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 the adults 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
Longevity with Varying Nutrition

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 27°C, 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, Leximia 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
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 \textit{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 \textit{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 ($\pm$ 0.49 d) (Table 1). For individuals provided water and the

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

DISCUSSION

Adult \textit{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 $\pm$ SD, minimum, and maximum recorded lifespan of \textit{Lucilia eximia} (Wiedemann) (Diptera: Calliphoridae) fed a standard colony diet of sugar and milk powder, lifespan ranged from 2 to 118 d with an average of 58.41 d ($\pm$ 27.79 d) (Table 1). Females and males on a standard diet lived an average of 57.27 d ($\pm$ 28.61 d) and 59.45 d ($\pm$ 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 ($F_{1,103} 121 = 3.2498, p < 0.001$). |
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...
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.

REFERENCES
1. Tomberlin, JK., Benbow, ME., Tarone, AM., Mohr, RM. Basic research in evolution and ecology enhances forensics. Trends in Ecology & Evolution. 2011; 26: 53-55.
2. Catts, EP., Goff, ML. Forensic entomology in criminal investigations. Annual Review of Entomology. 1992; 37: 257-272.
3. Amendt, J., Krettek, R., Zehner, R. Forensic entomology. Naturwissenschaften. 2004; 91(2): 51-65.
4. Hall, M., Wall, R. Myiasis of humans and domestic animals. Advances in Parasitology. 1995; 35: 257-334.
5. Singh, A., Singh, Z. Incidence of myiasis among humans—a review. Parasitology Research. 2015; 114: 3183-3199.
6. Azeredo-Espin, AML., Madeira, NG. Primary myiasis in dog caused by Phaenicia eximia (Diptera: Calliphoridae) and preliminary mitochondrial DNA analysis of the species in Brazil. Journal of Medical Entomology. 2014; 33: 839-843.
7. Galante, E., Marcos-Garcia, MA. Decomposer insects. In: Encyclopedia of Entomology. 2nd ed. ed J. L. Capinera. Springer. Boca Raton, FL. 2008; 1158-1169.
8. Shewell, GE. Calliphoridae. In: Manual of Nearctic Diptera, 2nd ed. Ed J.F. McAlpine. Canada Communication Group. Toronto, Canada. 1987; 1133-1145.
9. Boatright, SA., Tomberlin, JK. Effects of temperature and tissue type on the development of Cochliomyia macellaria (Diptera: Calliphoridae). Journal of Medical Entomology. 2010; 47: 917-923.
rodents in a secondary forest in Southeastern Brazil. European Journal of Entomology. 2008; 105: 691-696.

19. Guimarães, JH., Papavero, N., Prado, ÂPD. As miíases na região neotropical (identificação, biologia, bibliografia). Revista Brasileira de Zoologia. 1982; 1: 239-416.

20. Ipek, DNS., İpek, P. A case of traumatic myiasis in a domestic rabbit (*Oryctolagus cuniculus*) caused by Lucilia sericata. Türkiye Parazitoloji Dergisi. 2012; 36: 54.

21. Muñoz-García, CI., Lorenzo-Burgunder, D., Gumi-Castillo, G., Perelló-Undreiner, DB., Zenteno-Nava, E., Orozco-Gregorio, H. Canine myiasis by *Lucilia eximia* in North America. Trop Biomedicine. 2016; 33: 494-499.

22. Cansi, ER., Demo, C. Occurrence of myiasis in pets from the Federal District, in Brazil. Acta Scientiae Veterinariae. 2011; 39: 982-986.

23. Sanford, MR., Whitworth, TL., Phatak, DR. Human wound colonization by *Lucilia eximia* and *Chrysomya rufifacies* (Diptera: Calliphoridae): myiasis, perimortem, or postmortem colonization. Journal of Medical Entomology. 2014; 51: 716-719.

24. Sukontason KL, Chaiwong T, Piangjai S, Upakut Chaiwong T, Piangjai S, Upakut. Omomatidia of blow fly, house fly, and flesh fly: Implication of their vision efficiency. Parasitology Research. 2008; 103: 123-131.

25. Sherman, RA. Wound myiasis in urban and suburban United States. Archives of Internal Medicine. 2000; 160: 2004-2014.

26. Pace, RC., Talley, JL., Crippen, TL., Wayadande, AC. Filth fly transmission of *Escherichia coli* O157: H7 and *Salmonella enterica* to lettuce, Lactuca sativa. Annals of the Entomological Society of America. 2017; 110: 83-89.

27. Hollingsworth, MJ., Burcombe, JV. The nutritional requirements for longevity in *Drosophila*. Journal of Insect Physiology. 1970; 16: 1017-1025.

28. Muse, W., Adedoja, D., Ajala, A., Adepoju, O., Aloro, O., Suleman, T., Survival and starvation resistance of the blowfly *Chrysomya chloropyga* (Wiedemann) (Diptera: Calliphoridae). 2012; Academia.edu/7778530/. Accessed November 2020/

29. Ujvari, B., Wallman, JF., Madsen, T., Whelan, M., Hulbert, AJ. Experimental studies of blowfly (*Calliphora stygia*) longevity: a little dietary fat is beneficial but too much is detrimental. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 2009; 154: 383-388.

30. Abou Zied, EM., Gabre, RM., Chi, H. Life table of the Australian sheep blow fly *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). Egypt Journal of Zoology. 2003; 41: 29-45.

31. Rueda, LC., Ortega, LG., Segura, NA., Acero, VM., Bello, F. *Lucilia sericata* strain from Colombia: Experimental colonization, life tables and evaluation of two artificial diets of the blowfly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), Bogotá, Colombia. Strain. Biological Research. 2010; 43: 197-203.

32. Partridge, L., Green, A., Fowler, K. Effects of egg-production and of exposure to males on female survival in *Drosophila melanogaster*. Journal of Insect Physiology. 1987; 33: 745-749.

33. Mackerras, MJ. Observations on the life-histories, nutritional requirements and fecundity of blowflies. Bulletin of Entomological Research. 1933; 24: 353-362.

34. Davila, EL., Brundage, A. Comparison of the longevity of *Cochliomyia macellaria* (Fabricus) and *Chrysomya rufifacies* (Macquart) adult males (Diptera: Calliphoridae). Instars: A Journal of Student Research. 2019; 5.

35. Gabre, RM., Adham, FK., Chi, H. Life table of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae). Acta Oecologica. 2005; 27(3): 179-183.

36. Baumgartner, DL. Review of *Chrysomya rufifacies* (Diptera: Calliphoridae). Journal of Medical Entomology. 1993; 30(2): 338-52.

37. Collins, CR., Brundage, A. Adult Longevity of *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) by Sex. Instars: A Journal of Student Research. 2017; 3.
38. Mohr, RM., Tomberlin, JK. Development and validation of a new technique for estimating a minimum postmortem interval using adult blow fly (Diptera: Calliphoridae) carcass attendance. International Journal of Legal Medicine. 2015; 129: 851-859.
39. Greenberg, B. Nocturnal oviposition behavior of blow flies (Diptera: Calliphoridae). Journal of Medical Entomology. 1990; 27: 807-810.
40. Payne, JA. A summer carrion study of the baby pig Sus scrofa Linnaeus. Ecology. 1965; 46: 592-602.
41. Wells, JD. A forensic entomological analysis can yield an estimate of postmortem interval, and not just a minimum postmortem interval: An explanation and illustration using a case. Journal of Forensic Sciences. 2019; 64: 634-637.