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ORIGINAL ARTICLE

# Competition for food resources affects time-of-death estimation variables in the forensic-relevant fly species *Lucilia sericata* and *Calliphora vicina*

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## Abstract

In forensic entomology, larval density and competition for food resources among the first cadaver-colonizing insects can affect the accuracy and reliability of the estimated time of death as the minimal postmortem interval (mPMI). This study evaluated the impact of intra- and interspecific food resource competition within and between the forensically relevant fly species *Lucilia sericata* and *Calliphora vicina* on life history traits relevant to estimating mPMI. Intraspecific competition assays proceeded with 25, 50, 100, and 300 larvae on 25 g of beef liver. Likewise, interspecific competition experiments proceeded at three food resource levels (5 g, 50 g, and 150 g of beef liver) with 30 larvae (15 of each species). Intraspecific assays revealed a maximum increase of 4.3 °C above the ambient temperature in the larval masses of both species. In both species, larval size decreased with increasing larval density. Larval stage-specific mortality for *L. sericata* occurred predominantly between instars LII and early LIII, whereas in *C. vicina*, LI and LII instar larvae experienced the highest mortality. In the interspecies assays, the length and weight of adults of both species differed markedly, with *L. sericata* outcompeting *C. vicina*, demonstrating that food was the limiting resource determining adult sizes. This study provides valuable insights to enhance the accuracy of time-of-death estimates in forensic entomology contexts. This study unravels the intricate interplay of food resource availability, competition, and environmental factors in shaping the developmental dynamics of *L. sericata* and *C. vicina* at intra- and interspecific levels. A possible inverse relationship between larval density and survival rates may reflect the influence of competition and food availability, underscoring their relevance in understanding larval behavior and improving mPMI estimations. Additionally, the study reveals the advantage of *L. sericata* when competing under extreme food scarcity conditions, as evidenced by its survival rates and adult sizes. Considering the uniqueness of each forensic case and the interspecific competition for resources and microhabitats, analyzing the temperature of the mixed larval mass and the relative proportions of each species can enhance the accuracy of mPMI determinations.

**Keywords:** Calliphoridae; forensic entomology; time of death; larval mass; larval density.

## 1. Introduction

One of the significant contributions of forensic entomology (FE) to legal medicine investigations is the estimation of the approximate time of death or minimal postmortem interval (mPMI) [1, 2]. Additionally, FE helps clarify the cause of death by detecting toxic substances in insects feeding on the corpse [3, 4] and assessing whether a body has been relocated [1].

Two of the methods employed to estimate the mPMI using entomological evidence focus on (i) determining necrophagous insect life states and stages [2, 5], assuming that these insects were the first to colonize the corpse, will help infer the time elapsed since the moment of death; and (ii) the analysis of cadaveric entomofauna succession, which includes the study of insect and arthropod waves associated with decomposition stages [1, 2, 6].

Based on the first method mentioned, dipterans from the family Calliphoridae serve various forensic objectives [7–12]. Their significance lies in the fact that they are generally the first insects to detect gases originating during the decomposition process, quickly colonizing corpses to feed and complete their life cycles [1, 12, 13]. Thus, calliphorids are fundamental indicators for estimating the mPMI.

A behavioral characteristic of larvae from this family is their tendency to form clusters or larval masses on the corpse, which, through metabolic action, can raise the temperature of the larval mass above ambient temperature, protecting the larval mass and increasing development chances [14]. When larval development is complete, the larvae disperse from the mass and, in most cases, move away from the corpse to find a dry, dark place to become pupae and reach the adult stage [14].

While various studies demonstrate the use of FE to estimate the mPMI, this estimation can be affected by the intra- and interspecific competitive ability of colonizing species, generating a certain degree of dominance among dipteran larvae, which may depend on the amount of available food, ambient temperature, population size, and the temperature of large larval masses, among other variables [13]. Consequently, some species may adopt strategies to obtain sufficient nutrients and ensure reproductive success. These strategies directly affect crucial features for establishing the mPMI, such as larval length, width, and developmental time. Omitting these factors may lead to errors when estimating the mPMI [14–17]. Therefore, it is essential to consider these variables to ensure a more precise and reliable estimation of the PMI in the context of forensic entomology. Thus, research addressing the effects of larval density on parameters commonly used for mPMI estimation remains necessary.

With these data, reference studies can integrate these effects, making entomology an increasingly precise and valuable biological tool for the judicial system, resolving real cases as needed [10, 12, 18]. Despite research in Colombia on the life cycle and population parameters of the species *Lucilia sericata* (Meigen, 1826) and *Calliphora vicina* Robineau-Desvoidy, 1830 under controlled laboratory conditions [19, 20], to date, no investigations have been carried out in the country addressing the effect of larval competition among Calliphoridae family member species. This underscores the need to conduct research in this field and consider the possible implementation of such studies. Expanding research in this area will advance FE in Colombia and consolidate more comprehensive and internationally applicable knowledge.

In the present study, we evaluated the intra- and interspecific competition effects of *L. sericata* and *C. vicina*, with food quantity as the main variable, to identify potential implications on mPMI estimation. Additionally, we aimed to generate knowledge in forensic entomology that benefits the scientific community and the judicial system. The relevance of these findings lies in their potential to enhance the precision and reliability of forensic entomology, thereby strengthening its utility in resolving real cases and impacting society positively.

## 2. Materials and Methods

### 2.1. Study Area and Species

Calliphoridae, *L. sericata* and *C. vicina*, adults were collected on the campus of the Pedagogical and Technological University of Colombia (UPTC), located in the municipality of Tunja, Boyacá (5°32′56.9″ N, 73°21′16.0″ W). The specimens were transported to the Medical and Forensic Entomology Laboratory at UPTC to establish colonies under controlled laboratory conditions (27 °C ± 5 °C, 70 % RH ± 10 %, and a 12:12 photoperiod). Species were identified using an illustrated Calliphoridae key for Colombia published by [21]. Live specimens were placed in 45 × 45 cm Gerber cages with 200 g of beef liver for oviposition and 230 mL of 25 % sugar water as a carbohydrate source. The study was conducted with F2 generation first-instar larvae (LI) obtained for intra- and interspecific competition assays.

### 2.2. Density-Dependent Growth-Mortality and Development Rates – Intraspecific assay

Larvae of the same species were subjected to four individual-density treatments in 600 mL glass jars, all containing 25 g of beef liver as food. The four treatments consisted of 25 (25/25 g), 50 (50/25 g), 100 (100/25 g), and 300 (300/25 g) first-instar larvae (LI) placed in growth jars, with 10 replicates for each case. To record larval lengths and observe how larval density affected development, the number of larvae that successfully transitioned from one larval stage to another was recorded daily, along with the number of insects that reached the adult stage. Within each growth jar, second-instar larvae (LII), early third-instar larvae characterized by a full intestine (LIII-1), and late third-instar larvae, characterized by an empty intestine (LIII-2) were sacrificed and preserved by immersion in water at 70 °C for 5 s, followed by immersion in cold water for 5 s, and finally placement in 70 % alcohol [18]. Specimens from each developmental stage and larval density treatment were measured using a digital vernier caliper to assess the effect of density on larval body length. A single researcher conducted all measurements to minimize potential human error during data collection.

Furthermore, to identify possible thermal differences related to larval density and developmental stage, daily temperature recordings of the larval masses for each density and stage were conducted using a digital probe thermometer. Similarly, laboratory room temperature was monitored using a thermo-hygrometer. Subsequently, to assess the effect of larval density on the size and body weight of the adults, pre-pupae were transferred to individual 600 mL glass jars with a 5 cm sawdust layer to allow for adult emergence. The emerged adults were sacrificed, recording their body length and weight.

### 2.3. Inter-specific Competition for Food Resource

To assess the effect of inter-specific competition on the morphometric variables of adults under different levels of food availability, three treatments were established in 600 mL glass jars with 5 g, 50 g, and 150 g of liver. Each jar received 15 first-instar larvae (LI) of *L. sericata* and 15 of *C. vicina*, totaling 30 larvae. Upon the emergence of adults from both species, body length and weight were recorded. Each treatment had three replicates.

### 2.4. Statistical Analysis

Descriptive statistics were performed for both species in R studio. The Kruskall Wallis, Tukey and Mann-Whitney U statistical tests were conducted with Python software (version 3.7.0) to assess differences between treatments, larval sizes, larval mass temperatures, sex ratios, adult size and weight, and food quantity.

### 2.5. Results

The colonies of *C. vicina* and *L. sericata* were successfully established under the laboratory conditions of the Medical and Forensic Entomology Laboratory at UPTC (27 °C ± 5 °C, 70 % HR ± 10 %, and a 12:12 photoperiod).

### 2.6. Intra-specific Density-Dependent Growth-Mortality and Development Rates

### 2.7. Larval Mass Temperature

Larval mass temperatures were recorded from the larval stage LII onwards for both species, disregarding LI stages because all larvae remained dispersed without forming larval masses. As of LII larvae, all treatments with *L. sericata* experienced a temperature increase, ranging from 1.9 °C to 2.1 °C above room temperature. This increase was more pronounced in treatments with a higher number of individuals (100 and 300), where a maximum temperature of 25.4 °C occurred for the 100/25 g treatment and 27.1 °C for the 300/25 g treatment (**Table 1**). In contrast, for *C. vicina* LII larvae, temperatures rose between 1 °C and 4.3 °C above room temperature. For this species, the highest temperature was recorded in the replicas of the 100/25 g treatment, reaching a larval temperature of 30.2 °C compared to the room temperature of 25.9 °C (Table 1).

Regarding the LIII-1 treatments, for *L. sericata*, there was a temperature increase in the larval mass ranging from 1.4 to 4.3 °C above room temperature. The highest temperature was observed in the 300/25 g treatment, where the larval masses reached 27.4 °C ± 1.01 SD, compared to the room temperature of 23.1 °C. On the other hand, for *C. vicina*, maximum temperatures experienced slightly smaller increases, ranging between 0.8 and 1.1 °C above room temperature. The only exception was the 25/25 g treatment, where the maximum larval temperature was 0.3 °C lower than the room temperature. In contrast, for LIII-2, there was an increase in temperature between 0.9 and 3.9 °C above room temperature for *L. sericata*, while for *C. vicina*, the temperature of larval masses increased between 0.8 and 2.1 °C. In both cases, the maximum temperature was recorded in the 300/25 g treatments (Table 1).

When applying the Mann-Whitney U test, larval mass and room temperatures differed notably. In the case of *C. vicina*, significant differences ($P < 0.05$) were observed in most treatments, except for the LIII-1 stage in the 100/25 g treatment ($P > 0.05$). These results emphasize the importance of considering temperature in the study, since *C. vicina* larval aggregates developed at temperatures significantly above those of the environment. On the other hand, *L. sericata* larval mass temperatures varied across treatments and developmental stages. Significant differences ($P < 0.05$) were found in the LII and LIII-1 stages for the 25/25 g and 100/25 g treatments. For the 50/25 g treatment, significant differences were only found between larval masses and ambient temperature in LIII-1. In the 300/25 g treatment, significant differences were found in LIII-1 and LIII-2. Overall, these findings indicate that the formation of larval masses and competition for resources under low food availability generate a considerable thermal increase compared to ambient temperature.

**Table 1.** Maximum temperatures recorded in larval masses for the species *Lucilia sericata* and *Calliphora vicina* across four variable intra-specific larval densities (treatments), namely 25, 50, 100, and 300 larvae/25 grams of food. LII: Second-instar larvae, LIII-1: Early third-instar larvae, LIII-2: Late third-instar larvae, Room: Room temperature of the laboratory, Max. larval: Maximum temperature recorded in larval masses, ±: Standard deviation. Numbers in bold refer to the highest temperatures recorded during the experiments. Note: Temperature data for the first larval instar were not recorded as larval masses were not formed during this stage.

| Larval instar | Treatment (Larval density) | Temperature (°C) | | | |
|---|---|---|---|---|---|
| | | *Lucilia sericata* | | *Calliphora vicina* | |
| | | Room | Max. larval | Room | Max. larval |
| **LII** | 25 | 24.4 | 26.4 ± 0.7 | 24.6 | 25.6 ± 0.34 |
| | 50 | 24.6 | 26.5 ± 0.79 | 24.6 | 26.8 ± 0.71 |
| | 100 | 23.3 | **25.4 ± 0.63** | 25.9 | **30.2 ± 1.03** |
| | 300 | 25 | **27.1 ± 0.9** | 23.5 | 26.4 ± 0.81 |
| **LIII-1** | 25 | 22.9 | 25 ± 0.96 | 26 | **25.7 ± 0.56** |
| | 50 | 22.9 | 25.4 ± 0.6 | 27 | 27.8 ± 0.75 |
| | 100 | 25 | 26.4 ± 0.9 | 27.2 | 28.2 ± 0.68 |
| | 300 | 23.1 | **27.4 ± 1.01** | 24 | 25.1 ± 0.29 |
| **LIII-2** | 25 | 24.6 | 25.9 ± 0.73 | 24.8 | 24.8 ± 0.51 |
| | 50 | 24.6 | 25.8 ± 0.69 | 24.8 | 26.6 ± 0.89 |
| | 100 | 24.1 | 25 ± 0.58 | 24.5 | 25.3 ± 0.35 |
| | 300 | 23.1 | **27 ± 0.79** | 23.5 | **25.6 ± 0.76** |

The Kruskal-Wallis test was conducted to compare larval mass temperatures between treatments within each developmental stage. In *L. sericata*, no significant differences were found in LII ($P > 0.05$), whereas in LIII-1 and LIII-2, differences were identified between treatments ($P < 0.05$). In contrast, in *C. vicina*, all larval temperature differed significantly across treatments within larval stages (LII, LIII-1, and LIII-2) ($P < 0.05$). These results suggest that larval aggregate temperatures in *L. sericata* remained relatively constant in LII, despite differences in food availability between treatments, unlike *C. vicina*, where the temperature varied between treatments in all stages.

### 2.8. Density-dependent larval development time – intra-specific assay

The egg stage of *L. sericata* lasted approximately 18 h, while that of *C. vicina* was around 12 h. For *L. sericata* in the 25/25 g treatment, the larval development time took up to 4 days, while in the other treatments, the duration of the larval stage was 5 days. On the other hand, *C. vicina* completed its larval development in 4 days in the 25/25, 50/25, and 100/25 g treatments. However, in the 300/25 g treatment, larval development lasted 5 days.

### 2.9. Density-dependent larval size – intra-specific assay

Larval length increased from LI to LIII-2 across all treatments in both species, as expected. In *L. sericata*, the 50/25 g treatment during LIII-2 stood out for having larvae reaching lengths close to 12 mm, surpassing the average lengths of this stage in all treatments. However, a decrease in the average length of this species was noted as larval density increased in different treatments. In the treatment with the highest larval density (300/25 g), a drastic reduction in the average length of *L. sericata* larvae (8 mm) was observed throughout larval development (LI to LIII-2), compared to the other lower-density treatments, clearly falling below the average seen in the other treatments. Overall, these results indicate that high larval density is associated with a marked reduction in average length from the early stages of development in *L. sericata* (**Table 2**).

*C. vicina* larvae decreased in length as their density increased. However, in the 25/25 g, 50/25 g, and 100/25 g treatments, the size reduction occurred mainly at instar LIII-1. In the 300/25 g treatment, the length decrease was evident at the LII instar, with larval sizes below 10 mm, contrasting with the average length of about 15 mm across larval densities for this instar. Under extreme overcrowding conditions (300/25 g), both fly species are strongly affected, revealing ample length variation at all developmental stages. The Kruskal-Wallis test in both species revealed statistically significant differences ($P < 0.05$) in larval length at all stages (LI, LII, LIII-1, LIII-2), indicating that larval growth progresses significantly during maturation under the experimental conditions (Table 2).

The Mann-Witney U compared larval lengths between *L. sericata* and *C. vicina* at each larval stage in all treatments. In most of these comparisons, significant differences were identified ($P < 0.05$). However, a high similarity was observed between the larval lengths at LIII-1 of both species under the 25/25 g treatment (U: 164.0, $P > 0.05$).

### 2.10. Density-dependent survival and development – intra-specific assay

*L. sericata* and *C. vicina* survival tended to decrease as their larvae progressed from one stage to another. In the 25/25 g treatment, *L. sericata* showed a gradual decrease in the number of larvae from LI to LII, followed by a significant decline from LII to LIII-1, indicating high mortality at that point (**Fig. 1a**). Although a decrease in mortality occurred between LIII-1 and LIII-2, there was notable variability among replicates, as evidenced by wide standard deviations at these stages. On the other hand, *C. vicina* experienced a decrease in survival at each stage, with mortality being particularly pronounced from LI to LII (Fig. 1e).

In the 50/25 g treatment, *L. sericata* exhibited a gradual decrease in survival from LI to LIII-2 (Fig. 1b), with low variability among replicates. On the other hand, C. vicina showed high mortality from LI to LII, followed by a stabilization in survival with ample variability among replicates (Fig. 1f).

At a larval density of 100/25 g, *L. sericata* (Fig. 1c) experienced high larval mortality and ample variability among replicates compared to *C. vicina* (Fig. 1g). Specifically, during larval stages, *L. sericata* exhibited higher mortality than *C. vicina*. These findings suggest that under conditions of intermediate larval density (100/25 g), *L. sericata* endured a marked intra-specific competitive pressure, resulting in lower survival compared to *C. vicina*, which showed lower larval mortality at the same density.

**Table 2.** Larval lengths recorded for the species *Lucilia sericata* and *Calliphora vicina* across four variable intra-specific larval densities (treatments), namely 25, 50, 100, and 300 larvae/25 grams of food. LI: First instar larva, LII: Second instar larva, LIII-1: Early third instar larva, LIII-2: Late third instar larva. ±: Standard deviation.

| Species | Treatment (Larval density) | Average larval length (mm) per developmental stage | | | |
|---|---|---|---|---|---|
| | | LI | LII | LIII-1 | LIII-2 |
| *Lucilia sericata* | 25 / 25g | 1.44 ± 0.3 | 3.57 ± 0.9 | 8.08 ± 0.7 | 9.98 ± 0.8 |
| | 50 / 25g | 1.82 ± 0.4 | 3.92 ± 0.6 | 7.75 ± 0.7 | 11.14 ± 0.9 |
| | 100 / 25g | 0.57 ± 0.2 | 2.62 ± 0.4 | 7.29 ± 2.1 | 9.13 ± 1.5 |
| | 300 / 25g | 0.55 ± 0.3 | 3.42 ± 2.0 | 6.70 ± 1.0 | 7.13 ± 1.5 |
| *Calliphora vicina* | 25 / 25g | 3.31 ± 0.6 | 15.00 ± 1.2 | 8.35 ± 1.6 | 15.26 ± 0.6 |
| | 50 / 25g | 3.56 ± 0.8 | 15.76 ± 0.7 | 9.69 ± 1.7 | 14.49 ± 0.6 |
| | 100 / 25g | 3.51 ± 0.9 | 13.68 ± 1.3 | 10.86 ± 1.8 | 12.28 ± 1.2 |
| | 300 / 25g | 4.67 ± 0.8 | 9.91 ± 1.91 | 13.93 ± 1.0 | 14.93 ± 1.0 |

Regarding larval mortality rates under food restriction conditions, in *C. vicina* 59.2 % of individuals failed to complete their larval development, whereas in *L. sericata*, this percentage was of 53.9 %. Although the difference in mortality between both species was close, the data suggest a slightly more effective adaptation of *L. sericata* to food scarcity, which could explain its higher larval survival compared to the other species under study. It is important to note that *L. sericata* exhibited its highest mortality between larval stages LII and LIII-1, while *C. vicina* experienced it between LI and LII. This variation could be attributed to increased intra-specific competition for limited food resources during the early larval stages and subsequent adaptation to an environment with restricted resources.

### 2.11. Male to female Ratios – intra-specific assay

From the 2,850 larvae of each species with which the experiments commenced, a total of 1,315 individuals of *L. sericata* successfully reached the adult stage, with a sex ratio of 48.4 % males to 51.6 % females. Regarding *C. vicina*, 1,163 adults emerged, with a sex ratio of 57.5 % males and 42.5 % females. It is worth noting that sex ratio biases could be related to specific reproductive strategies and behaviors of each species. However, there is no evidence that larval density affects the ratio of males and females in these species. But, food restrictions may have an impact on the quality of life of developing adults.

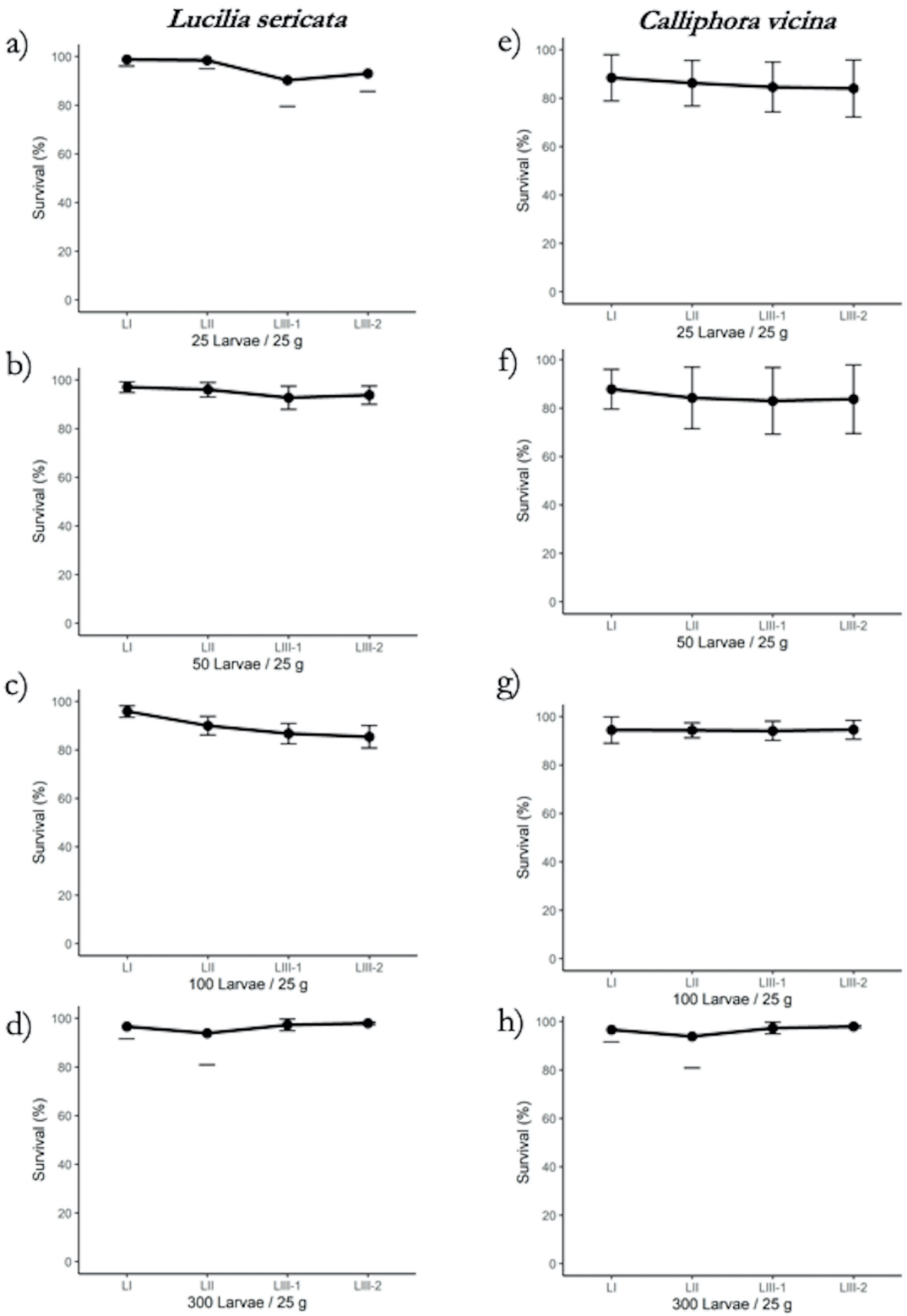


**Figure 1.** Intra-species *L. sericata* and *C. vicina* larval survival across developmental stages under four different food resource availabilities. *L. sericata*: **a)** 25 larvae/25 g of food, **b)** 50 larvae/25 g, **c)** 100 larvae/25 g, and **d)** 300 larvae/25 g. *C. vicina*: **e)** 25 larvae/25 g, **f)** 50 larvae/25 g, **g)** 100 larvae/25 g, and **h)** 300 larvae/25 g.

## 3. Interspecific competition for food resources

### 3.1. Survival and development of adults competing for food resources – interspecies assay

*L. sericata* outcompeted *C. vicina* in the interspecific adult emergence experiment under strong food resource competition conditions (30 /5 g) with 35 *L. sericata* individuals emerging vs. 10 *C. vicina* individuals. Under medium (30 /50 g) and low (30 /150 g) food resource competition conditions, no species outcompeted the other. At the intermediate food resource competition level, 41 *C. vicina* and 39 *L. sericata* individuals completed their development, and under the least food competition pressure, 40 individuals of each species reached adulthood. Considering these results, the interaction between the two species under limited food resources elicited *L. sericata's* competitive advantage over *C. vicina*.

### 3.2. Adult body size – interspecies assay

**Table 3** presents the average length and weight of *C. vicina* and *L. sericata* adults emerging under three food availability levels. At the lowest food availability level (30/5 g, i.e., highest level of food resource competition) adults of both species showed very low average lengths and weights in both sexes, with a tendency for larger sizes in females. In contrast, in the treatment with the highest food availability (30/150 g), an increase in body size was observed. When comparing the adults from the treatment with the highest food availability (less competition for food) with the adults from the treatment with the least resources, *C. vicina* females from the highest food availability treatment were larger by, on average, 2.21 mm ($\pm$ 0.65 mm), while males were 2.08 mm ($\pm$ 0.37 mm) larger. Similarly, in *L. sericata*, a size increase was recorded for females and males (2.8 mm $\pm$ 0.33 mm and 1.94 mm $\pm$ 0.3 mm, respectively). These results suggest a food availability effect on adult size and weight, with adult individuals of both species being smaller and of variable weights when larvae were grown under high food restriction conditions.

The Tukey test revealed statistically significant differences in the length and weight of *L. sericata* and *C. vicina* adults under low vs. medium and low vs. high food resource availability conditions. However, there were no significant differences between medium and high food availabilities. Body size and weight differences between the low and the other food availability levels suggest that the limiting factor was food restriction under the 30/5 g treatment. Adult body traits, such as length and weight, are likely determined by dietary limitations experienced by the developing larvae.

## 4. Discussion

The larval gregarious behavior, from hatch to the LIII instar, favors the formation of larval masses and is a remarkable feature of this investigation. Such larval aggregates can generate high temperatures, a well-known phenomenon that protects the larvae from unexpected ambient temperature drops [22–24], and that can accelerate larval development and potentially reduce predation [15, 25–27]. Furthermore, aspects such as food availability, larval density, and ambient temperature influence larval development [14, 28, 29]. Various studies emphasize the importance of considering the effects of larval activity and fluctuating temperatures when estimating mPMI in forensic cases [9, 15, 16, 30]. Our results support these implications, as larval mass temperatures differed from those of room temperature due to high larval activity and competition for resources during development.

**Table 3.** Average length and weight of *Lucilia sericata* and *Calliphora vicina* adults emerging from interspecific competition assays under high (30 / 150 g), medium (30 / 50 g), and low (30 / 5 g) food resource availabilities.

| Species | Treatment (Food availability level) | Adult length (mm) | | Adult weight (mg) | |
|---|---|---|---|---|---|
| | | Female | Male | Female | Male |
| *Lucilia sericata* | 30/5g | 5.47 ± 0.52 | 5.24 ± 0.48 | 2.3 ± 0.40 | 2.2 ± 0.48 |
| | 30/50g | 8.14 ± 0.40 | 6.92 ± 0.6 | 6.3 ± 0.51 | 5 ± 1.31 |
| | 30/150g | 8.27 ± 0.33 | 7.41 ± 0.3 | 6.4 ± 0.50 | 5.2 ± 0.51 |
| *Calliphora vicina* | 30/5g | 6.8 ± 0.25 | 6.24 ± 0.59 | 5.6 ± 1.03 | 5.2 ± 0.97 |
| | 30/50g | 8.76 ± 0.49 | 8.48 ± 0.58 | 9 ± 0.72 | 8.5 ± 0.70 |
| | 30/150g | 9.01 ± 0.65 | 8.32 ± 0.37 | 9.4 ± 0.98 | 8.9 ± 0.72 |

Under conditions of low food availability and consequently higher competition (100/25 g and 300/25 g), larval sizes of both fly species were affected. Our findings are consistent with previous research on *L. sericata* [31, 32] and other Calliphoridae species [28, 33, 34]; these studies reported that an increase in larval density was related with a reduced size and average weight of pupae and adults, leading to lower species survival across different developmental stages. However, in *C. vicina*, high variability in larval length was observed among intraspecific treatments, which could be related to factors beyond competition for food.

Our data support these findings by showing a relationship between the elevated temperatures reached by larval masses, increased larval density, and reduced food availability in experimental treatments [14, 15, 25, 26, 34]. Additionally, it is demonstrated that competition for food, resulting from its limited availability, influences the individual size of larvae, being less evident in treatments with low competition, as shown in food competition models evaluated by De Jong [35], individuals in high population densities try to acquire the most food to ensure their survival; consequently, in this case, adults with lower weight and lower-than-average survival rates are observed. Upshot highlights the importance of considering competition and food availability when studying larval mass behavior and development, as these variables can affect larval body size, development time, and, ultimately, mPMI estimates in cases involving entomological evidence.

When studying competition between *L. sericata* and *C. vicina* under conditions of extreme food scarcity (30/5 g), *L. sericata* performed better, given its higher survival. However, the resulting adults were considerably smaller compared to those of *C. vicina*. These findings align with the study by Hans & VanLaerhoven [30], which assessed the impact of interactions between these species, reporting that the presence of *C. vicina* can affect the size and larval survival of *L. sericata*, influencing mPMI estimates. Furthermore, temperature significantly impacts larval development and growth in both species. Collectively, these studies highlight the role of ecological factors such as competition, limited resources, and temperature on growth and developmental parameters of forensic importance.

On the other hand, the results of this study suggest that competition for food resources does not affect fly sex ratios. However, it is relevant to consider that competition for food may influence the reproductive success of adults; nevertheless, this requires further investigation.

In Colombia, *L. sericata* and *C. vicina* have been identified as early colonizers of cadavers in the initial stages of decomposition, particularly in urban areas at high altitudes [36–39]. Additionally, both species may coexist on the same cadavers [37], thus suggesting potential interspecific competition for available resources during the early phases of decomposition. Under the conditions of this study, we did not observe interspecific predation or cannibalism in either species despite these strategies having been reported in other Calliphoridae species under trophic stress conditions [40–45].

From an ecological perspective, laboratory studies possess limitations, such as the inability to adequately assess phenomena like predation, especially by species that actively feed on dipteran larvae in cadavers, such as *Oxelytrum discicolle* (Brullé, 1836) (Coleoptera: Staphylinidae) [37, 46, 47]. Nevertheless, although the results are subject to the inherent limitations of laboratory experimentation, they remain relevant as they permit the evaluation of factors such as competition for space and food, as well as the gregarious behaviors of larvae, thus laying the groundwork for future field-based research [48].

The results of this research provide key insights into the influence of food resource availability and intra- and inter-specific competition on the development of *L. sericata* and *C. vicina*. The study also highlights the increase in larval mass temperature relative to ambient temperature, the latter being the reference commonly used for calculating accumulated degree days (ADD), which are fundamental for estimating the minimum postmortem interval (mPMI) [8]. These findings emphasize the importance of considering environmental factors and species competition in the context of forensic entomology, as their integration may improve the accuracy of mPMI estimations and, consequently, enhance the value of this discipline in criminal investigations.

Future studies should explore the interaction of *L. sericata* and/or *C. vicina* with native species also associated with early cadaveric phenomena, such as *Sarconesia magellanica* (Le Guillou, 1842) or *Compsomyiops verena* (Walker, 1849) (Diptera: Calliphoridae), as these species are relevant in forensic contexts [37, 49–51] and are found in the same localities in Colombia [52, 53]. Moreover, as suggested by Ivorra *et al*. [54], other studies could assess species responses under variable experimental conditions, such as temperature variation, interspecific proportions (20:80, 40:60), and competition between different larval instars. Such factors are necessary to deepen our understanding of competition mechanisms among necrophagous species commonly found on cadavers.

## 5. Conclusions

This study unravels the intricate interplay of food resource availability, competition, and environmental factors in shaping the developmental dynamics of *L. sericata* and *C. vicina* at intra- and interspecific levels. A possible inverse relationship between larval density and survival rates may reflect the influence of competition and food availability, underscoring their relevance in understanding larval behavior and improving mPMI estimations. Additionally, the study reveals the adaptability of *L. sericata* in interspecific competition under extreme food scarcity, influencing survival rates and adult sizes. Considering the uniqueness of each forensic case and the interspecific competition for resources and microhabitats, analyzing the temperature of the mixed larval mass and the relative proportions of each species can enhance the accuracy of mPMI determinations.

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## 7. Conflict of interest

The authors have no competing interests to declare.

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**La competencia por recursos alimenticios en las especies de moscas con interés forense *Lucilia sericata* y *Calliphora vicina* afecta las variables de estimación del tiempo de muerte**

**Resumen:** En entomología forense, la densidad larval y la competencia por recursos alimenticios entre los primeros insectos en colonizar un cadáver pueden afectar la precisión y confiabilidad de la estimación del tiempo de muerte como intervalo post mórtem mínimo (mPMI por su sigla en inglés). En este estudio se evaluó el impacto de la competencia intra e interespecífica por recursos alimenticios en dos especies de moscas de relevancia forense, *Lucilia sericata* y *Calliphora vicina*, enfocándose en rasgos del ciclo de vida clave para la estimación del mPMI. Los ensayos de competencia intraespecífica se realizaron con 25, 50, 100 y 300 larvas sobre 25 g de hígado de res. Los experimentos de competencia interespecífica se llevaron a cabo con tres niveles de recurso alimenticio (5, 50 y 150 g de hígado de res) y 30 larvas (15 de cada especie). En los ensayos intraespecíficos, las masas larvales de ambas especies alcanzaron un aumento máximo de temperatura de 4.3 °C por encima de la temperatura ambiente. En ambas especies, el tamaño larval disminuyó con el aumento de la densidad de larvas. En *L. sericata*, la mayor mortalidad se presentó entre los estadios larvales LII y principios de LIII, mientras que en *C. vicina*, las mayores tasas de mortalidad ocurrieron en los estadios LI y LII. En los ensayos interespecíficos, la longitud y el peso de los adultos de ambas especies mostraron diferencias marcadas, siendo *L. sericata* competitivamente superior a *C. vicina*. Esto indica que la disponibilidad de alimento fue el factor limitante que definió el tamaño alcanzado por los adultos. Este estudio ofrece información valiosa para mejorar la precisión en la estimación del tiempo de muerte en contextos de entomología forense. Además, el estudio muestra cómo interactúan la disponibilidad de alimento, la competencia y los factores ambientales en el desarrollo de *L. sericata* y *C. vicina*, tanto a nivel intraespecífico como interespecífico. La posible relación inversa entre la densidad larval y la supervivencia sugiere que la competencia y la disponibilidad de alimento influyen directamente en el comportamiento larval y, por tanto, en la precisión de las estimaciones del mPMI. Adicionalmente, el estudio evidenció la ventaja competitiva de *L. sericata* bajo condiciones extremas de escasez alimentaria, como se refleja en sus tasas de supervivencia y tamaños adultos. Dado que cada caso forense es único y que existe competencia interespecífica por recursos y microhábitats, el análisis de la temperatura de las masas larvales mixtas y de la proporción relativa de cada especie puede mejorar la precisión en la determinación del mPMI.

**Palabras Clave:** Calliphoridae; densidad larval; entomología forense; masa larval; tiempo de muerte.

## A competição por recursos alimentares nas espécies de moscas de interesse forense Lucilia sericata e Calliphora vicina afeta as variáveis de estimativa do tempo de morte

**Resumo:** Na entomologia forense, a densidade larval e a competição por recursos alimentares entre os primeiros insetos a colonizar um cadáver podem afetar a precisão e a confiabilidade da estimativa do tempo de morte, representada pelo intervalo pós-morte mínimo (mPMI, na sigla em inglês). Este estudo avaliou o impacto da competição intra e interespecífica por recursos alimentares em duas espécies de moscas de relevância forense, *Lucilia sericata* e *Calliphora vicina*, com foco em características do ciclo de vida essenciais para a estimativa do mPMI. Os ensaios de competição intraespecífica foram realizados com 25, 50, 100 e 300 larvas em 25 g de fígado bovino. Os experimentos de competição interespecífica foram conduzidos com três quantidades de recurso alimentar (5, 50 e 150 g de fígado bovino) e 30 larvas (15 de cada espécie). Nos ensaios intraespecíficos, as massas larvais de ambas as espécies apresentaram um aumento máximo de 4.3 °C acima da temperatura ambiente. Em ambas as espécies, o tamanho larval diminuiu com o aumento da densidade de larvas. Em *L. sericata*, a maior mortalidade ocorreu entre os estágios larvais LII e início de LIII, enquanto em *C. vicina*, os maiores índices de mortalidade foram observados nos estágios LI e LII. Nos ensaios interespecíficos, o comprimento e o peso dos adultos de ambas as espécies diferiram significativamente, sendo *L. sericata* competitivamente superior a *C. vicina*. Isso indica que a disponibilidade de alimento foi o fator limitante que determinou o tamanho final dos adultos. Este estudo fornece informações valiosas para aprimorar a precisão na estimativa do tempo de morte em contextos de entomologia forense. Além disso, mostra como a disponibilidade de alimento, a competição e fatores ambientais interagem no desenvolvimento de *L. sericata* e *C. vicina*, nos níveis intra e interespecíficos. A possível relação inversa entre densidade larval e taxa de sobrevivência sugere que a competição e a disponibilidade de recursos influenciam diretamente o comportamento larval e, consequentemente, a precisão das estimativas do mPMI. Adicionalmente, o estudo evidenciou a vantagem competitiva de *L. sericata* sob condições extremas de escassez alimentar, como demonstrado por suas taxas de sobrevivência e tamanhos adultos. Considerando que cada caso forense é único, e que há competição interespecífica por recursos e micro-habitats, a análise da temperatura das massas larvais mistas e da proporção relativa de cada espécie pode melhorar a presicão na determinação do mPMI.

**Palavras-chave:** Calliphoridae; densidade larval; entomologia forense; massa larval; tempo de morte.

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