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Original Article

Chrysomya rufifacies: Developmental Analysis Over Range of Temperature

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ABSTRACT

Forensic entomology deals with the study of insects used in criminal investigations. Insects are drawn from the beginning to the decaying carcass and deposit their eggs. Forensic investigators can easily identify the cause of death, the postmortem index, and any changes in the posture of the dead body by observing the insect larval growth and population. **Objective:** To increase students' interest in pursuing careers related to forensic entomology, this study aims to determine the usefulness and viability of forensic entomology in Pakistan. This also creates an understanding of insects' lifestyles, awareness about beneficial insects, and the development of critical thinking. Forensic entomology plays a role in situations where the body has been dead for a while. **Methods:** The Maggot Blow Fly (*Chrysomya rufifacies*) was studied to determine the time since death using insects as evidence. The research at the University of Punjab, Lahore, Pakistan focused on the fly's life cycle and development under different temperature conditions. The aim was to establish the accuracy of using the fly and its life cycle to estimate postmortem interval. **Results:** The following conditions were observed: temperature (humid) 19°C-23°C and cold temperature (dry) 16°C-22°C. Room temperature includes Cold Temperature (Humid) at 23°C -28°C and Dry at 22°C - 26°C. **Conclusions:** *Chrysomya rufifacies* employed in this study have shown that the growth cycle is affected by changes in temperature, and these characteristics may be used to predict the minimal period of death.

INTRODUCTION

Forensic entomology is a field of forensic science that involves the study of insects in relation to legal investigations [1]. The use of insects in forensic investigations dates back to ancient China, where flies and maggots were used to determine the time of death of a corpse. However, the modern application of forensic entomology began in the early 20th century with the work of French entomologists [2]. Forensic entomology has many applications in criminal investigations, including determining the time of death, identifying the location of a crime scene, and establishing the presence of drugs or

toxins in a body [3]. Insects can provide valuable information about a crime scene, as they can be attracted to and feed on decomposing bodies, and their life cycles are relatively predictable and well-studied. Moreover, to find out the postmortem interval, different insects that feed, grow, and feed on dead bodies provide a valid specimen for toxicological investigations [4, 5]. One of the most important applications of forensic entomology is determining the time of death. This is done by examining the insect activity on a corpse, which can estimate the time since death. The insects attracted to a corpse can be

classified into primary, secondary, and tertiary colonizers. Primary colonizers, such as blowflies and flesh flies, are the first insects to colonize a corpse and are most useful in determining the time of death [6, 7]. Secondary colonizers, such as beetles and mites, arrive after the primary colonizers and can also provide information about the time since death. Tertiary colonizers, such as rodents and birds, are attracted to a corpse after it has been decomposing for some time and are less useful in determining the time of death. [8, 9]. The life cycle of insects is also important in forensic investigations. The stages of insect development, including egg, larva, pupa, and adult, are relatively predictable and can be used to determine the time since the colonization of a corpse [10, 11]. The temperature and humidity of the environment can also affect the rate of insect development, which must be considered when estimating the time since death [12]. Forensic entomology can also be used to identify the location of a crime scene. Insects found on a body can be used to determine where the body was located before death [13]. For example, if a body is found in a forest, the type of insects found on the body can indicate whether the body was moved to the forest after death or whether the person died in the forest. Forensic entomology can be used to establish the presence of drugs or toxins in a body. [14, 15]. Some insects are attracted to certain drugs or toxins and can accumulate them in their bodies. By analyzing the insects found on a body, forensic scientists can determine whether the person was exposed to certain drugs or toxins [16]. The accuracy of the time of death estimate can be affected by many factors, including the presence of drugs or toxins in the body, the temperature and humidity of the environment, and the presence of other animals that may interfere with insect activity [17]. In addition, the accuracy of the time of death estimate decreases as the time since death increases and insect activity becomes more complex and unpredictable [18].

METHODS

Meat from the beef shop is collected randomly. About 3 kg of beef was collected, and the flies were cared for until they were fully grown. Some portions of meat are kept in an open environment, subject to the collection and growth of flies, at the Centre of Applied Molecular Biology, University of The Punjab, Lahore. After this, flies were collected using fine nets from the prescribed place and kept in a box in the laboratory. Different flies have shown attraction to this exposed meat in the environment. Maggot Blow Fly (*Chrysomya rufifacies*) was part of this study. These flies were placed inside the jar along with beef meat. All these flies are reared in different conditions. Jars filled with flies were under observation at different temperature

conditions, like cool temperature, cold temperature (humid) 19°C–23°C, and cold temperature (dry) 16°C–22°C. Room temperature includes humid conditions at 19°C–23°C as well as dry conditions at 16°C–22°C. Observations were carefully noted down daily. Counting larvae at first appearance was done until the larval stage converted into the pupal stage. Insect Collection Kit, including the fly's stage. Insects for collecting larvae, bottles, plastic boxes, and cups for specimens. A thermometer is used for measuring temperature, a camera for taking pictures, and a log book. Figure 1 [19, 20].

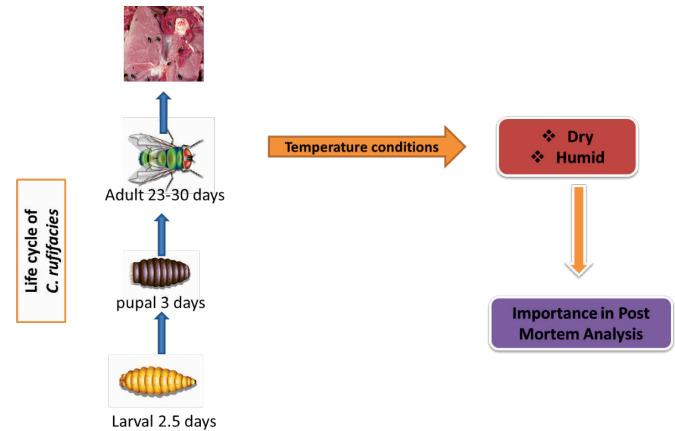


Figure 1: Shows the growth of *Chrysomya rufifacies* species on the meat

RESULTS

Flies like *Chrysomya rufifacies* are most commonly used in forensic entomology. Various effects of temperature significantly affect the growth and development of flies [16]. However, closer examination has resulted in the speeds up of larval growth to increasing temperature and developmental rate of the pupal stage. At cold temperature (Dry), 16 °C–22 °C, the insect collection jar consisted of mature flies and was placed at room temperature on October 21, 2022 (Table 1). The eggs were discovered to have been deposited on October 24, 2022. The first instar larva was spotted on the third day following incubation. After 4 hours, point counting was completed. After completing 78 hours, the reading included a larva and pupa (Figure 2).

Table 1: Day-to-day observation of the flies at cold temperatures (Dry), 16°C–22°C

Observing Date	Day-to-Day Readings of <i>Chrysomya rufifacies</i>
21 October, 2022	5 flies placed in a jar
22 October, 2022	No activity by 4 Flies, but 1 Dead
23 October, 2022	2 Adult flies dead
24 October, 2022	Eggs laid; 1 fly got dead
25 October, 2022	Death of 1st Adult fly, 1st instar (1.95mm)
26 October, 2022	Growth of Second Instar 8.5 mm
27 October, 2022	16 mm growth of third instar
28 October, 2022	Pupal Stage

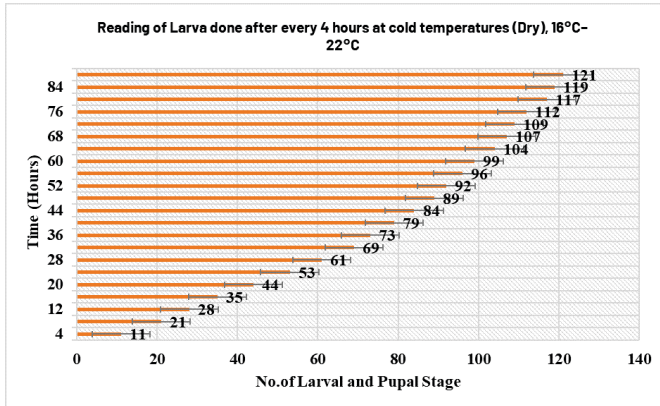


Figure 2: Reading of Larva done after every 4 hours at cold temperatures (Dry), 16°C-22°C

At cold temperature (Humid) 19 °C -23 °C, the insect collection jar consisted of mature flies and was placed at room temperature on November 5, 2022 (Table 2). The eggs were discovered to have been deposited on November 7, 2022. The first instar larva was spotted on the third day following incubation. After 4 hours, point counting was completed. After completing 78 hours, the reading included a larva and pupa. The final reading indicated the pupal stage at 84 hours (Figure 3).

Table 2: Day-to-day observation of the flies

Observing Date	Day-to-Day Readings at cold temperature (Humid) 19 °C -23 °C
5 November 2022	5 flies were placed in a jar
6 November 2022	No activity by 4 Flies, but 1 Dead
7 November 2022	Eggs laid, 2 Adult flies dead
8 November 2022	2 fly dead, the first instar about 2mm
9 November 2022	The second instar is about 7mm.
10 November 2022	The third instar is about 16 mm
11 November 2022	Pupal stage

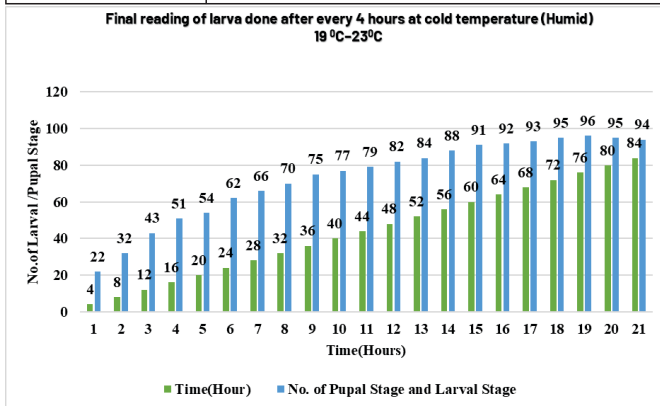


Figure 3: Final reading of larva done after every 4 hours at cold temperature (Humid) 19°C-23°C

The insect collection jar consisted of mature flies and was placed at room temperature Cold Temperature Dry at 22°C -26°C on November 5, 2022. The eggs were discovered to have been deposited on November 10, 2022 (Table 4). The

first instar larva was spotted on the sixth day following incubation. After 4 hours, point counting was completed. After the completion of ninety hours, the reading included a larva and pupa. The final reading indicated the pupal stage at 102 hours (Figure 4).

Table 3: Day-to-day observation of the flies

Observing Date	Day-to-Day Reading
5 th November 2022	4 flies were placed in a jar
6 th November 2022	No activity
7 th November 2022	2 flies dead: No Activity shown
8 th November 2022	Eggs laid; no activity shown
9 th November 2022	2 flies again dead
10 th November 2022	First instar about 1.9 mm
11 th November 2022	The second instar is about 6.5 mm.
12 th November 2022	The second instar grows up to 12 mm.
13 th November 2022	Third instar up to 16 mm
14 th November 2022	Pupal stage

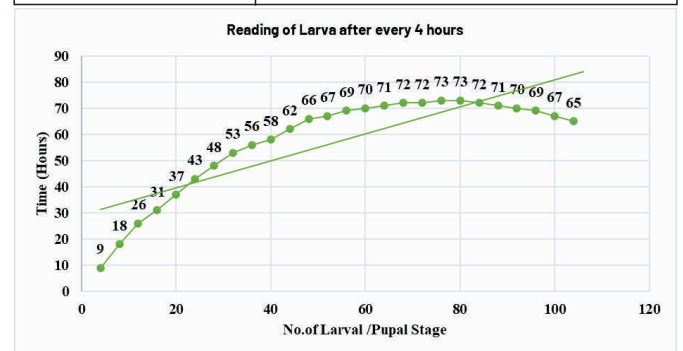


Figure 4: Final reading of the larva done after every 4 hours

The insect collection jar consisted of mature flies and was placed in cold temperature dry at 22°C -26°C on November 5, 2022. The eggs were discovered to have been deposited on November 10, 2022 (Table 5). The first instar larva was spotted on the sixth day following incubation. After 4 hours, point counting was completed. After ninety hours, the reading included a larva and pupa. The final reading indicated the pupal stage at 96 (Figure 5) [15].

Table 5: Day-to-day observation of the flies at cold temperatures dry at 22 °C -26 °C

Observing Date	Day-to-Day Reading
5 th November 2022	5 flies were placed in a jar
6 th November 2022	No activity was shown; one fly died.
7 th November 2022	One fly is dead, and no activity is shown.
8 th November 2022	No activity shown
9 th November 2022	Laying of eggs, 2 flies again dead.
10 th November 2022	First instar about 2.9 mm
11 th November 2022	The second instar is about 8.5 mm.
12 th November 2022	The second instar grows up to 13 mm.
13 th November 2022	Third instar up to 16 mm
14 th November 2022	Pupal stage

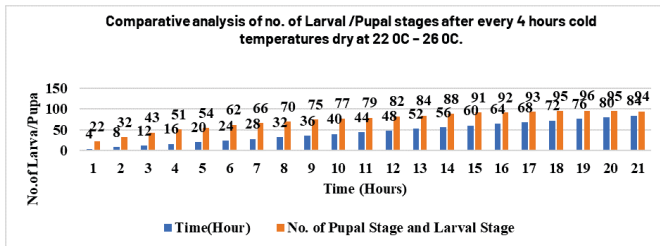


Figure 5: Comparative analysis of no. of Larval/Pupal stages after every 4 hours cold temperatures dry at 22°C-26°C

DISCUSSION

One of the primary determinants in estimating the time of death is the growth progression of insects such as flies. In field scenarios, various temperature conditions have significantly influenced the size and development of instars in *Chrysomya rufifacies* [21]. The impact of temperature on insect life cycles has been extensively studied, as evidenced by multiple investigations [22, 23, 24]. Insects such as *P. sericata*, *P. regina*, *Chrysomya rufifacies*, and *Cochliomyia macellaria* developed at slightly different rates under varying temperatures compared to constant conditions. Distinct temperature effects on these flies have been documented, indicating pronounced impacts on the lifecycle and developmental pace within nine genera of the Calliphoridae family [24, 26, 27]. From a Pakistani forensic entomology perspective, insects, especially blowflies from the Calliphoridae family, serve as significant indicators [28, 29]. A previous study examined entomological data from 54 human cadavers across different seasons, revealing that *Calliphora vicina* was only present during winter and spring [30, 31]. In contrast, *Chrysomya megacephala* and *Chrysomya rufifacies* displayed growth throughout the year, with degradation timelines influenced by climatic factors and mirroring seasonal temperature fluctuations [32]. Climatic conditions, including rainfall and warmth, played a pivotal role in the decomposition rate, with rain and warm conditions accelerating the process. At the same time, winter temperatures and cold weather slowed it down by retarding dipterous larval growth [33, 34]. Given the complex interplay of environmental factors, calculating the postmortem interval (PMI) necessitates meticulous consideration of temperature variations. Insect development is significantly impacted by ambient temperatures, with higher temperatures leading to accelerated growth [37]. Cold weather has been shown to delay or even prevent hatching, while warmer conditions expedite the process. Incorporating environmental variables such as geographical location, temperature, and meteorological conditions in forensic literature databases is imperative [37,38]. Moreover, investigating these environments could yield intriguing results considering

that forensic examinations often encompass indoor settings. The environment's temperature plays a crucial role in insect development, with elevated temperatures corresponding to accelerated growth rates [39].

CONCLUSIONS

This study aims to enhance students' understanding of insects' lifestyles, forensic entomology, and critical thinking. The study found that temperature and humidity significantly impact the growth and development of blowflies, leading to more offspring. The study also found that humidity impacts the colonization cycle, with blow flies thriving in environments with high temperatures and humidity levels. The course of study used insects in forensic investigations as evidence and serve as a model for similar nationwide studies. The findings also influence the calculation of the precise postmortem interval (PMI) during investigations. The study demonstrates that variations in temperature and humidity significantly impact larval stages' development patterns and that environmental factors and medication use also impact the growth and colonization of *Chrysomya rufifacies*.

Authors Contribution

Conceptualization: AI, RF, MM¹, MM², SA

Methodology: RF, MA

Formal Analysis: SA

Writing-review and editing: SA, MW, MA, RM, FY

Author have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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