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Original article

The Effect of Temperature on the Accuracy of Postmortem Interval Estimation Based on the Larval Growth of the Green Bottle Fly *Lucilia sericata* Using Isomegalen Diagrams

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Abstract
Forensic entomology provides essential tools for estimating the post-mortem interval (PMI), particularly when traditional pathological methods are limited. This study investigates the larval development of *Lucilia sericata* (green bottle fly) under realistic environmental conditions to evaluate the influence of temperature on PMI estimation. Two rabbit carcasses were monitored during February 2025, with daily temperatures ranging from 14.65°C to 15.5°C. Larvae were collected, measured, and analyzed using isomegalen charts to determine developmental durations. Results indicated that even a minor difference of 1°C between sampling periods significantly affected total larval development, with Sample A requiring 235 hours and Sample B 198 hours to reach maximum lengths. The hatching period remained relatively stable (30–32 hours), suggesting early developmental stages are less sensitive to small temperature variations. A clear difference was observed between temperature conditions based on larval growth patterns derived from the isomegalen chart. The findings indicate that relying on local temperature data, larval measurements, and population-specific developmental references plays a crucial role in improving the accuracy of PMI estimation. Incorporating precise environmental and experimental data enhances the reliability of forensic entomology as an effective tool in legal investigations.
Keywords. Forensic Entomology, *Lucilia Sericata,* Isomegalen Diagram**,** Postmortem Interval, Temperature Effects.

Introduction

Forensic entomology is a pivotal discipline in contemporary criminal investigations, providing reliable scientific tools for estimating the post-mortem interval (PMI), particularly when traditional pathological approaches reach their [1-3]. This field utilizes the analysis of insect colonization patterns and developmental rates on decomposing remains, enabling precise estimation of the minimum post-mortem interval (minPMI). Among carrion-associated insects, blow flies of the family Calliphoridae, especially *Lucilia sericata* (green bottle fly), are recognized as primary colonizers of decomposing bodies [4,5]. Their rapid and predictable development, coupled with the ease of identifying life stages, renders their eggs, larvae, and pupae critical biological indicators for PMI estimation. These developmental stages can be systematically analyzed using life-cycle tables, isomegalen and isomorphen diagrams, and accumulated degree day/hour (ADD/ADH) models [6- 8].

Development of *L. sericata* is influenced by several environmental factors, with temperature being the most crucial determinant of growth rate and developmental [9,10,8]. Elevated temperatures generally accelerate larval and pupal development, resulting in shorter developmental periods, whereas lower temperatures retard growth and may prolong minPMI estimations [6,7]. Other factors, including humidity, larval density, and substrate type, also significantly modulate growth rates and survival [11-13]. Recent studies have demonstrated that developmental rates and durations of *L. sericata* can vary considerably among geographically distinct populations, even under comparable thermal conditions. These population-specific differences highlight that reliance on generalized developmental data may introduce errors in forensic estimations, emphasizing the necessity of integrating region-specific datasets into forensic models [14,15].

Despite extensive research on the life cycle of *L. sericata*, knowledge gaps persist regarding the effects of extreme temperatures and diverse tissue types on larval growth and survival. Recent investigations underscore the importance of combining temperature-dependent developmental data with isomegalen diagrams to enhance PMI estimation accuracy [16,7,8]. Additionally, larval aggregation behavior and its influence on microclimatic temperature and survival represent critical considerations in practical forensic analyses [13,9].

The present study aims to evaluate the effect of temperature on the accuracy of PMI estimation based on *L. sericata* larval growth using isomegalen diagrams. Specifically, it focuses on: (1) documenting larval developmental stages on different rabbit tissue types, (2) analyzing temperature-dependent growth patterns, and (3) providing experimental data to support practical forensic applications. The findings are expected to contribute region-specific developmental data, improve PMI estimation accuracy, and reinforce the forensic relevance of *L. sericata* as a reliable biological indicator.

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Materials and Methods

Materials

The following materials and tools were employed to ensure accurate and scientifically controlled experimental procedures: two medium-sized rabbit carcasses; airtight plastic containers lined internally with plastic sheets to prevent fluid leakage; a natural soil layer to simulate environmental conditions; sterilized forceps; 75% ethanol; airtight glass storage tubes; Petri dishes; paper tissues; sugar and yeast as nutritional substrates for larval rearing; a precision ruler; a notebook and digital camera for documentation; a light microscope; pure acetone; and a paper-based isomegalen chart. The selection and use of these materials followed established protocols commonly applied in forensic entomology research to preserve specimen integrity and ensure accurate developmental measurements [11,12,13,6,1,8].

Methods

The study was conducted during the winter season on February 7 and 17, 2025, in the Aradah area of the Souq Al-Juma neighborhood, located in the northeastern part of Tripoli, Libya, along the Mediterranean coast, approximately 50 m from the shoreline. The geographic coordinates of the study site were recorded at the intersection of longitude and latitude lines (32°52′56.0″N,13°17′31.7″E). Daily ambient temperatures were systematically recorded, as temperature represents a critical factor influencing the developmental rate of *Lucilia sericata* larvae and is fundamental for estimating the minimum post-mortem interval (minPMI) using isomegalen diagrams [10,7].

The rabbit carcasses were carefully prepared and placed individually inside plastic containers lined with internal plastic sheets. A layer of natural soil was added to the bottom of each container to simulate environmental conditions while minimizing fluid loss. Each container was covered with a perforated plastic lid to allow ventilation while preventing access by scavengers. The carcasses were monitored daily, and visible stages of decomposition and insect colonization—particularly by members of the family Calliphoridae—were documented.

Larvae were collected using sterilized forceps to avoid contamination or physical damage. To preserve larval morphology and prevent post-fixation contraction, specimens were briefly immersed in lukewarm water (60–70 °C) before being immediately fixed in 75% ethanol. This procedure follows standard forensic entomology protocols for larval preservation and measurement accuracy [4,5,8].

A subset of live larvae was transferred to Petri dishes lined with moistened paper tissues to maintain humidity. Controlled amounts of sugar and yeast were provided as nutritional sources to support continued larval development. The Petri dishes were maintained near a stable heat source to provide moderate and consistent warmth, facilitating normal larval growth, pupation, and adult emergence. Upon emergence, adult flies were euthanized using pure acetone to ensure rapid death without compromising external morphological characteristics. Adult specimens were then examined microscopically to confirm species identification based on established taxonomic features [9,16].

Larval lengths were measured precisely using calibrated millimeter ruler (precision ±0.01 mm and documented photographically. The collected morphometric data were subsequently analyzed using isomegalen charts to determine the time required for larvae to reach specific lengths at recorded ambient temperatures. This approach enabled the estimation of the minPMI under realistic environmental conditions [6,8]. Overall, the applied methodology integrates the combined effects of temperature, humidity, larval density, and substrate type on larval development and is consistent with both classical and contemporary forensic entomology studies [10-13] [1,7].

Results

The growth of *Lucilia sericata* larvae was analyzed using an isomegalen diagram to estimate developmental times under realistic environmental conditions.

Sample A (7–16 February 2025)

The daily mean temperature during the collection period averaged approximately 14.65°C, rounded to 15°C for standardized analysis, as shown in (Table 1).

Table 1. Daily mean temperatures for Sample A

Date	Temperature (Min–Max, °C)	Daily Mean (°C)
7 Feb	13–18	15.5
8 Feb	12–15	13.5
9 Feb	12–16	14.0
10 Feb	11–17	14.0
11 Feb	10–17	14.0
12 Feb	9–20	14.5
13 Feb	10–19	14.5
14 Feb	13–18	15.5

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15 Feb	13–20	16.5
16 Feb	11–18	14.5
Overall Mean	—	14.65 ≈ 15

Different larval lengths were used to analyze growth according to the isomegalen diagram, as summarized in (Table 2).

Table 2. *Lucilia sericata* larval growth data for Sample A

Larval Length (mm)	Larval Stage	Egg Laying Time	Larval Development	Total (hours)
4	Second	32 h	2 days 11 h	91
5	Second	32 h	3 days	104
8	Third	32 h	5 days 9 h	161
9	Third	32 h	5 days 15 h	167
12	Third	32 h	6 days 16 h	192
14	Third	32 h	8 days 11 h	235

Sample B (17–24 February 2025)

The daily mean temperature during the collection period averaged approximately 15.5°C, rounded to 16°C for standardized analysis, as shown in (Table 3).

Table 3. Daily mean temperatures for Sample B

Date	Temperature (Min–Max, °C)	Daily Mean (°C)
17 Feb	12–20	16.0
18 Feb	12–20	16.0
19 Feb	12–20	16.0
20 Feb	11–19	15.0
21 Feb	12–18	15.0
22 Feb	11–19	15.5
23 Feb	12–19	15.5
24 Feb	12–19	15.5
Overall Mean	—	15.5 ≈ 16

Various larval lengths were used to analyze growth according to the isomegalen diagram, as presented in (Table 4).

Table 4. *Lucilia sericata* larval growth data for Sample B

Larval Length (mm)	Larval Stage	Egg Laying Time	Larval Development	Total (hours)
4	Second	30 h	2 days	78
5	Second	30 h	2 days 12 h	90
8	Third	30 h	3 days 12 h	114
12	Third	30 h	6 days	144
13	Third	30 h	6 days 11 h	185
14	Third	30 h	7 days	198

Discussion

The present study provides a comprehensive analysis of the larval development of the green bottle fly, *Lucilia sericata*, using isomegalen charts to estimate the post-mortem interval (PMI) under realistic environmental conditions. The findings clearly demonstrate the pivotal role of ambient temperature in regulating larval growth rates and overall developmental duration, highlighting the necessity of incorporating accurate, site-specific thermal data into forensic entomology models.

A comparison between Sample A (mean temperature ≈ 15°C) and Sample B (mean temperature ≈ 16°C) revealed that a temperature difference of only 1°C resulted in a pronounced reduction in total larval development time. Larvae in Sample A required up to 235 hours to reach maximum recorded lengths, whereas those in Sample B completed development within 198 hours. This observation aligns with previous studies reporting the high sensitivity of *L. sericata* development to thermal variation, even within narrow temperature ranges [6,7,14,17].

Interestingly, the egg hatching period remained relatively stable between the two samples (30–32 hours), suggesting that early developmental stages may be less responsive to minor temperature fluctuations than

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later larval instars. Similar trends have been reported in earlier investigations, indicating that post-hatching growth phases exhibit greater thermal plasticity [10, 16].

From a forensic standpoint, the observed discrepancy of approximately one day in larval development duration has substantial implications for PMI estimation. Even small temporal deviations may critically influence forensic interpretations, particularly in cases involving advanced decomposition or limited corroborative evidence [2,3,18,19]. These findings reinforce the importance of integrating continuous environmental monitoring with morphological and developmental assessments to improve the accuracy of PMI calculations.

The effective application of isomegalen charts in this study further supports their value as reliable tools for estimating larval age when combined with precise ambient temperature data. This approach aligns with classical forensic entomology frameworks emphasizing temperature-dependent developmental models for PMI estimation [6, 4, 5,20].

Moreover, the results highlight the necessity of utilizing locally calibrated developmental datasets. Variations in temperature, substrate type, and rearing conditions may collectively influence larval growth patterns, underscoring the limitations of relying solely on generalized reference data. Recent studies documenting population-specific developmental variability among geographically distinct *L. sericata* populations further support this recommendation [14,15,21,22].

Overall, this study emphasizes that accurate PMI estimation requires a multidisciplinary approach combining meticulous field observations, controlled experimental data, and appropriately selected developmental charts. Neglecting environmental factors or applying non-local reference data may result in under- or overestimation of PMI, potentially affecting the reliability of forensic conclusions [9,13].

Conclusion

The findings reaffirm the fundamental influence of ambient temperature on the larval development of *Lucilia sericata* and highlight the forensic value of integrating local environmental data into entomological analyses. Such an approach enhances the precision of minimum PMI estimation and strengthens the evidentiary contribution of forensic entomology in legal investigations.

Author Contributions

All authors contributed to the study's conception and design. All authors are equal in material preparation, data collection, and analysis. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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