ABSTRACT

The most common application of forensic entomology involves estimating a portion of the postmortem interval (PMI), which usually assumes that blow flies (Diptera: Calliphoridae) do not oviposit nocturnally. Research objectives were to (1) investigate blow fly nocturnal oviposition in relation to sunrise and sunset in Michigan; (2) evaluate abiotic variables postulated to affect blow fly oviposition; and (3) conduct laboratory experiments testing blow fly activity under complete darkness. In 2006, nocturnal oviposition was evaluated in relation to sunset by exposing pigs to fly colonization at 1-h intervals, beginning 2 h before and ending 2 h after sunset. This test was replicated in 2007; however, replicate pigs were placed in the field 2 h after sunset, and hourly observations were made into the following morning. Oviposition was never observed at night. In a laboratory experiment, Lucilia sericata (Meigen), never oviposited on liver hanging above or placed directly on the ground in a completely dark room. Another dark room laboratory study documented that adult flies launched into the air could not fly. This study documents that the probability of nocturnal oviposition on pig carcasses in Michigan was extremely low to nonexistent. These results should be considered when estimating a portion of the PMI in forensic entomological investigations.

KEY WORDS

nocturnal oviposition, postmortem interval, forensic entomology, nocturnal flight
between 0.6 and 0.8 lux. Nocturnal oviposition (from 2200 to 0300 hours) occurred in 33% of trials, which was identical to the findings of Greenberg (1990). This study supported the hypothesis that the colonizers flew rather than crawled to the illuminated food.

Williams (2002) master’s thesis examined the taxonomic occurrence of nocturnal oviposition in southern African blowflies. Her work found only one night oviposition by L. sericata in the field. In the laboratory, L. sericata, Chrysomya putoria (Wiedemann), Chrysomya chloropyga (Wiedemann), and Chrysomya albiceps (Wiedemann) oviposited in complete darkness an average of 30% of the time.

Baldridge et al. (2006) evaluated the effect of light on blow fly ovipositional behavior in Texas using a variety of baits. Nocturnal oviposition occurred only once in >200 h of nocturnal bait presentation. The only oviposition that occurred was within 1 h after sunset. No flies were observed between 2200 and 0600 hours.

Amendt et al. (2007) conducted the most recent study on this topic; it occurred in Munich, Germany, and used hedgehog carcasses and beef liver as bait. Oviposition never occurred at night in 51 field trials but was reported in dark conditions in 33% of laboratory trials.

Woodridge et al. (2007) studied the flight patterns of L. sericata and Calliphora comitatoria L. in reported darkness. Using a wind tunnel, they found that fly activity was correlated with light intensity, and the probability of oriented flight leading to oviposition on a corpse in the dark was low. However, they were not able to conclude with certainty that the wind tunnel was absolutely dark.

To our knowledge, the fundamental question of whether or not any insect can fly under complete darkness has never been definitively answered in entomological literature. We postulate that insect flight under total darkness would be extremely unlikely, given the requirements of optical input to the brain to stabilize and direct flight through the well-known optomotor response system (Gotz 1968, Kennedy 1977, David 1979, Kennedy et al. 1981). However, if flight under complete darkness was to be found in the Insecta, a dipteran would be the most likely candidate because of the theoretical possibility for generating information for flight stabilization from inertial data derived from the halteres functioning as gyrosopes.

The objectives of this study were to (1) describe nocturnal oviposition in relation to sunrise and sunset in a rural Michigan setting; (2) evaluate abiotic variables postulated to affect ovipositional timing, magnitude, and species composition when oviposition occurred after sunrise; and (3) conduct controlled laboratory experiments testing blow fly activity under complete darkness. We postulated that blow flies would not oviposit under natural conditions after dark in a rural setting and that the initial timing, duration, and magnitude of oviposition occurring after sunrise would vary with temperature, light, wind, humidity, and rainfall.

Materials and Methods

2006 Field Season Experiment. During the summer months of 2006 and 2007, six pigs weighing 25 ± 10 kg were obtained from the Michigan State University swine research facility (SRF). Each pig was euthanized by SRF using lethal injection at ~1600 hours and was transferred to a black plastic garbage bag immediately after death. Each bag was tightly tied off and placed into a second bag to prevent insect access. The pigs were immediately transported to the Michigan State University entomological field research center, ~0.8 km from the SRF and stored inside a barn for ~4 h. A hobo temperature data logger (Onset Company, Pocasset, MA) (recording at 1-min intervals) was placed next to the bagged pigs in the barn and moved with the pigs into the field for the duration of the experiment.

Table 1. Nocturnal oviposition studies found in the primary forensic entomology literature, providing the design, major results, and species studied.

<table>
<thead>
<tr>
<th>Location</th>
<th>Bait</th>
<th>Platform</th>
<th>Exposure</th>
<th>Results</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greenberg (1990)</td>
<td>Chicago, Illinois</td>
<td>Rats, ground beef</td>
<td>No</td>
<td>0100-0400, 2100-2400</td>
<td>33% trials nocturnal oviposition</td>
</tr>
<tr>
<td>Faucherre et al. (1999)</td>
<td>Swiss Jura Mountains</td>
<td>Fresh Meat</td>
<td>No</td>
<td>12 d in a dark cave, 2200-0300</td>
<td>Oviposition after 12 d</td>
</tr>
<tr>
<td>Singh and Bharti (2001)</td>
<td>Punjab, India</td>
<td>Mutton</td>
<td>Yes, 2 m, wood</td>
<td>33% trials nocturnal oviposition</td>
<td>C. vicina, Chrysomya megacephala, and Chrysomya rufifacies</td>
</tr>
<tr>
<td>Williams (2002)</td>
<td>South Africa</td>
<td>Rats, pork</td>
<td>No</td>
<td>2 h in the dark</td>
<td>31-35% laboratory</td>
</tr>
<tr>
<td>Baldridge et al. (2006)</td>
<td>Central Texas</td>
<td>Rats, mice, pigs</td>
<td>Yes, 0.6 m, wood</td>
<td>1800, 2100, 2400 and 0600</td>
<td>One time in 200 h of bait presentation</td>
</tr>
<tr>
<td>Amendt et al. (2007)</td>
<td>Munich, Germany</td>
<td>Hedgehog</td>
<td>Window sill, 2.5 m</td>
<td>2230 until 0430 (field) 2100-0700 (lab)</td>
<td>Twice in the laboratory, zero in the field</td>
</tr>
</tbody>
</table>
Two wooden 15-cm-high platforms were erected 16 m apart in a grassy field 183 m from the barn. An adhesive gel (Tanglefoot, Tanglefoot Company, Grand Rapids, MI) was generously applied to each leg of both platforms to capture any crawling insects. Beginning 2 h before sunset, one pig was removed from the barn and transported to the field, removed from the plastic bag, and placed on a platform facing north. After 1 h of exposure, the pig was removed and replaced with another pig from the barn. This process was repeated every hour until 2 h after sunset for a total of five sequential 1-h intervals.

After the 1-h exposure, the pigs were carefully examined for fly eggs; any present were removed using a small paintbrush and placed in a styrofoam cup containing a piece of beef liver on top of a moistened paper towel (Tarone and Foran 2006). Examination involved a thorough inspection of the body, with a concentration on mucus membranes and orifices. After the last pig was examined, the styrofoam cups were transported to the laboratory, where hatched larvae were fed 28 g of beef liver each day until reaching the third larval instar postfeeding stage, after which they were preserved in 70% ethanol and identified to species (Stoganovich et al. 1962).

To understand the local fly species composition in the area during the experiment, a separate pig was placed in the field on the second platform 2 h before sunset and left to colonize for 1 wk. A wood-framed cage wrapped with chicken wire was placed over this pig to prevent large predators and scavengers from disturbing the carcass. Adult flies were collected once, 7 d after the initial experiment. These flies were pinned and identified to species using Whitworth (2006).

**2007 Field Season Experiment.** The experimental design of 2006 was repeated in 2007 except for the following conditions: (1) three replicate pigs were obtained for each of eight trials; (2) replicate pigs were deployed from 2 h after sunset until oviposition occurred after sunrise the following morning; and (3) the pigs were 10 m apart and examined every hour after sunset until sunrise and thereafter every half hour. Egg collection and rearing were performed as in 2006, except adult flies also were collected from the three pigs during the morning of each trial.

A regression model was developed to predict egg number from egg mass during the 2007 field season to determine the magnitude of oviposition during each trial (Fig. 1). Using a laboratory strain of *L. sericata*, 16 egg masses (*N* = 2–250) were counted and weighed to 0.001 mg on a Sartorius balance.

In both 2006 and 2007, a Hobo temperature data logger and a TES-1336 data logging light meter (TES Electrical Company, Taipei, Taiwan) were placed between the platforms, and both recorded at 1-min intervals for the duration of each trial. Sunset and sunrise were determined using the Weather Channel website (www.weather.com), which is consistent with National Oceanic and Atmospheric Administration (NOAA) data.

**Laboratory Tests for Evidence of Flight and Oviposition in Darkness.** These experiments were conducted under standard laboratory conditions in an empty 2 by 4-m photographic dark-room, whose single, sealed door opened into an unlit laboratory where a solid, closed door led into a dimly lit hallway. Four layers of tightly woven black cloth were wedged against the bottom of the dark-room door to compensate for a seal judged possibly imperfect. Investigators with fully dark-adapted vision were able to detect no trace of light from any position within this dark room when the 60-W tungsten bulb available for illuminating this room was unlit.

The first experiment tested the rates of *L. sericata* egg deposition under darkness or light on 28 g of beef liver contained in a plastic flat bottomed weighing dish placed in a basket set either on the floor or suspended 22 cm above the floor by string connected to the ceiling. Ten laboratory-reared flies (F:M ratio = ~60:40) at a time were enclosed in a 473-ml glass jar. After 1 h of acclimation either in darkness or lightness, the jar was set on its side on the floor within 30 cm of the basket and the lid removed. Each trial ran overnight for 8 h. Eggs on the liver were counted the next morning. Five replicates were accumulated for each combination of illumination and basket elevation.

**Fig. 1.** Relationship between egg mass weight and the number of eggs using *Lucilia sericata* as a model (*R*² = 0.98; *y* = 3276*x* + 17.78).
To test whether *L. sericata* (0.03 ± 0.01 g) might be able to fly under complete darkness, we elected to launch them into the air in complete darkness and determine whether and how their settling pattern deviated from that of inanimate objects of similar size, shape, and mass. To this end, plastic flies (0.39 ± 0.02 g) were purchased from a local hobby shop. One plastic fly at a time was placed into a 1-cm-diameter, 10-ml glass vial held at eye level of a standing investigator whose back was near the sealed darkroom door. Using a practiced forward thrust followed by an abrupt stop, 15 plastic flies were singly launched so that all fell directly on the floor where their positions could be recorded. Immediately thereafter, the same procedure was repeated but in the darkness and using 15 dark-adapted *L. sericata* females. For 15 s after each live fly was tossed, the investigators listened diligently for any sounds of sustained or intermittent flights. Then, the light was turned on and the landing position of that female noted.

**Data Analysis.** The probability of nocturnal oviposition was analyzed using a χ² test. Using data from both field seasons, the probability of observed diurnal oviposition was compared with observed nocturnal oviposition that occurred in both the field and laboratory experiments. To determine the influence of abiotic factors on oviposition, we used forward and backward multiple stepwise linear regression using SAS (SAS Institute 1998).

**Results**

**Nocturnal Oviposition.** In the 2006 field season, oviposition occurred 2 h before sunset in 50% of the trials, 1 h before sunset in 75% of the trials, and at sunset in 50% of the trials. Oviposition never occurred...
1 or 2 h after sunset (Table 2; Fig. 2). No insects were found crawling up to or on the platform supporting the pigs in either field season.

In 2007, adult flies were never observed after sunset, and oviposition was never documented on any of the pig replicates in any of the trials. Flies were first observed, on average, 50 ± 40 min after sunrise and oviposition documented 4 h later. The earliest time flies arrived and oviposited was 8 min and 3.5 h after sunrise, respectively (Table 3). On average, one of three pigs was found to have diurnal oviposition in 2007.

No oviposition occurred at night, but oviposition did occur in 33% of the trials during the daylight. Using this 33% oviposition rate as the probability of oviposition at any time (day or night), the probability of nocturnal oviposition was significantly lower than oviposition during daylight hours ($\chi^2 = 10.67; \text{df} = 1; P < 0.01$).

### Factors Influencing Daylight Oviposition

During 2006, oviposition was most often observed for *Lucilia coeruleiviridis* (Macquart) before sunset and *P. regina* at sunset (Fig. 3). However, *C. vomitoria* comprised ≈20–35% of the eggs collected from all trials pooled. *L. coeruleiviridis* was the most frequent species ovipositing in early summer compared with *C. vomitoria* at the end of the season (Fig. 3). *P. regina* was the most frequent adult fly collected from pigs 1 wk of exposure among all trials (Fig. 3). The species composition changed not only by time interval within a day but also among dates (Fig. 3). *P. regina* was the most prevalent blow fly ovipositing at sunset.

In 2007, abiotic factors were evaluated to understand their influence on oviposition magnitude, defined by the number of oviposited eggs. Forward multiple stepwise linear regression found that wind was negatively ($F = 6.72; \text{df} = 7; r = 0.53; P = 0.04$) and light was positively ($F = 7.61; \text{df} = 7; r = 0.75; P = 0.03$) related to egg number in independent models. When temperature, light, wind, humidity, and rainfall were incorporated into the same model, there was not a significant relationship ($F = 2.35; \text{df} = 7; r = 0.85; P = 0.32$). We originally postulated that all environmental variables would influence oviposition and covary, so backward stepwise linear regression was used to include all variables in the same model and sequentially remove the least important variable. The final significant model included wind, temperature, and humidity ($F = 7.78; \text{df} = 3; r = 0.85; P = 0.038$), but not light.

Pearson correlation analysis between abiotic factors determined that temperature and humidity were positively correlated ($n = 8; r = 0.95; P = 0.03$), as well as temperature and light ($n = 8; r = 0.96; P = 0.02$). This accounts for the discrepancies among the forward and backward stepwise linear regressions because, whereas wind and light are both important individually, the significance of light depends on the temperature and humidity.

*Lucilia sericata* comprised the highest percentage of blow fly species eggs collected from pigs during summer 2007; however, this varied by date (Fig. 4). *P. regina* was the most common adult fly species when averaged across the season, but this also varied by date (Fig. 4). Similarly, *L. coeruleiviridis* was most abundant in the early 2006 summer, but the community was replaced almost entirely by *L. sericata* in July and August and then by *C. vicina* and *P. regina* in September. Also, *P. regina* adults were found in most trials, and although eggs of *L. sericata* were most often collected right after sunrise, *P. regina* was the most prevalent adult collected. Adult species richness was higher than the richness from identified larvae reared from eggs. For example, the Sarcophagidae and *Pollenia rudis* (F.) (Muscidae) were only collected as adults (Fig. 4). *P. rudis*, a known parasite of earthworms, was most likely attracted to the carcass incidentally.

### Laboratory Flight and Oviposition in Darkness

No eggs were deposited in complete darkness either when liver was hanging above or sitting on the floor. In the light, however, 87 total eggs were produced in four of the five trials (80%) when the liver was elevated. By comparison, 58 total eggs were produced in three of the five trials (60%) where the liver was placed on the floor and lighted. The average probability of depositing some eggs under any lighted conditions (70%) was significantly higher than under
darkness ($0\%: \chi^2 = 7.0; df = 1, P < 0.01$). In lighted trials, all flies were absent from the jars in the morning compared with an average of 80% flies in dark trials, which suggested inactivity because there was an average of only two flies left in the jar per trial in total darkness.

All launched plastic flies landed on the floor of the dark room; their collective resting positions roughly constituted a circle 1 m in diameter. However, only 2 of 15 real flies tossed into darkness landed among the surrogates. The dispersion pattern for the live flies was wider than the dark room and extended $\sim 1$ m up the side walls. In no case was the sound of wings sustained; rather, it was brief and never reinitiated after a perceived landing.

**Discussion**

In 22 separate trials that included field and laboratory conditions, nocturnal oviposition never occurred. These findings contradict those of Greenberg (1990) and Singh and Bharti (2001), where both reported nocturnal oviposition in 33% of their trials, as well as Faucherre et al. (1999), who recorded nocturnal oviposition after 12 d in a completely dark cave (1999) and Williams (2002) (MSc thesis) who found one incidence in South Africa of nocturnal oviposition in the field by *L. sericata*. However, our findings are consistent with Amendt et al. (2007) and Baldridge et al. (2006), where the latter authors recorded oviposition only once at $\sim 30$ min after sunset. This is in agreement with our field results in 2006 that documented oviposition only when there was still ambient light during sunset but never during dark hours. These data suggest that if a body is exposed 2 h after sunset, it will not be colonized until the following morning or later, which is consistent with what Smith (1986) concluded in his manual on forensic entomology. Furthermore, our laboratory studies suggest that *L. sericata* is inactive in total darkness. No eggs were laid on liver in the darkness, even though it was accessible by crawling. This is most likely because of the inactivity of the flies in the darkness.

Our data from launching flies into total darkness seem to provide evidence that Diptera may not successfully fly under total darkness. Woodridge et al. (2007) made this argument previously; however, they provided indirect rather than direct proof. Although our data do establish flight behavior to be a bit more sophisticated than the trajectories of similarly shaped inanimate objects, these results suggest little more than a bit of path extension that could be attributed to
wing-flapping or limited gliding. Such behaviors might suggest limited flight stabilization. However, there was no evidence of orientation to sensory cues distant from the insect’s body. The behaviors inferred from this limited experiment can hardly be classified as successful flying. Rather, the results suggest that blow fly flight in total darkness is not sufficient to actively move to find sites for oviposition.

In the 2007 field season, the earliest that flies appeared at the carcass was 8 min after sunrise, but oviposition by those flies did not occur for at least another 3 h. This is important in PMI estimations because it should not be assumed that flies will oviposit immediately after sunrise, even though they may visit a carcass. Light has been shown to be positively correlated with oviposition for other cyclorrhapha flies, such as the blueberry maggot *Rhagoletis mendax* (Curran), but negatively correlated in the case with the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Smith and Prokopy 1981, Burk 1983). Wind speed alone was negatively correlated with oviposition in *Cotesia rubecula* (Marshall) wasps (Keller 1990). The combination of wind speed, temperature, and humidity had the greatest influence on oviposition magnitude among all the abiotic factors in this study, suggesting that abiotic factors may interact to influence daylight oviposition on carcasses. It should be noted that on some trial dates, temperatures were low in the evening (e.g., 16 August 2006 dropped to 12.4 and 13.8 °C, 1 and 2 hours after sunset, respectively), and whereas oviposition was still possible at these temperatures, this factor may have limited the flight activity and subsequent oviposition by some blow flies during these dates. We also observed a low level of oviposition overall during daylight hours and at times with temperatures that are generally optimal for oviposition. We are not sure why this occurred, but many environmental factors seem to play a role in fly oviposition activity at any one site. Our results provide important information for further in depth studies into the effects of abiotic variables on blow fly oviposition.

*Phormia regina* has been reported to arrive later on remains than other blow flies, such as *L. sericata* and *C. vicina* (Denno and Cothran 1976, Lord and Burger 1984, Hall and Doisy 1993, Byrd and Castner 2000). Anderson and VanLaerhoven (1996) collected adult *P. regina* within 24 h of death, but eggs were not laid until 48 h after death. The results of our 2006 field season showed that *P. regina* adults and eggs were collected <24 h after death, but before or during sunset time periods, and never after dark.

This study differs from previous ones on several important points. First, with the exception of a few trials in the experiments by Baldridge et al. (2006), this...
is one of the first studies to use pig carcass models, shown to be the best model for human bodies (Goff 2000, Byrd and Castner 2000, Schoenly et al. 2007). Second, one of us (K.Z.) remained at the field site through the duration of the trials and made continual, hourly observations as opposed to leaving the bait overnight and documenting oviposition the next morning. This allowed for important observations about blow fly behavior to be recorded throughout the night, namely the time of the adult fly’s arrival, their disappearance around sunset, and the fact that it took at least 3 h after sunrise to oviposit after appearing near the carcass. In addition, species composition of adults and larvae were recorded in both field seasons, and the most prevalent adult species were not necessarily the species found to have oviposited. This indicated that adults may be attracted to the carcass, but oviposition conditions may not be optimal at that particular time or for that particular species. If adults are collected from a body and one is trying to estimate portions of the PMI, it should not be assumed that any eggs collected are of the same species.

A criticism of the study of Greenberg (1990) was that perhaps flies crawled rather than flew to the bait at night. Our laboratory studies did not support this hypothesis. Another criticism of Greenberg (1990) was that there was a streetlight nearby and the light could have affected the fly’s behavior; our findings suggest that street light may have been necessary for oviposition. We found that a significant relationship existed between light levels in the field and the magnitude of oviposition. We also found that no nocturnal oviposition occurred in field studies that took place in a rural environment, with little artificial lighting. If there is enough artificial ambient light, nocturnal oviposition may be possible, and future studies should test oviposition under controlled lighting conditions in the field.

In conclusion, we showed that nocturnal oviposition is highly improbable even under favorable weather, temperature, and light conditions in a mid-Michigan habitat. In criminal investigations using insects to help narrow the PMI interval, it should be noted that there are time intervals immediately after sunset and sunrise where oviposition is also unlikely. When oviposition does occur after sunrise, identified adult flies associated with a corpse may not be the same species ovipositing at that time. Both of these factors should be considered when developing entomological-based PMI estimates during criminal investigations.

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