Effects of environmental temperature on oviposition behavior in three blow fly species of forensic importance

Abstract

A number of factors are known to affect blow fly behavior with respect to oviposition. Current research indicates that temperature is the most significant factor. However temperature thresholds for oviposition in forensically important blow flies have not been well studied. Here, the oviposition behavior of three species of forensically important blow fly species (*Calliphora vicina*, *Calliphora vomitoria* and *Lucilia sericata*,) was studied under controlled laboratory conditions over a range of temperatures (10 to 40°C). Lower temperature thresholds for oviposition of 16°C and 17.5°C were established for *C. vomitoria* and *L. sericata* respectively, whilst *C. vicina* continued to lay eggs at 10°C. *C. vomitoria* and *L. sericata* both continued to lay eggs at 40°C, whilst the highest temperature at which oviposition occurred in *C. vicina* was 35°C. Within these thresholds there was considerable variation in the number of surviving pupae, with a general pattern of a single peak within the range of temperatures at which eggs were laid, but with the pattern being much less distinct for *L. sericata*.

Keywords

Forensic entomology; Calliphoridae; minimum post-mortem interval; *Calliphora*; *Lucilia*

1. Introduction

Developmental stages of key insect species found on corpses can be used to estimate the minimum post-mortem interval (mPMI). As primary colonizers of human remains, blow flies are frequently used in these forensic calculations [1-2]. The developmental stages of colonizing insects reached at the time of the discovery of human remains will be the result of two main components: the time between death and the arrival of the colonizers, and the developmental rates of resulting larvae and pupae. Both of these components will vary, depending on habitat and environmental conditions. However, the vast majority of research to date has focused on the effect of environmental factors (mainly temperature) on developmental rates, and determining lower temperature thresholds of development for specific blow fly species [3-19]. Relatively little work has investigated environmental effects on the timing of colonization. Therefore, currently there is a substantial lack in our understanding of how environmental parameters may affect a key component determining the relationship between mPMI estimates and the actual time since death.

The colonization interval (the time between death and colonization) of an exposed corpse can vary from minutes to days for blow flies. Previous research has demonstrated that abiotic factors, including temperature, humidity, solar radiation, rainfall, wind, and light levels can influence blow fly oviposition behavior [20-25]. In particular, temperature has been recognized as one of the most important influences [20, 23-24]. Temperatures will determine the geographical ranges and densities of blow fly species [26], as well as influencing their patterns of activity. For example, warmer nocturnal temperatures have been found to encourage blow flies to oviposit at night [20, 27], and Berg & Benbow [22] found that the abundance of diurnal blow fly oviposition significantly increases when temperatures exceed 20°C on the previous night.

It is generally accepted that colonization by necrophagous flies occurs when air temperatures are between 10°C and 30°C [20, 28-29] although there are exceptions [30-32]. However, little is known about specific oviposition temperature thresholds for individual blow fly species. In addition, it is likely that oviposition behavior will show regional variation within species, a further complication which could have significant impacts on the utility of mPMI estimation [33] in estimating actual post-mortem interval (PMI).

The time taken for blow fly species to colonise a corpse is likely to be a function of the probability of oviposition which, in turn, will be affected by environmental temperature, solar

radiation and the temperature of the corpse. How the probability of oviposition taking place changes with environmental temperature has not been well defined for blow fly species. The local environment at the corpse will also influence the probability of survival of the eggs laid, an aspect which could also be of potential significance for forensic entomology, as differential survival of species will be key in determining the later community structure of colonizers [34]. In this study we attempt to define the temperature ranges over which oviposition occurs for three blow fly species of forensic importance. In addition, we determine how the probability of oviposition changes within these ranges, as well as the probability of the survival of eggs laid.

2. Materials and Methods

Insect culturing

Colonies of laboratory-bred *Lucilia sericata* (Meigen), *Calliphora vomitoria* (Robineau-Desvoidy) and *Calliphora vicina* (Linnaeus) were reared in Bugdorm cages (60cm^3) in the insectary at the University of Derby at 20°C ± 3°C, under a 16:8h light: dark photocycle. Each species was bred on a rolling basis from wild-caught parents; the first generation used in the experiment was F1/F2. Flies were fed sugar and water *ad libitum* and provided with porcine liver on emergence and until eggs were laid, providing resources for vitellogenesis and oviposition, following Barnes and Gennard [35]. All adult flies used in these experiments were between 1 and 5 weeks old. Flies for the experiments were only taken from colonies which had demonstrated the ability to oviposit at the time of the experiment.

Oviposition studies

Experiments were conducted from June 2014 to January 2016. Each replicate consisted of 20 adult male flies and 20 adult female flies of the same species being placed in a meshed cubic cage (length = 30 cm) lined with sawdust. Flies in each cage were provided with 55g ± 0.5g porcine liver, a container of granulated sugar, and a container of water. Cages were placed in an insect growth chamber (IGC) (Fitotron® SGC 120), allowing temperature and humidity to be controlled. Relative humidity was maintained at 55% ± 5% and there was constant light provided by full spectrum light bulbs (4 x 36W fluorescent tubes per shelf, max intensity ~170 µmol m^{-2} s^{-1}).

The first run for each species was conducted at 20°C (the temperature at which the adult flies had been reared, and the approximate center of the temperature ranges at which oviposition

was generally accepted to occur in these three species [20, 29]. For each subsequent run, a new cage of flies was used, and the temperature inside the chamber adjusted to the required temperature (sequential 5°C intervals either side of 20°C within the range of 10°C to 40°C until the flies would no longer oviposit). Three replicates for each species were run at each temperature (10°C, 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C). Where oviposition was occurring at one temperature and not at the next, finer increments of 2.5°C and 1°C were then used. In each case the temperature remained constant for 24 hours, after which the presence/absence of eggs was recorded and a new run was undertaken. New adults from the stock populations were used for each replicate.

In addition to recording the presence or absence of eggs after 24 hours, the quantity of eggs (or sometimes larvae at higher temperatures) was estimated. This was done by placing a Petri dish lid with a marked grid (1 cm cells; Figure 1) over the top of the liver and counting the number of squares which contained at least one egg whilst observing from directly above. Although this method does not quantify the number of eggs exactly, it was used as accurately counting the eggs would have required disturbing the eggs. Egg masses are known to generate a structural framework that could influence the distribution of microbes which can potentially alter developmental and survival rates. We therefore avoided complete counting of the eggs as this would have disturbed these processes and potentially affected the key criteria which we were investigating.

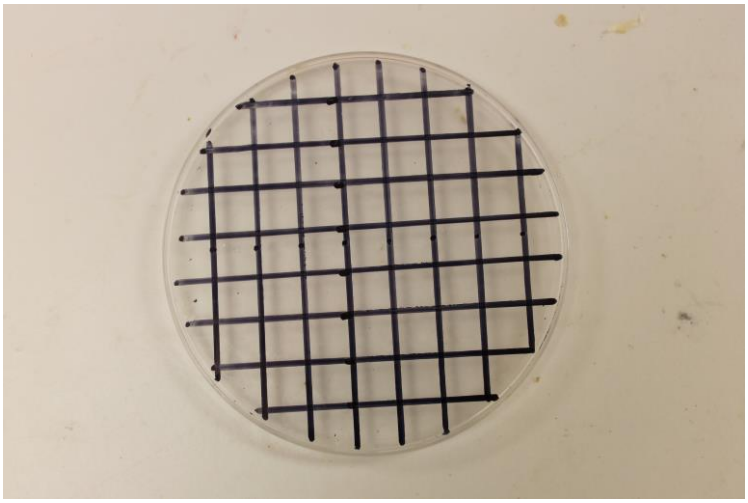

Figure 1: Photograph of the petri dish lid (19cm diameter) with a marked grid of 1cm squares, used to estimate the quantity of eggs laid on the liver

Survival studies

At the end of the 24 hr oviposition period, the liver with the eggs or larvae was carefully transferred onto a thin layer of sawdust (pupation substrate) within clear, plastic tanks and put in a controlled environment (16:8h light: dark cycle, 20 °C ± 3°C temperature). Eggs were reared through to the pupal stage and the resulting number of pupae in each cage was recorded.

Statistical analysis

The presence / absence of eggs for each species was modelled using generalized linear regression models with logit link function and binomial error distributions (logistic regression). The presence / absence of eggs was treated as the dependent variable and the temperature as the independent variable. Given the reasonably complex patterns of the number of squares with at least one egg in, and of the number of surviving pupae, with increasing temperature, both were modelled for each species using a generalized additive model (GAM; [36]). The number of squares/surviving pupae was fitted as a smoother function of temperature. For both the logistic regressions and the GAMs, model assumptions were assessed using residual diagnostics following Wood [36] and Zuur et al. [37]. All analyses were conducted in the R statistical programming software [38], and the GAMs were developed using the package mgcv [39]. Model visualizations were produced using the visreg package [40].

3. Results

The lowest temperatures at which oviposition occurred in *C. vomitoria* and *L.* sericata were 16^{o}C and 17.5^{o}C respectively, whilst *C. vicina* continued to lay eggs at 10^{o}C. Both *C. vomitoria* and *L. sericata* were able to lay eggs at 40^{o}C, the highest temperature tested. The highest temperature for oviposition in *C. vicina* was 35°C. Temperature had a significant effect on the probability of egg laying taking place in all three blow fly species (*C. vicina*, LR = 29.42, $p < 0.001$; *C. vomitoria*, LR = 22.49, $p < 0.001$; *L. sericata*, LR = 21.39, $p < 0.001$; Figures 2 and 3). Temperature was also a significant variable in determining the number of cm^2 squares containing at least one egg for all species (*C. vicina*, F = 23.13, $p < 0.001$, explained deviance = 71.1%; *C. vomitoria*, F = 12.88, $p < 0.001$, explained deviance = 64.6%; *L. sericata*, F = 10.35, $p < 0.001$, explained deviance = 67.5%; Figures 2 and 3). However, temperature significantly affected the number of pupae recorded for the *Calliphora* species, but not for *L. sericata* (*C. vicina*, F = 8.52, $p < 0.001$, explained deviance = 64.6%; *C. vomitoria*, F = 4.54, $p < 0.001$, explained deviance = 52.9%; *L. sericata*, F = 1.48, $p = 0.181$, Figure 4).

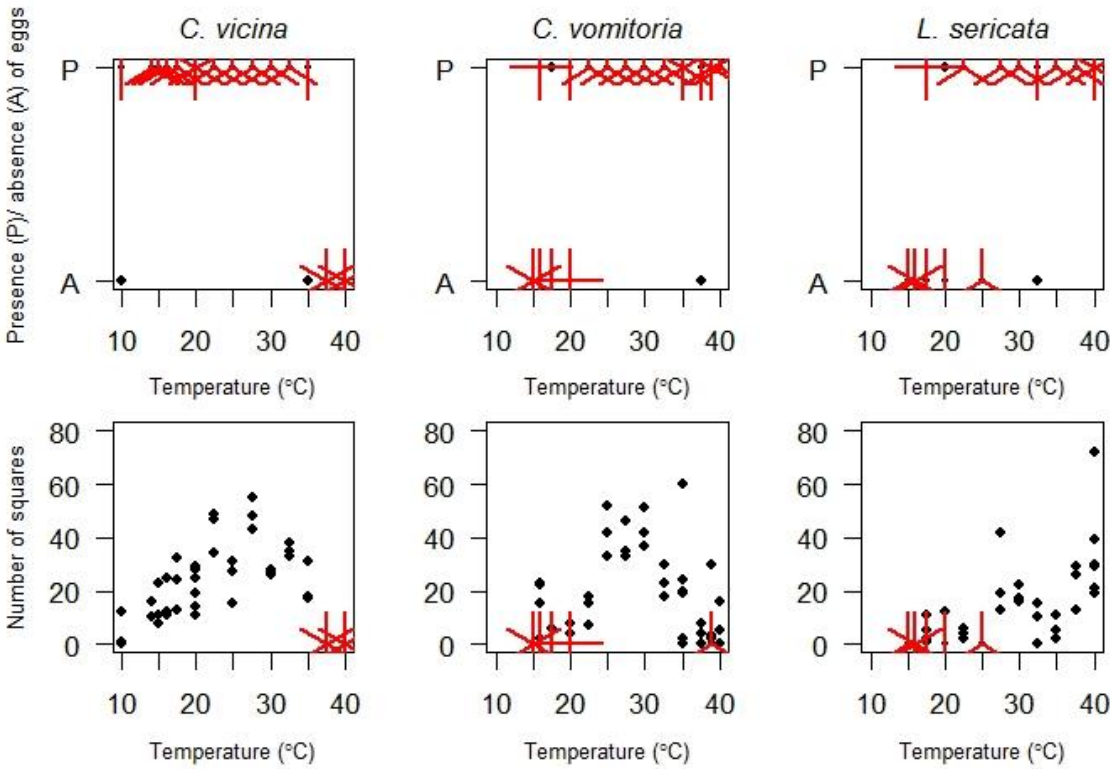

Figure 2: Sunflower plots of the experimental results showing the presence or absence of eggs with increasing temperature (top row), and the number of cm^2 counting squares containing at least one egg at increasing temperatures (bottom row) for each of the blow fly species (columns). The number of arms at each point indicates the number of results. For example, where there are four arms this represents four data points in the same position. A single point represents a single case.

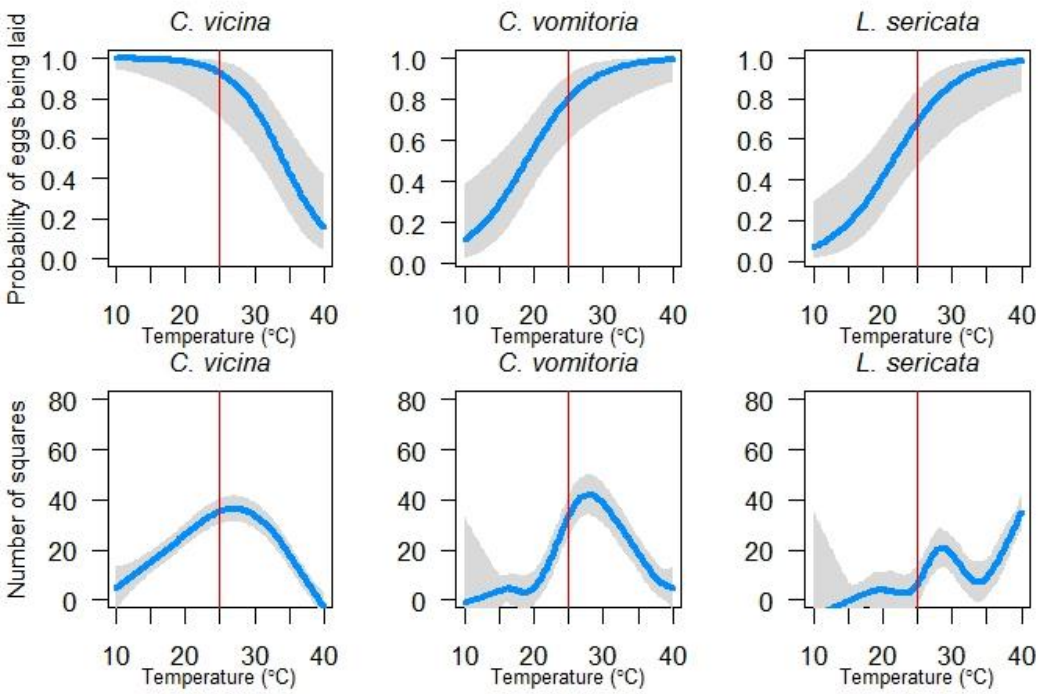

Figure 3: Model visualizations of the logistic models predicting the probability of presence of eggs with increasing temperature (top row), and the generalized additive models predicting the number of cm^2 counting squares containing at least one egg at increasing temperatures (bottom row) for each of the blow fly species (columns). Grey margins indicate 95% confidence intervals. In each plot a vertical (red) line is shown which marks the position of 25°C, to act as a guide for comparing model results between species.

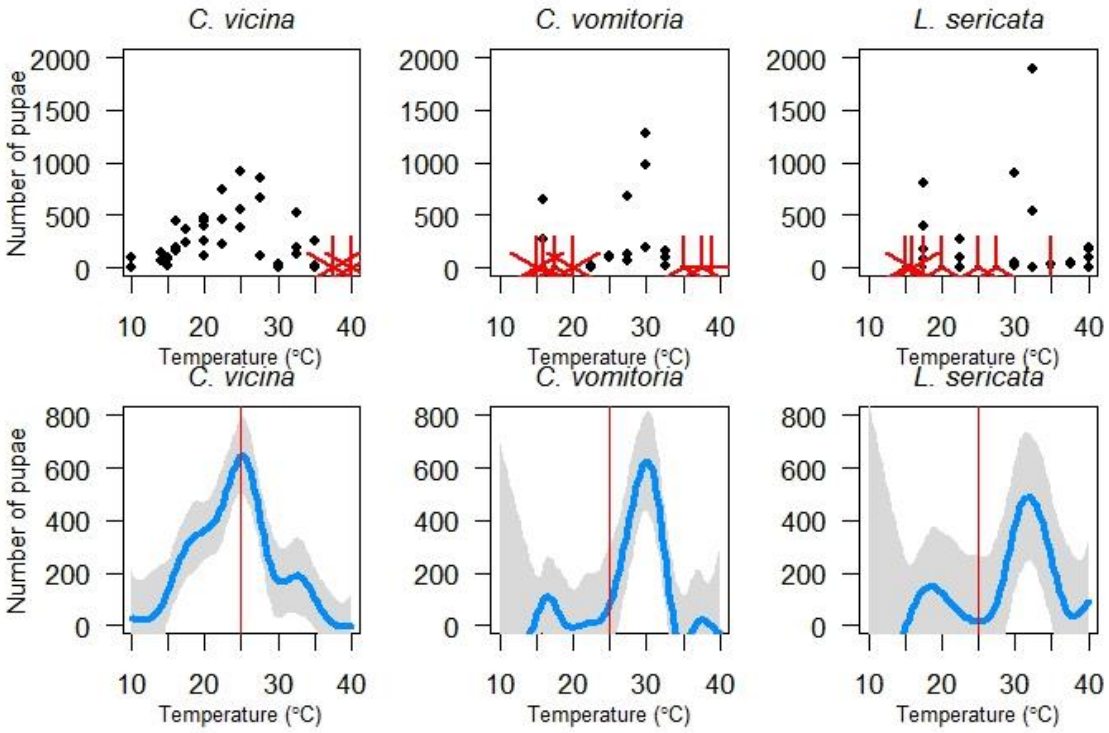

Figure 4: Sunflower plots of the experimental results showing the number of surviving pupae (top row), and the generalized additive models predicting the number of surviving pupae for each of the blow fly species (columns). Grey margins indicate 95% confidence intervals. In each plot a vertical (red) line is shown which marks the position of 25 ºC, to act as a guide for comparing model results between species. In all plots, the temperature represents the temperature at which the eggs were laid. Following the laying period (24 hr) the egg masses were transferred to an environment of 20°C ± 3°C, under a 16:8h light: dark cycle.

4. Discussion

Temperature had a significant effect on the probability of eggs being laid in all three blow fly species in this study. *C. vicina* oviposited at lower temperatures (10 °C, the lowest temperature tested to 35 °C), and *C. vomitoria* (from 16 °C to 40 °C, the highest temperature tested) and *L. sericata* (from 17.5 °C to 40 °C) at higher temperatures. The results for *C. vicina* are consistent with field observations of this species ovipositing at temperatures of 10°C and below [32]. Note that the lower threshold for oviposition for *C. vicina*, and the upper threshold for *L. sericata* and *C. vomitoria*, were not reached within the range of temperatures used in this study.

The greater probabilities of oviposition occurring at the low end of the temperature range used in this study with *C. vicina* compared to the other two species, matches patterns in abundance and activity in other studies. In Germany, *L. sericata* has been associated with corpses over a short, warm period of the year from May to October, *C. vomitoria* from April to November, whereas *C. vicina* was found to be active all year round [41]. Hwang & Turner [33] found activity of *C. vomitoria* and *L. sericata* restricted to the summer months, whilst *C. vicina,* although being most active in summer months, was active from much earlier in the year (April). However, our results contrast with those of Hwang and Turner [33] when comparing results for *C. vomitoria* and *L. sericata*. Our results suggest that the transition from high to low probability of oviposition occurs at slightly lower temperatures for *C. vomitoria*. However, Hwang & Turner [33] found *C. vomitoria* activity restricted to mainly June and August, whereas activity for *L. sericata* started later (August) and continued into the colder months (October). We need to be cautious in our interpretation of this difference due to our relatively small data set and the estimated contrast between the oviposition behaviors of the two species in our study being small. However, the habitat preferences of the two species may explain the apparent differences between our results and those of Hwang & Turner [33]. *L. sericata* is associated with urban environments [26, 33] which will tend to be warmer and therefore potentially maintain preferred temperatures into colder months compared with the rural areas with which *C. vomitoria* is associated [26, 33]. This is supported by Hutchinson [42] who conducted a study in South East England, and found *C. vicina* and *C. vomitoria* to be active in low numbers during the winter months at an urban study site, but not at their rural pasture site.

We must also recognize that in the wild temperatures will fluctuate, and the extent and consistency of these fluctuations will vary over time, particularly between seasons. However, in our experiment the flies had 24 hours to acclimatize to a constant temperature and oviposit, whereas in the wild they would have several days, possibly allowing flies to oviposit over greater temperature ranges than found in our study.

The temperature at which the eggs were laid had a significant effect on how many pupae developed for *C. vicina* and *C. vomitoria,* but not for *L. sericata.* The upper temperature threshold for oviposition and puparial survival in *C. vicina* in this study was similar to observations from Donovan et al. [17] who found that all larvae reared at 35°C died before pupariation. Development studies for several species of Calliphoridae, including *C. vicina, C. vomitoria* and *L. sericata* [9, 43] tend to be conducted at 20°C or above, which is believed to be favorable or close to optimum for these species [14]. This makes it difficult to compare

patterns of oviposition and numbers of pupae in this study with most developmental studies. However, for *L. sericata*, Grassberger and Reiter [11] reported development rates at temperatures from 17°C to 34°C, indicating that complete development was possible within this range, which coincides with the occurrence of oviposition and patterns of pupae counts in this study. It is important to note that our study differed in methodology compared with these other studies [9,11,14,17,43]. In our study we varied the temperature at which oviposition occurred, and then reared eggs to pupation at room temperature (20°C ± 3°C), whereas the developmental studies varied the temperatures at which larvae and pupae developed.

Identifying potential causes underlying the distributions of pupae at the different temperatures in this study is hampered by not having exact counts for the number of eggs (due to the requirement of not disturbing them) and due to the period of development, following the initial 24 hr oviposition period, occurring at a consistent single temperature (20 °C). Potentially the number of surviving pupae could be fully explained by the number of eggs laid. The distributions of the number of squares containing at least one egg and the number of pupae match closely for *C. vicina*. However, the range of temperatures over which there are high pupal survival numbers in *C. vomitoria* is considerably narrower than the range over which there were high numbers of squares containing eggs. This strongly suggests that survival rates decreased rapidly at the edges of the temperature range at which this species showed oviposition behavior. There are two potential reasons for this difference in this study, which cannot be partitioned out. Firstly, the temperature at which the eggs were laid affected the survival rates. Secondly, the temperature change between when the eggs were laid and when they were developing could have affected development and survival. The results for *L. sericata* suggest that the spatial distribution of eggs laid may be important in determining survival rates. At 32.5°C, there were low numbers of squares containing eggs, whereas the largest number of surviving pupae was recorded at this temperature. This suggests that large numbers of eggs were laid in highly aggregated patterns, resulting in greater survival rates. However, as we did not record the exact number of eggs laid, our data are not strong enough to allow us to draw a firm conclusion on this. This may explain why *L. sericata* was the only species for which temperature was not a significant predictor of the number of surviving pupae. However, the spatial distribution of eggs in the experiments was not consistent, and eggs were also laid erratically on other substrates at higher temperatures. This may also have had an effect on survival rates of eggs.

The lowest temperatures at which egg laying took place in the three species tested were higher than the lower development thresholds recorded by other authors. During controlled laboratory studies, Wall et al. [4] recorded the lower development threshold temperature (DTT) for *L. sericata* as 9°C. In comparison, our study *L. sericata* did not lay eggs below 17.5 °C suggesting that oviposition behavior is restricted to temperatures which are considerably greater than those at which the eggs are capable of developing. *C. vicina* laid eggs over the range of 10 °C to 35 °C. However, the number of squares containing eggs and the number of surviving pupae showed marked peaks centered around 25 °C, with very low numbers at the edges of the range. Similarly, results for *C. vomitoria* showed reduced number of squares containing eggs and number of surviving pupae towards the ranges at which eggs were laid, although there was greater variation towards the lower oviposition temperature threshold.

The developmental stages of colonizing insects reached at the time of the discovery of human remains will be a function of the time between death and the arrival of the colonizers, oviposition behavior, and the developmental rates of the resulting larvae and pupae. Results from this study indicate that temperature will strongly affect oviposition behavior in blow fly species of forensic importance. Importantly, the pattern of oviposition occurring in *C. vicina* was very different compared with the other two species, generally occurring at the lower temperatures. However, our results and comparison with experiments estimating DDTs, suggest that for all three species the range of temperatures over which oviposition occurred was well within the survival tolerances of the eggs. In addition, results indicate that the number of eggs laid, and the survival rates, are likely to vary considerably within the range of temperatures at which oviposition occurs, generally being high over a much more restricted range of temperatures. Previous work [3-5,7-9,11,12,17] has established that temperature will affect development rates and therefore mPMI estimates. Our work suggests that temperature is also an important determinant of the colonization interval and will lead to variation in colonization times between species. The extent of the colonization interval is important from a forensic perspective as its length will affect the estimate of time of death relative to the mPMI. However, the effects of other factors such as habitat configuration and solar radiation, and their potential interaction effects with temperature on colonization rates, will need to be studied and quantified before colonization intervals by blowflies can be reliably estimated. In addition, the sharp transition between oviposition occurring and not occurring at the upper temperature threshold for *C. vicina* compared with the more variable transitions at the lower thresholds for

C. vomitoria and *L. sericata*, suggest that data from some species may allow for greater accuracy in mPMI estimates than other data from species over particular temperature ranges.

In this study we have quantified oviposition behavior over a wide range of temperatures for three blow fly species of forensic importance, providing information on how colonizing rates of corpses by these species may be affected by environmental temperature. This provides valuable information for the forensic entomology community, but it is important that the results are viewed within their full context. There is considerable variation in the distribution of blow fly species between habitats, as well as differences between the effects of temperature on blow fly behavior within the same species between geographical regions. This study was conducted using flies collected in the midlands of England, and therefore the use of the thresholds and ranges found should not be extrapolated more widely. However, the broader ecological patterns suggested by this study, such as the nested nature of the distribution of the number of eggs laid and their survivorship, within the range of temperatures at which eggs were laid, are likely to represent more general relationships driven by natural selection. Finally, we have examined only one environmental variable here, whereas other variables such as humidity, wind speed and the presence of rain have been shown to influence oviposition behavior, and offer the potential for interaction effects with temperature. Patterns of the effects of the closely related variables environmental temperature, solar radiation and the temperature of the corpse are likely to be particularly important to determine.

5. Conclusion

Temperature significantly affected the probability of oviposition occurring in all three blow fly species, with *C. vicina* having greater probabilities at the lower temperatures and *C. vomitoria* and *L. sericata* having greater probabilities at the higher temperatures. The temperature at which oviposition took place also significantly affected the number of surviving pupae in the *Calliphora* species, but this was not the case for *L. sericata.* These results suggest that temperature will be a significant factor in determining colonization rates of a corpse by blow fly species through its effect on oviposition behavior and will therefore impact on the difference between estimations of mPMIs and the actual time since death. Further work is needed to determine the extent of regional variations in temperature - oviposition relationships and the nature of potential interaction effects between temperature and other abiotic variables, in order to provide more robust guidance on the extent to which mPMI estimates may vary from the actual time since death.

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