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Observations on the life cycle and developmental stages of *Lucilia cuprina* (Order: Diptera; Family: Calliphoridae) on Rohu carp

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Abstract
Lucilia cuprina (Wiedemann, 1830) (Class: Insecta; Order: Diptera; Family: Calliphoridae) is a blowfly of forensic importance belonging to the family Calliphoridae. In India, researchers have identified 119 species of Calliphoridae belonging to 30 genera across 9 subfamilies. They play an essential role in the decomposition process, making them an invaluable resource in forensic science. They play a valuable role as pollinators of some plants in horticulture and can increase seed yields. The life cycle and developmental stages of *L. cuprina* on Rohu carp (*Labeo rohita*) were studied from 15.03.2024 to 01.04.2024. Like other dipterans, it undergoes four stages of growth: egg, larva, pupa, and adult.

Keywords: Diptera, calliphoridae, life cycle, developmental stages

Introduction

Lucilia cuprina (Wiedemann, 1830) (Diptera: Calliphoridae) is a blowfly of forensic importance belonging to the family Calliphoridae. The Calliphoridae family is a cosmopolitan group of calyptrate flies, consisting of nearly 1,500 recognized species globally (De Carvalho and Mello-Patiu, 2008) [11]. The Indian representatives of the Calliphoridae family include 63 species across 18 genera (Nandi, 2004; Singh and Sidhu, 2004; Singh and Sidhu, 2007) [21, 25, 26]. In India, researchers have identified 119 species of Calliphoridae belonging to 30 genera across 9 subfamilies (Bharti, 2011) [3]. Its wide distribution is due to movement patterns and the travelling of humans and livestock within the last century. Hoffman (1886) [17] identified insects and other arthropods as forensic indicators during mass exhumations in Germany and France. Forensic entomology is the analysis of insect evidence for forensic and legal purposes (Amendt *et al.*, 2007) [1]. It causes myiasis in humans and livestock, particularly in sheep (Heath & Bishop, 2006) [15], and commonly thrives around garbage and decaying flesh (Byrd & Castner, 2010) [6].

In Ghana, the genus *Lucilia* has been reported insect of forensic importance (Combey *et al.*, 2017) [8]. It has also been reported that calliphorid flies serve as pollinators of mango (*Mangifera indica*) (Dag & Gazit, 2000; Sung, 2006) [9, 27]; and as important decomposers (Ramos- Pastrana *et al.*, 2018, Meira *et al.*, 2020) [23, 19] and are used in the treatment of necrotic ulcers through maggot debridement therapy (Mumcuoglu *et al*. 1999; Williams *et al.*, 2008; Martelet *et al.*, 2009; Du Plessis & Pretorious, 2011; Williams & Villet, 2014) [20, 28, 18, 12, 29]. The larvae of *Lucilia sericata* used in wound therapy help reduce infection and enhance tissue nutrition, promoting faster wound healing (Borkataki et al., 2019) [4].

Forensic entomology is an emerging field in India, although it is in its early stages. Only a few scientists were initially engaged in this field; however, the number has grown over time. Despite this increase, the number of studies conducted in forensic entomology in India is relatively low (Sharma & Singh, 2016; Amin & Sinha, 2018) [24, 2]. To our knowledge, no detailed work on the developmental stages of *L. cuprina* on Rohu carp *(Labeo rohita)* has been recorded, and thus, this study aimed to understand the various observations during different developmental life stages. Hence, efforts have been made to give detailed information and photo plates on their life stages.

Materials and methods

The present study was conducted in Sodepur (22.7040809° Latitude and 88.3797656° Longitude), North 24 Parganas, West Bengal, India, from 15.03.2024 to 01.04.2024. The fresh Rohu carp (*L. rohita*) were bought from the local fish market. The length and breadth of each Rohu carp are about 15-17 cm and 3-3.5 cm, respectively, and each weigh about 230-250 g. Three sets containing two Rohu carps were kept in separate plastic containers. (20x10x5 cm) on 15.03.2024 at 10 AM and placed in the swampy, shady area in our locality to protect them from other animals. Observations were done every day in the morning, afternoon, and evening for 30 minutes. The blowfly (*L. cuprina*) started interaction with the opening surface of Rohu carp on 16.03.2024. The female flies laid eggs on the opening surface of the carp's body. After the emergence of the 1st instar larva concerning the entire attractant that was kept in a plastic container, the larval stage was kept aside in a small plastic container and scaling was done. The above process has been repeated many times to keep track of all the instar stages of blowfly development. A few newly hatched larvae were collected from the common carp of Rohu, which had been placed in the experimental setup and kept inside the sterilized container covering the net for gradual aeration.

The larvae of *L. cuprina* were fed using Rohu carp as their food source. The developmental stages of the blowfly (*L. cuprina*) on Rohu carp were examined from both taxonomical and ecological perspectives. Specimens were carefully collected using fine forceps for taxonomic analysis. Some were euthanized with ether, preserved in 70% alcohol, and transported to the laboratory for future examination.

Results and discussion

Calliphoridae includes a diverse group of calyptrate flies with a worldwide distribution, featuring species that are ecologically, veterinary, medically, and forensically

important. Forensic entomologists can utilize insect evidence to estimate the developmental time of insects and track the succession of carrion insects to determine the time since death. They have a significant role in human medicine because their larvae heal chronic injuries and act as scavengers, consuming rotting organic matter. Like all flies, *L. cuprina* is holometabolous, which means it undergoes complete metamorphosis." (Byrd *et al.*, 2001) [5]. *L. cuprina* has a complete life cycle consisting of the egg, larva, pupa, and adult stages. Date-wise detailed observations are shown in Table 1. Adult females may lay 100–200 eggs in a cluster on Rohu carp. Ootheca-like structures of dipteran blowfly (*L. cuprina*) were found in the Rohu carp body on 16-03-2024. The eggs are approximately 1mm long, are typically laid in clusters, and hatch between 12 to 24 hours after oviposition. The eggs are elongated, yellowish-white oval-shaped structures. Whereas Clark *et al.* (2006) [7] reported that a female adult blowfly (*L. sericata*) will oviposit between 150-200 eggs per batch, with a total of over 2,000 eggs in her lifetime. The cryptic information about the developmental stages of *L. cuprina* is shown in Figure 1. The eggs of most species will hatch within 12 to 24 hours, depending on the specific species and temperature. Larval development may progress rapidly at warmer temperatures. The entire developmental stages were completed at temperatures ranging between 22°C and 33°C (Table 1). The body temperature of larvae affects their growth rate, which depends on environmental conditions such as ambient temperature and the heat generated by maggot aggregations (Gruner *et al*., 2007) [14].

Observing the life cycle of *L. cuprina* on Rohu carp (*L. rohita*) provides insight into the species' adaptability and its role in decomposing animal matter. Grassberger & Reiter (2001) [13] studied the life cycle of a population of *L. sericata* in Vienna (Austria) at temperatures ranging from 15°C and 34°C. An increase in temperature causes an increase in metabolic activity and reduces the development time (Bharti, 2011) [3]. It has also been reported that the relationship between insect development and temperature has been well-established (Higley & Peterson, 1994) [16]. The 1st instar larvae lack functioning mouth parts, and then the body increases in size to produce a majority of the digestive enzymes that liquefy a large amount of tissue compared to the 1st instar. Day & Wallman (2006) [10] reported that at 24°C, *L. cuprina* took an average time of 48 hrs to reach the 1st ecdysis, 72 hrs to reach the 2nd ecdysis, 96 hrs to begin wandering, and 144 hrs to begin pupation. Nigam *et al.* (2010) [22] reported that they produce secretions with digestive enzymes that aid in breaking down their food. During pupation, the larvae create multiple layers of chitin that form a hardened outer shell. Pupae are elongated oval capsules with tough brown skin. The pupa does not feed, but the pupal casing becomes darker over time to change from rice-like larvae into adult flies. The adults possess large, distinct reddish-brown eyes.

The status of forensic entomology in India is quite promising, and it is important to emphasize this field for future researchers. Forensic entomology plays a pivotal role in criminal investigations, utilizing insects and their evidence to estimate the postmortem interval (PMI) and other critical aspects. They are likely the most important insects, not only because they occur in large numbers but also because they are among the earliest groups to locate corpses. They are highly beneficial insects in the decomposition process, making them invaluable resources in the field of forensic science.

Table 1: Date-wise observation of different developmental stages of *L. cuprina*

Developmental stages		Size (in mm)	Date of collection	Time of collection (AM/PM)	Temperature (°C)
Larval stages	Stage 1	1mm	17-03-2024	06:45 PM	28°C
	Stage 2	2.1mm	18-03-2024	12:05 PM	31°C
	Stage 3	2.8mm	19-03-2024	10:15 AM	24°C
	Stage 4	5mm	19-03-2024	02:00 PM	33°C
	Stage 5	6mm	20-03-2024	09:00 PM	26°C
Pupal stages	Stage 1	7mm	22-03-2024	09:45 AM	22°C
	Stage 2	6.5-7mm	22-03-2024	02:00 PM	33°C
	Stage 3	8.9mm	22-03-2024	09:00 PM	27°C
Adult stage	Adult	9-10mm	24-03-2024	01:45 PM	32°C

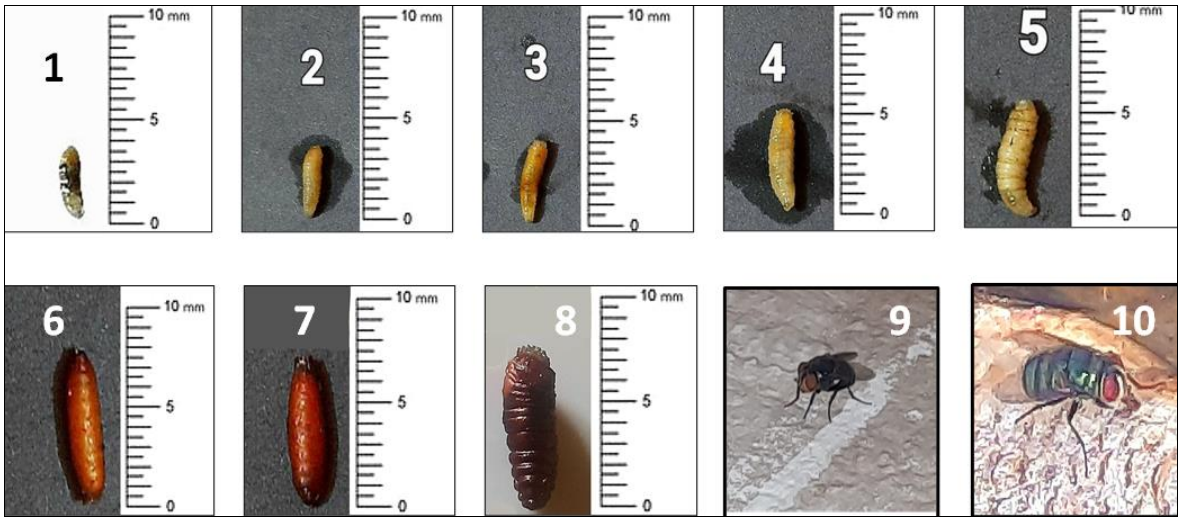

Fig 1: Showing the cryptic information about the developmental stages of *L. cuprina* recorded from the experimental setup (Plate 1-5: 1st, 2nd, 3rd, 4th, and 5th instar larva respectively); Plate 6-8: Pupal stages); (Plate 9 & 10: Adult females)

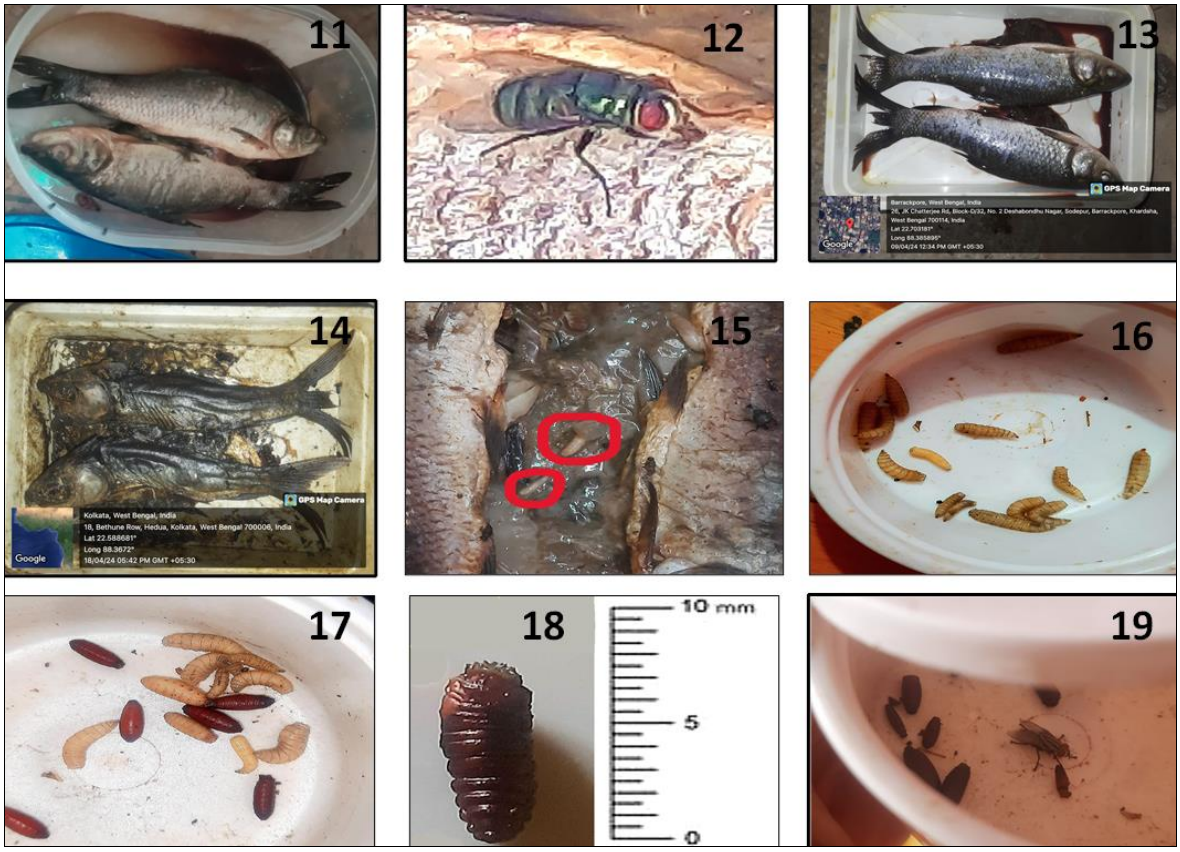

Fig 2: (Plate 11 & 12: Blowfly interaction with the opening surface of Rohu carp); (Plate 13: The initial stage of decomposition); (Plate 14 & 15: Last skeletal stages of decomposition with ample maggot infestations); (Plate 16: The collection of the instar (maggots) from the Rohu carp and placing it in a sterilized container); (Plate 17: Within 24-48 hrs the instar soon initiate moulting showing the reddish brown colour indicate the pupation stage of the blowfly); (Plate 18: Pupal casing becomes darker over time); (Plate 19:The emergence of adult female blowfly).

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