

## FECUNDITY OF NATURALLY BLOODFED *CULISETA MELANURA*

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**ABSTRACT.** Naturally bloodfed *Culiseta melanura* were collected annually from resting boxes in and around a swamp in Oswego County, NY from 1982 to 1989. Females were held individually in a laboratory until they oviposited. Except in 1982, every other female was provided a 10% dextrose solution. Females were classed as alive or dead following oviposition and female size was based on abdomen length, measured after oviposition. Egg rafts from each female were held individually and the numbers of larvae and unhatched eggs were counted. Fecundity (number of eggs laid per female) was based on number of larvae plus unhatched eggs. Rafts from 2,120 females averaged 129 eggs and 106 larvae per raft. Rafts from females that were alive following oviposition were significantly larger and produced more larvae than those of females that died following oviposition. Availability of sugar influenced female survival but not egg raft production. Size and fecundity of females decreased from May through September. These differences were attributed to the temperature and larval density of breeding crypts. Seasonal changes in size may influence the vector efficiency of *Cs. melanura*.

### INTRODUCTION

Fecundity can be defined as the number of eggs laid (Jalil 1974). Studies on the fecundity of mosquitoes have generally used laboratory-reared or colonized mosquitoes and, as described by Jalil (1974), have led to confusion in determining the relationship between body size and body weight and the number of eggs produced. The paucity of information on fecundity of natural mosquito populations can be attributed to an inability to collect large numbers of naturally blooded or gravid females (Service 1993).

The mosquito *Culiseta melanura* (Coquillett) is the primary vector of eastern equine encephalitis (EEE) virus (Morris 1988). Diurnal resting shelters adequately sample males and blooded and non-blooded females and have been used in population and virus studies of this species (Morris and Srichongse 1978, Morris 1984). Lorenz et al. (1990) used females collected from shelters to study the relationship between size and parity and demonstrated a decline in size by date of collection. Since fecundity is considered a function of size (Bock and Milby 1981), a study to relate these biological parameters would contribute to the knowledge of the population dynamics of this species. This report relates the size of this species to fecundity and relates these parameters to survival through oviposition, access to sugar, and time of year for naturally bloodfed females.

### MATERIALS AND METHODS

Specimens were collected from diurnal resting shelters (Morris 1981) by closing the shelter with a lid wetted with 2-3 ml chloroform. After ≈5 min-

utes, anesthetized mosquitoes were transferred to a 120-ml glass vial with a plastic snap cap. Diurnal resting shelters were placed at 6 sites in the vicinity of Toad Harbor Swamp, West Monroe, Oswego County, NY (Morris et al. 1980). Specimens were collected from one site in the swamp interior, 3 sites on the swamp edge and 2 upland sites from May to September 1982-89.

Collections were transported to the Shad Slade field station where they were placed in a 15-cm<sup>3</sup> Gerberg cage and allowed to revive for approximately 30 minutes. Blooded or gravid female *Cs. melanura* were aspirated to individual, cylindrical, wire mesh cages (15 × 5.5 cm). The tops and bottoms of the wire cages were fitted with 15 × 60-mm plastic petri plates. A female was put into each cage through a hole bored through the petri plate top and sealed with a cork. Sets of 15 cages were placed on end in enamel trays containing distilled water 4-5 cm deep and the trays were covered with Masonite<sup>®</sup> panels with holes large enough to fit the cages. This arrangement provided females with the preferred dark oviposition and resting site (Morris et al. 1980).

In 1982, females were not provided with sugar. Beginning in 1983, sugar was provided to every other female by replacing the cork with a cottonball soaked in 10% dextrose. Females were held at ambient temperatures at the field station. Cages were checked daily for egg rafts. Cages containing rafts were removed from the trays and the egg rafts were individually transferred to 1-dram glass vials containing 3 ml distilled water. Each ovipositing female was classed as dead or alive.

Vials containing rafts were checked daily for the presence of larvae. Larvae were counted within 2 days of eclosion. Raft remnants were examined microscopically and the number of unhatched eggs was counted. Raft size is the sum of larvae plus unhatched eggs. Percent hatch is the number of lar-

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Table 1. Summary statistics of naturally bloodfed, ovipositing *Culiseta melanura*, 1982–89.

Dates of oviposition	n	Abdomen length (mm)			Eggs per raft		Larvae per raft	
		Mean	SE	CV	Mean	SE	Mean	SE
Jun 4–Sep 27, 1982	523	3.0	0.01	8.8	124.7	2.0	94.3	2.5
May 25–Sep 12, 1983	367	3.1	0.01	7.2	152.6	2.7	126.5	3.3
Jun 18–Sep 20, 1984	240	3.0	0.01	7.1	124.5	3.1	102.8	3.5
Jun 10–Aug 21, 1985	337	2.8	0.01	7.5	113.1	2.3	93.0	2.7
Jun 4–Sep 10, 1986	395	3.2	0.01	8.0	113.1	2.3	97.3	2.6
Jun 1–Aug 12, 1987	153	3.2	0.02	9.3	148.1	3.5	129.4	4.4
May 31–Aug 25, 1988	29	3.0	0.03	6.3	171.2	9.3	147.7	13.6
Jun 19–Sep 5, 1989	76	3.2	0.02	6.8	154.6	4.8	140.7	6.0
Total	2,120	3.0	0.01	9.1	128.9	1.1	106.1	1.3

vae divided by raft size. Rafts were considered nonviable if they did not hatch within 7 days of oviposition. The eggs of these rafts were also counted and included in the totals for raft size. Size was determined by measuring the abdomen from the apex of postnotum to the apex of the VIII abdominal segment. Measurements were taken to the nearest 0.125 mm.

Data were analyzed using version 6.08 of the Statistical Analysis System (SAS Institute 1989) at Syracuse University. Means were calculated for abdomen length and eggs and larvae per raft. Analyses were performed for alive/dead status, sugar presence/absence, all combinations, year, month, and week of oviposition. Statistical methods included general linear models (GLMs) with Duncan's means groupings, Student's *t*-test, chi-square, regression, and Spearman correlations.

## RESULTS AND DISCUSSION

**Fecundity:** An average of 129 eggs per raft (range 1–308) was calculated from 2,120 rafts (Table 1). This falls on the low side of raft sizes reported for *Culiseta*. Frohne (1953) reported 93 for *Cs. impatiens* (Walker). Others reported include 130 for *Cs. incidens* (Thomson) (Barr et al. 1986), 191 for *Cs. inornata* (Williston) (Pappas and Pappas 1982), 197 for *Cs. morsitans* (Theobald) (Wallis and Whitman 1968), and 280 for *Cs. longiareolata* (Macquart) (van Pletzen and van der Linde 1981). Our estimate of fecundity was based on egg

raft size for all females regardless of hatching success, status following oviposition, or access to sugar.

**Hatching success:** Of the 273,283 eggs oviposited, 77.6% hatched, producing an average of  $106.1 \pm 1.3$  larvae per raft (Table 1). Ninety-five percent ( $n = 2,011$ ) of all rafts had eggs that hatched (larvae > 0). Excluding the 109 rafts from which no eggs hatched, the hatching success was 81.8%. Rafts with hatched eggs were significantly larger ( $132.4 \pm 1.0$  eggs/raft) than rafts without hatched eggs ( $64.4 \pm 4.9$  eggs/raft) (*t*-test,  $P < 0.001$ ).

Hatching success could be related to egg raft formation or female insemination rates. The smaller size of rafts with no hatched eggs suggests that these rafts were malformed and did not incubate properly. Morris (1984) indicated that a majority of female *Cs. melanura* were inseminated within days of emergence and attributed the finding of 10 infertile parous females to diagnostic errors. But 14% ( $n = 15$ ) of nonhatched rafts in our study had egg counts at or above the mean (range 129–238 eggs/raft), suggesting that at least some *Cs. melanura* were not inseminated at the time of blood-feeding.

**Status:** The 1,454 (69%) females that survived oviposition produced larger egg rafts and more larvae than females that did not survive oviposition (Table 2). Morris (1984) estimated the daily survival rate for *Cs. melanura* as 0.89 and that only 20% of females survived to take a second blood-meal. Since death is associated with oviposition in nature (Corbet and Danks 1975), and oviposition is probably a significant cause of mortality, the estimate of egg raft size should include both females that survived and those that died at oviposition.

**Sugar:** The number of females that were alive following oviposition was approximately equal for those with ( $n = 721$ ) and without ( $n = 732$ ) access to sugar. For those that died, the number with access to sugar was significantly smaller ( $n = 47$ ) than those without access to sugar ( $n = 619$ ,  $\chi^2 = 357$ ,  $P < 0.001$ ). For those that lived, the number of eggs per raft and number of larvae hatched were equal for the 2 groups (Table 2, GLM, Duncan grouping,  $P = 0.05$ ). For those that died, egg raft size was significantly smaller for the group with

Table 2. Analysis of means for eggs, larvae, and abdomen length (mm) by survival and availability of sugar for 2,120 ovipositing *Culiseta melanura*, 1982–89.

Status	Sugar	n	Eggs	Larvae	Abdomen (mm)
Alive	No	733	135.0a <sup>1</sup>	117.5a	3.02bc
Alive	Yes	721	137.4a	121.3a	3.05ab
Dead	No	619	113.8b	77.1b	2.97c
Dead	Yes	47	101.9c	76.8b	3.10a

<sup>1</sup> Means with different letters are significantly different (GLM, Duncan's groupings,  $P \leq 0.05$ ).

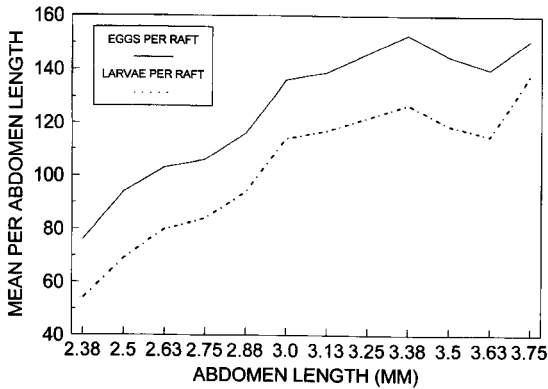


Fig. 1. Mean fecundity (solid line) and larvae (broken line) per egg raft for 2,110 ovipositing *Culiseta melanura* by abdomen size class (mm).

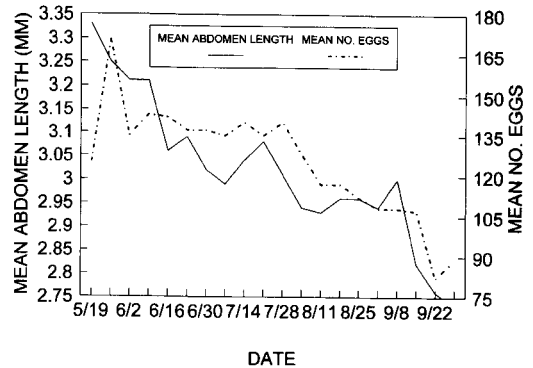


Fig. 2. Weekly mean abdomen length (solid line) and fecundity (broken line) of 2,120 ovipositing *Culiseta melanura*, May through September.

access to sugar than for those without sugar and was significantly smaller than for both groups that survived oviposition (Table 2). The sugar feeding cycle for *Cs. melanura* was determined to be 7–19 days and the ovarian cycle 5–14 days, indicating that *Cs. melanura* may take sugar following a blood meal (Morris 1984). Nasci and Edman (1984) observed that most gravid *Cs. melanura* contained nectar. Our results demonstrate that the availability of sugar after a blood meal influenced survival but did not influence egg production, in contrast to the findings of Nayar and Sauerman (1975), who stressed the importance of sugar for both fecundity and survival.

**Size:** The mean abdomen length was  $3.0 \pm 0.01$  mm (Table 1), with a range of 2.0–4.13 mm. Although abdomen lengths were normally distributed, this parameter probably was not the best estimate of size. There were differences in abdomen lengths among females by access to sugar suggesting that the availability of sugar influenced abdomen size (Table 2). Kalpage and Brust (1974) also reported a direct correlation between body size and fecundity for *Aedes atropalpus* (Coquillett).

**Size and fecundity:** There was a trend for raft size and larvae to increase with increasing abdomen length;  $r = 0.3$  for both raft and larvae. The correlation was  $r = 0.8$  between raft size and number

of larvae. The relationship between mean raft size and larvae for 2,110 females in abdomen size classes between 2.38 and 3.75 mm is illustrated in Fig. 1. This figure excludes the smallest and largest size classes, which contained 5 females each. The optimum for *Cs. melanura* was a female with an abdomen length of 3.75 mm that produced an egg raft with 151 eggs of which 92% hatched. The median ( $n = 491$ ) was a female of 3.0 mm that produced an egg raft of 136 eggs with 84% hatching success.

**Seasonal fecundity:** To test for seasonal relationships, data were analyzed by month of oviposition. Females ovipositing in May were significantly larger than those that oviposited during all other months (Table 3). Means for each subsequent month from June through August were significantly smaller ( $P < 0.05$ ) than for the preceding month. Females ovipositing in September were the same size as females ovipositing in August. Fecundity was highest in June but not significantly different than in May and July. Fecundity in August was significantly lower than in June and July, and in September fecundity was lower than all other months. The seasonal change in size and fecundity by week is illustrated in Fig. 2. This trend for decreasing size over season is similar to the findings of Lorenz et al. (1990) who used wing length as a measure of size. Similarities in population structure

Table 3. Monthly and brood means of abdomen length (mm), eggs, and larvae for 2,120 ovipositing *Culiseta melanura*, 1982–89.

Month of oviposition	n	Abdomen length	Eggs	Larvae	Brood <sup>1</sup>	Abdomen length	Eggs	Larvae
May	33	3.3a <sup>2</sup>	127.1ab	91.2bc	Spring	3.2	137.6	114.8
June	500	3.1b	138.4a	116.4a				
July	645	3.0c	137.1a	114.5a				
August	701	2.9d	121.9b	99.8b	Summer	3.0	126.0	103.2
September	241	2.9d	107.8c	82.8c				

<sup>1</sup> Spring brood females were significantly larger than summer brood females for all three parameters (*t*-test,  $P < 0.001$ ).

<sup>2</sup> Means with different letters are significantly different (GLM, Duncan's groupings,  $P \leq 0.05$ ).

between *Cs. melanura* in central New York and in the coastal areas studied by Lorenz et al. (1990) can be inferred from the coefficients of variation reported by them and herein (Table 1). However, they attributed seasonal size differences mainly to the temperature at which larval development occurs. Bock and Milby (1981) also related differences in size and fecundity in wild-caught, laboratory-fed *Culex tarsalis* Coq. to temperature. But larval density is also a factor in adult size (Jalil 1974, Nasci 1988, Reisen et al. 1984) and may contribute to the seasonal differences observed in *Cs. melanura*.

*Culiseta melanura* overwinters in the larval stage (Joseph and Bickley 1969) and only 3rd and 4th instar larvae are found in April and May in central NY (Howard, unpublished data). Adult emergence begins in May and 1st instar larvae are not found until around June 1 with a second brood of adults occurring in early July. All instars can be found thereafter (Woodrow and Howard 1994). Although Joseph and Bickley (1969) attribute the staggered appearance of adults from crypts to extended larval development, we believe that more than one female will oviposit in a crypt, resulting in increased larval densities and irregular adult emergence. We attribute the decreasing size of females over the season to the fact that second brood larvae develop at higher temperatures (L. A. Patrican, unpublished data) and larval densities than overwintering larvae. Thus it is reasonable that second brood adults are smaller and less fecund than first brood adults.

**Size and vector status:** The role of *Cs. melanura* in the endemicity of EEE virus is established. While adults are present from May through September, there is a distinct seasonality to the transmission of EEE virus in temperate areas associated with the second brood of adults in July (Scott and Weaver 1989). In central NY, the seasonality of EEE virus is similar, with the earliest evidence of EEE virus occurring in late June and 68% ( $n = 136$ ) of isolations occurring in July and August (Howard et al. 1994). When the data from our study were analyzed by brood, 1st-brood females, those ovipositing in May and June, were significantly larger (Table 3,  $t$ -test,  $P < 0.001$ ) than 2nd brood, those ovipositing in July–September. Lorenz et al. (1990) found no difference in parity rates between first and second brood *Cs. melanura*, indicating that the vector capacity for this species was similar throughout the year. The influence of the observed decrease in size of *Cs. melanura* on the seasonal appearance of EEE virus has not been investigated.

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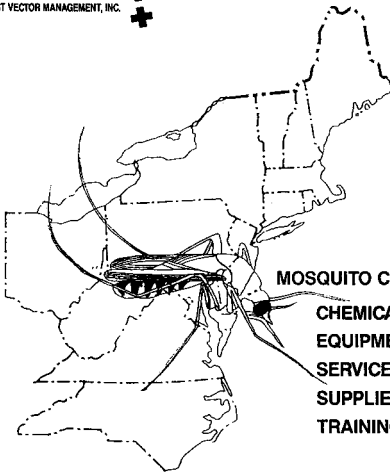
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