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Studies on the larval growth of forensically important flesh fly *Sarcophaga dux* Thompson 1869 (Diptera: Sarcophagidae) under outdoor ambient temperatures from Central India

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Abstract

The Larval Growth of flesh fly *Sarcophaga dux* Thompson 1869. (Diptera: Sarcophagidae) was studied in outdoor ambient temperatures in two seasons, Spring and summer in Jagdalpur, District-Bastar, Chhattisgarh, India. The duration of growth of immature stages of the species, from first instar until adult emergence, was 26 days and 17 days, during spring and summer seasons respectively. The mean larval length measured for first instar, second instar, third instar and puparia were 4 mm, 12 mm, 19 mm and 9 mm respectively. From the present study it was concluded that, in this species low temperature delayed larval growth, whereas high temperature accelerate the larval growth, if sufficient food is available.

Keywords: Forensic entomology, Diptera, Fleshfly, *Sarcophaga dux*, larval growth.

Introduction

Forensic entomology is defined as the use of insects and other arthropods in criminal and other legal investigations has been growing importance during last decades. Numerous articles have been published on the subject, Bharti and Singh^[1], Catts and Goff^[2], Suri Babu *et al*^[3], Suri Babu^[4] and some books like those of Smith^[5], Catts and Haskell^[6] Byrd and Castner^[7], and Greenberg and Kunich^[8] were published with in the last 30 years. A reliable application of forensic entomology was mostly laid down during the first half of 20th century by taxonomists interested in those insect species of forensic importance belong to two principal dipteran families like Calliphoridae (Blow flies) and Sarcophagidae (Flesh files). The Sarcophagidae flies are a large family comprising over 2600 species and representatives of this family are found throughout the world with most species occurring either in tropical or warm temperate regions, Byrd and Castner^[7]. The Indian representatives belong to 117 species under 38 genera of three subfamilies, Nandi^[9]. In India the first studies on the larval growth of *Sarcophaga ruficornis* (Fabricius, 1794.) was carried out by Singh *et al*^[10]. In the present study the larval growth of *Sarcophaga dux* (Thomson, 1869) was carried out under uncontrolled outdoor ambient temperature in two seasons in Jagdalpur, District Bastar, Chhattisgarh, India at 19°05'N and 82°01'E at about 566 m mean sea level. In five cases larvae of *S. dux* was collected from different human carrions from Jagdalpur and its surroundings. The aim of this article is to provide the data of larval development of forensically important flesh fly *S. dux* to those forensic entomologists who are working with investigating agencies on the estimation of post mortem interval (PMI) which are involved in criminal and suspicious cases.

Materials and Methods

The present study was conducted during two seasons, in spring and summer in the months of February, March and June, year 2015. The first instar larval of *S.dux* were obtained by baiting outside on the terrace of Regional Forensic Science Laboratory building situated in Jagdalpur, District Bastar, Chhattisgarh, India at 19°05'N and 82°01'E at about 566 m mean sea level. During rearing 24 hours old decayed goat meat pieces were provided to the flies in a plastic container for larviposition.

We do not know the species of the larvae until the emergence of adult flies. About 40 small plastic container were setup in which each container one first instar larva was transferred and reared them providing small goat meat pieces. The top of each plastic container was wrapped with mosquito net cloth using a rubber band. From the reared larvae two larvae of each stage were separated and preserved in 70% alcohol for larval instar stage determination. When the larvae attained third instar it was transferred in to small plastic container with bottom of 3 cm thick layer of mixture of wet sawdust and soil was filled to prevent desiccation and to provide suitable place for the prepupa for pupation. The plastic containers were closed with perforated lids to facilitate air circulation to the immature stages and to prevent escaping newly emerged flies from the container. The different instars were observed daily two times at 6 AM and 6 PM and adults were collected and get identified by the experts of Zoological Survey of India, Kolkata, West Bengal, India. During rearing few other species of family Calliphoridae and Muscidae flies were also emerged simultaneously which were sorted out. Thus the pure culture of *S. dux* was achieved and maintained. The developmental data was recorded by observation of each stage daily during Spring and Summer seasons year 2015. The temperature and relative humidity data were procured from The Government Agronomy observatory, Shaheed Gundadhoor college of Agriculture and Research Station, Kumaravand, Jagadalpur.

Results

The data was collected and analyzed to know if there was any relationship among larval developmental days of *S. dux* with ambient temperature and relative humidity. The 1st, 2nd and 3rd instar larva grown up to 4 mm, 12 mm and 19 mm length and they spent 2,2 and 3 days at mean temperature 22.4, 23.5 and 24 °C. The prepupa measured 9 mm and spent 3 days at mean temperature 22.20 °C, whereas pupa spent 18 days at mean temperature 23.20 °C. It took 1st instar larva to adult fly emergence 26 days duration at mean temperature 23.1± 0.33 °C, range 22.2-24 °C and mean relative humidity 62.1±1.61% range 61-65.8% (N=30). During the months of February and March of spring season. The 1st, 2nd and 3rd instar larvae grown up to 4mm, 12mm and 19mm and they spent 2,2 and 2 days at mean temperature 35.10, 34.3 and 33.3 °C. The prepupa spent 2 days at mean temperature 37.1 °C. The duration of pupa was 9 days at mean temperature 26.4°C. The duration of 1st instar larva to adult fly was 17 days with mean temperature 35.2±3.34 °C with range 26.4-42.1 °C with relative humidity 46.30±5.82% Range 34-67.1% (N=30) during the month of June Fig 1,2,3.

The findings of present studies revealed the fact that the duration of larval and pupal stages of *S. dux* is directly related to the ambient temperature and humidity. The low temperature and high humidity delayed the larval and pupal development where as high temperature and low humidity accelerate the larval and pupal development in this flesh fly.

Table 1: Growth pattern of rearing of *S. dux* in the spring and summer of 2015 (N=30)

Stage	Length (mm)	Time spent in stage (Days) in spring season	Time spent in stage (Days) in summer season
1 st Instar Larvae	6	2(25-26/02/2015)	1(03/06/2015)
2 nd Instar larvae	12	2(27-28/02/2015)	1(04/06/2015)
3 rd Instar Larvae	19	3(01-03/03/2015)	2(05-06/06/2015)
Prepupa	9	3(04-06/03/2015)	2(07-08/06/2015)
Pupa - Adult emergence	P(9)-A(10)	16(07-25/03/2015)	11(09-19/06/2015)
Total Days		26 Days for emergence of adult Fly	17 Days for Emergence of adult Fly

Table 2: Average Temperature during each stage of rearing

Sl No.	Variation in Temperature* on each stage of rearing of <i>S. dux</i>		
	Stage	Spring (°C)	Summer (°C)
1	1 st Instar Larvae	22.4	35.1
2	2 Instar Larvae	23.5	34.3
3	3 rd Instar Larvae	24	33.3
4	Prepupae	22.2	42.1
5	Pupae – Adult emergence	23.2	26.4
6	Range	22.2-24	26.4-42.1
7	Mean (X̄)	23.1	35.2
8	± SE	0.338	3.343

Table 3: Average Relative humidity during each stage of rearing

S. NO.	Stage	Variation in Relative humidity* on each stage of rearing of <i>S. dux</i>	
		Spring (%)	Summer (%)
1	1 st Instar Larvae	63	42.5
2	2 nd Instar Larvae	64.3	34
3	3 rd Instar Larvae	56.5	38
4	Prepupae	65.8	49.8
5	Pupae-Adult emergence	61	67.1
6	Range	61-65.8	34-67.1
7	Mean(X̄)	62.1	46.3
8	±SE	1.611	5.829

Discussion

The rearing was conducted outdoor at the mean ambient temperature of 23.10 °C and mean relative humidity 61.1% in spring and mean ambient temperature of 35.20 °C and 46.3% mean relative humidity in summer season. Gunn [11] reported that the eggs of this genus fly hatch in the reproductive track of female fly and it lays first instar larvae. All the first instar larvae were collected within a duration of one hour after the decayed meat was placed outside for baiting. It has been reported that the viviparous females of Sarcophagids do not deposit all their larvae in the same carcasses like blowflies, rather spreading them among several carcasses, Galonte [12]. While comparing the life cycle of flesh fly *S. dux* and blow fly *Chrysomya megacephala* and *Ch. rufifacies*, the former spend more than 50% of their immature life cycle in the pupation period, Kumara, *et al* [13]. The present study also support this statement.

The developmental duration hours for *S. dux* from first instar larval until adult emergence was 307.0 ± 3.0 hours at the fluctuating temperature of 28.9 ± 1.2 °C in Malaysia, Kumara *et al* [13]. In Saudi Arabia, a study conducted by Misned [14] at a constant temperature of 28 °C and found the development of *S. dux* from first instar until adults emergence was 393.9 ± 20.9 hours. Sukontasan *et al* [15] reported that they reared this species under natural ambient temperature of 26 ± 2 °C and found prepupae began at 72 hours in summer season. Amoudi *et al*. [16] Studied the life cycle of *Sarcophaga ruficornis* and reported at constant temperature of 28 °C and the duration of total development up to adult 436.8 ± 15.4 hours. Singh *et al* [10] reported that the life of cycle of *Sarcophaga ruficornis* was observed and reported that it completed life cycle within 15 days during summer season with temperature ranged between 20-42 °C and relative humidity between 14-54% with no rain fall.

Galloway *et al* [17] studied the life cycle of in *Sarcophaga cesseraster* during spring season in Southern Arizona and concluded that temperature and sunlight greatly influenced the duration of larval growth, whereas cloudy weather prevent larviposition in this species.

Sukontasan *et al* [15] examined the development rates of *S. dux* in Northern Thailand during the year 2002-2008 under natural ambient temperature 24 to 28 °C and indicating relatively rapid larval development in summer (March-June) the prepuae initiated at 72 hours, where as in raining season (July – October), 72 or 90 hours and in winter 96 hours. Singh and Bharati [18] reported nocturnal larviposition by the flesh flies *Sarcophaga albiceps* (Meigen, 1826) and *Sarcophaga hitripes* (Wiedemann, 1830) in Patiala, Punjab, India, contrary to this statement in the present study it was observed that both nocturnal and diurnal larviposition performed by *S. dux*.

Chakraborty *et al* [19]. Studied on the life cycle of *S. dux* on a dead reptilian carcass in the month of May in Kolkata, India at $38 \pm 3^{\circ}\text{C}$ ambient temperature with relative humidity 18-53% and reported the fly completed the life cycle in 312 hours. Rawat *et al* [20] reported that in *S. dux* short day photo periods and low temperature below 20 °C induced pupal diapause.

Adhikari *et al* [21] studied the life history of *Sarcophaga ruficornis* and the developmental time from 1st instar larva to adult fly 28 ± 2 days at 28 ± 1 °C in the month of February, 26 ± 2 days at 30 ± 1.33 °C in the month of March, 24 ± 1 days at 31 ± 2 °C in the month of April and 23 ± 1 days at 32 ± 2 °C

in the month of May. They observed the life cycle of the same species and found longer duration in the month of February than in the month of May. Similar observations were also made in the present study.

The present study reveled the fact that the life cycle of *S. dux* was directly related to the ambient temperature, the low temperature delayed the larval growth, whereas the high temperature decreased the period of larval and pupal development, if sufficient food is available to the immature stages of this flesh fly.



Fig 1: first instar larvae of *S. dux* on goat meat pices.



Fig 2: first instar larva of *S. dux*



Fig 3: Second instar larva of *S. dux*



Fig 4: third instar larva of *S. dux*



Fig 5: prepupa of *S. dux*



Fig 6: pupa of *S. dux*



Fig 7: Adult fly of *S. dux*

Conclusion

It is concluded from the present study that the duration of larval and pupal stages of flesh fly *sarcophaga dux* is directly related to the ambient temperature and humidity. The low ambient temperature and high humidity delayed the larval and pupal development, whereas high temperature and low humidity accelerate the larval and pupal development if sufficient food is available to this flesh fly. The data of the present study, the life cycle of *S.dux* in two seasons, spring and summer is useful to those forensic entomologists who are working on the estimation of postmortem interval (PMI) which are involved in criminal and suspicious cases.

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