1. Morphological parameters of different developmental stages of *Chrysomya saffranea* in different seasons are showed in Figures 1.2 to 1.4.

In summer, the total developmental time spent in the feeding stages was 66 h (2.75 days) and in rainy season 80 h (3.33 days) indicating a delay of more than half a day from the time spent in summer, while in winter the time spent in the feeding stages was 102 h (4.25 days) indicating a delay of about one day compared to the rainy season and two days compared to the time spent in summer. The developmental time spent in the post feeding and the pupal stages in summer was 135 h (5.63 days) and in rainy season it was 157 h (6.54 days) which showed a delay of about one day from the time spent in summer, while in winter 212 h (8.83 days) ware spent, indicating a delay of about three days from the time spent in summer and about two days from the time spent in the rainy season.

The total life cycle duration of *Chrysomya saffranea* in summer was completed in 220 hrs (9.17 days) when the average temperature ranged from 30.5°C to 33.2°C and the average humidity ranged from 12% to 19%, but in rainy season it was completed in 259 h (10.79 days) when the average temperature ranged from 25.6°C to 28.9°C and the average humidity ranged from 50% to 65%, while in winter it was completed in 341 h (14.21 days) when the average temperature and humidity ranged from 17.8°C to 24.4°C and 17% to 28% respectively. Likewise, the shortest life cycle duration was recorded in summer, then followed by the rainy season and the longest life cycle duration was in winter season.

This result supported the previous study on Lucilia sericata (Diptera: Calliphoridae), when it was reared in different temperatures of 15°C, 17°C, 19°C, 20°C, 21°C, 22°C, 25°C, 28°C, 30°C and 34°C, and the shortest life cycle duration of 259 h was observed in 34°C, while the longest duration of 842 h was observed in 17°C but in 15°C, the period recorded up to post feeding was 340 h and there are no records of emergence of the adult in this lowest temperature [24].

Similarly in the previous study on 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 [25].

These results are in agreement with the data obtained in the present study. 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 [26].

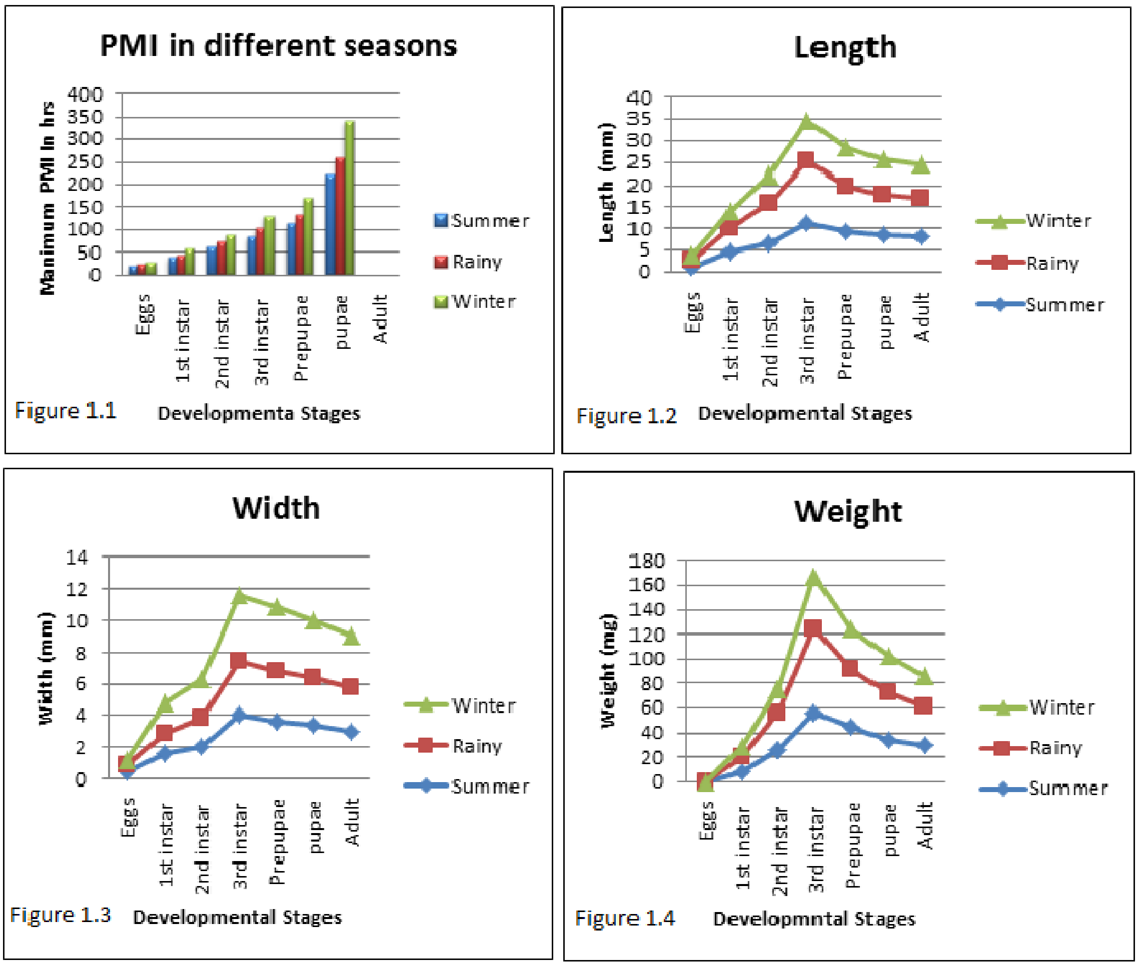

Figure 1: PMI in h and morphological parameter of different developmental stages in different seasons. 1.1: PMI in h in different seasons; 1.2: Length of developed stages in different seasons; 1.3: Width of developed stages in different seasons; 1.4: Weight of developed stages in different seasons.

Similar study on the developmental time of forensically important blow fly species (Diptera: Calliphoridae) Phormia regina (Meigen, 1826) at different constant minimum and maximum threshold temperatures (8°C to 32°C) reported that no development occurred at 12°C and below but in 14°C the total life cycle was of 45 days and at 26°C the total life duration was 13 days, while at 32°C the total life cycle was of 11.3 days [27].

Warren and Anderson, [28] compared the immature development of (Diptera: Calliphoridae) Protophormia terraenovae (Robineau-Desvoidy, 1830) at fluctuating temperatures of 4-28 and 9°C to 23°C and at their mean constant temperature 16°C, they reported that the development was fastest at the greater fluctuation and slowest at the constant temperature and showed similar percentages of development time in each stage. They conclude that the fluctuations above the mean enhance the developmental rate relatively more than temperatures below the mean can reduce the developmental rate.

Another study on the developmental rate of forensically important (Diptera: Calliphoridae) species *Chrysomya albiceps* at different constant temperature 15°C, 20°C, 25°C, 30°C and 35°C reported that at 15°C all pupae failed to develop to adult but the total life cycle at 20°C was about 18.5 days, while at 25°C, 30°C and 35°C the total life cycle duration were 14.1 days, 9.8 days and 9.2 days respectively, These results support that the shortest life cycle was at high temperature and longer duration was at lower temperature [29].

Stages	Developmental stages	PMI in Hours in different seasons		
		Summer	Rainy	Winter
P-value=8.10E-06	Eggs	19	22	27
	1st instar	39	44	57
	2nd instar	61	72	87
	3rd instar	85	102	129
	Prepupae	112	130	169
	pupae	220	259	341
	Adult			
	Seasons	P-value=0.010		

Table 2: PMI in hours of different developmental stages in different Seasons with the P-value at level P ≤ 0.05.

Stages	Developmental stages	Length (mm) in different seasons		
		Summer	Rainy	Winter
P-value=1.42E-05	Eggs	1.34 ± 0.05	1.46 ± 0.05	1.13 ± 0.02
	1st instar	4.5 ± 0.08	5.5 ± 0.16	4 ± 0.26
	2nd instar	6.7 ± 0.22	9.4 ± 0.02	6 ± 0.13
	3rd instar	11.2 ± 0.24	14.2 ± 0.23	9.2 ± 0.26
	Prepupae	9.3 ± 0.11	10.5 ± 0.21	8.7 ± 0.17
	pupae	8.5 ± 0.04	9.4 ± 0.11	8 ± 0.12
	Adult	8 ± 0.13	9 ± 0.15	7.4 ± 0.22
	Seasons	P-value=0.0006		

Table 3: Length of different developmental stages in different Seasons with the P-value at level P ≤ 0.05.

Higher temperatures generally support egg hatching and accelerate maturation of larvae which can double their size in few h and reduce the development time of Diptera. Once the Calliphoridae larvae have reached maximum length at the peak of feeding, they tend to decline progressively to about 75% of the length in the Calliphorid pre-adult cycle spent in post feeding and pupation [30,17].

The data obtained on PMI, length, width and weight of (Diptera: Calliphoridae) *Chrysomya saffranea* in the present study during three seasons (summer, Rainy and winter) were statistically analyzed for analysis of variance (ANOVA) and the P-value obtained are shown in Tables 2-5.

Stages	Developmental stages	Width (mm) in different Seasons		
		Summer	Rainy	Winter
P-value=8.13E-11	Eggs	0.4 ± 0.06	0.4 ± 0.06	0.4 ± 0.06
	1st instar	1.6 ± 0.24	1.9 ± 0.24	1.3 ± 0.14
	2nd instar	2 ± 0.11	2.5 ± 0.11	1.8 ± 0.24
	3rd instar	4 ± 0.24	4.2 ± 0.14	3.4 ± 0.14
	Prepupae	3.6 ± 0.22	4 ± 0.22	3.2 ± 0.14
	pupae	3.4 ± 0.33	3.6 ± 0.23	3 ± 0.21
	Adult	3 ± 0.16	3.3 ± 0.24	2.7 ± 0.13
	Seasons	P-value=2.42E-07		

Table 4: Width of different developmental stages in different Seasons with the P-value at level P ≤ 0.05.

Stages	Developmental stages	Weight (mg) in different Seasons		
		Summer	Rainy	Winter
P-value=1.59E-06	Eggs	0.20 ± 0.03	0.21 ± 0.03	0.20 ± 0.03
	1st instar	8.3 ± 0.64	12.6 ± 0.14	7.6 ± 0.23
	2nd instar	25.5 ± 0.02	30.2 ± 0.02	20.1 ± 0.12
	3rd instar	56.1 ± 0.33	68.1 ± 0.31	42.2 ± 0.22
	Prepupae	44.6 ± 0.21	47.5 ± 0.16	32.2 ± 0.16
	pupae	33.7 ± 0.34	38.2 ± 0.14	30.4 ± 0.24
	Adult	29.5 ± 0.06	32.3 ± 0.16	24.3 ± 0.06
	Seasons	P-value=0.0014		

Table 5: Weight of different developmental stages in different Seasons with the P-value at level P ≤ 0.05.

On the basis of the P- values, it can be stated that there was significant variation in PMI, length, width and weight of *Chrysomya saffranea* (Diptera: Calliphoridae) species among the various growth stage as well as among the three seasons.

Thus, it can be concluded that different growth stages as well as different seasons were responsible for the variation in the morphological parameter of this species. The values obtained during ANOVA analysis clearly indicated significant variation among duration of PMI of different stages, length, width and weight of all the three species.

Conclusion

From the previous studies and our recent study we can conclude that the longest life cycle duration was in winter when the temperature was low followed by rainy season while the shortest period was observed in summer, indicating that the warmer temperature accelerates the development and growth rate and shorts the life cycle, while the cooler temperature slows-down the growth rate and extends the life cycle duration of different stages. And the analyzed data have been showed a significant variation in the life cycle durations and in the morphological parameters of the different stages in the different seasons.

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References

1. Smith KGV (1986) A manual of forensic entomology. The British Museum (Natural History) and Cornell University Press, London, UK.
2. Williams KA, Villet MH (2006) A history of southern African researchogy. South African Journal of Science 102: 59-65
3. Byrd JH, Castner JL (2001) Insects of forensic importance, Forensic entomology: the utility of arthropods in legal investigations. CRC, Boca Raton, FL pp: 43-79.
4. Archer M (2007) Forensic entomology. Australian Police J 61: 66-70.
5. Donovan SE, Hall MJR, Turner BD, Moncrieff CB (2006) Larval growth rates of the blowfly, Calliphora vicina, over a range of temperatures. Med Vet Entomol 20: 106-114.
6. Abd-AlGalil FMA, Zambare SP, Mashaly AMA (2016) First record of Chrysomya saffranea (Diptera: Calliphoridae) of forensic importance in India. Tropical Biomedicine 33: 102-108.
7. Spradbery JP (2002) A manual for the diagnosis of screw-worm fly, Department Agriculture, Fisheries and Forestry, Canberra, Australia.
8. Gomes L, Gomes G, Oliveira HG, Sanches MR, Von Zuben CJ (2006) Influence of photoperiod on body weight and depth of burrowing in larvae of Chrysomya megacephala (Fabricius)(Diptera, Calliphoridae) and implications for forensic entomology. Rev Bras entomol 50: 76-79.
9. Gomes L, Gomes G, Oliveira HG, de Carvalho QMM, Von ZCJ (2008) Post-feeding larval dispersal in blowflies during summer and winter seasons in southeast Brazil. Acta Entomologica Sinica 51: 1099-1128.
10. Gomes L, Gomes G, Von ZCJ (2009) The influence of temperature on the behavior of burrowing in larvae of the blowflies, Chrysomya albiceps and Lucilia cuprina, under controlled conditions. J Insect Sci 9: 14.
11. Wigglesworth VB (1965) The principles of insect physiology. Methuen publishers, London, New York.
12. Archer MS, Elgar MA (2003) Effects of decomposition on carcass attendance in a guild of carrion-breeding flies. Med Vet Entomol 17: 263-271.
13. Sukontason K, Piangjai S, Siriwattanarungsee S, Sukontason KL (2008) Morphology and developmental rate of blowflies Chrysomya megacephala and Chrysomya rufifacies in Thailand: application in forensic entomology. Parasitol Res 102: 1207-1216.
14. Charabidzé D, Bourel B, Gosset D (2011) Larval-mass effect: characterisation of heat emission by necrophageous blowflies (Diptera: Calliphoridae) larval aggregates. Forensic Sci Int 211: 61-66.
15. Rivers DB, Ciarlo T, Spelman M, Brogan R (2010) Changes in development and heat shock protein expression in two species of flies (Sarcophaga bullata [Diptera: Sarcophagidae] and Protophormia terraenovae [Diptera: Calliphoridae]) reared in different sized maggot masses. J Med Entomol 47: 677-689.
16. Rivers DB, Thompson C, Brogan R (2011) Physiological trade-offs of forming maggot masses by necrophagous flies on vertebrate carrion. Bull Entomol Res 101: 599-611.
17. Campobasso CP, Di Vella G, Introna F (2001) Factors affecting decomposition and Diptera colonization. Forensic Sci Int 120: 18-27.
18. Clark MA, Worrell MB, Pless JE (1997) Postmortem changes in soft tissues. Forensic taphonomy: the postmortem fate of human remains. CRC press, Florida, USA pp: 151-164.
19. Nielsen BO, Nielsen SA (1976) (Calliphoridae) and vacuum packed ham. Indicators for pest, plant protection. Environmental protection 49: 113-115.
20. Nafte M (2000) Flesh and Bone: An introduction to forensic anthropology. Carolina Academic Press, Durham, NC, USA.
21. Myskowiak JB, Doums C (2002) Effects of refrigeration on the biometry and development of Protophormia terraenovae (Robineau–Desvoidy) (Diptera: Calliphoridae) and its consequences in estimating post-mortem interval in forensic investigations. Forensic Sci Int 125: 254-261.
22. Howe RW (1967) Temperature effects on embryonic development in insects. Annu Rev Entomol 12: 15-42.
23. White RS, Aubertin D, Smart J (1940) The fauna of British India, including remainder of the oriental region: Diptera VI, Family Calliphoridae. Taylor and Francis, London, UK.
24. Grassberger M, Reiter C (2001) Effect of temperature on Lucilia sericata (Diptera: Calliphoridae) development with special reference to the isomegalen-and isomorphen-diagram. Forensic Sci Int 120: 32-36.
25. Abd-AlGalil FM, Zambare SP (2015) Effects of temperature on the development of Calliphorid fly of forensic importance Chrysomya megacephala (Fabricius, 1794). Indian J Appl Res 5: 767-769.
26. Abd-AlGalil FM, Zambare SP (2015a) Effect of temperature on the development of Calliphorid Fly of forensic importance, Chrysomya rufifacies (Macquart, 1842). IJAR 3: 1099-1103.
27. Nabity PD, Higley LG, Heng-Moss TM (2006) Effects of temperature on development of Phormia regina (Diptera: Calliphoridae) and use of developmental data in determining time intervals in forensic entomology. J Med Entomol 43: 1276-1286.
28. Warren JA, Anderson GS (2013) Effect of fluctuating temperatures on the development of a forensically important blow fly, Protophormia terraenovae (Diptera: Calliphoridae). Environ Entomol 42: 167-172.
29. Al-Misned FA, Amoudi MA, Abou-Fannah SS (2003) Development rate, mortality and growth rate of immature Chrysomya albiceps (Wiedemann) (Diptera: Calliphoridae) at constant laboratory temperatures. Journal of King Saud University Science 15: 49-58.
30. Greenberg B (1991) Flies as forensic indicators. J Med Entomol 28: 565-577.