A SURVEY OF POTENTIAL OVERWINTERING SITES OF *CULEX TARSALIS* COQUILLETT IN MINNESOTA [1]

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INTRODUCTION. The association of *Culex tarsalis* Coquillett with the transmission of the western equine encephalitis (WEE) virus in nature has led to speculation that this virus in its more northern ranges might survive the severe winters in infected hibernating female *C. tarsalis*. Unfortunately, to date there is little literature dealing with the specific sites in which *C. tarsalis* is capable of overwintering. A recent article by Rush *et al.* (1958) nicely summarizes the previous findings; since this article should be consulted in the original by those not familiar with the subject, its content will not be dealt with in detail here. It will suffice to note that: (1) food storage cellars in western Nebraska have harbored a few *C. tarsalis* until late in April; (2) information gathered from abandoned mines in Colorado and northern Utah did not indicate that these sites were especially favorable for overwinter survival of this species; and (3) several studies in California are not entirely applicable to the more rigorous climate of the north central United States. Rush and co-workers established rock piles as good hibernation sites in Washington, but they admit that this does not answer the question for the large plains areas where such habitats are not in abundance. In a study carried out in Colorado on the relation of soil temperature inversion to *C. tarsalis* emergence from hibernation, Bennington *et al.* (1958a) reported the recovery of a few *C. tarsalis* that had successfully overwintered in wild animal burrows and in artificially constructed shafts in the ground.

Aside from the suggestions given by the above findings, the question as to specifically where *C. tarsalis* hibernates in an area such as the northern plains states is unanswered. This study of potential overwintering sites of the mosquito in Minnesota has therefore been pursued the past three winters and it represents but one aspect in a much broader investigation on the bionomics of *C. tarsalis*. In spite of the gathering evidence that this species of mosquito does not take a blood meal before entering hibernation and hence would not harbor the WEE virus over the winter months (Bennington *et al.*, 1958b) and in spite of the inability of the present authors to observe any habitats containing successfully overwintering *C. tarsalis*, the information reported here offers data on the overwintering of mosquitoes in a geographical area for which very little is known. Furthermore, these findings tend, at least for the Minnesota region, to disagree with a statement, in a review by Jenkins (1950), that female *C. tarsalis* overwinter in protected places such as buildings, caves, and cellars.

The only previous reports of collections of hibernating mosquitoes in Minnesota are those of Owen (1937) relating the recovery of *Anopheles punctipennis* and *C. pipiens* in mid and late winter from artificial sandstone caves in the St. Paul area and of Wallace (1943) relating the capture and colonization of *C. pipiens* from a similar habitat. Owen also found *A. earlei* and *A. quadrimaculatus* in caves in the late fall, but none survived the very severe winter that followed.

METHODS. Beginning in October, 1957, excursions were made to various representative areas in and adjacent to Minnesota, with care being taken to choose regions most likely to have a sufficient population of *C. tarsalis* to give the best chance of discovery of hibernating female

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mosquitoes. A total of 27 artificial and natural caves as well as 35 other types of shelters consisting of empty outbuildings, chicken houses, rock piles, mushroom houses, log piles, vacant houses, potato houses, pig houses, and root cellars was inspected for their mosquito content. These 35 miscellaneous structures were at or near St. Paul, St. Peter, or Moorhead, Minnesota. Observations were made in all of these potential habitats at some time between the first of October and the middle of April.

Caves 1–6 (see Table 1) were all small artificial sandstone caves located in the Minnesota River valley near St. Peter, Minn. It might be noted at this point that the authors have arbitrarily classified as small caves those whose depth does not exceed 50 feet and whose volume is not greater than 2,500 cubic feet, as medium caves those whose depth lies between 50 and 250 feet with volumes not exceeding 25,000 cubic feet, as large caves those whose depths are variable but whose volumes are 25,000 to 100,000 cubic feet, and as very large caves those which contain at least a mile or more of tunnels and have a volume in excess of 100,000 cubic feet.

Caves 7–11 were situated along the St. Croix River in the Stillwater area; all were artificial sandstone caves, the first four being of medium size and the fifth being a very large, many-chambered commercial cave. Caves 12–18 were artificial sandstone caves, several of them in commercial use, in the St. Paul area. Caves 13 and 15 were small; cave 16 medium; caves 12 and 14 large. No estimates were made of 17 and 18. Cave 19, near Harmony, Minn., was a very large, well-maintained commercial natural limestone cave with several miles of passageways. Caves 20 and 21 were also natural limestone caves in the southeastern part of Minnesota; the former was only a large cavity in the hill adjacent to the much larger cave 21. The latter has an explored depth of well over two miles and is known as Mystery Cave. The remaining six were artificial sandstone caves. That at Ninninger, Minn., number 22, was medium-sized. Caves 23 and 24 were at Diamond Bluff, Wisconsin. The former was a large cave excavated for storage and supplied with artificial heat and the latter an unheated medium-sized cave higher up the same hill. Caves 25–27 were all very large and complex caves used as "sand mines." They invariably had several miles of spacious passageways.

The number of field workers varied from one to five and the time devoted to collecting or counting mosquitoes ranged from a few minutes to an hour (occasionally an hour and a half or longer). Since the emphasis was on numbers of species and individuals present at each visit, sufficient time was spent at each habitat to insure that most of the visible mosquitoes were counted and all or a portion of them were collected. The effort applied can understandably be correlated with the size of the cave and the numbers of mosquitoes present. To claim that all mosquitoes were accounted for would be hazardous, but it is believed that probably very few, if any, *C. tarsalis* were overlooked in the course of this survey. Searching was done by flashlight; all collected specimens were gently aspirated and placed in specially prepared ice cream cartons for transport alive to the laboratory where specific identification was made. Certain of the habitats were re-visited at intervals after the first inspection, both during the same winter as well as in the succeeding one or two winters.

In order to obtain information relative to the environmental conditions which prevailed in the various habitats, the temperature and often the relative humidity were recorded at the time of each visit. Also, a recording thermometer was placed in cave 2 at St. Peter and this provided a continuous reading of temperature over a period of 18 months.

Results. In an effort to find hibernating *C. tarsalis*, 103 visits were made to 27 different caves. A summary of the findings from each cave is given in Table 1. The dimensions stated for each cave are for length, width, and height, respectively. The date denotes the specific winter and

TABLE 1.—Mosquito species observed in caves of the Minnesota region during fall and winter

Cave	Size (in feet)	Winter and inspections	*Culex tarsalis*	*Culex pipiens*	*Anopheles* species	Other	Total
1	18 x 13 x 5	1957–58 (4)	–	7	26	–	33
		1958–59 (3)	–	19	11	–	30
		1959–60 (1)	–	15	33	1 [a]	49
2	21 x 12 x 7	1957–58 (4)	–	9	36	–	45
		1958–59 (3)	–	241	48	–	289 [b]
		1959–60 (1)	–	75	12	1 [c]	88
3	27 x 11 x 8	1957–58 (4)	7	26	79	–	112
		1958–59 (3)	1	168	18	–	187
		1959–60 (1)	4	368	57	–	429
4	15 x 6 x 8	1957–58 (4)	1	7	36	–	44
		1958–59 (3)	3	55	89	–	147
		1959–60 (1)	1	199	60	–	260
5	22 x 10 x 6	1957–58 (4)	–	25	25	–	50
		1958–59 (3)	1	7	20	–	28
		1959–60 (1)	2	170	134 [d]	–	306
6	34 x 12 x 12	1957–58 (4)	1	5	20	1 [a]	27
		1958–59 (3)	1	238	105	–	344
		1959–60 (1)	2	161	110	–	273
7	50 x 14 x 12	1957–58 (5)	–	209	–	–	209
		1958–59 (1)	–	56	–	–	56
		1959–60 (1)	–	9	–	–	9
8	50 x 16 x 15	1957–58 (6)	–	710	4	1 [a]	715
		1958–59 (2)	–	77	1	–	78
		1959–60 (1)	–	130	1	–	131 [b]
9	104 x 14 x 12	1957–58 (5)	–	978	18	–	996
		1958–59 (2)	–	58	2	–	60
10	234 x 8 x 10	1957–58 (7)	–	1707	4	–	1711
		1958–59 (1)	–	320	–	–	320
11	Very large	1957–58 (1)	–	13	–	–	13
12	90 x 40 x 15	1957–58 (2)	–	201	–	–	201
13	15 x 3 x 6	1957–58 (2)	–	103	1	–	104
14	Large	1957–58 (2)	–	–	–	–	0
15	12 x 12 x 8	1957–58 (2)	–	55	–	–	55
16	110 x 14 x 12	1957–58 (2)	–	164	–	–	164
17	No estimate	1957–58 (1)	–	1	–	–	1
18	No estimate	1957–58 (1)	–	–	–	–	0
19	Very large	1957–58 (1)	–	–	–	–	0
20	80 x 30 x 20	1957–58 (1)	–	4	1	–	5
21	Very large	1957–58 (1)	–	–	9	–	9
22	100 x 14 x 8	1957–58 (1)	–	51	–	–	51
23	1500 x 10 x 8	1957–58 (2)	–	8	30	–	38
24	250 x 8 x 9	1957–58 (2)	–	79	8	–	87
25	Very large	1957–58 (1)	–	–	–	–	0
26	Very large	1957–58 (1)	–	18	29	–	47
27	Very large	1957–58 (1)	–	2	–	–	2
Total		103	24	6748	1027	4	7803

[a] *Culex territans.*
[b] An additional 400–600 mosquitoes estimated here—none *C. tarsalis.*
[c] *Uranotaenia sapphirina.*
[d] Includes one *A. quadrimaculatus.*

the number in parentheses the visits to the cave during that period; the figure given for each mosquito count represents the largest population of a species known to have been present that season. It is recognized that at times it is difficult to distinguish with certainty between *C. pipiens* and *C. restuans*. Of the 6,748 specimens stated to be *C. pipiens* in Table 1, approximately half were confirmed microscopically in the laboratory as being this species. On the strength of this, the black-legged *pipiens*-like mosquitoes which were left in the caves have been regarded as *C. pipiens* in the accompanying tabulation. Although three species of *Anopheles* were found, only one of the 1,027 specimens was *A. quadrimaculatus*. The remainder consisted of *A. punctipennis* and *A. earlei*, the former consistently outnumbering the latter by a ratio of at least 10:1. In the interest of simplicity, no distinction between these two species has been made in the table.

In the three years of this study, almost 8,000 mosquitoes were observed in caves. Of this number, only 24 were *C. tarsalis*. Furthermore, this species was found solely in four caves situated near St. Peter and the latest date of occurrence for each year was Nov. 14, 1957, Nov. 28, 1958, and Oct. 29, 1959. In these and in a number of other caves successfully overwintering populations of *C. pipiens, A. earlei*, and *A. punctipennis* were present through the period of January to April.

Temperature data derived from the thermograph in cave 2 showed that the temperature reached its lowest point (34–36° F.) during the January and February period and then gradually rose to its highest point (59–63° F.) during the July to September period. Additional observations made in other caves substantiated the fact that temperatures in caves in which *C. pipiens* and *Anopheles* species overwintered never dropped below freezing, but at midwinter generally remained between 34° and 42° F. Several caves, such as numbers 15 and 16 in St. Paul, were open and exposed; in these, even though the initial *C. pipiens* population in late October numbered 55 and 164, respectively, none was alive in January when temperatures within had fallen to 22–25° F.

The walls of most caves are quite wet, often having water droplets on them and at times actively dripping water. Pools of water on the floors are not uncommon. The relative humidity appears to be near the saturation point. However, in this study, measurements using a sling psychrometer, a dew-point apparatus, and/or an electric hygrometer indicated relative humidities lower than expected. These readings ranged from 45–92 percent during the November through March period, with an unexpectedly high number of readings in the lower half of this range.

Only 4 of the 35 miscellaneous structures examined for mosquitoes yielded any specimens. These were as follows: (1) 123 *C. pipiens* from a mushroom house in St. Paul on Oct. 24, 1957; this building, however, is pasteurized every three months and would not be suitable for overwinter survival; (2) 100 *C. pipiens* from the basement of an occupied house in Minneapolis, Jan. 23, 1958; (3) 4 *A. punctipennis* from an abandoned log house at Bay City, Wisc., Jan. 28, 1958, and (4) 1 *A. punctipennis* in an outcrop on the side of a hill near Diamond Bluff, Wisc., on Oct. 26, 1957.

DISCUSSION. While it is apparent from the three-year study just completed that many Minnesota caves are suited to the overwintering of mosquitoes, especially *C. pipiens, A. punctipennis*, and *A. earlei*, it is equally obvious that *C. tarsalis* did not select such sites for hibernation. Only 24 of 7,803 mosquitoes encountered in caves were *C. tarsalis* and all of these were limited to a series of small sandstone caves near St. Peter. Furthermore, no specimens of *C. tarsalis* were found in these caves later than November 28. This would seem to indicate that, although a small number of this species may enter caves in this area each fall, these habitats do not offer them a successful overwintering site. However, it should be pointed out that these same caves are suitable hibernation sites for other species. Appreciable numbers of *C. pipiens,* an important vector of the St. Louis encephalitis virus, along with lesser numbers of *A. punctipennis* and *A. earlei* were observed to be alive on dates from the middle of January to the middle of April. This substantiates the observations of other workers who have found that the overwintering requirements of *C. pipiens* and certain *Anopheles* species seem to differ from those of *C. tarsalis.* When one considers the relative paucity of caves in southern and western areas of the state where *C. tarsalis* reaches its highest populations, this result is not too surprising.

From an inspection of Table 1, it is apparent that there was a disproportionately low number of *C. tarsalis* in comparison with the other species. This is especially evident when one considers the relative abundance of these species in summer light trap collections. In the St. Paul-Stillwater area, *C. tarsalis* is exceeded only by *Aedes vexans* in number while *C. pipiens* and *Anopheles* species are distinctly lower in rank. All other things being equal, if caves were the favored hibernation site of *C. tarsalis,* one might expect to find large numbers of this species in such situations. Since no *C. tarsalis* were found in caves except in the St. Peter area and since they occurred there only in limited numbers and only up to late November, one may conclude that, in Minnesota, caves are not hibernation sites for this species.

Keener (1952) reported the overwinter survival of some *C. tarsalis* in fruit cellars in western Nebraska and Dow *et al.* (1956) referred to unpublished observations made by Smith in similar habitats in Montana. Support for this type of habitat as being important in Minnesota is lacking, since an examination of structures such as root cellars, outbuildings, chicken houses, and potato houses failed to reveal any *C. tarsalis.* In Washington, rock piles have been established as potential overwintering sites (Rush *et al.,* 1958). This has not been confirmed in Minnesota, due to negative findings in the few rock piles investigated to date and to the scarcity of such a habitat type.

The possibility still exists that *C. tarsalis* can hibernate in such relatively inaccessible sites as rodent burrows. The results of Bennington *et al.* (1958a) in Colorado tend to support this possibility. Unfortunately, at the present time insufficient evidence is available for the Minnesota area. If indeed the female of this species does hibernate in Minnesota, it can be said rather definitely that some naturally occurring habitat, as yet uninvestigated, must offer the optimum conditions necessary for overwintering. In this study, caves and a variety of man-made structures have so consistently given negative results for *C. tarsalis,* and at the same time positive findings for other mosquito species, that it is the opinion of the authors that in Minnesota such habitats only rarely afford overwintering protection for *C. tarsalis.*

SUMMARY. In a three-year survey of potential overwintering sites of *C. tarsalis* in the Minnesota area, 103 visits to 27 different caves during the fall and winter months yielded a total of 7,803 mosquitoes. These consisted of 24 *C. tarsalis,* 6,748 *C. pipiens,* 3 *C. territans,* 1,026 *A. punctipennis* and *A. earlei,* 1 *A. quadrimaculatus,* and 1 *U. sapphirina.* All of the *C. tarsalis* were confined to a series of four sandstone caves in the St. Peter area and none was found later than November 28. Investigation of 35 structures other than caves resulted in the capture of only 223 *C. pipiens* and 5 *A. punctipennis.* The consistent inability to find any *C. tarsalis* in caves after November 28, coupled with the successful hibernation in these habitats of *C. pipiens,* an important vector of St. Louis encephalitis virus, and at least two other species, has led the authors to conclude that caves and man-made structures only rarely afford hibernation sites for *C. tarsalis* in the Minnesota region.

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