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ORIGINAL ARTICLE

Nutrition and Water Deprivation Negatively Impacts Adult Longevity of *Lucilia eximia* (Diptera: Calliphoridae)

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ABSTRACT

Introduction: Forensic entomology is the utilization of arthropod science in legal practice. Blow flies (Diptera: Calliphoridae) are a prevalent family in medicolegal investigations due to their colonization of vertebrates, including living or deceased humans. Longevity of insects associated with legal investigations is an important life-history trait that could be useful in determining a minimum time of colonization (TOC) interval. *Lucilia eximia* (Wiedemann) (Diptera: Calliphoridae) is known to colonize remains and cause myiasis; however, this species is understudied. The purpose of this study was to investigate the longevity of *L. eximia* adults with and without resources. **Methods:** *Lucilia eximia* adults were placed in six different mesh cages at a 1:1 sex ratio, where flies in three cages were fed and provided water, while those in the remaining cages were not. Cages were placed in a walk-in incubator set to 24°C, 60% RH, and a 14:10 L:D. Mortality was recorded daily. **Results:** Males and females did not live significantly longer than each other within either treatment. Life span of adults provided resources was 58.41 ± 27.79 d, while deprived individuals lived 1.61 ± 0.49 d. Rate of mortality was nearly 6X greater for those deprived of food and water than those provided such resources. **Conclusions:** Access to food and water impacted adult longevity. Forensic entomologists could potentially estimate time since adult emergence based on mortality proportion of adults present in relation to environmental conditions and access to food in an enclosed environment (e.g., building). However, such a method will need to be validated.

KEYWORDS: nutrition ecology, longevity, resource availability, medicolegal investigations, forensic entomology

INTRODUCTION

Forensic entomology is the application of arthropod science in legal practice [1] and is typically used in cases concerning contamination of stored products or human structures [2], as well as abuse, neglect, or death of humans or other vertebrates [2, 3] (e.g., myiasis [4,5,6]). Because insect development is partially regulated by temperature [3], estimating the age of immature insects collected from living or deceased humans can be used to determine when colonization occurred [1, 3]. Thus, published development data sets are essential for making such calculations [3]. Blow flies (Diptera: Calliphoridae) are some of the first arthropods to colonize vertebrate remains [7]. To date, there are over 1,000 calliphorid species comprising approximately 150 genera, many of which remain understudied [8].

Adult blow fly longevity is an important life-history trait to record as related to forensic investigations. Longevity could provide the time of colonization (TOC) of dead adults found at a location assuming that they were produced from offspring that developed on the remains [9]. Longevity information is also necessary in studies focused on quantitative aging techniques, such as cuticular hydrocarbon [10] or pteridine fluorescence [10] analyses- both of which are relevant to forensic entomology. Such applications require

baseline data on the lifespan of the targeted species. Fecundity has been shown to negatively correlate with longevity, potentially due to the energy required for reproduction [11]. Consequently, longevity data could potentially be used to provide further insight into the overall pattern of reproductive exhaustion by individuals of a targeted species.

Lucilia eximia (Wiedemann) (Diptera: Calliphoridae) is found primarily in South America [12, 13] and the southeastern United States (primarily Texas and Florida, USA) [14]. Similar to other blow fly species, *L. eximia* is known to oviposit on vertebrate remains [15, 16, 17, 18] and human food waste [19]. This species has also caused myiasis on domestic rabbits (*Oryctolagus cuniculus*) [20] and other domesticated animals [21, 22]. In addition, *L. eximia* caused urogenital myiasis of a human male in Texas, USA [23]. Due to colonization of living and deceased vertebrates, *L. eximia* is a forensically important blow fly species [16, 17]. However, beyond the information outlined above, little is known about *L. eximia* when compared to other blow flies. The aim of this study was to determine the longevity of *L. eximia* with or without food and water. Information on longevity will provide greater understanding of the previously unknown life-history strategies of this species and improve understanding of their utility in forensic entomology.

MATERIALS AND METHODS

Colony Maintenance

Lucilia eximia larvae were obtained from chick (*Gallus gallus domesticus*) and rat (*Rattus norvegicus*) carrion at the Texas A&M University Farm in Snook, Texas (30.552278°N, 96.424519°W), and Coulter Airfield in Bryan, TX, USA (30.7161°N, 96.3330°W) during a study in August 2019. Adults were allowed to emerge in BugDorm© 30 x 30 x 30 cm (Taiwan) insect cages at the Forensic Laboratory for Investigative Entomological Sciences (FLIES) at Texas A&M University (College Station, TX, USA). A colony of *L. eximia* has been maintained without being supplemented with wild-type individuals for more than eight generations.

Colony adults were provided with a standard diet consisting of a 1:3 ratio of granulated sugar to milk powder, as well as water *ad libitum*. The colony was maintained at 27ºC, 70% RH, with a 14:10 L:D cycle. To stimulate egg production, 6 ml bovine blood on a Kimberly – Clark Kimwipe (Irving, TX, USA) in a plastic 30 ml cup were provided to adult flies 7, 9, 11, and 13 d post emergence. Approximately 25 g of bovine liver were provided to adult flies on 17, 19, 21, and 23 d post emergence as an oviposition site. Both the blood and the liver were left in the cage for 48 h before replacement. Liver with eggs or larvae present were placed in large (946 ml) glass mason jars containing a 16 cm layer of vermiculite. Larvae were provided with additional liver as needed until the wandering third instar. Approximately one week after eggs being collected and placed in the jar, vermiculite and wandering larvae were transferred into aluminum pans (20 x 12 x 5 cm) and placed into insect-rearing BugDorm© cages to pupate. Resulting adults from generations eight through ten were either used in the experiment described below or to maintain the colony.

Experiment Design

Three trials were conducted with each trial representing a generation. In the first trial, *L.eximia* pupae were individually separated into 60 ml condiment cups on a 2 cm layer of vermiculite. Singular pin holes were punched into the lid of each cup to allow ventilation and stored in the walk-in incubator set to 24°C, 60% RH, and a 14:10 L:D. Pupae were monitored every 24 hr for emergence. Preliminary data indicated a low percentage of emergence (<50%); therefore, to improve emergence in trials two and three, pupae were kept in aluminum pans (20 x 12 x 5 cm) with vermiculite and placed in a 30 x 30 x 30 cm BugDorm cage as previously described and checked every 24 hr for emergence. Emergent adults were sexed via interocular spacing [24] and sorted into six 30 x 30 x 30 cm wire mesh cages (Bioquip, USA). Each cage housed approximately 20 adults of the same age, apart from the first trial where each cage housed approximately 14 adults (all at a 1:1 male: female) of the same age. All cages were kept in the walk-in incubator previously described; cage locations were moved haphazardly to prevent placement effects. Flies in three cages (i.e., technical replicates) were deprived of both food and water, while

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those in the remaining three cages were provided with a 1:3 ratio of granulated sugar to milk powder in a plastic 90 mm petri dish and water from glass jars with paper towels to prevent drowning *ad libitum* (approximately three times per week). Mortality was recorded every 24 hr. Resulting dead adults were removed, and sex determined.

Statistical Analyses

Using the Shapiro-Wilks Test, data were determined to be non-normally distributed ($p < 0.0001$). Due to an inability to transform the data into a normal distribution using standard methods (i.e., Log, Square Root), a Kruskal Wallis test was used to determine if sex, treatment (standard diet or starved), trial, and/or cage impacted the longevity of *L. eximia* individuals. Significant results were then followed up using a Dunn post-hoc test with a Bonferroni correction, and alpha being set to 0.05. A One-way Analysis of Covariance (ANCOVA) was conducted to compare rate of mortality per day between standard diet and starved treatments. All assumptions associated with ANCOVA were met prior to performing the analysis. All statistical analyses were done in R version 4.0.3 (R Team 2020).

RESULTS

Adult access to food and water significantly increased longevity, as flies on the standard diet lived over 36x longer on average than the flies under dietary stress ($p < 0.0001$) (Figure 1). Individuals under dietary stress lived one to two days with an average of 1.61 d (± 0.49 d) (Table 1). For individuals provided water and the standard colony diet of sugar and milk powder, lifespan ranged from 2 to 118 d with an average of 58.41 d (± 27.79 d) (Table 1). Females and males on a standard diet lived an average of 57.27 d (± 28.61 d) and 59.45 d (± 27.18 d) respectively. Lifespan between sexes within a treatment was not statistically significant ($p = 0.626$). Trial ($p = 0.856$) and cages ($p = 0.758$) were not significant factors. Mortality rates (proportion of individuals dead/day) for both standard diet and starved individuals had a strong correlation with time ($R^2 = 0.9744$ and 1 respectively) (Figure 1). In addition, linear equations for standard diet ($y = 0.0098x - 0.0631$) and starved ($y = 0.6053x - 0.2106$) treatments were significantly different from each other ($F_{1,103}$ 121= 3.2498, $p < 0.001$).

DISCUSSION

Adult *L. eximia* provided with food and water far outlived adults deprived of these resources. Adults lived an average of 58.41 d, while males and females within treatment did not have statistically significant differences in life span. It is important to emphasize that this study was conducted under climate-controlled conditions. Provided the flies have access to food and water and are protected from natural predators, these data serve as an estimate for longevity of this population in a constant indoor climate. Indoor conditions with protection from natural predators as simulated in this study are experienced commonly by blow flies as they have been documented causing myiasis of patients in medical facilities [5, 25], as well as serving as a vector of pathogens to people in urban settings [26].

Table 1 Average ± SD, minimum, and maximum recorded lifespan of *Lucilia eximia* (Wiedemann) (Diptera: Calliphoridae) fed a standard diet and provided water or starved and without water for male, female, and adults overall

	Overall		Male		Female	
	Standard Diet	Starved	Standard Diet	Starved	Standard Diet	Starved
Average Life Span (Days) ± STDEV	58.41 ± 27.79	1.61 ± 0.49	59.45 ± 27.18	1.57 ± 0.50	57.27 ± 28.61	1.65 ± 0.48
Minimum Life Span (Days)	2	1	2	1	2	1
Maximum Life span (Days)	118	2	118	2	112	2

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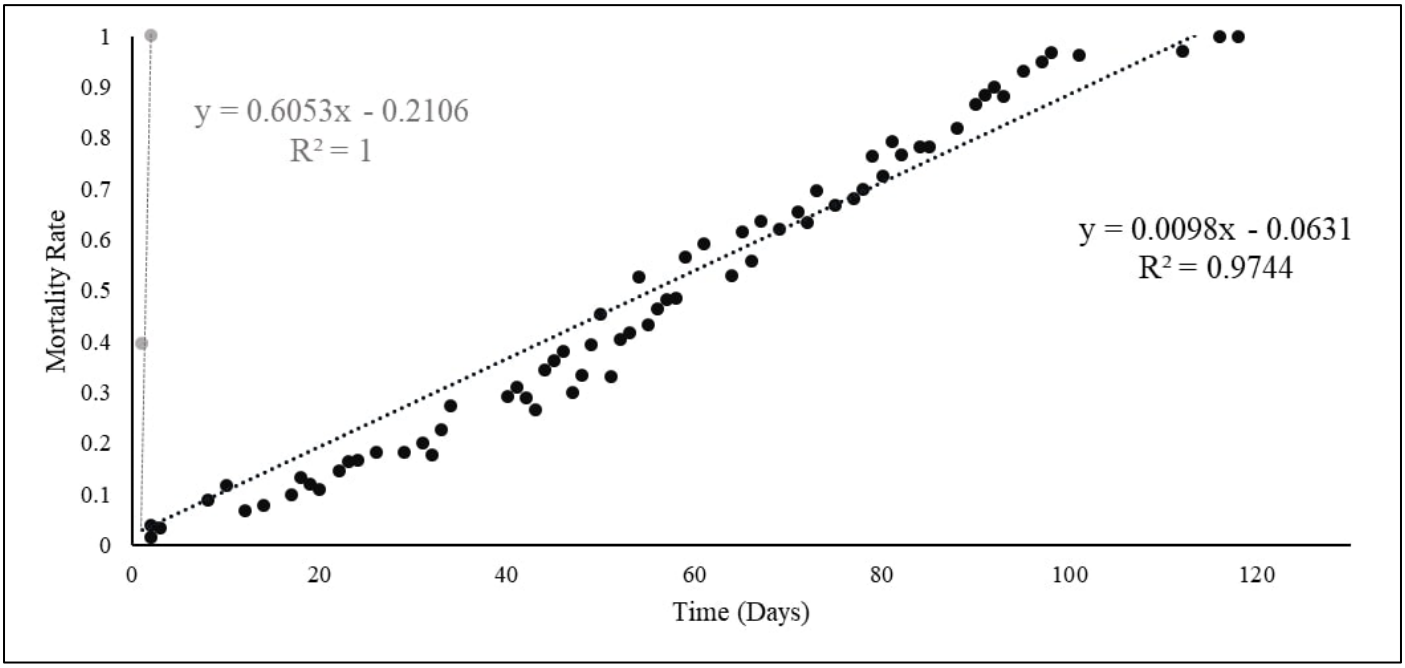

Figure 1 Morality rate of *Lucilia eximia* per day. Standard diet mortality is denoted in black, while the starved treatment is denoted in grey. Trend line is based on the denoted linear equation for each treatment and their respective R^2 values

Deprivation of food and water was predictably a limiting factor for longevity, as established in studies with *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) [27]. This trend has also been observed in blow flies, with starved populations of *Chrysomya chloropyga* (Wiedemann) (Diptera: Calliphoridae) never surviving past three days on average even when provided with food prior to starvation [28]. This study was unique from others [such as 27, 28] in that the starved treatments were also deprived of water and may explain why *L. eximia* in this study lived up to 2 d shorter than *D. melanogaster* [27] and 1 d shorter than *Ch. chloropyga* [28]. While this study highlights nutritive stress through starvation, nutrient deficiencies in adult blow fly diets can also have negative effects on longevity. *Calliphora stygia* (Fabricius) (Diptera: Calliphoridae) life span varied significantly when provided diets that varied in dietary fat [29]. Therefore, more research needs to be conducted regarding the factors that contribute to variability in longevity across forensically important blow flies.

Lucilia eximia longevity did not vary by sex. This result was unexpected as other blow flies have been identified to have differences in longevity between males and females. For example, both *Lucilia cuprina* (Wiedemann) [30] and *L. sericata* (Meigen) (Diptera: Calliphoridae) [31] females lived longer than males. Discrepancies in longevity among sexes could potentially be driven by a difference in nutritional requirements for reproduction, fat storage in immature stages, or intersex interactions and sexual conflict [29]. However, reproductive costs and male harassment were shown to contribute to a decreased female longevity in *D. melanogaster* [32]. These factors are potentially less significant for *L. eximia* than closely related flies. Though, further research should be conducted. Flies in this study were not provided blood meal, which is necessary for egg production in many blow fly species [33]. Perhaps this nutritional deficiency and subsequent absence or reduction of mating events in this study removed the physiological cost of reproduction and eliminated factors contributing to discrepancies among sex in these flies.

Compared to other blow fly species, individuals from the *L. eximia* population examined in the current study lived longer overall. For example, *L. eximia* lived up to 46.69 d longer on average than what has been reported for individuals from a population of *L. cuprina* from Australia [30]. This trend is apparent for other populations of other *Lucilia* spp. where *L. eximia* lived up to 30.75 d longer than *L. sericata* [31]. When compared to *Cochliomyia macellaria* (Fabricius) and

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Chrysomya megacephala (Fabricius), species that *L. eximia* overlaps geographically, temporally, and has been observed co-colonizing carrion [15], *L. eximia* tends to live longer. *Lucilia eximia* outlived the only known dataset for *C. macellaria* by up to 30.75 d [34], and *Ch. megacephala* by 34.15 d [35]. These data indicate *L. eximia* may employ different life-history strategies that allow for coexistence with known blow fly competitors by occupying alternative temporal niches. However, longevity is likely not the only indicator for coexistence with other blow fly species. *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) has displaced *L. eximia* within five years of its introduction in Costa Rica [36], despite *L. eximia* living up to 25.02 d longer [37].

Longevity data could be important in a forensic context as evidence. Currently, less is known about adult, rather than larval, biology in a forensic context. This discrepancy is largely due to TOC estimates traditionally focus on the period of insect activity (PIA) after colonization has already occurred, known as the post-colonization interval (post-CI) [1]. Adult biology and behavior have been shown to provide insight to the PIA based on studying the pre-colonization interval (pre-CI) [38]. For example, colonization events may be delayed due to environmental temperatures such as photoperiod, temperature, and humidity [38]; however, blow flies have been known to oviposit at night if they happen to be placed near remains in artificial light, which can impact estimations of TOC by up to 12 h [39]. This method is useful for determining the complete PIA prior to or coinciding with larval activity [1]; nevertheless, data from this study could prove useful when dealing with decedents in late stages of decomposition (see [40] for descriptions for stages of decomposition). Assessing adult mortality relative to living adults present at remains in enclosed environments combined with environmental conditions (e.g., temperature, RH, presence or absence of food) could potentially be used to determine the time to pass since first adult emergence, a key part of the post-CI. For example, if there is an enclosure at room at approximately 24°C with available food and water and 50% of adult *L. eximia* found in the vicinity were dead, using the linear model for standard diet ($y = 0.0098x - 0.0631$), approximately 57.46 d would be added to the calculated accumulated development time (from egg to eclosion) for *L. eximia.* Alternatively, if no food or water are present approximately 1.17 d would be added to the accumulated development time based on the alternative linear model. Validation of these models and more research examining individuals from other populations needs to be done to better understand the utility of this method. The shortened longevity of blow flies deprived of food and water as seen here may explain the lack of blow fly activity in combination with elevated thermal stress in cases where the remains are located in nutrient poor and arid environments (such as [41]). By further studying the effects of age on the behavior and physiology of *L. eximia* and other forensically relevant blow fly species, more information can be gleaned about the pre- and post-CI, in addition to the factors which effect blow fly attraction and acceptance of carrion resources as a fly ages.

CONCLUSIONS

Lucilia eximia lived significantly longer when fed and provided water. Data indicate the population of this species has a longer lifespan than other blow fly species. Additionally, unlike other blow fly species, there was no difference in lifespan between male and female *L. eximia* within a treatment. Furthermore, generating linear models by utilizing mortality rates may be a useful tool when evidence of completed lifecycles are found on remains kept in enclosures. Under more specific dietary conditions, longevity of this species will vary, so more studies are warranted.

Conflict of Interest

Authors declare none.

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Authors Contributions

SJS designed and directed the project, SMG, OC, KD, and SJS conducted the research. SJS conducted statistical analyses, and SMG led the writing of the manuscript. JKT is the principal investigator and provided the material, space, and oversight for this research. All authors provided critical feedback and helped shape this manuscript.

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