

Foraging by Food Deprived Larvae of *Neobellieria bullata* (Diptera: Sarcophagidae)

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ABSTRACT: Traditional entomological methods of estimating postmortem interval from developmental stages of fly larvae associated with the body are based on the premise that older larvae are not recruited from the surrounding environment. We found that food deprived second and third instar larvae of the fleshfly, *Neobellieria bullata* Parker, can locate beef liver over a distance of 33 cm, apparently by using chemical cues, and can crawl to the food within 90 min. The implications of these results are discussed with respect to methods of estimating postmortem interval by calculating rates of fly larvae development.

KEYWORDS: forensic science, forensic entomology, postmortem interval, *Neobellieria bullata* Parker

Determining the developmental stage of Diptera larvae found on or near decomposing bodies is often used as a means of estimating the postmortem interval (PMI) (1-8). Current methods (2,7,8) assume that larvae present on, or in the vicinity of, the body originated from eggs or first instar larvae deposited on that body by adult flies. However, if a subpopulation of larvae present on the body are significantly more advanced in development than the main population, a forensic entomologist may be presented with data which is difficult to interpret. Researchers have offered various explanations for the presence of anomalous larvae. Suggesting, for example, that the older larvae resulted from an accelerated growth rate due to elevated temperatures in specific regions of the corpse (2,7,9), or from the presence of certain drugs (10,11) or are the product of a few eggs laid during a relatively short, early window of favorable weather (3,6). Another possibility rarely addressed is that young maggots may crawl to the corpse from nearby overcrowded or depleted food sources such as dead animals, garbage, or faeces.

If young larvae regularly abandon unfavorable sites and search for fresh food sources, quantifying this behavior would increase the precision of estimating PMI. Although dispersal behavior of postfeeding Diptera larvae is well documented (13,14) and dispersal of immature maggots from depleted food sources has also been recorded (15), we are not aware of any studies on the foraging behavior of immature maggots. Here we report the results of our initial study on foraging behavior of immature larvae *Neobellieria bullata* Parker. We found that late second and early third instar

larvae of *N. bullata* deprived of food and placed in full light begin actively foraging within 15 min. The larvae were able to detect a concealed food source over a distance of at least 33 cm and crawl to the food within 90 min.

Materials and Methods

Pupae *N. bullata* were obtained from Carolina Biological Supplies and the resulting adults were used to initiate a colony. The flies were reared in an environmental chamber at 30°C, a relative humidity greater than 70%, and a daily cycle of 12h light and 12h dark. Adults were provided with crushed banana, beef liver, and water, as needed. Larvae were provided with fresh beef liver every two days. Larvae to be used in tests were reared in mass so that all were of roughly similar age.

Experiments were conducted in two terrariums, each with interior dimensions of 40 × 24 × 35 cm in height. A 2 to 3 cm layer of moist potting soil was placed in each terrarium to serve as a favorable substrate for movement and provide a retreat for maggots that burrowed. The soil was moistened regularly over the course of the observations. A series of reference marks were made on the sides of the glass near the soil. The marks were placed at 8-cm intervals along the long axis of the terrarium, dividing it into five equal sections. Sections were numbered 1 to 5. The two terrariums could be placed in the environmental chamber such that sections 1 to 5 either ran from left to right or from right to left. To control for any bias of the larvae for one side of the environmental chamber, the left/right orientation of the terrariums was assigned at random for each test. To allow observations of the maggot foraging behavior, tests were performed with continuous light. The potting soil was discarded and the terrariums were washed after each test to prevent any accumulation of chemical cues.

Second and early third instar larvae were weighed with an electronic scale prior to testing. Preliminary tests showed that larvae larger than 0.04 mg engaged in little or no foraging in continuous light, although they would forage in darkness. We also observed that larvae smaller than about 0.02 mg appeared to have great difficulty crawling across the soil, perhaps because of difficulties in maintaining a favorable water balance under continuous light. Consequently, only larvae with masses in the range of 0.02 to 0.04 mg were tested. Twenty larvae were used for each test. Experimental and control conditions were run simultaneously. To begin an experiment, 2 groups of 10 larvae were placed in separate terrariums. In each case, the 10 larvae were released in section 1 and covered with a small (4 by 8 cm) moist cloth. Two additional moist cloths were placed in section 5 at the opposite end of each terrarium. In one terrarium, the experimental condition, the second cloth concealed approximately 30 g of beef liver. In the other

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terrarium, there was no liver or any other food under the second cloth. The distance between the release point in section 1 and the cloth in section 5 was at least 33 cm. Number of larvae present in each of the five sections was recorded at 15, 30, 60, 90, 170, and 310 min after the test started. Regular monitoring of larval activity allowed all individuals to be located at each count, including larvae that had burrowed. Larvae were discarded after each experiment. Because the experiment was replicated six times, a total of 120 different larvae were tested, 60 for the experimental and 60 for the control conditions.

Results and Discussion

Figure 1 shows that when liver was present, *N. bullata* larvae began to leave the release site within 15 min and that the first larvae managed to crawl to the food within 90 min. On average, 6.7% (i.e., 4 of 60) of the larvae managed to reach the liver in 90 min, 15.0% (9 of 60) in 170 min, and 36.7% (22 of 60) within 310 min. By this time, only 8.3% (5 of 60) still remained near the release point in section 1. In contrast, Fig. 2 shows that none of the *N. bullata* larvae in the control condition had crawled to section 5 at the opposite end of the terrarium within 90 min, or even within 170 min. Even after 310 min, only 5.0% (3 of 60) of the control larvae had reached section 5, although 78.3% (47 of 60) still remained either near or under the moist cloth in section 1. A paired *t*-test for two sample means showed that the difference of the two experimental conditions were significant at 310 min, with $N = 6$, $df = 5$, $t = 7.8637$, and $P < 0.001$.

Inspection of the data showed that two replicates of the experimental condition which exhibited the least amount of movement also had the greatest proportion of larvae near the mass limit of 0.04 mg. Because larger larvae are less likely to forage in continuous light, these two replicates could have biased the results towards a slower mean rate of larvae movement. However, excluding the two replicates (reducing sample size to 4 groups of 10) had only a modest effect on the results. After 310 min, nearly half (19 of 40) of the larvae in the reduced sample had crawled to the liver and none remained behind in section 1.

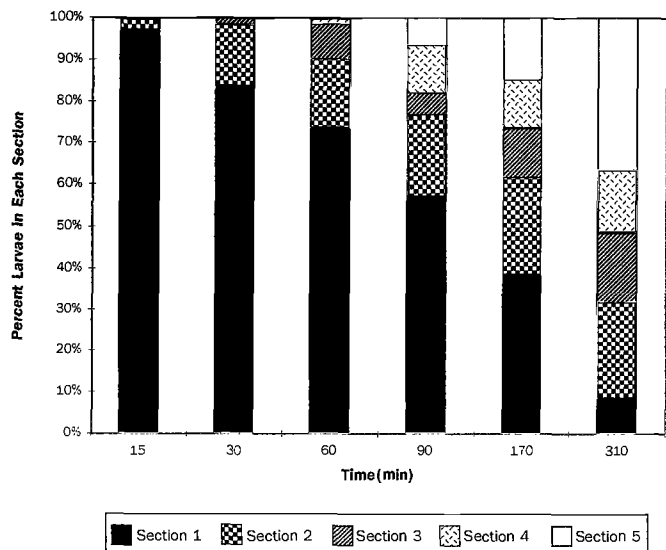


FIG. 1—Movement *N. bullata* larvae with liver present. Percentage of larvae $N = 60$ in each section at 15, 30, 90, 170, and 310 min after start of experiment.

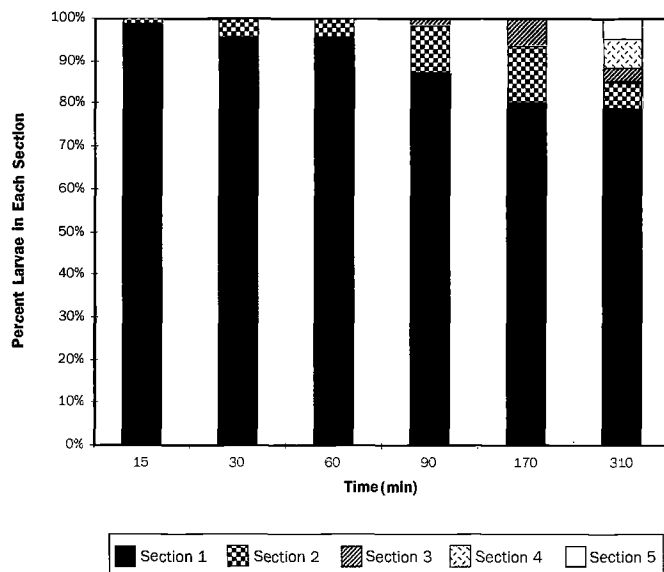


FIG. 2—Movement of *N. bullata* larvae with liver absent. Percentage of larvae *N. bullata* ($N = 60$) each section at 15, 30, 90, 170, and 310 min after start of experiment.

Foraging behavior exhibited by *N. bullata* in the experimental conditions indicated that the larvae were able to detect when liver was present at the opposite end of the terrarium. Although larvae showed little initial activity after being placed in section 1, within 15 min, they began to emerge from under the damp cloth and move about in the vicinity of the release point. Larvae movement became increasingly oriented after 30 min and they began to converge on the concealed liver. The behavior of the larvae indicated that they were following a chemical gradient, presumably odors emanating from the liver. Foraging larvae lifted their heads and moved them back and forth, crawled a few centimetre, more or less in the direction of the liver, and then repeated the process. They also burrowed frequently, presumably in response to light. As they closed on the liver, foraging larvae oriented with greater precision and spent less time burrowing. When foraging larvae entered section 4 of the terrarium and were within about 12 cm of the liver, they were able to proceed directly to the food. Under conditions of complete darkness, foraging *N. bullata* larvae would presumably spend less time burrowing and reach the liver faster.

Although this was a limited study and the data should be considered preliminary, our findings indicate that active foraging by Dipteran larvae has the potential to present a serious problem for crime scene investigators. In conditions of full light about 7% of larvae *N. bullata* in the weight category of 0.02 to 0.04 mg were able to crawl across 33 cm of soil in 90 min and nearly 37% had reached the liver in 310 min. Because the presence of even a single anomalous maggot on a body can compromise attempts to estimate PMI, noting possible sources of migrants (e.g., dead mice, faeces, etc.) in the immediate vicinity of the body is essential.

A review of the literature and a discussion with a practicing forensic entomologist did not uncover any cases in which larval movement from a depleted food source to a body had been identified in a practical forensic situation. Although foraging *N. bullata* in our study were limited to a distance of 33 cm, maximum distance that the larvae could crawl to reach carrion under realistic conditions is unknown. Consequently, the practical forensic significance of the observed foraging behavior by immature maggots is unknown in natural situations in which carrion is more widely

distributed and patchy in occurrence. Until we understand the adaptive significance of the observed foraging behavior in *N. bulatta* larvae, statements about maximum distances amount to simple speculation. Another problem is that although *Sarcophagidae* (flesh flies), such as *N. bulatta*, occur on carrion, *Calliphoridae* (blow flies) are usually more important in forensic situations, particularly in cooler temperate regions (1,3,8). Foraging tactics of immature *Calliphorid* larvae may be different than those of *N. bulatta*. However, larvae of certain forensically important *Calliphoridae*, such as *Phaenicia* (= *Lucilla* in Europe) *sericata* (Meigen) and most species of *Lucilla* are saprophagous and can develop in dung (8). Therefore, if the larvae are attracted to carrion, any faeces present in the vicinity of a body may serve as a source of foraging maggots. In summary, our findings argue for further research to determine: The range of environmental conditions in which immature fly larvae from the surrounding environment are likely to be recruited to a body, differences in foraging tactics among larvae of forensically important species, maximum distances over which maggots can detect carrion and maximum distances that maggots are likely to move to reach a fresh food source.

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