

# OBSERVATIONS ON *CULISETA MELANURA* (COQUILLET) IN RELATION TO ENCEPHALITIS IN SOUTHERN MARYLAND

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**INTRODUCTION.** Repeated isolations of eastern and western equine encephalitis viruses have been made from *Culiseta melanura* (Coquillett) in the southern and eastern United States. Both viruses were isolated from this species in Louisiana (Chamberlain *et al.*, 1951 and Kissling *et al.*, 1955), New Jersey (Holden *et al.*, 1954 and Chamberlain *et al.*, 1958), Alabama (Stamm *et al.*, 1962) and in Massachusetts (Feemster *et al.*, 1958 and Hayes *et al.*, 1961).

In Maryland, a number of outbreaks of eastern equine encephalitis have occurred, corresponding chronologically with those observed in the other Middle Atlantic States (Byrne *et al.*, 1959). According to Byrne all attempts to isolate the virus from mosquitoes collected in areas of EEE outbreaks yielded negative results. *C.*

*melanura* was not included among the mosquitoes collected and was believed to be uncommon in the state (Newson, 1959). During the spring of 1964, an extensive survey was made in southern Maryland to locate the breeding habitats of *Culiseta melanura*. The Pocomoke Cypress Swamp near Pocomoke City, Maryland, was recognized as an ideal breeding habitat for this species and was therefore chosen for this study.

This report is essentially a continuation of the efforts at identifying the arthropod and vertebrate hosts of EEE in the Chesapeake Bay area (Byrne *et al.*, MS). It presents results of preliminary investigations on the ecology of *Culiseta melanura* in the Pocomoke Swamp, and the virus isolations obtained. Additional information is given on the associated mosquitoes of this habitat.

**DESCRIPTION OF STUDY AREA.** Pocomoke Cypress Swamp is situated on the

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Delmarva (Delaware, Maryland, Virginia) peninsula in Worcester County, Maryland. It lies approximately 2.5 miles southwest of Pocomoke City and about the same distance north of the Maryland-Virginia State line (Figure 1). This

large bodies of fresh water whose levels fluctuate with total precipitation and the condition of the Pocomoke River which flows along its northern and western boundaries. There are numerous pockets in the soil, earth cavities, tree roots and

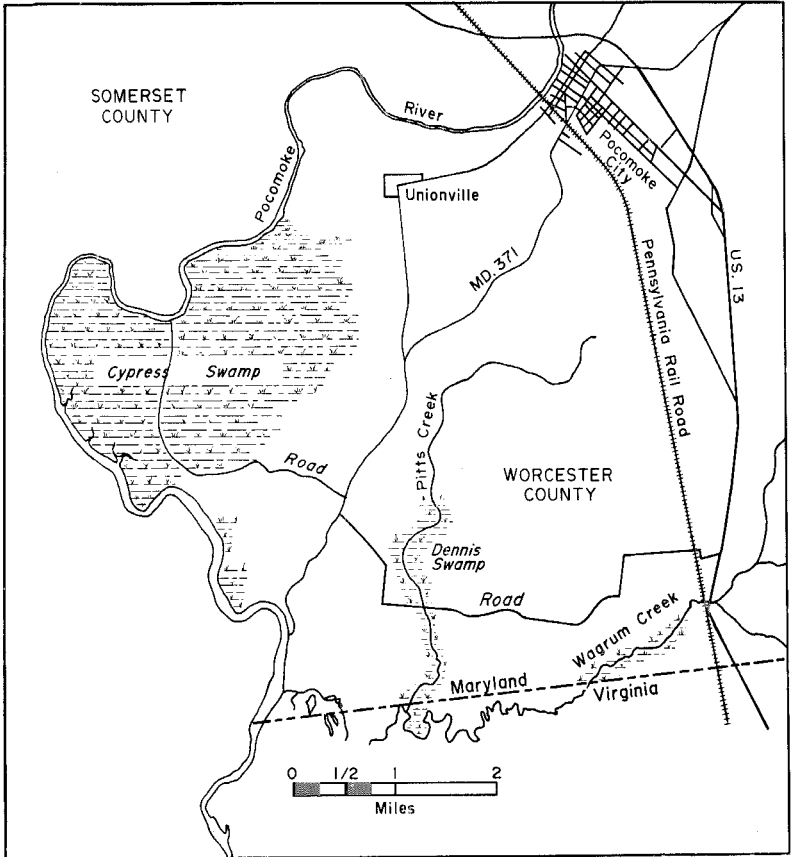


FIG. 1.—The study area of Pocomoke Cypress Swamp, Worcester County, Maryland.

swamp is about 3,000 acres in area and supports mixed vegetation of three types. The broadleaf type is represented by red maple and black gum; the conifers by loblolly pine, cedar and cypress, and the ericaceous shrubs chiefly by bayberry. On high ground, mature mixed oaks and pines are present. This swamp contains

stump holes where *C. melanura* larvae are present. The bottoms of these breeding sites range from 5 to over 10 feet in depth from the ground level at different locations in the swamp.

**MATERIALS AND METHODS.** Several methods were used for collecting mosquitoes from this freshwater swamp. Eight bat-

tery-operated Chamberlain light traps were set up at selected sites about 5 feet above the ground. Ten red resting boxes, similar to those used by Burbutis and Jobbins (1958), were placed on the ground with the open side directed westward. Three modified Magoon bait traps (Magoon, 1935) were also used; two with raccoons and the other with chicks. Each trap was 48" x 36", with a saddle roof 36" high or 32" to the eaves. Light traps and resting boxes were operated twice weekly. Bait traps were operated continuously and the mosquitoes were collected from them three times a week. All collections were made in the early morning between 0700 and 0830 hours. Mosquitoes were transported to the laboratory in jars covered with wet towels and placed in insulated boxes. In the laboratory they were sorted, identified and pooled for virus isolation. Engorged specimens were separated and frozen for later identification of the blood meal source. The precipitin test was used for this purpose utilizing ten antisera prepared in rabbits against: pig, deer, raccoon, reptile, cow, dog, horse, man, bird (chicken) and rodent (rat). The procedure followed was similar to that used by Thompson, *et al.* (1963).

An experiment was conducted to determine the flight range and dispersal pattern of *Culiseta melanura* using material collected from light traps. Females were dusted with green fluorescent powder<sup>2</sup> and released soon after at a designated point in the same habitat. Additional light traps and resting boxes, previously laid out at known distances from the point of release, were put in operation for 24 hours following the release. A long-wave ultraviolet lamp (3660 Angstroms) was used to detect marked individuals. The detailed plan of this experiment will be discussed elsewhere (Atwood, MS).

Additional information on the daily flight and biting cycles of *Culiseta melanura* was obtained in late September.

Human biting collections were made on a single night for 15-minute periods at 3-hour intervals. At the same time periodic collections were obtained by battery operated New Jersey light traps equipped with switch timers. These timers were set in advance in such a way that when one trap was shut off the other was started.

A hygrothermograph housed in a standard weather shelter recorded the temperature and relative humidity in the swamp.

RESULTS AND DISCUSSION. Fifteen mosquito species were collected during 1964 in the Pocomoke Cypress Swamp. These included both fresh and salt water breeding mosquitoes. *Culiseta melanura* was the dominant species, comprising 89 percent of the over 26,000 mosquitoes collected (Table 1). *Culex salinarius* and

TABLE 1.—Mosquito fauna of Pocomoke Cypress Swamp, Maryland as indicated by all collecting methods, 1964.

Species	Females collected number	Per cent of total catch
<i>Culiseta melanura</i>	23,895	89
<i>Culex salinarius</i>	985	4
<i>Aedes vexans</i>	945	3
<i>Anopheles bradleyi-crucians</i>	359	1
<i>Aedes canadensis</i>	338	1
<i>Aedes sollicitans</i>	258	1
<i>Aedes taeniorhynchus</i>	63	<1
<i>Aedes cantator</i>	13	<1
<i>Uranotaenia sapphirina</i>	11	<1
<i>Psorophora ferox</i>	10	<1
<i>Anopheles punctipennis</i>	9	<1
<i>Aedes triseriatus</i>	7	<1
<i>Culex territans</i>	2	<1
<i>Anopheles quadrimaculatus</i>	1	<1
<i>Mansonia perturbans</i>	1	<1
Total catch	26,897	100%

*Aedes vexans* represented 4 and 3 percent of the fauna respectively, while none of the remaining species exceeded 1 percent.

Light trap collections indicated the continuous presence of *Culiseta melanura* in the swamp throughout the season. The earliest collections of this species were obtained late in May and the latest were taken early in October. During the period

<sup>2</sup> United States Radium Corp., Morristown, New Jersey.

between June and October five peaks of activity were observed; occurring in mid-June, mid-July, mid-August, early September and early October (Figure 2). It is

averaged 226 females per trap-night early in September, the highest in the season. The continuous presence of this species and the noticeable overlapping of broods

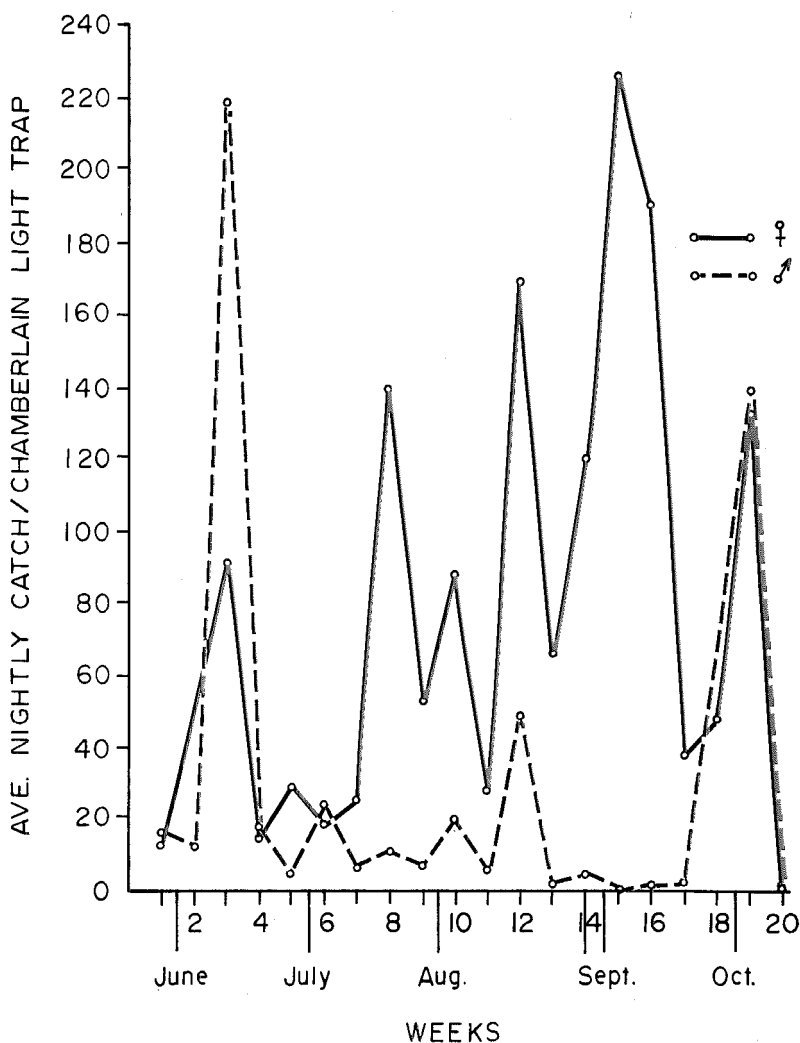


FIG. 2.—Light trap collections of *Culiseta melanura* in Pocomoke Cypress Swamp, Md., June–October 1964.

interesting to note the steady increase in the population size of *C. melanura* as the season advanced. The population density

suggest that this species is multivoltine at this latitude in Maryland.

In the swamp both the air temperature

and relative humidity appeared uniform throughout the study period (Figure 3). The mean weekly temperature ranged between 67° and 73° F. during the entire season except for a slight rise in early August when the mean temperature reached 83° F. The mean weekly relative humidity ranged from 67 to 76 percent but declined considerably toward the end of the season when it averaged 47 percent in mid-October.

Chamberlain light traps appeared very efficient in sampling populations of *Culiseta melanura* and in obtaining large numbers of mosquitoes for arbovirus surveillance (Table 2). Fewer adults were captured in the resting boxes, which appeared in agreement with the observations made in New Jersey by Burbutis and Jobbins (1958). Only two females were collected from one of the raccoon-baited traps, none were captured in the one with chicks. It is difficult to explain why the chick bait trap failed to attract *C. melanura*, a species believed to be predominately an avian feeder (Hayes, 1961). This may be due to competition from the wide variety of animals available in the area which pro-

TABLE 2.—Variation in species composition of mosquito catches obtained by different methods from Pocomoke Cypress Swamp, Maryland, 1964.

Species	Percentage of total catch		
	Chamberlain light trap	Bait trap	Resting box
<i>Culiseta melanura</i>	89	1	98
<i>Culex salinarius</i>	4	2	1
<i>Aedes vexans</i>	3	5	<1
<i>Aedes sollicitans</i>	1	7	..
<i>Aedes canadensis</i>	1	35	..
<i>Anopheles bradleyi-crucians</i>	1	45	<1
<i>Aedes taeniorhynchus</i>	<1	1	<1
<i>Aedes cantator</i>	<1	1	..
<i>Uranotaenia sapphirina</i>	<1	..	..
<i>Psorophora ferox</i>	<1	2	..
<i>Anopheles punctipennis</i>	<1	..	..
<i>Aedes triseriatus</i>	<1	1	..
<i>Culex territans</i>	<1	..	..
<i>Anopheles quadrimaculatus</i>	<1	..	..
<i>Mansonia perturbans</i>	<1	..	..
Number of collections	292	114	356

vide a much more accessible source for blood meals.

*Culiseta melanura* also dominated the mosquito catch obtained from resting

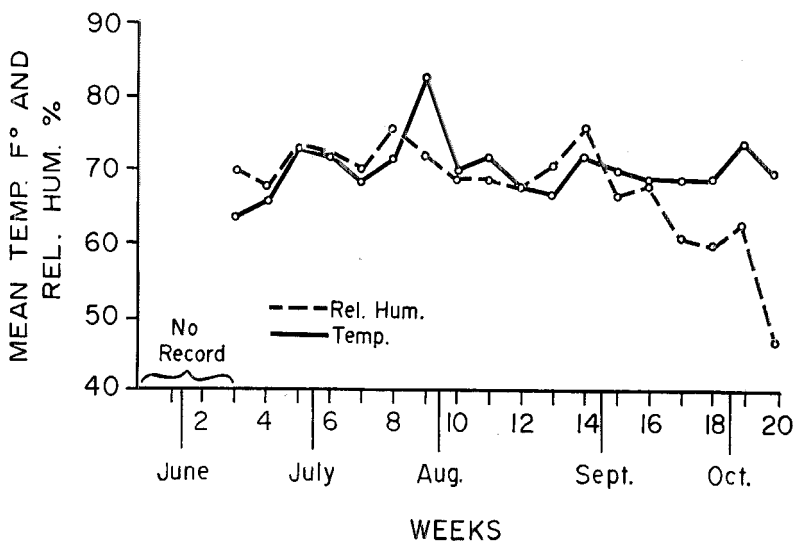


FIG. 3.—Temperature and relative humidity records of Pocomoke Cypress Swamp, Md. during 1964.

boxes. Weekly collections from these boxes indicated the continuous presence of this species in the swamp with one peak of activity occurring in mid-June (Figure 4). Because of the small num-

obtained from resting boxes than from light traps. Blood-fed females did not exceed 2 percent of the total mosquito catch in light traps while nearly one-fourth (24 percent) of the females col-

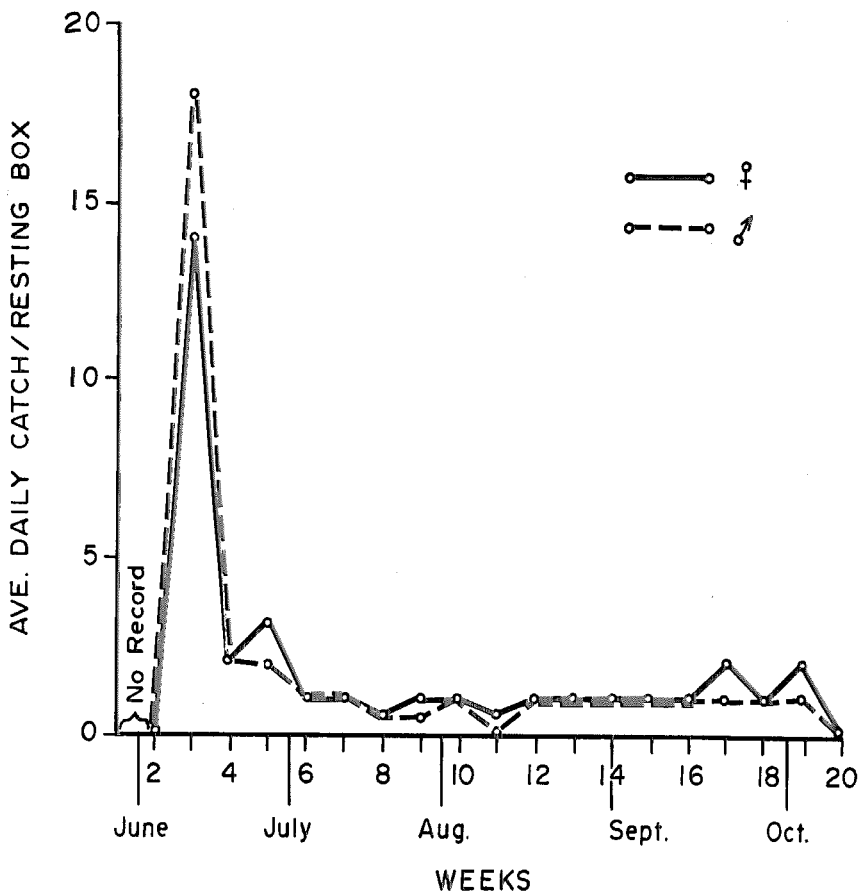


FIG. 4.—Resting box collections of *Culiseta melanura* in Pocomoke Cypress Swamp, Md., June–October 1964.

bers captured, these boxes failed to show the four subsequent peaks indicated by light trap collections. Failure of resting boxes to yield large catches of *C. melanura* might be due to competition with natural resting sites common in the swamp. A larger percentage of engorged females was

lected from resting boxes contained blood.

Only 48 of the 130 engorged *C. melanura* tested by the precipitin method gave positive reactions. Failure of the others to react with antisera used in this test may be attributed to the rapid digestion of the protein serum of the blood meal

beyond the point of being identifiable, or lack of specific antisera representing other animals available in this and adjacent habitats.

The precipitin tests indicated that *C. melanura* fed on pig, deer, raccoon, reptile, cow, dog, horse, and man in descending order (Table 3). Three of these

TABLE 3.—Source of blood meals of *Culiseta melanura* collected from Pocomoke Cypress Swamp, Md., during 1964 as determined by the precipitin test.

Antiserum	Positive reactions	Per cent* positive
Pig	13	27
Deer	11	23
Raccoon	8	17
Reptile	8	17
Cow	5	10
Dog	1	2
Horse	1	2
Man	1	2
Bird	0	0
Rat	0	0

\* Based on a total of 48 specimens which gave positive reactions.

hosts—deer, raccoon and reptiles, are present in the swamp while the others are found on farms located within a 2-mile radius from this habitat. The feeding of *C. melanura* on farm animals suggests possible movements of this species between the swamp and adjacent farms. No *C. melanura* appeared to have fed on birds or rodents, contrary to the observations made by Jobbins *et al.* (1961) in New Jersey and those by Hayes (1961) in Massachusetts. It is possible that testing more engorged specimens might provide additional information about the host preferences of this species.

A few blooded specimens other than *C. melanura* were also tested. One of five females of *Culex salinarius* yielded positive reactions with antireptile serum. Single specimens of *Aedes canadensis* and *Aedes vexans* were negative.

The single 24-hour observation made on the flight activity of *C. melanura*, using New Jersey light traps equipped with switch timers, showed that both males

and females were active during the night and occasionally in the daytime. The peak of activity occurred between 1930 and 2230 hours, when approximately 50 percent of the total catch (64) was collected. No females were collected during the interval of 0730–1330 hours, and no males were captured from 0430 through 1030. Similar records obtained from two New Jersey light traps operated alternatively for 12-hour periods support the previous observation. Approximately 94 percent of the total catch (137) was collected between 1630 and 0430 hours while the remaining 6 percent was obtained during the 0430–1630 interval.

Only a single attempt was made to study the flight range and dispersal pattern of *C. melanura*. Ninety-four marked females, or about 6 percent of the total number (1532) released, were recaptured within a 24-hour period. Since a relatively small number was released and a limited number of light traps were employed in this trial, it was difficult to establish the limits of the flight range. However, marked female *C. melanura* were recaptured 15 hours after release in the most distant light trap, located  $\frac{1}{8}$  mile east of the release point.

On a single occasion, human biting collections were made from one person in the swamp at regular intervals over a period of 24 hours. No *C. melanura* were collected biting man during that period even though light trap collections in operation in the general vicinity indicated considerable flight activity of that species. A single specimen of *Aedes vexans* was aspirated off a human arm at 0430 hours.

Over 25,000 mosquitoes representing six species collected from the swamp were pooled and sent to the Department of Virus Diseases, Walter Reed Army Institute of Research, for virus isolation. Twelve virus strains were isolated from pools of *C. melanura* (Buescher and Yuill, 1964). This was the only mosquito species yielding virus (Table 4). Eastern equine encephalitis virus was isolated from two pools collected by light traps on

TABLE 4.—Virus isolations from female mosquitoes collected on Pocomoke Cypress Swamp, Md. during 1964.

Species	Total specimens	No. pools tested	No. pools positive
<i>Aedes canadensis</i>	50	2	0
<i>Aedes sollicitans</i>	75	3	0
<i>Aedes vexans</i>	925	22	0
<i>Anopheles</i>			
<i>bradleyi-crucians</i>	350	14	0
<i>Culex salinarius</i>	850	16	0
<i>Culiseta melanura</i>	23,075	245	12
Total	25,325	302	12

September 1. Western equine encephalitis virus was isolated from one pool of mosquitoes collected by the same method on September 30. Viruses were also isolated from nine additional pools of this species collected by light traps and resting boxes during the period between mid-July and mid-September. The identity of these viruses has not yet been determined.

**SUMMARY.** In a field investigation conducted at Pocomoke Cypress Swamp, near Pocomoke City, Maryland during 1964, *Culiseta melanura* was the dominant mosquito among the fifteen species collected. Between mid-June and mid-October, five peaks of activities were observed for this species with a marked increase in the female population during September. Resting boxes yielded a larger percentage of engorged females than light traps. Magoon traps baited with raccoons and chicks proved unsuccessful in attracting this species.

Among the blood meal sources are some domesticated animals present within a 2-mile radius from this habitat. Females marked with fluorescent powder were captured  $\frac{1}{8}$  mile from the point of release within 15 hours. Both males and females of this species fly occasionally during the day but the peak of flight activity occurred at night between 1930 and 2230 hours.

Two strains of eastern equine encephalitis virus and one of western equine

encephalitis were isolated from *Culiseta melanura* collected in September from this swamp by light traps. Nine additional virus strains were isolated from the same species; their identity is not yet determined.

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