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# Effects of Different Temperature and Humidity Regimes on Reproduction and Development of *Lucilia sericata* (Meigen,1826) Female Populations

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## Abstract

**Aim to study:** The development of animals such as *Lucilia sericata* (Diptera: Calliphoridae), which are unable to regulate their body temperature metabolically and instead maintain a constant temperature by absorbing heat from their surroundings (i.e. poikilotherms), has been extensively described using the temperature collection model. This study aimed to investigate oviposition tendency and oviposition development times and ideal temperature and humidity values for mass rearing of the green bottle fly *L. sericata*, which was studied in the laboratory at three different constant temperatures (25 °C, 30 °C, and 35 °C) and three different constant humidities (35% relative humidity (R.H.), 50% R.H., and 65% R.H.).

**Material and methods:** The humidity was fixed at each experimental temperature to determine the maximum egg- laying and development time at different temperatures, and the number of days and degrees required to complete each stage was determined. The temperature was fixed in the different “”””””” “humidity experiments, and the insectarium was examined under controlled conditions in a 12:12 (L:D) photoperiod cycle.

**Results:** A significant difference was obtained between the number of *L. sericata* eggs laying at different temperature values ($\chi 2=21.143$, $p<0.05$). A significant difference was found between the number of *L. sericata* eggs laying at different humidity values ($\chi 2=17.913$, $p<0.05$).

**Conclusion:** A temperature of 35°C and 50% humidity were identified as the optimal breeding conditions for the oviposition tendency of *L. sericata* flies in a specific insect house under laboratory conditions.

**Keywords:** Egg-laying, Growth and development, Humidity, *Lucilia sericata*, Temperature.

# Farklı Sıcaklık ve Nem Rejimlerinin *Lucilia sericata* (Meigen,1826) Dişi Popülasyonlarının Üreme ve Gelişimi Üzerine Etkileri

## Öz

**Çalışmanın amacı:** *Lucilia sericata* (Diptera: Calliphoridae) gibi vücut sıcaklığını metabolik olarak düzenleyemeyen ve bunun yerine çevrelerinden ısı emerek sabit bir sıcaklığı koruyan hayvanların (yani poikilotermler) gelişimi, sıcaklık toplama modeli kullanılarak kapsamlı bir şekilde tanımlanmıştır. Bu çalışmanın amacı, laboratuvarda üç farklı sabit sıcaklıkta (25 °C, 30 °C ve 35 °C) ve üç farklı sabit nemde (%35 R.H., %50 R.H. ve %65 R.H.) çalışılan yeşil şişe sineği *L. sericata*'nın yumurtlama eğilimini ve yumurtlama gelişim sürelerini ve kitle yetiştiriciliği için ideal sıcaklık ve nem değerlerini araştırmaktır.

**Materyal ve yöntemler:** Farklı sıcaklıklarda maksimum yumurtlama ve gelişme süresini belirlemek için her bir deney sıcaklığında nem sabitlenmiş ve her bir aşamanın tamamlanması için gereken gün sayısı ve dereceler belirlenmiştir. Farklı nem deneylerinde sıcaklık sabit tutulmuş ve insektaryum 12:12 (L:D) fotoperiyot döngüsünde kontrollü koşullar altında incelenmiştir.

**Bulgular:** Farklı sıcaklık değerlerinde yumurtlayan *L. sericata* yumurta sayıları arasında anlamlı bir fark bulunmuştur ($\chi 2=21.143$, $p<0.05$). Farklı nem değerlerinde yumurtlayan *L. sericata* yumurta sayıları arasında anlamlı bir fark bulunmuştur ($\chi 2=17.913$, $p<0.05$).

**Sonuç:** 35°C sıcaklık ve %50 nemin, laboratuvar koşullarında belirli bir böcek barınağında *L. sericata* sineklerinin yumurtlama eğilimi için en uygun üreme koşullarını oluşturduğu tespit edilmiştir.

**Anahtar kelimeler:** Yumurtlama, Büyüme ve gelişme, Nem, *Lucilia sericata*, Sıcaklık.

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## Introduction

One of the main themes in biology is the understanding of how ecological conditions affect the evolution of an organism's body size and development time (Oudman et al., 1991; Plaistow et al., 2004; Gomez-Mestre & Buchholz, 2006). From a developmental point of view, it is difficult for organisms, especially during metamorphosis, to survive to adulthood with limited resources. According to their biological life cycles, their ability to maintain the developmental rate balance in their periodic phases has been a subject of curiosity and research (Wilbur & Collins, 1973). The balance between body size relative to age is an important criterion in the physiological process of the development of holo-metabolic insects. Physiological differences or some parameters involved in development are influential on the evolution of life histories. Minimum survival, weight and critical weight are examples of threshold parameters for development (Mirth & Riddiford, 2007). The distribution and characteristics of Calliphoridae species vary among populations worldwide, with variable body sizes and developmental times related to ecological conditions and responses. *Lucilia sericata* (Diptera: Calliphoridae), which is dependent on nutrient-rich substrates such as carrion and biological waste, can be stretched by rearing under laboratory conditions to achieve superiority *L. sericata* is sensitive to environmental factor changes in laboratory rearing conditions such as temperature, humidity, photoperiod, and food type (Clark et al., 2006; Tarone & Foran, 2006).

*Lucilia sericata*, also known as green bottle fly and green meat fly, is also known as myiasis flies because *L. sericata* is not a parasite of humans in its adult stage. In their larval stages, they settle in different anatomical regions of humans (Faulde et al., 2001) and animals such as sheeps, cats, dogs and carrion crows (Crystal and Ramirez, 1975; East and Eisemann, 1993; Dik et al., 2012) and cause myiasis. It causes traumatic Myiasis in livestock in many European, African and Asian countries (Dik et al., 2012). It is reported to be a serious problem in Hungary (Farkas et al.,1997). In our country, it is reported that miyasis cases caused by *L. sericata* are mostly encountered in Konya and Kırıkkal in Central Anatolia (Dik et al., 2012). *Lucilia sericata* is typically one of the first flies to reach the corpse after death. It is a biological marker in forensic entomology and entomotoxicology Forensic entomologists use blow fly development to estimate a postmortem interval (Byrd & Castner, 2010; Tomberlin et al., 2011a).

At the same time, in the treatment of chronic wounds that are slow to heal, facultative *L. sericata* larvae are used medicinally in the first and second stages of development to perform micro debridement as an alternative to surgical intervention (Gazi et al., 2021). In addition to the use of live larvae of this fly in wound treatment, secretions obtained from larvae are also employed in wound treatment. With regard to this, the antibacterial activities of secretions obtained from larvae have been investigated in both in vitro and in vivo environments (Zerek et al. 2019). Myiasis is associated with Post Mortem Interval (PMI) predictions and the development of success in a non-healing wound. Researchers have noted that members of Calliphoridae have different minimum developmental ranges between species and within species (Kamal, 1958; Greenberg, 1991; Grassberger & Reiter, 2001). This may result from genetic variation or environmental factors in laboratory rearing conditions (Clark et al., 2006; Tarone & Foran, 2006). Variation in minimum development times implies variation in age estimates and requires species-specific optimization. In this regard, studies on *L. sericata* larval stage development show that the minimum development time varies depending on the

variability of the rearing temperature or relative humidity factor (Tarone & Foran, 2011).

Temperature is an important factor not only for adults but also for all developmental stages in the life cycle. However, for egg laying, hatching, survival and the ability to transition to the next larval stage, humidity, light, nutrient amount and variety are also essential environmental factors along with temperature (Lefebvre & Pasqueraul, 2004). Temperature, humidity, and nutrient content are the main factors, but population density in laboratory-rearing conditions also regulates development. For example, when larval density is high and food is limited, larvae rapidly pass through three distinct stages due to food competition and pupate before the normal process. However, due to the larvae's excessive feeding activities and rapid metabolism within the aggregate, it has been determined that the ambient temperature rises further, and the development time is shortened to the extent that it exceeds the maximum life temperature (Campobasso et al. 2001; Kotze et al., 2016; Pruna et al., 2019).

According to Campobasso et al. (2001) and Ash and Greenberg, (1975), some Calliphoridae species enter larval or pupal diapause in response to seasonal changes, and their development stops. Pupal development is faster at high temperatures than at low temperatures (West, 1951). In a study on the effect of different temperatures on the development of *L. sericata*, it was reported that pupal entry was not observed at 15 °C, pupal stage constituted about 46% of the total developmental stage at 20 ºC, 42% at 25 ºC and 44% at 30 ºC (Grassberg & Reiter, 2001). Adult emergence occurs early at low temperatures, and small adult individuals with underdeveloped wings and smaller-than-normal appearances are formed. Therefore, the optimum temperature is also essential during the pupation period, and typical development occurs at a controlled optimum temperature (Grassberg & Reiter, 2001; Danks, 2013).

Humidity acts as a stimulus for the oviposition of Calliphorid adult females (Ashworth & Wall, 1994). It has been reported that Calliphorid females do not lay eggs unless their ankles are in contact with water and moist areas. The eggs dry up without moisture (Demirsoy, 2001). A suitable humidity environment is essential for egg laying and egg hatching. An environment with high humidity and high temperature decreases the activity of the flies and prepares for a faster death. Therefore, an environment with the possibility of evaporation increases resistance to high temperatures (Merrit, 1980). Calliphorids are typically very sensitive to ambient humidity levels at all life cycle stages, but when humidity is too high, the larvae leave the food medium, and larval development stops (Payne, 1965). Optimum humidity about temperature is essential in determining a precise developmental time. Between certain humidity limits, the growth rate of the larvae varies linearly with humidity. For larvae applied to the wound to treat non-healing wounds, the moist wound environment is ideal for the larvae to develop (Merrit, 1980).

The objective of this study is to identify the optimal temperature and humidity values for the development of *L. sericata*, a green bottle fly of forensic and medical importance, under laboratory conditions. Furthermore, the study aims to develop a standardisation protocol that can be applied consistently across different laboratories. The results of this study will ensure the continuity of the culture of *L. sericata* under laboratory conditions.

## Material and Methods

### Culture Supply with *Lucilia sericata* Colonies

A colony of *L. sericata* was established from pupae provided by the Biotherapy Laboratory of

Istanbul Cerrahpaşa University in October 2021. The obtained *L. sericata* pupae were transformed into adult individuals within 7-10 days in specially prepared 45x45x45 cm wire cages surrounded with tulle. Adult individuals were fed *ad libitum* with 20% sugar water medium and *ad libitum* food in 250 ml Erlenmeyer flasks. To feed the flies, one end of the cage was covered with cotton wool to reach the bottom of the bottle. The number of flies in a cage was maintained at approximately 500 to 1000 flies per cage under a pre-optimised day/night photoperiod consisting of 28ºC ambient temperature, 50% RH humidity and a 12:12 (L:D) hour light cycle. On the fifth day after the emergence of adult flies, 30 g of chicken liver per day in petri dishes was added to the cages for ovary development. One week later, 50 g of chicken liver was fed twice weekly for egg laying on the 12th or 13th day. Following the laying eggs of the female flies, the collected eggs were sterilized and incubated on liver agar medium prepared in petri dishes. After two days, the larvae in these petri dishes were transferred to the nutrient-agar medium in larger, approximately 15 cm diameter petri dishes to eliminate food competition by providing the massively growing larvae with a more extensive habitat and the required amount of nutrients. One part of the collected eggs was reserved for the experimental plan and targeted studies, and the other was used for colony maintenance. The larvae require a dry, calm, and shaded environment during the transition from the prepupal to the pupal stage of their development. This environment was created using plastic or glass jars (15x10x25 cm) and sawdust. During metamorphosis, a hole was made in the lid of the container or glass jar, and this part was covered with gauze to allow the air necessary for the larvae's life to enter and prevent the larvae from getting out of the container. Following a 3-5 day incubation period, petri dishes containing sterilized larvae at maximum feeding stage 3 were placed in the plastic container or jar on the sawdust. The larvae were expected to move away from the nutrient medium into the sawdust, and the petri dish was removed when they left the medium. The larvae were incubated in the fly-rearing room until they appeared as pupae, and the pupae were removed from the sawdust and placed in a new fly cage. The formation of the working groups was based on the selection of groups comprising 100-200 individuals, which had remained intact throughout the larval-rearing phase. This approach was deemed necessary to ensure the accurate evaluation of the targeted data. For each value of all studies, two repetitions were performed. In each repetition, the numerical data of the findings obtained from the determined number of groups (different numbers of study groups were formed depending on the target study) were collected, then divided by the number of groups, and the average obtained was recorded as the finding of that repetition study. The findings obtained from 2 repetitions were summed and divided by 2, averages were taken, and standard deviations were calculated.

**Experimental Design**

**Determination of differences in egg laying of *Lucilia sericata* at various temperature regimes**

In order to ascertain the egg-laying differences of *L. sericata* at varying temperature values, a standardisation was established in accordance with the reproductive potential, with experiments conducted at three distinct temperatures: 25 °C, 30 °C and 35 °C. Due to the adaptation of the flies to the laboratory, the temperature to be studied was fixed at °C, and the humidity value was fixed at the favorable humidity value, considering the possibility of humidity change depending on the temperature. A total of 6 study groups (100 females and 100 males in each study group) were formed, 2 for each temperature value and optimum humidity corresponding to this temperature. In order to stimulate egg and sperm formation in adults, they were fed with sugar

water in the first two days after emerging from pupae, and on the third day, the liver was renewed at 12 hours and fed with 50 g of liver for 24 hours. The days of oviposition, maximum oviposition days, and total number of eggs per cage were determined. The regular liver release process was carefully followed to avoid deviating from the experimental result data. In order to separate and count the eggs adhered to each other after spawning, the packets were placed in sterile falcon tubes filled with 9-10 ml distilled water and shaken gently by hand for 1-2 minutes until the separation process was realized. After separating the eggs, they were filtered through filter paper and counted directly if they were less dense or photographed if they were more dense and counted on the photograph using "AutoCAD Select Similar Programme" on the computer, and their numbers were determined. To calculate the average per cage, 6 study cages were divided into groups of 2 and labeled as L1, L2, and L3 for *L. sericata*. At the end of 12 hours in the first feeding, the total number of eggs per cage of *L. sericata* in the L1 group was recorded. Three or four feedings were made for the continuation of the cycle. At the end of a study repetition, the sums of the data obtained from the L1, L2, and L3 groups during the repetition were divided by the number of data taken (number of days of data obtained), and the number obtained was recorded as the total number of eggs per cage in one repetition. At the end of 2 study replicates, the means of the replicate findings were calculated with their standard deviations and recorded as the total number of eggs for the temperature value studied.

**Determination of egg-laying differences of *Lucilia sericata* at various humidity regimes**

Three different humidity values of 35%, 50%, and 65% were selected to determine the egg-laying differences of *L. sericata* at different humidity values, and the temperature of the laboratory environment was fixed at a pre-optimized temperature of 35°C. A total of 6 study group cages (100 females and 100 males in each cage), 2 for each humidity value, were established. The first spawning days, minimum and maximum spawning days, average of all eggs laid in one repetition per cage, and average life span were determined separately for *L. sericata* at the determined humidity values. The mathematical calculation of the target studies planned to obtain the study data was carried out by applying the same methods and mathematical calculations we followed in different temperature parameters.

**Statistical Analyses**

IBM SPSS Statistics 24.0 package program was used for statistical analysis of the data obtained in the study. Friedman's test method, which is a non-parametric method and an alternative to the repeated measures ANOVA method, was used to compare the egg-laying numbers at temperature and humidity values. This method had a significant difference in temperature or humidity values analyzed with the Wilcoxon signed-rank test, a non-parametric method, and an alternative to the dependent group's t-test method. A comparison was made at the $P<0.05$ significance level for statistical analyses.

## Results

The present study was evaluated to develop a standardization of *in vitro* temperature and humidity parameters for *L. sericata* (Diptera: Calliphoridae) species used in larval wound treatment under laboratory cultivation conditions, and the following results were obtained.

It was concluded that abiotic factors influenced the larval culture, growth, and development of *L. sericata* under laboratory conditions. At different temperatures of 25 ºC, 30 ºC, and 35ºC and constant humidity of 50%, adult life spans of *L. sericata* range between 18-30 days, first egg-

laying days between the 8th and 14th days and maximum egg-laying days between the 12th and 20th days. Days and the highest number of eggs laid between the 12th and 20th days showed that temperature is an essential criterion in spawning ability and fertility, and at 35%, 50%, and 65% different humidity values, 35 $^{o}$C constant temperature, the adult life span of *L. sericata* ranging between 24-30 days, first egg laying days ranging between the 8th and 15th days, first egg laying days ranging between the 14th and 22nd days.

It was concluded that the humidity factor is a second important criterion in regular reproduction and reproductive potential; in order to provide medical larvae of specific standards in laboratory conditions, the ambient humidity should not be above 60-65% if the ambient temperature is fixed at 35 $^{o}$C in order to prevent pathogen-induced contamination. Suppose it is desired to increase the reproductive potential of adults according to medical needs and to obtain medical larvae in a shorter time. In that case, choosing the temperature at 35$^{o}$C is appropriate by fixing the ambient humidity at 50%.

**Effects of 25 $^{o}$C, 30 $^{o}$C, and 35 $^{o}$C Temperature Regimes on Reproduction and Development in *Lucilia sericata* Female Populations**

Understanding how ecological conditions drive the evolution of body size and development time is a significant theme in biology (Gomez-Mestre & Buchholz, 2006). Based on the literature we reviewed before the study, we have analyzed the reproduction of Diptera: Calliphorid flies in the insectarium. The temperature conditions for reproduction and development are generally 25$^{o}$ C and above, and the potential for reproduction and development decreases at lower temperatures (Davies & Hobson, 1935; Mumcuoğlu et al., 2001; Blystone & Hansen, 2014). In our targeted study, depending on the physical characteristics of the insectarium in laboratory conditions, the temperatures suitable for insectarium conditions where reproductive behavior is observed in species-specific breeding and regular reproduction is observed were studied. In light of the literature data (Mumcuoğlu et al., 2001; Tachibana & Numata, 2001; Mohd et al., 2005; Polat et al., 2010; Barnes & Gennard, 2013), the ambient constant humidity value was taken as 45% during the study in order to determine the efficiency in oviposition rates at the determined critical base temperatures.

Whether there is a significant difference between *Lucilia sericata* egg-laying numbers at different temperature values was compared with the Friedman test, a non-parametric method. As a result of this analysis, the temperature values between which there was a significant difference were compared pairwise using the Wilcoxon signed-rank test, a non-parametric method.

A significant difference was found between the number of *L. sericata* eggs laying at different temperature values ($\chi 2$=21.143, P<0.05). Accordingly, when it was analyzed between which temperature values there was a difference, there was a difference between the number of eggs laying at all temperature values. The mean number of egg-laying at 35 °C was higher than the mean number of egg-laying at 30 °C and 25 °C, and the mean number of egg-laying at 30 °C was higher than the mean number of egg-laying at 25 °C (Figure 1). When the reproductive activity at all three temperatures studied under controlled conditions for *L. sericata* species was evaluated, eggs were obtained with regular reproduction in general. In the laboratory environment, stress factors (loudness, high population of flies in the cages, the density of objects around the cages, aromatic odor, for example) were encountered, negatively affecting the egglaying of flies during the egg-laying period. As a result of the trials at all three temperatures determined for the target

study, it was observed that egg-laying efficiency was low in the presence of these stress factors. In order to ensure laboratory line continuity and to establish standardization, all stress factors in the environment were eliminated. At 25 °C, spawning occurred 5-6 hours after the Petri dishes with chicken or beef liver were placed in the cages, while at 30 °C, the first spawning occurred within 2-3 hours, and at 35 °C, the first spawning occurred 1 hour after the Petri dishes with food were placed in the cages. On the other hand, it was observed that the adults were more mobile in the cages as the temperature values increased (Figure 1).

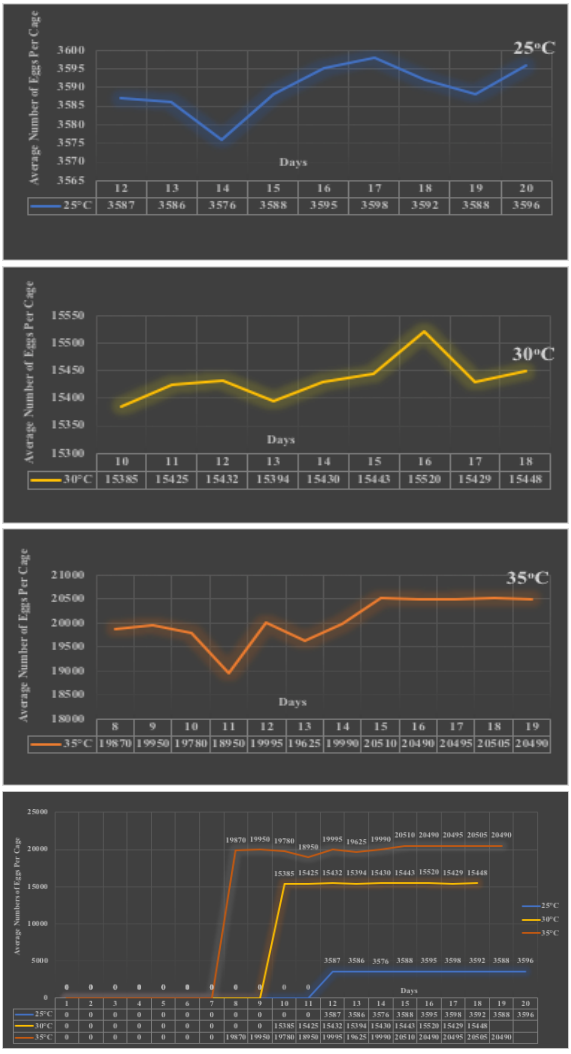


**Figure 1.** Time-dependent mean egg-laying trend of *L. sericata* at different temperatures

It was observed that *L. sericata* females usually clustered in the surface area of the lungs in the cage for reproduction, chose to lay eggs between the pieces of lungs, in the moist parts of the upper parts of the liver, and did not lay eggs in the veined and oily parts. It was observed that egg laying slowed down with drying due to the decay of the liver, so the liver was moistened by wetting from time to time. When the egg-laying potential of fresh liver and rotten lider was examined, it was determined that egg-laying was higher in moist areas where the amount of liquid was dense in rotten or near-rotten liver.

## Effects of 35%, 50%, and 65% Humidity Regimes on Reproduction and Development in *Lucilia sericata* Female Populations

The study was carried out at three different humidity values: 35% (±2), 50% (±2) and 65% (±2). Based on the literature information that we reviewed before the study, the insectarium was used for the reproduction and development of Diptera: Calliphorid flies in the insectarium for reproduction, development, and colonization Davies and Hobson (1935); Mumcuoğlu et al. (2001); Tachibana and Numata (2001); Mohd et al. (2005); Barnes and Gennard, (2013); Shefa et al. (2013); Thyssen et al. (2013); Blystone and Hansen, (2014) and Evans (1935), which showed that development is faster and eggs hatch faster in the range of *L. sericata* > 50% relative humidity and that higher relative humidity conditions are beneficial. The reproductive potential was evaluated at humidity levels below 45% relative humidity to determine the lower limit of humidity at which oviposition occurs in *L. sericata* species, and humidity levels below 35% relative humidity, which was determined to be the lower limit, was not studied. The maximum limit humidity value was determined according to our pre-study experiments to ensure the adaptation of the flies to the climate chamber (insectarium). According to the literature, most Calliphorid species are

reported to perform optimally between 80-90% relative humidity (Engelmann, 1970) for oviposition and oviposition rates. However, due to the high level of condensation in the environment when the humidity value is above 70% in the laboratory environment, the contamination situation related to the formation of fungi in the adult cages and the mortality of the cultures due to this, the maximum limit humidity value was determined as 65% and higher humidity values were not studied. In order to determine the effects of 35%, 50%, and 65% humidity regimes on the reproduction and development of *L. sericata* female populations, the temperature value at which we fixed the laboratory environment at different temperature values of 25 ºC, 30 ºC, and 35 ºC, determined according to our findings obtained from our study.

Whether there is a significant difference between *L. sericata* egg-laying numbers at different humidity values was compared with the Friedman test, a non-parametric method. As a result of this analysis, the difference between the humidity values was compared pairwise using the Wilcoxon signed-rank test, a non-parametric method.

A significant difference was found between the number of *L. sericata* eggs laying at different humidity values ($\chi 2=17.913$, $p<0.05$). Accordingly, when the humidity values were analyzed, there was a difference between the number of eggs laying at 35% humidity and those laying at 50% and 65% humidity values. The average number of eggs laying at 50% and 65% humidity values was higher than 35%. However, there is no significant difference between the number of egg-laying larvae at 50% and 65% humidity values ($p>0.05$).

## Discussion

The literature reported that the developmental stages of fly species should be graded in hours, as in Greenberg's study in 1985. Because insects are cold-blooded, they cannot keep their body temperature constant. Their activity, growth, and development rates depend on the environment's temperature, which must be at or above the minimum life temperature for insects to grow. Insects require a certain amount of heat to move from one stage of development to the next. There is usually a threshold temperature for each species, which is reported to be 15ºC for necrophagous species, and there is little data available for larval development below this temperature (Maria & Queiroz, 1996). Another study reported that carrion flies' reproduction and development is generally slower at lower temperatures (Byrd & Butler, 1996; Anderson & Cervenka, 2001). *L. sericata* eggs are often laid in areas 2-4 cm above the skin where the temperature is 28-34 °C (Cragg, 1956; Wall et al., 1992). At these temperatures in sheep fleece, the eggs hatch within 10-12 hours and the larvae feed in 2-3 days in three developmental stages (Wall et al., 1992). The critical abiotic factor limiting hatching success and the initiation of myiasis in the host is humidity (Davies & Hobson, 1935). In accordance with the results of previous studies and the data outputs obtained from our own study, the number of eggs decreased with increasing ambient humidity (Table 1 and Figure 2).

Short days and low temperatures were synergistically effective in inducing diapause in larval generations of *L. sericata* (Tachibana & Numata, 2004). In a larval culture study, Anderson (2001) found that larvae entered diapause at the prepupal stage under laboratory conditions at 15.8 ºC. Niedeeregger et al., (2010) showed that larvae developed from *L. sericata* at 13 ºC did not pupate similarly. In their study, Grassberg & Reiter (2001) noted that *L. sericata*

adults did not emerge at temperatures below 15 °C. Our study's findings, in which we examined the reproductive potential of *L. sericata* species at 25 °C, 30 °C, and 35 °C temperature regimes, parallel these literature data. It was determined that the egg-laying efficiency and egg density of *L. sericata* species increased in direct proportion to the temperature, and they showed more reproductive potential at high-temperature values than at low-temperature values (Table 2).

**Table 1.** The egg-laying tendency of *Lucilia sericata* at different humidity values

| Humidity range (%) | Time of first ovulation | Maximum ovulation days range | Average number of eggs in each cage | Average life |
|---|---|---|---|---|
| **35%** | Days 13-15 | Days 19 to 22 | 4935 pieces | Days 24 to 26 |
| **50%** | Days 10-11 | Days 18 to 22 | 17495 pieces | Days 28 to 30 |
| **65%** | Days 8-9 | Days 14 to 19 | 11324 pieces | Days 29 to 31 |

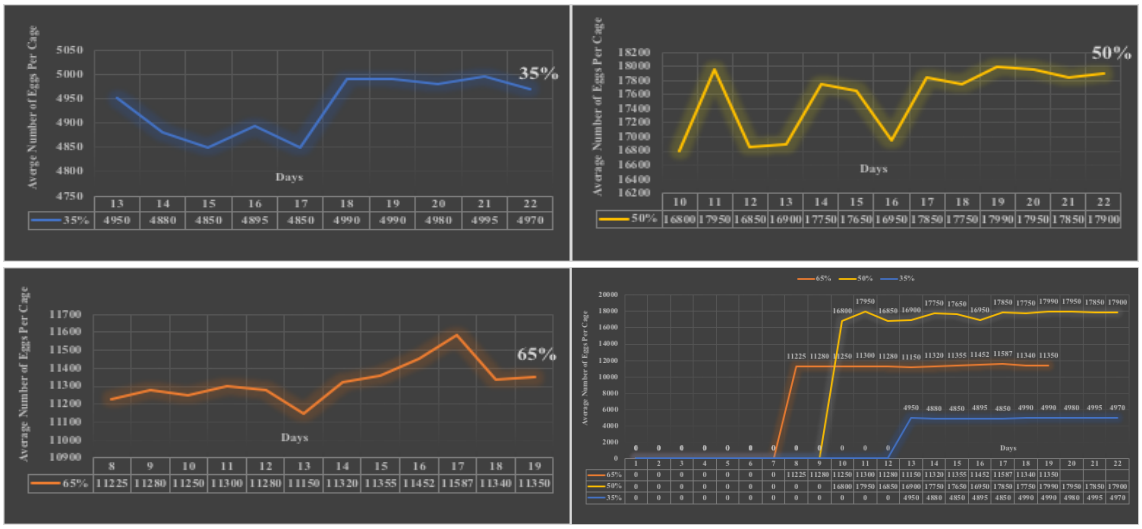


**Figure 2.** Time-dependent mean egg-laying trend of *L. sericata* at different humidity values

**Table 2.** The egg-laying tendency of *L. sericata* at different temperatures

| Temperture | Time of first ovulation | Maximum ovulation days range | Average number of eggs in each cage | The end of the culture |
|---|---|---|---|---|
| **25 °C** | Days 12-14 | Days 16 to 20 | 3490 pieces | Days 22 to 24 |
| **30 °C** | Days 10-12 | Days 15 to 19 | 15319 pieces | Days 26 to 28 |
| **35 °C** | Days 8-9 | Days 12 to 19 | 20561 pieces | Days 28 to 30 |

Cragg (1956) noted that *L. sericata* prefers warmed surfaces and does not lay eggs below 30 °C and on shaded surfaces. Niedeeregger et al., (2010) found that rearing at fluctuating temperatures between 5 ºC and 29 ºC did not improve *L. sericata* because periods of 5 ºC in the climate chamber prevented larvae from hatching. In our study, *L. sericata* was found to lay eggs at 25 ºC, which is not consistent with Niedeeregger et al., (2010) and Cragg (1956). However, when the number of eggs laying at 25 ºC, 30 ºC, and 35ºC was evaluated according to the results of the Friedman test ($p<0.05$), the temperature of 30 ºC and above at 35 ºC (Table 3) indicates a high level of reproductive potential in parallel with the increase in temperature and this respect, it is by Cragg (1956).

According to Rognes, (1991), the life span of Calliphoridae adults was reported to be between 14-21 days. Our research on *L. sericata* species found that adults lived 22-24 days at 25 ºC with 45% constant humidity, 26-28 days at 30 ºC, and 28-30 days at 35 ºC. Our findings for *L. sericata* species are parallel with those of Rognes (1991). Unlike the literature, *L. sericata* adult life span was one week longer at 30 ºC and 35 ºC, and the long life span of the adults at these temperature values is due to the consistent constancy of temperature and humidity values, which are among the environmental factors preferred by the species, as well as the regular food substrate. Insects are exposed to multiple abiotic factors in laboratory environments as well as in natural environments. Our literature review before the study revealed that humidity's effects are related to temperature, that changes in humidity accompany temperature changes, and that they can affect insect physiology, affecting the development, life span, and oviposition of many insects. (Ludwig, 1945; Engelmann, 1970; Norhisham et al., 2013). For this reason, the ambient temperature was fixed at 35 ºC for the humidity values to be determined.

**Table 3.** Friedman Test Table between *L. sericata* Egg Laying Numbers at Different Temperature Values

| Temperture | N | X±ss | Rank Mean | Chi-Square | p | Difference** |
|---|---|---|---|---|---|---|
| **(1)25 °C** | 18 | 1395,67±1800,29 | 1,44 | 21,143 | ,000* | 1 with 2,3 |
| **(2)30 °C** | 18 | 7717±7940,77 | 1,94 | | | 2 with 3 |
| **(3)35 °C** | 18 | 12231,11±10046,68 | 2,61 | | | |

It has been reported that low relative humidity (R.H.) conditions can cause detrimental effects such as decreased egg production, egg drying, and even egg mortality (Buxton, 1932; Guarneri et al., 2002) and that flies lay eggs in the direction of clustering to withstand low humidity (Davies, 1948; Hans, 2016). Norhisham et al, (2013) observed a high egg mortality rate at 20% relative humidity and found that dehydration at low R.H. caused the egg's chorion and embryo to shrink.
In determining the upper and lower humidity limit values in our study, the literature information (Davies & Hobson, 1935; Mumcuoğlu et al., 2001; Tachibana & Numata, 2001; Mohd et al., 2005; Barnes & Gennard, 2013; Shefa et al., 2013; Thyssen et al., 2013; Blystone and Hansen, 2014) was taken into consideration that the optimum humidity in the environment varies between 45% and 60% on average. The lower humidity value limit was determined to be 35%, with the idea that egg production would occur due to the high temperature fixed in the study. The upper limit of the humidity value in our study was not studied at higher humidity values due to

mortality in cultures due to increased contamination when the humidity value was 65-70% in the laboratory environment in our preliminary experiments.

In the laboratory environment with 35% humidity, it was found that the mating efficiency of the adults in the cage decreased and caused mortality. At the same time, it was determined that the low humidity in the laboratory environment also affected the food left in the cages, and the surfaces of the lungs started to dry rapidly. It was observed that there was spawning when released into the cages, but spawning decreased with drying. It was determined that the first spawning activity started later than the other humidity values studied.

According to Holmes et al, (2012), humidity is essential for ovarian maturation and egg eclosion in Diptera. In *L. sericata*, Davies & Hobson, (1935) stated that the moisture value of the environment and food are essential criteria affecting oviposition. Uvarov, (1931) states that the reproductive rate increases at higher relative humidity percentages. It was observed that spawning started earlier at 50% humidity, and reproductive potential was maximum at this value. The earliest spawning was observed at 60% humidity, but a decrease in reproductive potential was detected. Our findings regarding humidity values determined that reproductive potential did not increase directly with humidity values. We think the decrease in reproductive potential at 60% humidity is due to the formation of fungus in the cages according to the insectarium area due to the constant temperature and death of the cultures with contamination.

According to our findings (Table 4), there is a difference between the number of egg-laying at 35% humidity and the number of egg-laying at 50% and 60% humidity ($\chi 2=17.\ 913$, $p<0.05$). The average number of eggs laying at 50% and 60% humidity exceeds 35%. However, there was no significant difference between the number of egg-laying larvae at 50% and 60% humidity values ($p>0.05$).

**Table 4.** Friedman Test Table between *L. sericata* Egg Laying Numbers at Different Humidity Values

| Humidity (%) | N | X±ss | Rank Mean | Chi-Square | p | Difference ** |
|---|---|---|---|---|---|---|
| **(1)%35** | 18 | 1810,79±2436,1 | 1,42 | 17,913 | ,000* | 1 with 2,3 |
| **(2)%50** | 18 | 9181,05±8955,58 | 2,47 | | | |
| **(3) %65** | 18 | 7152,05±5612,84 | 2,11 | | | |

It was observed that the females of the species were also sensitive to relative humidity saturation. When there was not enough humidity in the environment for reproduction, they waited to lay their eggs, and the eggs laid were found to hatch due to dehydration. Humidity changes an organism's oviposition rate and total egg production (Ludwig, 1945). In our study, our findings (Table 4) on egg-laying rates at constant temperature and different humidity values within the species indicate that humidity affects reproductive potential and are in parallel with the literature data.

Each species has a humidity range considered optimal for specific physiological processes such as oviposition (Ludwig, 1945). This range is

reported to vary between 45% and 60% for *L. sericata* females to lay their eggs (Davies &Hobson, 1935; Mumcuoğlu et al., 2001; Tachibana & Numata, 2001; Mohd et al., 2005; Polat et al., 2010; Barnes & Gennard, 2013; Shefa et al., 2013; Thyssen et al., 2013; Blystone & Hansen, 2014). Our study observed that *L. sericata* reproduced at three humidity values of 35%, 50%, and 65%. We believe that oviposition was observed, albeit low, at 35% humidity, which does not comply with the literature, which we determined as the lower limit because we fixed the temperature, another influential factor in oviposition, at the appropriate value.

## Conclusion

In conclusion, this research on the mass rearing of *L. sericata* flies may be an alternative method to estimate the relationship between environmental factors of temperature and humidity and time in fecundity and the development of necrophagous species under laboratory-rearing conditions. However, parameters such as photoperiod (Nabity et al., 2006) and tissue type of rearing substrate (Day and Wallman, 2007) may affect the development time of fly fecundity and oviposition. In addition, model research for rearing the species under laboratory conditions that cover the extreme limits of the temperature and humidity range, taking into account parameters such as insectarium (fly rearing room) area, internal and external factors inside the insectarium, and fly population density, may contribute. Our evaluations of our limited study are promising. However, optimized temperature and humidity ranges can be determined with further research in wide ranges to ensure colony culture continuity and mass production under laboratory conditions.

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### Ethical Statement

This study does not present any ethical concerns.

### Author Contributions

Writing-Original Draft: N.P., Writing- review & Editing: N.P., S.M., M.K.

### Conflict of Interest

The authors declared that there is no conflict of interest.

### Data Availabilty Statement

No datasets were generated or analysed during the current study.

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