DEVELOPMENTAL STUDY OF *LUCILIA CUPRINA* AT A CONSTANT INDOOR TEMPERATURE

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Developmental Study of Lucilia Cuprina at a Constant Indoor Temperature

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Forensic entomology is the scientific study of arthropods in legal investigations. *Lucilia cuprina* is a carrion feeding blowfly and a primary colonizer commonly used to calculate postmortem interval (PMI) and time of colonization (TOC) to aid in medicolegal death investigations. PMI is the time elapsed since a person's death while TOC is the time elapsed since arthropods colonized carrion. Forensic entomologists identify exact species found on or near carrion, study research on the development rate of specific arthropods, and determine the temperature of the crime scene to estimate the rate of growth of arthropods found on carrion. By doing so, entomologists can determine the approximate time insects colonized carrion, also known as TOC. This information can assist law enforcement in cross referencing alibis and creating a more accurate timeline before the victim's death.

In this study, *L. cuprina* were taken from an existing colony at the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility at Texas A&M University to assess the development rate. The number of hours required for completion of each life stage (excluding egg) was recorded under the constant temperature of 25°C, typical of an indoor crime scene. The 1st instar larval stage was found to be the shortest stage for an average of about 6

hours while the 3rd instar post-feeding stage was the longest larval stage for an average of about 73.3 hours. However, *L. cuprina* were in their pupae stage for about 165.5 hours, which was longer than when they were in their larval stage. With the data collected from this research, forensic entomologists from the Southern parts of the United States, specifically Texas, will have a development data to reference to when aging *L. cuprina* in indoor crime scenes.

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CHAPTER I

INTRODUCTION

Carrion supports a wide variety of organisms as it goes through each stage of decomposition. Insects are attracted directly to remains as they use them for both a protein source and as an oviposition medium for their offspring (Anderson 2000). The flesh on a carcass is an excellent resource for which larvae feed on to maximize growth and development, as well as provides a source of shelter from the environment (Archer and Elgar 2003). The family Calliphoridae (Diptera), contains a genus of flies commonly referred to as blowflies, which are often the first colonizers of a body soon (minutes to hours) after death (Tomberlin et al. 2011, Goff 1993, Anderson 2000). Blowflies play a predominantly scavenger role and are largely responsible for the disposal of carrion. In 1965, Payne isolated insects and studied the stages and rates of decomposition of pigs. He found that insects undoubtedly hasten the rate of decomposition by the digestive juices they secrete, and by the mechanical processes of burrowing and consuming the carcass (Payne 1965).

Insects also provide evidence that can be used to determine how long arthropods have been present on remains (Goff 1993). This time period is often portrayed as postmortem interval (PMI), which can be inferred as time since death given certain assumptions (Tomberlin et al. 2011). PMI is often calculated by estimating the Accumulated Degree Days or Hours (ADD or ADH, respectively), which represent a specific number of thermal units that are necessary for blowfly larvae to reach a specific development stage (Reibe et al. 2010). However, recent publications suggest that a time of colonization (TOC) estimate may be a more accurate description of what forensic entomologists are actually able to calculate, as opposed to a PMI

estimate (Tomberlin et al. 2011) since a forensic entomologist is not trained on estimating the decomposition of a body and thus cannot be sure how long it was exposed before insect colonization (Wells 2018).

Nevertheless, since calliphorids are among the first colonizers of carrion, they are therefore one of the primary and most accurate forensic indicators for estimating forensically important timelines (e.g., TOC or PMI) (Grassberger and Reiter 2001, Stevens and Wall 1996). The lifecycle of a blow fly, specifically *L. cuprina*, contains four main stages: egg, larvae, pupae, and adult; however, the larvae stage can further be broken down into three stages: 1st instar, 2nd instar, and 3rd instar (Byrd and Allen 2001). Larvae grow over time and peak in size towards the end of the 3rd instar, right before they start to pupate and shrink (Kotzé et al. 2015).

The development of blowflies varies from one geographic location to another due to genetic drift and trait selections that favor local adaptations (Conner and Hartl 2004). Other studies have found differences in development rate of blowflies of the same species from different geographic locations due to factors such as temperature and local climate (Owings et al. 2014, Gallagher et al. 2010). However, there are few published articles on the developmental rate of *L. cuprina* in the southern regions of the United States, specifically in an indoor environment in Texas. Therefore, a detailed study of *L. cuprina* at a constant temperature of 25°C was conducted due to potential differences in development sets from *L. cuprina* in different geographic locations.

CHAPTER II

METHODS

Colony Maintenance and Experiment Design

An existing colony of *Lucilia cuprina* provided flies for this experiment and was maintained in the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility at Texas A&M University. Flies were kept in a climate-controlled room set to 25°C ± 2°C, 50 ± 5% relative humidity (RH), and a 14:10 light:dark cycle. Adult flies were fed a 50:50 composition of sugar:powdered milk mix and provided water *ad libitum*.

For the experiment, eggs were collected from the colony and isolated into separate mason jars containing vermiculite to rear out the larvae. Upon pupation, 500 pupa were removed and placed in individual condiment cups with vermiculite to prevent breeding of newly emerged flies. Condiment cups were checked twice a day for emergence and sex was determined by observing the ocular distance of adult blow flies. Roughly 100 adults of each sex were separated into a cage ($27cm^3$, BioQuip BugDorm) containing water, a mixture of sugar and powdered milk, and bovine blood. After 8 days, cups containing 10g of beef liver were placed in the cage at 8am as an oviposition medium for females. Female blowflies were given 4 hours to oviposit their eggs before egg cups were removed. Eggs were monitored every 8 hours until they hatched. Once larvae were present, 200 larvae were counted and placed in a mason jar containing approximately 350g of sand and 20g of beef liver. This process was repeated for 4 jars for replication. Larvae were provided an additional 10g of beef liver daily until pupation was observed.

Data Collection

After larvae were separated into mason jars, three were randomly sampled (i.e., removed) every 8 hours. During sampling, three larvae were blanched in boiling water to ensure development stopped, and then stored in 80% ethanol for preservation until they were processed. Larvae were sampled from each jar until initial pupation was observed. After initial pupation, pupae were continuously monitored at 8-hour increments until initial emergence was observed. Larvae were aged by examining the spiracles on the posterior end, as seen in Figure 1. The life stages used to categorize larvae were 1st instar, 2nd instar, 3rd instar feeding, and 3rd instar post-feeding (wandering) stage. Figure 2 below illustrates how 3rd instar larvae were differentiated between feeding and post-feeding. This concluded the experimental portion of the study.

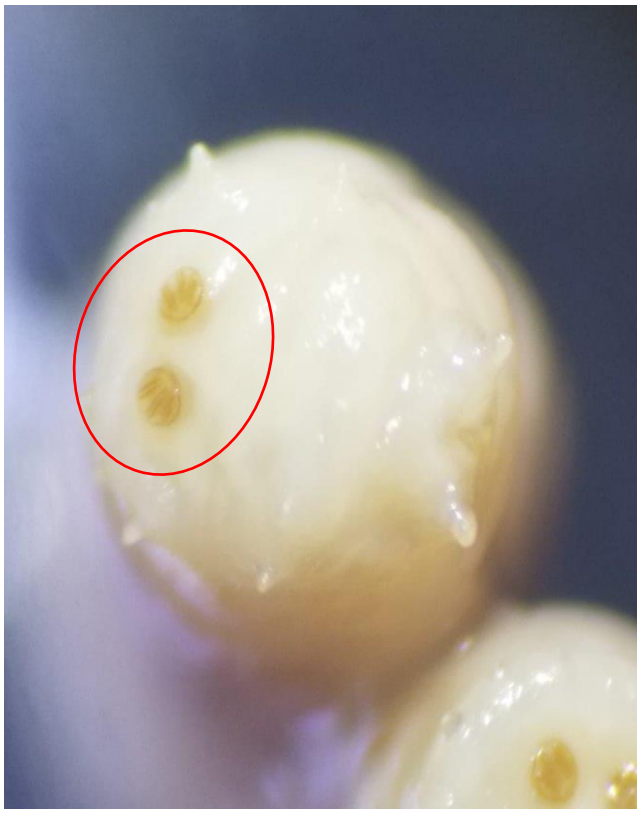

Figure 1. Posterior end of a larva in 3rd instar as can be defined by the three posterior spiracles in each circular region.

Figure 2. Left larva depicts a 3rd instar in its feeding stage while the right larva depicts a 3rd instar in its post-feeding stage.

Statistical Analysis

R studio was used to determine whether there was a jar effect for this study through a 1-way Analysis of Variance (ANOVA).

CHAPTER III

RESULTS

Figure 3 shows the average time *L. cuprina* remained at each life stage. *L. cuprina* were in their 1st instar, 2nd instar, 3rd instar feeding, 3rd instar post-feeding, and pupae stage for roughly 6.0, 15.3, 10.5, 73.3, and 165.5 hours, respectively. The shortest larval stage was the 1st instar stage while the longest was the 3rd instar post-feeding stage. Furthermore, the standard deviation for 1st instar, 2nd instar, 3rd instar feeding, 3rd instar post-feeding, and pupae was 2.8, 6.2, 3.7, 13.6, and 7.9 h, respectively. The standard deviations are shown below as error bars in Figure 3.

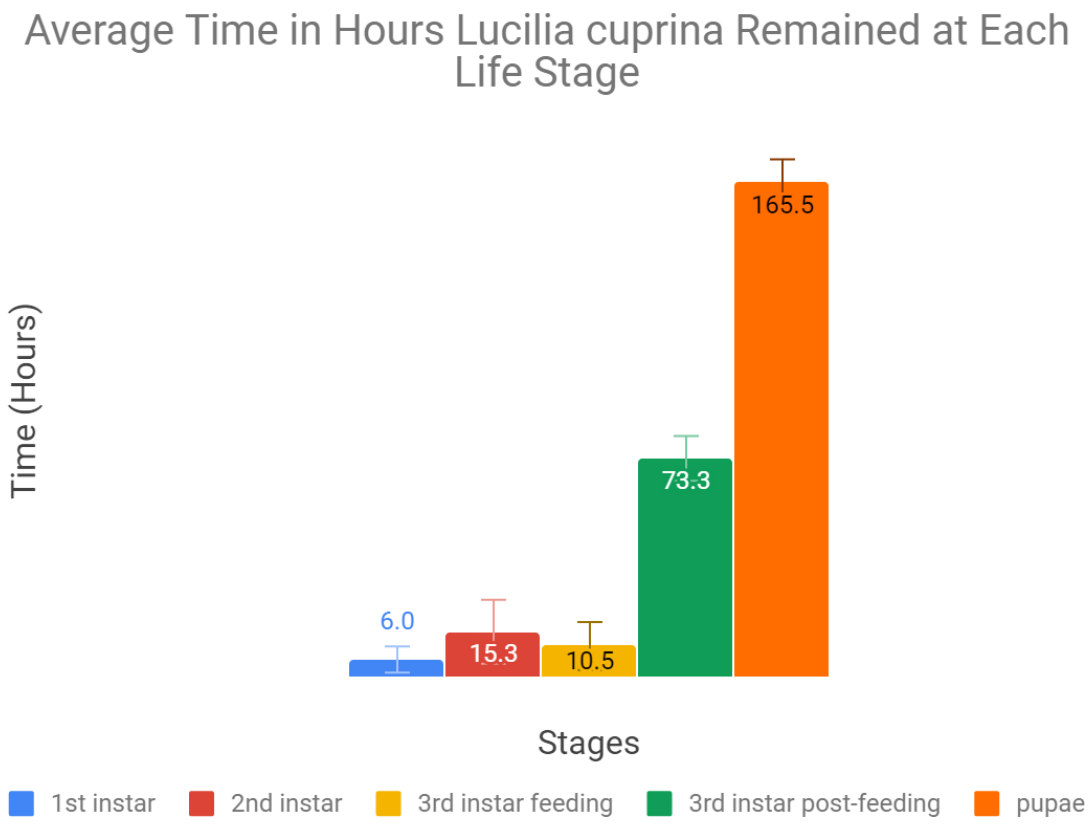

Figure 3. Average time in hours *L. cuprina* remained in each stage. Hour 0 being the moment larvae were isolated into their respective mason jars.

As seen in figure 4 below, it took 121.1, 102.1, 123.4, and 94.8 hours for jars 1-4, respectively, to reach initial pupation. Initial pupation was noted when at least 1 pupa was observed in a mason jar.

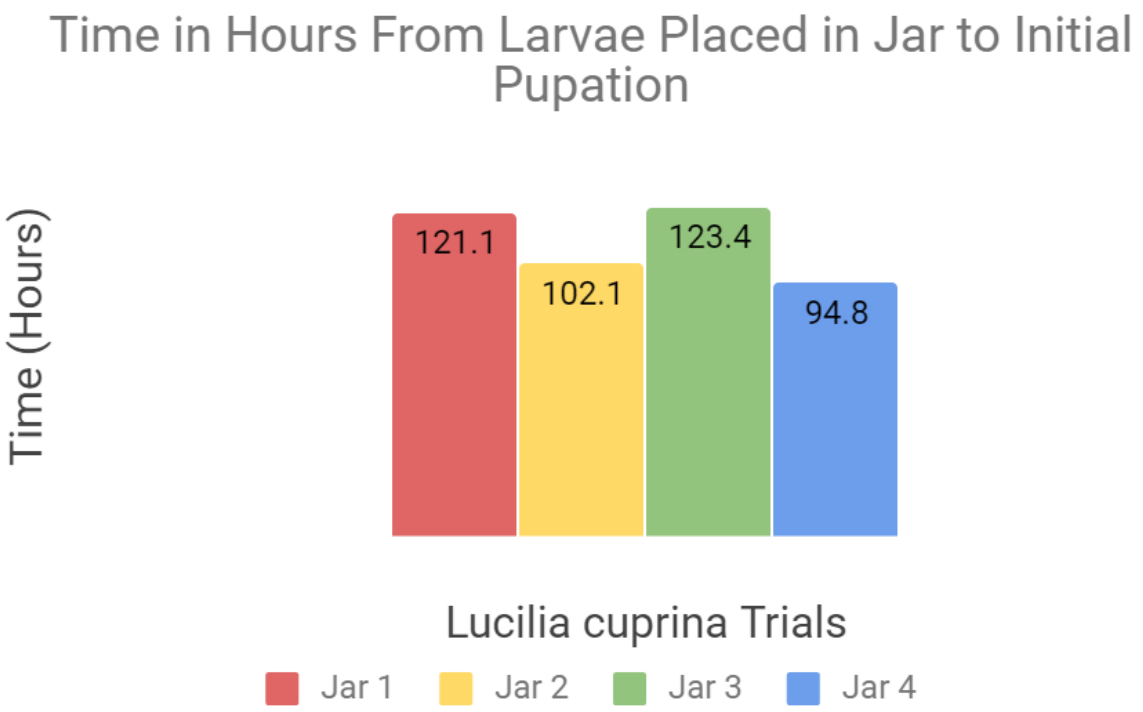

Figure 4. Time it took in hours for *L. cuprina* to reach initial pupation for each jar. Hour 0 being the moment larvae were isolated into their respective mason jars.

In Figure 5 below, *L. cuprina* in jars 1-4 took 159.0, 167.1, 160.0, and 176.0 hours, respectively, to reach initial emergence from initial pupation. Initial emergence was indicated when at least 1 adult fly was observed in the mason jar.

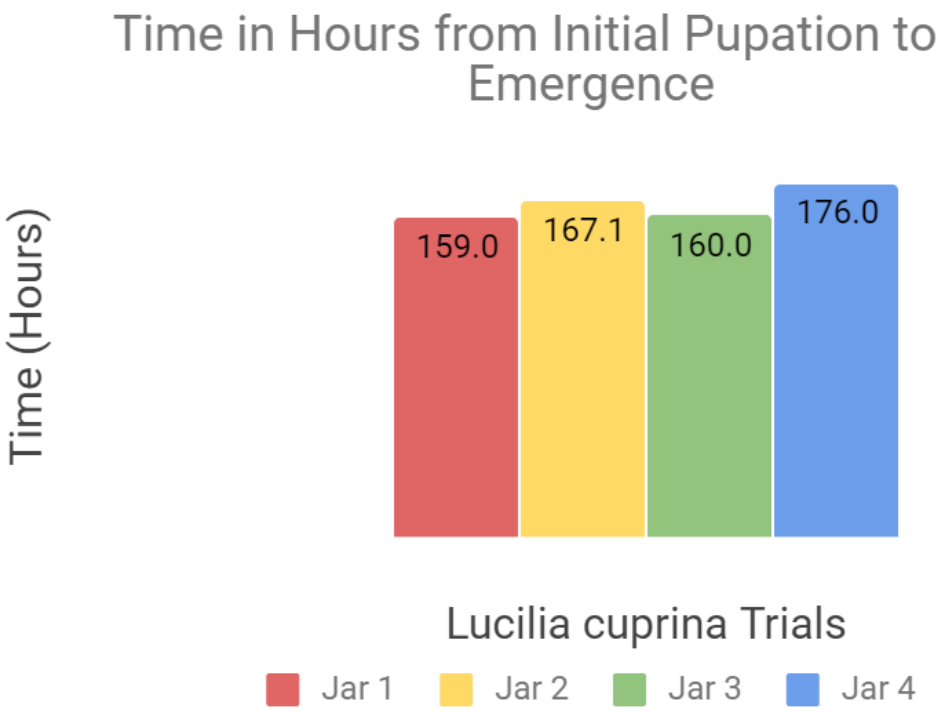

Figure 5. Time it took in hours for *L. cuprina* to reach initial emergence from initial pupation for each jar.

No statistical effect of jars on development time for larvae up to pupation (Figure 4; P = 0.472) and during pupation (Figure 5; P = 0.279) were found.

CHAPTER IV

DISCUSSION

Forensic Applications

The Centers for Disease Control and Prevention (CDC) stated that in 2015, 74% of all deaths in the United States were caused by heart disease, cancer, chronic lower respiratory disease, accidents, strokes, Alzheimer's disease, diabetes, influenza and pneumonia, kidney disease, and suicide (Heron 2017). A majority of these deaths occurred indoors in hospitals and homes just as a majority of homicides occurred indoors (Anderson 2010). Furthermore, temperature is the most important variable affecting the decomposition of a body (Mann et al. 1990). In other words, the ADD/ADH of blow flies is dependent on the specific temperature of the crime scene. Therefore, forensic entomologists obtain temperature and weather data of outdoor crime scenes by collecting data from nearby weather stations (Amendt et al. 2011). Fortunately, temperature is easier to obtain for indoor cases since it is usually kept at a constant temperature with an A/C unit. With this in mind, *L. cuprina* colony and larvae were kept in a constant temperature of 25°C to represent an indoor crime scene.

The second most important variable affecting the decomposition of a body is insect activity (Mann et al. 1990). Depending on accessibility of these bodies, necrophagous insects can quickly colonize and deposit their eggs on corpses within a matter of minutes to hours. Therefore, forensic entomologists are able to estimate a TOC from the largest larvae found on carrion because the largest larvae are expected to be the oldest and one of the first larvae on carrion.

Regardless of the geographic barrier, the development rate of *L. cuprina* larvae from Figure 3 corroborated the patterns observed from other *L. cuprina* development studies around the world (O'Flynn 1983, Day and Wallman 2006). O'Flynn conducted research in Queensland, Australia over a range of temperatures from 15°C-34°C. On the other hand, Day and Wallman conducted their study in Wollongong, NSW, Australia at a temperature-controlled room of 25°C ± 3.5°C. Data from both papers used ADD rather than ADH; however, when converting the data collected in this study into ADD, the development rate amongst the studies were about 1-3 days off. Patterns were similar from these studies in that the 1st instar stage was very brief while the 3rd instar stage was the longest larval stage. Furthermore, *L. cuprina* were in their pupae stage longer than their larval stage, which can be observed by comparing Figures 4 and 5 together.

According to evolutionary theory, the survival of individual and species occur because growth and learning takes place in their early lives (Sluckin 2018). In a study conducted by Marshall et al. (2010), the survival rate of Pacific oyster (*Crassostrea gigas*) larvae corresponded to faster growth rate (Marshall et al. 2010). In another study, the growth of Tobacco hornworm (*Manduca sexta*) larvae from about 1mg to 10g in under three weeks helped them experience a wider range of temperatures throughout the day for higher survivability (Kingsolver et al. 2011). Owings et al. (2014) conducted a study and determined that larger *Cochliomyia macellaria* larvae corresponded to larger adults where larger blow flies benefited from increased female fertility and wing surface area (Owings et al. 2014). From the ideas of these development studies of other species, we can speculate that the *L. cuprina* 1st instar stage is the shortest larval stage because their survivability depends on faster growth. By growing in size, 1st instar larvae become more mobile, less vulnerable to predators (e.g., fire ants), and can better compete with other larvae and species on the same food source.

Limitations

The greatest limitation we faced was sampling larvae three times a day. Since sampling took place every 8 hours, this created a greater probability of missing instar transitions during development. This is most likely why the 2nd instar stage was longer than the 3rd instar feeding stage in Figure 3. Furthermore, the data for the 1st instar includes a 4-hour window of error of emergence, since egg cups were taken out 4 hours after being placed inside the cages. Therefore, eggs could have been laid anytime from time 0-4 hours. Another limitation of this study was the sample size. We were only able to sample 3 larvae per 8-hour window due to the low density (200 larvae) of larvae in each jar. Having a smaller sample size limited the data we were able to analyze and was an inaccurate representation of the larvae in the jars.

Future Applications

Future studies may include the study of *L. cuprina* under several temperatures ranging from ~15ºC to ~35ºC. This range of temperatures include the known lower and upper temperature thresholds of *L. cuprina*, which can influence behavior and development (Richards et al. 2009). Developmental data sets at different temperatures are crucial because investigators handle deaths in different regions and times of the year where temperatures are known to differ. Future studies may also include more sample times throughout the day to obtain greater resolution in development time estimates and to minimize the chances of missing instar developmental growth between stages.

CHAPTER V

CONCLUSION

Forensic entomologists must know three key pieces of information to accurately determine the TOC: the species of insects found on the body, the rate of development of the immature arthropods found on the body, and the temperature of the crime scene (Amendt et al. 2011). Incorrect information of any of the three listed above can greatly skew an entomologist's TOC estimate, which could result in wrongfully convicting an innocent person to jail.

Entomologists must keep in mind that in a real indoor crime scene, the body may not be accessible for colonization right away due to closed doors and windows. Knowing this, the PMI and the TOC may be minutes, hours, or even days apart. Therefore, forensic entomologists use blow fly development data to determine the TOC rather than the PMI since entomologists are trained on the development of arthropods rather than the decomposition of the body.

There are few articles on the development study of *L. cuprina* in Texas, specifically at an indoor setting. This study was conducted to provide extra information needed for entomologists to accurately determine the TOC for forensic applications. Regardless of the limitations, this study provides a valuable reference on the ADH of *L. cuprina* at a constant indoor temperature of 25°C from 1st instar to initial emergence of adult blow flies. By comparing the data collected in this study with the data collected in O'Flynn's (1983) and Day and Wallman's (2006) study, it is observed that the ADD differ by over a day. This emphasizes the importance geographic location has on the developmental rate of the same species.

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