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Research Article

The Influence of Heat Analysis on the Hatching Rate of Lucilia sericata (*Diptera: Calliphoridae*) Eggs Using Polynomial Regression Method

Emre Deymenci[1], M. Erdem Isenkul[2*] and Erdal Polat[3]

[1]*Istanbul University-Cerrahpasa, Institute of Forensic Sciences and Legal Medicine, Istanbul, Türkiye*
[2]*Istanbul University-Cerrahpasa, Faculty of Engineering, Department of Computer Engineering, Istanbul, Türkiye*
[3]*Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, Istanbul, Türkiye*
[*]*Corresponding author*

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Abstract

This study examines the effects of temperature on the hatching rates of *Lucilia sericata*, a species important for forensic entomology and medical biology. Using a polynomial regression approach, we investigated the relationship between extreme thermal conditions and egg emergence rates to provide insights that could enhance applications in postmortem interval (PMI) estimation and ecological modeling. Eggs were subjected to controlled temperature treatments ranging from 0°C to 41°C at 1°C intervals, maintaining 45% relative humidity. Observations were conducted at 24, 48, and 72-hour intervals, with additional incubation at 30°C for those subjected to extreme temperatures. Findings revealed no larval emergence at temperatures below 10°C or above 40°C. However, within a 14°C to 38°C range, hatching rates reached their peak at 28°C (89.7%), indicating optimal developmental conditions. Our polynomial regression model demonstrated a high degree of accuracy ($R^2 > 0.70$) in predicting hatching rates across varying temperatures, thus establishing a predictive framework for forensic and ecological applications. The results emphasize the potential for this thermal modeling approach to enhance forensic entomological practices by providing refined developmental timelines for *L. sericata*, a species widely used in PMI estimation. The findings also underscore the species' ecological sensitivity to temperature fluctuations, which may inform future ecological distribution models.

1. Introduction

Arthropods, comprising approximately 70–80% of Earth's animal species, represent an essential component of biodiversity, with estimates suggesting over 10 million species [1, 2]. Despite the vast number of identified arthropod species, only a fraction holds significance in medicine, veterinary science, and forensic entomology [3]. Within this context, *Lucilia sericata* (Diptera: Calliphoridae), commonly known as the green bottle fly, has garnered substantial interest due to its dual relevance in medical and forensic applications. For instance, the larvae of *L. sericata* are used in Maggot Debridement Therapy (MDT) for treating non-healing wounds [4, 5], while in forensic science, the species is crucial in postmortem interval (PMI) estimation for determining the time since death in legal cases [6–8].
Temperature and humidity are critical factors that significantly influence the life cycles of arthropods, including *L. sericata*. Being ectothermic organisms, their metabolic rates and development processes are directly dependent on environmental conditions [1, 2]. Studies have shown that temperature fluctuations can either accelerate or decelerate the life cycle of *L. sericata*, affecting their development and survival [9, 10]. Wang et al. identified optimal temperature ranges for *L. sericata* development under constant conditions but did not address the effects of abrupt environmental changes or extreme temperatures [11]. Similarly, Hans et al. highlighted how fluctuating temperatures influence

***Email addresses and ORCID numbers*: edeymenci@dogus.edu.tr, 0000-0003-1455-1674 (E. Deymenci), eisenkul@iuc.edu.tr, 0000-0003-0856-2174 (M.E. Isenkul), erdalp@iuc.edu.tr, 0000-0002-3944-6443 (E. Polat)**
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oviposition behavior, underscoring the need for dynamic models [12]. Cervantès et al. examined low-temperature effects on the life cycle but provided limited insights into developmental recovery mechanisms at extreme conditions [13]. These studies collectively emphasize the necessity for more comprehensive models that capture the effects of both low and high thermal extremes. Given the species' importance in forensic contexts, understanding the precise thermal conditions that optimize or hinder its development is essential for accurate PMI estimations and can contribute valuable data for forensic entomology [7, 14].

While existing studies have established general temperature ranges affecting *L. Sericata*'s development, they often lack specificity in modeling the effects of extreme thermal conditions. Most prior research has relied on linear or quadratic models, which inadequately capture the non-linear relationship between temperature and hatching rates, especially at the thermal extremes. Furthermore, there is limited discussion on how abrupt environmental changes influence egg viability, an issue particularly relevant in forensic scenarios. Recent reviews, such as those by Matuszewski [8] and Niederegger et al. [14], have called for refining forensic entomology models to incorporate regional and environmental variability. However, these frameworks often overlook the impact of thermal extremes on developmental timelines, creating a critical gap that our study aims to address. Addressing these gaps is critical for enhancing the accuracy and applicability of forensic entomological models.

2. Materials and Methods

This study was conducted at Istanbul University-Cerrahpasa, Center for Traditional and Complementary Medicine Practice and Research (IU-C, GETAT).

2.1. Preparation of the medium

To cultivate an optimal environment for *Lucilia sericata* egg incubation, a nutrient-rich medium was prepared using sterilized chicken liver. The use of a controlled culture medium, rather than raw tissue, was chosen to maintain consistency across experimental conditions and to minimize potential contamination, which could impact egg development and hatching rates.

The preparation process involved dissolving 20 g of bacteriological agar agar in 650 ml of distilled water, followed by heating until fully dissolved. Subsequently, 500 g of finely blended chicken liver was mixed into the agar solution to ensure homogeneity. This mixture was then divided into 250 ml portions and autoclaved at 121°C for 15 minutes to achieve sterilization. The sterilized culture medium was stored at +4°C until use to preserve its nutrient quality and prevent microbial growth, which could alter the experimental outcomes (9,13). This controlled medium provided a consistent nutrient source for the eggs, allowing for standardized observations of hatching rates across varying temperature treatments.

2.2. Egg collection procedure

The eggs were obtained by utilizing plastic containers placed within 30×30×30 cm cages. These containers were populated with 1000–1100 *Lucilia sericata* flies of the species. Each container contained 25–30 g of chicken liver, selected due to its nutrient richness, promoting consistent egg-laying behavior under controlled conditions, as supported by previous studies on suitable media for *Lucilia sericata* [9, 15]. The flies were allowed to remain in contact with the liver for Figure 2.1-A). Following this period, eggs adhered to the liver surface by the adult flies (Figure 2.1-B) were carefully collected using a soft-bristled brush (Figure 2.1-C). The careful use of a soft-bristled brush minimized potential mechanical damage to the eggs, a step that is critical in studies where egg integrity can influence hatching rates and experimental outcomes.

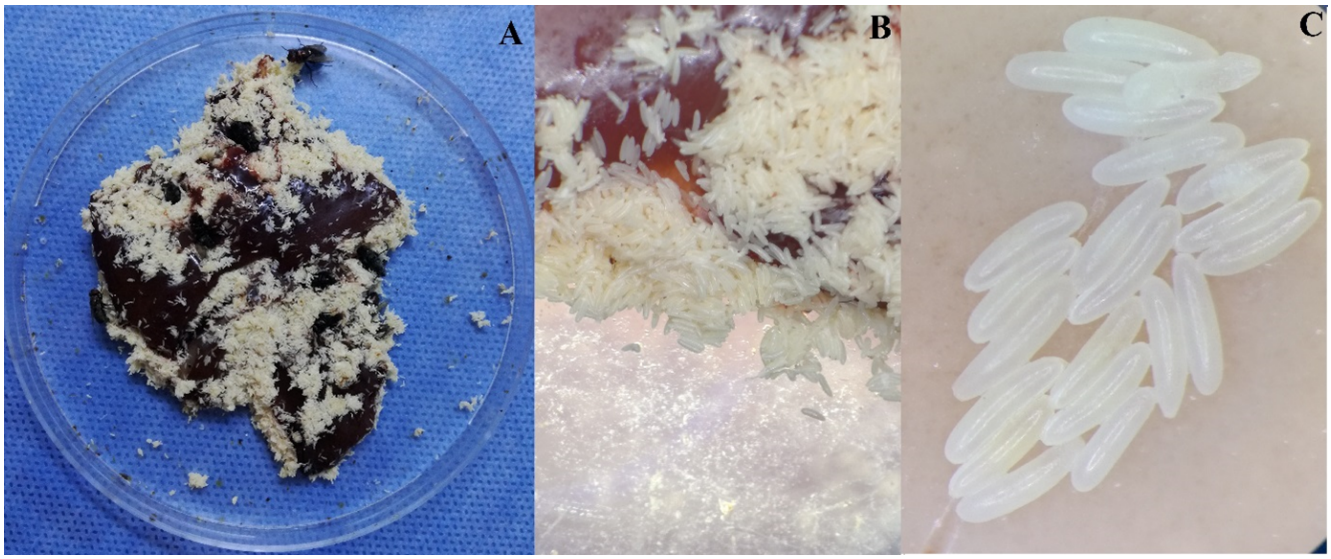

Figure 2.1: Egg collection procedure. (A) Contacted adult flies on the liver for a duration of 3.5–4 hours, (B) Eggs adhered to the liver surface, (C) Carefully collected eggs using a soft-bristled brush.

The collected eggs were transferred into Petri dishes for counting. Under a stereo microscope (Olympus SZX10), 100 eggs were carefully enumerated and placed into individual culture vials for further analysis. To ensure consistency, the culture vials were separated into two groups based on incubation conditions. One group was subjected to a cooling incubator (Elektro-mag® M7040R), while the other was

placed in a regular incubator (Nüve EN 400). This setup allowed for controlled exposure to temperature gradients, ranging from 0°C and incrementally increasing to 41°C, with relative humidity consistently maintained at 45%, aligning with protocols for minimizing environmental variability [9].
Observations were conducted at intervals of 24, 48, and 72 hours after each temperature adjustment. Following the temperature ramping process, the eggs were then transferred to a separate incubator set at a constant temperature of 30°C for 24 hours to evaluate larval emergence rates. This temperature was chosen because 30°C represents the optimal developmental condition for *Lucilia sericata*, as identified in previous studies, ensuring consistent and reliable baseline observations. Each experimental condition was replicated three times to validate the findings. Additionally, a control group maintained in a standard incubator at 30°C with 45% relative humidity served as a baseline for comparing hatching rates [4].

2.3. Temperature calibration and reproducibility

To ensure the reproducibility of the experimental setup, all temperature-controlled incubators were calibrated prior to the experiments using a certified precision thermometer (±0.1°C accuracy). Calibration was repeated weekly throughout the experimental period to verify consistent performance across all trials. Additionally, humidity levels were monitored and maintained using automated controls to eliminate variability (Table 2.1).

Temperature (°C)	Mean (±SD)
5	0.0 ± 0.0
10	0.0 ± 0.0
15	67.0 ± 13.7
20	83.3 ± 5.3
23	83.3 ± 5.3
26	73.7 ± 8.7
29	84.3 ± 2.1
32	85.7 ± 3.4
35	78.3 ± 7.1
38	68.0 ± 9.4
39	7.7 ± 8.8
40	0.0 ± 0.0

Table 2.1: Hatching rates (%) of *Lucilia sericata* eggs at different temperatures (°C). Values are expressed as mean ± standard deviation (SD).

The incubation procedure was standardized by employing the same type of containers, egg densities, and incubation times across all experimental groups. For further validation, each experimental condition was replicated three times, and the data from replicates were subjected to correlation analysis to confirm consistency. A high degree of reproducibility was observed, with correlation coefficients exceeding 0.90 among replicates.

2.4. Data analysis

The data analysis for this study was conducted using the Python programming language and relevant statistical libraries, enabling precise control over data processing and analysis [16]. Specifically, a cubic polynomial regression model was applied to investigate the relationship between temperature (independent variable) and the hatching rate of *Lucilia sericata* eggs (dependent variable). Polynomial regression, particularly cubic regression, is often used to capture non-linear relationships within biological datasets, where the response variable does not follow a simple linear trend [5]. This choice aligns with existing methodologies used to model complex interactions in entomological studies, allowing for a more accurate representation of the thermal effects on egg hatching rates [9] .
The cubic polynomial regression model is expressed as:

$$F = ax^3 + bx^2 + cx + d$$

where F represents the hatching rate, x denotes temperature, and a, b, c, and d are coefficients optimized through the regression analysis [17]. This model was chosen due to its ability to accommodate the observed non-linear patterns, especially at temperature extremes, which can significantly affect egg viability. The regression coefficients were calculated for each experimental setup to fit the model, yielding a high coefficient of determination ($R^2 > 0.70$) across all conditions, indicating a robust model fit (Table 2.2).

Experiment	PHE Equation	df	R^2
1	$-0.010T^3 + 0.414T^2 + 0.108T - 13.875$	8	0.83
2	$-0.009T^3 + 0.395T^2 + 0.351T - 14.428$	8	0.92
3	$-0.010T^3 + 0.423T^2 + 0.050T - 13.248$	8	0.86

Table 2.2: Equations, degrees of freedom (df), and coefficients (R^2) of the relationship between the percentage of hatched eggs of *Lucilia sericata* (PHE) and temperature (T) across three experiments.

To assess consistency across trials, a correlation analysis was performed among replicate datasets. Correlation coefficients greater than 0.90 (Figure 2.2) indicated strong agreement between trials, demonstrating the reliability of the experimental setup and the robustness of the findings. Each temperature condition was replicated three times, and the average hatching rate across replicates was used for the regression model to ensure that the data reflected consistent trends and minimized experimental variability.

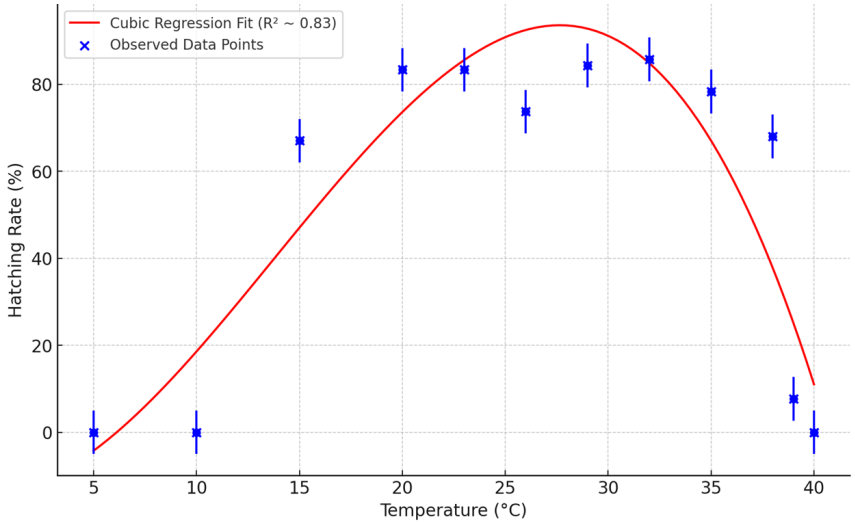

Figure 2.2: Hatched eggs (%) diagram of *Lucilia sericata*. The percentage of hatched eggs was calculated as the mean of three experiments and plotted against the temperature. Each point represents a measurement, with error bars indicating the Standard Deviation (SD) for each milestone. The red curve corresponds to the regression line ($R^2 = 0.83$).

The regression model provided a predictive formula for estimating hatching rates across different temperatures, supporting its potential application in forensic and ecological models. This predictive accuracy is essential for developing reliable reference data for *L. sericata* in postmortem interval estimation and ecological studies, where temperature effects on development are crucial [18]. The results from this statistical approach align with previous studies emphasizing temperature as a critical factor in the life cycle of *L. sericata* and other arthropods [7].

3. Results

The results demonstrated that *Lucilia sericata* eggs showed varying hatching rates across a broad temperature range, with notable thresholds observed at both low and high extremes. For temperatures at the lowest end of the scale (0°C, 5°C, 7°C, and 10°C) and the upper end (39°C and 41°C), no larval emergence was observed after an initial 24-hour incubation period, indicating a critical range where egg viability is significantly compromised. However, when transferred to a 30°C incubator for an additional 48 hours, a low rate of larval emergence was observed for eggs previously incubated at 11°C, 12°C, and 13°C, yielding hatching rates of 5.3%, 25.7%, and 26.7%, respectively. This outcome suggests that while low temperatures may delay development, subsequent warming can partially restore hatching viability.

For temperatures within the range of 14°C to 18°C, eggs initially showed no larval emergence within the first 24 hours. However, at the 48-hour mark, significant hatching rates were recorded. Specifically, eggs incubated at 14°C had a hatching rate of 61.7%, 15°C yielded 67.0%, 16°C showed 63.0%, 17°C had 69.0%, and at 18°C, the hatching rate reached 80.7%. These results suggest a gradual increase in viability as the temperature approaches the optimal range, reflecting the critical role of thermal regulation in *L. sericata* development [5, 9].

In the temperature range of 19°C to 38°C, eggs exhibited consistent and rapid larval emergence within the first 24 hours of incubation. The overall average hatching rate across this range was recorded as 80.3%, with the highest hatching rate observed at 28°C (89.7%), indicating an optimal developmental condition at this specific temperature. This peak supports the hypothesis that *L. sericata* has a well-defined thermal preference for egg development, as previously noted in entomological studies [14, 18].

Detailed statistical analysis was conducted to validate the experimental findings and ensure consistency across trials. A correlation analysis among the three experimental replicates demonstrated a strong correlation ($\rho > 0.90$) across all temperature conditions (Figure 2), indicating high reproducibility and reliability of the setup. This strong correlation supports the methodological consistency across trials, enhancing the robustness of the results.

For each temperature condition, a cubic polynomial regression analysis was performed to model the relationship between temperature and hatching rate. The choice of the cubic regression model was based on its ability to accurately capture the non-linear trends characteristic of biological processes, particularly at thermal extremes. Linear and quadratic models were also evaluated; however, they failed to fit the data adequately, especially in the temperature ranges where sharp changes in hatching rates occurred. The cubic model provided the best fit, with R^2 values consistently exceeding 0.70 across all experiments, demonstrating its suitability for representing the complex relationship between temperature and hatching viability.

Overall, these findings emphasize that temperatures below 10°C and above 40°C effectively inhibit larval emergence, while the intermediate range of 14°C to 38°C supports development, with the optimal temperature pinpointed at 28°C. These observations align with previous studies, including those by Cervantès et al. [13], which identified similar thermal tolerances in *L. sericata*. This range is particularly relevant in forensic entomology, where precise temperature modeling is crucial for postmortem interval estimation, as developmental rates can be significantly influenced by environmental conditions.

The experimental findings contribute valuable data to the field, confirming that *L. sericata*'s egg hatching rates are highly sensitive to temperature changes. These insights could support further research in forensic and ecological modeling, providing reliable reference data on *L. sericata*'s developmental thresholds under variable thermal conditions.

4. Conclusion

The findings from this study underscore the profound influence of temperature on the hatching rates of *Lucilia sericata* eggs, affirming the importance of thermal conditions in the developmental biology of ectothermic organisms, particularly those of forensic interest. Our results revealed that egg hatching is severely restricted at both low ($< 10°C$) and high ($> 40°C$) temperatures, while intermediate ranges (14°C to 38°C) provide viable conditions, with an optimal hatching rate observed at 28°C. These thermal thresholds are crucial in the context of forensic entomology, where *L. sericata* is widely used to estimate postmortem intervals (PMI), as temperature-driven development directly influences the species' life cycle timing [13].

In our study, eggs incubated at temperatures below 10°C displayed no hatching activity even after 24 hours, aligning with previous findings that low temperatures inhibit development in *L. sericata* and similar blowfly species. The absence of larval emergence at these low temperatures indicates a dormancy-like state, suggesting that *L. sericata* eggs can endure extended periods in suboptimal conditions without initiating development until exposed to warmer temperatures. In forensic scenarios, this dormancy characteristic can inform PMI estimations by accounting for periods when development is halted due to cold conditions. For example, in outdoor crime scenes during winter, our findings highlight the necessity of adjusting PMI models to incorporate potential delays caused by low temperatures. This characteristic may serve as a natural survival strategy in colder climates, although it poses limitations in forensic applications where accurate PMI estimations are required. The delay in hatching observed in our study at these low temperatures supports the need for temperature adjustments in forensic models to account for such dormant phases.

Conversely, the marked reduction in hatching rates at high temperatures ($> 40°C$) highlights the adverse effects of thermal extremes on egg viability, confirming that upper thermal thresholds can rapidly lead to non-viability. Previous research has similarly shown that extreme heat imposes stress that disrupts cellular processes in *L. sericata*, compromising survival. For cases in regions with extreme heat, such as arid climates, our findings suggest that forensic practitioners must consider the rapid inhibition of egg viability above 40°C. This is particularly important when estimating PMIs in environments exposed to prolonged high temperatures, where larval emergence may be absent due to thermal stress.

The intermediate temperature range (14°C to 38°C) showed notable viability, with a significant increase in hatching rates as temperatures approached 28°C. The highest hatching rate (89.7%) observed at this temperature aligns with previous studies identifying similar optimal conditions for *L. sericata* development [13]. This optimal temperature likely represents the species' natural preference, enhancing metabolic processes that support rapid development and survival. In forensic practice, these results could be applied to more accurately model *L. sericata*'s life cycle in cases where environmental temperature falls within this range, providing a reliable baseline for PMI estimations.

Additionally, our cubic polynomial regression model proved effective in capturing the non-linear relationship between temperature and hatching rate, achieving an $R^2 > 0.70$ across all trials. The model's predictive accuracy reinforces its potential for application in forensic models, where non-linear responses to temperature are frequently observed [9]. By offering a detailed, temperature-dependent hatching framework, this model addresses the critical need for precise developmental data in forensic entomology. For example, in scenarios where a body has been moved between environments with drastically different temperatures (e.g., from a cold outdoor location to a warm indoor setting), the delay and subsequent recovery in larval emergence documented in our study can provide critical evidence for reconstructing the timeline of events. This data can aid forensic practitioners in adjusting PMI estimations based on specific temperature profiles encountered at crime scenes, thereby enhancing the reliability of entomological evidence [7].

The study's findings also hold ecological significance, as they provide insights into how *L. sericata* populations may respond to fluctuating temperatures in natural environments. The thermal tolerance range identified here may contribute to broader ecological studies, particularly those assessing the impact of climate change on insect development and distribution. As global temperatures rise, understanding how *L. sericata* adapts to extreme conditions could prove valuable in predicting population shifts and ecosystem dynamics, especially in regions where this species plays a role in decomposition processes and nutrient cycling [12, 19].

In conclusion, this study reinforces the significance of temperature in *L. sericata* egg hatching and provides a foundational model for predicting hatching rates under various thermal conditions. The results emphasize the critical role of temperature in forensic applications, where accurate PMI estimation relies on reliable developmental timelines. By integrating temperature-driven developmental thresholds and their practical implications, our findings support the development of enhanced forensic entomological models for diverse environmental scenarios. While this study provides valuable insights, certain limitations should be noted. The experiments were conducted under controlled laboratory conditions with a fixed relative humidity of 45%, which may not fully replicate natural environmental variability. Additionally, the study focused solely on temperature as the independent variable, without considering potential interactions with other factors such as humidity, light exposure, or substrate type. These variables could significantly influence hatching rates and should be explored in future research.

Future studies could expand on this work by investigating the combined effects of temperature and other environmental factors, such as humidity and substrate variability, on egg viability and developmental rates. Longitudinal studies examining population-level variations in thermal tolerance across different geographic regions could also provide insights into how *L. sericata* adapts to diverse climates. Furthermore, integrating advanced statistical techniques or machine learning approaches may enhance the predictive power of the models, enabling even more accurate PMI estimations.

Article Information

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