THE DIAPAUSE IN ADULT FEMALE *CULEX TARSALIS* COQUILLETT (DIPTERA: CULICIDAE)

E. E. BENNINGTON,[1] C. A. SOOTER,[2] AND HAROLD BAER[2]

One of the most fascinating aspects of the encephalitides is their regular seasonal appearance and disappearance. If the viruses are resident during the winter, a most logical suspect would be *Culex tarsalis,* the summer vector, acting as both vector and reservoir. In the laboratory encephalitis viruses are retained throughout life by this mosquito which hibernates as the adult female. Under laboratory conditions they live at least three months, or long enough to survive a winter and transmit virus. However, wild *Culex tarsalis* may have sufficiently different habits so that this phenomenon does not occur under actual field conditions in the Greeley, Colorado study area. The impact of natural conditions upon wild *tarsalis* is evident in the diapause or cessation of blood engorgement in fall broods. The prerequisites of *Culex tarsalis* for hibernation were investigated to determine whether this species does provide a mechanism for over-wintering viruses in nature.

It was apparent from mosquito collections made in 1954 that blood engorgement dropped rapidly in late summer, that fat bodies developed much later and that some fat bodies were developed with no blood meals. The investigation reported here was part of an ecologic study of population dynamics in relation to virus isolations and host preferences. It was carried out in 1955 by collecting weekly segments of a resting site population to find out how long these mosquitoes live, how fat bodies are developed, and to determine the extent to which re-engorgement takes place.

[1] United States Department of Health, Education and Welfare, Public Health Service, Bureau of State Services, Communicable Disease Center, Atlanta, Georgia.

[2] United States Department of Health, Education and Welfare, Public Health Service, Bureau of State Services, Communicable Disease Center, Encephalitis Investigations Unit, Greeley, Colorado.

METHODS. A natural resting site was chosen for this investigation because of an apparent species preference (Brookman, 1950; Mortensen, 1953; Loomis and Green 1954). This site had been a consistently good collecting site throughout previous summers. It was the east end of an old bridge across the St. Vrain River near Platteville, Colorado. Each Monday morning the mosquitoes were collected from the end of the bridge to the first row of supports. When the population numbered in thousands, collections were made of one-half, one-quarter, or one-eighth of the area and estimates of total numbers were made.

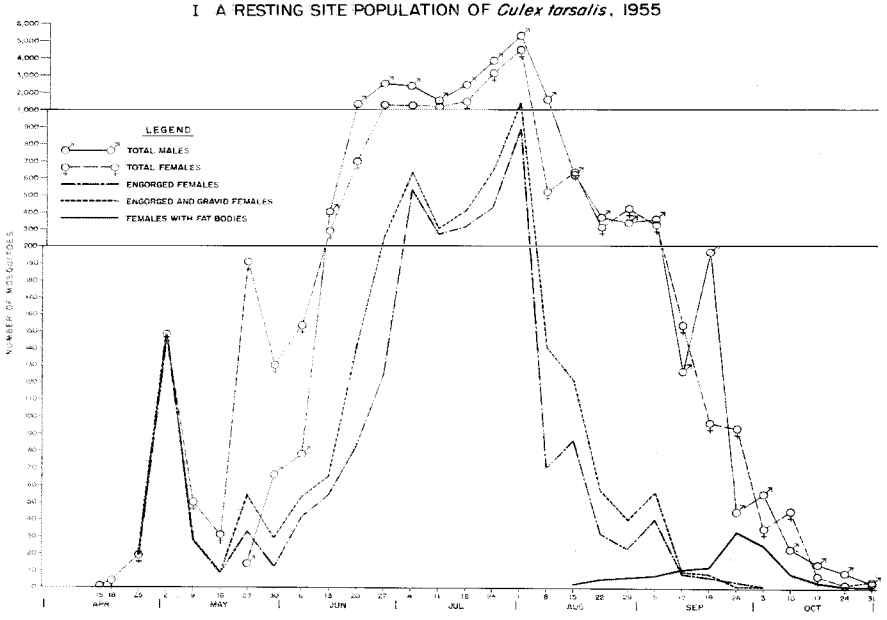
The collections were made with a large, reservoir type aspirator which caused little damage to the mosquitoes as they were carefully blown into pint-size ice cream cartons, and covered with wet towels. Upon return to the laboratory, they were lightly anesthetized with ether and carefully separated into five groups: males and blood engorged, gravid, fat, or deplete females. Numbers in each group as well as total numbers were recorded. The females presumed to be newly emerged had well defined white and dark markings which appeared in sharp contrast to the rubbed and faded appearance of older specimens. The other mosquitoes were separated as to their engorgement, gravid or fat status by the size and color of the abdomen. Fat bodies were identified by Sudan III stains.

From each weekly collection 200 females and 100 males were randomly selected, their categories recorded, and then they were allowed to revive in small cages so that any which were unable to fly could be replaced. These selections were placed in separate 18″ x 18″ x 18″ cages which were built in batteries, of wood and wire screen. These cages were constructed be-

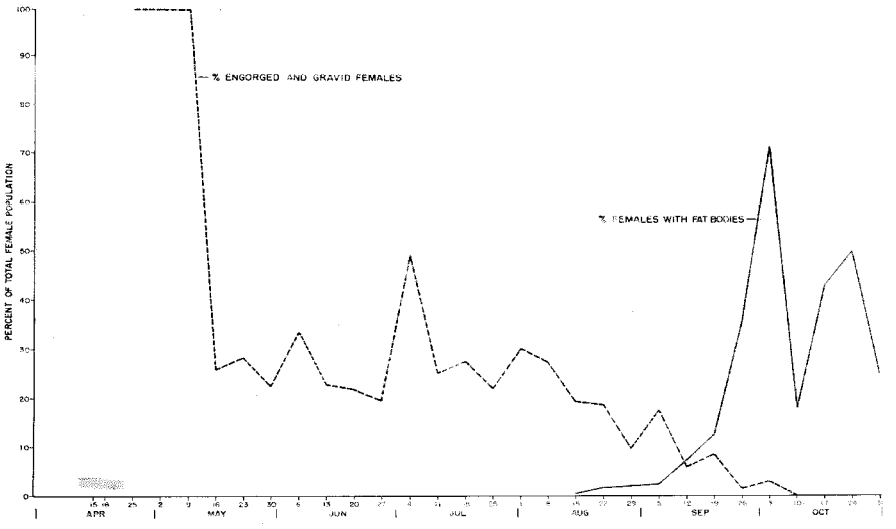
neath a small shaded bridge over permanent water which had proven to be an excellent natural resting place. Each was provided with an 8" x 10" pan of water and with finger bowls containing carbohydrate in the form of 10 percent sugar soaked pads. Each cage was labeled by weeks of collection from April 15 to October 10, was checked daily for egg rafts, was checked regularly for sugar solution and was provided one night each week until September 20 with an immobilized pigeon. A special row of cages was not supplied with a blood source or sugar. Three cages were used for emergence of wild larvae. The egg rafts were collected to determine the extent of reengorgement.

OBSERVATIONS AND RESULTS. The records of the resting place collections when plotted (see graph I) showed interesting changes in the mosquito population as the season progressed. As would be expected, there was an early peak of emerging hibernators in May and another much greater peak in the female population in midsummer which dropped very rapidly in late summer. The curve indicating the numbers of engorged females in this population was almost identical with the early peak of the total female population but dropped off steadily to a peak lower than, but occurring at the same time as, the second peak of total female population. Then, about August 1 there was a sudden dramatic drop in engorgement to very low numbers, a minor peak about September 5 followed by a continued drop to almost none by October. When expressed as a percentage of the total female population, (see graph II) the engorgement rate dropped unevenly but steadily from almost 100 percent in May to less than 10 percent when fat body development began, about August 15, and continued to drop to a zero point in the first week of October. A sudden drop in the total mosquito population of males and females followed that

I A RESTING SITE POPULATION OF *Culex tarsalis*, 1955



II A RESTING SITE POPULATION OF *Culex tarsalis*, 1955



of engorgement but lagged by 30 to 40 days.

Fat body development began very slowly about August 15, proceeded to a peak about September 25, and declined by October 30.

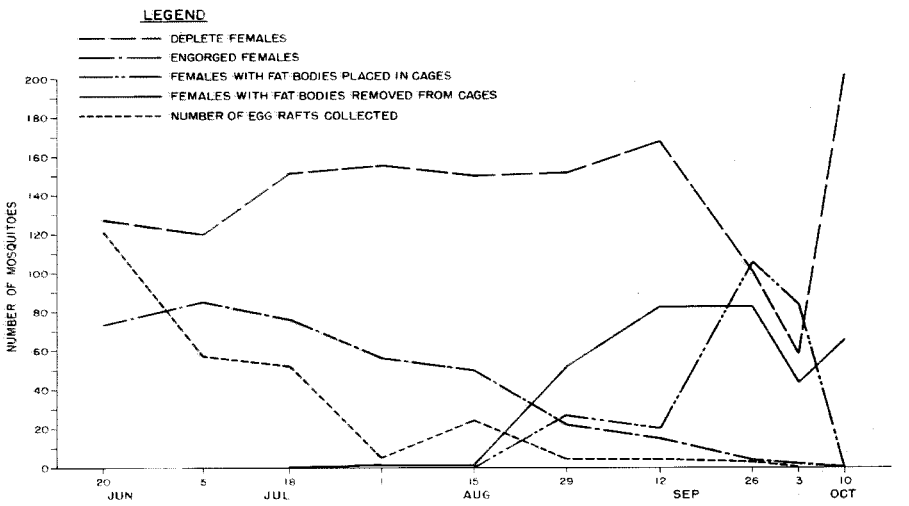
The male population began abruptly about May 22, rose quickly to remain equal to or greater than the female population except for four minor changes in September and October.

The records of the weekly population segments which were placed in cages are interesting in regard to longevity and fat body development. The recovery of egg rafts was much lower than expected and indicated that these females under the conditions imposed rarely re-engorged. Oviposition occurred at a progressively lower rate from early May to October.

The last recorded wild hibernators from the previous season were observed in the cages on May 30, June 22 and on June 30, a total of 39, 49, and 50 days in each cage. Likewise the greatest longevity recorded for females from the summer collections was 49, 66, 59, 57, 56, 53, 40, 44, 37, 70 and 71 days in each consecutive cage beginning with June; some of the later ones going into hibernation. Although some of these females lived 36–71 days at least half of them died in most cages in two weeks or less except in fall collections where longevity was increased. The greatest longevity for males was 34, 34, 31, 37, 48, 17, 23, 30 and 58 days in each cage beginning with July. The die-off time was not estimated for males but half of them were dead in less than two weeks. The high male population in late season probably indicated late emergence rather than increased longevity.

Graph III shows the composition of the weekly collections of females with respect to date, engorgement and fat body development as they were placed in each cage. The time and number of egg raft collections are indicated as well as the number of females which had developed fat bodies at the time when all of the mosquitoes were collected from each cage in October. The cages for the weekly

III DEVELOPMENT OF FAT BODIES IN CAGED *Culex tarsalis* BY WEEKS OF COLLECTION



collections were treated alike while two indicated by CHO and NO CHO, in which deplete, newly emerged females were placed, were treated differently. One cage marked NO CHO was provided only water to determine whether the fat source might be larval, the other, marked CHO was provided with 10 percent sugar in place of water in cotton pads. The females with only water died while 15 of the other group developed fat bodies with only carbohydrate.

The observations on female *tarsalis* in the cages revealed that fat body development began when the engorgement rate dropped to a very low figure and continued after no further blood engorgement could be induced in wild mosquitoes. The last blood source was offered September 20 because no blood was taken in any cage on that date. Fat body development occurred during the same time in the caged *tarsalis* as it did in mosquitoes taken from natural resting places. Prior to fat body development, many females in the cages and in resting places were observed to have engorged on some clear fluid which seemed to be a precursor of fat development. This fluid in the caged mosquitoes was the 10 percent sugar solution. Dissection revealed that fluid-filled individuals have no fat bodies or ovarian development and as they became fewer in numbers the proportion of those with fat bodies increased. All stages of development between fluid and discrete fat bodies were demonstrable.

When the mosquitoes were collected from the cages in October, more females with fat bodies were recovered from mid- and late summer groups than were originally placed in the cages while the cages with late fall collections produced somewhat fewer "fat" females than were originally introduced. Fat body development was in all cases considerably in excess of blood engorgement. Of the 100 deplete females placed in cage 20 which was never provided with a blood source, 65 were recovered with fat bodies.

DISCUSSION. Three very significant phases occurred in late season *Culex tar-*

salis populations in the Greeley, Colorado study area. They were (1) the refusal of fall broods to take blood, (2) the development of fat bodies without blood meals, and (3) the act of hibernation. The first two are prerequisite to the latter and are of most interest here. The drop in engorgement has been noted by others (Brookman 1950, Mortenson 1953) who pointed out that in California where true hibernation does not take place, there was decreased biting activity in the fall, the proportion of engorged females fell off rapidly, and many were fluid-filled. This drop in activity and blood seeking was not shared by *Culex quinquefasciatus* (Brookman 1950) or by *Culex pipiens* in the same area (Loomis and Green 1954).

Those *tarsalis* collected in late fall and winter from resting places, which would probably correspond to those truly hibernating further north, showed no evidence of blood meal or gravid condition from December through January (Mortenson 1953, Loomis and Green 1954). Brookman (1950) stated that in his winter collections the females were with few exceptions deplete and contained undeveloped ovaries. Some had fat bodies, and nearly all of them contained spermatozoa.

The observations in California on the drop in engorgement, development of fat bodies and the depletion of most winter resting females agree very well with those obtained in a more northern climate of Colorado. Some hibernation could well take place in California and not be noticed since in Colorado there is a fall dispersal at the time of fat body formation as evidenced by the collection of hundreds of females in abandoned gold mines.

The reluctance of *tarsalis* females to take blood in late summer has been at least partly responsible for early failures to colonize this species (Brookman 1950, Brennan & Harwood 1953, Lungstrom 1954) which is essentially eurygamous with a genetic stenogamic strain (Brookman 1950, Brennan & Harwood 1953). The final colony achieved by Brennan & Harwood (1953), and from which other colonies have come, seems to be stenogamic and to have other differences. Very few develop fat bodies in the fall and their ability to hibernate is questionable. They readily take blood meals in the fall and quite readily bite man in daytime. The lack of a diapause in the colony mosquitoes is mentioned by Brennan, Rush and Hubert (1954).

Wild *tarsalis* from the Greeley area, where fall broods very seldom take blood, readily develop fat bodies. They do not bite man except at dusk and hibernate successfully. It is possible that there are variants in this species as have been noted in others (Bates 1949, Richards 1941) and that differences in behavior observed in different areas may be due to selective action of extrinsic environmental factors.

The sharp drop in engorgement rate which preceded the population drop would suggest that *Culex tarsalis* is a rather unlikely overwintering host for encephalitis viruses. The sudden drop in population is a result of a very high mortality which occurs after the drop in engorgement and at a time when female mosquitoes should take the infective blood meal in order to overwinter a virus. Since individual females live at least 70 days in late season, some infected mosquitoes do without doubt live into November and December or longer in mild climates. No *tarsalis* females, however, have been found infected upon emergence from hibernation.

Some females taken in the Platteville resting place which showed only a trace of blood were dissected and found to contain a few developed ova. Since a few egg rafts were found into October, it is possible that all females taking blood oviposit (no female dissected for fat bodies had a trace of ova) and those which do not take blood—late broods—take carbohydrate and develop fat bodies to hibernate while the others probably die. Evidence for late broods is that (1) larvae were present, (2) females were predominantly unrubbed and sharply marked and (3) males which are shorted-lived usually far outnumbered the females.

From the above it seems evident that in the Greeley, Colorado area *Culex tarsalis* females from late summer and fall broods mate, take plant juice, and enter a diapause with respect to blood feeding to develop fat bodies. These females in areas of severe winters disperse to hibernate under ground, and emerge the next spring at the time of the soil temperature inversion to take their first blood meal. This habit is similar to that described for *Culiseta impatiens* and other single-brooded northern species of mosquitoes (Frohne 1953).

Frohne (1953) observed that *Culiseta impatiens, Culex territans* and *Anopheles earlei (occidentalis* of Frohne, 1953), which are many-brooded in the United States and Southern Canada, are single-brooded in Alaska and Northern Canada. In the northern climate the life cycle is characterized by refusal of newly emerging adult females to take a blood meal. They mate, take plant juice and rest (diapause) before the females enter obligatory hibernation. The biting in these species in the north is limited to emerging hibernators. It is interesting to compare *Culex tarsalis* with these species where they are many-brooded and tempting to speculate that they all impose hibernation between mating-and-fat body formation and engorgement-and-oviposition. If this is true, none of these species could be an overwintering vector or reservoir of vertebrate disease. The diapause which occurs in northern mosquitoes is entirely different from Swellengrebel's "gonotropic dissociation" in which blood is digested to form fat bodies, none being used for ovarian development (Frohne 1953).

This investigation is being continued at the Greeley Field Station to determine whether it is possible for any wild *tarsalis*, after having once taken blood and oviposited, to take carbohydrate and develop fat bodies. In two successive years large groups of engorged females have been placed in cages as previously described but have died before a fair conclusion could be drawn.

SUMMARY. A population of *Culex tarsalis* from a natural resting place near Platteville, Colorado, was studied throughout the 1955 season. Segments of this population in cages revealed that this species developed fat bodies without blood. Other observations indicate that in this species fall broods emerge, mate, take carbohydrate and the females enter a diapause to develop fat bodies. These prepared females hibernate and emerge the next spring to take their first blood meal for ovarian development. This diapause in late broods of *tarsalis* suggests that the species is a very unlikely overwintering mechanism for encephalitis viruses. The results of this study agree closely with observations recorded by other workers on the life habits of *C. tarsalis* and several northern mosquito species.

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