

CALLIPHORA VICINA AS A FORENSIC BIOINDICATOR: INTEGRATING DEVELOPMENTAL BIOLOGY, THERMAL MODELING, AND MOLECULAR TOOLS FOR ACCURATE POSTMORTEM INTERVAL ESTIMATION

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ABSTRACT

In forensic investigations, *Calliphora vicina*, a member of the Calliphoridae family, is essential, especially when estimating post-mortem intervals (PMI). Because of its consistent growth stages, *C. vicina*, one of the first insects to colonize decaying remains, provides important chronological markers. It is particularly useful in forensic casework because of its durability in a variety of environmental circumstances, preference for cooler climates, and comprehensive developmental schedules. Its dependability in forensic applications has been further improved by recent developments in temperature-based development modeling and molecular identification. Accurate forensic interpretations depend on an understanding of the biology, ecology, and developmental factors of *C. vicina*, which remains a primary focus of forensic entomology research.

Keywords: *Calliphora vicina*, Postmortem interval estimation PMI, Forensic Entomology, Larval development, Temperature dependent growth.

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INTRODUCTION

The blue bottle fly, or *Calliphora vicina*, is a forensically relevant species of blowfly that is frequently used to estimate the post-mortem interval (PMI) [1]. Its existence, life stages, and behavior offer vital hints for forensic investigations because it was among the first to colonize decaying remains. Early arrival at carcasses, tolerance for lower temperatures (activity thresholds as low as 13–16 °C), and consistent development patterns under a range of environmental variables make this species especially desirable. Beyond PMI estimate, *Calliphora vicina* has forensic significance. It is an important species in forensic entomology because of its interactions with microbial communities on decomposing tissue, reaction to toxicological chemicals, and climate tolerance. Furthermore, studies have shown that the presence of bacteria

(such as *Staphylococcus aureus* and *Escherichia coli*) can affect the growth of maggots, underscoring the necessity of examining these interactions for precise forensic interpretations. This study examines the development of the Calliphoridae family on two organs that differ in their biochemical makeup but are commonly exposed in suspicious death cases. The first targets of necrophagous insects are humid tissues, such as the mouth, nose, eyes, ears, genitalia, etc.[2]. Because the sampling of specimens for the entomological study does not require movement, transportation, or any other action that could change or de-nature a forensic inquiry, pig tongues and eyes were selected. The pig's eye and the human eye are comparable. The conjunctiva, eyelids, and external mucus create a moist environment that is ideal for the growth of necrophagous insect larvae. Its thin

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wall facilitates penetration and permits the removal of the vitreous humor within the eye, which helps to maintain the nutrition and humidity necessary for the growth of necrophagous insects. In terms of calories, the tongue has 271 calories and the eye has 60 calories for every 100 grams of body weight. From a biological perspective, the pig eye has 27 g of proteins, 2 g of lipids, and the remaining portion is blood plasma. The pig tongue has 64 g of proteins, 18.6 g of lipids, and 0 carbs. In order to propose possible directions of data use in forensic expertise in the calculation of the post-mortem interval, the goal of this work is to gather preliminary data regarding the development, under laboratory conditions, of *Calliphora vicina* on two nutritional substrates (pig's tongue and eye), which differ in terms of anatomical structure and biochemical composition/nutrients.

BIOLOGY OF CALLIPHORA VICINA

Calliphora vicina can be identified by its ruby eyes, black thorax with whitish hairs, and metallic blue abdomen [3]. Although adults, which range in length from 6 to 12 mm, are frequently mistaken for other species of blow flies, accurate morphological or genetic identification is essential in forensic settings.

Table 1: Taxonomy of *Calliphora vicina*.

Taxonomy	
Kingdom	Animalia.
Phylum	Arthropoda
Category	Insecta
The Order	Diptera
Family	Calliphoridae
Genus	Calliphora
Species	<i>C. vicina</i>

Morphological Identification

Adults of *Calliphora vicina* have unique characteristics that help distinguish them from related species like as *Lucilia sericata* and *Calliphora vomitoria*.

The fly has a length of 8–14 mm and has unique coloration patterns. In contrast to the duller *Calliphora vomitoria*, the abdomen is a vivid metallic blue with a faint violet sheen, and the thorax is black to dark gray with faint blue specks [4]. In

contrast to *C. vomitoria*, which has black cheeks, a crucial diagnostic characteristic is the orange to reddish-orange cheeks (jowls). The legs are black with bright blue dots, and the wings are clear with somewhat darker leading margins [5].

LIFE CYCLE

Calliphora vicina's (blue bottle fly) life cycle

In forensic entomology, the life cycle of *Calliphora vicina* is essential for calculating the post-mortem interval (PMI). It goes through four separate stages of metamorphosis (holo-metabolism), same like other blowflies: egg, larva (maggot), pupa, and adult. Since each stage's duration varies greatly with temperature, developmental data is crucial for PMI computations [6].

The Egg Stage

It Lasts for 12 to 24 hours. The little (1.5–2 mm), oval, creamy white eggs are usually laid in bunches on young carcasses, especially close to wounds or natural openings like the mouth, nose, or eyes. Temperature affects hatching time: eggs hatch in 12–18 hours at optimal temperatures (20–25°C), but it might take up to 24–48 hours at lower temperatures (10–15°C).

Larval Stage

The larvae feed on decomposing tissue and go through three instars during a period of 3–10 days. Worm-like, transparent larvae with tiny mouth hooks for eating are produced during the first instar, which lasts for around a day. They reach 5–10 mm in the second instar (1-2 days), when their cream-colored tracheal trunks become more noticeable and they have more movement. The last and biggest larval stage prior to pupation is the third instar, which lasts for two to seven days. With a length of 10–17 mm, the third instar is the largest larval stage. The posterior spiracles, or breathing organs, are a crucial trait of this stage because they have a particular three-slit pattern that is crucial for species identification. The larvae go through a wandering phase as they get ready to pupate, during which they cease eating and move away from the body in search of a suitable place to mature into pupae. This behavior ensures the right conditions for metamorphosis while assisting them in avoiding competition and predation.

Table 2: Comparative morphology of forensically important blowflies.

Features	<i>Calliphora vicina</i>	<i>Calliphora vomitoria</i>	<i>Lucilia sericata</i>
Cheek Color	Reddish-orange	Black	Pale or yellowish
Abdomen Color	Bright metallic blue	Dull blue/green	Brilliant green
Size (Length)	8–14 mm	10–14 mm	6–10 mm
Thorax	Dark gray with bluish tint	Dark gray	Golden-green sheen
Activity Threshold	Active at 13–16°C	Less cold-tolerant	Prefers warmer climates
Forensic Importance	Early colonizer in cool climates	Slower development in cold	Common in summer cases

Table 3: Larval development duration of *Calliphora vicina* at various temperatures.

Temperature (°C)	Total Larval Development Time (Days)
10°C	10–14 days
15°C	7–10 days
20°C	4–6 days
25°C	3–4 days
30°C	2–3 days (may have reduced survival)

Pupal Stage (Seven to Fourteen Days)

The mature larvae change into dark brown, barrel-shaped puparia during pupation, which are frequently concealed in dry, protected areas close to the decaying cadaver or buried in dirt. The larva transforms into an adult fly by rearranging its tissues inside the protective puparial shell. Temperature affects how long this stage takes; at 20°C, it takes 10–12 days, but at 25°C, it takes 7–9 days.

Stage of Adulthood (Emergence and Reproduction)

When the fly reaches adulthood, it breaks out of the puparium, hardens its exoskeleton, and spreads its wings. Rapid sexual maturity is attained, and mating usually starts three to five days following emergence. In the wild, adult *Calliphora vicina* can live for two to four weeks, during which time females can produce several batches of 150 to 200 eggs each, allowing the population to grow quickly. They are essential to decomposition ecology and forensic investigations because of their high reproductive rate and predilection for fresh carrion.

Figure 1.1 Life cycle of *Calliphora vicina*

FORENSIC RELEVANCE OF LIFE CYCLE (POSTMORTEM ESTIMATION)

In forensic investigations, the life cycle of the blue bottle fly, *Calliphora vicina*, offers vital hints for calculating the postmortem interval (PMI) [7]. Fresh egg clusters (1.5–2 mm, creamy white) discovered in body holes or wounds suggest that death happened within the last 0–24 hours. Hatching starts 12–18 hours later at 20–25°C, or up to 48 hours later in colder climates. Usually, 7–10 days after death, the larvae enter the wandering phase, during which they depart the body to pupate. The presence of new egg-laying adults may imply recolonization and a longer PMI, whereas empty puparia indicate adult emergence and a minimum PMI of roughly 14 days [1]. Environmental elements such as temperature (warmth speeds up development) and location (interior corpses attract flies later than outdoor ones) must be taken into account in these estimations. A PMI of 10–14 days, for instance, might be suggested by the discovery of third instar larvae (15 mm) at 20°C with pupae close by (7 days larval development plus 7 days pupation). Even in winter, *C. vicina* is especially useful for PMI estimation due to its cold tolerance [8].

LABORATORY METHOD FOR ESTIMATING PMI

Using *Calliphora vicina* to estimate postmortem interval (PMI) requires careful specimen collection

and regulated rearing procedures [1]. In order to gather adult flies for possible DNA analysis, field collection starts with baited traps filled with decaying meat or chicken put close to crime scenes. The flies are then frozen or stored in 70% ethanol. Adults are kept in mesh cages with a 12:12 light-dark cycle, 25°C, 60–70% humidity, and food that includes sugar and water, as well as liver or meat to encourage oviposition, in order to maintain the colony [9]. The first step in monitoring the development of eggs and larvae is to place gravid females with fresh tissue (such as pork liver) in containers and meticulously record the time it takes for them to lay eggs ($t=0$ for PMI calculations). After that, the eggs and larvae are raised in environmental chambers at regulated temperatures (such as 20°C or 25°C). Under 25°C settings, hatching times are usually observed around 12 to 24 hours. In order to measure body length and inspect posterior spiracles, 10 specimens per developmental stage are sacrificed at predetermined intervals (e.g., every 5 hours) during routine larval sampling [1]. The distinctive three-slit pattern is used to validate the identification of *C. vicina* species. Mature larvae in the wandering phase are moved to dry vermiculite to start pupation in order to conduct investigations on pupation and adult emergence. The pupariation period is regularly monitored by researchers; dark brown puparia usually appear 3–10 days after oviposition. Empty puparia, which indicate a minimum PMI of roughly 14 days, are essential indicators of adult emergence, and the pupal stage duration is meticulously documented (usually 7–9 days at 25°C). Accurate PMI calculation in forensic investigations requires exact documentation of the full life cycle under controlled laboratory circumstances, which is made possible by our all-inclusive technique. Researchers use degree-day (ADD) or degree-hour (ADH) models, which use the formula to quantify accumulated thermal units.

$$ADH = (\text{temperature} - \text{threshold temperature}) \times \text{time.}$$

$$ADH = \sum (\text{Temperature} - \text{Threshold temp.}) \times \text{Time}$$

(the threshold for *C. vicina* is about 10°C) [10].

By taking into consideration microclimate changes like burial circumstances or indoor versus outdoor exposure that may alter insect growth rates, these thermal accumulation models are validated by comparing development rates of specimens collected in the field with those raised in laboratories. In order to guarantee precise species

identification, which is essential for trustworthy PMI measurement, quality control procedures combine morphological and molecular techniques. While microscopic analysis of important morphological traits (such cheek coloration and spiracle morphology) provides complementary verification, DNA barcoding directed at the cytochrome c oxidase subunit I (COI) gene enables conclusive species confirmation [11]. Researchers use strong modeling techniques, such as maximum likelihood estimation and linear regression, to statistically analyze developmental data and produce accurate PMI forecasts.

CONCLUSION

In forensic entomology, the blue bottle fly (*Calliphora vicina*) is an essential biological indicator that provides accurate postmortem interval (PMI) estimations due to its regular embryonic stages and growth patterns that are reliant on temperature. Forensic investigations favor this species because to its early colonization of remains, cold tolerance, and unique physical characteristics (such as reddish-orange cheeks and a metallic blue abdomen). In order to further improve PMI

estimations, controlled laboratory research employing substrates such as pig tongue and eye show how nutritional composition and microclimate affect larval development. Thermal accumulation models (ADH/ADD), DNA barcoding for species confirmation, and statistical studies to take environmental factors into account are important techniques. By integrating these approaches, forensic entomologists can provide accurate PMI estimates, even in challenging conditions. Future research should explore interactions between *C. vicina* and microbial communities, as well as the impact of toxins on development rates, to enhance forensic interpretations.

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REFERENCE

1. Brown K, Thorne A, Harvey M. *Calliphora vicina* (Diptera: Calliphoridae) pupae: a timeline of external morphological development and a new age and PMI estimation tool. *International journal of legal medicine*. 2015 Jul;129(4):835-50. DOI: 10.1007/s00414-014-1068-z
2. Obafunwa JO, Roe A, Higley L. A review of the estimation of postmortem interval using forensic entomology. *Medicine, Science and the Law*. 2025 Jan;65(1):52-64. <https://doi.org/10.1177/00258024241275893>
3. Talha Arshad, Muhammad Naeem. Forensic entomology as an advanced tool in crime investigation. *Forensic Insights and Health Sciences Bulletin*, 2023;1(2):50-4. <https://doi.org/10.56770/fi2023114>.
4. Morris GA, Considine DB, Dessler AE, Kawa SR, Kumer J, Mergenthaler J, Roche A, Russell III JM. Nitrogen partitioning in the middle stratosphere as observed by the Upper Atmosphere Research Satellite. *Journal of Geophysical Research: Atmospheres*. 1997 Apr 20;102(D7):8955-65. <https://doi.org/10.1029/97JD00073>
5. Mukhopadhyay N, Jain D, Tripathi A, B AK, Bhaskar P. A comprehensive insight into the pharmaceutical potential of *saussurea obvallata*. *Current Pharmacology Reports*. 2024 Dec;10(6):349-59. DOI: 10.1007/s40495-024-00370-2
6. Salanitro LB, Massaccesi AC, Urbisaglia S, Peria ME, Centeno ND, Chirino MG. *Calliphora vicina* (Diptera: Calliphoridae): Growth rates, body length differences, and implications for the minimum post-mortem interval estimation.
7. Abbasi E. A review of the advances in insect evidence analysis for estimating postmortem interval and detecting drug toxicity. *Journal of Forensic and Legal Medicine*. 2025 Dec 30:103059. <https://doi.org/10.1016/j.jflm.2025.103059>
8. López-García J, Martín-Vega D. Survival of *Calliphora vicina* (Diptera: Calliphoridae) embryos under cold temperature conditions: forensic implications. *Medical and Veterinary Entomology*. 2025 Mar;39(1):33-40. <https://doi.org/10.1111/mve.12749>
9. Day DM. Development of immature blowflies and their application to forensic science (Doctoral dissertation, University of Wollongong).
10. Donovan SE, Hall MJ, Turner BD, Moncrieff CB. Larval growth rates of the blowfly, *Calliphora vicina*, over a range of temperatures. *Medical and veterinary entomology*. 2006 Mar;20(1):106-14. <https://doi.org/10.1111/j.1365-915.2006.00600.x>
11. Rolo EA, Oliveira AR, Dourado CG, Farinha A, Rebelo MT, Dias D. Identification of sarcosaprophagous Diptera species through DNA barcoding in wildlife forensics. *Forensic Science International*. 2013 May 10;228(1-3):160-4. <https://doi.org/10.1016/j.forsciint.2013.02.038>