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Effect of seasonal variations on the life cycle of *Lucilia cuprina* (Wiedemann, 1830) (Diptera: Calliphoridae)

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Abstract
Australian sheep blowfly, *Lucilia cuprina* (Diptera: Calliphoridae) is a fly of medical and veterinary importance. 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. Effect of seasonal variations on its life cycle duration and morphological parameters *L. cuprina* have been studied. Results shows that temperature and humidity affect the development and morphology of the maggots, in summer life cycle of the fly is completed in 218 hrs. (9.083 days), in rainy and winter season life cycle is completed in 301 hrs. (12.56 days) and 274 hrs. (11.41 days). The purpose of this study is to investigate seasonal and regional differences in carrion decomposition patterns and carrion blow fly communities from Osmanabd district (MH).

Keywords: Diptera, calliphoridae, blowflies, Lucilia, forensic, decomposition

Introduction

Lucilia cuprina is commonly known as the sheep green bottle. It breeds in carrion, almost continuously throughout the year [43]. Fly-strike, also known as cutaneous myiasis is a serious problem for both the New Zealand and Australian sheep industries. The disease is predominantly caused by the fly, *L. cuprina*. Fly-strike is not only a cost to the farming enterprise, but it is also considered a major animal welfare issue; especially with the practice of mulesing to prevent fly-strike coming under scrutiny from animal welfare organizations [18].
L. cuprina is often used as a very helpful tool to aid medical and forensic professionals. It is known to be one of the first flies to occupy a corpse upon its death. Once it lands on a corpse, it continues in the formation of its next generation by laying its eggs. Its larva, pupa and finally the adult follow the eggs. Forensic professionals may form a postmortem interval by the life stage found on the corpse. *L. cuprina*, although it is a worldwide pest, is very climate specific. It occupies dryer climates. A forensic investigator may conclude that a corpse has been relocated from its original location if it is found in a moist climate with *L. cuprina* on it. Medical doctors for Debridement therapy have used the maggots of *L. cuprina* for patients who suffer from wounds that are healing slowly [24]. The maggots cleanse the wound by eating the dead and infectious skin and preventing gangrene and further infection.
L. cuprina, like all flies are holometabolous, meaning they go through a complete metamorphosis [1]. Flies have four stages of growth like other dipterans: egg, larvae, pupa, and adult. Adult *L. cuprina* arrive early on carrion, appearing hours or even minutes after death. There, on the fresh body, they lay their eggs. The eggs then hatch into larvae, which begin to feed and grow. After about five days, larvae enter the pupal stage. This is said to be an inactive stage, although many changes occur during this part of the flies' life cycle. The pupa does not feed, but rather uses the time inside the casing to change from rice like larvae into an adult fly with wings and six legs. The whole process can take anywhere from eleven to twenty-one days depending on environmental conditions including temperature and nutritional availability. In most cases, warmer temperatures and better nutrition lead to a faster life cycle.
Forensic Entomology may use the insect evidence to estimate the developmental time of insects whilst also observing the carrion insect succession to estimate time since death. As death may occur a variable amount of time prior to colonisation the entomologist will estimate the Minimum PMI (mPMI) by determining the earliest period the body was colonised by insects to time taken for the collected specimens to reach a certain developmental stage [36, 7]

as the Time of Colonisation (TOC) had also defined this time. The maximum PMI (maxPMI) can be calculated from when the person was last seen alive to the discovery of the body [37]. Larval development is dependent on temperature [4], and every species has a slightly different growth rate [10, 17, 30]. Higley *et al.*, [20] concluded that the source of temperature data used for degree-day calculations was likely the most significant single source of error. Therefore, data from the nearest recording site need to be directly compared to maximum and minimum temperatures from the death scene to allow for possible adjustment in the estimates [1].

Developmental data for primary blowflies provide the most accurate means of estimating the PMI using arthropod information [12]. It is presumed that the first individuals that arrives at, and lay eggs in a corpse do so within hours after death [8]. Developmental times from oviposition to emergence might differ depending on many factors. Geographic variations and climate conditions can influence adaptations explaining the differences in the development at different temperatures. High temperatures accelerate the growth and development, whereas low temperatures slow it down. Alternatively, the rearing in field conditions allows a more precise approximation to the conditions of growth in a real forensic case. However, more studies are necessary to understand the behavior during the development of the immature stages, like the degree-days estimates and the knowledge of the intrinsic and extrinsic factors that could affect the development of the forensically important species [25]. In order to make these estimates, forensic entomologists rely on laboratory development data for the species in question. Given that colonization by many of these arthropods occurs after death, these estimates are synonymous with the minimum postmortem interval (mPMI). The need for development data for these forensically important species from various eco-regions is necessary, as they might be significantly different [31, 3].

The time duration, ambient humidity and temperature are the most important factors in the developmental stage of arthropod species. Insects are poikilothermic organisms. The growth rate of blow fly larvae is highly dependent on temperature as their body temperature changes with ambient temperature. For insect development, a relationship between ambient temperature and duration of developmental processes is long known. There is a temperature zone where the development rate is optimal; furthermore, temperature thresholds exist below or above that optimum where no development will take place. This research is also important to the field of forensic entomology, which is the application of arthropod science in the judicial system. Forensic entomologists assist in criminal cases by estimating the time of insect colonization of human or other animal's remains. To date, no seasonal or regional studies have been conducted in this area. This affords an excellent opportunity to make a significant contribution to the baseline database used in forensic entomology.

Materials and methods

Collection of the flies

Present study was carried out in the Osmanabad district of Maharashtra state, India, during 2015 to 2016.The fresh liver sample was purchased from the local slaughterhouse. Partially putrefied liver/meat was exposed in the air and within few minutes, the flies were attracted. Similarly, maggots and adult flies were collected from different dead bodies of different animal. Flies and maggots were collected with the help of insect net. Collected flies were brought to the laboratory and reared in the laboratory conditions. Adults were fed daily on fresh liver and honey mixed in water. Fresh liver was used as oviposition site for females.

Maintenance of the pure culture

Maggot culture was provided with fresh liver as a food until the prepupae stage. Prepupae were kept in 500ml beakers containing dry soil, which is required for the pupation. Adult flies emerged out from pupae were reared in a separate rearing box of size 22× 12×10 inches in dimensions' length × width × depth respectively and regular culture was maintained [44]. After the collection of the samples, pure cultures for each species was obtained by separating eggs or larvae of one female and cultured them for identification and further experiment. Morphological identification of the fly was done by using various published identification keys. [5, 35, 39, 40, 41, 42]

Observations

Life cycle of the fly was studied throughout the year to observe and record the effect of seasonal variation on its life cycle and morphological parameters. Five replicates were taken for each get the perfect results.

Morphological characters were photographed by using 16.0 Megapixel Nikon Coolpix Optical Zoom Digital Camera. Adults and maggots were studied morphologically with the help of stereo-zoom microscope and light microscope Magnus Trinocular Microscope. Temperature and humidity of the rearing room was recorded throughout the experiment. Daily maximum and minimum temperature and humidity data was recorded three to four times a day. Digital hygrothermometer was used to record temperature and humidity. In stastical analysis standard deviation was performed for each seasonal data.

Ethical statement

The Government of India or any of the State Government of Maharashtra as an endangered or threatened species restricting or regulating its collection and observation have not notified *L. cuprina* under any act or laws and rules. No permits were required, for collecting the larvae or flies from the different regions of Osmanabad district.

Observation Tables

Table 1: Duration of different life cycle stages of *L. Cuprina* in summer season and PMI in hours.

PMI in Hours	Develop-mental stages	Length (mm)	Weight (mg)	Width (mm)	Temperature (0C)			Humidity (%)		
					Max.	Min	Record	Max	Min	Record
0	Eggs	1.4 ± 0.04	0.51±0.02	0.6 ± 0.06	39.2	28.4	33.8	38	22	30
23	1st Instar	4.7 ± 0.01	8.2 ± 0.39	1.2 ± 0.11	40.2	28.6	34.4	34	18	26
50	2nd Instar	8.9 ± 0.27	20.3 ± 1.54	2.3 ± 0.39	39.6	28.6	34.1	37	17	27
86	3rd Instar	14 ± 0.81	51.2 ± 1.10	3.5 ± 0.11	38.4	26.8	32.6	37	19	28
116	Prepupae	12.1±0.28	42.8 ± 0.66	3.8 ± 0.33	38.1	25.5	31.8	39	21	30
218	Pupae	9 ± 0.76	36 ± 0.06	3.6 ± 0.47	37.4	25.4	31.4	33	19	26
218 (9.083days)	Adult	8 ± 0.03	30 ± 0.85	3.5 ± 0.17	37.6	25.6	31.6	35	17	26

Table 2: Duration of different life cycle stages of *L. Cuprina* in rainy season and PMI in hours.

PMI in Hours	Developmental stages	Length (mm)	Weight (mg)	Width (mm)	Temperature (0C)			Humidity (%)		
					Max	Min	Record	Max	Min	Record
0	Eggs	1.2 ± 0.06	0.47 ± 0.04	0.4 ± 0.06	29.6	28.6	29.1	88	64	76
23	1st Instar	4.2 ± 0.64	7.4 ± 0.29	1 ± 0.76	30.1	29.1	29.6	82	66	74
47	2nd Instar	7.8 ± 0.22	18.3 ± 1.33	1.8 ± 0.18	30.2	29.2	29.7	78	66	72
88	3rd Instar	13.4±0.35	46.2 ± 1.15	2.7 ± 0.63	28.6	26.4	27.5	81	63	72
161	Prepupae	11.6±0.28	40.8 ± 1.56	3.5 ± 0.61	29.6	26.8	28.2	77	57	67
301	Pupae	8.8 ± 0.51	34 ± 1.12	3.4 ± 0.50	28.2	7.6	17.9	79	57	68
301 (12.56 days)	Adult	8.2 ± 0.63	27 ± 0.26	3.3 ± 0.11	30.3	28.1	29.2	78	56	67

Table 3: Duration of different life cycle stages of *L. Cuprina* in winter season and PMI in hours.

PMI in Hours	Developmental stages	Length (mm)	Weight (mg)	Width (mm)	Temperature (0C)			Humidity (%)		
					Max	Min.	Record	Max.	Min.	Record
0	Eggs	1.6±0.04	0.54±0.05	0.7±0.08	28.5	26.4	27.45	66	50	58
24	1st Instar	5.3±0.33	9.8±1.23	1.7±0.14	27.3	26.3	26.8	72	48	60
49	2nd Instar	9.5±0.81	25.2±1.15	3±0.47	29.2	24.2	26.7	65	31	48
91	3rd Instar	16± 0.30	58.5±1.34	4.2±0.55	29.6	24.6	27.1	72	33	52.5
139	Prepupae	13.3±0.86	45.6±0.38	4±0.13	28.6	22.6	25.6	53	31	42
274	Pupae	9.2±0.54	38.7±1.76	3.8±0.23	27.4	21.8	24.6	46	34	40
274 (11.41days)	Adult	8.5 ± 0.93	33.1±0.87	3.6±0.35	27.4	20.8	24.1	41	37	39

± Standard deviation of five values.

ults and Discussion

The eggs of *L. cuprina* hatched after 18 hours in summer season when the average temperature and relative humidity were 33.8 ^{0}C and 30%, and hatched after 22 hrs. in rainy season when the average temperature and relative humidity were 27.45 ^{0}C and 58%, while in winter season hatched after 28 hrs. when the average temperature and relative humidity were 29.1 ^{0}C 76%. When the temperature was high, the incubation period was less and when the temperature was low, the incubation period was more.

The first instar larvae of *L. cuprina* in summer season remained for 23 hrs. when the average temperature and relative humidity were 34.4 ^{0}C and 26%, while in rainy season spent 23 hrs. when the average temperatures were 29.6 ^{0}C and average relative humidity were 74%, while in winter season spent 24 hrs. when the average temperatures and relative humidity were 26.8 ^{0}C and 60% respectively. The sizes of the first instar larvae in summer season were bigger than the sizes in winter season and smaller than the sizes in rainy season.

Th econd instar larvae developed in 27 hrs. in summer sea on when the average temperature and relative humidity were 34.1 ^{0}C and 27% and spent 24 hrs. in rainy season when the average temperature and relative humidity were 29.7 ^{0}C and 72%, while in winter season when the average temperature and relative humidity were 26.7 ^{0}C and 48% 2nd instar larvae spent 25 hrs. The sizes were nearly doubled because all instar larvae were vigorous feeders. The third instar larvae spent 36 hrs. when the average temperature and relative humidity were 32.6 ^{0}C and 28% in summer season and spent 29 hrs. in rainy season when the average temperature and relative humidity were 27.5 ^{0}C and 72%, while in winter season 3rd instar spent 42 hrs. when the average temperature and relative humidity were 27.1 ^{0}C and 52.5%. The sizes of maggots reach the maximum level and started migrate away from the media for pupation.

The prepupae remained for 30 hrs. in summer season when the average temperature and relative humidity were 31.8 ^{0}C and 30%, while in rainy and winter season prepupae spent 35 and 48 hrs. respectively when the average temperature and relative humidity in rainy and winter seasons were 28.2 ^{0}C, 7% and 25.6 ^{0}C and 42% respectively. The prepupae initially started searching for safe and dry place for pupation. The sizes of prepupae shrunk because they stopped feeding at this stage. The sizes of prepupae in rainy season were bigger than the sizes in summer and winter seasons, which indicate that the temperature and relative humidity in rainy season was more favorable for the larval development. Pupae in summer season spent 102 hrs. when the average temperature and relative humidity were 31.4 ^{0}C and 26% and spent 135 hrs. in rainy season when the average temperature and relative humidity were 17.9 ^{0}C and 68%, while in winter season when the average temperature and relative humidity were 24.6 ^{0}C and 40% pupae spent 135 hrs. The sizes of pupae in rainy season were bigger than the sizes in summer and winter.

Adult emerged out from the pupae after 218 hrs. in summer and 274 hrs. in rainy season, while in winter season emerged after 301 hrs. The size of adult varied in different seasons, in rainy season the sizes were bigger than the sizes in summer and winter seasons. The time spent in feeding and post-feeding stages varied in different seasons. In summer, season total time spent in larval or feeding stages was 86 hrs. (2.79 days) and in rainy season were 91 hrs. (3.42 days) means delay by about one day from the time spent in summer, while in winter season were 76 hrs. (4.21 days) indicating delay of about one day from rainy season and two days from summer season.

The total life cycle of *L. cuprina* in summer season was completed in 218 hrs. (9.08 days) when the average temperature was 31.6 ^{0}C and average humidity was 26%, in rainy season completed in 301 hrs. (12.54 days) when the average temperature was 29.2 ^{0}C and average humidity was 67%, while in winter season was completed in 274 hrs. (11.4 days) when the average temperature and humidity were 24.1 ^{0}C and 39% respectively. Larval development is dependent on temperature Bowler K., and Terblanche J.S [6], and every species has a slightly different growth rate [12, 10, 30].

Laake *et al* [22] have studied the environmental and wound temperatures, being slower at low temperatures, although true diapause does not occur, influence the rate of development of the immature stages. As per Parman D.C. [29] this effect is most pronounced in the off-host pupal stage, which can vary from 1 week to 2 months' duration depending on the season, Thus, the complete life cycle of fly may take 2–3 months in cold weather, whereas in temperate conditions with an average air temperature of 22 ^{0}C, it is completed in about 24

days James, [21], and in tropical conditions averaging 29 °C it is completed in about 18 days, Tarone and Foran [32]. Thus, the complete life cycle of fly may take 2–3 months in cold weather Parman [29], whereas in temperate conditions with an average air temperature of 22 °C, it is completed in about 24 days, James, M. T. [21], and in tropical conditions averaging 29 °C it is completed in about 18 days Thomas and Pruett [34]. Survival is uniformly high if eggs are held in a saturated atmosphere at 15-40 °C, but falls off sharply with a decrease in ambient humidity, or at temperatures higher than 40 °C Vogt and Woodburn [37]. Fly eggs hatch from eight hours to three days, depending on temperature, after they have been deposited on the skin of the sheep Tellum & Bowles [32].

Flies spend a major part of their life cycle in the soil. Post-feeding the third instar larvae burrow into the soil to pupate [24]. This stage is dependent on soil temperature. At 30 °C the pupal stage takes about six days, whereas at 15 °C it takes 25 days Foster et al. [14]. The adult fly then emerges. Adults feed on plant and animal material and the females require protein before they can develop and lay mature eggs Tellam and Bowles [32]. Foster et al. and Vogt and Woodburn [14, 38] have explained that the egg survival and development rates are maximal around 35 °C. Eggs usually hatch within 12-24 hrs, if the oviposition site remains moist [24]. The duration of post feeding larval stage is highly variable. During summer, the median time drop off to pupariation is 2 days and ranges between 1 and 4 days as explained by Dallwitz and Wardhaugh, Mackerras [9, 24]. The time to pupariation increases with decreasing temperature in autumn.

Pupal development rates increase linearly between 15 °C (25 days) and 30 °C (6days) as studied by Foster et al., [14]. Pupae held under fluctuating or constant temperature regimes show similar development rates and survival between 15 and 30 °C, respectively, whereas under fluctuating conditions pupae can survive short exposure to -10 °C or 46 °C. Survival of pupae, exposed daily for 7 hours to 38 and 0 °C, was 78 and 98%, respectively. Dallwitz [9]. Foster et al. and Vogt and Woodburn [14, 37] also have studied the period of emergence of flies from a single cohort of post feeding larvae varies from 4 days in summer to about seven weeks in spring.

Maria Maria C. Velez Marta Wolff, [25] has explained the developmental times from oviposition to emergence might differ depending on many factors. Geographic variations and climate conditions can influence adaptations explaining the differences in the development at different temperatures. High temperatures accelerate the growth and development, whereas low temperatures slow it down. Alternatively, the rearing in field conditions allows a more precise approximation to the conditions of growth in a real forensic case. However, more studies are necessary to understand the behavior during the development of the immature stages, like the degree-days estimates and the knowledge of the intrinsic and extrinsic factors that could affect the development of the forensically important species.

Mali K. H. [23], obtains similar results life cycle of *Lucilia cuprina* was completed in 226 hrs. in summer, 251hrs.in winter and 298 hrs.in summer season. First instar larvae emerged after 18hrs.in summer, 22 hrs. in rainy season and 21 hrs.in winter. Second instar larvae were developed after 39 hrs. in summer, 43 hrs.in rainy season and 42 hrs.in winter season. After 64 hours second instar larvae molted into third instar in summer season, 70 hrs.in rainy season and on 65 hrs. of the development second instar molted into third instar larvae in winter season. Larvae spent 127 hrs. in prepupa stage in summer, 158 hrs.in rainy season and 135 hrs.in winter season. Pupa stage lasts for 127 to 144 hrs. in summer,139 to 298 hrs.in rainy season and 135 to 251 hrs.in winter season.

Conclusion

Carrion decomposition studies conducted in various geographic locations and among seasons within one geographic location are a necessity in developing a baseline data set for use in the field of forensic entomology. Seasonal and regional differences in carrion decomposition patterns and carrion blow fly communities affect the PMI. Geographic variations and climate conditions can influence adaptations explaining the differences in the development at different temperatures. High temperatures accelerate the growth and development, whereas low temperatures slow it down.

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