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TECHNICAL NOTE

Robert S. Baldridge,[1] *Ph.D.; Susan G. Wallace,*[2] *Ph.D.; and Ryan Kirkpatrick,*[3] *B.S.*

Investigation of Nocturnal Oviposition by Necrophilous Flies in Central Texas*

ABSTRACT: The need to accurately estimate the postmortem interval (PMI) has prompted research into factors affecting fly oviposition (i.e., oviposition and/or larviposition) on a corpse. Research efforts have focused on whether or not diurnally active flies oviposit during nighttime hours. This study reports that nocturnal oviposition (defined as occurring between 2100–0600 h CDST (Central Daylight Savings Time)) did not occur on freshly killed white rats or mice, on beef (fresh or aged up to 48 h), on freshly thawed pigs, nor, usually, on thawed pigs that were aged for up to 48 h. Limited oviposition did occur between 2100 and 2120 h on one bloated pig at a lighted rural site. Necrophilous flies were present and active at lighted and dark sites (urban and rural) before and immediately after sunset, but fly activity on the bait ceased within 50 min postsunset and did not resume until after 0600 h. These observations support other studies reporting that diurnally active flies do not oviposit during the nighttime.

KEYWORDS: forensic science, forensic entomology, nocturnal oviposition, Central Texas

A more thorough knowledge of oviposition behavior by necrophilous flies should allow for a more precise characterization of the postmortem interval (PMI) in forensic investigations. This study adds to the existing data regarding the oviposition behavior of diurnally active necrophilous flies and whether or not these flies will oviposit/larviposit during nighttime hours or postpone oviposition until the next day. Nocturnal oviposition, if it occurred, would affect the PMI estimation. Previous research regarding fly oviposition during nocturnal hours, in dark and lighted environments, has produced disagreement regarding this behavior. Greenberg (1) and Singh and Bharti (2) reported that such nocturnal oviposition did occur on a variety of nonhuman media. However, Haskell et al. (3), Tessmer et al. (4), and Spencer (5), reported no nocturnal oviposition on nonhuman media.

Materials and Methods

Experiments were designed to determine if nocturnal oviposition/larviposition occurred and, if it did, was it influenced by a site being lighted or not. All sites were located in the Central Texas area, and all rearing of fly life stages was conducted in the laboratory in the Biology Department at Baylor University. All times were recorded as Central Daylight Savings Time (CDST). The protocol for this study was approved by the Animal Care and Use Committee at Baylor University, Waco, Texas.

In 1993, urban sites were used. One site was beneath a mercury vapor security light (= "lighted" site) while the other site, without any artificial light (= "unlit" site), was in a dense, mature riparian woodland and was blocked from the security light from dusk to dawn.

Freshly killed white rats (*Rattus rattus* (Linnaeus); 14 males average weight = 113 g; 20 females average weight = 151 g) were cut ventrally to expose thoracic and abdominal viscera. The rats were placed in ZipLoc® (S. C. Johnson and Sons, Inc., Racine, WI) bags half-full of vermiculite, with the top edges rolled back to expose oviposition sites (anus/genitalia, nose, eyes, ears, wounds). The bag was placed inside a wire cage, about 0.6 m above the ground, atop a wooden platform treated with Tangle Foot® (The Tanglefoot Company, Grand Rapids, MI) to prevent red imported fire ants (*Solenopsis invicta* Buren) from accessing the carcasses and affecting oviposition (6).

A fresh rat was presented at 1800, 2100, 2400, and 0600 h on each of nights June through August. After the 3-h exposure, the bagged rat was removed from the cage, attendant flies dislodged, and the bag was sealed and labeled. Carcasses were stored in a closed styrofoam ice chest until taken to the laboratory the next morning. No ventilation holes were made in the closed bags in the field as it was observed that flies oviposited through these tiny holes.

The number of each species of fly observed on a rat was recorded at 15-min intervals throughout each 3-h period and temperature, relative humidity, and wind speed data were recorded at 30-min intervals during the same period. Data were not recorded between 2400 and 0600 h, as no flies had been observed on carcasses during this period, and oviposition, if it occurred, would be determined from rearing of eggs/larvae deposited on the rat presented during that period. Observations of the carcasses were resumed at 0500 h the following morning.

In 2003, two different urban sites were selected. One lighted site was in a grassy, open area beneath a mercury vapor security light. An unlit site was in the same area but isolated from any artificial light sources. Freshly killed male white rats ($n = 6$, average weight = 147 g) and mice (*Mus musculus* (Linnaeus), males (aver-

[1] Department of Biology, Baylor University, Waco, TX 76798.
[2] Department of Sociology and Anthropology, Baylor University, Waco, TX 76798.
[3] Department of Entomology, College Station, TX 77843.

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age weight = 27 g); one female (27 g)) were presented at the sites during four nights (June–August, 2200–0500 h). Either one rat or two mice were presented nightly at each of the sites. The open Ziploc® bag containing the rodent(s) was placed at ground level, inside a plastic dish half filled with soapy water, inside a wire cage. The soapy water prevented red imported fire ants from accessing the carcass. Two control rats were exposed from 0900 to 1500 h on two different dates during the study period and sticky boards were used to collect flies attracted to the rats for identification purposes.

Samples (100 g each) of ground sirloin beef (fresh and "aged" at ambient temperature for 24 and 48 h before presentation) were presented during three nights in July, 2003, at a rural site. Each sample was placed in an open glass jar (10-cm opening) suspended 60 cm above the ground. Bait was presented between 2300 and 0500 h (a) in a dark area away from area lights, (b) under a bank of 4 60-W white frosted incandescent bulbs suspended 60 cm above the sample, and (c) beneath a mercury vapor security lamp 4 m above the sample. A control sample was exposed on the first day of the study between 0700 and 1200 h, and flies were collected on sticky boards placed within 0.6 m of the samples. All samples were removed at the end of the exposure period and taken to the lab.

Three white/blue-speckled male Yorkshire cross pigs (frozen then thawed immediately before presentation) were used at the same rural site, June 29–July 1, 2003. The heart had been surgically removed from each pig, leaving a wound in the thorax and blood on the carcass. Recently thawed pigs are attractive to necrophilous flies (7).

One pig ("control," 19 kg weight) was placed on the ground on three consecutive days between 0800 and 1900 h. At 1900 h, flies/eggs/larvae were sampled from the pig, and all flies were removed. The pig was double-bagged in new plastic bags, secured in a clean ice chest (sealed with duct tape), and stored in an unrefrigerated building on the site. A 2-h "rest period" followed before two test pigs were put out at 2100 h. One pig (19.5 kg) was placed on the ground under a mercury vapor security light; another (18.25 kg) was placed on the ground in a dark area away from any artificial lights. Both were exposed from 2100 to 0300 h on the same night. Four sticky traps were placed within 0.6 m of each pig to collect flies attracted to the carcass. At 0300 h, any flies/eggs/larvae on each pig were removed and the pigs double-bagged together in new plastic bags, placed in a clean ice chest (sealed with duct tape), and stored in an unrefrigerated building distant from the "control" pig storage site. Environmental data were recorded, as in 1993, at each site at 2100 and 2300 h, and the presence of flies at each pig was recorded at 10-min intervals between 2100 and 0300 h.

Results and Discussion

Although diurnally active flies were found in urban and rural study areas in 1993 and 2003, and oviposited on bait "controls" in the study areas, nocturnal oviposition was recorded only once during some 200 h of bait presentation. No flies were seen on any bait after 2200 h or before 0600 h.

In 1993, *Phaenicia coeruleiviridis* (Macquart), *Cochliomya macellaria* (Fabricius), *Neobellieria bullata* (Parker), and *P. cuprina* (Wiedemann) were attracted to freshly killed rats up to 50 min postsunset but did not oviposit. *P. coeruleiviridis* occurred with *N. bullata* at both study sites but was more common in the "unlit" site while *N. bullata* was more common in the "lighted" site. Fly activity ceased on the rat carcasses within an hour after sunset and no oviposition occurred between 2100 and 0600 h, even though flies were active at the sites during this period. Sunset times (8) on collection dates ranged from 2038 h in June to 2006 h in August.

No flies oviposited on freshly killed rats or mice placed at ground level between 2200 and 0500 h in an urban area in the summer of 2003, even though the "control" baits were attractive to fly species (*N. bullata*, *P. cuprina*, *P. coeruleiviridis*, *Cynomyopsis cadaverina* (Robineau-Desvoidy), and *Hydrotea* sp.) during the daytime. Neither fresh nor aged beef (24- or 48-h) attracted flies in lighted or dark rural situations between 2300 and 0500 h, but fresh beef was attractive to ovipositing fly species during the daytime (*Musca domestica* (Linneaus), *P. coeruleiviridis*, *C. macellaria*, and *N. bullata*). Sunset times on collection dates ranged between 2037 h (July), 1958 h (August), and 1941 h (September).

One of two pigs (fresh to bloat) placed in a lighted rural site experienced oviposition by *P. coeruleiviridis*, *C. macellaria*, and *M. domestica* (a total of 120 eggs; 2100–2120 h). Sunset time on the collection dates occurred at 2036 h. *Chrysomya rufifacies* (Macquart), *Hydrotea* sp., and *N. bullata* were also attracted to, but did not oviposit on, the pig. It is possible that the advanced state of decay of the pig influenced its attractiveness to the flies (9).

Flies attracted to carcasses (corpses) during the daytime may remain in the area, oviposit during a narrow window of time postsunset and then remain at the site without ovipositing until the next morning. Nocturnal environmental conditions during our studies relative to air temperature (range: 19–36°C), relative humidity (range: 33–94%), and wind speed (less than 10 kph) appeared within normal ranges for oviposition. More refined laboratory assessment of these variables, and their possible effect(s) on oviposition behavior, seems warranted.

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Additional information and reprint requests:
Robert S. Baldridge, Ph.D.
Department of Biology
Baylor University
Waco, TX 76798
E-mail: Robert_Baldridge@baylor.edu