Nocturnal oviposition behavior of forensically-important Diptera in Central England

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ABSTRACT

Timing of oviposition on a corpse is a key factor in entomologically-based minimum post mortem interval (mPMI) calculations. However, there is considerable variation in nocturnal oviposition behavior of blow flies reported in the research literature. This study investigated nocturnal oviposition in Central England for the first time, over 25 trials from 2011 to 2013. Liver baited traps were placed in an urban location during control (diurnal) and nocturnal periods and environmental conditions were recorded during each 5 hour trial. No nocturnal activity or oviposition was observed during the course of the study indicating that nocturnal oviposition is highly unlikely in Central England.

Keywords: forensic science; forensic entomology; nocturnal activity; Calliphoridae; minimum postmortem interval; England

Forensic entomology uses the colonization times of specific insect species to estimate the minimum Post Mortem Interval (mPMI); the time between insect colonization and discovery of the corpse. Blow flies (Diptera: Calliphoridae) are commonly the initial colonizers of human remains and are therefore most utilized in these estimations. It is generally assumed that Calliphoridae only oviposit during daylight so that, if a victim is killed at night, the body will not be colonized before the following morning (1). However, if nocturnal oviposition does take place this assumption could lead to an error in the estimation of a mPMI by as much as 12 hours, a significant amount of time in relation to an investigation.

To date, there is variability in the published literature relating to nocturnal activity and oviposition behavior. Whilst some studies support the observation that oviposition does not occur at night (1-7), others have found clear evidence that it is possible (8-14). Laboratory work conducted to investigate conditions facilitating nocturnal oviposition has also proved inconclusive. For example, Zurawski et al. (6) demonstrated that *Lucilia sericata* did not oviposit nocturnally and provided evidence that Diptera were unable to fly in the dark. However, Amendt et al. (3) reported that nocturnal oviposition by *L. sericata* took place in two out of six studies. Therefore it is evident that further work needs to be conducted to clarify the situation.

Temperature is recognized as one of the most important factors affecting diurnal blow fly oviposition (15-17), and it is generally accepted that colonization by necrophagous flies occurs between air temperatures of 12°C and 30°C (18-19). Other environmental factors also influence oviposition activity, with low humidity, light or no rainfall, high light levels and a wind speed of less than 10 kilometers per hour (kmph) being commonly reported as favorable conditions for oviposition to occur (6-7, 10, 17). However, even when environmental conditions in nocturnal studies are conducive to oviposition, this behavior is not always observed. Other factors such as variation in bait type and methodology cannot be used to explain the differences in results to date, as all studies recording nocturnal oviposition recorded diurnal oviposition, demonstrating that the bait and method utilized were fit for purpose. Some studies have used artificial lights and reported that, they have positively influenced oviposition activity in some cases (11) but this was not true in others (2, 4-5, 7-8, 10, 14). Such substantial differences between studies suggest that that geographical area is a strong determinant of nocturnal blow fly behavior. If this is the case, for a forensic entomologist to make a robust assessment of colonization times, it is important that reference nocturnal oviposition data are collected from the specific areas in which entomological data are being applied to mPMI calculations.

To date, there are very few colonization data that can be applied to UK casework (15, 20). This study examined nocturnal oviposition in Calliphoridae in the Central Midlands, England for the first time and determined which environmental factors had the most significant effect on blow fly activity and oviposition. These results will improve the interpretation of fly behavior in relation to mPMI estimations (21), enabling practitioners to make more informed decisions about when colonization could have taken place.

Methods

Activity sampling

This study was conducted in an urban environment in south Derbyshire, U.K. from July to December 2011 (20 sampling days), and a secondary sampling period ran from July to August 2013 (5 sampling days). Each experiment used 100g of fresh liver as bait, as this has proven successful in trapping Calliphoridae in previous studies (7, 13-14, 22-23). Pieces of lamb liver were placed into separate sealed bags to prevent contamination by insects prior to exposure. Bait was placed in a trap suspended from a bird feeding station 1.61m from the ground in an open area free from artificial light (Figure 1). The trap was based on a modified cone trap (24), which allowed specimens to be obtained in good condition for identification and provided an inexpensive and reproducible design for these experiments. The bait was placed in the trap for five hours during daylight (always between 10am and 6pm) to confirm fly activity and oviposition. This was followed by a freshly baited trap being presented for five hours during complete darkness within the same 24 hour period, in order to assess nocturnal activity and oviposition. A wireless 2.4GHz wildlife video camera (IR Night vision 380 TVL, Spycameracctv.com) was used to record presence or absence of blow fly activity around the bottle trap (Figure 1). The camera had six infra-red LED lights enabling it to record nocturnal activity, using a wavelength of 940nm. Wall and Smith (25) stated that Diptera are not attracted to colors with a reflectance greater than 580nm. Therefore, it was assumed that the infra-red lighting did not affect fly behavior in this study although there was no experimental data to demonstrate this. The camera was also used to show whether insects crawled or flew to the bait, thus addressing the criticisms by later authors of the Greenberg study (8). The traps were collected at the end of each five hour period and the presence or absence of eggs recorded. Any adult flies caught were pinned and identified to species. No attempt to rear the eggs was made in this investigation.

Environmental conditions

Environmental conditions were recorded during each 5 hour sampling period in order to assess their effects on blow fly oviposition and activity. The ambient air temperature (°C) and humidity (%) were recorded at hourly intervals using a data logger (Tinytag TGP-4500, Chichester, U.K.) for the duration of exposure. Rainfall was categorized as either no rainfall, light rainfall or heavy rainfall, and the phase of the moon was recorded as a potential indication of light levels during nocturnal experiments. Wind speed data (kmph) was taken from the nearest Met Office weather station at Nottingham Watnall (25), approximately 16 km from the study site.

Statistical analyses

Separate statistical analyses were conducted treating presence/absence of blow fly activity and oviposition as dependent variables and environmental parameters as potential explanatory variables. Additive generalized linear models with a logit link function (logistic regression) were generated, and backwards stepwise selection was carried out to find the minimum adequate model. Additive independent variables were temperature, humidity, wind speed (all continuous) and rainfall (ordinal). Prior to the application of generalized linear regression, independent variables were assessed for levels of correlation. Temperature and humidity were found to be highly negatively correlated (Spearman = -0.85) therefore, humidity was regressed on temperature using linear regression, and the residuals were calculated. The residuals from this regression were used in the generalized linear models in place of humidity values. Assumptions of the generalized linear regression framework were assessed using standard residual diagnostics (27). All statistical analyses were performed using the statistical programming language R (28).

Results

No blow fly activity or oviposition was recorded during any of the 25 night time experiments in 2011 or 2013. Blow fly activity around the bottle traps was recorded during daylight hours on 16 of the 20 days in 2011. *Calliphora vicina* individuals were trapped on seven occasions throughout August, October and November in 2011 with *Calliphora vomitoria* only being caught once on 25th August. All flies were female. *Lucilia* species were recorded on camera during both years but never trapped for species identification. Blow fly activity did not occur on 12th August 2011, nor during the last three weeks of the trials (from 20th November through until 4th December 2011) (Table 1). In 2013, blow fly activity was recorded during the day on 4 of the 5 days but did not occur on 23rd August 2013 when there was light rain, a mean humidity of 61% and a wind speed of 11kmph.

The ranges of values for diurnal experiments were 7.4 – 31.8°C, 26.7-98.1% relative humidity (RH), no to light rain fall and wind speed of 6-21 kmph. For nocturnal experiments the range was 1-16.1°C, 65.2-100% RH, no to heavy rain and wind speed of 3-24kmph.

Oviposition was recorded during 9 of the 20 daytime experiments in 2011 and 1 of the 5 daytime experiments in 2013 (Table 1) indicating the bottle traps were fit for purpose and there were gravid blow flies in the area. Oviposition was associated with higher temperature and lower humidities (raw humidity values), but it must be remembered that temperature and humidity were highly correlated. The minimum adequate model for activity contained only one significant single term (Temperature; LRT = 10.18, $p < 0.001$). This was also true of the minimum adequate model for oviposition (Temperature; LRT = 4.501, $p = 0.034$).

Recent work by Berg & Benbow (7) demonstrated that the abundance of diurnal blow fly oviposition can increase when temperatures exceed 20°C the night before. This study did not record the temperatures outside of the 5 hour sampling periods. However, mean temperature data was collected from the nearest Met Office weather station (Nottingham Watnall [25], approximately 16km away from the experimental site) for the 24 hour period preceding diurnal experiments to assess if there was a significant correlation between preceding temperature and activity or oviposition. The results demonstrated that mean temperature from the preceding 24 hour period did not have a significant influence on activity. However, the minimum adequate model for diurnal oviposition indicated there was a potential significant interaction between temperature of the preceding 24 hour period and oviposition (Temperature; LRT = 3.65, p = 0.055).

Discussion

Daytime activity and oviposition were recorded on 20 and 10 of the 25 days respectively. No nocturnal blow fly activity or oviposition was recorded during any of the 25 nocturnal sampling periods conducted in 2011 and 2013. These results support the theory that blow flies do not visit bait to oviposit during the hours of complete darkness (2-7, 14).

Blow fly activity and oviposition were recorded on baits exposed during daylight hours in the same location demonstrating the presence of gravid blow flies. During the 25 diurnal sampling periods only 10 occurrences of oviposition were recorded despite high blow fly activity filmed around the bottle traps the majority of the time. This could have been an underestimate due to the presence of wasps recorded feeding on the bait and possibly any eggs present in both 2011 and 2013.

Logistic regression indicated that temperature was the only significant variable affecting both diurnal activity and oviposition; both generally occurring at warmer temperatures, above 14°C and 17°C respectively. This corresponds to previous reports of 14-15°C as the minimum air temperature needed for blow fly activity (29-30). Oviposition was recorded within the temperature range 12.2°C to 29.9°C, within a humidity range from 26.7% to 76.6%, when there was light or no rain and with wind speed ranging from 6 to 18kmph

(Table 1). It is generally accepted that necrophagous flies will oviposit between an air temperature of 12°C and 30°C (18-19), and the results from the present study support this theory. In addition, results indicated that the probability of oviposition occurring increased with increasing ambient temperature, in accord with previous studies conducted in the UK (15, 20), North America (6) and South Eastern Australia (17).

An assessment of the range of mean environmental conditions indicates that nocturnal temperatures were below the minimum oviposition threshold temperature suggested by the current model (17°C) predicted from the diurnal data set. However, the nocturnal temperature range (1 - 16.1°C) overlaps with temperatures reported in previous studies where nocturnal oviposition both occurred (9) and did not occur (3-7, 14) indicating that other factors and/or regional differences do influence oviposition activity. Whilst there is overlap between the diurnal and nocturnal temperature ranges in the current study, the nocturnal humidity range is restricted to the higher humidities, and this may have been a negative influence on oviposition. However, the finite sample of weather conditions experienced during nocturnal experiments in this study means that further work is required for a more complete understanding of how environmental conditions affect nocturnal oviposition.

Calliphora vicina was caught on seven occasions and *C. vomitoria* was trapped only once. Previous work indicates that *C. vicina* is dominant in urban environments in the UK (24) and this ratio supports this observation. Camera footage showed that *Lucilia* species were active around the bait but none were trapped enabling them to be identified to species level. Previous work utilizing an almost identical trap design successfully trapped *Lucilia* species in South East England (24) indicating that it was not the trap design in these experiments influencing this result. *C. vicina* activity was recorded from the beginning of July up until

Mid-November, which is in accordance with the seasonal activity recorded in other regions of the UK, flies generally disappearing in late autumn but being seen flying on sunny winter days (24, 32). Oviposition was recorded up until mid-November but it is not known whether these eggs were viable or which species of blow fly was responsible. *C. vicina* has been recorded ovipositing at temperatures around 12°C (31), whereas *Lucilia* species oviposit at higher ambient temperatures of more than 27°C (29-30). Therefore it was possible both genera contributed to the eggs recorded on liver baits throughout the July-November period, but this could not be confirmed as eggs were not reared to adulthood to confirm this.

There is evidence to suggest that the temperature preceding insect colonization is important. For example, Berg & Benbow (7) demonstrated that the abundance of diurnal blow fly oviposition increased when temperatures had exceeded 20°C the night before. Therefore, as a follow up to the original analysis the mean temperature from 24 hours preceding the diurnal experiment in this study was included in the starting logistic regression model. Interestingly, the model indicated that high temperatures in the previous 24 hour period had a negative effect on whether oviposition was recorded the next day.

In conclusion, this study suggests that nocturnal oviposition is unlikely to occur in Central England and demonstrates that temperature is a significant factor in determining diurnal oviposition behavior. A minimum oviposition temperature threshold of 17°C was suggested by the logistic regression model, which may explain the lack of nocturnal oviposition considering the mean nocturnal temperature range (1 - 16.1°C) experienced. However, there were other potentially influential environmental factors (humidity, wind speed and rainfall), for which no significant effects were detected, but this could have been due to the lack of contrasting combinations of environmental conditions experienced in this study. In addition, temperature in the preceding 24 hours may have a significant influence on oviposition, with oviposition less likely to occur on days following warm periods. These results suggest that blow fly oviposition is strongly determined by temperature, but also suggest that there may be more complex environmental interactions, thus providing a baseline for future study. Further investigation, particularly in relation to interactions, is markedly needed in order to build a more precise understanding of the conditions affecting oviposition in different regions. This is particularly the case in countries such as the UK where published data are currently unavailable.

References

1. Anderson GS. Wildlife forensic entomology: determining time of death in two illegally killed black bear cubs. J Forensic Sci 2009;44:856-859.

2. Tessmer JW, Meek CL, Wright VL. Circadian patterns of oviposition by necrophagous flies (Diptera: Calliphoridae) in Southern Louisiana. Southwest Entomol 1995;20:439-445.

3. Amendt J, Zehner R, Reckel F. The nocturnal oviposition behaviour of blowflies (Diptera: Calliphoridae) in Central Europe and its forensic implications. Forensic Sci Int 2008;175:61-64.

4. Stamper T, Debry RW. The nocturnal oviposition behaviour of carrion flies in rural and urban environments: methodological problems and forensic implications. Can Soc Forensic Sci J 2007;40:173-182.

5. Stamper T, Davis P, DeBry RW. The nocturnal ovipositing behaviour of carrion flies in Cincinnati, Ohio. J Forensic Sci 2009;54:1450-1452.

6. Zurawski KN, Benbow ME, Miller JR, Merritt R. Examination of nocturnal blow fly (Diptera: Calliphoridae) oviposition of pig carcasses in Mid-Michigan. J Med Entomol 2009;46:671-679.

7. Berg MC, Benbow ME. Environmental factors associated with *Phormia regina* (Diptera: Calliphoridae) oviposition. J Med Entomol 2013;50:451-457.

8. Greenberg B. Nocturnal oviposition behaviour of blow flies (Diptera: Calliphoridae). J Med Entomol 1990;29:807-810.

9. Singh D, Bharti M. Further observations on the nocturnal oviposition behaviour of blow flies (Diptera: Calliphoridae). Forensic Sci Int 2001;120:124-126.

10. Baldridge R, Wallace S, Kirkpatrick R. Investigation of Nocturnal Oviposition by Necrophilous Flies in Central Texas. J Forensic Sci 2006;51:125-126.

11. Kirkpatrick RS, Olson JK. Nocturnal light and temperature influences on necrophagous, carrion-associating blow fly species (Diptera: Calliphoridae) of forensic importance in Central Texas. Southwest Entomol 2007;32:31-36.

12. Singh D, Bharti M. Some notes on the nocturnal larviposition by two species of *Sarcophaga* (Diptera:Sarcophagidae). Forensic Sci Int 2008;177:e19-e20.

13. Pritam H, Jayaprakash P. Nocturnal Oviposition Behaviour of Necrophagous Dipterans in Kelantan, Malaysia. J Forensic Sci 2009;54:1135-1140.

14. George KA, Archer MS, Toop T. Nocturnal colonization behaviour of blowflies (Diptera: Calliphoridae) in Southeastern Australia. J Forensic Sci 2013;58:S112-S116.

15. MacLeod J. The climatology of blowfly Myiasis. II.-oviposition and daily weather indices. B Entomol Res1949;40:179-201.

16. Faucherre J, Cherix D, Wyss C. Behaviour of *Calliphora vicina* (Diptera: Calliphoridae) under extreme conditions. J Insect Behav 1999;12:687-690.

17. George KA, Archer MS, Toop T. Abiotic environmental factors influencing blowfly colonisation patterns in the field. Forensic Sci Int 2013;229:100-107.

18. Erzinçlioğlu, Z. Blowflies, Naturalists' Handbooks 23. Slough: The Richmond Publishing Co. Ltd, 1996.

19. Gennard DE. Forensic Entomology: An Introduction, 2nd rev.ed. Chichester: Wiley-Blackwell, 2007.

20. Pitts KM, Wall R. adult mortality and oviposition rates in field and captive populations of the blowfly *Lucilia sericata*. Ecol Entomol 2004;29:727-734.

21. Tomberlin JK, Mohr R, Benbow ME, Tarone AM, VanLaerhoven S. A Roadmap for bridging basic and applied research in forensic entomology. Annu Rev Entomol 2011;56:401-421.

22. Davies L. Species composition and larval habitats of blowfly (Calliphoridae) populations in upland areas in England and Wales. Med Vet Entomol 1990;4:61-68.

23. Brundage A, Bros S, Honda JY. Seasonal and habitat abundance and distribution of some forensically important blow flies (Diptera: Calliphoridae) in Central California. Forensic Sci Int 2011;212:115-120.

24. Hwang C, Turner BD. Spatial and temporal variability of necrophagous Diptera from urban to rural areas. Med Vet Entomol 2005;19:379-391.

25. http://wow.metoffice.gov.uk/sitehandlerservlet?requestedAction=READ&siteID=3029. Last accessed 8th April 2014.

25. Wall R, Smith K. Colour discrimination by the sheep blowfly *Lucilia sericata.* Med Vet Entomol 1996;10:235-240.

26. Zuur AF, Ieno EN, Smith GM. Analysing Ecological Data. New York: Springer, 2007.

28. R Development Core Team R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. See http://www.R-project.org, 2011. Last accessed 8th April 2014.

29. Cragg JB. The olfactory behaviour of *Lucilia* species (diptera) under natural conditions. Ann Appl Biol 1956;44:467-477.

30. Reibe S, Madea B. How promptly do blowflies colonise fresh carcasses? A study comparing indoor with outdoor locations. Forensic Sci Int 2010;195:52-57.

31. Davies L, Ratcliffe GG. Development rates of some pre-adult stages in blowflies with reference to low temperatures. Med Vet Entomol 1994;8:245-254.

32. Cruickshank I, Wall R. Population dynamics of the sheep blowfly *Lucilia sericata:* seasonal patterns and implications for control. J Appl Ecol 2002;39:493-501.

No Additional Information and Reprint Requests Section

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Table 1: A summary of the mean environmental conditions recorded during each five hour diurnal experimental period in 2011 and 2013 stating if oviposition was recorded. The minimum and maximum conditions under which oviposition was recorded are bolded.

Date	Mean ambient temperature (°C)	Mean humidity (%)	Rain fall	Wind speed (kmph)	Oviposition
15/07/11	**29.5**	**26.7**	none	14	Yes
22/7/11	21.1	54.0	light	8	No
28/07/11	29.9	38.3	none	**6**	Yes
05/08/11	28.3	48.4	none	13	No
12/08/11	20.3	78.1	light	6	No
19/08/11	28.6	28.3	none	13	Yes
25/08/11	21.8	48.6	light	8	Yes
01/09/11	26.9	37.2	none	13	No
16/09/11	20.2	57.9	none	14	No
24/09/11	21.6	57.1	none	16	No
01/10/11	28.4	39.1	none	11	Yes
08/10/11	14.4	84.8	none	10	No
16/10/11	17.6	66.3	none	11	Yes
23/10/11	18.9	61.1	none	**18**	Yes
30/10/11	17.5	**76.6**	none	16	Yes
06/11/11	16.5	55.7	none	14	No
13/11/11	**12.2**	56.3	none	16	Yes
20/11/11	6.9	98.1	none	6	No
27/11/11	4.3	60.9	none	21	No
4/12/11	3.0	76.9	light	21	No
20/07/13	23.6	60.7	none	14	Yes

26/07/13	29.2	35.7	none	11	No
27/07/13	31.8	37.2	none	10	No
23/08/13	24.1	61.0	light	11	No
24/08/13	19.1	83.3	none	13	No