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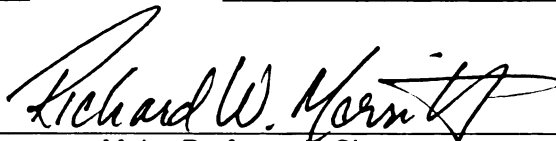
BLOW FLY OVIPOSITION (DIPTERA:
CALLIPHORIDAE) IN MID-MICHIGAN IN RELATION TO
SUNRISE AND SUNSET

presented by

KRISTI NICHOLE ZURAWSKI

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Entomology



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**BLOW FLY OIVPOSITION (DIPTERA: CALLIPHORIDAE) IN MID-
MICHIGAN IN RELATION TO SUNRISE AND SUNSET**

By

Kristi Nichole Zurawski

A THESIS

**Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

“Blow fly oviposition (Diptera: Calliphoridae) in Mid-Michigan in Relation to Sunrise and Sunset”

By

Kristi Nichole Zurawski

The objective of this research was to determine whether blow flies of mid-Michigan demonstrate nocturnal oviposition, and if so, under what conditions. In two different field seasons blow fly oviposition was evaluated in relation to sunset or sunrise. Two laboratory studies examined oviposition under controlled conditions. During 2006, pigs were exposed to fly colonization in one hour intervals, beginning two hours before sunset and ending two hours after sunset, to determine the occurrence of initial oviposition relative to sunset. In 2007, pigs were placed in the field two hours after sunset and oviposition was recorded into the following morning. In 2006, oviposition only occurred in intervals before sunset, but never after dark. During 2007, no oviposition took place from two hours after sunset to sunrise. A chi square- test using data from both summers was used to quantify the probability of nocturnal oviposition, which was significantly less than the observed oviposition rate during daylight hours ($\chi^2=10.67$; d.f. 1; $p<0.01$). A laboratory experiment using bait either hanging 22 cm above or directly on the ground in a completely dark room found that oviposition never occurred. Another study observed *Lucilia sericata* (Meigen) flight activity in the dark and in 13 out of 15 trials blow flies glided rather than fell to the ground when forced to fly. Based on my studies, when using insects to help narrow the PMI interval in criminal investigations, nocturnal oviposition should be considered to occur at very low probabilities or not at all.

ACKNOWLEDGEMENTS

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Brian Bugajski deserves a significant amount of credit for help in the field, keeping me sane, building the cages and platforms used in the experiments, and allowing maggots to be grown on his workbench in the garage. This project wouldn't have happened without his support.

Finally, I would like to thank to my Mom and Dad who have supported me both financially and emotionally for the past 25 years. I wouldn't have pursued this dream without their encouragement.

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Introduction

Forensic entomology uses data derived from insects to assist the criminal justice system (Catts and Haskell, 1990; Byrd and Castner 2000; Greenberg 1991). This field dates back to 13th century China when flies were used to assist in solving a murder involving the use of a farmer's sickle. The day after the murder the investigator asked the workers to come together and lay their sickles on the ground. Blow flies were drawn to one of the sickles and the science of forensic entomology was born (Sung 1981). The science has substantially developed since then, and continues to attract leading entomologists into the field.

There are three main types of forensic entomology: urban, stored product pests, and medico-legal (Catts and Goff 1992). Urban forensic entomology involves insects that affect man-made structures and other aspects of the human environment (Catts and Haskell, 1990). Stored product entomology involves insects that infest stored commodities in some way (Catts and Haskell, 1990).

Medico-legal forensic entomology is the use of insects in determining the amount of time that has passed since insect colonization, usually within a time period that is within a few hours of the postmortem interval (PMI) (Catts and Haskell, 1990). Insect colonization can be used in cases of suicide, homicide and other violent crimes, including cases of neglect. Many factors can affect the PMI (Catts 1992; Hall and Doisy 1993), such as temperature (Ames and Turner 2003), disturbance to the body, chemicals (Goff 1993), weather (Mann *et al.* 1990) and nocturnal oviposition (Greenberg 1990; Singh and Bharti 2001; Baldridge *et al.* 2006; Amendt *et al.* 2007). Several studies have addressed

the probability of nocturnal oviposition under a variety of conditions, demonstrating some disagreement among reported findings (Table 1).

The PMI can be calculated using a system of accumulated degree hours (ADH), or accumulated degree days (ADD). Based on life history characteristics and larval development times of different fly species, ADH (or ADD) is a measure of thermal energy required for insect larvae to reach a specific life stage. The ADH's can be applied to determine approximate time since death, when fly oviposition initially occurred (Kamal 1958; Anderson 2000; Byrd and Allen 2001). Calculating an ADH usually assumes nocturnal oviposition does not occur (Catts and Haskell 1990). If blow flies do indeed oviposit nocturnally, these calculations could be affected by up to 12 hours, which could be the difference between convicting or acquitting a suspect based on alibis (Greenberg 1990).

Greenberg (1990) conducted the first study to examine nocturnal oviposition. The study was conducted in Chicago, Illinois, using rats and ground beef as bait (Greenberg 1990). The species that reportedly oviposited during the night were *Lucilia sericata* (Meigen), *Calliphora vicina* (Robineau-Desvoidy) and *Phormia regina* (Meigen). Nocturnal oviposition (from 0100 to 0400) occurred in 33% of trials, and some oviposition was reported on rat carcasses one hour after sunset but not after. The experimental design of this study raised a few questions. First, it was conducted in an urban setting, having substantial artificial lighting. Second, the food source was placed on the ground near bushes that may have allowed flies to walk rather than fly to the bait.

A second relevant study by Singh and Bharti (2001) examined nocturnal oviposition of blow flies-in Punjab, India, by placing mutton on a 2 meter high wooden

platform. Oviposition occurred five times at ambient light intensities between 0.6-0.8 lux. The species reported to oviposit were *C. vicina*, *Chrysomya megacephala* (Fabricius) and *Chrysomya rufifacies* (Macquart). Nocturnal oviposition (from 2200 to 0300) occurred in 33% of trials, which was identical to Greenberg's (1990) findings. This study supported the hypothesis that the colonizers flew rather than crawled to the illuminated food.

Baldrige *et al.* (2006) evaluated the effect of light on fly ovipositional behavior in Texas using a variety of baits. Nocturnal oviposition occurred only once in over 200 hours of nocturnal bait presentation. The only oviposition occurred on a pig within an hour of sunset. Flies were not observed between 2200 and 0600.

Amendt *et al.* (2007) conducted the most recent study in Munich, Germany using hedgehogs and beef liver as bait. Oviposition never occurred at night in 51 field trials, but was reported to occur under darkened conditions in 33% of laboratory trials (Amendt *et al.* 2007).

Woodridge *et al.* (2007) conducted a study on the flight patterns of *L. sericata* and *Calliphora vomitoria* (Linnaeus) under 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.

The objectives of this study were to: (1) describe nocturnal oviposition in relation to sunrise and sunset in a rural Michigan setting; and (2) evaluate abiotic variables that were hypothesized to affect oviposition timing, magnitude and species composition after sunrise. The hypothesis was that blow flies would not be found to oviposit under natural conditions after dark and that the initial timing, duration and magnitude of oviposition

occurring after sunrise would vary based on environmental conditions such as temperature and precipitation.

Chapter One: “Blow fly oviposition (Diptera: Calliphoridae) in Mid-Michigan in Relation to Sunrise and Sunset”

Abstract-

The most common application of forensic entomology involves establishing a post mortem interval (PMI) to aid investigators in determining the time since initial insect colonization of a corpse. In most instances, it is calculated on the assumption that blow flies (Diptera: Calliphoridae) do not oviposit during the night. The objective of this research was to determine whether blow flies of mid-Michigan demonstrate nocturnal oviposition, and if so, under what conditions. In two different field seasons (summers of 2006 and 2007), blow fly oviposition was evaluated in relation to sunset or sunrise. In addition, two laboratory studies examined oviposition under controlled conditions.

During summer of 2006, pigs were exposed to fly colonization in one hour intervals, beginning two hours before sunset and ending two hours after sunset, to determine the occurrence of initial oviposition relative to sunset. In 2007, pigs were placed in the field two hours after sunset and oviposition was recorded into the following morning. Temperature and light conditions were monitored during both experiments. In the first summer, oviposition only occurred in intervals before sunset, but never after dark. During the second summer, no oviposition took place from two hours after sunset to sunrise. On average, adult flies arrived at the carcasses 50 min after sunrise but did not oviposit until at least four hours later. A chi square- test using data from both summers was used to quantify the probability of nocturnal oviposition, which was significantly less than the observed oviposition rate during daylight hours ($X^2=10.67$; d.f. 1; $p<0.01$).

A laboratory experiment using bait either hanging 22 cm above or directly on the ground in a completely dark room found that oviposition never occurred. A second laboratory study observed *Lucilia sericata* (Meigen) flight activity in the dark and in 13 out of 15 trials blow flies glided rather than fell to the ground when forced to fly. Based on my studies, when using insects to help narrow the PMI interval in criminal investigations, nocturnal oviposition should be considered to occur at very low probabilities or not at all.

Introduction-

Forensic entomology uses data derived from insects to assist the criminal justice system (Catts and Haskell, 1990; Byrd and Castner 2000; Greenberg 1991). Medico-legal forensic entomology uses insects to determine the amount of time that has passed between death and insect colonization, referred to as the postmortem interval or PMI (Catts and Haskell, 1990). Many factors can affect the PMI (Hall and Haskell 1995; Catts 1992; Hall and Doisy 1993), such as temperature (Ames and Turner 2003), disturbance to the body, chemicals (Goff 1993), weather (Mann *et al.* 1990) and nocturnal oviposition (Greenberg 1990; Singh and Bharti 2001; Baldridge *et al.* 2006; Amendt *et al.* 2007). The PMI can be calculated using a method of accumulated degree hours (ADH), or accumulated degree days (ADD). Based on life history characteristics and larval development times of different fly species, ADH is a measure of thermal energy required for larvae to reach their present life stage. The ADH's can be applied to determine approximate time since death (Catts 1992; Anderson 2000; Byrd and Allen 2001). Calculating an ADH usually assumes that blow flies do not oviposit at night (Catts and Haskell 1990). If blow flies oviposit nocturnally, these calculations could be affected by

up to 12 hours, which could be the difference between convicting or acquitting a suspect, based on alibi's (Greenberg 1990). Several studies have addressed the probability of nocturnal oviposition under a variety of conditions, demonstrating some disagreement among reported findings (Table 1).

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hours of nocturnal bait presentation. The only oviposition that occurred was within an hour after sunset. Flies were not observed between 2200 and 0600.

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Woodridge *et al.* (2007) conducted a study on the flight patterns of *L. sericata* and *Calliphora vomitoria* (Linnaeus) in supposed 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 so we studied blow fly flight activity in complete darkness.

The objectives of this study were to: (1) describe nocturnal oviposition in relation to sunrise and sunset in a rural Michigan setting; and (2) evaluate abiotic variables that were hypothesized to affect oviposition timing, magnitude and species composition after sunrise. The hypothesis was that blow flies would not oviposit under natural conditions after dark and that the initial timing, duration and magnitude of oviposition occurring after sunrise would vary based on environmental conditions such as temperature and precipitation.

Methods-

2006 Field Season Experiment-

Six pigs weighing an average of 25 kg were obtained from the Michigan State University Swine Research Facility. Each pig was euthanized by lethal injection at approximately 1600 hours and transferred to a black plastic garbage bag immediately

following 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, approximately 0.8 km from the Michigan State University Swine Research Facility and stored inside of a barn for approximately 4 h. A Hobo[®] temperature data logger (set 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.

Two wooden platforms, 15 cm high were erected 16 m apart in a grassy field 183 m from the barn (Figure 1). Tanglefoot[®], an adhesive paste, was generously applied to each leg of both platforms to capture any crawling insects. Beginning two hours before sunset, one pig was removed from the barn and transported to the field. Pigs were removed from the bags and placed on a platform facing north. After one hour of exposure the pig was removed and replaced with another pig from the barn. This process was repeated every hour until two hours after sunset for a total of five 1-hour time intervals.

After exposure, the pigs were carefully examined for the presence of fly eggs, which 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 2007). After the last pig was examined, the styrofoam cups were transported to the laboratory. Hatched larvae were fed 28g of beef liver each day until reaching the third larval instar post-feeding stage, after which they were preserved in 70% EtOH and identified to species (Stoganovich *et al* 1962).

In 2006 and 2007, a Hobo[®] temperature data logger and a TES-1336 Data logging light meter were centrally located between the platforms. Both data loggers logged at 1-min intervals for the duration of each trial. Sunset and sunrise were determined using the

Weather Channel website (www.weather.com), which coincides with data on NOAA's website, but the readings were closer to the site.

During this first summer a pig was placed in the field on the second platform two hours before sunset. A wood-framed cage wrapped with chicken wire was placed over this pig. The pig was left exposed for one week, and the cage prevented predators from contacting the carcass. Adult flies were collected in the days following the initial experiment to acquire information about the local species that were present at the time of the experiment. These flies were later pinned and identified to species using the taxonomic key of Whitworth (2006).

2007 Field Season Experiment-

The same 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 exposed from two hours after sunset until oviposition occurred after sunrise the following morning; and 3) the pigs were placed on 15 cm high platforms that were 10 m apart, examined every hour after sunset until sunrise, and thereafter every half hour. Examination involved a thorough inspection of the body, with a concentration on mucus membranes and orifices. Egg collection and rearing were performed as in 2006, except adult flies 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 in order to determine the magnitude of oviposition during each trial (Figure 2). In five separate instances, a piece of beef liver was placed into a cage that contained a laboratory strain of *L. sericata*, and after 2 h the liver was removed from the

cage and the egg masses were collected, separated into sixteen different egg counts ranging from 5 to 250 g, and weighed on a Sautorius balance.

Laboratory Experiment on Oviposition-

Ten adult flies in a 60:40 female/male ratio of laboratory reared *L. sericata* were placed into a 473 mL glass mason jar and then into a light tight box for 1 h to acclimate to darkness. After 1 h, the jar was set on its side on the floor in a completely dark room (1.2 m by 4.2 m), and the lid was removed. A weighing dish containing 28 g of beef liver was either placed in a small basket that was hung from the ceiling of the room 22 cm above the floor or directly on the floor. The flies were left overnight for eight h and the liver was checked in the morning for eggs. Each experiment was repeated five times and five trials also were performed with the lights on for both bait placements.

A second observational laboratory experiment involved 15 *L. sericata* adults that were acclimated to darkness and placed into 10mL glass tubes with a piece of cotton covering the opening. The flies were then held at eye level, and launched into the air by flicking the tube. After 30 seconds the lights were turned on and the flies were located and collected. Prior to launching the live flies, 15 plastic flies were launched to form an area where flies would land if they fell straight to the ground as opposed to where they would land if they were actively flying or simply gliding.

Analysis-

The probability of nocturnal oviposition was analyzed using a chi square test. Using data from both field seasons, the probability of observed diurnal oviposition was compared to observed nocturnal oviposition that occurred in both the field and laboratory experiments. T-tests were used to evaluate environmental and climatic differences

between dates when oviposition occurred compared to dates when there was none.

Pearson Product Moment correlation analyses were used to evaluate the relationship between the number of eggs oviposited and the environmental variables. The relative species composition of blow fly adults and larvae were to describe the community composition across the field seasons.

Results-

Nocturnal Oviposition 2006 Field Season

Oviposition occurred 2 hours before sunset in 50% of the trials, 1 hour before sunset in 75% of the trials, and at sunset in 50% of the trials. Oviposition did not occur 1 and 2 hours after sunset (Table 2, Figure 3). No insects were found crawling up the platform and on to the pigs in 2006 or 2007.

2007 Field Season

Adult flies were never observed after sunset, and no nocturnal oviposition was documented on any pig in any trial. Flies were first observed on average 50 minutes after sunrise and oviposited 4 hours later. The earliest that flies arrived and oviposited were 8 minutes and 3 ½ hours after sunrise, respectively (Table 3). The average lux reading in trials when oviposition occurred was 19193 compared to 11805 when oviposition was not seen. Average temperatures were also higher when oviposition occurred (32° C) than when it did not (23° C).

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, the probability of nocturnal oviposition was significantly lower than oviposition during daylight hours ($X^2 = 10.67$; d.f. = 1, $p < 0.01$).

Laboratory

In both laboratory experiments no oviposition occurred in the dark. Under lighted conditions, oviposition occurred in 60% of the trials when the bait was hanging from the ceiling and 80% when on the floor. This resulted in an average 70% probability of oviposition under laboratory lighted conditions which was significantly greater than oviposition under dark conditions ($X^2 = 7.0$; d.f. = 1, $p < 0.01$).

Factors influencing oviposition:

2006 Field Season:

In the hours before sunset *Lucilia coeruleiviridis* (Macquart) was the most common blow fly species found ovipositing compared to *P. regina* which was the most common species found to oviposit during the sunset time interval (Figure 4); however, *C. vomitoria* made up from about 20 – 35% of the eggs collected during the trials. *L. coeruleiviridis* was the most frequent species ovipositing in early summer compared to *C. vomitoria* at the end of the season (Figure 5). *P. regina* was the most frequent adult fly collected from pigs after one week of exposure among all trials (Figure 6). The species composition changed not only by time interval, but also by the date (Figures 3-5). *P. regina* was the most prevalent blow fly ovipositing at sunset.

2007 Field Season:

On days when oviposition occurred, temperature was significantly greater ($t = 3.46$; d.f. = 6; $p = 0.014$) than days when no oviposition occurred (Figure 7). Light was also significantly greater ($t = 9.81$; d.f. = 6; $p < 0.001$) on days when oviposition (Figure 8).

There were no statistically significant differences in percent relative humidity ($t=0.71$; d.f.= 6; $p=0.50$), wind speed ($t= 1.62$; d.f.= 6; $p=0.16$), or precipitation ($t=1.21$; d.f.=6 ; $p=0.27$), between dates of no oviposition and those with detected oviposition (Figures 9-11. Correlation analyses were found to be insignificant between number of eggs and temperature ($\rho= -0.05$; d.f. 3; $p=0.47$), light ($\rho= 0.37$; d.f. 3; $p=0.29$), wind speed ($\rho= -0.34$; d.f. 3; $p=0.31$), relative humidity ($\rho= 0.038$; d.f. 3; $p=0.48$), rainfall ($\rho= 0.45$; d.f. 3; $p=0.25$), and time of oviposition ($\rho= -0.62$; d.f. 3; $p=0.15$).

L. sericata made up the highest percentage of blow fly species eggs collected from pigs during the 2007 summer; however, this varied by date (Figures 12, 13). *P. regina* was the most common adult fly species when averaged across the season; but this also varied by date (Figures 14, 15). Similar to the 2006 summer, *L. coeruleiviridis* was most abundant early in the summer, but the community changed to almost entirely *L. sericata* in July and August and then was replaced by *C. vicina* and *P. regina* in September. There was a higher species richness in the adults collected, compared to the eggs (Sarcophagidae and *Pollenia rudis* (Fabricius) were only collected as adults) (Figures 12-15). It is interesting to note that *P. rudis* is a known parasite of earthworms and most likely was at the carcass incidentally, but was present at the study site. *P. regina* adults were found in all trials except on 16 August 2007 when only *L. sericata* was collected; however, *Cochliomyia macellaria* (Fabricius) eggs were found in that trial. Although *L. sericata* had the highest oviposition rate after sunrise, *P. regina* was the most prevalent adult collected.

Laboratory:

When stimulated for flight, flies were observed gliding to the ground, rather than flying directly, or simply falling. In 15 trials, 13 flies glided which suggested that they

may have some control over movement when provoked to fly but not enough to take off into flight. The other two flies simply fell to the ground. If flies will not initiate flight when stimulated in the dark, it is unlikely that they will fly to a carcass at night. If flies are not flying, then the likelihood of oviposition is very low. The flies' behavior was consistent between trials suggesting an innate reaction.

Discussion:

In twenty-two separate trials, including both field and laboratory conditions, nocturnal oviposition was not found to occur once. These findings are in contrast to those of Greenberg (1990) and Singh and Bharti (2000), where both reported nocturnal oviposition in 33% of their trials. However, it is consistent with the findings of Amendt *et al.* (2007) and Baldrige *et al.* (2006). Baldrige *et al.* (2006) recorded oviposition only once and it was approximately 30 min after sunset. This agrees with my field results in 2006 that showed oviposition occurred when there was still ambient light during sunset, but not during the dark hours. These data suggest that if a body is exposed after sunset, it will not be colonized until the following morning or later which is consistent with what Smith (1986) described in his manual on forensic entomology. My laboratory results suggest that under dark conditions, flies do not engage in active flight, providing a plausible reason why nocturnal oviposition was not documented in this field study, or others (see above), under naturally dark conditions (i.e., no artificial lighting).

P. regina has been reported to arrive later on remains than other blowflies, such as *L. sericata*, and *C. vicina* (Hall and Doisy 1993; Byrd and Castner 2001; Lord and Burger 1984; Denno and Cothran 1976). Anderson and VanLaerhoven (1996) collected adult *P. regina* within 24 h of death, but eggs were not laid until 48 hours after death. The results

of my 2006 field season demonstrated that *P. regina* adults and eggs were collected less than 24 hours after death, but before or during sunset time periods and never after dark.

In the 2007 field season, the earliest that flies appeared at the carcass was 15 min after sunrise, but oviposition by those flies did not occur for at least another 3 hours. This is important in PMI calculations because it should not be assumed that flies will oviposit immediately following sunrise. The abiotic factors of temperature and light were significantly higher in trials where oviposition occurred, compared to those trials when it did not (Table 4, Figures 7, 8). Temperature and light have been shown to have be positively correlated with oviposition for other flies such as the blueberry maggot *Rhagoletis mendax* (Curran), but negatively correlated with the Carribean fruit fly *Anastrepha suspensa* (Loew) (Smith and Prokopy 1981; Burk 1983). Wind speed, humidity and precipitation were not statistically significant between trials with and without oviposition, although all average values were lower in trials with oviposition (Table 4). The relationship between number of eggs laid and abiotic factors were evaluated and no significant associations were found. However, because of the low sample size (i.e., number of trial of no oviposition), the statistical power was low and may have limited my ability to detect significant differences in these variables.

This study differs from previous studies on several important points. First, with the exception of a few trials by Baldridge *et al.* (2006), this is one of the first studies to use pig carcass models, which have been shown to be the best substitute for humans (Goff 2000, Byrd and Castner 2001, Schoenly *et al.* 2007). Second, I 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 blowfly behavior to be recorded, namely the time of adult fly's arrival, their disappearance around sunset, and the fact that it took at least 3 hours after sunrise to oviposit after appearing at 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 that were most often 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. If eggs are collected from a body and one is trying to determine the PMI, they should not assume that eggs are the same species as the adults that were collected at the same time.

A criticism of Greenberg's (1990) study was that perhaps flies had crawled to oviposit on the bait instead of flying to it. My laboratory studies did not support this hypothesis since no oviposition occurred under darkness when bait was placed directly on the floor. Another criticism of Greenberg (1990) was that there was a streetlight nearby and the light could have affected the fly's behavior. My findings suggest that the streetlight may have been necessary for oviposition in the Greenberg (1990) study. I found that a significant relationship exists between light levels in the field and whether or not oviposition occurs. I also found that no nocturnal oviposition occurred in field studies that took place in a rural environment with little nearby artificial lighting. If there is enough artificial ambient light, then nocturnal oviposition may be possible. Future studies should test oviposition under controlled lighting conditions in the field.

The results of this study suggest that if a body is exposed to the environment after sunset, it will not be colonized on average until at least three hours after sunrise the following morning, provided temperature and light conditions are favorable. In

conclusion, my studies demonstrate that nocturnal oviposition is unlikely and improbable even under favorable weather, temperature and light conditions. 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 unlikely, and when oviposition does occur after sunrise, that identified adult flies associated with a corpse may not be the species ovipositing at that time. Both of these factors should be considered when developing entomological-based PMI estimates during criminal investigations.

APPENDIX A

TABLES

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

	Location	Bait	Platform	Exposure	Results	Species
Greenberg (1990)	Chicago, Illinois	Rats, ground beef	No	0100-0400 2100-2400	33% trials nocturnal oviposition	<i>Lucilia sericata</i> , <i>Calliphora vicina</i> and <i>Phormia regina</i>
Singh and Bharti (2001)	Punjab, India	Mutton	Yes, 2 meters, wood	2200- 0300	33% trials nocturnal oviposition	<i>C. vicina</i> , <i>Chrysomya megacephala</i> , and <i>Chrysomya rufifacies</i>
Baldrige et al. (2006)	Central Texas	Rats, mice, pigs	Yes, 0.6 meters, wood	1800, 2100, 2400 and 0600	1 time in 200 hours of bait presentation	<i>Cochliomyia macellaria</i> , <i>L. sericata</i> and <i>Musca domestica</i> .
Amendt et al. (2007)	Munich, Germany	Hedgehog	Window sill, 2.5 meters	2230 until 0430 (field) 2100-0700 (lab)	Twice in the laboratory, zero in the field	<i>L. sericata</i> (lab strain)

Table 2. Environmental conditions measured during the summer 2006 field trials when oviposition did and did not occur during each time interval. The species of fly that was found to oviposit are also given. T-2 = 2 h before sunset; T-1 = 1 h before sunset; T = Sunset; T+1 = 1 h after sunset; T+2 = 2 h after sunset.

Date	Time Interval	Light Reading (Lux)	Temperature (°C)	Time	Oviposition/Species
June 29	T-2	4700	22.33	2016	Yes, <i>Lucilia coeruleiviridis</i>
	T-1	100	15.95	2016	Yes, <i>L. coeruleiviridis</i>
	T	0.07	15.76	2217	None
	T+1	0.05	14.9	2315	None
	T+2	0.05	13.75	2408	None
July 19	T-2	5170	24.44	2009	None
	T-1	330	17.95	2109	None
	T	0.09	16.71	2208	None
	T+1	0.06	16.04	2310	None
	T+2	0.05	13.71	2407	None
August 2	T-2	6540	32.8	1953	None
	T-1	501	29.25	2048	Yes, <i>Lucilia sericata</i>
	T	0.11	28.76	2152	Yes, <i>L. coeruleiviridis</i> , <i>Phormia regina</i>
	T+1	0.12	23.48	2255	None
	T+2	0.12	21.66	2354	None
August 16	T-2	4300	26.19	1933	Yes, <i>Calliphora vomitoria</i>
	T-1	820	14.71	2034	Yes, <i>C. vomitoria</i>
	T	0.02	12.88	2136	Yes, <i>C. vomitoria</i>
	T+1	0.02	12.4	2232	None
	T+2	0.03	13.84	2333	None

Table 3. Environmental conditions during the summer of 2007 for the time of first fly appearance in the field as well as the time of oviposition. Predicted number of eggs of oviposition and species of those eggs is also given.

Date	Time of first fly Appearance (min)	Lux reading when flies appear	Temperature when flies appear (°C)	Time of oviposition (min)	Temperature at time of oviposition (°C)	Lux reading at oviposition	Predicted # of eggs of oviposition	Species
June 1	9	1797	16.71	None	-	-	-	-
June 15	27	2020	23.77	196	32.29	19160	64	<i>Lucilia sericata</i>
July 6	71	1927	26.98	324	29.75	18945	12	<i>L. sericata</i>
July 27	19	602	17.09	None	-	-	-	-
August 9	8	1149	17.67	None	-	-	-	-
August 17	59	4200	15.56	555	39.39	17860	22	<i>L. sericata</i> ; <i>Chochilomyia macelleria</i>
September 13	104	4980	8.78	294	27.96	20,000	30	<i>Calliphora vicina</i>
September 27	105	15250	13.56	300	31.71	20,000	46	<i>Calliphora vomitoria</i> ; <i>Phormia regina</i>
Mean	50.25	3990.625	17.515	333.8	19193	32.22		
Std Deviation	40.35	4785.51	5.67	132.99	886.28	4.36		

Table 4. Results of t-tests comparing abiotic factors in trials with and without oviposition. Bold p-values indicates significance.

Factors	t- value	Degrees of freedom (d.f.)	p- value
Temperature (°C)	3.46	6	0.014
Light Level (Lux)	9.81	6	<0.001
% Relative Humidity	0.71	6	0.503
Wind Speed (km/day)	1.62	6	0.157
Rainfall (cm)	1.21	6	0.273

APPENDIX B
FIGURES FOR PUBLICATION



Figure 1. An aerial map of research site from Google maps. The site was on the property of the Michigan State University Entomological Research Station, seen in the lower lefthand corner of the photo. GPS Coordinates are $42^{\circ}41'30.48''\text{N}$ and $84^{\circ}29'41.01''\text{W}$ with an elevation of 853 feet.

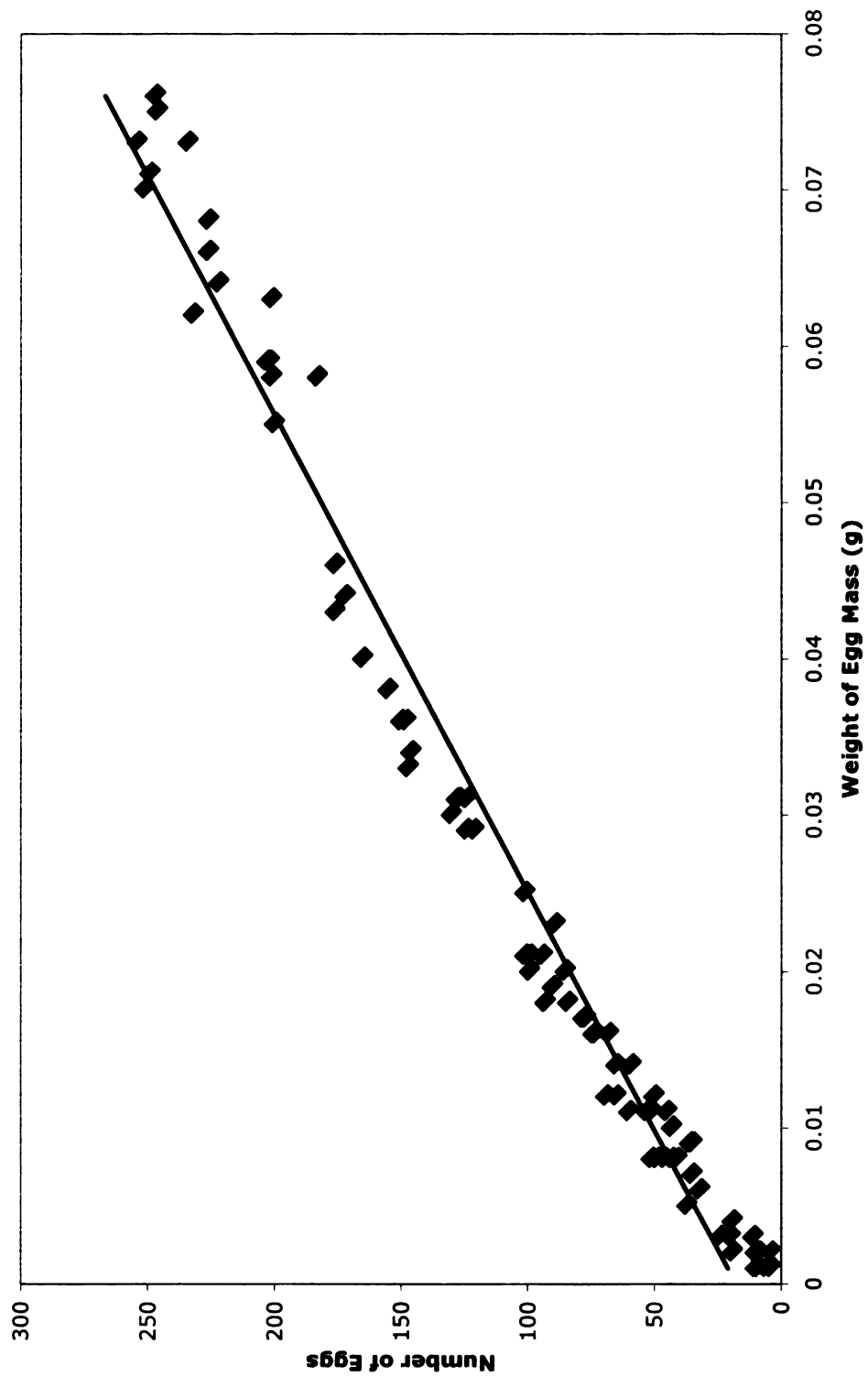


Figure 2. Linear regression model of *Lucilia sericata* egg mass weight and egg number. $R^2 = 0.98$. The equation for the line is: $y = 3275.9x + 17.778$.

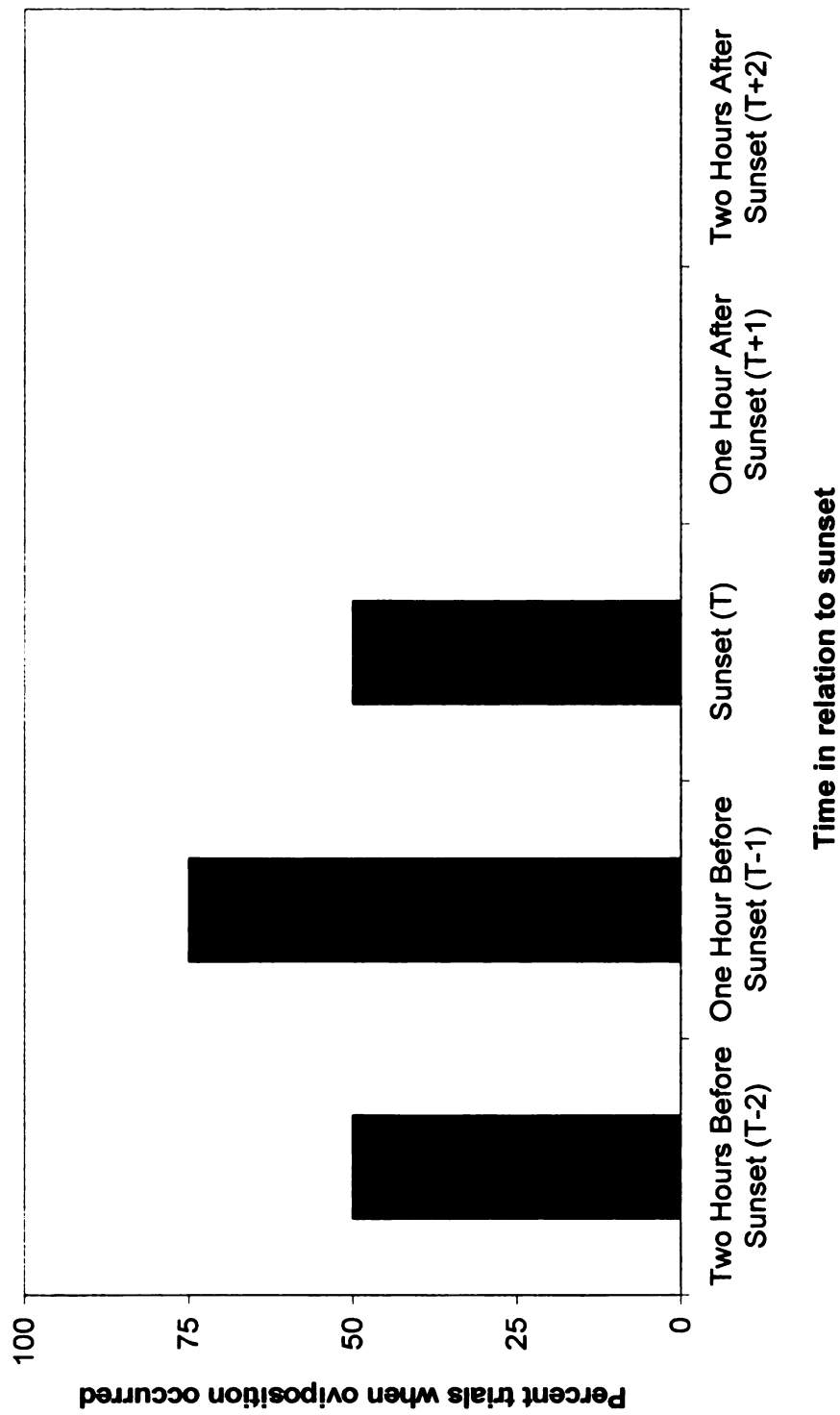


Figure 3. The percent oviposition during each time interval over the summer of 2006. Data were compiled from eggs collected during each of four trials over the summer.

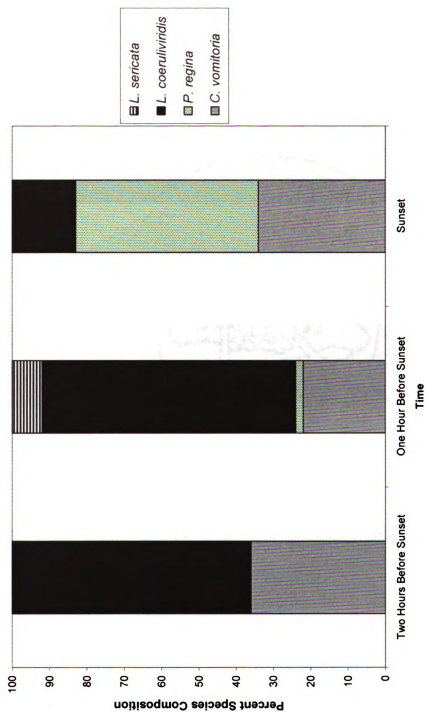


Figure 4. The percent of each species composition of eggs collected during three time intervals over the summer of 2006. Eggs were collected at the end of each one hour time interval and reared to the third larval post feeding instar for identification.

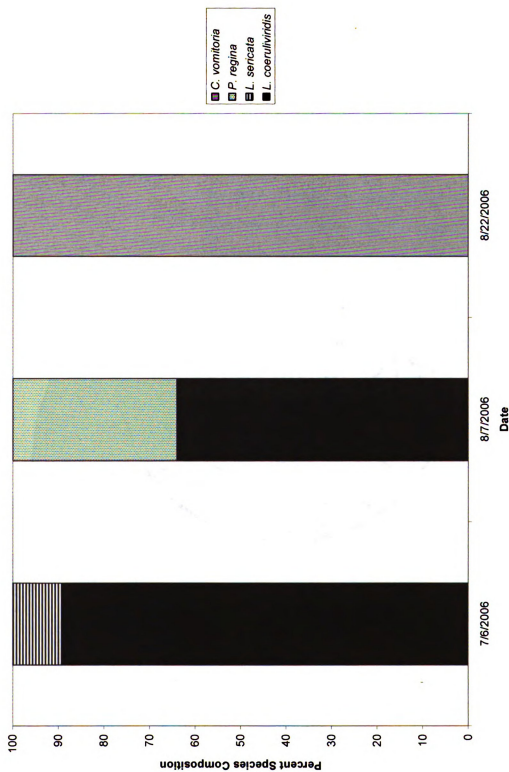


Figure 5. The percent of each species composition of eggs collected during three trials over the summer of 2006. Eggs were collected at the end of each one hour time interval and reared to the third larval post feeding instar for identification.

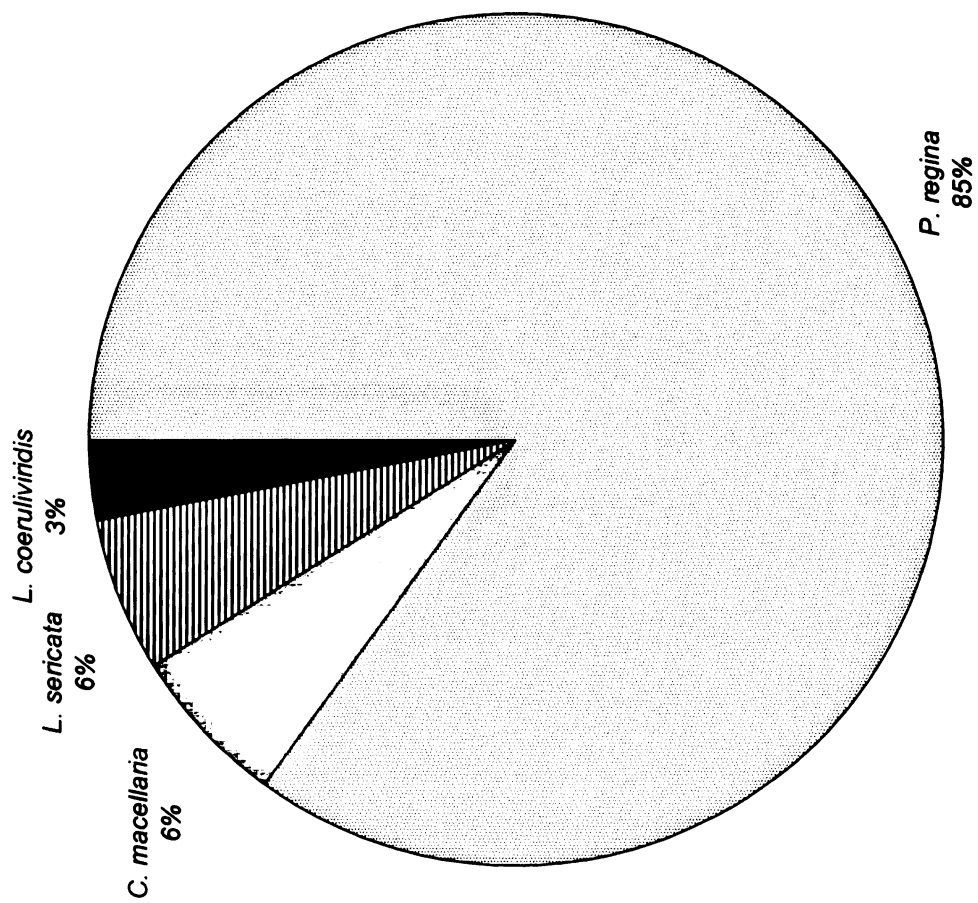


Figure 6. Percent species compositions of adults found on the control pig one week after trials in the summer of 2006.

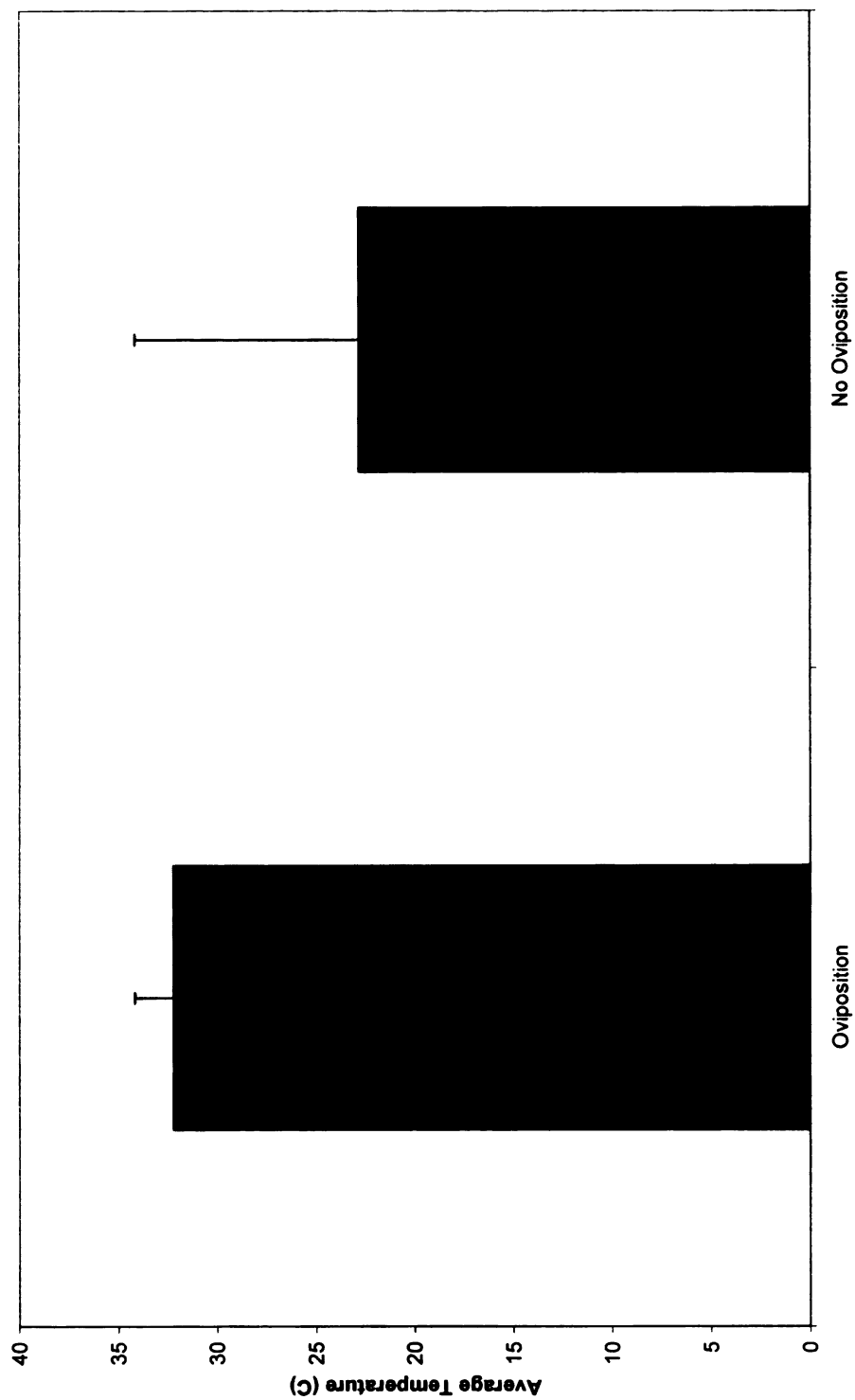


Figure 7. The average temperature (°C) (taken at conclusion of trial) where oviposition did and did not occur. Error bars represent plus and minus one standard deviation.

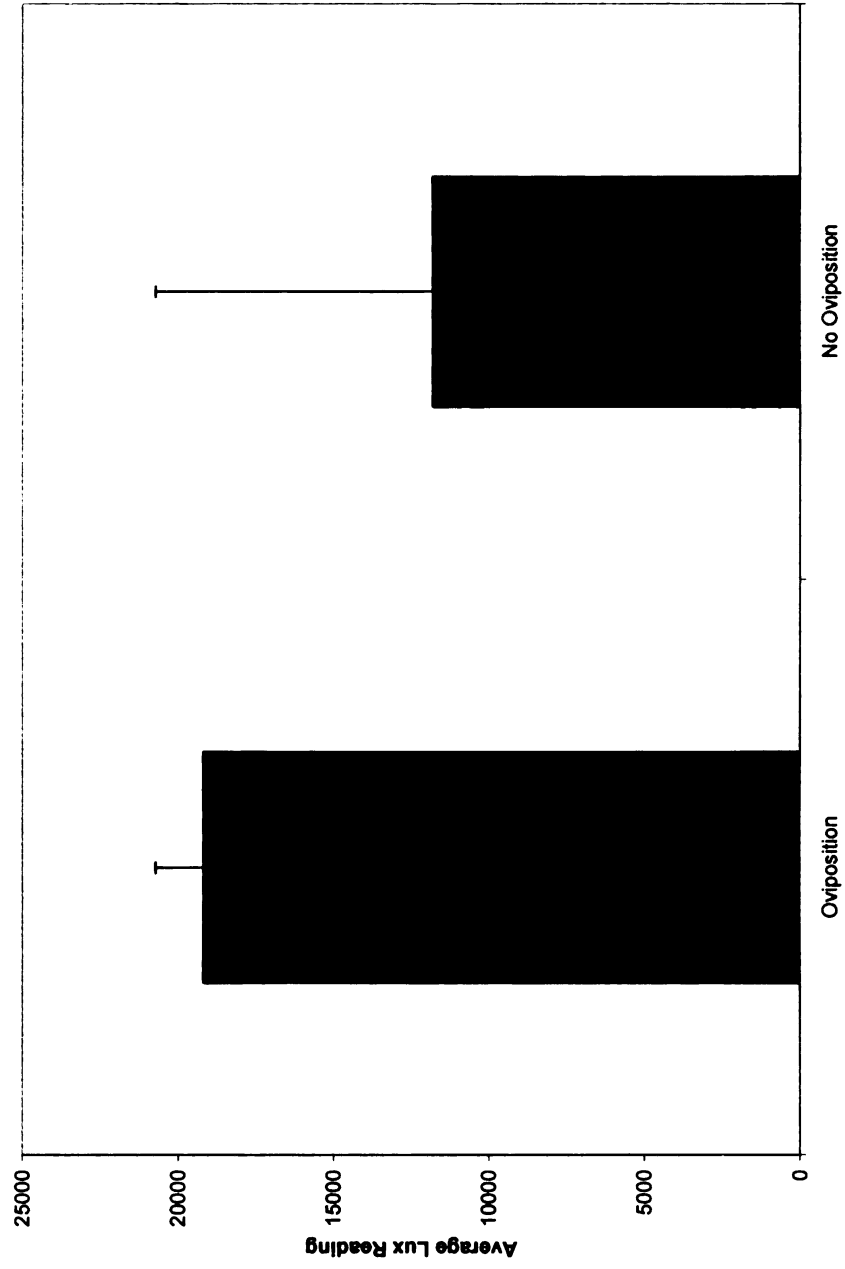


Figure 8. Average Lux readings (taken at conclusion of the trial) where oviposition did and did not occur. Error bars represent plus and minus one standard deviation.

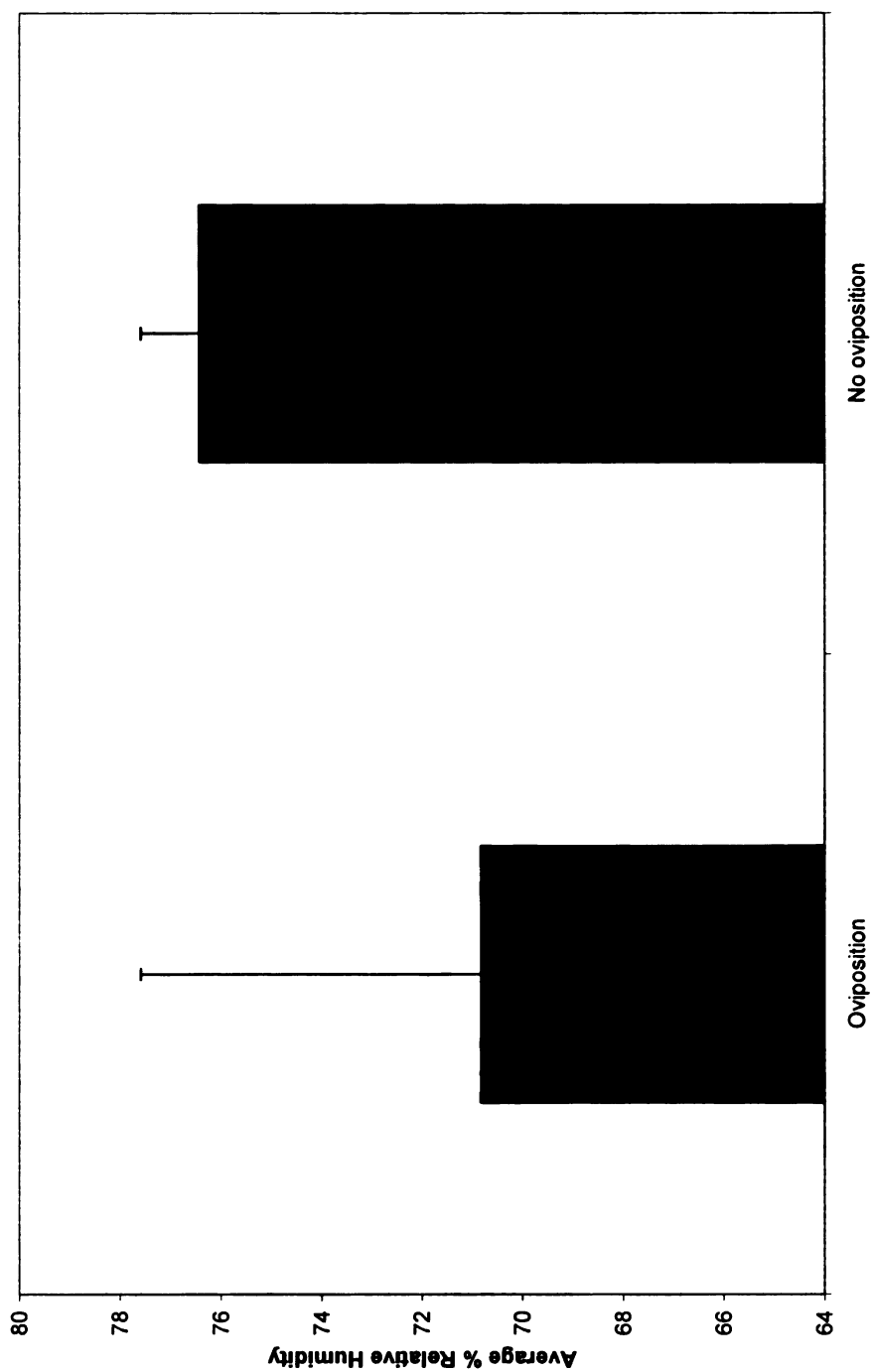


Figure 9. Average % Relative humidity for trial dates when oviposition did and did not occur. Error bars represent plus and minus one standard deviation.

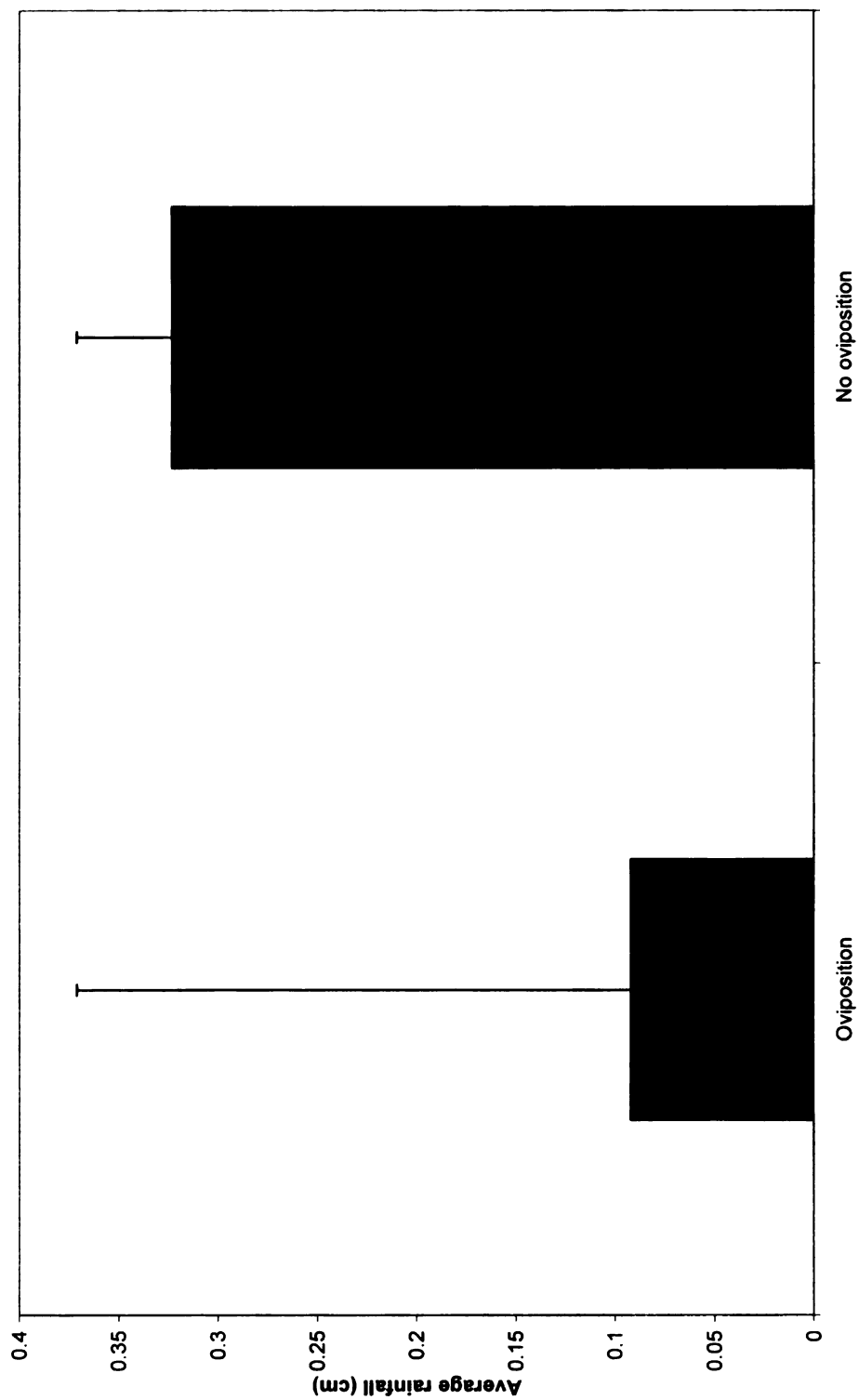


Figure 10. Average rainfall (cm) for trial days when oviposition did and did not occur. Error bars represent plus and minus 5 one standard deviation.

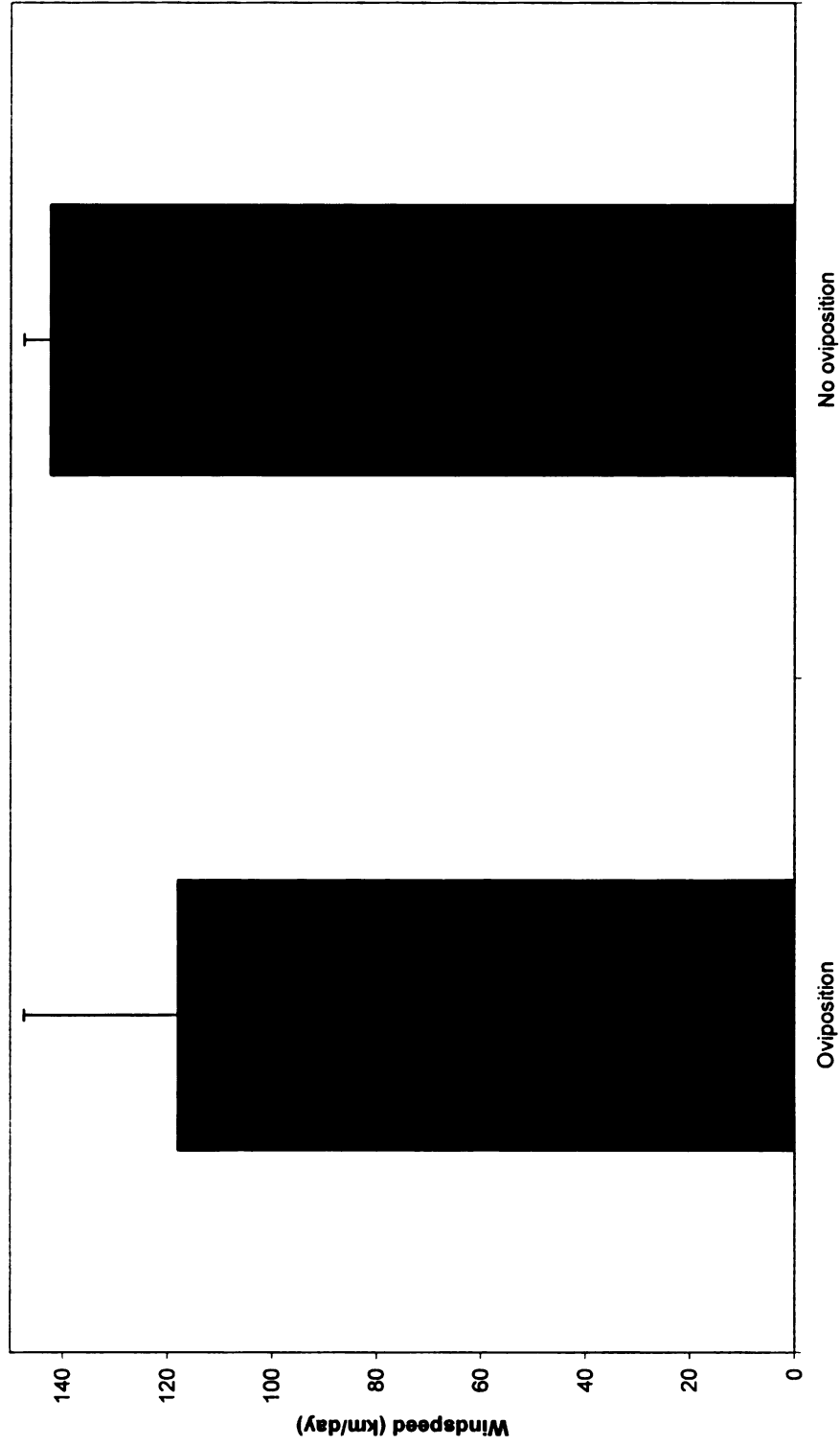


Figure 11. Wind speed (km/day) for trial dates when oviposition did and did not occur. Error bars represent plus and minus one standard deviation.

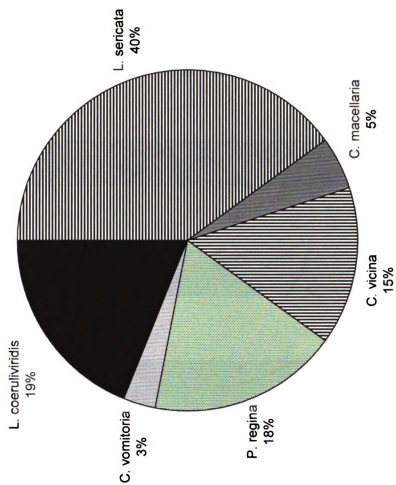


Figure 12. The percent species composition of all eggs collected from test pigs over the summer of 2007.

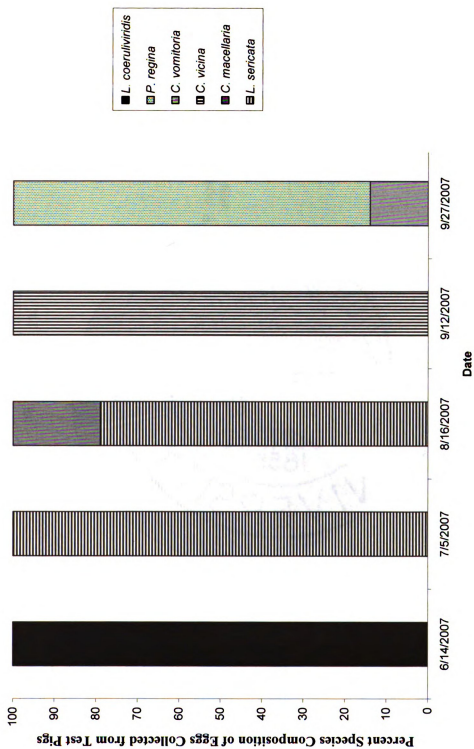


Figure 13. Species composition of eggs collected in five trials over the summer of 2007.

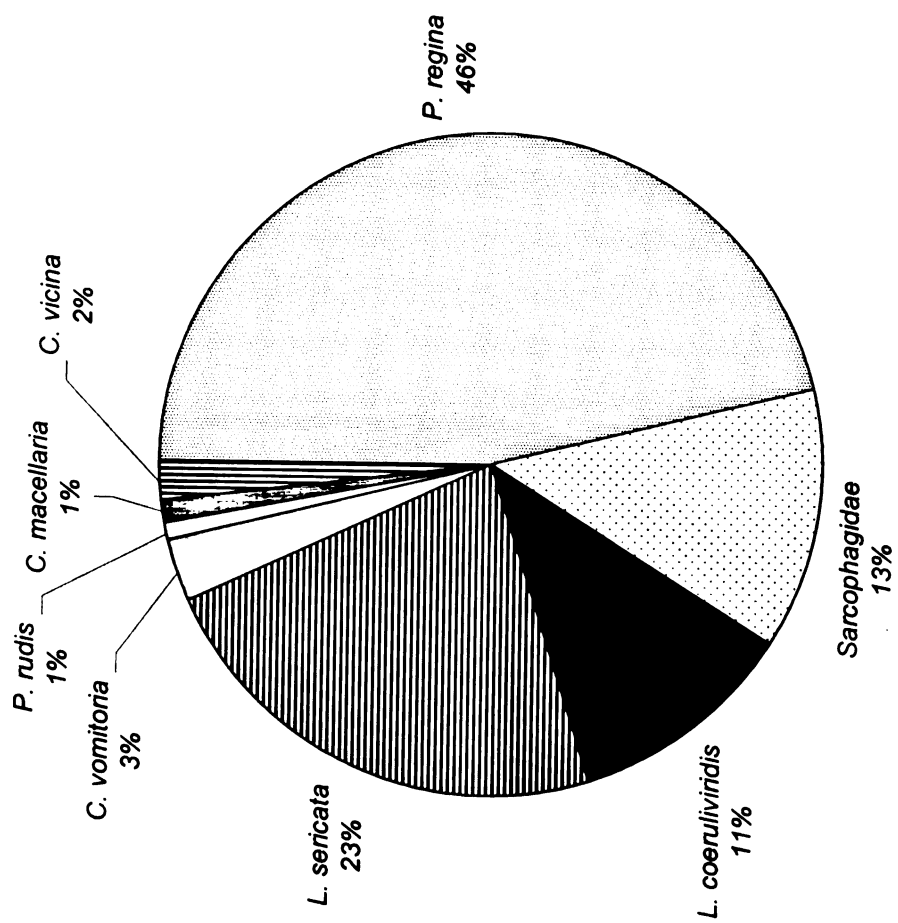


Figure 14. The percent species composition of adult flies collected during the summer of 2007.

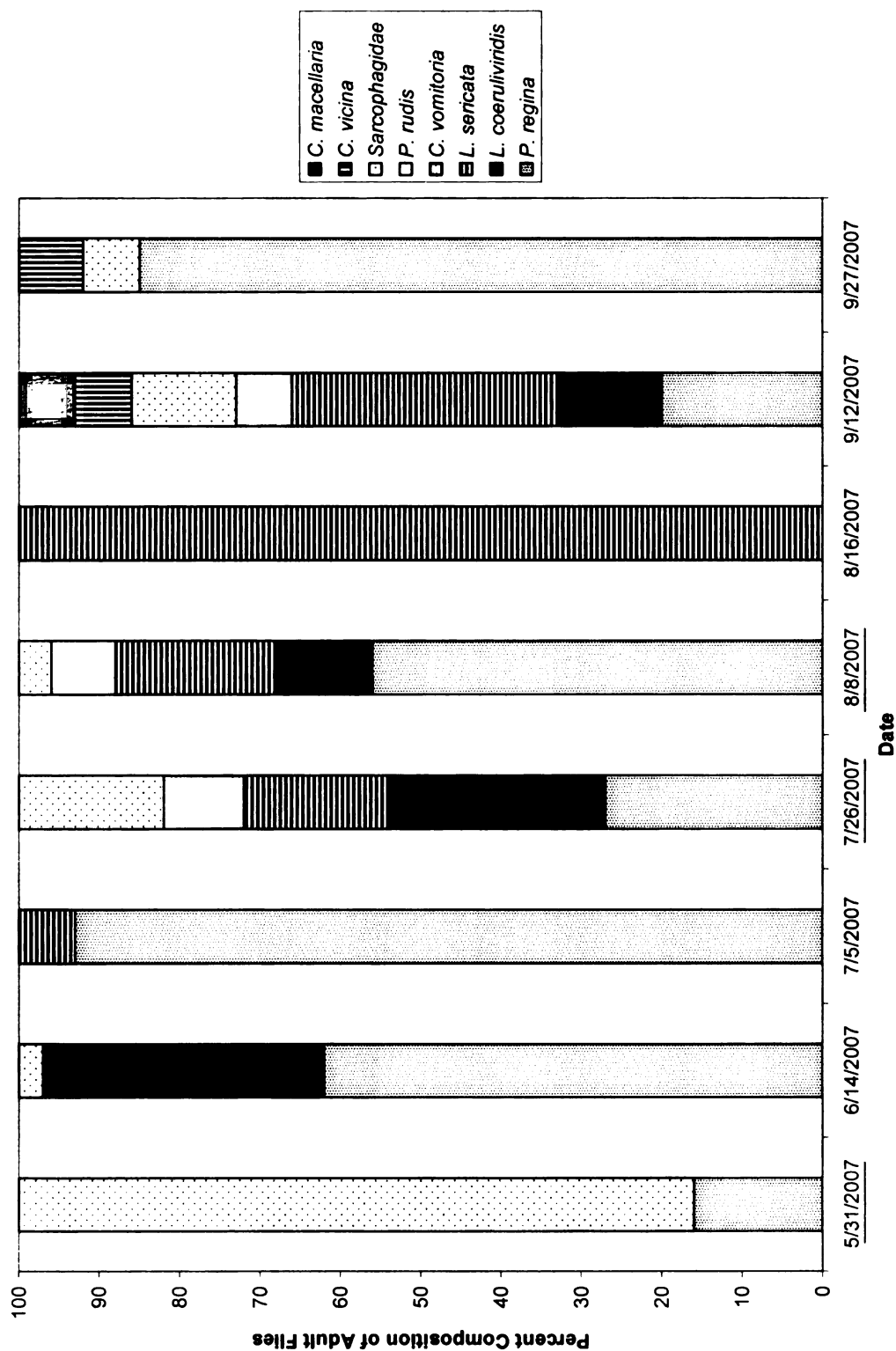


Figure 15. Percent composition of adult flies collected from test pigs in eight trials during the summer of 2007. Underlined dates indicate that the trial took place but oviposition did not occur.

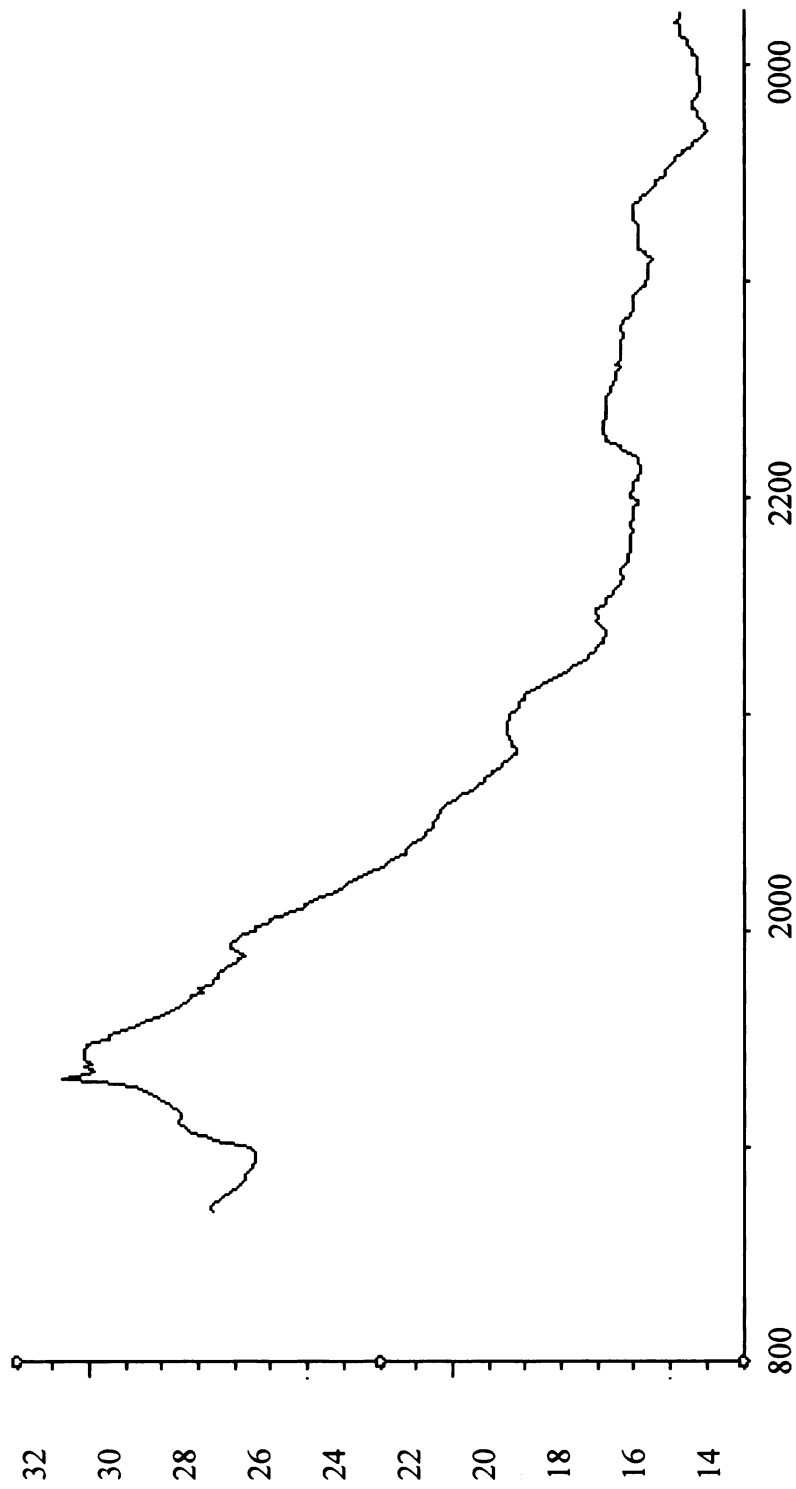


Figure 16. Graph depicting temperatures ($^{\circ}\text{C}$) in the field during the evening of June 29, 2006 during which oviposition occurred.

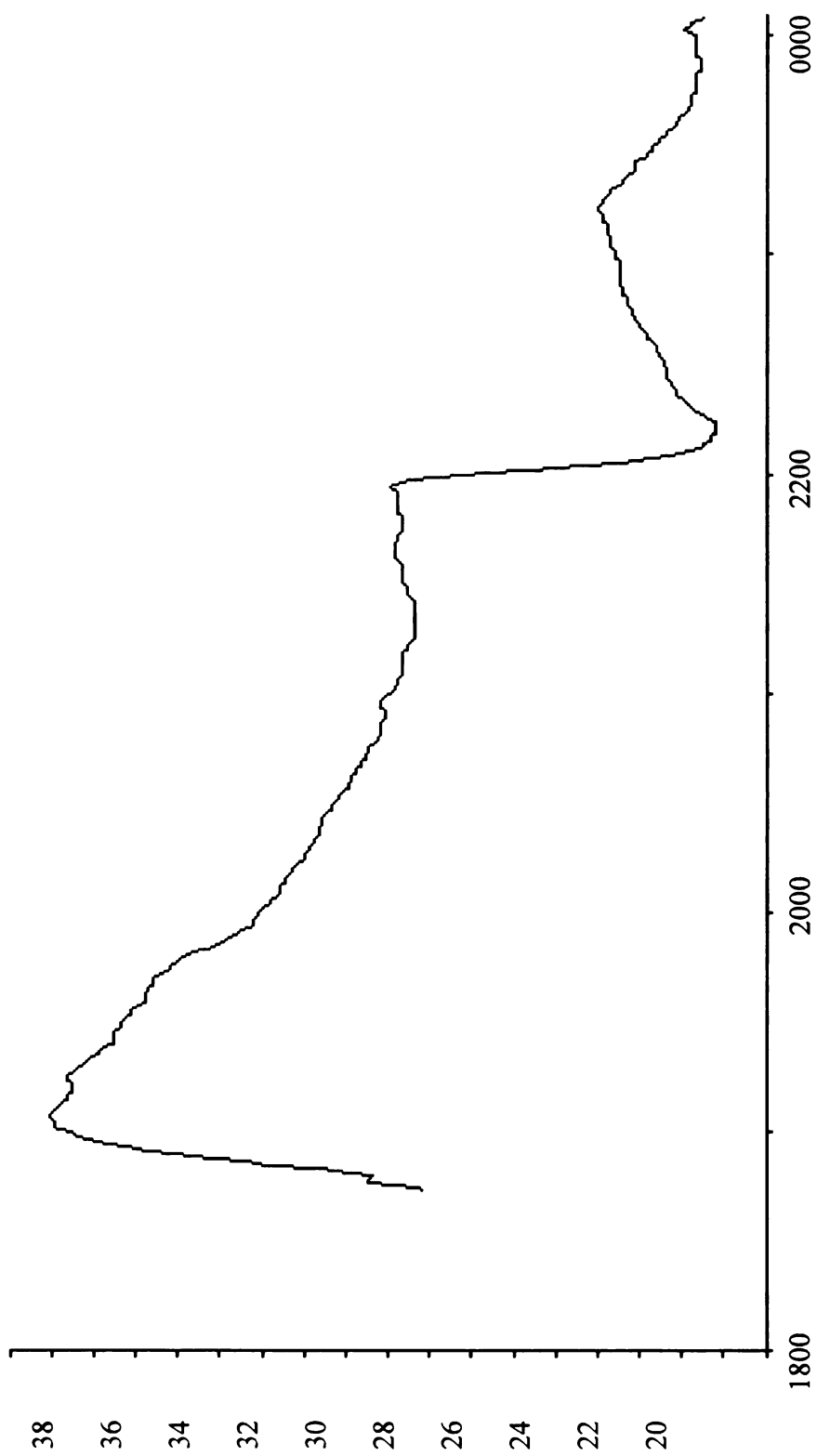


Figure 17. Graph depicting temperatures (°C) in the field during the evening of August 2, 2006 during which oviposition occurred.

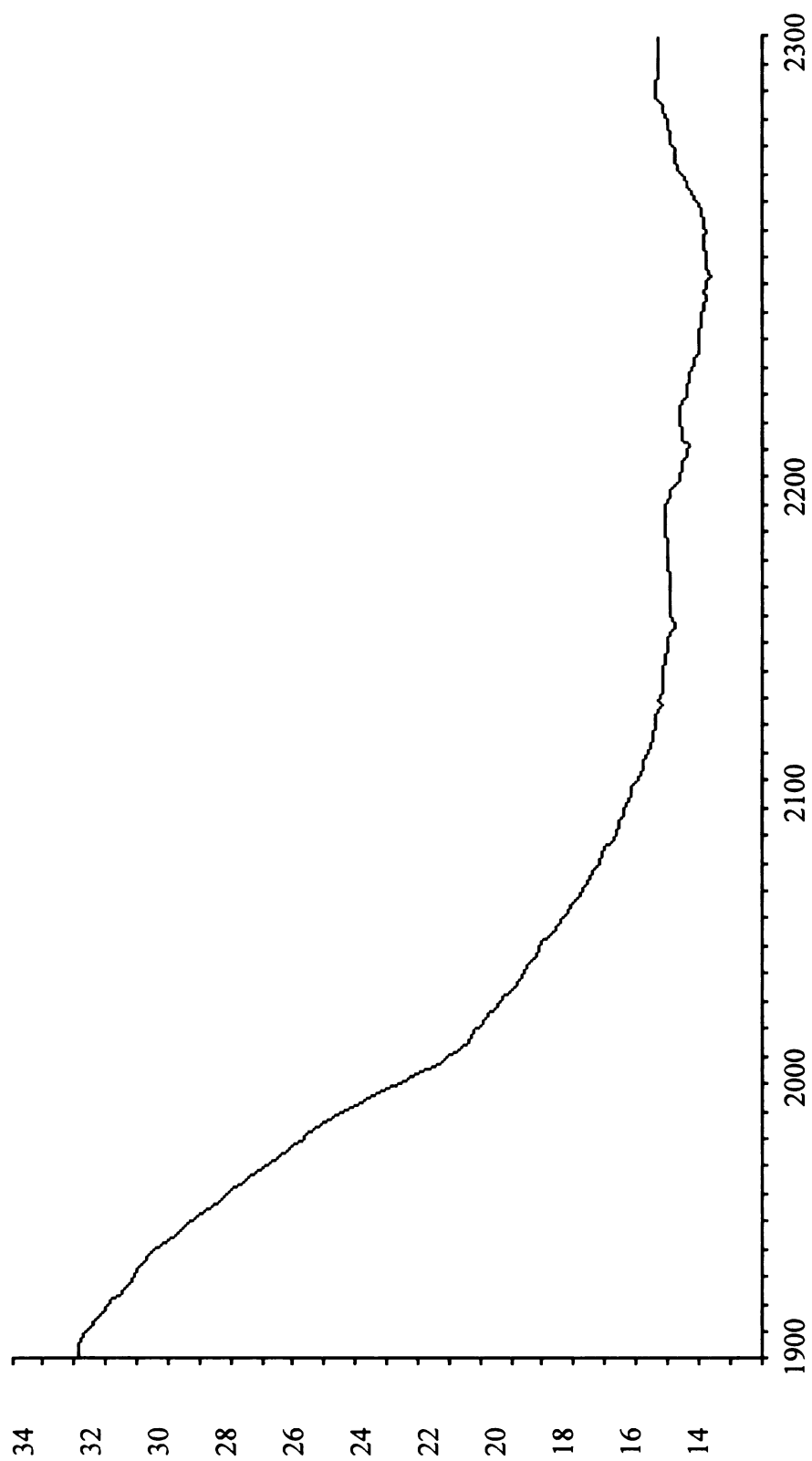


Figure 18. Graph depicting temperatures (°C) in the field during the evening of August 16, 2006 during which oviposition occurred.

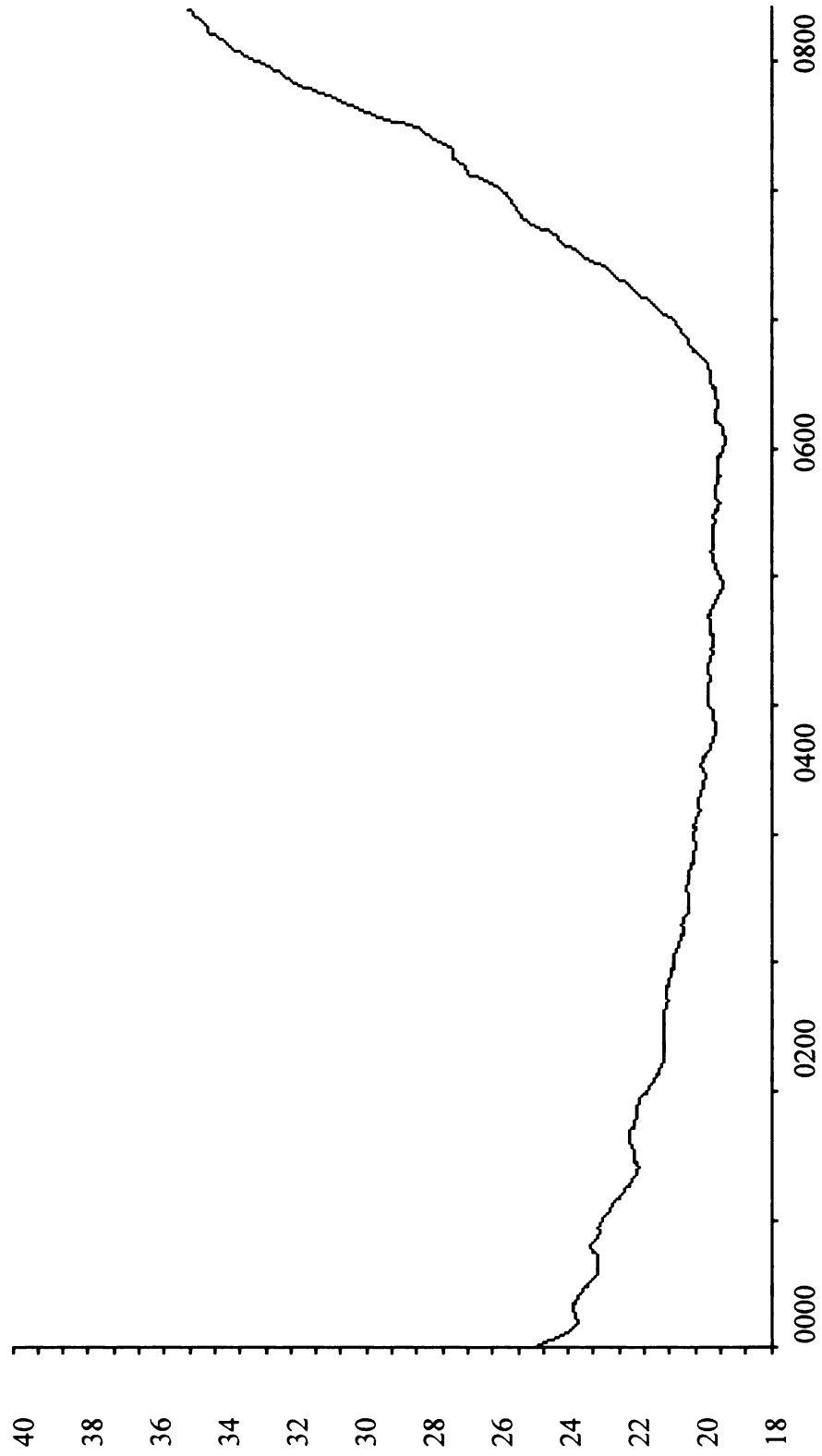


Figure 19. Graph depicting temperatures (°C) in the field during the evening of June 14, 2007 until oviposition occurred on June 15, 2007.

APPENDIX C

TEMPERATURE AND WEATHER DATA FOR THESIS

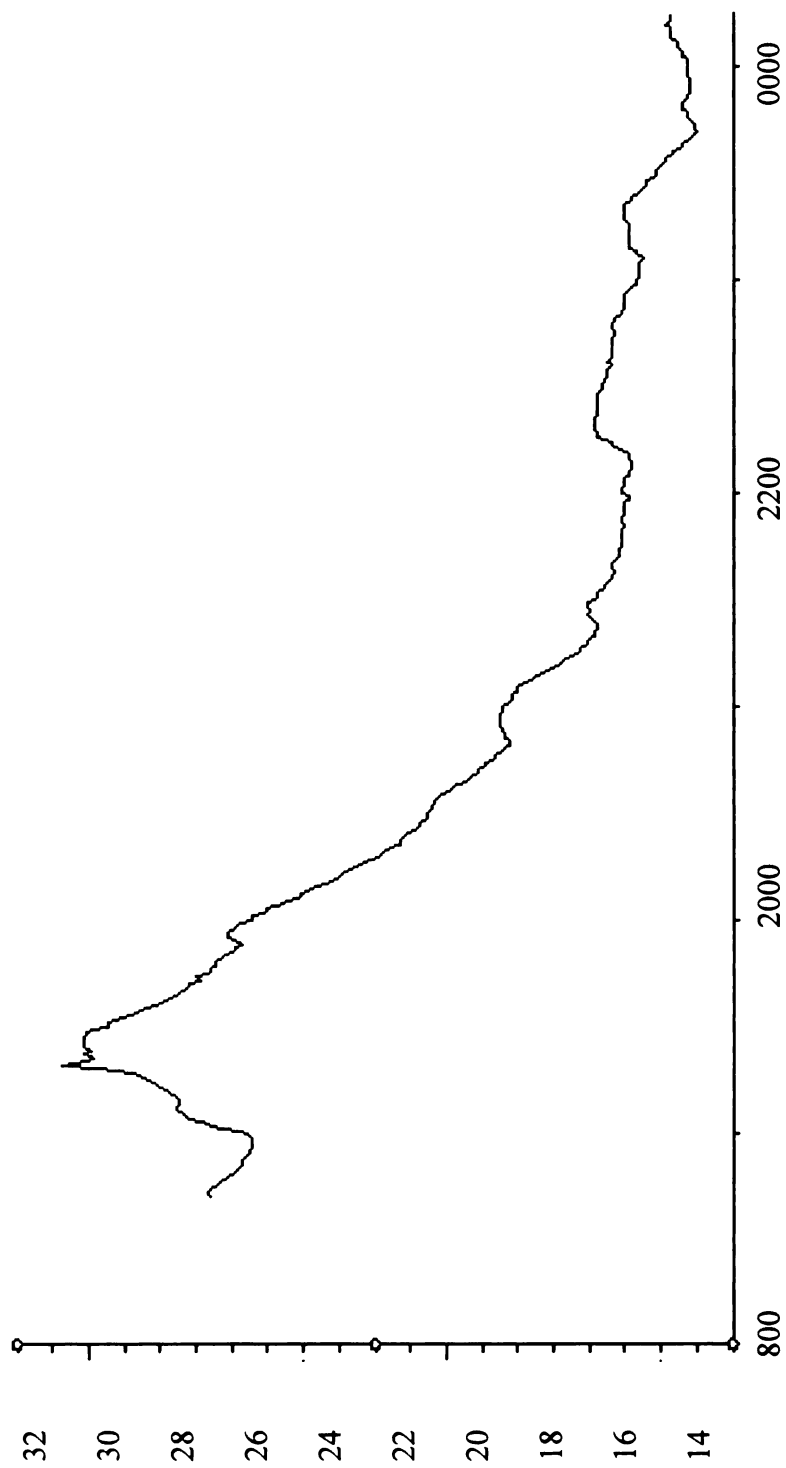


Figure 16. Graph depicting temperatures (°C) in the field during the evening of June 29, 2006 during which oviposition occurred.

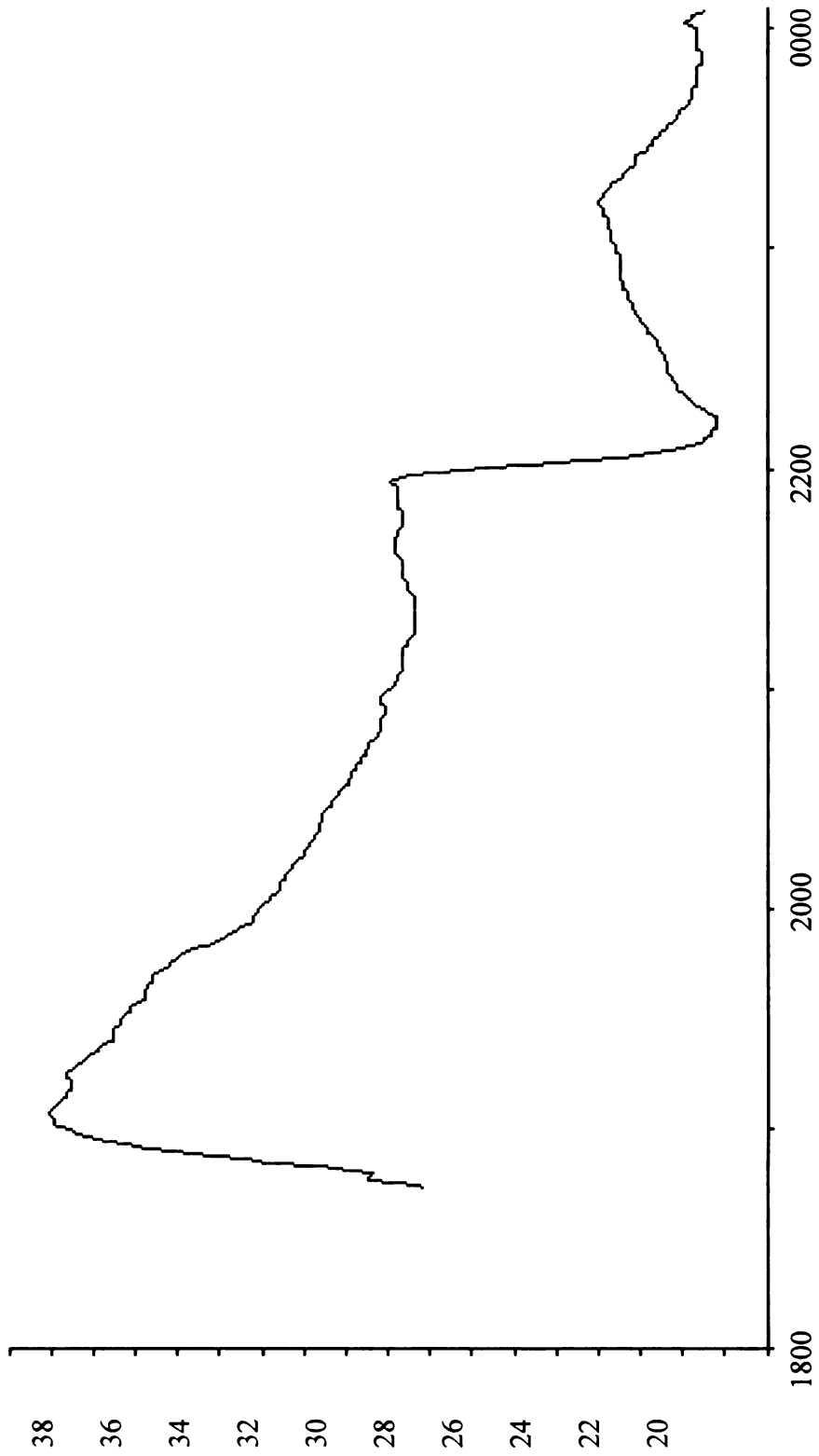


Figure 17. Graph depicting temperatures (°C) in the field during the evening of August 2, 2006 during which oviposition occurred.

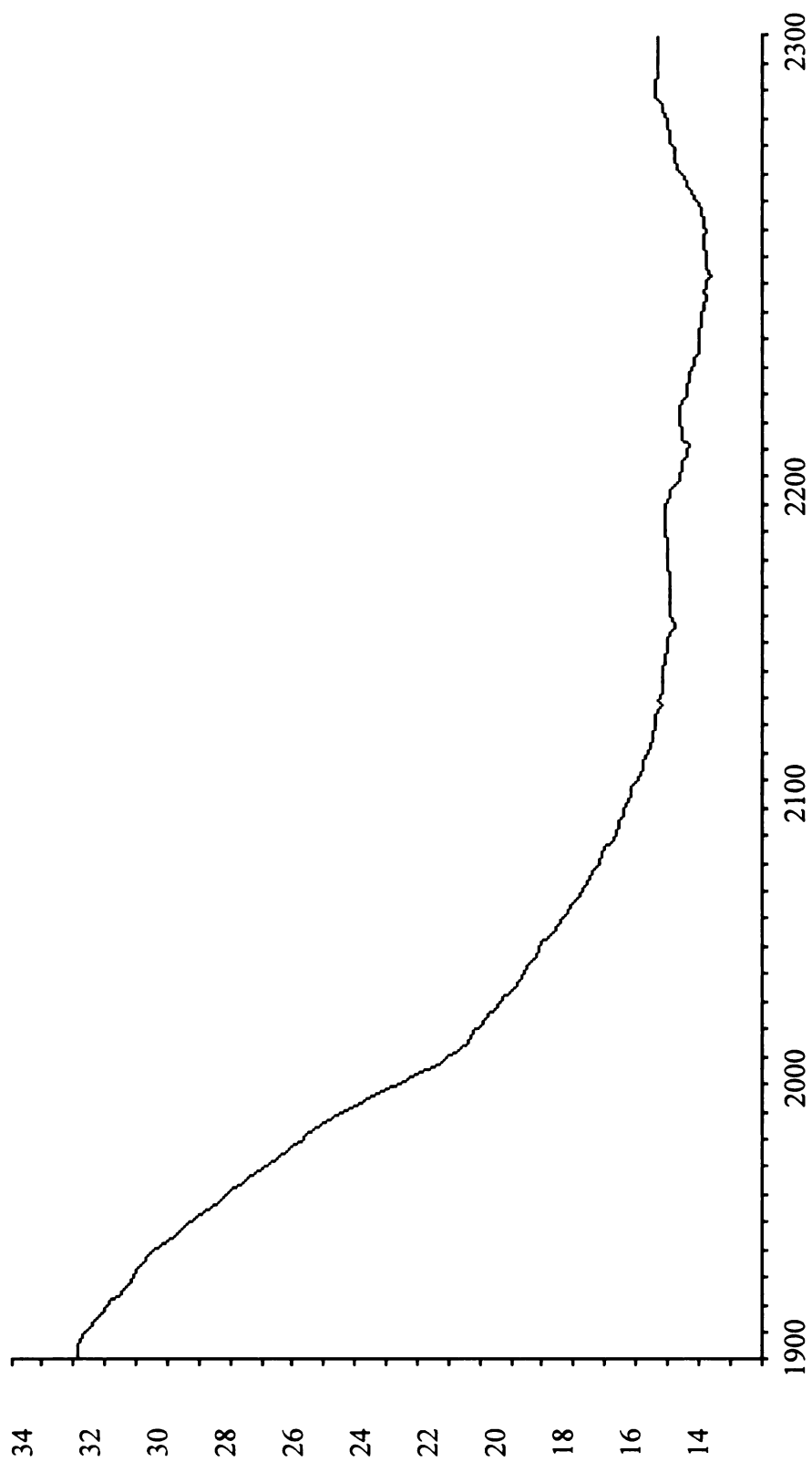


Figure 18. Graph depicting temperatures (°C) in the field during the evening of August 16, 2006 during which oviposition occurred.

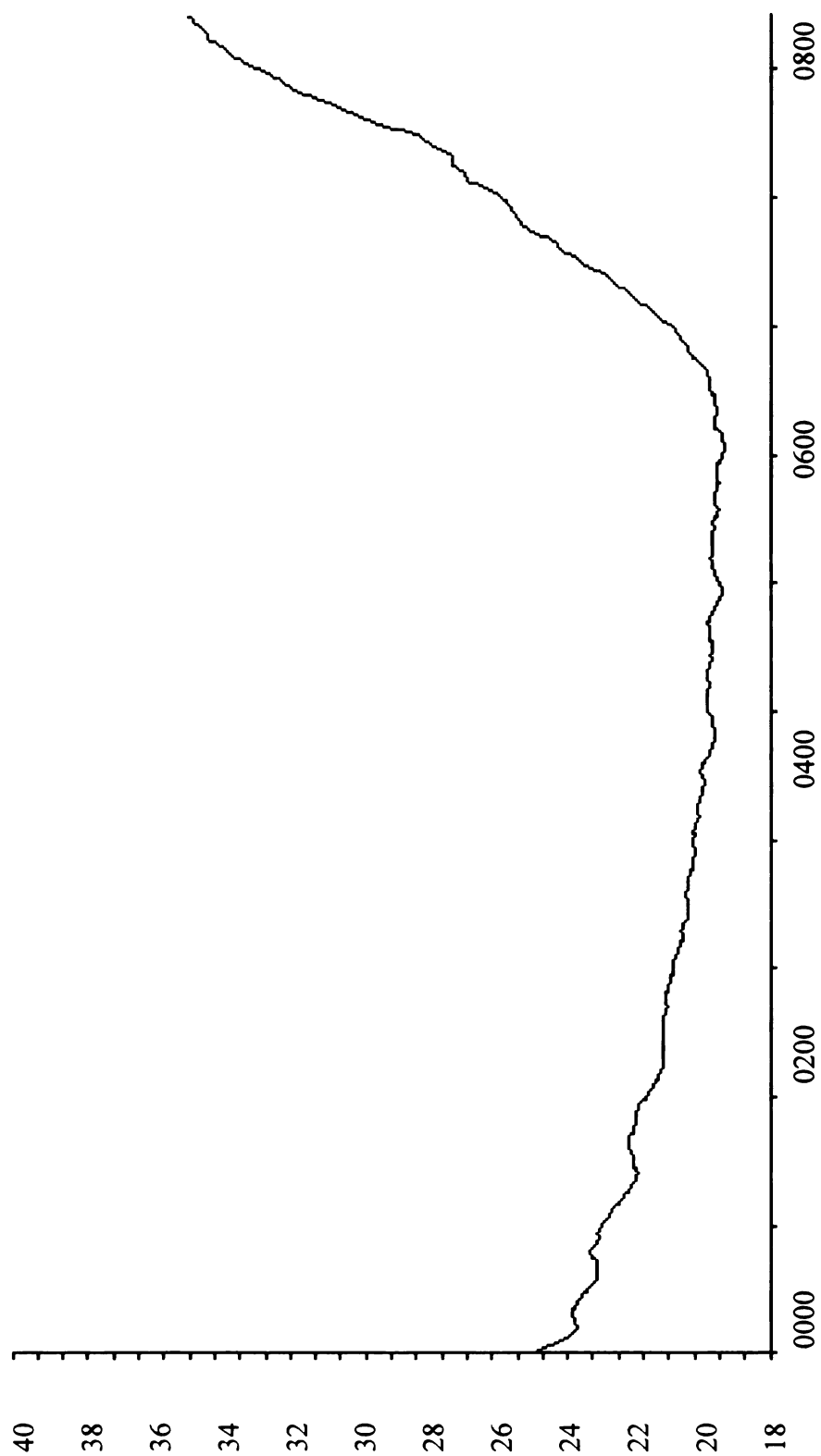


Figure 19. Graph depicting temperatures (°C) in the field during the evening of June 14, 2007 until oviposition occurred on June 15, 2007.

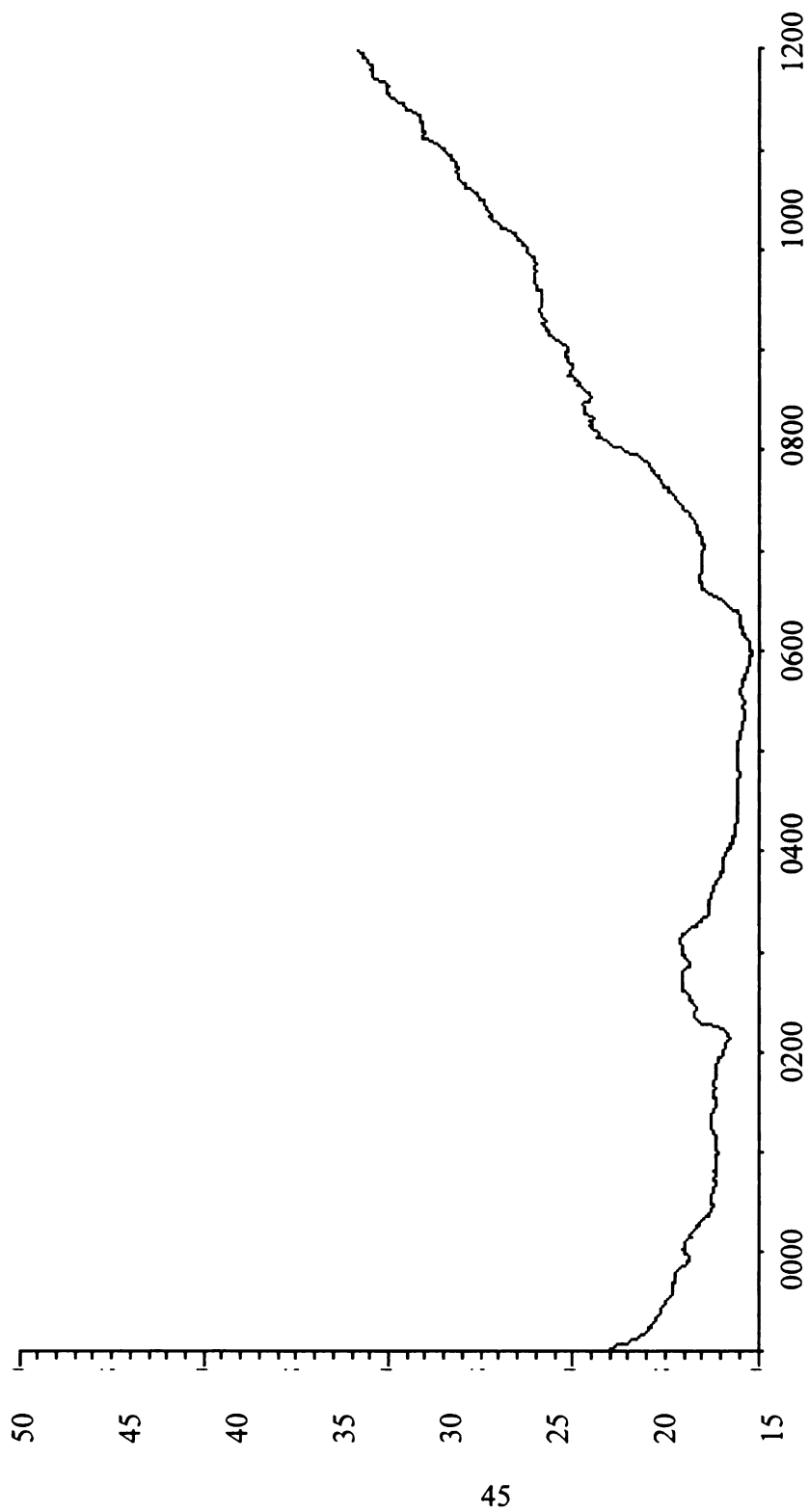


Figure 20. Graph depicting temperatures ($^{\circ}\text{C}$) in the field during the evening of July 5, 2007 until oviposition occurred on July 6, 2007.

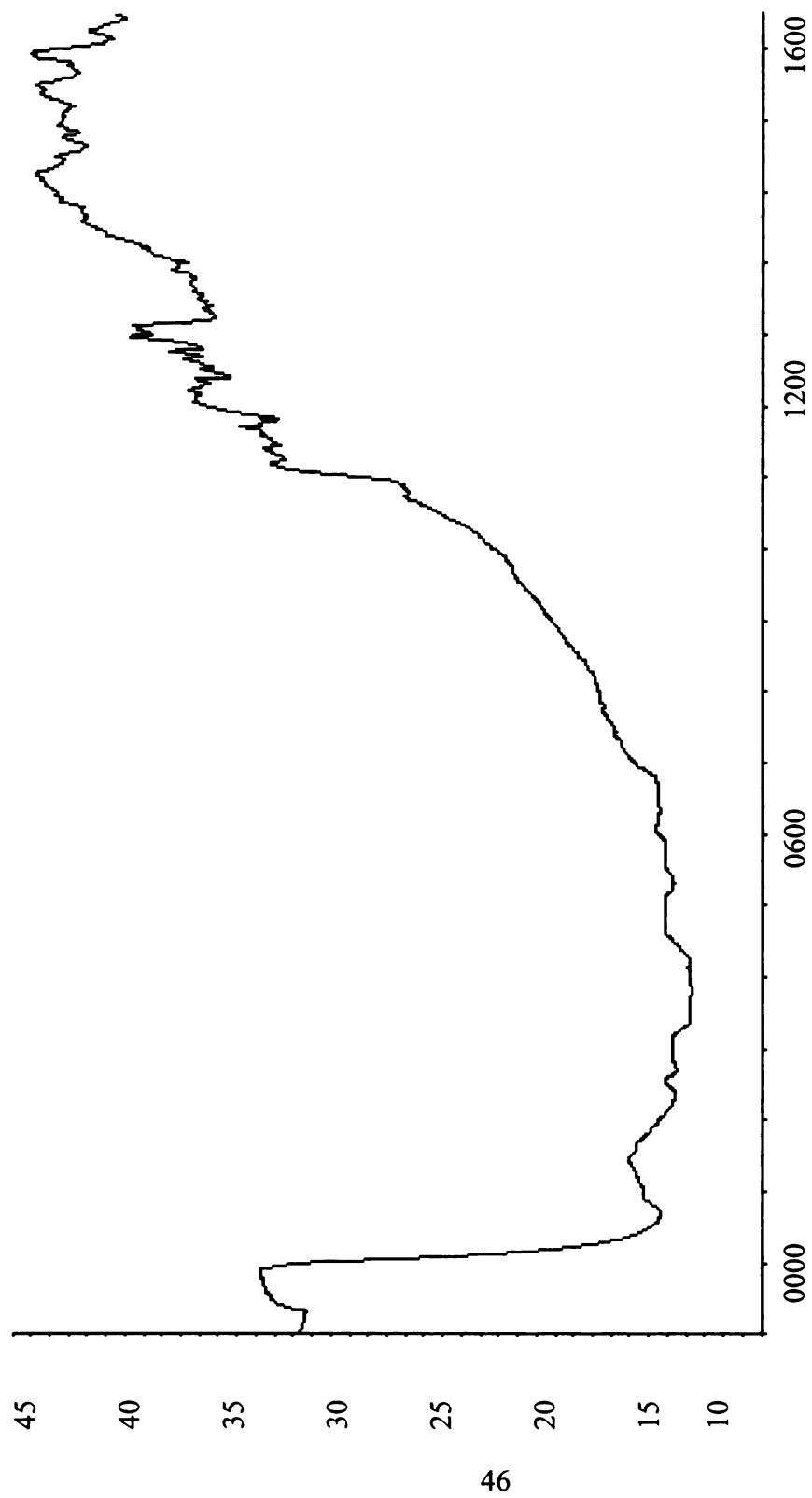


Figure 21. Graph depicting temperatures ($^{\circ}\text{C}$) in the field from the evening of August 16, 2007 until oviposition occurred on August 17, 2007.

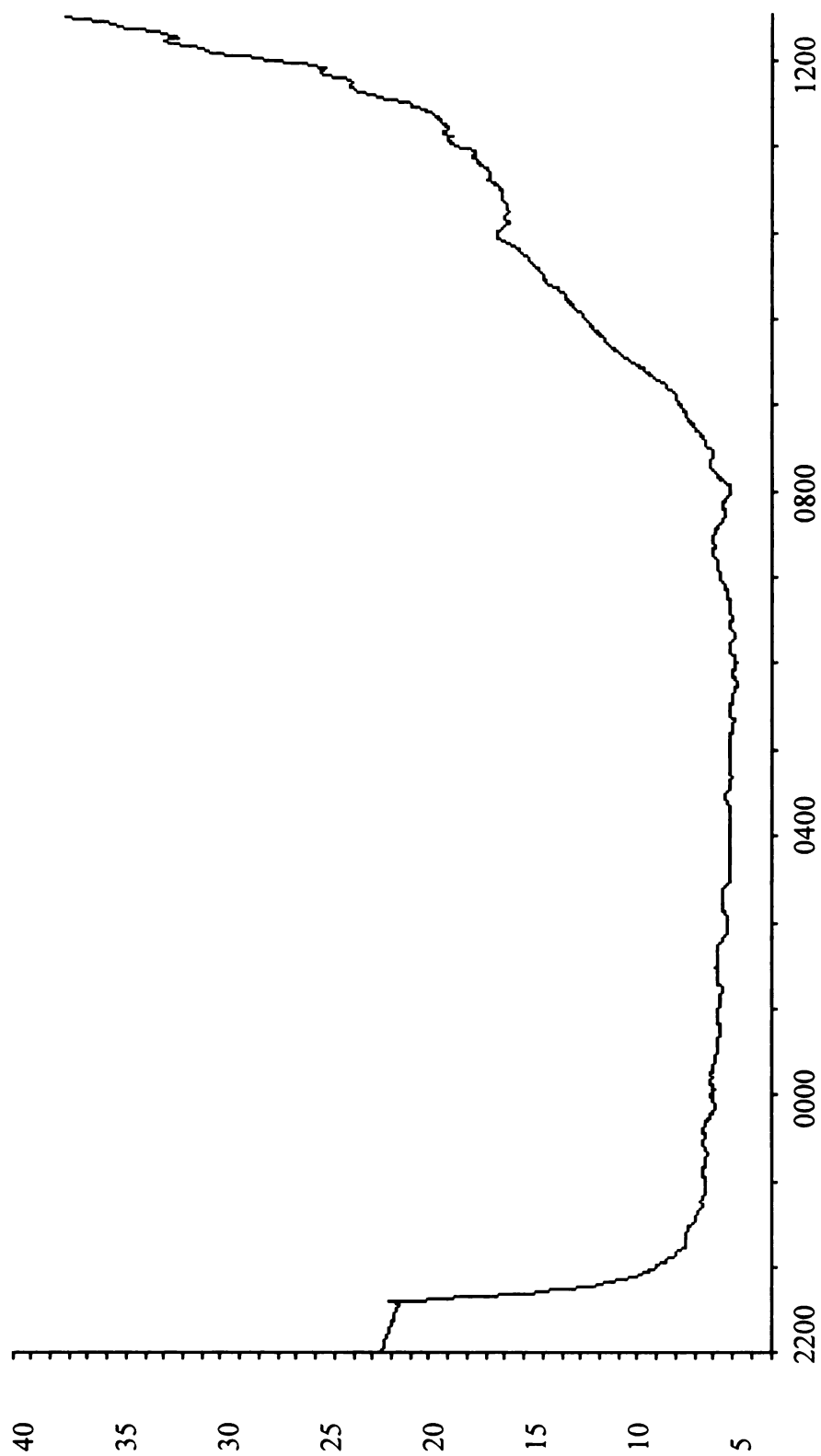


Figure 22. Graph depicting temperatures ($^{\circ}\text{C}$) in the field during the evening of September 12, 2007 and the morning of September 13, 2007.

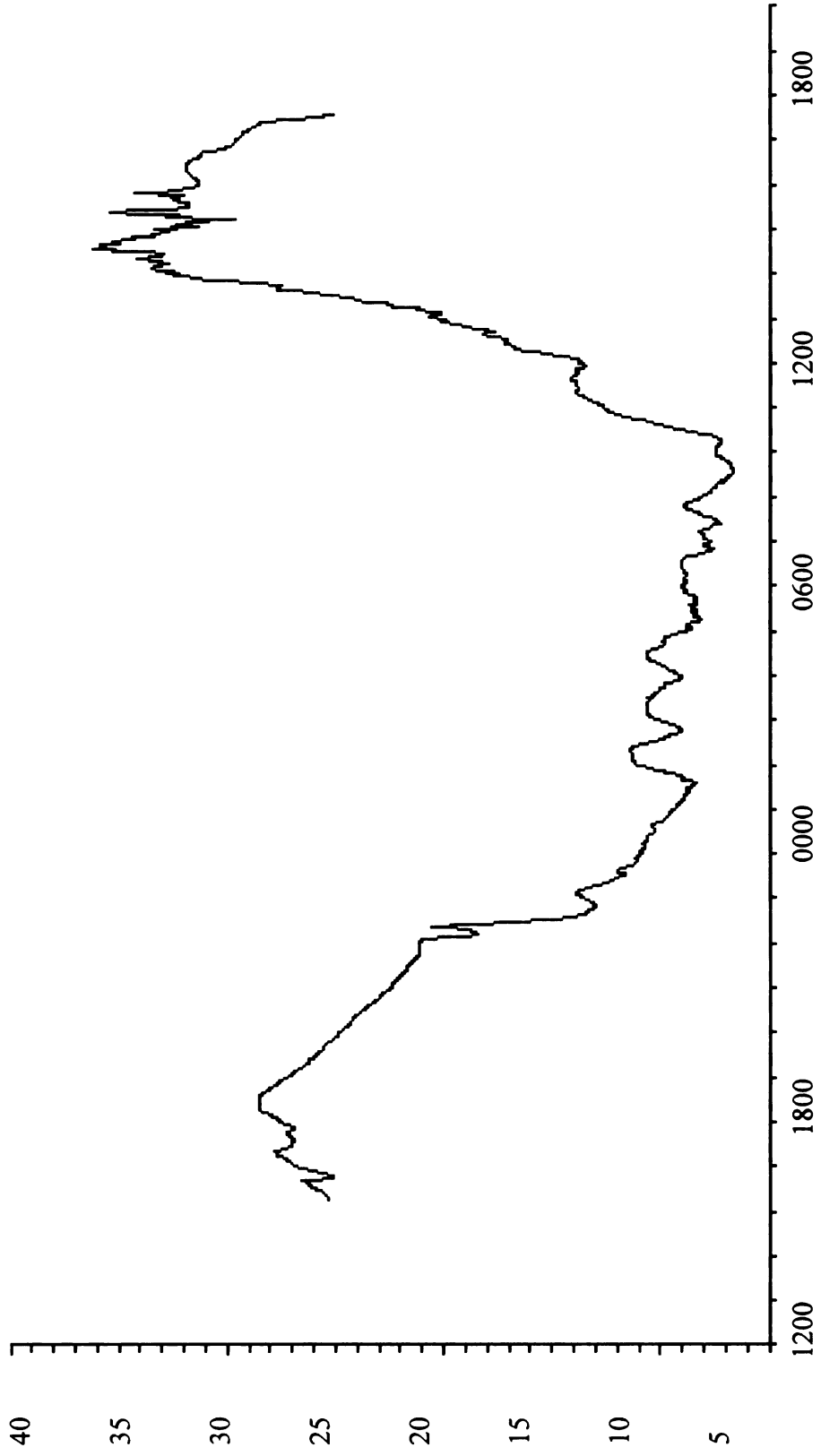


Figure 23. Graph depicting temperatures (°C) in the field during the evening of September 26, 2007 until oviposition occurred on September 27, 2007.

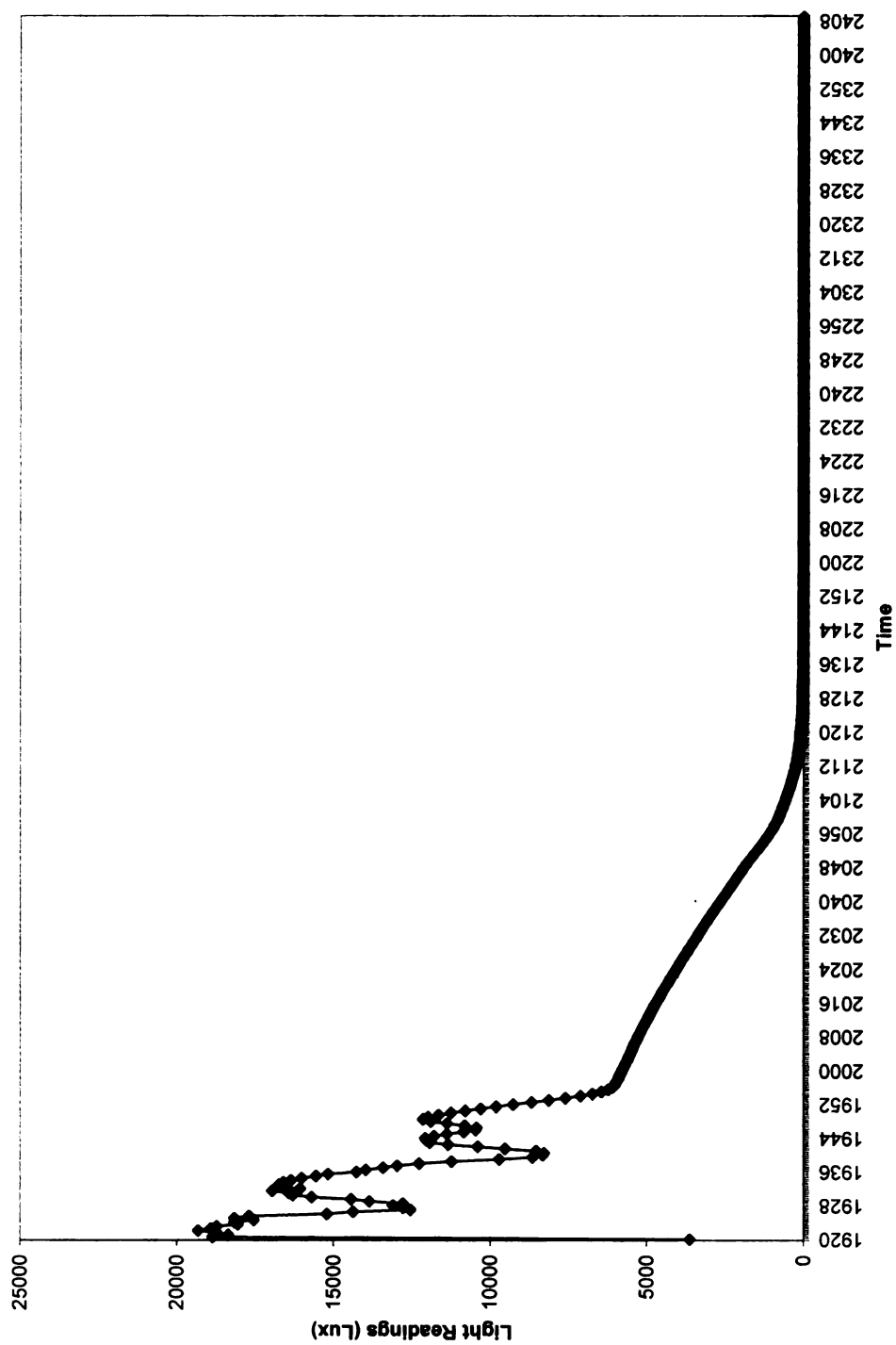


Figure 24. Light Readings for June 26, 2006 starting two hours before sunset and ending two hours after sunset.

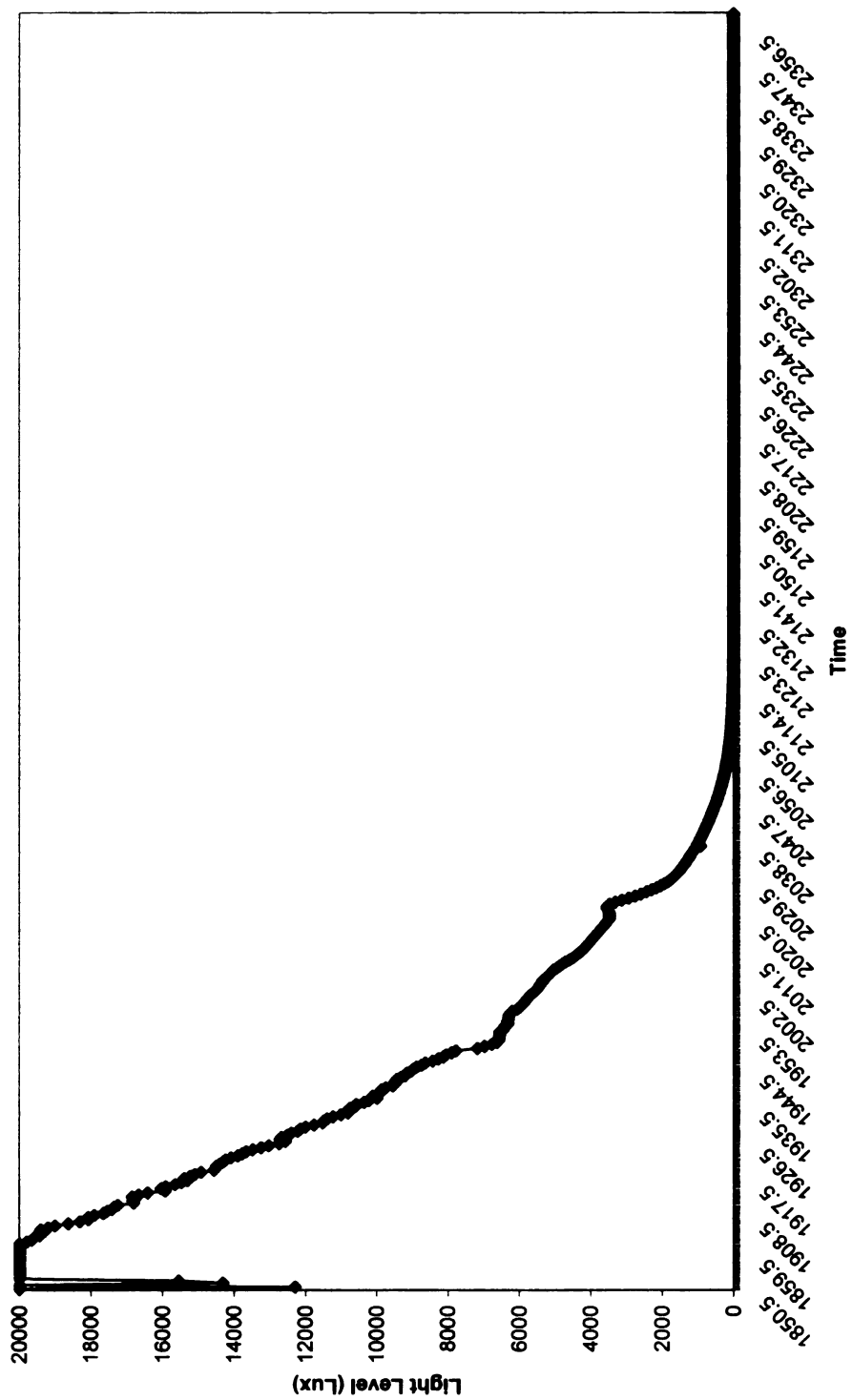


Figure 25. Light Readings for August 2, 2006 starting two hours before sunset and ending two hours after sunset.

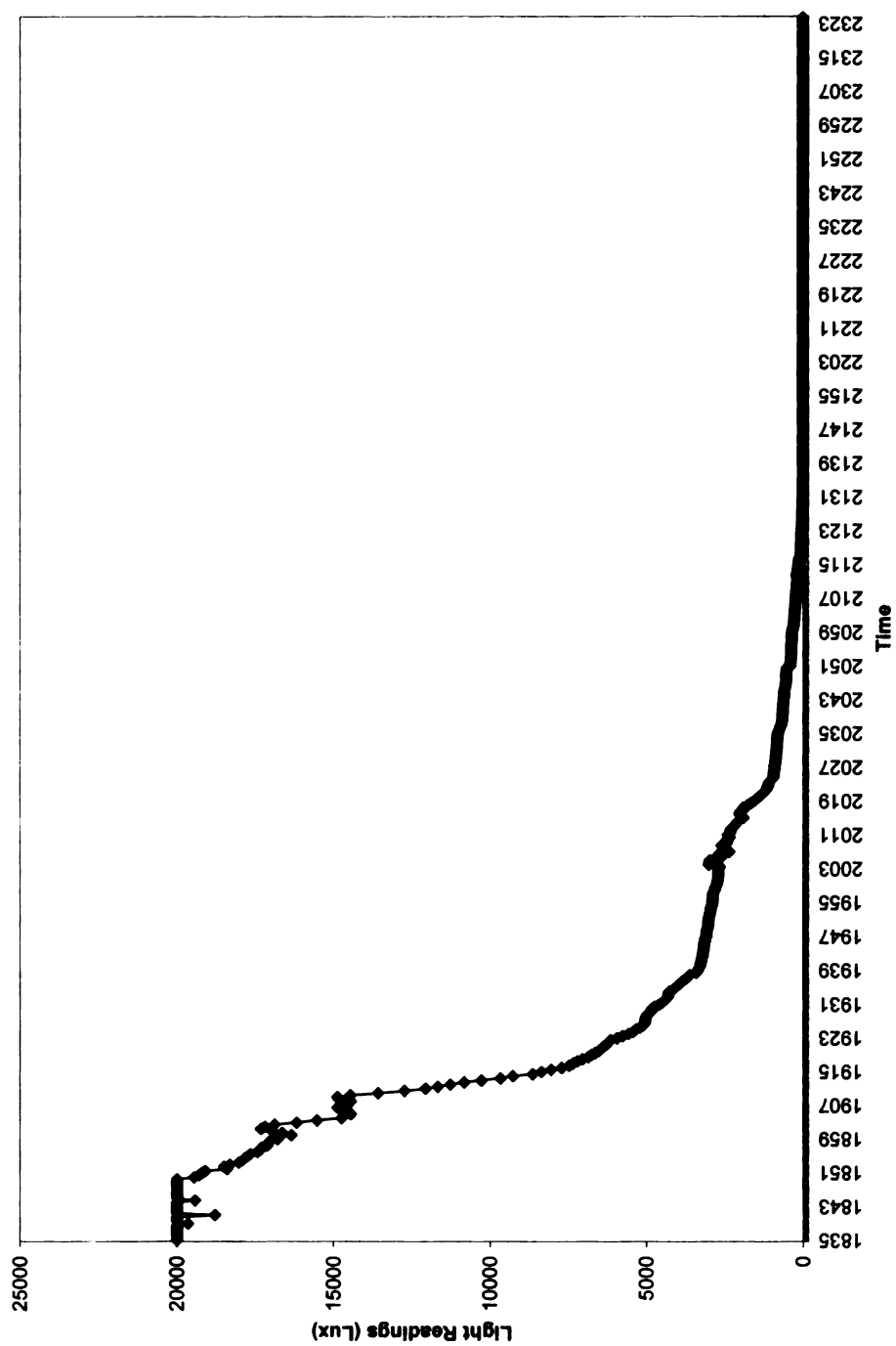


Figure 26. Light Readings for August 16, 2006 starting two hours before sunset and ending two hours after sunset.

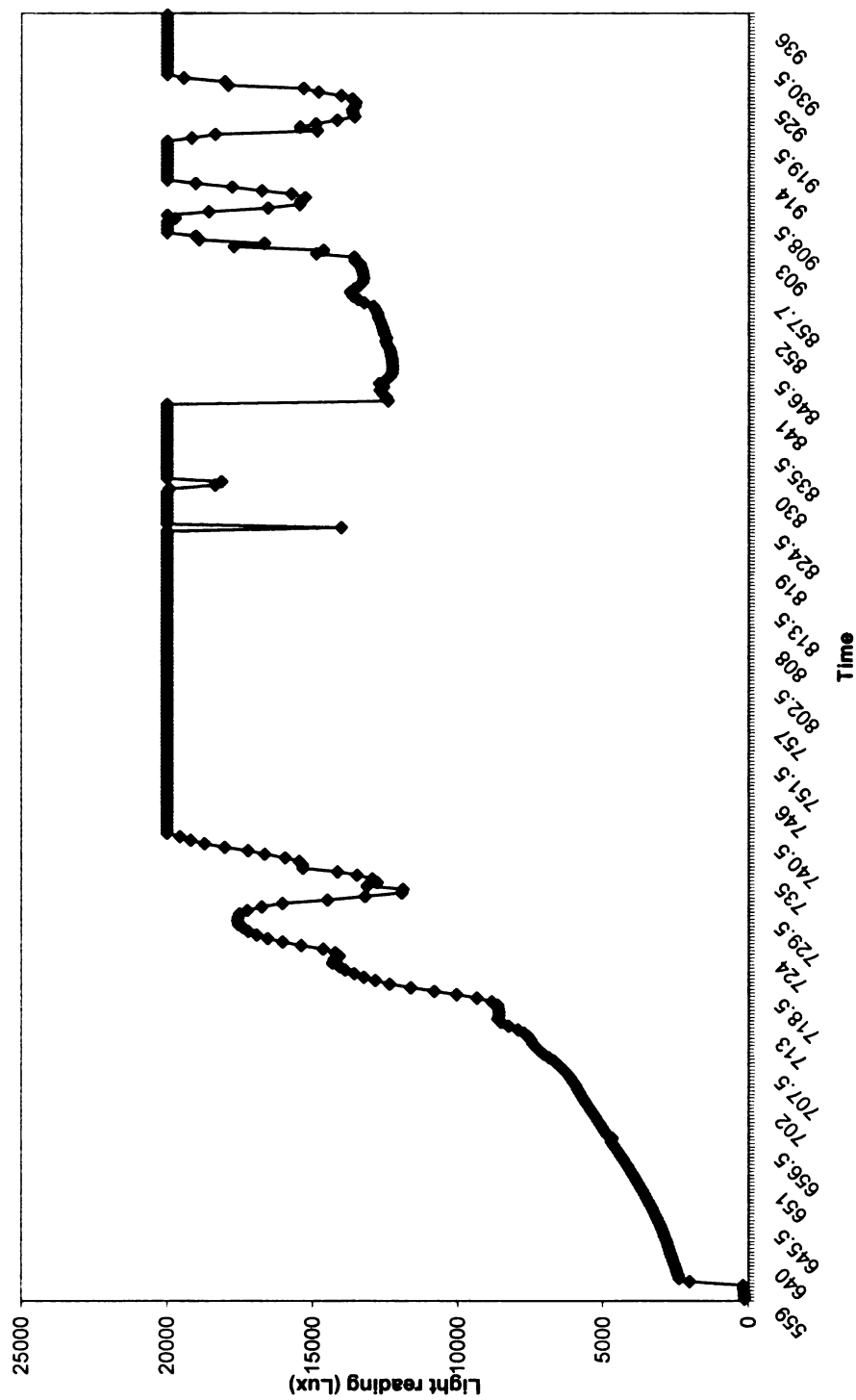


Figure 27. Light Readings for June 1, 2007 starting at sunrise and ending when oviposition occurred. Light readings are in Lux. The leveling off represents the upper threshold of the light meter (20,000 lux).

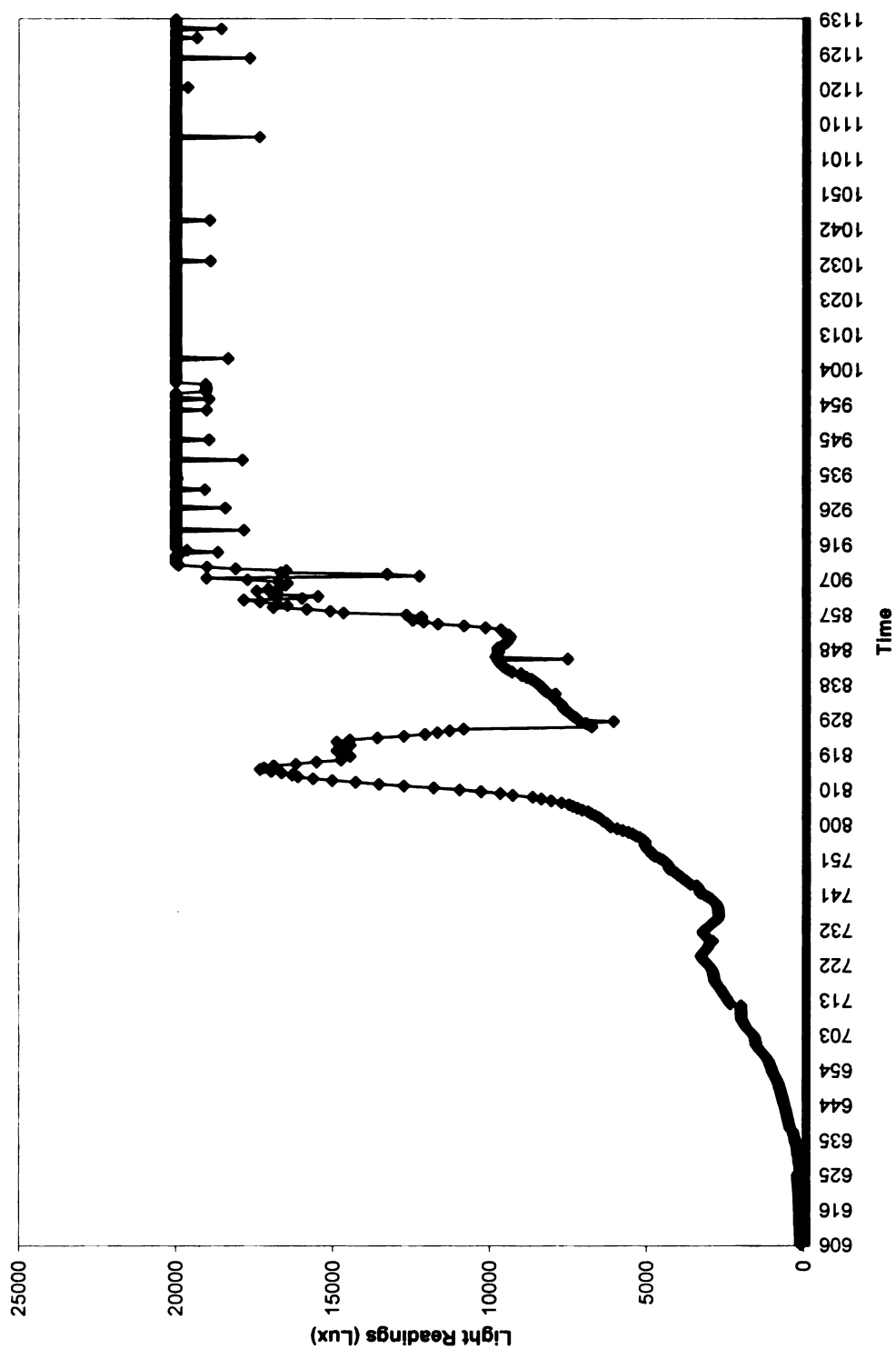


Figure 28. Light Readings for July 6, 2007 starting at sunrise and ending when oviposition occurred. Light readings are in Lux. The leveling off represents the upper threshold of the light meter (20,000 lux).

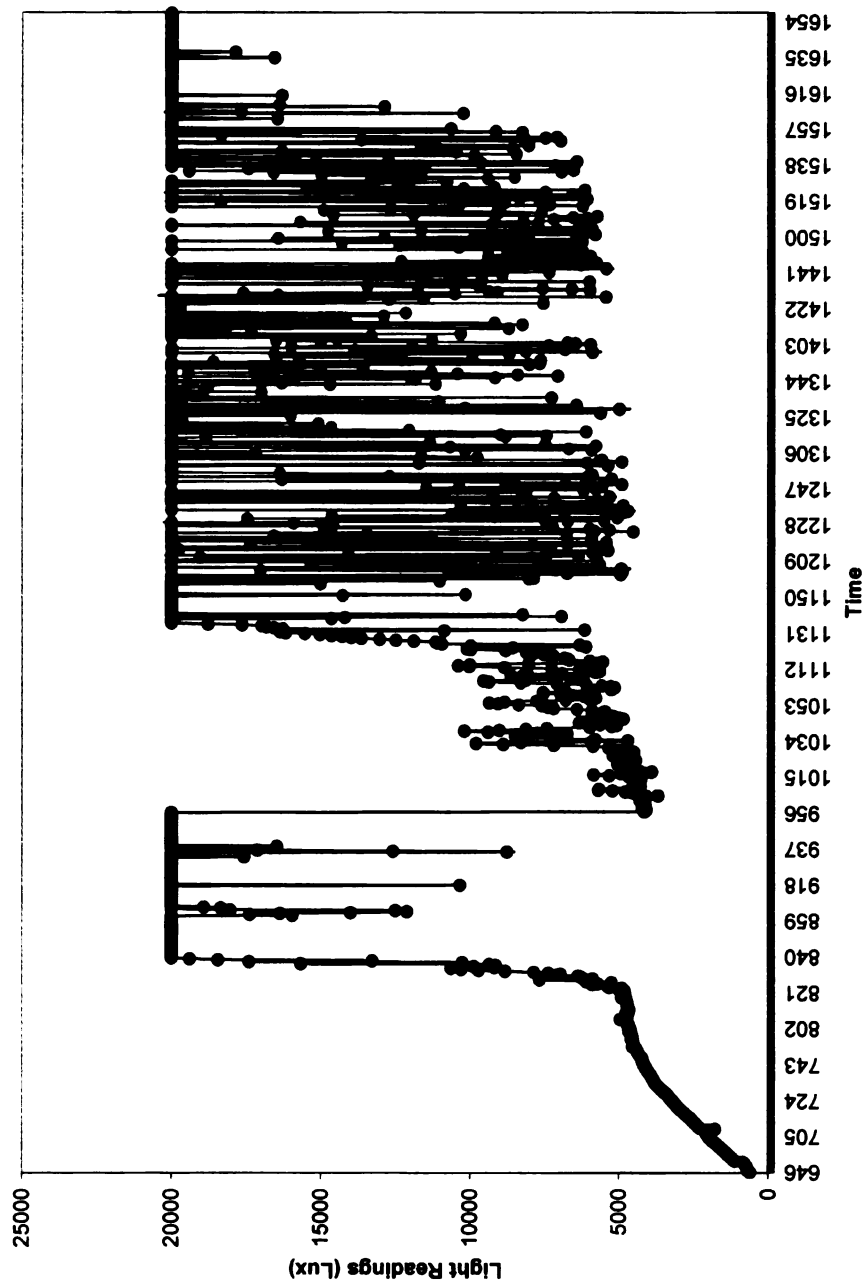


Figure 29. Light Readings for August 17, 2007 starting at sunrise and ending when oviposition occurred. Light readings are in Lux. The leveling off represents the upper threshold of the light meter (20,000 lux). The severe jumps in the data are due to cloud cover.

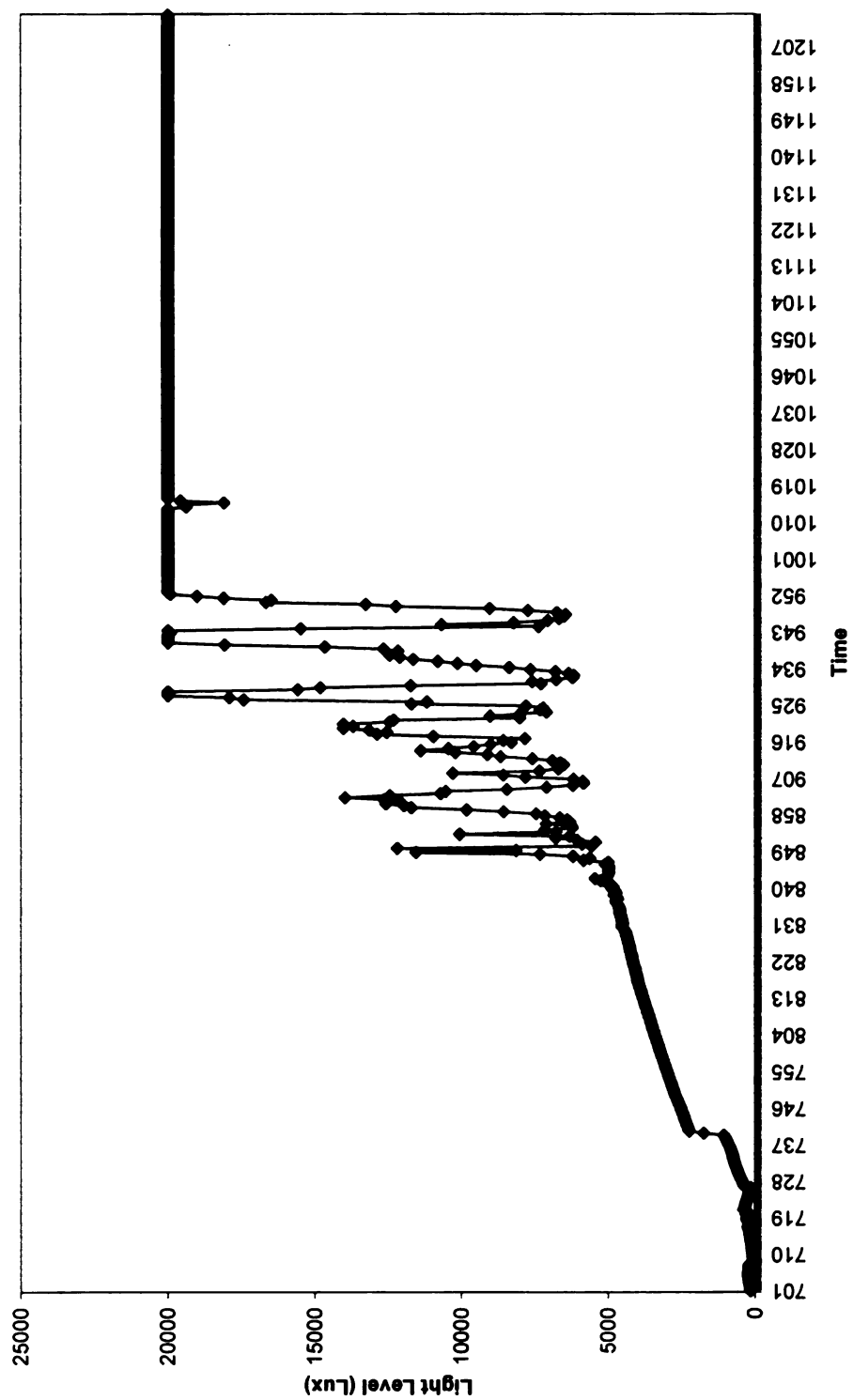


Figure 30. Light Readings for September 13, 2007 starting at sunrise and ending when oviposition occurred. Light readings are in Lux. The leveling off represents the upper threshold of the light meter (20,000 lux).

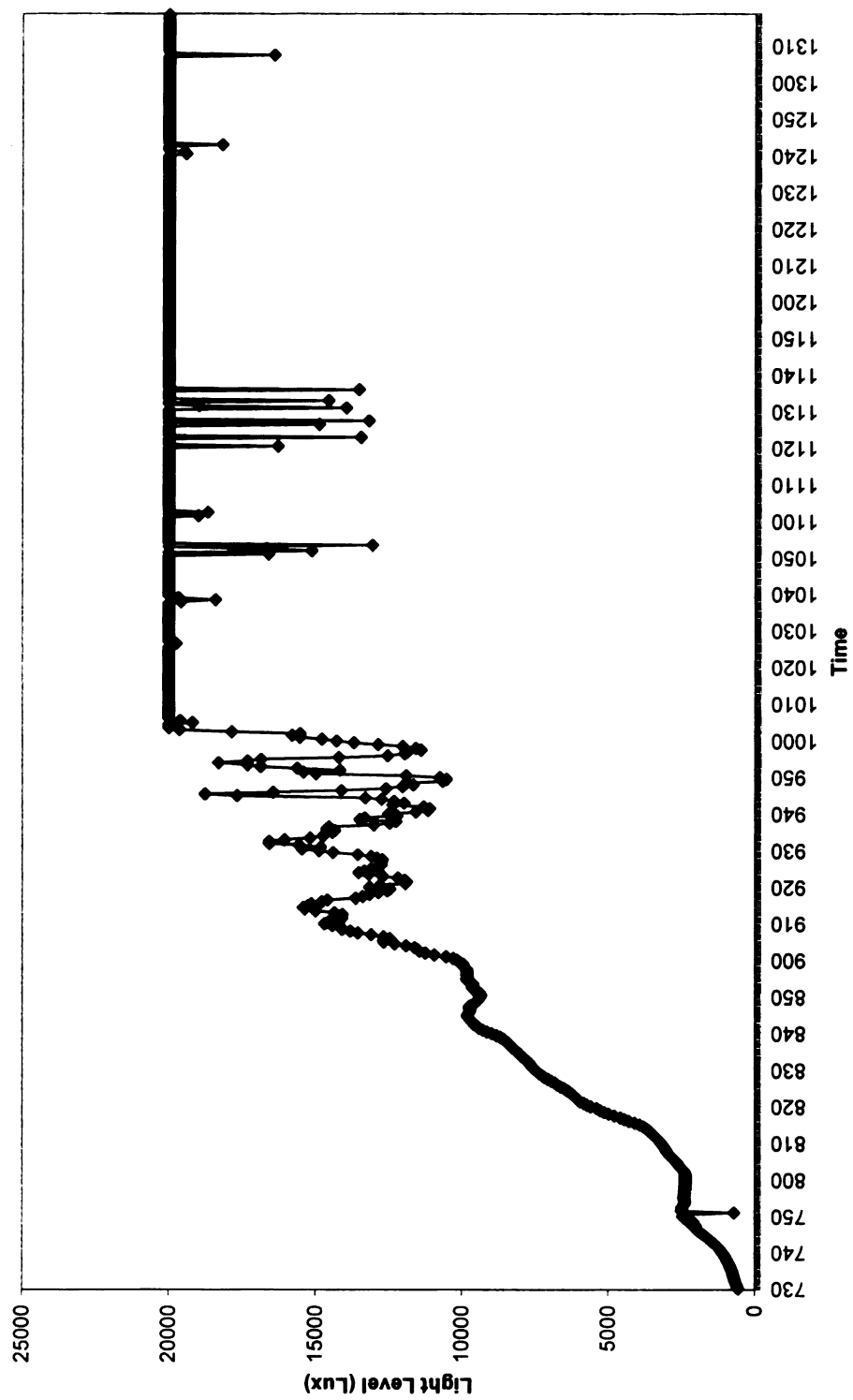


Figure 31. Light Readings for September 27, 2007 starting at sunrise and ending when oviposition occurred. Light readings are in Lux. The leveling off represents the upper threshold of the light meter (20,000 lux).

APPENDIX D

VOUCHER SPECIMEN INFORMATION FOR THESIS

Appendix D

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 2008-02

Title of thesis or dissertation (or other research projects):

"Nocturnal Oviposition Behavior of Blowflies in Relation to Sunrise and Sunset"

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name(s) (typed)

Kristi Zurawski

Date: March 2008

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24: 141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.

Copies: Include as Appendix 1 in copies of thesis or dissertation.

Museum(s) files.

Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

Appendix D

Voucher Specimen Data

Page 1 of 3 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of:							Museum where deposited
		Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	
<i>Phormia regina</i>	MSU campus; Mouth of Pig: 7-Aug-06	11							MSU
	MSU campus; Mouth of Pig: 22-Oct-07	15							MSU
	MSU campus; Entomology Farm: 1-Jun-06					2	1		MSU
	MSU campus; Entomology Farm: 7-Jul-06						1		MSU
	MSU campus; Entomology Farm: 10-Aug-06						2		MSU
	MSU campus; Entomology Farm: 22-Aug-06						1		MSU
	MSU campus; Entomology Farm: 15-Jun-07						2		MSU
	MSU campus; Entomology Farm: 6-Jul-07						3		MSU
	MSU campus; Entomology Farm: 27-Jul-07						1		MSU
	MSU campus; Entomology Farm: 27-Sep-07						1		MSU
<i>Lucilia sericata</i>	MSU campus; Mouth of Pig: 5-Jun-06	4							MSU
	MSU campus; Mouth of Pig: 9-Jul-07	9							MSU
	MSU campus; Entomology Farm: 1-Jun-06					1	1		MSU
	MSU campus; Entomology Farm: 6-Jul-07					2			MSU
	MSU campus; Entomology Farm: 27-Jul-07					1	1		MSU
	MSU campus; Entomology Farm: 9-Aug-07								MSU
	MSU campus; Entomology Farm: 17-Aug-07					1	1		MSU
	MSU campus; Entomology Farm: 13-Sep-07					2			MSU
	MSU campus; Laboratory Colony: 11-Feb-07					7	3		MSU
									MSU

Investigator's Name:

Kristi Zurawski

Voucher No. 2008-02

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Date:

10-Mar-08

Curator

Date

Appendix D

Voucher Specimen Data

Page 2 of 3 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of:							Museum where deposited
		Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	
<i>Lucilia coeruleiviridis</i>	MSU campus; Mouth of Pig: 5-Jul-06	11							MSU
	MSU campus; Mouth of Pig: 7-Aug-06	18							MSU
	MSU campus; Mouth of Pig: 16-Jul-07	15							MSU
	MSU campus; Entomology Farm: 1-Jun-07					1			MSU
	MSU campus; Entomology Farm: 15-Jul-07					4	1		MSU
	MSU campus; Entomology Farm: 27-Jul-07					1	1		MSU
	MSU campus; Entomology Farm: 9-Aug-07					2	1		MSU
	MSU campus; Entomology Farm: 13-Sep-07						1		MSU
<i>Calliphora vomitoria</i>	MSU campus; Mouth of Pig: 22-Aug-06	11							MSU
	MSU campus; Mouth of Pig: 2-Oct-07	3							MSU
	MSU campus; Entomology Farm: 27-Jul-07						1		MSU
<i>Calliphora vicina</i>	MSU campus; Entomology Farm: 13-Sep-07						1		MSU
	MSU campus; Mouth of Pig: 19-Sep-07	11							MSU
	MSU campus; Entomology Farm: 13-Sep-07								MSU
	MSU campus; Entomology Farm: 27-Sep-07								MSU

Investigator's Name:

Kristi Zurawski

Date

10-Mar-08

Voucher No. 2008-02

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator

Date

Appendix D

Voucher Specimen Data

Page 3 of 3 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of:							Museum where deposited
		Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	
<i>Cochliomyia macellaria</i>	MSU campus; Mouth of Pig: 21-Aug-07 MSU campus; Entomology Farm: 10-Aug-06 MSU campus; Entomology Farm: 13-Sep-07		4			1	1		MSU MSU MSU
<i>Pollenia rudis</i>	MSU campus; Entomology Farm: 9-Aug-07					2			MSU
Sarcophagidae	MSU campus; Entomology Farm: 1-Jun-06 MSU campus; Entomology Farm: 27-Jul-07					3	3		MSU MSU

Investigator's Name:

Kristi Zurawski

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