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Cover Page Footnote

This project was funded through the Pierce Cedar Creek Institute's Undergraduate Research Grants for the Environment (URGE) program. We thank the Pierce family's generosity in their support of this program. Sara Syswerda and the staff at Pierce Cedar Creek Institute deserve thanks for their support during the field trials. The College of Arts and Sciences at Valparaiso University also provided supplies for this project. Voucher specimens are stored in the Valparaiso University permanent collection.

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Effect of Habitat on Blow Fly (Diptera: Calliphoridae) Oviposition in Michigan

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Abstract

Forensic entomology applies data gained from insects to aid the criminal justice system. Blow flies (Diptera: Calliphoridae) are usually the first insects to arrive and oviposit (lay eggs) on carrion. Their quick appearance is the foundation for post mortem interval estimations, making any factors that influence their oviposition crucial. This study examined the effects of habitat (forest vs. prairie) on blow fly oviposition timing. Research was conducted in 2015 at Pierce Cedar Creek Institute in Barry County, Michigan. No significant differences were found in oviposition timing or frequency between prairie and forest habitats. Temperature and humidity were not correlated with oviposition timing, which contradicted previous studies. *Lucilia coeruleiviridis* (Macquart) was the dominant species found, comprising about 90% of collected specimens. The data confirm previous reports that *Lucilia* species are the first to arrive on carrion.

Keywords: Forensic Entomology, Prairie, Forest

Forensic entomology is the use of insects in the criminal justice system (Greenberg 1991, Amendt et al. 2007, Haskell and Williams 2008, Byrd and Castner 2009). Colonization by blow flies (Diptera: Calliphoridae) usually occurs within the first few hours after death and is used to estimate the postmortem interval (PMI) (Haskell and Williams 2008). The PMI is the period of time between death and corpse discovery. Establishing estimations of the PMI is an important contribution of forensic entomology to investigators. A number of different factors can influence blow fly oviposition (egg laying) such as weather (Mann et al. 1990), temperature (Ames and Turner 2003), chemicals (Goff 1993), and habitat (Matuszewski et al. 2013, Silahuddin et al. 2015).

This study examined the relationship between habitat and oviposition timing. Previous studies have reported differences in decomposition rates of blow fly breeding in different habitats (Cruickshank and Wall 2002, Vanin et al. 2008, Matuszewski et al. 2013, Silahuddin et al. 2015). Silahuddin et al. (2015) documented differences in decomposition rates between jungle, highland, and rural areas, with rural areas having the fastest rate of decomposition. Matuszewski et al. (2013) found *Lucilia sericata* (Meigen) bred exclusively in open habitats and Sarcophagidae species regularly bred in open habitats and rarely in forest habitats in Central Europe. A study done in England by Cruickshank and Wall (2002) found that *Lucilia* species were caught in warmer, more humid field sites. They also found that in the absence of odors, *L. sericata* aggregated near the hedgerow at the edges of farm fields. Vanin et al. (2008) studied *L. sericata* in northern Italy and found that the species does not show a habitat preference in rural regions with urban sprawl.

There is an absence of published data on blow fly habitat preferences in the United States (with the exception being rural vs urban studies), and this research provides data on the first blow flies to arrive in forest and prairie habitats in Michigan. Based on the results of previous studies (Cruickshank and Wall 2002, Matuszewski et al. 2013, and Silahuddin et al. 2015) the authors hypothesized that oviposition would occur faster in the prairie habitat than the forest habitat. Typically researchers use one dedicated field site for their studies, and it is possible that different habitats will have different oviposition times. It is important to understand the potential effects of the habitat where field trials are conducted, in order for the trials to be applicable in other situations.

Materials and Methods

Research was conducted from June 1 to August 10, 2015 at Pierce Cedar Creek

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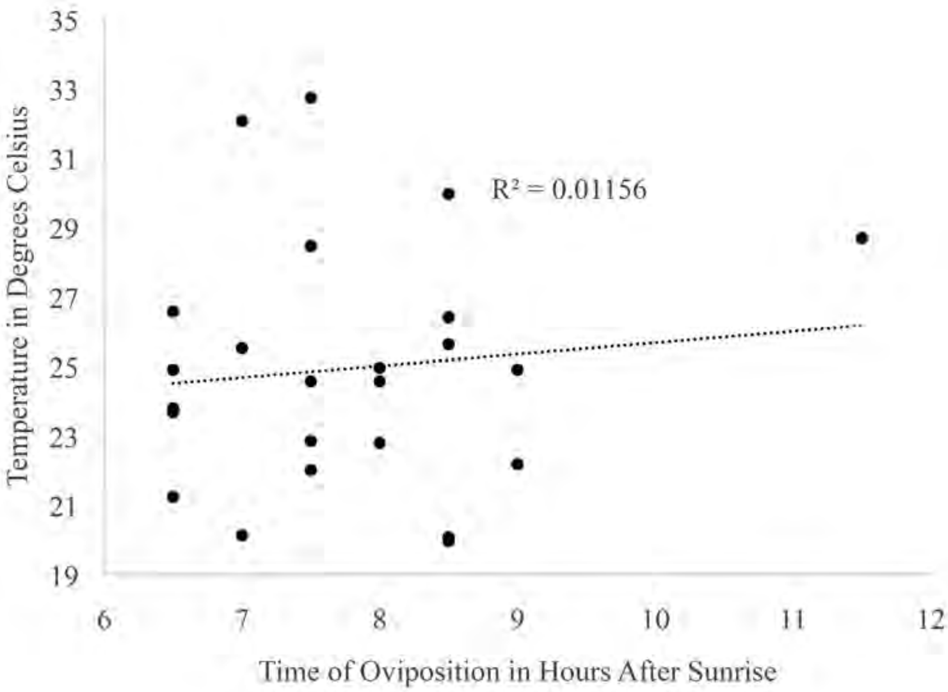

Figure 1. Ambient temperature at the time of oviposition is not correlated with the timing of oviposition in hours after sunrise (r^2=0.012, df=22, P=0.617).

Institute in Barry County, Michigan. Pierce Cedar Creek Institute is a 300 ha nature preserve that has forest, prairie, and wetland habitats, as well as two small lakes and two creeks running through the property. The Institute has a weather station on site (approximately 0.4 km from the field sites), that recorded the temperature and humidity data used in this study.

Field sites were located on three different trails at the Institute, and were chosen because of the close proximity of forest and prairie habitats to each other. Each field trial consisted of bait cups in a prairie and forest location along one of three trails. Ten bait cups were used for each field trial (n= 3 trials per trail). Cups were placed in forest (n = 5 replicate cups) and prairie (n = 5 replicate cups) habitats. Ninety bait cups total were used in the experiments (10 cups per trial × 3 replicates × 3 trails). Bait cups were used to attract blow flies for oviposition and consisted of a clear 0.47 L plastic cup with approximately 6.35 mm of vermiculite in the bottom and a foil cup with approximately 60 grams of aged (approximately 14 hours in a fume hood) chicken liver placed inside. The covered cups were placed on the ground at the sites 4 hours after sunrise, to allow the cups to acclimate to the ambient temperature. Zurawski et al. (2009) found the earliest that blow flies oviposited diurnally was three hours after sunrise, with an average of 5.6 hours after sunrise. Using the Zurawski et al. (2009) data to inform bait placement, trials began six hours after sunrise. The lids were removed from the cups and observations began. Every half hour the cups were checked for blow fly eggs, flesh fly larvae, adult flies and other insects. Once oviposition or larviposition was observed, the cup was covered, labeled, and removed from the field. Observations ended 12 hours after sunrise. Bait cups with eggs or larvae were placed in the fume hood and reared to the third larval instar stage and identified to species for Calliphoridae and family for Sarcophagidae (Stojanovich et al. 1962, Whitworth 2006).

Analysis: Data were analyzed using SPSS statistical software (SPSS 2009). Independent sample t-tests were used to examine significant differences in the number and timing of oviposition events for prairie and forest observation trials. The trails were also tested individually using independent sample t-tests. A one way ANOVA compared oviposition timing on the three trails. Separate correlations examined the relationship

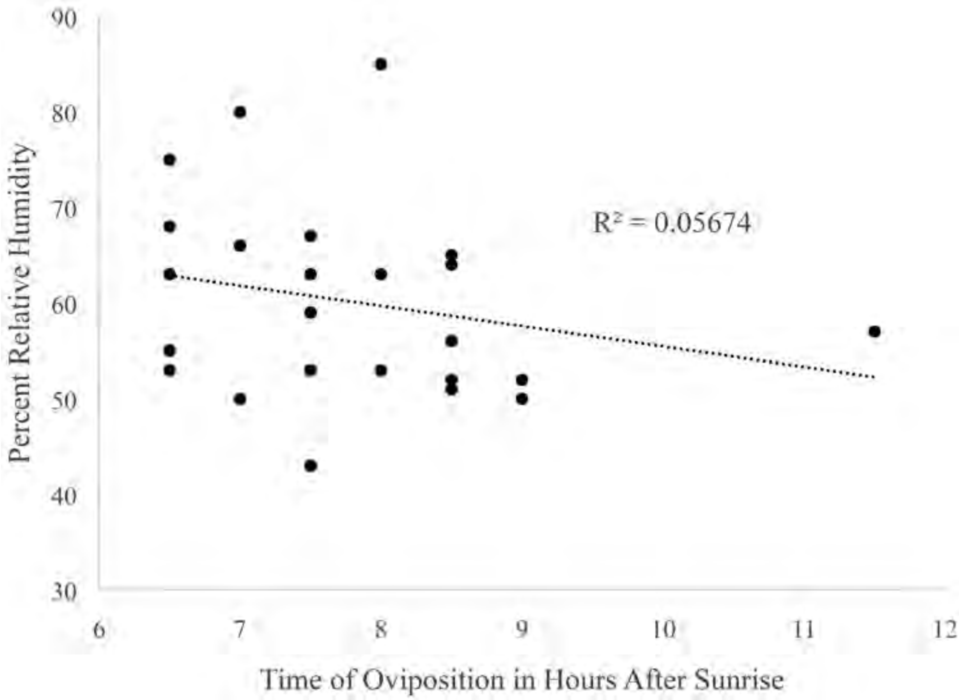

Figure 2. Humidity at the time of oviposition is not correlated with the timing of oviposition in hours after sunrise (r^2=0.057, df=22, *P*=0.262).

between the timing of oviposition and temperature (Fig. 1), and humidity (Fig. 2).

Results

A total of 6,267 maggots were identified from nine trials. *Lucilia coeruleiviridis* (Macquart) was the dominant species found, comprising around 90% of all collected specimens. Table 1 contains specific species information for all trials.

There was no significant difference (significance level P = 0.05) in oviposition timing between habitats when all combined prairie sites were compared to all combined forest sites, (t = –0.196, df = 84, P = 0.845). The three different trails were tested independently and all results showed no significant difference in oviposition timing (trail one: t = –0.143, df = 28, P = 0.888; trail two: t = .389, df = 24, P = 0.701; trail three: t = –0.736, df = 28, P = 0.468). A one way ANOVA and tukey posts hoc tests showed no significant difference in oviposition timing between any of the prairie and forest sites on the three trails (F = 1.24, df = 85 , P = 0.298).

Abiotic factors were examined for significant relationships to oviposition timing. There were no significant correlations between temperature and time of oviposition (r^2 = 0.012, df = 22, P = 0.617) (Fig. 1) or humidity and time of oviposition (r^2 = 0.057, df = 22, P = 0.262) (Fig. 2).

Discussion

This study had species composition results that were consistent with previous findings by Gruner et al. (2007) in Florida. Gruner et al. (2007) found *L. coeruleiviridis* to be the most abundant calliphorid species collected, comprising about 90% of the species collected on days 1–2 of the study in the summer. They reported that *L. coeruleiviridis* was always the first to arrive at the fresh carrion and the first to deposit eggs (Gruner et al. 2007). This is similar to results found at Pierce Cedar Creek Institute, where *L. coeruleiviridis* represented about 90% of species collected (Table 1), and were the first blow flies recorded ovipositing. Joy et al. (2006) also reported *L. coeruleiviridis* as the initial colonizers of carcasses in Virginia.

Lucilia species are known to be early arrivers at carrion and therefore important in forensic entomology investigations (Byrd and Castner 2009, Joy et al. 2006). However, they are not always the dominant species found in succession studies. Previous work by Haskell (1989) was done in Northwest

Table 1. Species composition for three trail locations. A total of 6,267 larva were identified from 9 trials. Data from the three replications at each field site are compiled.

	L. coeruleiviridis	*L. sericata*	*P. regina*	Sarcophagidae
Trail 1 Forest	97%	0	0	3%
Trail 1 Prairie	91%	0	0	9%
Trail 2 Forest	100%	0	0	0
Trail 2 Prairie	100%	0	0	0
Trail 3 Forest	90%	10%	0	0
Trail 3 Prairie	97%	0	0.80%	2.20%

Indiana, which has a similar climate and is geographically close to Pierce Cedar Creek Institute. He found that in the summer *Phormia regina* (Meigen) comprised 85% of the total specimens with the *Lucilia* species represented at 10% and *Cochliomyia macellaria* (Fabricius) 5% in Northwest Indiana. This is vastly different from the data found in this study, where *Lucilia* species dominated. It has been documented that *Lucilia* species will be the first to colonize and dominate species collections early, then their numbers will decline and *P. regina* or other species will become dominant (Joy et al. 2006). The Haskell (1989) study spanned multiple days, where the current study took place over six hours, which could account for the difference in overall species composition between the studies. Future work could document the difference in species composition over an extended time period in prairie and forest habitats.

The results of this study were similar to those found by Matuszewski et al. (2013) in terms of the habitat that Sarcophagidae were found. Our study found Sarcophagidae most often in the prairie habitat, and only in small numbers in one of the forest sites (Table 1). Matuszewski et al. (2013) also found Sarcophagidae most commonly in open habitats and only rarely in forest habitats. However, they found that *Lucilia* species only bred in open habitats, and our study found them in high numbers in all of the field sites (Table 1).

The prairie sites in the study were characterized by open fields and grasses (direct sunlight), as opposed to the forest sites that contained dense trees and shaded canopies. Previous studies by Joy et al. (2006) and Sharanowski et al. (2008) found conflicting results when they examined sunlight vs shaded habitats. Joy et al. (2006) found no difference in oviposition timing for carrion in the shade, versus direct sunlight, in West Virginia in the spring and fall. They did find that decomposition occurred faster in the direct sun habitats. They attributed this to temperature differences between the habitats. They also found that *L. sericata* dominated the species composition in the early days of the experiment, and there was not a difference in species composition between sunlight and shaded habitats. In contrast, Sharanowski et al. (2008) noted a greater species diversity in sunlit habitats when compared to shaded ones in Saskatchewan. The Pierce Cedar Creek study did not find a difference in species abundance or composition between habitats. The results of this study indicate that research trials conducted in prairie or forest habitats would be applicable for either habitat in reference to oviposition timing.

The researchers thought that oviposition timing would be tied to ambient weather conditions, since there appeared to be a shift towards earlier oviposition in the warmer month of July. In June oviposition tended to occur later in the day, specifically between 7.5 to 12 hours post sunrise. At the end of July, oviposition occurred between 6 and 9.5 hours after sunrise. When the temperature and humidity at the time of oviposition were plotted against oviposition timing, there were no significant correlations (Fig 1, 2). This is surprising, because blow flies are more active at warmer temperatures. Zurawski et al. (2009) found that blow flies became active earlier in the day and oviposited earlier in warmer temperatures. Therefore, researchers hypothesized that oviposition would occur earlier in the day when the temperature was higher. The data do not support this hypothesis, as there was not even a small correlation between the ambient temperature and time of oviposition ($P = 0.617$).

No significant differences were found in the timing of oviposition between prairie and forest sites, but this research provides three important pieces of information regarding blow fly oviposition timing. (1) Oviposition timing is critical to postmortem interval estimations, and this study found oviposition occurring the most frequently at 7.5 hours after sunrise. (2) Research conducted in prairie or forest settings should be applicable to either habitat, as long as other factors such as temperature are consistent.

(3) Temperature and humidity were not correlated with oviposition timing, contradicting previous studies that blow flies are more active at warmer temperatures (Joy et al. 2006, Zurawski et al. 2009). Future research should continue to examine factors that affect the timing of blow fly oviposition.

Acknowledgments

This project was funded through the Pierce Cedar Creek Institute's Undergraduate Research Grants for the Environment (URGE) program. We thank the Pierce family's generosity in their support of this program. Sara Syswerda and the staff at Pierce Cedar Creek Institute deserve thanks for their support during the field trials. The College of Arts and Sciences at Valparaiso University also provided supplies for this project. Voucher specimens are stored in the Valparaiso University permanent collection.

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