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# DETERMINATION OF THE IMPORTANT NATURAL POTENTIAL VECTORS OF DOG HEARTWORM IN MICHIGAN

by

Henry B. Lewandowski, Jr.

A DISSERTATION

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in partial fulfillment of the requirements

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## ABSTRACT

DETERMINATION OF THE IMPORTANT NATURAL POTENTIAL VECTORS OF DOG HEARTWORM IN MICHIGAN

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In Michigan, the dog heartworm, Dirofilaria immitis (Leidy), is now recognized as a serious pest. Cases of this disease are being reported with increased frequency. Because of a lack of field studies, little was known about the natural maintenance of this parasite in Michigan. The objectives of this study were to: 1) determine which species of mosquitoes in Michigan are attracted to dogs and are present in sufficient numbers to make them suspect as potential vectors of D. immitis; 2) determine which species may carry the parasite under natural conditions by examining field-captured mosquitoes for the presence of infective larvae; 3) determine if D. immitis develops to the infective stage in species of mosquitoes found to be the best potential natural vectors; and 4) transmit D. immitis from dog to dog to prove that Michigan strains of suspect mosquito species are capable of transmitting dog heartworm.

In 1974 and 1975 mosquitoes were collected in dog-baited and CDC miniature light traps. They were identified to species and over 43,000 were crushed in groups of 25 so that infective D. immitis could be isolated from field-captured specimens. Infective larvae of D. immitis and D. tenuis were stained for acid phosphatase activity to determine if this histochemical stain could be used to identify larvae obtained from field captured mosquitoes. Aedes stimulans, A. vexans, Anopheles

quadrimaculatus, Mansonia perturbans, and Culex pipiens were selected to study the development of D. immitis larvae in Michigan strains of these mosquito species. Transmission of dog heartworm to non-infected dogs was attempted with Aedes stimulans, A. vexans and Anopheles quadrimaculatus.

Field studies showed that Aedes cinereus, A. fitchii, A. stimulans A. triseriatus, A. trivittatus, Anopheles quadrimaculatus, A. walkeri, Culex pipiens and Mansonia perturbans were attracted to dogs and collected most frequently. These species appeared to be the best potential vectors of dog heartworm in Michigan. Laboratory studies showed that Anopheles quadrimaculatus to be a very efficient host of D. immitis larvae. A. vexans is also a suitable host while larvae complete development in Culex pipiens but this species is a very poor host. Aedes stimulans and Mansonia perturbans are unacceptable hosts of D. immitis larvae. The histochemical stain proved to have no value for the purpose of identifying infective larvae isolated from field-captured mosquitoes. Results of this study indicate that Anopheles quadrimaculatus, A. walkeri and Aedes vexans are likely to be the most important mosquitoes involved in the natural maintenance of D. immitis in Michigan

To Connie

## ACKNOWLEDGEMENTS

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# INTRODUCTION

One of the main causative agents of canine filariasis is Dirofilaria immitis (Leidy), commonly referred to as the dog heartworm. This parasite was described by Leidy in 1850 (Leidy, 1850) and is placed in the phylum Nematoda, superfamily Filarioidea.

Adult males and females live in the heart and pulmonary artery of canine hosts. Females produce active embryos called microfilariae which are found in a dog's circulatory system. Microfilariae, or first stage largae, are ingested by mosquitoes taking a blood meal from an infected dog. From the mosquito midgut, microfilariae migrate to the Malpighian tubule where they inhabit the distal cells of these excretory organs for approximately 6 or 7 days. These larvae break out of the cells to complete development in the lumen of the Malpighian tubules where the first and second molts occur. The third larval stage is infective to dogs and development to this stage requires about 12-14 days in suitable hosts. Infective larvae escape from the labium of the mosquito host while the infected insect takes a blood meal and enter the dog through the wound created by the mosquito proboscis. Apparently they are unable to penetrate the vertebrate host unless the skin is broken. Kume and Itagaki (1955) were the first to trace the development of D. immitis larvae in subcutaneous tissues of the dog. Two additional molts occur in the dog about 10 and 65 days after inoculation (Orihel, 1961). Soon

after the final molt young adults travel, via the circulatory system, to the heart and pulmonary artery of the definitive host.

Besides the usual occurence in dogs, various authors have found D. immitis in red foxes, beavers, coyotes, wolves, dingoes, gibbons, cats, seals, tigers, jaguars, sea lions and man. No human deaths have been reported due to D. immitis infections, however, and the dog appears to be the primary reservoir host.

D. immitis has a world-wide distribution and is known from 34 of the 48 continental United States. Renewed interest in this disease has been stimulated by an increased number of severe clinical cases being reported and a rapid northern spread of the infection (Otto, 1974). In Michigan, the reported incidence of dog heartworm has increased at an alarming rate.

## OBJECTIVES

Numerous researchers have studied the development of D. immitis in the laboratory or have isolated suspect larvae from some 80 species of field-captured mosquitoes. Of these, 24 are known to occur in Michigan. Because of the bio-ecological characteristics of individual mosquito species, the vectors of D. immitis must be studied on a local basis. Michigan does not have a state-wide mosquito control program and there is a threat of infection to Michigan dogs and humans. No field studies concerning dog heartworm transmission in Michigan have been reported and little is known about the natural maintenance of this parasite in the state. The lack of this basic knowledge and the increased interest in this problem were reasons to undertake this project.

The objectives of this study were to:

1) Determine which species of mosquitoes in Michigan are attracted to dogs and are present in sufficient numbers to make them suspect as potential vectors of D. immitis;
2) Determine by examining field-captured mosquitoes for the presence of infective larvae, which species may carry the parasite under natural conditions;
3) Determine if D. immitis develops to the infective stage in species of mosquitoes found to be the best potential natural vectors; and
4) Transmit D. immitis from dog to dog to prove that Michigan strains of suspect mosquito species are capable of transmitting dog heartworm.

# LITERATURE REVIEW

## Classification and Evolution

Dog heartworm, *Dirofilaria immitis*, was first found in the blood of dogs by Panthot in 1679 (Neumann and Maqueen, 1905). In 1850 Leidy (1850) described this parasite and in 1856, he named it *Filaria immitis* (Leidy, 1856). In 1911 Raillet and Henry (1911) created the genus *Dirofilaria* and *D. immitis* became the type species.

Chitwood (1969) placed this parasite in the phylum Nematoda and although the ranking of this taxon may be disputed, most authors agree that *D. immitis* is correctly classified in the superfamily Filarioidea, family Dipetalonematidae. Hawking and Worms (1961) reported that the chief attributes of filarial worms are the production of embryonated eggs or larvae by the female in the body of the vertebrate host, ingestion of the larvae by an arthropod in which two molts occur and, entry into another vertebrate while the arthropod is feeding.

Anderson (1957) contended that the Filarioidea and Spiruroidea evolved from a common ancestor which lived in the gut of its host and that this postulated ancestor established itself in the orbit where larvae were taken up by arthropods feeding on lacrymal secretions and then were transmitted to the eyes of other hosts. In the next phase of evolution Anderson suggested that adults became established in subcutaneous tissues but returned to the orbit to deposit their larvae.

Eventually adults pierced the skin to deposit their larvae in lesions which were attractive to hematophagous arthropods which ingested larvae at this site. Larvae then accumulated in the subcutaneous tissues, being accessible only to arthropods able to pierce the skin. Finally Anderson hypothesized that larvae went into the circulatory system, allowing adults to penetrate deeper into the host's tissues. It is to this stage of development that the dog heartworm has evolved.

## Vector Determination

Dog heartworm was known prior to the 1900's. It was not, however, until Manson's discovery in 1878 that Wuchereria bancrofti (Cobbold) developed in the mosquito that researchers began examining the possibility that other filarids might develop in mosquitoes. In 1900 Grassi and Noe (1900) experimentally demonstrated the development of D. immitis in the mosquito and this observation was further substantiated by Bancroft (1904). Since 1900 it has been suggested that fleas may also be vectors of dog heartworm (Breinl, 1920; Brown and Sheldon, 1940; Summers, 1943; Stueben, 1954) but in 1956 and 1957 the experimental evidence of Newton and Wright (1956, 1957) proved the flea to be the vector of another filarid, Dipetalonema reconditum (Grassi). Since the time of these publications the mosquito has been considered the sole vector of Dirofilaria immitis and the complete life cycle is now well understood.

## The Dog as a Host

Adult male and female D. immitis live in the heart and pulmonary artery (Kume and Itagaki, 1955; Otto and Bauman, 1959) where they feed

on blood (Bicknell et al., 1956). Otto (1974) reviewed the literature on heartworm in abnormal locations in the dog. These include the posterior vena cava, hepatic vein, liver trachea, esophagus, stomach, and (encysted in) the subcutaneous or intermuscular connective tissue. Generally only one worm was found in these unusual locations and rarely did these aberrant worms produce circulating microfilariae. Various authors (Bicknell et al., 1956; Crans, 1963; Otto and Jackson, 1969) have written about the affect of the parasite on the dog. Symptoms may be absent in light infections or may include coughing and quick loss of energy in moderate infections. In severe cases, dogs may be subject to dyspnoea, collapse, weight loss, ataxia, anaemia, edema of the lower limbs, enlarged heart, congestion of the lungs and liver, endocarditis, ascites, nephritis and sudden death.

Adult females may produce over 1000 microfilariae, or young embryos, each day and these circulate in the blood stream of the host. Underwood and Harwood (1939) transfused blood containing microfilariae from an infected dog to a 4-month old noninfected dog. These survived for over two years in the animal in the absence of any adult D. immitis infections. Kartman (1953c) found that transfused microfilariae were infective to Anopheles quadrimaculatus Say for a period of only 3 months after transfusion. Afterward the microfilariae seemed to lose their infectivity and failed to develop to the infective stage in the mosquito. He estimated the age of the microfilariae to be between 3 and 12 months at the time of transfusion.

## Development in the Mosquito

Microfilariae, or first stage larvae, are taken into the mosquito's midgut while the insect feeds on blood. Fewer microfilariae than expected are ingested in the amount of blood consumed (Kershaw et al., 1955), although the number ingested is quite variable. Gordon and Lumsden (1939) studied the filarid Foleyella dolichoptera Wehr and Causey in the frog Rana sphenocephala (Cope). They thought the variability in the amount of ingested microfilariae resulted either from different concentrations of microfilariae in various capillaries or whether or not blood was taken directly from a capillary or from a pool of blood formed from a broken capillary.

From the mosquito midgut, the microfilariae migrate to the Malpighian tubules. Kartman (1953b) found that this migration can occur within eight hours in susceptible mosquito host, but is mechanically inhibited by the clotting of blood in the mosquito midgut. In his experiments, twice as many microfilariae reached the Malpighian tubules of Aedes aegypti (L.) fed on blood to which an anticoagulant was added than when fed on blood without an anticoagulant. Kutz (1972) postulated that migration into the Malpighian tubules can occur within an hour after ingestion. Kartman (1953a and b) also found that in refractory mosquito hosts dead microfilariae passed to the hindgut, presumably for excretion, 48 hours after ingestion and also observed the loss of microfilariae from the anus of Anopheles quadrimaculatus Say during the act of feeding.

Taylor (1960) studied the development of D. immitis in Aedes aegypti. She reported that during the first 6 or 7 days, larval

development occurred inside the distal cells of the Malpighian tubules in "sausage" larvae. These larvae broke out of the cells to inhabit the lumen of the Malpighian tubules for the next 6 days. The first molt occurred about the tenth day of development and took place in the lumen of the Malpighian tubules. The first cast larval cuticle may not always be shed at this time. Finally Taylor noted that the second molt in this species of mosquito occurred between 13 and 17 days post infection after which infective larvae broke out of the Malpighian tubules and moved toward the head and proboscis. Burton (1963) observed infective larvae of D. immitis emerge from the antennae and palps of Aedes taeniorhynchus (Wiedemann) and Culex pipiens quinquefasciatus Say.

## Fate of the Infective larvae

While the infective mosquito feeds, the infective larvae escape from the proboscis and can be observed on the skin of the host. Hawking and Worms (1961) have cited several references for various filarids which indicate that penetration is possible only through broken skin. Emergence of the infective larvae of Brugia pahangi Buckley from Aedes togoi (Theobald) was shown to be unrelated to temperature, moisture or chemical stimuli, but appeared to be initiated by the mechanical bending of the labium (Lavoipierre and Ho, 1973). There is the possibility of spontaneous loss of infective larvae from the mosquito. Ho et al. (1974) reported significant loss of infective larvae from A. togoi deprived of a blood meal while on the other hand, Bemrick and Bemrick (1969) found no significant loss of larvae from infective Anopheles quadrimaculatus feeding on a sugar solution.

Controversy has arisen concerning the exact proboscis location from which the third stage larvae emerge. It is likely that variation exists but that the tip of the labium and the labial sheath are the usual places. Bancroft (1904) saw larvae emerge from the tip of the labium and Laviopierre (1958) also reported that this is the usual escape site. Grassi and Noe (1900) thought the bending of the labium ruptured the sheath, allowing the larvae to escape. More recently, McGreevy et al. (1974) observed larvae emerging from the tip of the labellae and the mid-portion of the labium. Occasionally, larvae continued to emerge after feeding had ended. Heavily infected mosquitoes had trouble feeding because the labium would not bend. In addition, McGreevy noted that fluid, possibly hemolymph, always escaped from the mouthparts along with the infective larvae but never escaped while noninfected mosquitoes were feeding.

## Development in the Dog

Once inside the dog the larvae molt twice before becoming mature adults. Kume and Itagaki (1955) showed that these larvae develop in the submuscular membranes, subcutaneous tissue, adipose tissue subserosa and muscles. Orihel (1961) found them in these areas during the first 80 days of development. He also noted that the first molt occurred in the dog about 9-12 days and the second molt 60-70 days after inoculation. Worms begin moving toward the heart via the circulatory system as soon as 67 days after inoculation (Kume and Itagaki, 1955). Microfilariae are not produced until 8-9 months after inoculation. No correlation has been found between the number of circulating microfilariae and the number of adult female worms (Hinman, 1935; Fowler et al., 1973).

Newton (1968) reported that a laboratory infected dog maintained the heartworm infection for over 7½ years.

## Daily and Seasonal Periodicity

Microfilariae circulating in the dog have an incomplete nocturnal periodicity. They are present in the peripheral blood at any point in a 24 hour period, but occur in greatest numbers between 6:00-12:00 P. M. Bicknell et al. (1956), among others, found a second increase in microfilaremia in the peripheral blood between 7:00-11:00 A. M. Ansari (1970) indicated that there is an active and a passive stage of periodicity. In the active stage microfilariae accumulate in the capillaries of the lungs, where oxygen is available to the larvae and conditions insure the survival of the individuals. In the passive stage, microfilariae are evenly distributed in the circulatory system and are subject to ingestion by susceptible mosquitoes. This insures survival of the species. Hawking (1956) demonstrated that the periodicity of D. immitis was related to oxygen tension. In 1967 (Hawking, 1967) he reported that under conditions of low oxygen tension (30-60 mm Hg) microfilariae were stimulated to initiate undulating movements sufficient to maintain their position is vessels less than 20 um in diameter (presumably in the lungs). No response was given when oxygen tension was higher and microfilariae were swept through the vessels. Otto (1969) has mentioned the possibility that the spleen may be important in the maintenance of periodicity, but Hawking (1962) has presented evidence to the contrary. A seasonal periodicity has also been demonstrated in which microfilariae occur less frequently in the peripheral blood during the colder months of the year (Eyles et al.,

1954; Kume, 1974; Sawyer, 1974) and Hawking (1967) suggests that daylength, in conjunction with hormonal balance may be responsible for this.

## Alternate Vertebrate Hosts

Besides dogs, D. immitis has been found in red foxes (Erickson, 1944; Stuht and Youatt, 1972), beavers (Foil and Orihel, 1975), coyotes (Gier and Ameel, 1959), wolves (Hartley, 1938; Coffin, 1944; Faust et al., 1941), dingoes (Otto, 1969), gibbons (Johnson et al., 1970), cats (Faries et al., 1974; Sharp, 1974; Donahoe, 1975), seals (Medway, 1975) and was reported from tigers, jaguars, and sea lions (Faust et al., 1941). Evidence indicates that the dog is the primary host (Otto, 1969) and that these other wild animals do not represent a substantial reservoir for the parasite. Numerous human cases also have been reported (Abadie et al., 1965; Brine et al., 1971; Moorhouse et al., 1971; Feld, 1973; and Martire et al., 1975). In humans, D. immitis tends to localize in the lungs where it becomes enclosed in a non-calcified cyst. Lesions as large as 5 cm have been reported. Symptoms may include chest pain, fever, cough, pleural thickening, and adhesions between the chest wall and lung (Feld, 1973). No deaths have been attributed to D. immitis infections in man. In only one case have circulating microfilariae been found and this case was further complicated because the patient also suffered from Lupus Erythematosis (Green, 1974). Microfilariae were found only on one occasion in the patient even though more than 50 additional blood samples were examined from this patient.

## Geographic Distribution

A near world-wide distribution for dog heartworm was reported in 1905 (Neumann and Maqueen, 1905). More recently, Kutz (1972) reported it from the United States, Europe, India, Burma, China, Japan, Australia, and various South Pacific Islands. In the United States reports are so numerous that it is impossible to discuss them individually. The most recent reported continental survey was conducted by Young (1955) (898 of 2337 questionnaires were returned by responding veterinarians). Survey results indicated that only 11 states, including 3 from which no veterinarians responded, had no diagnosed cases of heartworm (Figure 1A). Young's survey was conducted prior to the work of Newton and Wright (1956, 1957) which showed that in the United States at least 2 filarids occurred in dogs. Subsequent to Young's survey reports between 1956 and 1965 show 12 states in which D. immitis was diagnosed (Figure 1B) (Soltys, 1956; Currell, 1957; Durrer, 1957; Bailey, 1958; Wallenstein and Tibola, 1960; Healy and Kagan, 1961; Leash and Hanson, 1961; Crans, 1963; Thrasher et al., 1963; Groves and Koutz, 1964; Lillis, 1964; Schlotthauer, 1964; Mann and Bjotvedt, 1965). Between 1966 and 1976 heartworm was reported from 34 of the 48 continental states (Figure 1C) (Hirth et al., 1966; Marquardt and Fabian, 1966; Kravis, 1968; Thrasher et al., 1968; Butts, 1970; Joiner and Jardine, 1970; McGreevy et al., 1970; Zydeck et al., 1970; Mallack et al., 1971; Rabalais and Votava, 1972; Monson et al., 1973; Tritch et al., 1973; Graham, 1974; Alls et al., 1974, Jaskoski, 1974; Graham, 1975; Georgi et al., 1975; Sengbush et al., 1975).

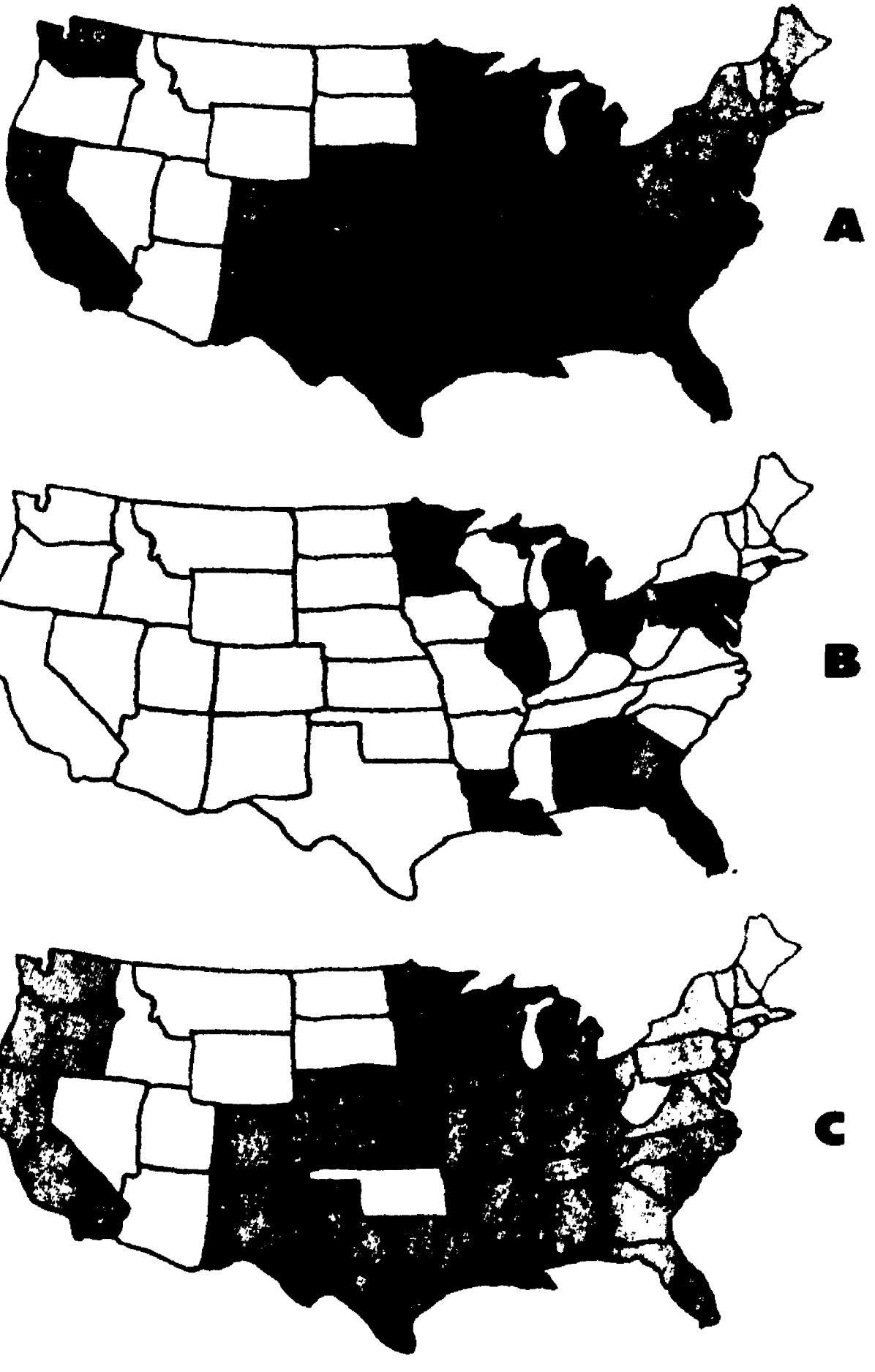
Several publications by Dr. Gilbert Otto outline the distribution of canine heartworm disease in the United States and these summarize

Fig. 1. Distribution of dog heartworm in the continental United States.

A) Results of Young's survey in 1955.

B) States reporting cases between 1956 and 1965.

C) States reporting cases between 1966 and 1976.


A
B
C

well the changing opinion on the spread of this disease. In 1949 (Otto, 1949) he wrote that the disease occurred on the Atlantic seaboard from New Jersey to Florida and around the coast to Texas but that the incidence of disease was markedly reduced inland, especially in the north. He considered the disease to be serious only in these coastal regions and said that the inland spread of the disease has not been demonstrated. In 1972 Otto (1972) reemphasized the importance of the disease along the east coast where reported infection rates were as high as 63%. In the midwest reports at that time indicated lower rates of infection but that heartworm was widespread. In 1974 (Otto, 1974) he wrote that the infection was recognized with increasing frequency in the middle Atlantic states and interest in the disease was stimulated by the increased number of severe clinical cases being reported in the northern states and the rapid northern movement of this infection which was once considered to have mainly a tropical and subtropical distribution.

## Importance in Michigan

Like so many other states, Michigan has had a rapid increase in the number of reported cases of canine heartworm disease during the past 25 years. An unpublished report by Newson and Stuht (1972, H. D. Newson, Michigan State University, personal communication) indicated that dog heartworm was present in 54 of 83 counties in Michigan. In total, from 1951 to May of 1972, 14,525 cases were reported by responding veterinarians. Forth-three and one half percent of these cases were reported from 1970 to mid-1972. Leash *et al*. (1961) screened 192 dogs at the Michigan State University Veterinary Clinic from mid-April to early August 1960. An infection rate of 2% was found. Worley (1964)

reported 5.7% of 123 dogs infected with D. immitis in southeastern Michigan. Zydeck et al. (1970) found 1.67% of 248 dogs with heartworm in Detroit, Michigan. Prouty (1972) reported infection rates of 22% of 880, 6% of 399, and 6% of 698 dogs in Belleville, Detroit, and Farmington, Michigan, respectively. An incomplete survey of veterinarians in the Lansing, Michigan area detected over 30 cases in the spring of 1974. A subsequent follow-up survey revealed an additional 83 cases reported during the same summer.

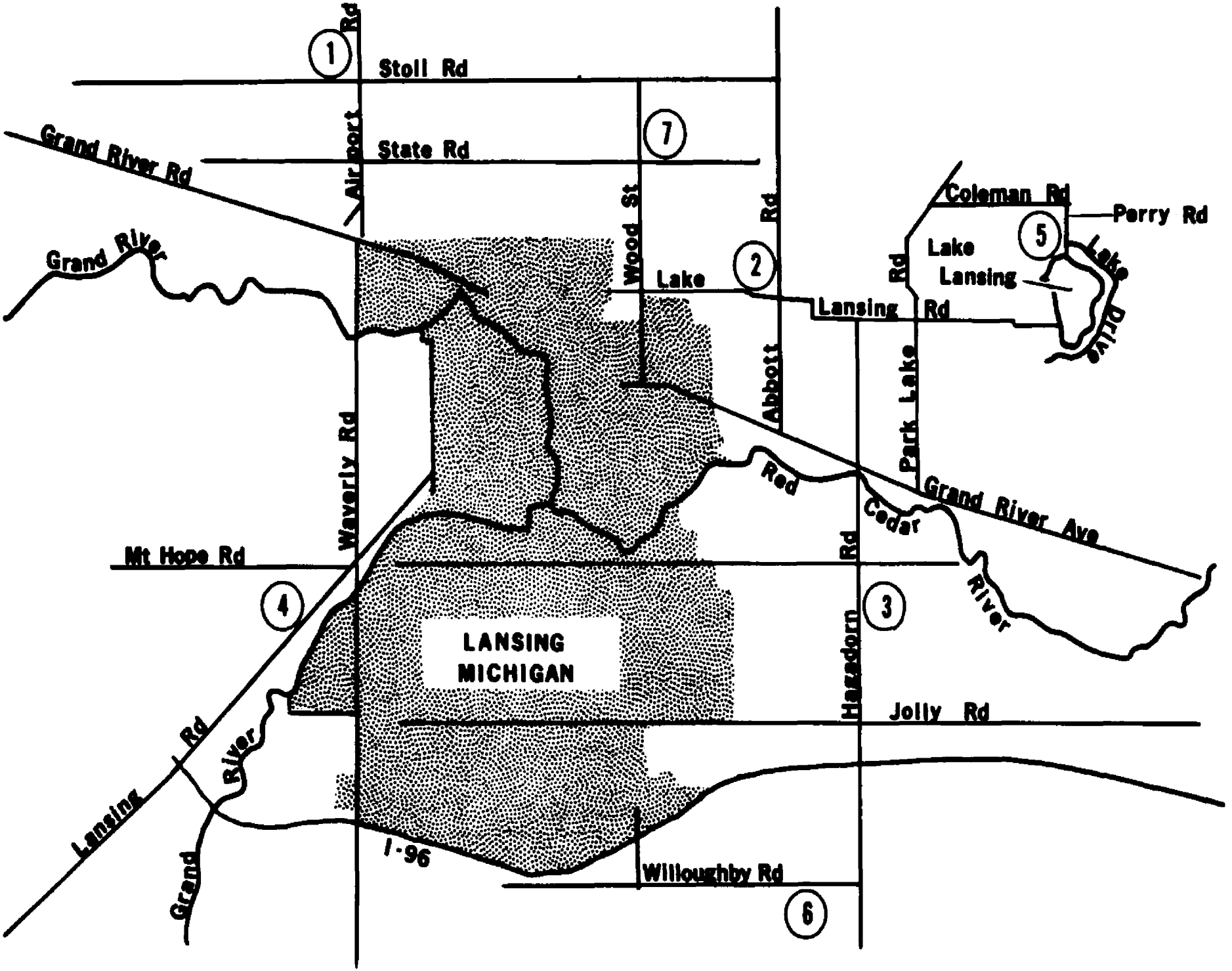
In addition to cases reported in Michigan dogs, Sharp (1974) reported that mortality in a cat was due to 7 worms in the pulmonary artery and right atrium. Stuht and Youatt (1972) found 11 of 39 red foxes examined harbored adult heartworms. These foxes were taken from the Shiawassee River State Game Area in Saginaw County. Dashiell (1961) reported a resident of Detroit, Michigan had been found, through x-ray examination, to have a nodular lesion caused by a nematode of the genus Dirofilaria. Its morphology and location in the lung suggested that it was D. immitis. The patient had visited South Carolina prior to diagnosis so it could not be proven that the infection was incurred in Michigan.

## MATERIALS AND METHODS

### Site Selection

Mosquitoes were collected at seven sites (Figure 2) in the Lansing, Michigan area in 1974, 1975, and 1976. Adult females were collected at Sites 1-5 in 1974 and 1975 and brought to the laboratory for identification and examination for the presence of infective D. immitis larvae. These sites (1-5) were selected because dogs at each of these private residences had been infected with D. immitis in the recent past and the chances of finding infective larvae in field-captured mosquitoes would presumably be increased if carrier dogs were still present in these particular areas. Collecting was done at Site 1 in 1974. Sites 2-5 were used for study in 1975 and dogs at each of these households were treated for D. immitis infections in 1974. Dogs at Sites 3 and 4 died from heartworm in 1974 and one dog at Site 2 was considered cured of heartworm in 1974 but in 1975 again developed a low microfilaremia. Because of his age and the severity of symptoms he was euthanized. In 1976, adult female and/or larval mosquitoes were collected at Sites 2, 5, 6, and 7 for use in laboratory studies. These sites were selected as the best local source of adult Aedes vexans (Meigen) (Site 2); A. vexans larvae and adult Mansonia perturbans (Walker) and Anopheles quadrimaculatus (Site 5); Aedes stimulans (Walker) larvae (Site 6); and Culex pipiens larvae (Site 7).

Fig. 2. Seven sites in the Lansing, Michigan area where adult and larval mosquitoes were collected in 1974, 1975 and 1976.


1
Stoll Rd
Airport Rd
7
State Rd
Grand River Rd
Grand River
Wood St
Rd
2
Lake
Lansing Rd
Coleman Rd
Perry Rd
5
Lake Lansing
Lake Dr
Rd
Abbott
Park Lake
Waverly Rd
Red Cedar
Grand River Ave
Mt Hope Rd
Rd
4
3
LANSING
MICHIGAN
Hagadorn
River
Jolly Rd
Lansing Rd
River
Grand
I-96
Willoughby Rd
6

## Description of the Sites

Site 1 was a kennel where 25-30 dogs were maintained. The immediate surrounding area was used for farming but nearby there also were several permanent ponds, marshy areas, a drain canal, and a large woodlot.

Site 2 was a more populated area where seven dogs were maintained. Several cases of heartworm were reported within a half mile radius of the site during 1975 and 1976. Within this area were large fallow fields and at least one woodlot. Close by was a large marshy area into which 2 drainage canals emptied.

Site 3 was mainly pastureland and included the Michigan State University horse barns. On the eastern side of the site was a pine woodlot behind which Herron Creek flowed through a marshy area.

In the immediate area around Site 4, at least 3 dogs were diagnosed with heartworm in 1974. This site was located west of the Grand River and east of a large pond. A dense woodlot between these waters flooded each spring and was subject to flooding after heavy rains.

Site 5 was located at the north tip of Lake Lansing about 250 feet from the lake. The surrounding area is marshy and at the collecting site itself, a small woodlot yielded as many as 15 different species of mosquitoes in a single night. This woodlot became flooded after heavy rains.

Site 6 was a low-lying woodlot flooded each spring by melting snow and overflow from the Mud Lake Drain. It provided an excellent source of early season snowpool mosquitoes.

Located at Site 7 were 4 sewage lagoons. Both terrestrial and emergent foliage around the periphery of the #1 pond provided enough cover for Culex pipiens to breed.

## Collection Methods

Adult mosquitoes were collected weekly or biweekly in 1974 and 1975 in the following manner. Collecting was done at night during the season for an approximate 12 hour period which included dusk and dawn. Biweekly collections were made at Sites 1, 4, and 5 (Figure 2) where both CDC miniature light traps and dog-baited traps were used. Weekly collections were made at Sites 2, and 3 (Figure 2), where only CDC miniature light traps were utilized. In 1976 adult mosquitoes were collected in CDC miniature light traps and larvae were collected with pint dippers. These adult and larval collections for laboratory studies, were made when mosquito species became available in the field.

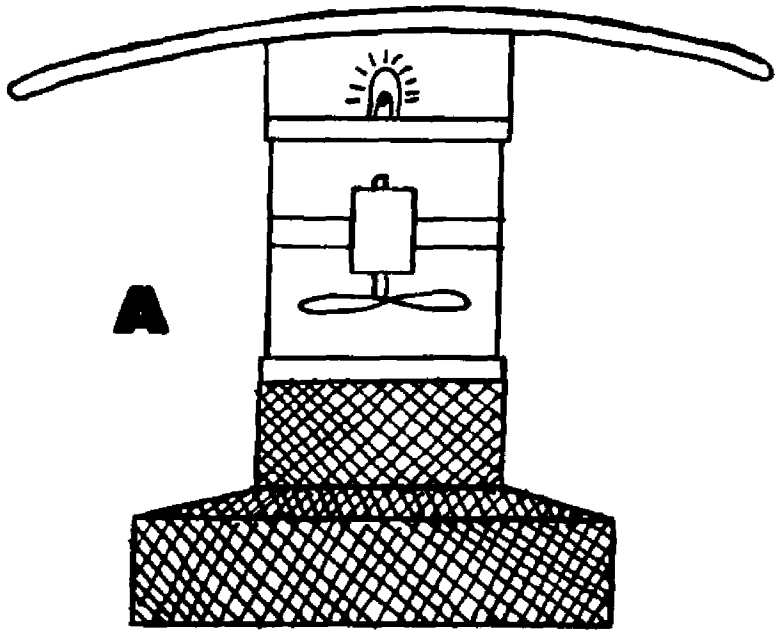
In 1974 and 1975, at Sites 1-5 (Figure 2) 3 CDC miniature light traps, baited with $CO_2$ (dry ice), were operated during all collecting periods. At Site 1 in 1974, 2 dog-baited traps (Figure 4) were used. One of these traps was placed in a large woodlot and the other placed in an open field situation. This latter dog-baited trap was relocated between the July 18 and July 24 collecting periods and placed closer to the kennels located at Site 1. It was hoped that this change would increase the catch of mosquitoes attracted to the dogs located in the immediate area. Only one dog-baited trap was used at Sites 4 and 5 in 1975.

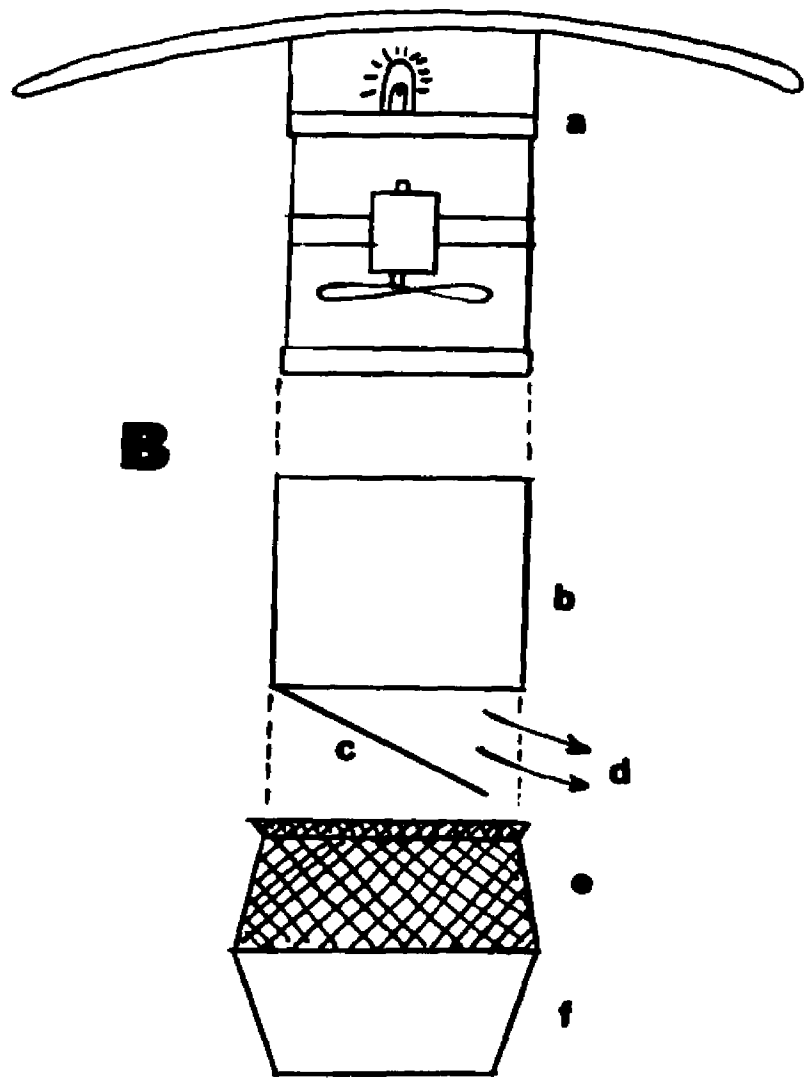
The CDC miniature light traps were modified for this study as shown in Figure 3, to increase the longevity of the captured mosquitoes.

Fig. 3. CDC miniature light trap.

A) Standard CDC trap with gauze mesh collecting bag.

B) Modified CDC trap with hardware cloth removed at (a) as suggested by Floore et al. (1971), pint ice cream container inserted at (b) with bottom (c) partially cut out to form a baffle to direct air flow (d) through the stockinette (e). Mosquitoes are held in the collecting chamber (f) which is a large ice cream container.


A


B
a
b
c
d
e
f

Herbert et al. (1972) and Miller et al. (1969) showed that $CO_2$ significantly increased the number of mosquitoes trapped so CDC miniature light traps baited with $CO_2$ were used to enhance the capture of mosquitoes needed for study in the laboratory. Although it was realized that no single trapping method is adequate for sampling mosquito populations, the CDC miniature light trap has proven its utility as a mosquito collecting device, especially where conventional AC electric power is not available. Arcuff (1976) felt the CDC miniature light trap was one of the best methods to provide a representative sample of mosquito populations. For purposes of this study, it was felt that the CDC miniature light trap was the best single collecting method available to capture high numbers of mosquitoes needed for study and at the same time provide a representative sample of mosquito species present at the collecting sites.

Dog-baited mosquito traps (Figure 4) were designed and constructed for this project. They were similar to the collapsable dog-baited trap of Villavasco and Steelman (1970) and although not collapsable, they were more portable. The louvers of the collecting boxes were modified as suggested by Bates (1949). The dog-baited traps were used to determine which mosquito species were attracted to dogs in the study areas. Although trapped mosquitoes were prevented from feeding on the dogs it was assumed their presence in the traps indicated the potential of these species to feed on dogs.

## Identification and Examination of Mosquitoes for the Presence of infective D. immitis larvae

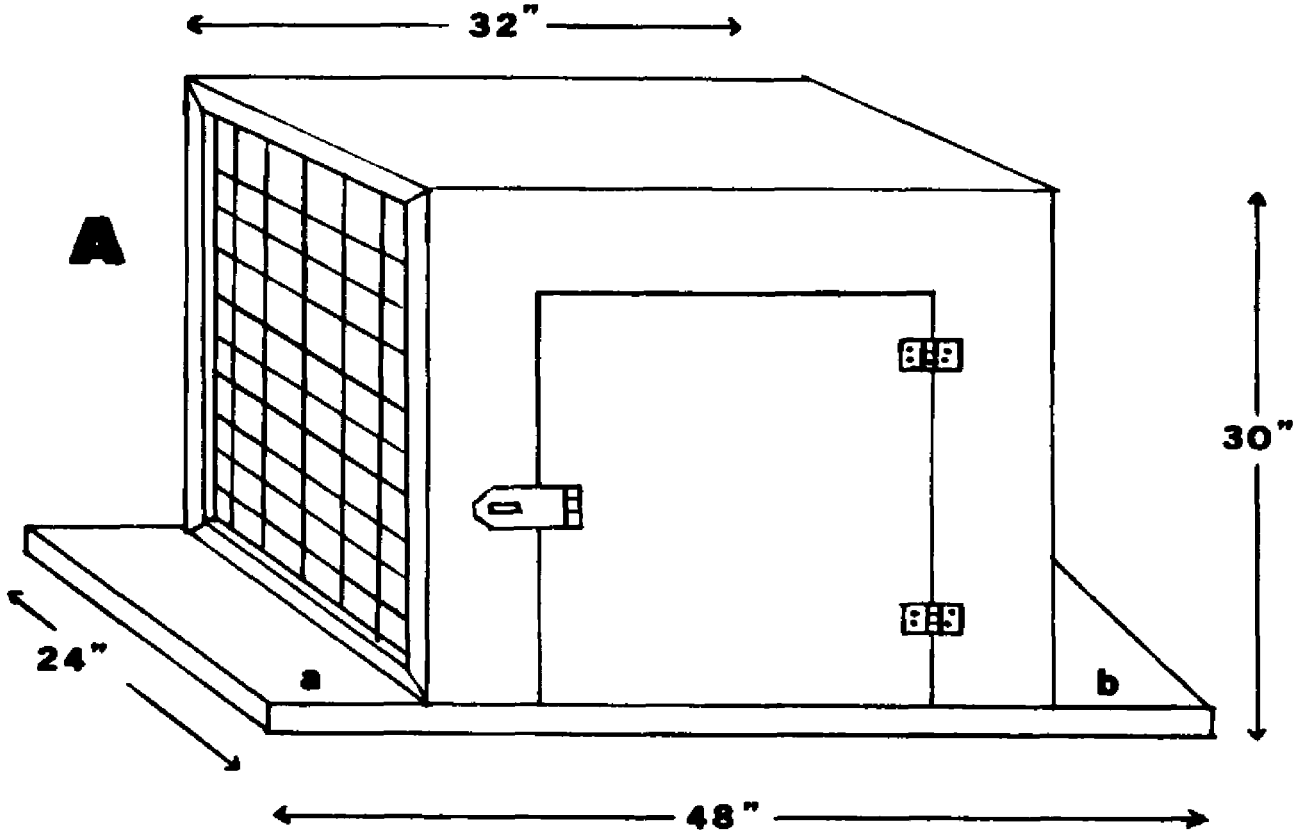
Adult mosquitoes captured in the field were brought to the laboratory in the removable end-boxes of the dog-baited traps and the

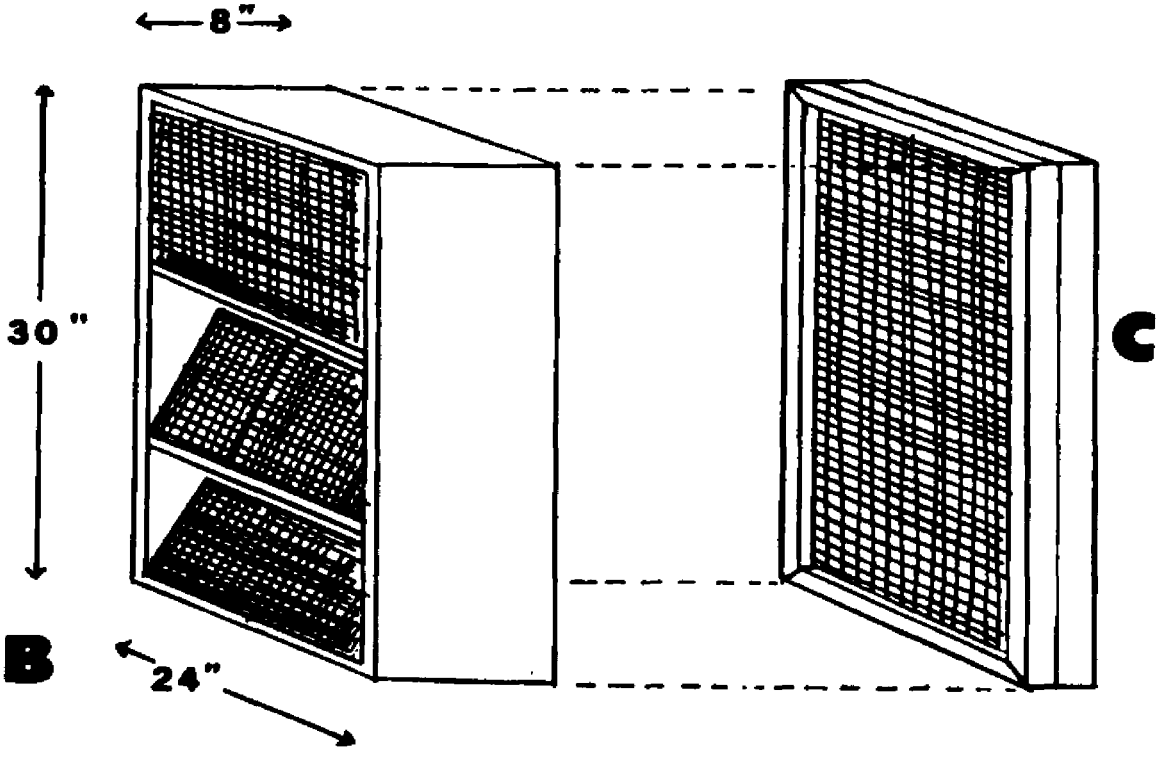
Fig. 4. Dog-baited trap.

A) Main compartment where dog is held. The wire screen is 1" hardware cloth.

B) Mosquito collecting chambers which set on the platform of the dog compartment at points (a) and (b).

C) Removable screened frame which is taken off to remove mosquitoes from the chamber or can be left off in the field to allow mosquitoes to feed on the experimental dog. The screen is aluminum window screen.


32"
A
30"
24"
a
b
48"


8"
30"
C
B
24"

collecting chambers of the light traps. These were placed in walk-in refrigeration rooms maintained at about 40° F. Mosquitoes were transferred to pint containers and kept refrigerated until they were identified and pooled during the same day the mosquitoes were brought to the laboratory. Mosquitoes were anesthetized with $CO_2$, generated from dry ice, and identified to species with the aid of a dissecting microscope. Each species was placed in pools of 25 or 30, (or less if too few had been captured) and kept in chilled containers.

Pools were examined for the presence of infective D. immitis larvae according to the method of Crans (1971). Pooled mosquitoes were crushed between two microscope slides and their remains placed in .9% saline. This mixture was placed in a plastic, disposable beverage container from which the bottom had been removed and replaced with a fine mesh gauze. It measured 7.0 cm high, 9.4 cm across the top and 3.4 cm across the bottom. This container was placed in a 60 x 35 mm crystallization dish with saline covering the gauze. Living, third stage larvae exited the mosquito bodies and fell through the gauze into the crystallization dish. After one hour, the saline with larvae and smaller mosquito parts was placed into a 60 ml separatory funnel. Here the larvae and debris were concentrated by gravitation for 30 minutes. From this funnel 5 to 6 ml of saline were drawn off and examined for third stage larvae. This technique seemed to be selective for extracting infective larvae free in the hemocoel of the mosquitoes because few mosquito parts, especially Malpighian tubules were present in the examined debris. This method was preferable to individual dissection of mosquitoes because large numbers of mosquitoes could be examined for the presence of infective larvae in relatively short time.

## Problems in Mosquito Identification

During the course of study, quick identification of living mosquito species was required. Members of two Aedes complexes were not always differentiated because the adults are, for practical purposes, indistinguishable. Aedes fitchii and A. stimulans, members of the Aedes stimulans complex (Barr, 1958) were, therefore, tabulated together in the results. Similarly, Aedes cinereus, although not a member of the A. communis complex (Barr, 1958), was confused with members of this group and was not positively identified in collections made before September of 1975. Because of this, A. cinereus was included with members of the A. communis complex in pre-September 1975 collections. A. sticticus and A. aurifer are members of the A. communis complex but these species were positively identified and tabulated separately throughout the study period.

## Differentiation of D. immitis Larvae

Infective larvae of Dirofilaris immitis cannot presently be differentiated from third stage larvae belonging to other species in this genus. Chalifaux and Hunt (1971) developed a histochemical stain which differentiated the microfilariae of Dipetalonema reconditum from Dirofilaria immitis. Larvae, presumed to be D. immitis, obtained from field-captured mosquitoes were stained by this method. These were compared with known third stage larvae of D. immitis and infective larvae of D. tenuis Chandler treated in the same manner.

Records were kept of the date, location and mosquito species from which larvae were obtained. Several other nematodes were observed in mosquitoes during the course of study and were readily distinguishable

from D. immitis larvae. The criteria used to identify larvae, assumed to be D. immitis, were size, as indicated by Taylor (1960) and Symes (1960), and shape and activity of the worms compared to observations of known specimens of D. immitis.

## Selection of Mosquitoes for Laboratory Studies

Mosquito species were selected for development and transmission studies based on 4 criteria: 1) those attracted to the dog-baited trap, 2) those most numerous in the Lansing, Michigan area based CDC miniature light trap and dog-baited trap collections, 3) those found to be harboring presumed infective D. immitis larvae in nature and, 4) those incriminated in the literature as being potential vectors of dog heartworm. Based on these 4 criteria Aedes stimulans, A. vexans, Mansonia perturbans, Anopheles quadrimaculatus and Culex pipiens were selected for laboratory study.

## Dirofilaria immitis Developmental Trials

Mosquitoes selected for study in the laboratory were given an infective blood meal. They were then held in an insectary maintained at 80° F and 80% relative humidity and dissected at various times or after their death to determine the developmental progress of D. immitis larvae.

## Obtaining the Infective Blood Meal

For D. immitis developmental studies, mosquitoes were infected by allowing them to feed directly on a Basset Hound known to be infected with dog heartworm, or through cow-gut membranes stretched over glass containers that contained infected dog blood. Blood was not warmed

during the membrane feeding trials. To obtain the infective blood meal directly from dogs or to attempt transmission, mosquito cages were placed over dogs held in a restraining chamber (Figure 5).

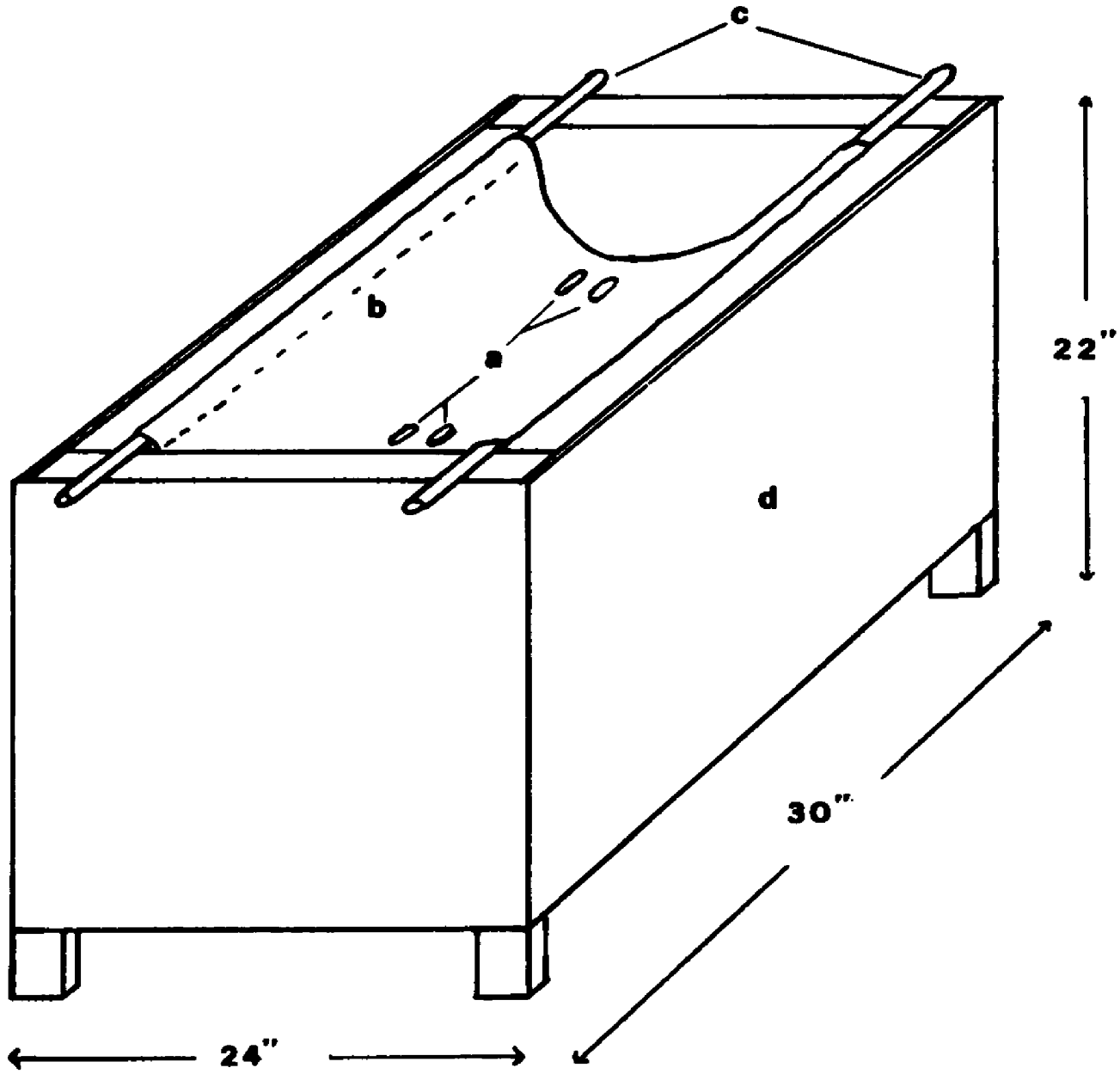
## Determination of Microfilaremia or Dilution of Infected Blood

Before mosquitoes were allowed to feed on dogs, microfilaremia was checked by the method of Seeley and Bickley (1974). A single, 3-5 ml blood sample was drawn within 1 hour prior to mosquito feeding and microfilaremia was determined within 16 hours after the time the blood sample was taken. Blood samples were refrigerated if microfilaremia determinations were delayed. Twenty µl subsamples were placed on a microscope slide, and diluted with a drop of normal saline tinted slightly with methylene blue. a 24 x 50 mm coverslip was placed on the slide and microfilariae were counted. Three to ten subsamples from each sample were examined. For membrane feeding trials, 3-5 ml of blood was drawn from the infected dog and always diluted with 12-18 ml of blood from a noninfected dog in order to reduce excessive mosquito mortality due to the high microfilaremia in the infected dog. Concentration of microfilariae was determined by the same method described above.

## Transmission Trials

For transmission studies, *Aedes stimulans*, *A. vexans* and *Anopheles quadrimaculatus* were allowed to feed on laboratory reared, parasite-free, purebred Beagle dogs obtained from a commercial supplier. A different dog was used as a recipient host for each mosquito species studied, for which transmission was attempted. Transmission attempts were made at least once daily until all mosquitoes died. Dogs were maintained for an

Fig. 5. Apparatus used to restrain dogs during mosquito feedings. Dog was strapped to canvas sling (b) with feet through holes (a) and supported by wooden dowels (c) resting on frame (d).


c
b
a
d
22"
30"
24"

appropriate amount of time after exposure to the bite of infected mosquitoes and then examined at necropsy for the presence of adult heartworms.

## RESULTS AND DISCUSSION

### Field Collections

A complete tabulation of dog-baited and CDC miniature light trap collections, is given in Appendices A-I. Data on light trap collections made at Site 1 are not presented because all mosquitoes from those collections were not identified and pooled. It was felt that those examined were not selected randomly and tabulation of these data could not be made without bias. Pertinent weather data are recorded in Appendices J-O.

The most likely vectors of dog heartworm were chosen from the dog-baited trap collections listed in Table 1. Host preferences for the mosquito species, except Culiseta impatiens (Walker), collected in these traps were reviewed by Edman (1971, 1974) and Tempelis (1975). Aedes and Anopheles prefer mammalian hosts, while Mansonia prefers mammals but readily feeds on birds. Culex pipiens L. prefers avian hosts and C. territans Walker prefers amphibians. Determination of host preferences in a given area is difficult for any particular species. Besides considering a mosquito's usual blood meal source, host availability must be considered. To illustrate, Tempelis et al. (1970) studied a population of Culex pipiens quinquefasciatus, primarily a bird-feeder, in Hawaii from which 31% had fed on dogs and large bovines when these mammals were the most abundant blood meal source in the area.

Table 1
1974 and 1975 Dog-baited Trap Collection Totals

| Species | Site 1 Open Area | Site 1 Woodland | Site 4 | Site 5 |
|---|---|---|---|---|
| **Aedes** | | | | |
| canadensis | 2 | 1 | 1 | 1 |
| cinereus | - | - | - | 46 |
| communis complex[a] | 4 | 7 | - | 11 |
| fitchii-stimulans | 9 | 51 | 2 | 9 |
| sticticus | - | - | - | 4 |
| triseriatus | 19 | 11 | 3 | - |
| trivittatus | - | - | - | 7 |
| vexans | 149 | 34 | 3 | 74 |
| **Anopheles** | | | | |
| quadrimaculatus | 92 | 5 | 4 | 51 |
| walkeri | 112 | 1 | - | 131 |
| **Culex** | | | | |
| pipiens | 168 | 184 | 1 | 9 |
| territans | - | - | - | 31 |
| **Culiseta** | | | | |
| impatiens | - | - | - | 3 |
| **Mansonia** | | | | |
| perturbans | 461 | 423 | 5 | 59 |
| TOTAL | 1,016 | 717 | 19 | 436 |

[a]Including Aedes cinereus and excluding A. aurifer and A. sticticus.

Because availability is so important, those mosquito species captured in the dog-baited traps were highly suspect as vectors of dog heartworm, especially those species captured most frequently. Only C. territans was not initially suspect as a dog heartworm vector because Tempelis (1975) wrote that this species feeds almost exclusively on amphibians. C. territans was only captured in the dog-baited trap at Site 5. During the season, many frogs were seen in the immediate collecting area and several times, frogs were found in the dog-baited trap. The preferred host of C. territans was abundant in the area. In spite of its presence in the dog-baited trap C. territans, most probably does not readily feed on dogs and is not a likely vector of D. immitis.

Table 1 shows that Aedes fitchii, A. stimulans, A. vexans, A. triseriatus (Say), Anopheles quadrimaculatus, A. walkeri Theobald, Culex pipiens and Mansonia perturbans were most abundant in dog-baited trap collections. These same species were also most abundant in CDC light trap collections (Table 2). Additionally, Aedes cinereus Meigen, A. sticticus (Meigen), and A. trivittatus Coquillett were abundant in light trap collections, and these species were also collected in the dog-baited trap at Site 5. These 11 mosquito species, because of their presence in dog-baited traps, and their abundance, indicated by CDC light trap collections, are initially the most suspect vectors of dog heartworm in the Lansing, Michigan area.

Univoltine Mosquitoes

In Michigan, A. fitchii, A. stimulans and Mansonia perturbans are thought to be univoltine as they are in Minnesota (Barr, 1958). Aedes fitchii and A. stimulans were collected until mid-August (Figure 6E)

Table 2
1975 CDC Miniature Light Trap Collection Totals

| Species | Site 2 | 3 - A | 3 - B | 4 | 5 |
|---|---|---|---|---|---|
| Aedes | | | | | |
| aurifer | - | - | - | 3 | 10 |
| canadensis | 1 | 2 | 3 | 108 | 36 |
| cinereus | - | 32 | 38 | 39 | 2,040 |
| communis complex[a] | 37 | 18 | 7 | 50 | 381 |
| dorsalis | - | 11 | 12 | 3 | 4 |
| fitchii-stimulans | 44 | 88 | 214 | 593 | 628 |
| flavescens | 1 | 49 | 1 | - | - |
| sollicitans | - | 1 | - | - | - |
| sticticus | 192 | 96 | 46 | 117 | 1,231 |
| triseriatus | 1 | 2 | 2 | 236 | 22 |
| trivittatus | 1,102 | 327 | 344 | 801 | 787 |
| vexans | 19,770 | 12,705 | 10,476 | 6,044 | 20,437 |
| Anopheles | | | | | |
| earlei | - | - | - | - | 27 |
| punctipennis | 34 | 18 | 36 | 65 | 37 |
| quadrimaculatus | 45 | 19 | 8 | 396 | 2,282 |
| walkeri | 55 | 300 | 55 | 134 | 5,391 |
| Culex | | | | | |
| erraticus | - | - | - | - | 9 |
| pipiens | 227 | 293 | 180 | 110 | 691 |
| restuans | - | - | 1 | 1 | 1 |
| salinarius | 8 | 15 | 4 | 1 | 19 |
| tarsalis | 1 | - | - | - | - |
| territans | - | - | 2 | - | 19 |
| Culiseta | | | | | |
| morsitans | - | - | - | - | 21 |
| impatiens | - | - | - | 1 | - |
| inornata | - | 1 | - | - | - |
| Mansonia | | | | | |
| perturbans | 375 | 87 | 53 | 137 | 1,024 |
| Orthopodomyia | | | | | |
| spp. | 1 | - | - | - | 2 |
| Psorophora | | | | | |
| ciliata | 2 | - | - | - | 1 |
| ferox | - | - | - | - | 1 |
| Uranotaenia | | | | | |
| sappharina | - | - | - | 14 | 3 |

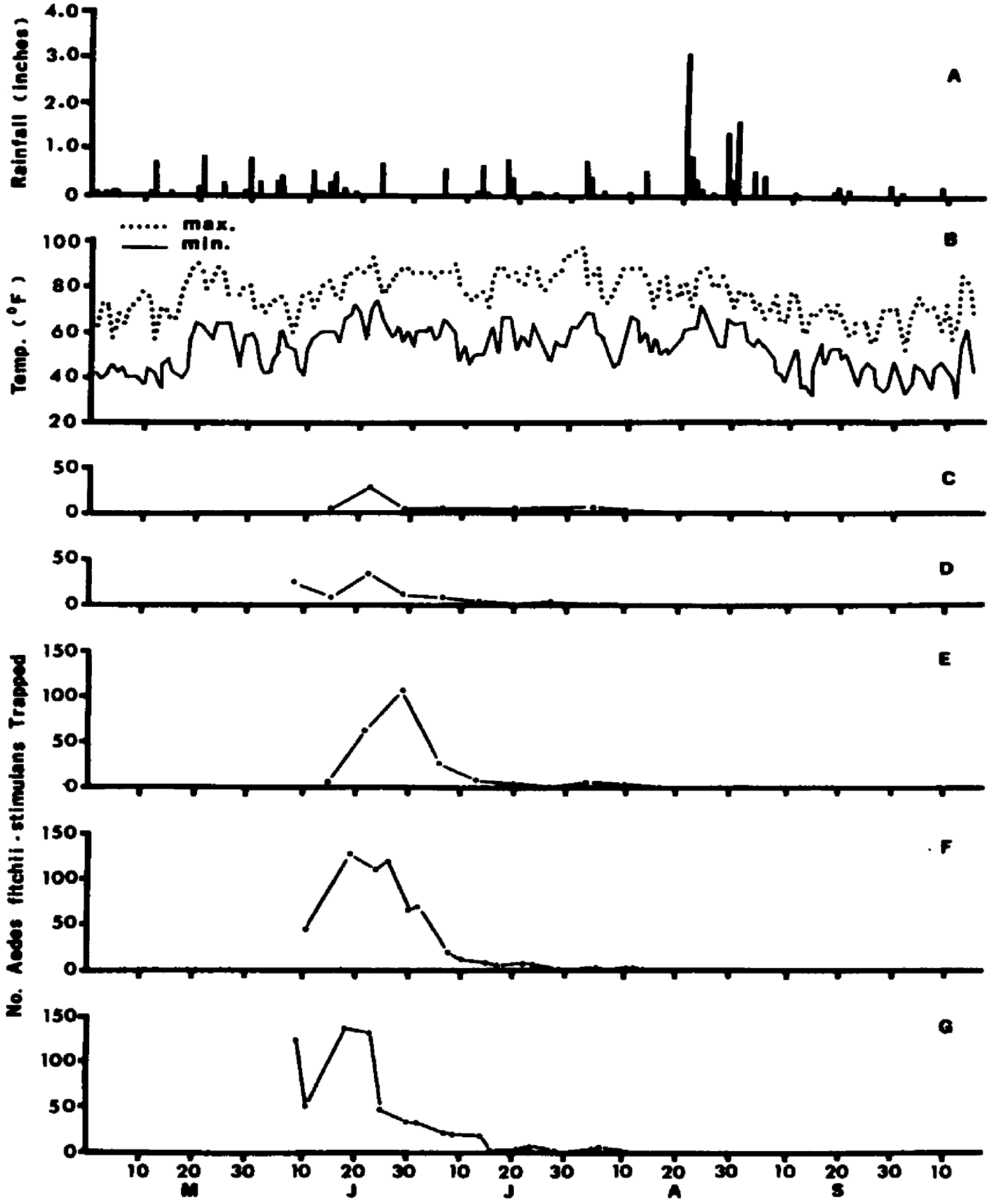
[a]Includes Aedes cinereus and excludes A. aurifer and A. sticticus.

Fig. 6. Seasonal incidence of Aedes fitchii-stimulans at several locations in the Lansing, Michigan area during June through early October, 1975. Collections made with CDC miniature light traps.

A) Rainfall in inches during the collecting period.

B) Daily maximum and minimum temperatures during the collecting period.

C-G) Collections at Sites 2, 3-A, 3-B, 4, and 5 respectively.


Rainfall (inches)
4.0
3.0
2.0
1.0
0
A
..... max.
—— min.
Temp. (°F)
100
80
60
40
20
B
50
0
C
50
0
D
No. Aedes fitchii - stimulans Trapped
150
100
50
0
E
150
100
50
0
F
150
100
50
0
G
10
20
30
10
20
30
10
20
30
10
20
30
10
20
30
10
M
J
J
A
S

indicating a potential life span of about 2 months or more under natural conditions. Collection data for these two species show them to be more abundant in their woodland habitat (Figures 6E, F, G; 8C) than in open areas (Figures 6C, D; 7C; 9C). If they are unwilling to fly out of a woodland situation their potential to transmit heartworm would be less in areas outside their preferred woodland habitat. From collection data shown in Figures 10C-G it would appear that Mansonia perturbans adults are also long-lived. They were captured from June 10 to September 10. Barr (1958), however, noted that in Minnesota, adults emerged over an extended period of time and this fact makes their longevity impossible to determine based only by their presence in trap collections. Almost equal numbers (slightly more in the open area) of M. perturbans were captured in the dog-baited traps in the open area and the woodlot at Site 1. This might indicate a willingness of this species to feed in either habitat, however, Figure 7H shows that in the open area, after peak emergence about July 18, incidence in this location falls sharply while in the woodlot trap (Figure 8H) this species was frequently captured after July 18. Collecting was done the same night at both locations. This might indicate a migration of M. perturbans into the woodlot and possibly a higher potential of heartworm transmission by this species in a woodland habitat. No experimentation was done, however, to prove that migration occurred or to determine another cause for this difference in abundance through time.

Multivoltine mosquitoes breeding in temporary or fluctuating water situations

Aedes cinereus, A. sticticus, A. triseriatus, A. trivittatus and A. vexans may produce more than one generation per year and often

Fig. 7. Important mosquito species collected in a dog-baited trap located in an open situation at Site 1 during June through August, 1974.

A) Rainfall in inches during the collecting period.

B) Daily maximum and minimum temperatures during the collecting period.

C-H) Seasonal incidence of Aedes fitchii-stimulans, A. vexans, Anopheles quadrimaculatus, A. walkeri, Culex pipiens and Mansonia perturbans.

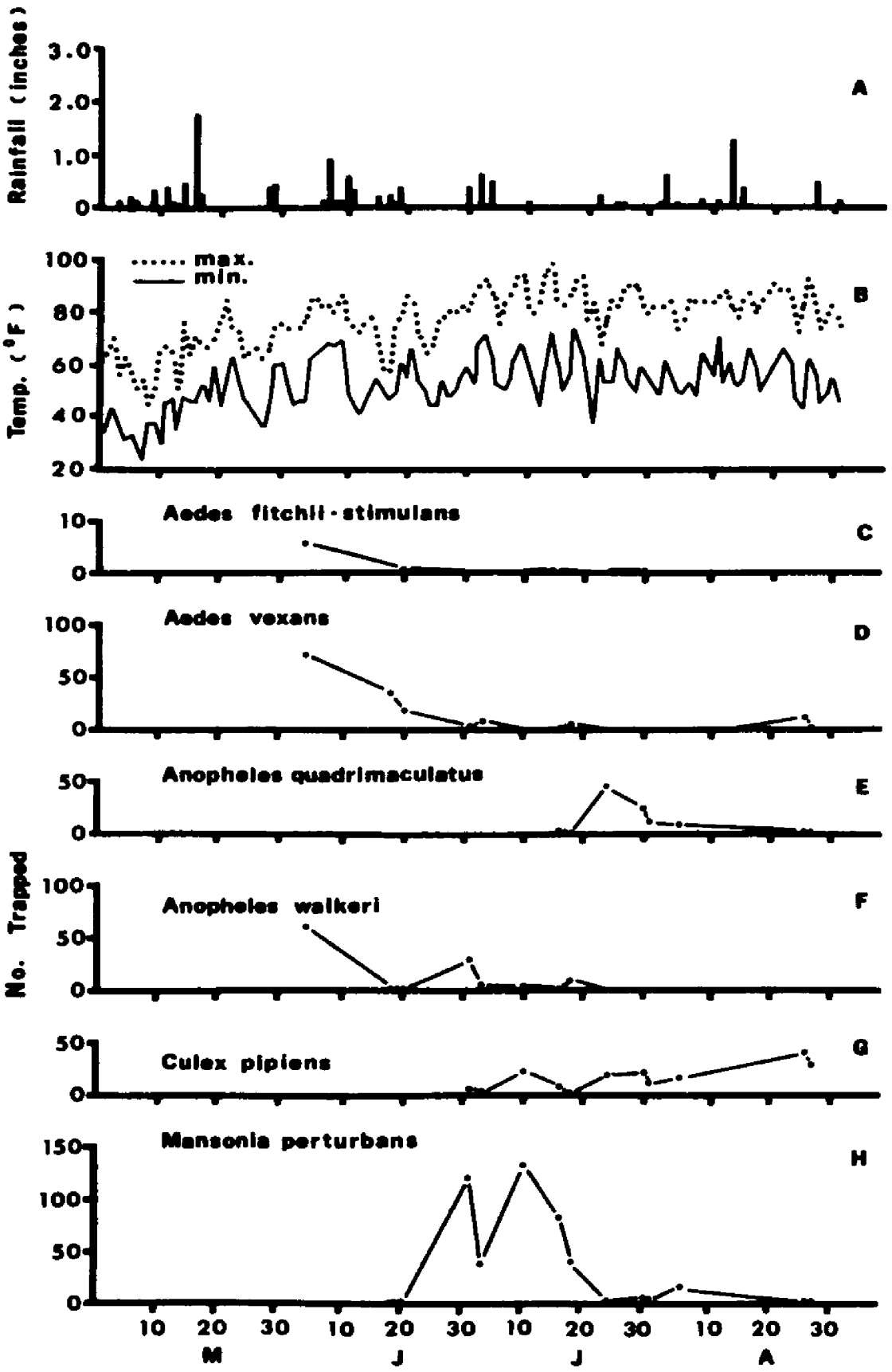

Rainfall (inches)
3.0
2.0
1.0
0
A
Temp. (°F)
100
80
60
40
20
..... max.
—— min.
B
No. Trapped
Aedes fitchii-stimulans
10
0
C
Aedes vexans
100
50
0
D
Anopheles quadrimaculatus
50
0
E
Anopheles walkeri
100
50
0
F
Culex pipiens
50
0
G
Mansonia perturbans
150
100
50
0
H
10 20 30 10 20 30 10 20 30 10 20 30
M J J A

Fig. 8. Important mosquito species collected in a dog-baited trap located in a woodland situation at Site 1 during June through August, 1974.

A) Rainfall in inches during the collecting period.

B) Daily maximum and minimum temperatures during the collecting period.

C-H) Seasonal incidence of Aedes fitchii-stimulans, A. vexans, Anopheles quadrimaculatus, A. walkeri, Culex pipiens and Mansonia perturbans.

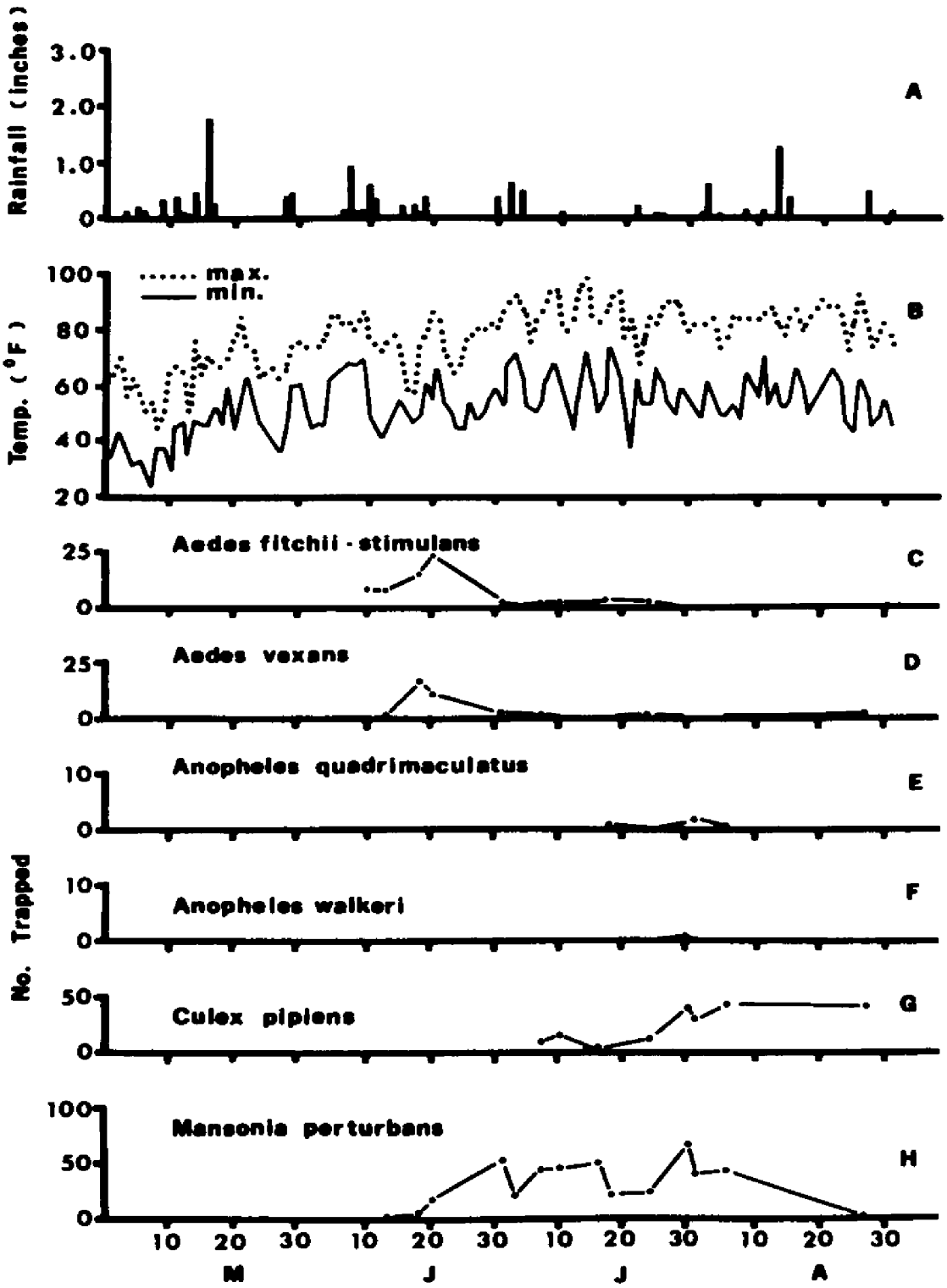

Rainfall (inches)
3.0
2.0
1.0
0
A
Temp. (°F)
100
80
60
40
20
..... max.
—— min.
B
No. Trapped
Aedes fitchii - stimulans
25
0
C
Aedes vexans
25
0
D
Anopheles quadrimaculatus
10
0
E
Anopheles walkeri
10
0
F
Culex pipiens
50
0
G
Mansonia perturbans
100
50
0
H
10 20 30 10 20 30 10 20 30 10 20 30
M J J A

Fig. 9. Important mosquito species collected in a dog-baited trap at Site 5 during June through early October, 1975.

A) Rainfall in inches during the collecting period.

B) Daily maximum and minimum temperatures during the collecting period.

C-H) Seasonal incidence of *Aedes fitchii-stimulans*, *A. vexans*, *Anopheles quadrimaculatus*, *A. walkeri*, *Culex pipiens* and *Mansonia perturbans*.

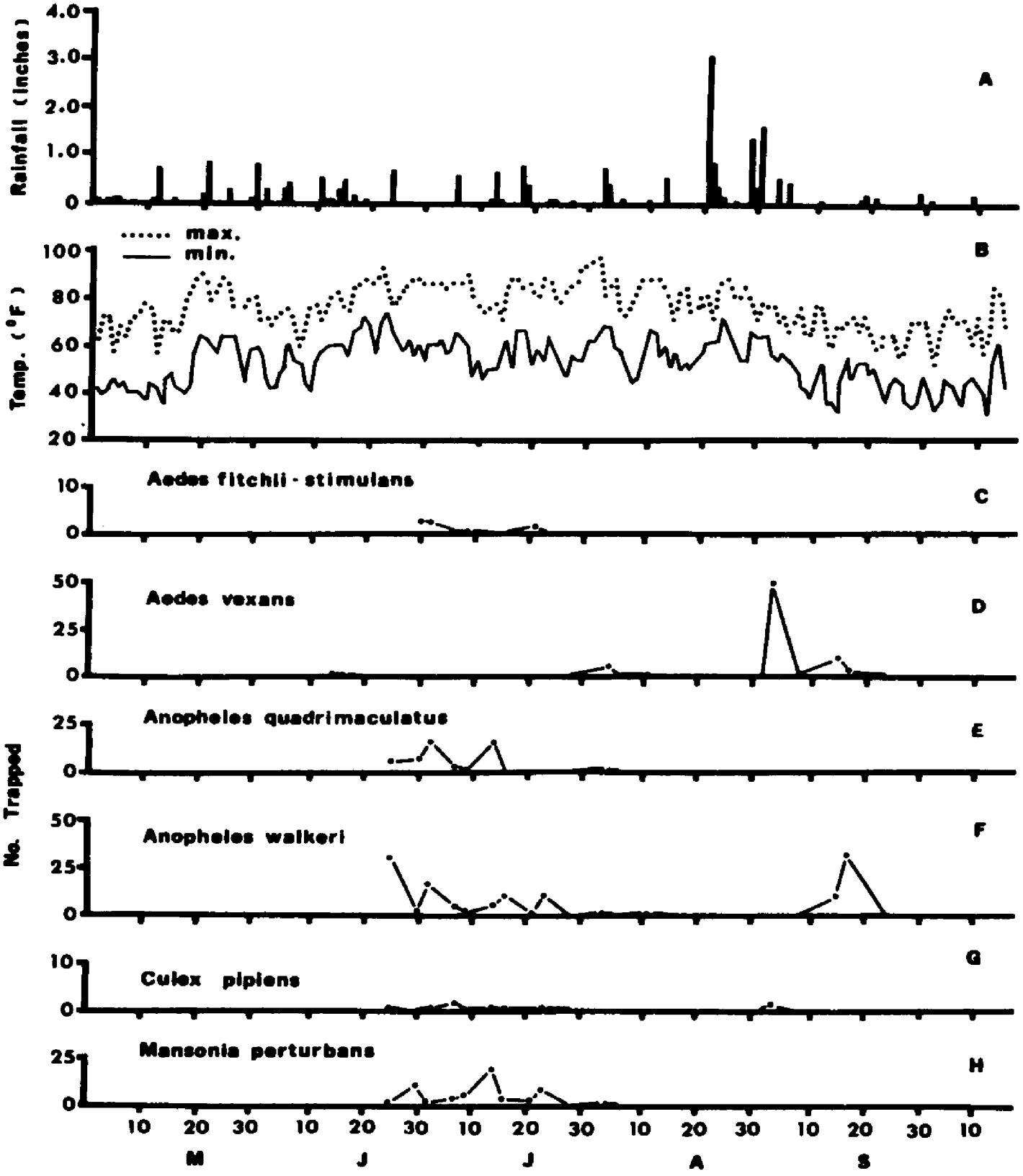
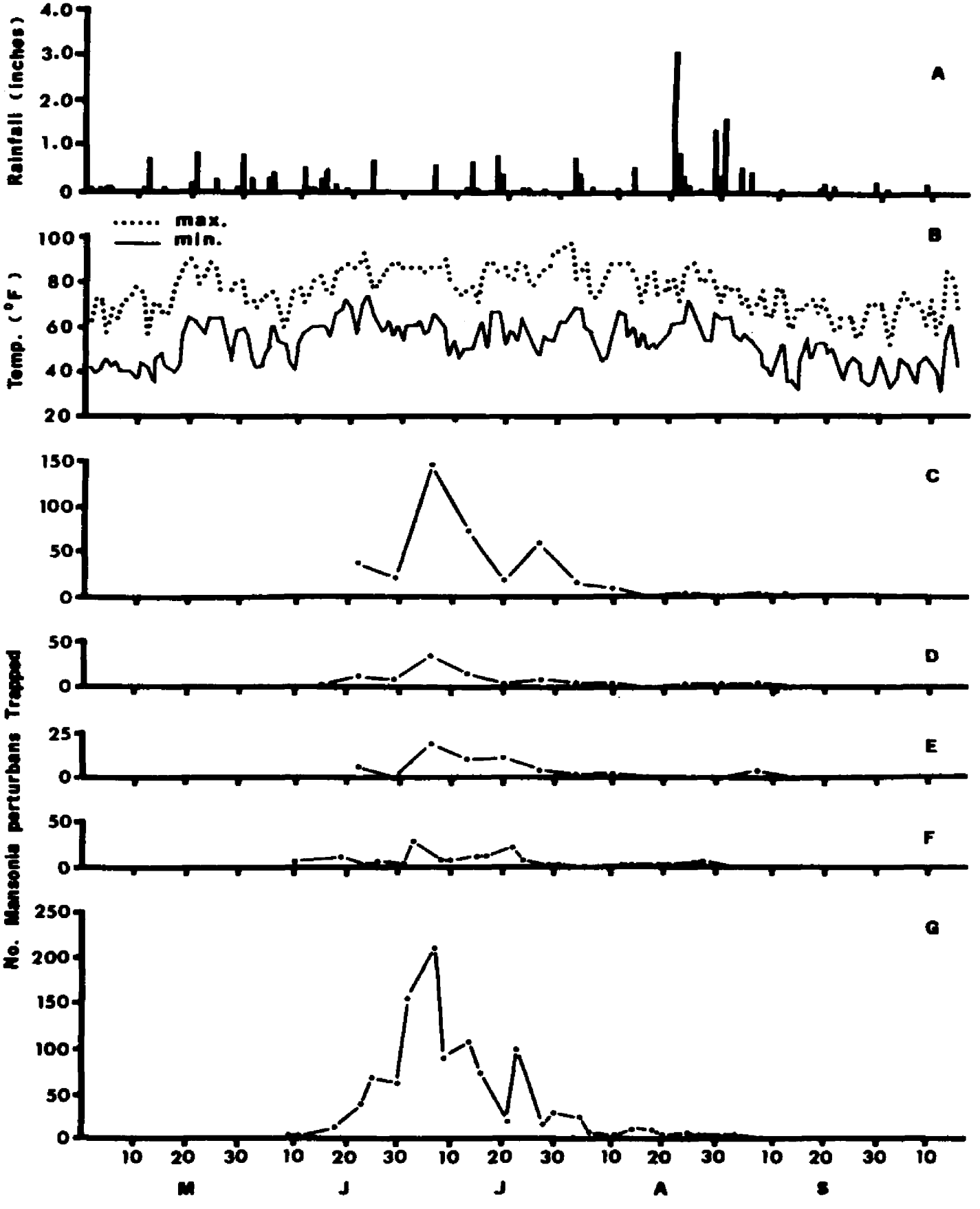

Rainfall (inches)
4.0
3.0
2.0
1.0
0
A
..... max.
—— min.
Temp. (°F)
100
80
60
40
20
B
No. Trapped
Aedes fitchii - stimulans
10
0
C
Aedes vexans
50
25
0
D
Anopheles quadrimaculatus
25
0
E
Anopheles walkeri
50
25
0
F
Culex pipiens
10
0
G
Mansonia perturbans
25
0
H
10 20 30 10 20 30 10 20 30 10 20 30 10 20 30 10
M J J A S

Fig. 10. Seasonal incidence of Mansonia perturbans in the Lansing, Michigan area during June through early October, 1975. Collections made with CDC miniature light traps.

A) Rainfall in inches during the collecting period.

B) Daily maximum and minimum temperatures during the collecting period.

C-G) Collections at Sites 2, 3-A, 3-B, 4, and 5 respectively.


Rainfall (inches)
4.0
3.0
2.0
1.0
0
A
max.
min.
Temp. (°F)
100
80
60
40
20
B
150
100
50
0
C
50
0
D
25
0
E
50
0
F
No. Mansonia perturbans Trapped
250
200
150
100
50
0
G
10 20 30 10 20 30 10 20 30 10 20 30 10 20 30 10
M J J A S

produce several broods in one season. Barr (1958) wrote that these 5 species can be expected to have an early season emergence in May or June. Subsequent emergences result from heavy rainfalls during the summer and even early fall if temperatures are favorable. Figures 11C-15C show that in 1975 these 5 species produced at least 2 broods: The annual early season brood shown by their abundance in June and a late summer brood in September resulting from heavy rains which occurred in mid-August. A third emergence may have occurred in early August, 1975 because Figures 11C-15C show a small peak during this time. Moderate rainfall in mid-July may have produced an additional brood but there is no other evidence to support this conclusion. The important point to make here is that these 5 species, by their seasonal occurrence are most important as potential vectors of heartworm during early summer and thereafter, following heavy rainfall. This generalization though, must be weighed carefully because A. triseriatus, although abundant only at Site 4 (Figure 13C) was collected each night that traps were set except on September 11 (This species was not collected after September 23, 1975 at that site.) Thus, when abundant, this species can be a continual pest and potential heartworm vector throughout the mosquito season. Similarly, A. trivittatus (Figure 14C) may be collected only during peak periods of abundance as it was at Site 2, or may be continually present as it was at Site 4. Likewise A. vexans showed peak periods of abundance at all sites similar to the peaks observed at Site 2 (Figure 15C). This was the species collected most frequently in the study area and although there were periods of peak activity, significant numbers of A. vexans were present throughout the mosquito

Fig. 11. Seasonal incidence of Aedes cinereus and some members of the A. communis complex. Excludes A. sticticus and A. aurifer, which were not distinguished from A. cinereus until September, 1975.

A) Rainfall in inches during the collecting period.

B) Daily maximum and minimum temperatures during the collecting period.

C) Seasonal incidence of A. cinereus collected with CDC miniature light traps at Site 5.

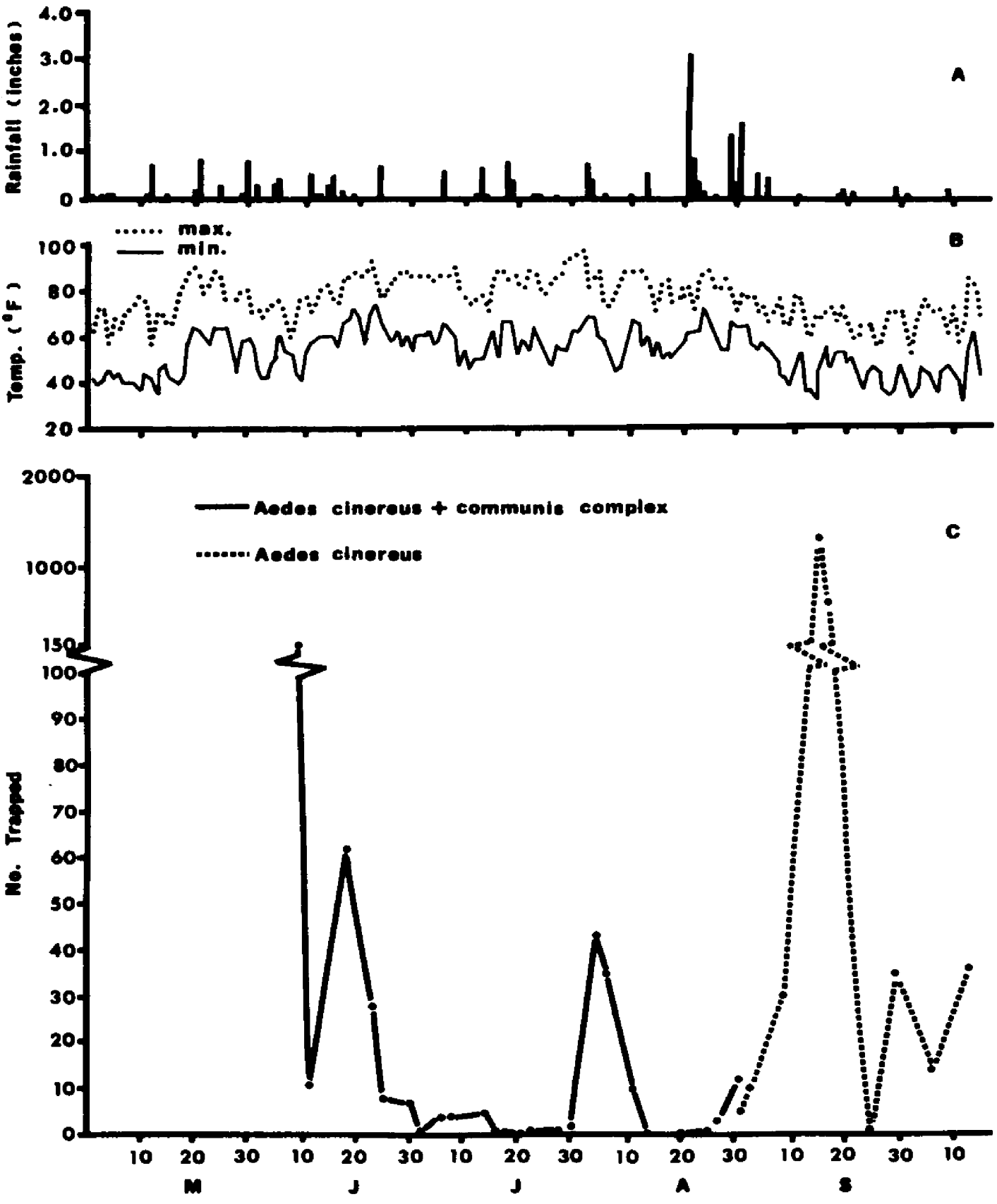

Rainfall (inches)
4.0
3.0
2.0
1.0
0
A
max.
min.
Temp. (°F)
100
80
60
40
20
B
Aedes cinereus + communis complex
Aedes cinereus
No. Trapped
2000
1000
150
100
90
80
70
60
50
40
30
20
10
0
C
10 20 30 10 20 30 10 20 30 10 20 30 10 20 30 10
M
J
J
A
S

Fig. 12. Seasonal incidence of Aedes sticticus at Site 5 in the Lansing, Michigan area during June through early October, 1975. Collections made with CDC miniature light traps.

A) Rainfall in inches during the collection period.

B) Daily maximum and minimum temperatures during the collection period.

C) Seasonal incidence of A. sticticus.

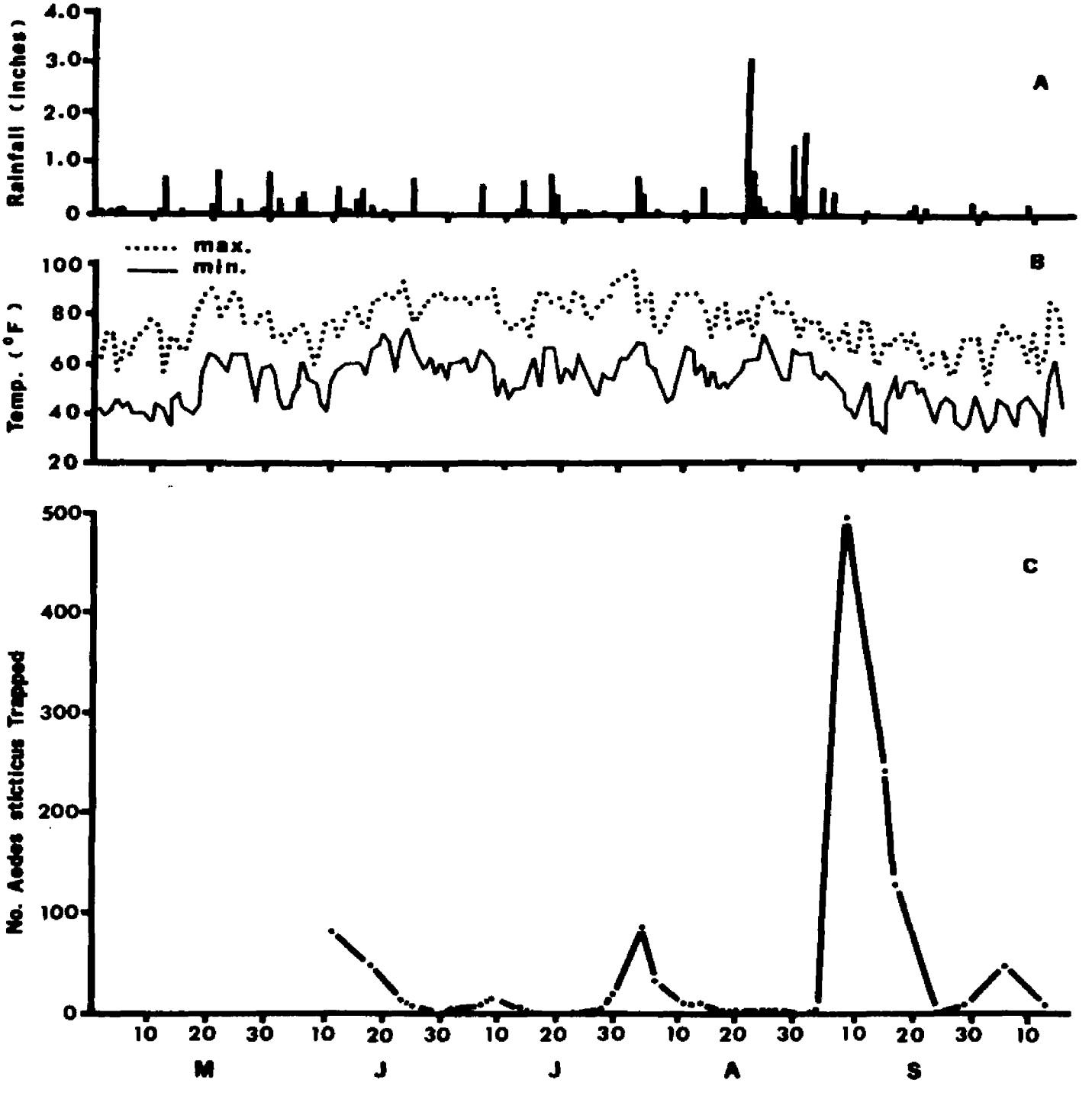

Rainfall (inches)
4.0
3.0
2.0
1.0
0
A
..... max.
—— min.
Temp. (°F)
100
80
60
40
20
B
No. Aedes sticticus Trapped
500
400
300
200
100
0
C
10 20 30 10 20 30 10 20 30 10 20 30 10 20 30 10
M J J A S

Fig. 13. Seasonal incidence of Aedes triseriatus at Site 4 in the Lansing, Michigan area during June through early October 1975. Collections made with CDC miniature light traps.

A) Rainfall in inches during the collecting period.

B) Daily maximum and minimum temperatures during the collecting period.

C) Seasonal incidence of A. triseriatus.

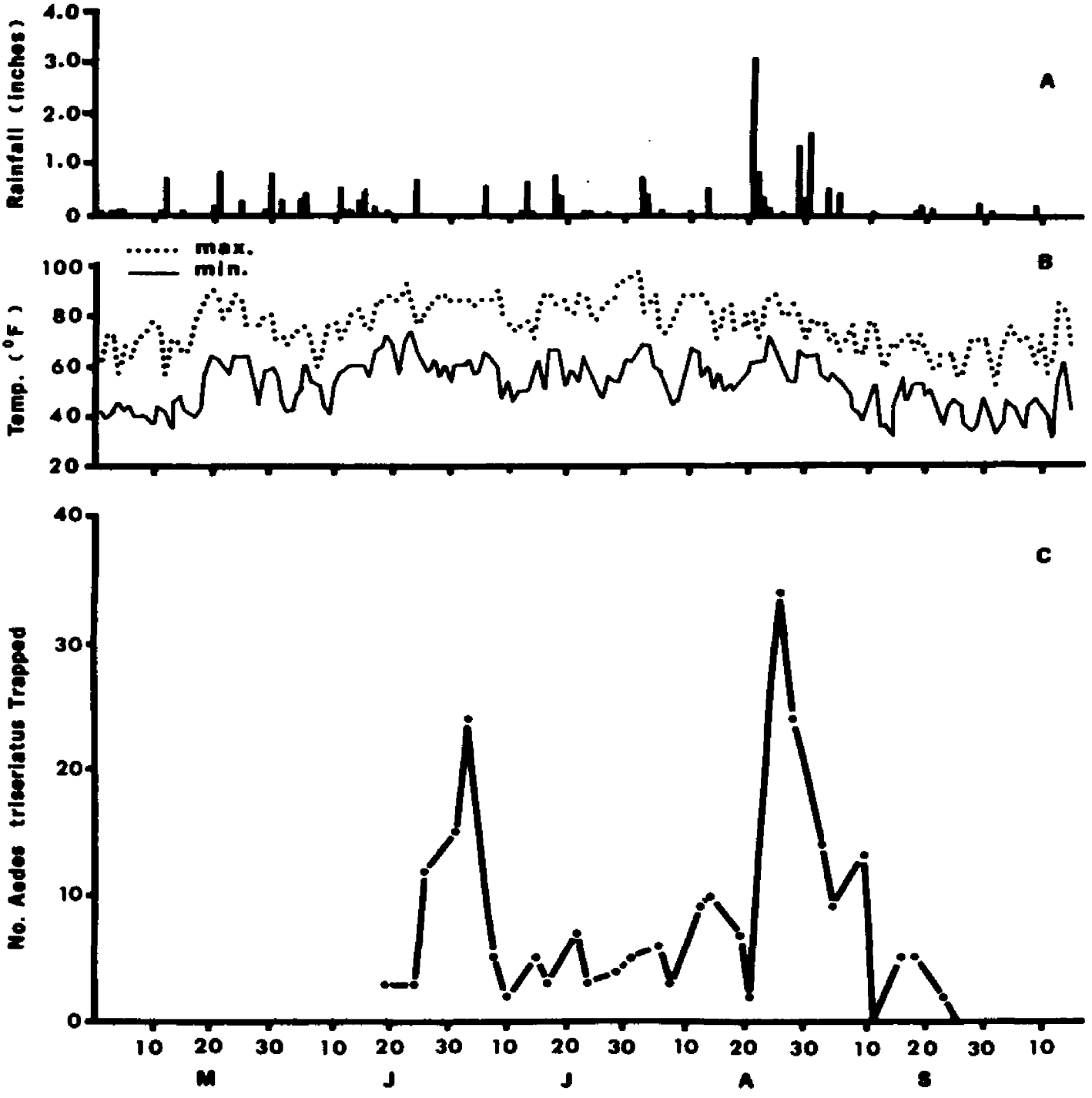

Rainfall (inches)
4.0
3.0
2.0
1.0
0
A
..... max.
—— min.
Temp. (°F)
100
80
60
40
20
B
No. Aedes triseriatus Trapped
40
30
20
10
0
C
10 20 30 10 20 30 10 20 30 10 20 30 10 20 30 10
M J J A S

Fig. 14. Seasonal incidence of Aedes trivittatus at 2 locations in the Lansing, Michigan area during June through early October, 1975. Collections made with CDC miniature light traps.

A) Rainfall in inches during the collection period.

B) Daily maximum and minimum temperatures during the collection period.

C) Collections at Sites 2 and 4.

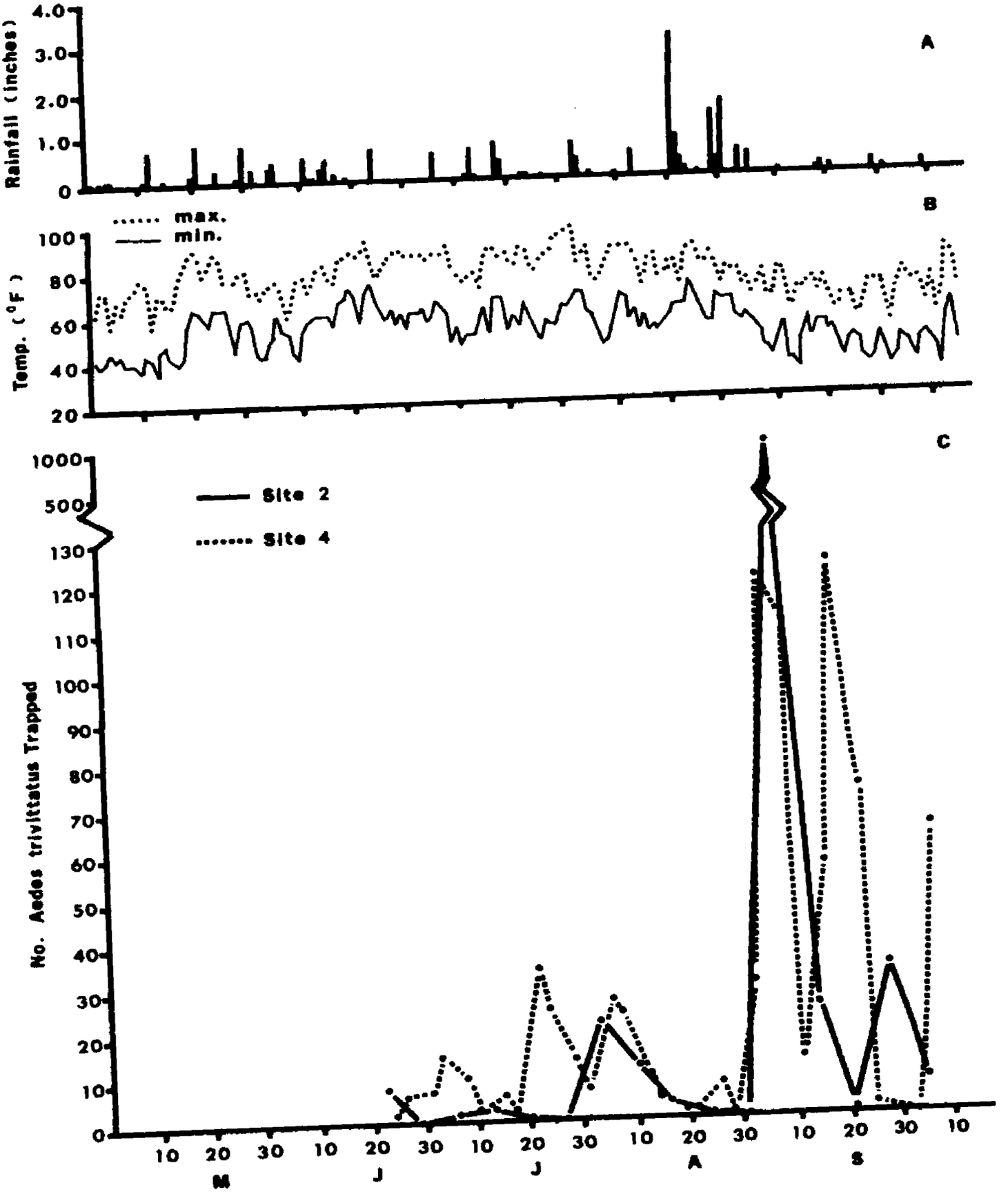
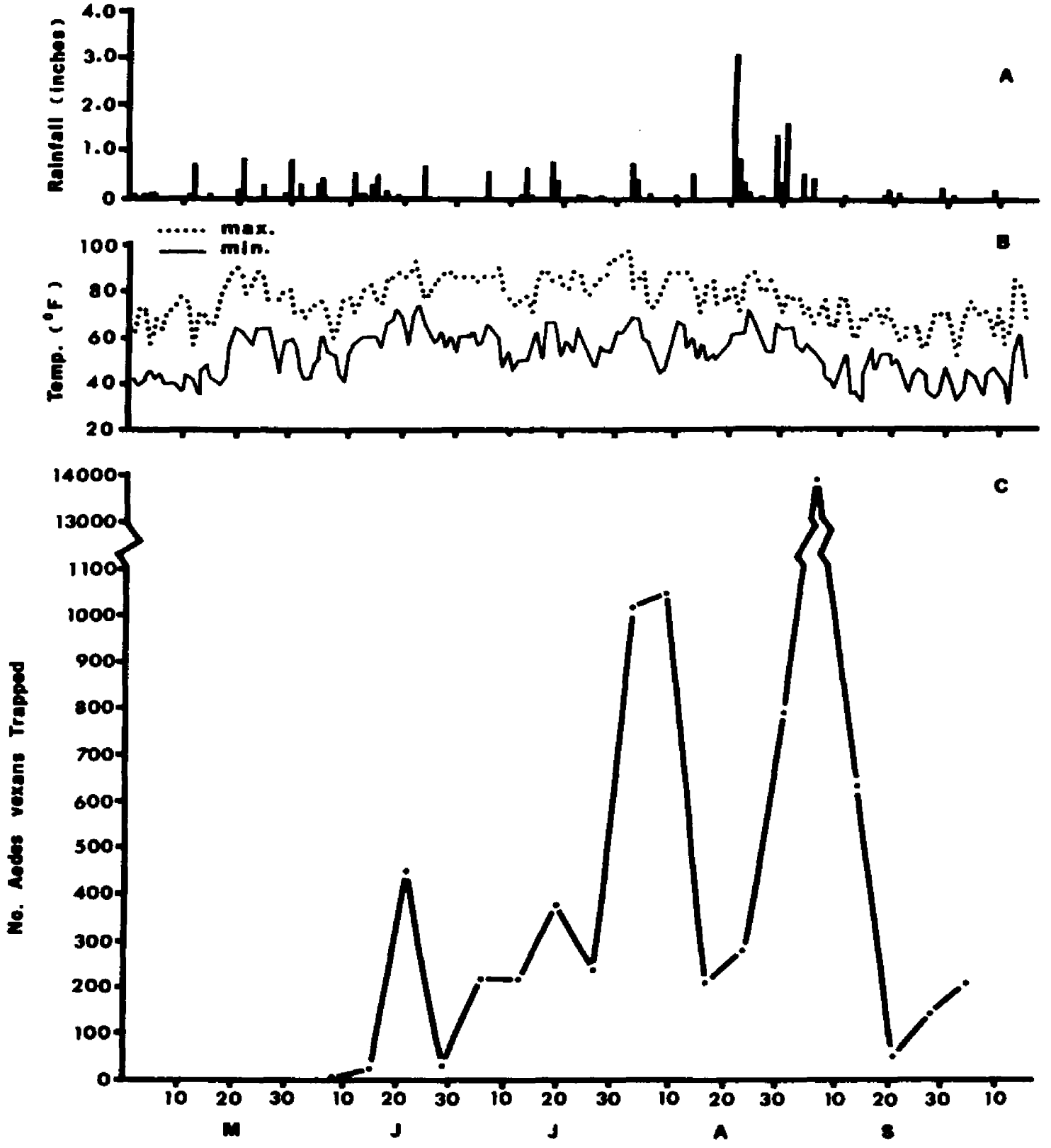

A
Rainfall (inches)
4.0
3.0
2.0
1.0
0
B
..... max.
—— min.
Temp. (°F)
100
80
60
40
20
C
—— Site 2
..... Site 4
No. Aedes trivittatus Trapped
1000
500
130
120
110
100
90
80
70
60
50
40
30
20
10
0
10 20 30 10 20 30 10 20 30 10 20 30 10 20 30 10
M J J A S

Fig. 15. Seasonal incidence of Aedes vexans in the Lansing, Michigan area during June through early October, 1975. Collections made in CDC miniature light traps.

A) Rainfall in inches during the collecting period.

B) Daily maximum and minimum temperatures during the collecting period.

C) Collections at Site 2.


Rainfall (inches)
4.0
3.0
2.0
1.0
0
A
max.
min.
Temp. (°F)
100
80
60
40
20
B
No. Aedes vexans Trapped
14000
13000
1100
1000
900
800
700
600
500
400
300
200
100
0
C
10 20 30 10 20 30 10 20 30 10 20 30 10 20 30 10
M J J A S

season. Thus this species could be involved in the transmission of D. immitis during the entire mosquito season.

A final interesting point concerns A. triseriatus. Barr (1958) contended that this species does not readily leave its woodland breeding site and is generally not attracted to light traps. Table 2 shows that in light trap collections at Site 4 it was one of the most frequently captured mosquitoes. Table 1 shows that A. triseriatus was captured at Site 1 more often in the dog-baited trap set in the open area than in an identical trap set in a woodlot. It may be that this mosquito leaves its breeding site more readily than is generally suspected, especially in areas, such as Site 1, where dense shrubs outside the woodlot, provide sufficient cover. This remains to be experimentally proven.

Multivoltine mosquitoes breeding in permanent water situations

Anopheles quadrimaculatus, A. walkeri and Culex pipiens may produce several generations per year. Collection data shown in Figures 7E, F, G; 8E, F, G; 9E, F, G; 16C-G; 17C-G; and 18C-G does not permit the determination of the number of generation produced in 1974 and 1975 because emergence of these species is likely to be continual during summer months and is not as dependent on heavy rainfall as are the multivoltine Aedes species. Heavy rainfall can, however, increase population numbers by providing additional breeding sites or enlarging existing ones. Significant rainfall in August, 1975 apparently caused population increases in Anopheles walkeri (Figures 9F and 17G) and Culex pipiens (Figure 18C) in September, 1975. Surprisingly, similar population increases of A. quadrimaculatus did not occur in September,

Fig. 16. Seasonal incidence of Anopheles quadrimaculatus in the Lansing, Michigan area during June through early October 1975. Collections made with CDC miniature light traps.

A) Rainfall in inches during the collecting period.

B) Daily maximum and minimum temperatures during the collecting period.

C-G) Collections at Sites 2, 3-A, 3-B, 4, and 5 respectively.

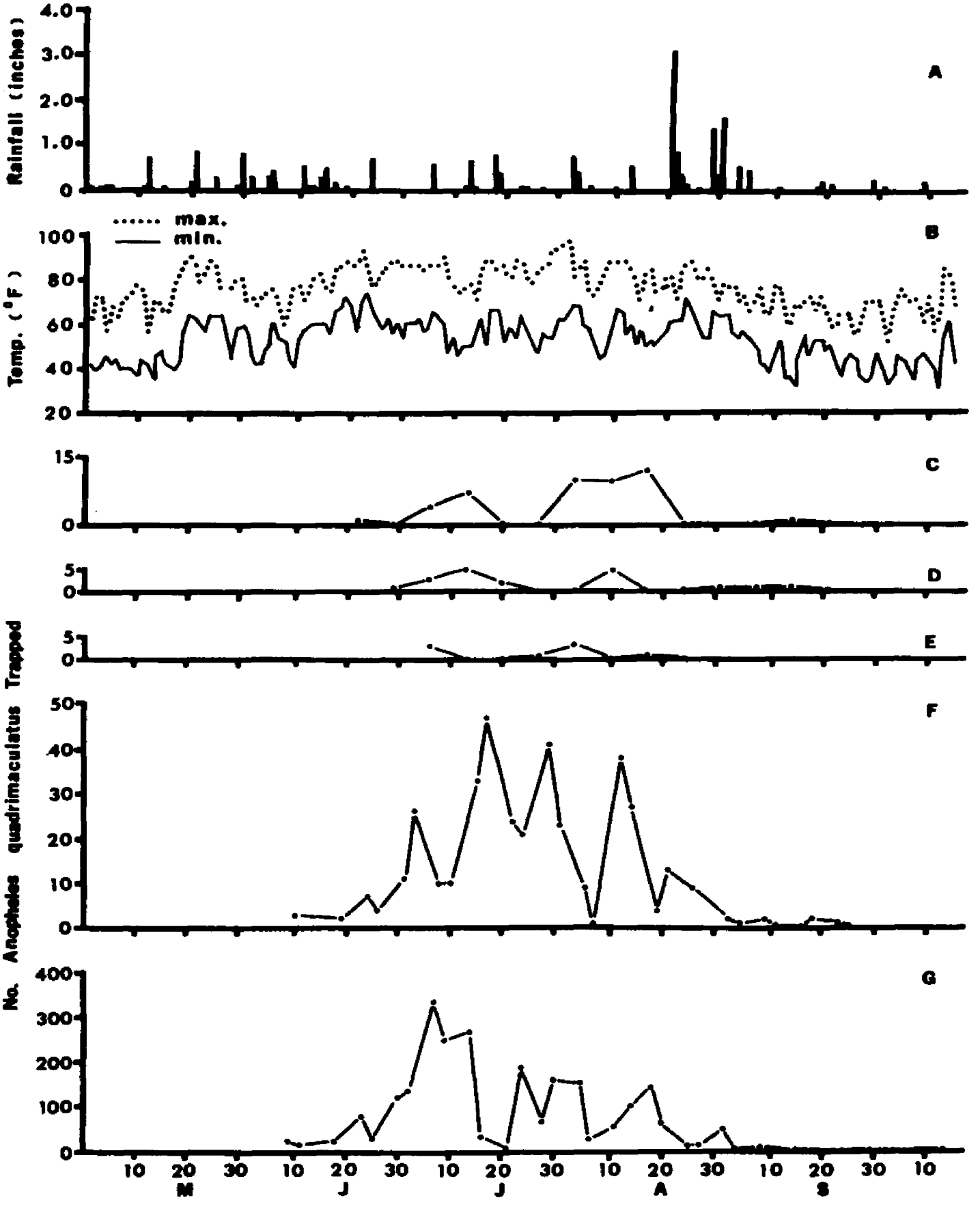

Rainfall (inches)
4.0
3.0
2.0
1.0
0
A
..... max.
—— min.
Temp. (°F)
100
80
60
40
20
B
15
0
C
5
0
D
5
0
E
No. Anopheles quadrimaculatus Trapped
50
40
30
20
10
0
F
400
300
200
100
0
G
10 20 30 10 20 30 10 20 30 10 20 30 10 20 30 10
M J J A S

Fig. 17. Seasonal incidence of Anopheles walkeri in the Lansing, Michigan area during June through early October 1975. Collections made with CDC miniature light traps.

A) Rainfall in inches during the collecting period.

B) Daily maximum and minimum temperatures during the collecting period.

C-G) Collections at Sites 2, 3-A, 3-B, 4, and 5 respectively.

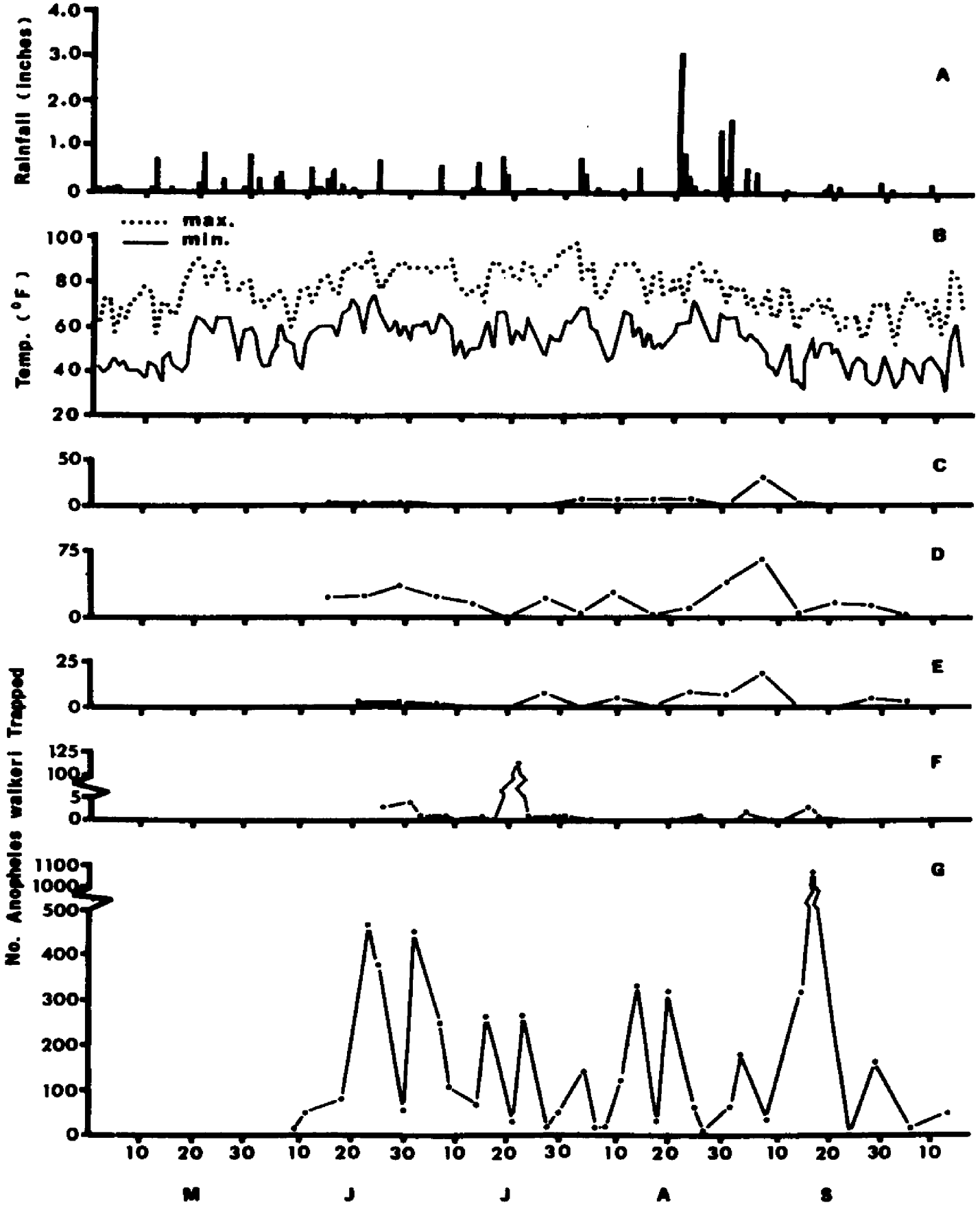
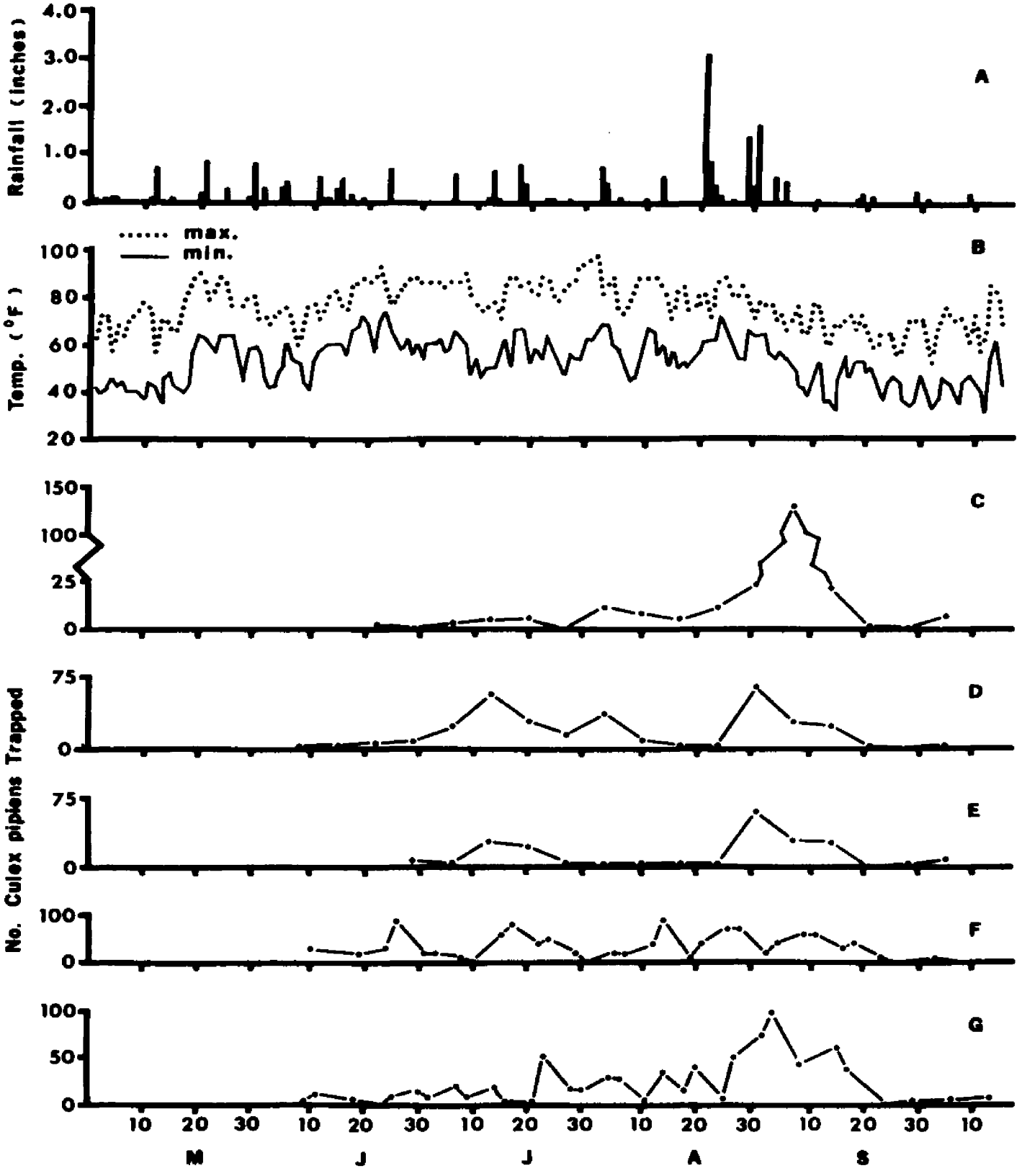

Rainfall (inches)
4.0
3.0
2.0
1.0
0
A
..... max.
—— min.
Temp. (°F)
100
80
60
40
20
B
50
0
C
75
0
D
25
0
E
125
100
5
0
F
No. Anopheles walkeri Trapped
1100
1000
500
400
300
200
100
0
G
10 20 30 10 20 30 10 20 30 10 20 30 10 20 30 10
M J J A S

Fig. 18. Seasonal incidence of Culex pipiens in the Lansing, Michigan area during June through early October, 1975. Collections made with CDC miniature light traps.

A) Rainfall in inches during the collecting period.

B) Daily maximum and minimum temperatures during the collecting period.

C-G) Collections at Sites 2, 3-A, 3-B, 4, and 5 respectively.


Rainfall (inches)
4.0
3.0
2.0
1.0
0
A
max.
min.
Temp. (°F)
100
80
60
40
20
B
No. Culex pipiens Trapped
150
100
25
0
C
75
0
D
75
0
E
100
0
F
100
50
0
G
10 20 30 10 20 30 10 20 30 10 20 30 10 20 30 10
M J J A S

1975 (Figures 9E and 16C-G). Table 1 and Figures 7D and E and 8D and E show A. quadrimaculatus and A. walkeri present in open area dog-baited trap collections at Site 1 but nearly absent from woodland collections at the same site. These species apparently do not readily enter the woodland habitat and would not be highly suspect as vectors of heartworm in such areas. On the other hand, C. pipiens was equally abundant in woodland and open area collections at Site 1. This species must be considered a potential vector of heartworm in both habitats. An interesting observation for which no explanation can be given is that at Site 1 in 1974, A. walkeri was collected early in the season (June 4-July 18) while A. quadrimaculatus was collected later in the season (July 16-August 27) (Figure 7E and F). During 1975 these species were collected concurrently (Figures 9E and F, 16C-G, 17C-G).

The conclusion to be drawn from the collection data discussed above is that these 11 discussed species, by their presence in dog-baited traps and abundance in dog-baited and CDC miniature light traps are the most likely vectors of dog heartworm, at least in the study area. Information is lacking concerning which of these species survives long enough, in nature, to support complete development of D. immitis larvae. Kutz (1972) found that under laboratory conditions at 70° F, 21 days were required for D. immitis to develop to the infective stage and reach the head and mouthparts in A. quadrimaculatus and development was arrested at 60° F. Jaskowski and Bickley (1976) found infective larvae in the head and mouthparts of Aedes canadensis (Theobald), held at 64.4° F, between 27 and 37 days after an infective blood meal. If approximately 27 days is required for complete development in an ideal

host, it is possible to speculate on the longevity required for a mosquito to support development of D. immitis under natural conditions. In Lansing, Michigan the average temperature (Environmental Data Service; Ashville, North Carolina) from 1936-1975 for May through September was 56.6, 66.2, 70.7, 68.9, and 61.8° F, respectively. Temperature may limit development of heartworm in the mosquito to June, July and August when average temperatures are around 70° F. It seems logical that the most likely vectors of dog heartworm would have to survive a minimum of 30 days, allowing time to search for blood meals. Certainly, development may not require 27 days under ideal conditions and some mosquito species may serve as vectors during warm seasons but not during cooler seasons. Assuming development is possible, the most likely vectors are those in which a good portion of females in the population have a longevity of approximately 30 days. Additionally, Crans and Feldlaufer (1974) wrote that the time of greatest danger for transmission is when population numbers are low. Obviously it is at this time when the population consists mainly of older females that have had at least one, possibly infective, blood meal.

## Examination of Mosquitoes for the Presence of Infective Larvae

Tables 3 and 4 list the mosquito species and numbers examined for nematode larvae in 1974 and 1975. Table 5 shows the site and date on which infective larvae, possibly D. immitis, were extracted from field captured mosquitoes. These nematodes, by their size, shape, and activity were indistinguishable from infective D. immitis larvae, and for the sake of discussion, it will be assumed that they were D. immitis.

Table 3
Mosquitoes Pooled in 1974 from CDC Miniature Light Trap and Dog-baited Trap Collections

| Species | # Pooled |
|---|---|
| **Aedes** | |
| canadensis | 9 |
| communis complex[a] | 24 |
| fitchii-stimulans | 122 |
| triseriatus | 53 |
| vexans | 2,209 |
| **Anopheles** | |
| punctipennis | 31 |
| quadrimaculatus | 422 |
| walkeri | 383 |
| **Culex** | |
| pipiens | 463 |
| territans | 2 |
| spp. | 10 |
| **Mansonia** | |
| perturbans | 2,492 |
| TOTAL | 6,220 |

[a]Includes Aedes cinereus and excludes A. aurifer and A. sticticus.

Table 4
Mosquitoes Pooled in 1975 from CDC Miniature Light Trap and Dog-baited Trap Collections

| Species | # Pooled |
|---|---|
| **Aedes** | |
| aurifer | 13 |
| canadensis | 150 |
| cinereus | 413 |
| communis complex[a] | 359 |
| dorsalis | 2 |
| fitchii-stimulans | 1,437 |
| flavescens | 51 |
| sticticus | 779 |
| triseriatus | 243 |
| trivittatus | 1,031 |
| vexans | 24,912 |
| **Anopheles** | |
| earlii | 33 |
| punctipennis | 137 |
| quadrimaculatus | 2,340 |
| walkeri | 3,462 |
| **Culex** | |
| erraticus | 1 |
| pipiens | 828 |
| restuans | 2 |
| salinarius | 14 |
| territans | 34 |
| **Culiseta** | |
| inornata | 1 |
| morsitans | 7 |
| **Mansonia** | |
| perturbans | 1,547 |
| **Orthopodomyia** | |
| spp. | 1 |
| **Uranotaenia** | |
| sappharina | 3 |
| TOTAL | 37,800 |

[a]Includes Aedes cinereus and excludes A. aurifer and A. sticticus.

Table 5
Nematodes, Possibly Dirofilaria immitis, Extracted from Mosquitoes

| Mosquito | Site[a] | Date | Pool Size | # Worms |
|---|---|---|---|---|
| Aedes vexans | 3 | June 30, 1975 | 25 | 12 |
| | 2 | July 20, 1975 | 25 | 3 |
| | 5 | August 26, 1975 | 25 | 20 |
| Anopheles quadrimaculatus | 1 | August 27, 1974 | 4 | 4 |
| | 5 | August 19, 1975 | 25 | 20 |
| Culex pipiens | 1 | August 26, 1975 | 12 | 33 |

There is additional evidence that the nematodes, extracted from Aedes vexans on July 20, 1975, may have been D. immitis larvae. After these larvae were found, dogs at Site 2 were screened for microfilariae. A single dog, thought to be cured of heartworm the previous year, again showed a low microfilaremia. An infected dog was at Site 2 during the time mosquitoes were being collected and examined for infective larvae.

Table 5 shows that larvae, possibly D. immitis, were recovered from mosquitoes collected at 4 of the 5 collection sites. These larvae were found most often in A. vexans while the highest number of larvae were found in Culex pipiens. Because of the low incidence of larvae found in mosquito pools, it is probable that nematodes emerged from a single individual of the pools. Results of the developmental trials, to be discussed later, show C. pipiens to be a poor host for D. immitis. Never was more than a single D. immitis larvae found in C. pipiens fed an infective blood meal. Thirty-three larvae were extracted from a pool collected in 1974 (Table 5). This pool of C. pipiens was collected at Site 1 in the dog-baited trap set in the woodland area. Filarid worms are common parasites of birds (Anderson and Freeman, 1969) and birds are the preferred host of C. pipiens. Anderson and Freeman (1969) found four genera of the family Onchocercidae in Ontario, Canada. Cardiofilaria Strom, is an especially common genus which they suggest is present throughout North America. Since this genus has been found in many different bird species, these authors suggest that the vector's feeding habits are not highly selective, and typical of a mosquito. At least one species of Cardiofilaria, from Ceylon, is known to be transmitted by mosquito. Furthermore, C. inornata (Anderson) a common

North American species is known from the woodcock, robin, olive-backed thrush, long-eared owl, sharp-skinned hawk, marsh hawk, raven and the oven-bird. This is not to suggest that the nematodes isolated from Culex pipiens were Cardiofilaria inornata but to bring out the fact that bird filarids are no doubt common in the study area and it appears likely that the nematodes found in Culex pipiens were not D. immitis.

Again, if we assume, based on the low numbers of larvae extracted from field-captured mosquitoes, that nematodes recovered from pools originated from a single individual in the pool, we can calculate infection rates in the population. Nematodes were obtained from A. vexans on 3 occasions from a total of 27,121 mosquitoes of that species. This indicates that 0.01% of the population may have been carrying D. immitis larvae. Similarly, nematodes were extracted from Anopheles quadrimaculatus on 2 occasions from a total of 2,762 pooled individuals. In this case the infection rate would be 0.072%. From these data it may be suggested that Anopheles quadrimaculatus carry proportionately more presumed D. immitis infective larvae under natural conditions and would be more important as a vector of dog heartworm than Aedes vexans.

## Differentiation of D. immitis Larvae

The histochemical stain developed by Chalifaux and Hunt (1971) to differentiate microfilariae of D. immitis and Dipetalonema reconditum proved to be of no value in identifying infective larvae from field-captured mosquitoes. Third stage larvae of D. immitis treated according to this method appeared similar to third stage larvae of D. tenuis prepared in the same manner. Orihel (1959) concluded that the

developmental stages of D. immitis and D. tenuis in the mosquito host are indistinguishable on a morphological basis. Thus, infective larvae isolated from field-captured mosquitoes could not be positively identified.

It is not surprising that the histochemical stain, to locate areas of acid phosphatase activity, did not react differently in these 2 Dirofilaria species. D. immitis and D. tenuis are closely related taxonomically and because both species have developmental stages in the Malpighian tubules of mosquitoes, their physiology is no doubt, very similar.

## Dirofilaria immitis Developmental Trials

Complete tabulation of D. immitis developmental trial results appear in Appendices P-FF. Microfilaremia of the infected dog or dilution of blood, for membrane feedings, at the time of the infective blood meals are listed for the various trials in Appendix GG. Results of the developmental trials will be discussed from Table 6.

### Results with Aedes stimulans

Aedes stimulans proved to be a poor host for D. immitis larvae. Infective larvae were never observed in the head and proboscis of this mosquito. Development appeared to be retarded because second stage larvae were observed in the Malpighian tubules as late as 26 days after the infective blood meal. Microfilariae did not become established in the Malpighian tubules of 16 of 19 (84.2%) mosquitoes dissected. Encapsulation was observed in every mosquito in which larvae did reach

Table 6
Summary of Developmental Trials

| Species | Trial | # Mosquitoes Taking an Infective Blood Meal | # Days Required for Development $L_3$ | | | # Mosquito Mortality until First $L_3$ Observed in Head | # Mosquitoes Dissected | # Mosquitoes Dissected with No Larvae in M. T. | # Mosquitoes Dissected with No Larvae | # Mosquitoes Dissected with some Encapsulation Observed |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $L_2$ | M. T.[a] | Head | | | | | |
| Aedes stimulans | 1 | 57 | NO[b] | 18 | ND[c] | - | 19 | 16 | 84.2 | 3 |
| Mansonia perturbans | 1 | 35 | ND | ND | ND | - | 4 | 4 | 100 | 0 |
| | 2 | 54 | ND | ND | ND | - | 4 | 1 | 25 | 3 |
| | 3 | 84 | ND | ND | ND | - | 23 | 22 | 95.7 | 1 |
| | 4 | 71 | 8 | ND | ND | - | 31 | 31 | 100 | 0 |
| Aedes vexans | 1 | 45 | 10 | NO | 12 | 68.9 | 10 | 6 | 60 | 3 |
| | 2 | 14 | NO | NO | NO | - | 2 | 0 | 0.0 | 0 |
| | 3 | 210 | 7 | 9 | 12 | 98.6 | 10 | 3 | 30 | 1 |
| | 4 | 212 | 7 | 10 | 12 | 97.6 | 7 | 1 | 14.3 | 3 |
| Anopheles quadrimaculatus | 1 | 10 | 9 | 11 | 13 | 70 | 3 | 0 | 0.0 | 0 |
| | 2 | 37 | NO | 10 | 14 | 89.2 | 5 | 0 | 0.0 | 0 |
| | 3 | 15 | NO | NO | NO | - | 2 | 0 | 0.0 | 0 |
| | 4 | 15 | NO | NO | NO | - | 2 | 0 | 0.0 | 0 |
| | 5 | 6 | 9 | NO | NO | - | 3 | 0 | 0.0 | 0 |
| Culex pipiens | 1 | 10 | NO | NO | NO | - | 4 | 4 | 100 | 0 |
| | 2 | 84 | 9 | 14 | NO | - | 45 | 40 | 88.9 | 0 |
| | 3 | 27 | 14 | NO | 15 | 22.2 | 23 | 19 | 82.6 | 0 |

[a]Malpighian tubules. [b]Stage not observed but may have occurred. [c]Development to this stage most likely did not occur.

the Malpighian tubules. No unencapsulated third stage larvae were observed. Yen (1938) found only 21 of 170 mosquitoes of this species alive 10 days after taking an infective blood meal. Of these 21, only 10 (47%) had developing larvae. Retardation of development was also reported by Yen and infective larvae were found in 3 mosquitoes but never in the head. Encapsulation was common but Yen did not observe encapsulated third stage larvae. Yen reported that this mosquito is a likely potential vector of dog heartworm in Minnesota but the results of the present study indicate that the local Michigan strain of A. stimulans is not a natural vector of dog heartworm.

Results with Mansonia perturbans

Mansonia perturbans proved to be an unacceptable host for D. immitis larvae. Second stage larvae were seen in only one individual and infective larvae were never seen. Microfilariae did not become established in an average of 80.2% of the individuals dissected and encapsulation of larvae was always observed in individuals in which microfilariae did reach the Malpighian tubules. Yen (1938) had similar results with this species. He found only 1 of 8 mosquitoes taking an infective blood meal had microfilariae in the Malpighian tubules. All microfilariae were dead and one was encapsulated. Results indicate that the local Michigan strain of M. perturbans is not a vector of dog heartworm under natural conditions.

Results with Aedes vexans

Aedes vexans proved to be an acceptable host for D. immitis larvae. Under laboratory conditions infective larvae reached the head and

mouthparts of infected mosquitoes in as little as 12 days. Mortality of infected mosquitoes was very high; only 22 of 481 individuals (4.6%) in 4 trials, lived long enough (12 days) for infective larvae to reach the labium. Additionally, microfilariae did not become established in an average of 34% of the mosquitoes of this species taking an infective blood meal and encapsulation of larvae was common although not every larva was encapsulated in any individual mosquito. Hu (1931) wrote that 80.3% of A. vexans taking an infective blood meal became infected. He felt that relatively few larvae (10.6 larvae/mosquito) became established although one mosquito had 33 developing larvae. Similarly, Jankowski and Bickley (1976) reported 78.9% of 78 individuals (held at 80° F) became infected after an infective blood meal with an infection rate of 10.6 larvae/infected individual. Yen (1938) found that 25 of 129 (19.3%) lived long enough for D. immitis to complete development to the infective stage and all of these 25 mosquitoes harbored infective larvae. Infective larvae reached the labium in as little as 13 days and one specimen contained 76 infective larvae. Encapsulation of larvae was common. Yen considered this mosquito to be a likely vector. Bemrick and Sandholm (1966) found 72.4% of A. vexans infected after taking an infective blood meal and 84.6% of these 113 mosquitoes harbored larvae after 16-18 days. Over half of these were carrying infective larvae in the head and body cavity. The present study confirmed the results of these previous studies. A. vexans is a suitable host for D. immitis larvae and is most likely involved in the natural maintenance of this parasite.

Results with Anopheles quadrimaculatus

Laboratory experiments during the present study showed Anopheles quadrimaculatus to be an excellent host for dog heartworm larvae. All individuals taking an infective blood meal became infected. Infective larvae reached the head and mouthparts of this mosquito in 13 post-prandial days. Mortality up to this point of development for 5 trials (78/83 mosquitoes) was 94% indicating that A. quadrimaculatus is a better host for D. immitis larvae than Aedes vexans, especially considering that Anopheles quadrimaculatus was fed directly on an infected dog and thus carried a heavier parasite load. Kutz (1972) noted a mortality of 75.3% after 13 postprandial days in a laboratory colony maintained under similar conditions. Phillips (1939) reported 100% of 90 mosquitoes infected after taking an infective blood meal. Infective larvae were found in 72 of these 11-18 days later. The remaining 18 died or were sacrificed. He found an average of 40 larvae per mosquito. Kartman (1953b) found all of 210 A. quadrimaculatus infected after taking an infective blood meal. Infective larvae reached the labium in as little as 14 postprandial days. Kartman did find a negligible amount of encapsulated larvae (Microfilariae, 0.09%; first stage larvae, 0.2%) and some degenerate first and second stage larvae but A. quadrimaculatus still proved to a very acceptable intermediate host for D. immitis. Similarly, Keegan et al. (1968) reported successful development of D. immitis in A. quadrimaculatus. They worked with fewer individuals and found 2 out of 11 individuals to harbor infective larvae after 12-18 days. Assuming that A. quadrimaculatus is not limited by its longevity, this species appears to be an efficient host for D. immitis and is most

likely the species most important in the natural maintenance of this infection in the Lansing, Michigan area.

Results with Culex pipiens

Laboratory studies showed that Culex pipiens is a possible vector of dog heartworm in nature. Development of larvae in this mosquito did not appear to be retarded and no encapsulation was observed. It is not an efficient host, however, because microfilariae did not become established in the Malpighian tubules in 87.5% of 72 mosquitoes dissected. Never was more than a single larva, at any stage, observed in any individual. Only one infective larva was seen in the proboscis of one mosquito during all 3 of the developmental trials. Similarly, Hu (1931) found only 27.4% of 182 mosquitoes infected after taking an infective blood-meal but found larvae able to complete development to the infective stage. Kartman's (1953b) infectivity results were similar to these but he found infective larvae in the proboscis of C. pipiens in as little as 10 days after the infective blood meal. Because C. pipiens is not an efficient host and because it normally feeds on birds, it is not likely that this species plays an important role in the natural maintenance of D. immitis infections. Apparently however, assuming its longevity is adequate, it may serve as a vector of dog heartworm under natural conditions.

During the course of this investigation, two phenomena occurred which have a bearing on the suitability of a mosquito as a vector of heartworm: encapsulation of larvae and elimination of microfilariae before they become established in the Malpighian tubules. Encapsulation was observed in Aedes stimulans, A. vexans and Mansonia perturbans.

Kartman (1953b) found consistant encapsulation of D. immitis microfilariae in Aedes aegypti but felt this had little bearing on the ability of this mosquito to act as a vector of dog heartworm because only 12% of the microfilariae were encapsulated. In the present study, encapsulation prevented Aedes stimulans, A. vexans and Mansonia perturbans from acting as an efficient host for D. immitis larvae.

Complete loss of larvae from mosquitoes which took an infective blood meal, was noted in Aedes stimulans, A. vexans and Mansonia perturbans and Culex pipiens in which this was observed in an average of 84.2, 34.8, 80.2, and 90.5% of these species, respectively. Kartman (1953b) observed this phenomena in Culex pipiens and C. quinquefasciatus and Yen (1938) reported it in Aedes trivittatus. It is not known if digestive enzymes work against dead microfilariae killed by another substance or whether digestive enzymes act directly on living microfilariae. In the present study, inability of larvae to reach the Malpighian tubules proved to be an important factor in preventing Aedes stimulans and Mansonia perturbans and limiting the ability of Culex pipiens to act as an efficient host of dog heartworm.

Kartman (1953b), among others have shown that, different geographical strains of a mosquito species may show variation in their efficiency to act as a host for D. immitis larvae. In the present study development of D. immitis larvae in Michigan strains of mosquitoes were compared with the results reported with other strains in the United States. Because the results of the present study so closely parallel the results of these previous studies, it might be suggested that, at least in the continental United States, a species shows little geographic variation in its ability to support development of D. immitis larvae.

It must be understood, however, that these comparisons are difficult to make. In the past, some authors have considered a species a suitable host if development appeared normal or if infective larvae were observed. Realistically, development cannot be considered complete until infective larvae migrate to the labium of the mosquito. Additionally, techniques among authors vary. One important difference among various studies is the parasite load received by the mosquito at the time of the infective blood meal. Villavaso and Steelman (1970) have shown that there is a direct correlation between mortality and parasite load. Seeley and Bickley (1974) reported development of D. immitis larvae was complete only in one of three United States strains of Culex salinarius. It remains then, that host efficiency must be determined locally to determine the vector potential of any mosquito species.

## Transmission Trials

Complete observations of the transmission trials are given in Appendices HH-JJ.

Aedes stimulans was allowed to feed on experimental dog MW 75. Of 57 mosquitoes known to have taken an infective blood meal 15 survived 16 postprandial days when transmission attempts began. All 15 mosquitoes fed on the clean dog during the first two transmission attempts. 248 days after the final transmission attempt a necropsy was performed. This dog was not infected with D. immitis. These results are to be expected because developmental trials with a Michigan strain of

A. stimulans indicated that this species is not a suitable host for dog heartworm larvae.

Aedes vexans was allowed to feed on experimental dog ER 54. Eighteen of 481 mosquitoes known to have taken an infective blood meal survived at least 12 postprandial days when transmission attempts began. Only one of these mosquitoes is known to have fed on the clean dog. This mosquito died several days later. Dissection indicated that this individual was not infected with D. immitis. A necropsy was performed on dog ER 54 173 days after the final transmission attempt. This dog was not infected with dog heartworm. Although A. vexans appears to be a suitable host for D. immitis, this species is difficult to work with in the laboratory. While in the confines of the cages used in this study it did not readily feed on dogs. Likewise, A. vexans does not readily breed in small cages. It may be that the behavior of this mosquito is altered in typical mosquito rearing cages. It cannot be concluded that A. vexans is an unsuitable vector of D. immitis. Further experimental evidence is required to access the importance of this mosquito as a vector of D. immitis in Michigan.

Anopheles quadrimaculatus was allowed to feed on experimental dog HT 05. Seven of 83 mosquitoes, known to have taken an infective blood meal, survived 13 postprandial days when transmission attempts began. On two occasions, several mosquitoes landed on the experimental dog and engaged in probing activity. These mosquitoes did not appear to have taken any blood. Developmental trials indicated that all A. quadrimaculatus taking an infective blood meal became infected. McGreevy et al. (1974) noted that heavily infected mosquitoes had trouble feeding because the labium, filled with infective larvae, would

not bend. Similarly, during Newton's transmission experiments, mosquitoes had trouble feeding and only 8 of 55 were known to have taken any blood, yet transmission was accomplished. In spite of the failure of these transmission attempts, A. quadrimaculatus must be considered an excellent potential vector of D. immitis in Michigan.

## GENERAL DISCUSSION

Barnett (1960) has outlined 4 criteria for the incrimination of an arthropod as a vector of disease. These were generalized by James and Harwood (1969) as follows:

"1) Demonstration of feeding or other effective contact with the host under natural conditions.

2) A convincing biological association in time and/or space of the suspected arthropod species and occurrence of clinical or subclinical infection in the host.

3) Repeated demonstration that the arthropod under natural conditions, harbors the infectious agent in the infective stage.

4) Transmission of the agent under controlled conditions."

The present study attempted to meet 3 of these criteria. Criterion 2 is very difficult to demonstrate with a disease such as dog heartworm primarily because of the present method of screening for the disease which is through various kinds of examination for microfilaremia. Generally, 8-9 months pass before microfilariae are produced and it may not always be possible for a dog owner to recall where a dog has been over an extended period of time. Second, unless annual or biannual blood screening is performed, an asymptomatic infection may go undetected for several years and it may be impossible to estimate the

time the infection was contracted. Third, unless mosquito surveys were ongoing during the time of suspected transmission, one can only speculate on which mosquito species may have been present at the time of transmission.

As mentioned earlier, 24 suspected mosquito vectors of D. immitis are known to occur in Michigan. Their importance will be discussed in relation to 3 of Barnett's criteria.

Aedes

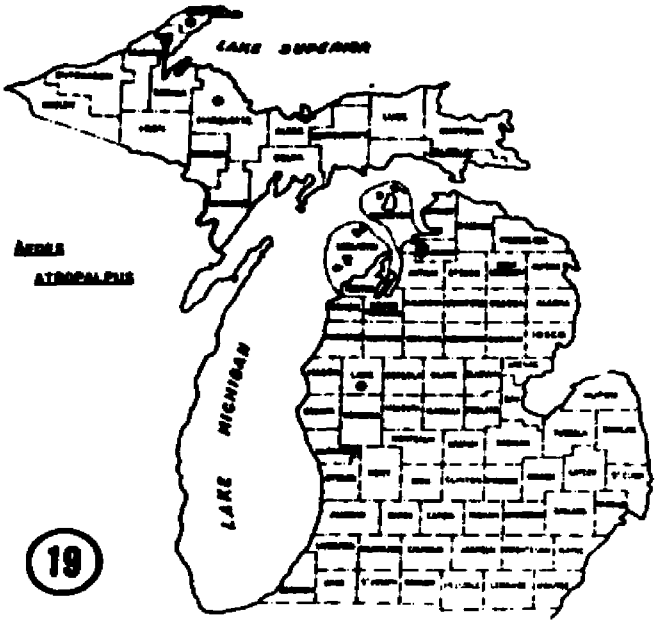
Aedes spp. typically prefer mammalian hosts (Tempelis, 1975). It will be assumed that all Aedes discussed here would take a blood-meal from an available dog and satisfy Barnett's criterion 1.

Aedes atropalpus Coquillett was studied by Keegan et al. (1968). In their report this species readily fed on dogs and 74.3% of 31 mosquitoes examined harbored infective larvae. Infective larvae have not been isolated from field-captured specimens nor are their any reports of transmission of heartworm involving this species. It was not collected in the Lansing, Michigan area and for this reason it is not likely to be a vector in this area. Figure 19 shows the reported distribution of this mosquito in Michigan. Because it has been shown to be capable of supporting complete development of D. immitis it may have some importance as a vector in the upper and northern half of the lower penninsula of Michigan.

Aedes canadensis (Theobald) was seldom trapped at Sites 1-5 (Table 1 and 2). Crans (personal communication) using CDC miniature light traps found this species to be very abundant in New Jersey. Because of the high incidence (about 2%) of these mosquitoes harboring presumed,

Figs. 19-24. Reported distribution of Aedes atropalpus, A. canadensis, A. cinereus, A. fitchii, A. excrucians and A. punctor, respectively, in Michigan[a].

[a]Distribution of mosquitoes illustrated in Figures 19-42 was compiled from the following reports: Irwin (1941, 1942), Newson and McGroarty (personal communication), Obrecht (1949), Sabrosky (1946), and Zavortink (1973).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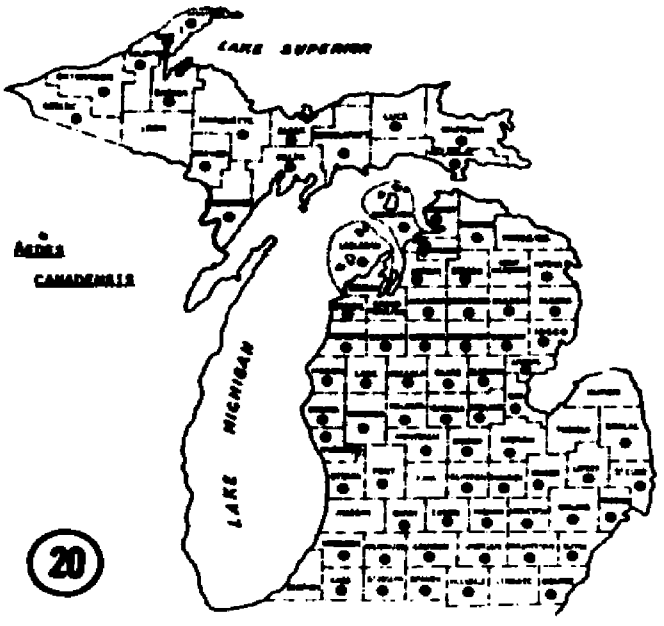
LAKE MICHIGAN
AEDES ATROPALPUS
19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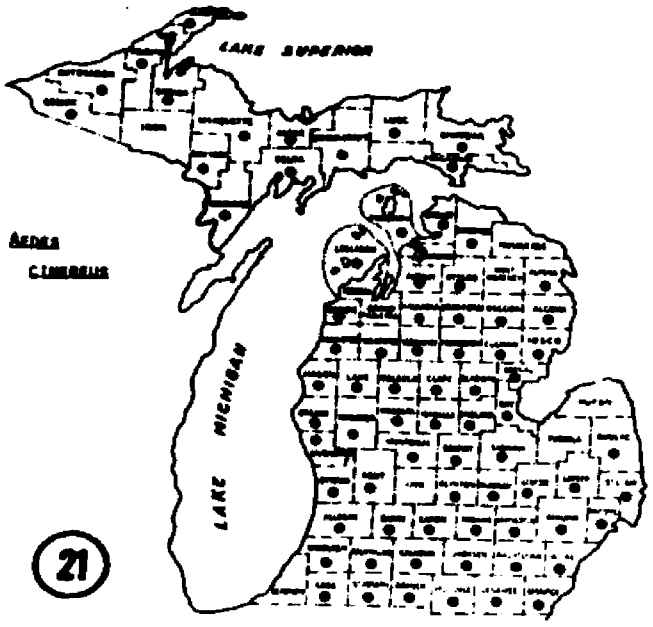
LAKE MICHIGAN
AEDES CANADENSIS
20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LAKE MICHIGAN
AEDES CINEREUS
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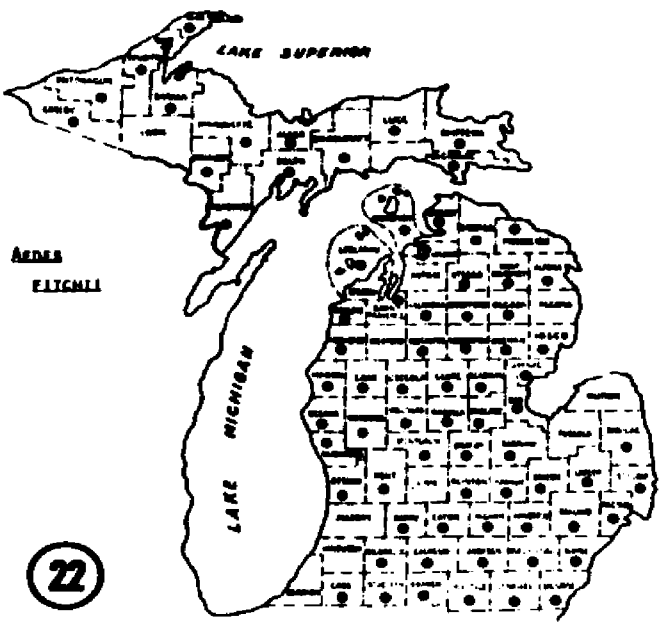
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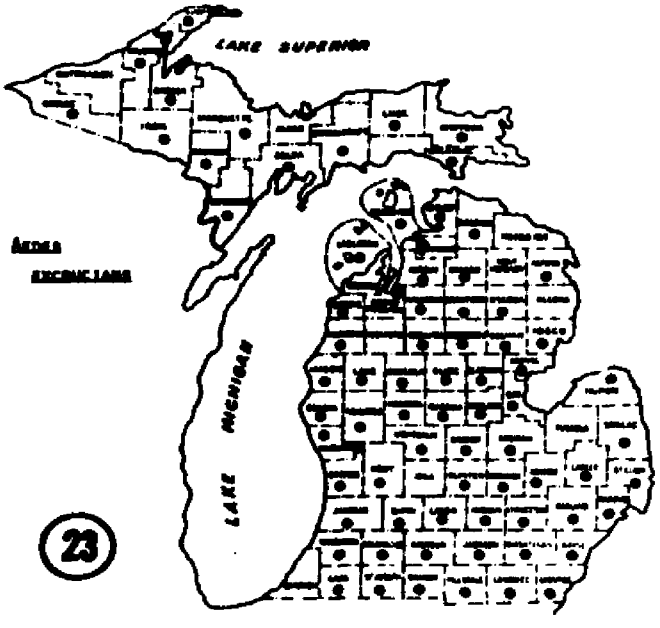
LAKE MICHIGAN
AEDES FITCHII
22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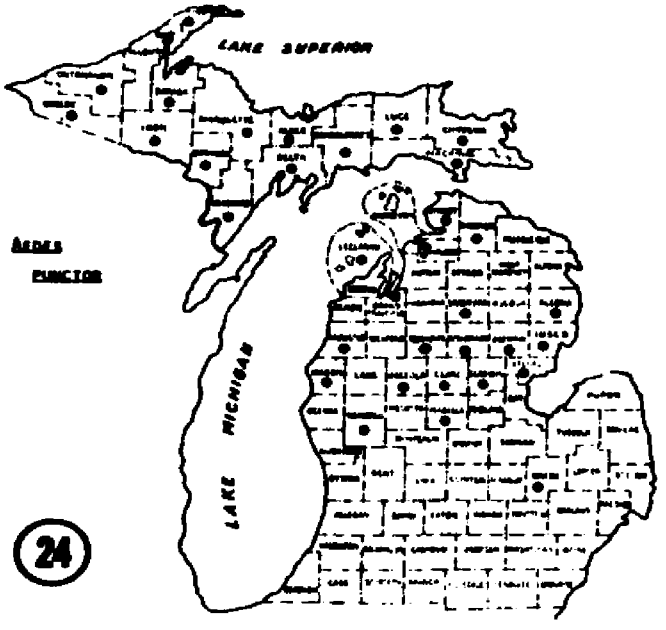
LAKE MICHIGAN
AEDES EXCRUCIANS
23


LAKE SUPERIOR
LAKE MICHIGAN
AEDES PUNCTOR
24

D. immitis, infective larvae he considered this species to be the primary vector of D. immitis in this state. Hu (1931) and Yen (1938) both showed that A. canadensis was able to support the development of D. immitis to the infective stage. Yen found infective larvae in the proboscis of this species 13 days after the infective blood-meal. Jankowski and Bickley (1976) found this species to feed readily on dogs during the course of their experiments. Additionally, Morris and De Foliart (1971) estimated that 19.1% of female A. canadensis searched for more than one blood meal indicating that their life-span may be adequate to allow this species to be a natural vector of D. immitis. In Michigan, this species has been reported from nearly every county (Figure 20). A. canadensis must be considered a good potential vector of dog heartworm in Michigan, primarily in its woodland habitat where females generally remain (Jankowski and Bickley, 1976).

Aedes cinereus Meigen was not positively identified from trap collections until September, 1975. The increased incidence of this mosquito in the field at this time (Figure 17) demonstrate its potential to occur in large numbers and Morris and De Foliart found 36.8% of a Wisconsin population to seek more than one blood-meal indicating some potential for an extended life-span. Phillips (1939), under laboratory conditions, found D. immitis developed to the infective stage in this mosquito in 73 of 120 mosquitoes and infective larvae were able to reach the proboscis 12 days after the infective blood meal. Yen (1938) worked with fewer mosquitoes and observed some encapsulation of developing larvae. Maturation to the infective stage did occur and he considered A. cinereus to be highly susceptible to D. immitis infections. A. cinereus, because of its widespread distribution and apparent

suitability as an intermediate host in Michigan (Figure 21), must be considered a potential vector of dog heartworm, in this state.

Aedes excrucians (Walker) was not collected in the Lansing, Michigan area during this study. Phillips (1939) found infective larvae of D. immitis in the labium of this mosquito 15 days after it took an infective blood-meal. Because of its widespread distribution (Figure 23) A. excrucians has potential to be a vector of dog heartworm in Michigan. However its importance as a vector is probably very minor, at least in the Lansing area where its density is apparently very low.

Aedes fitchii (Felt and Young) as shown in Figure 6, was collected from early June through Mid-August. It was readily attracted to the dogs during this study. Carpenter and Nielson (1965) found A. fitchii to go through as many as 4 gonotrophic cycles and live as long as 53 days in nature. Its longevity would make this species an ideal host for D. immitis. Bemrick and Sandholm (1966) found A. fitchii to support larval development of dog heartworm to the infective stage but these larvae were never found outside the Malpighian tubules. They did not consider development complete in this species. Until further evidence is obtained, A. fitchii should not be considered an important vector of dog heartworm in Michigan, in spite of its widespread distribution (Figure 22).

Aedes punctor (Kirby) was not collected in the Lansing, Michigan area. In Europe, Roubaud 2nd Collas-Belcour (1937) found infective larvae of D. immitis were able to migrate to the labium of this mosquito. No further information has been reported about this mosquito and therefore it must be considered a potential vector of dog heartworm where it occurs in Michigan (Figure 24).

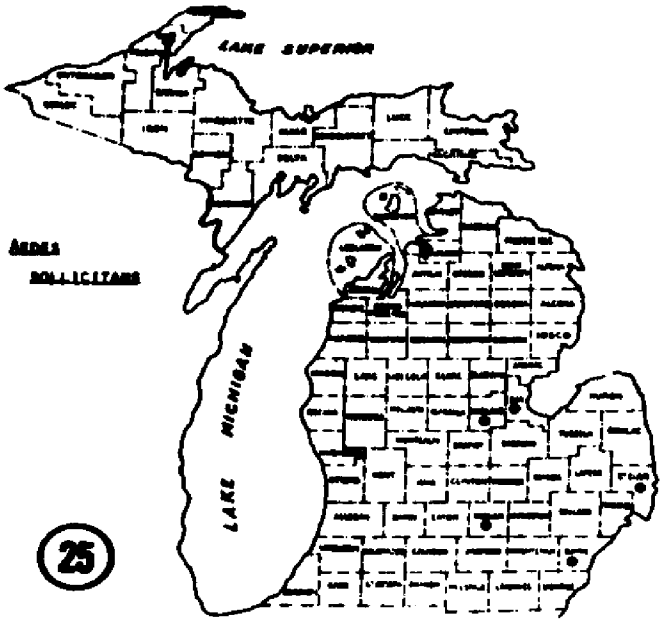
Only one specimen of Aedes sollicitans (Walker) was collected during the course of this project but it has now been collected in 5 Michigan counties (Figure 25). Hu (1931) found this salt marsh mosquito able to support the development of D. immitis to the infective stage. This was confirmed by Summers (1943) and Keegan et al. (1968) who observed third stage larvae in the head of this mosquito. It seems unlikely that this mosquito has much importance in the Lansing area, but it has become quite a nuisance in Marysville, Michigan (H. D. Newson and D. L. McGroarty, Michigan State University, personal communication) and it may be an important vector of dog heartworm in this and other areas where populations of this species are abundant.

Aedes sticticus (Meigen) which is distributed throughout Michigan (Figure 26). It may occur locally in large numbers (Figure 12). Bemrick and Sandholm (1966) did not consider larval development of D. immitis to be complete in this species because infective larvae were not observed outside the Malpighian tubules. It appears unlikely then that this species is a vector of dog heartworm in Michigan.

Aedes stimulans (Walker) has a widespread distribution in Michigan (Figure 27). It does not appear to be a vector of dog heartworm because in the present study microfilariae did not reach the Malpighian tubules of all individuals taking an infective blood meal. Furthermore those larvae which did reach the Malpighian tubules were subject to encapsulation and retardation of development in this species.

Phillips (1939) first experimented with the tree-hole breeding mosquito Aedes triseriatus (Say). His data indicates that this species is an excellent host for D. immitis. Infective larvae migrated to the proboscis of this species in as little as 9 days after the infective

Figs. 25-30. Reported distribution of *Aedes sollicitans*, *A. sticticus*, *A. stimulans*, *A. triseriatus*, *A. trivittatus*, and *A. vexans*, respectively, in Michigan.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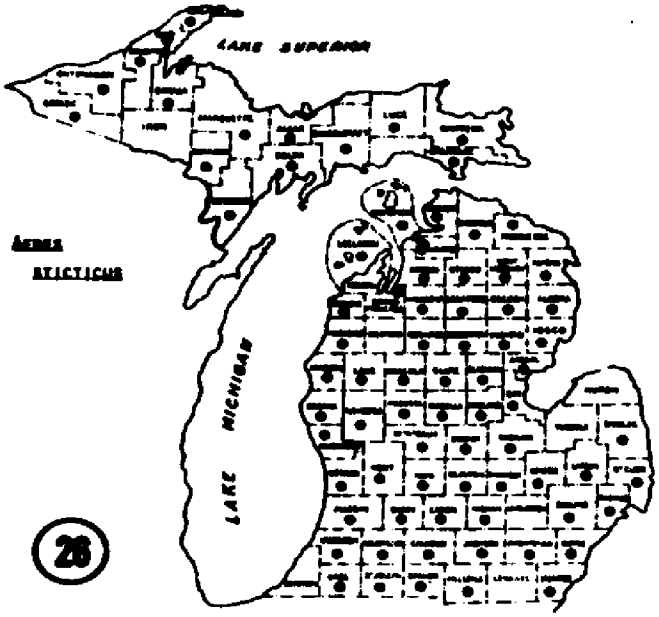
LAKE MICHIGAN
25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LAKE MICHIGAN
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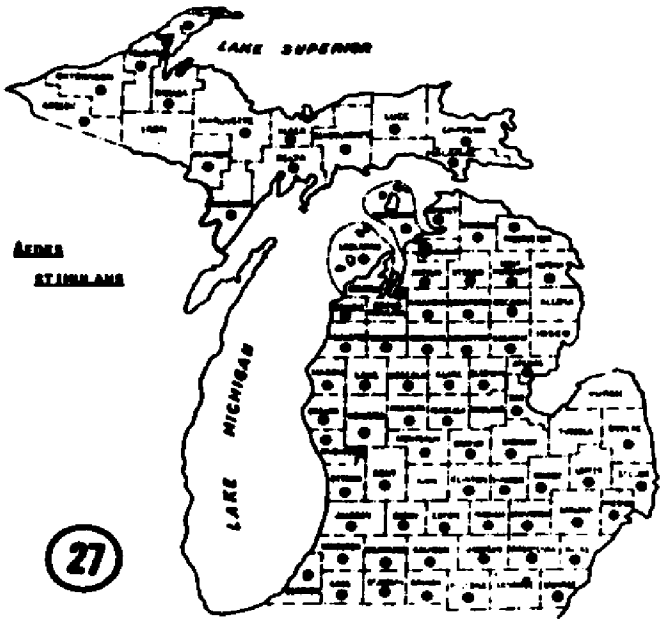
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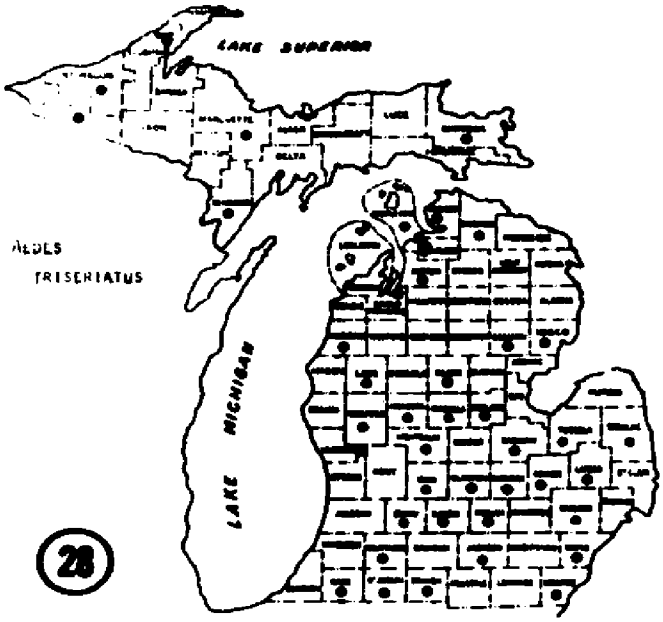
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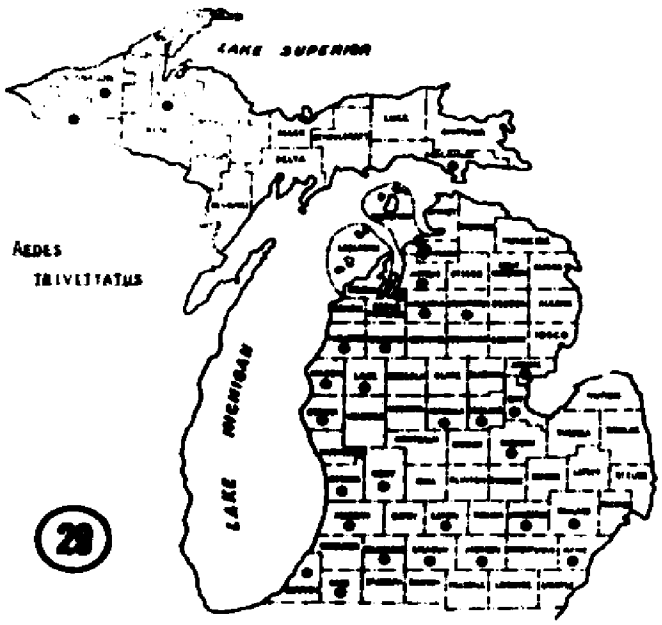
28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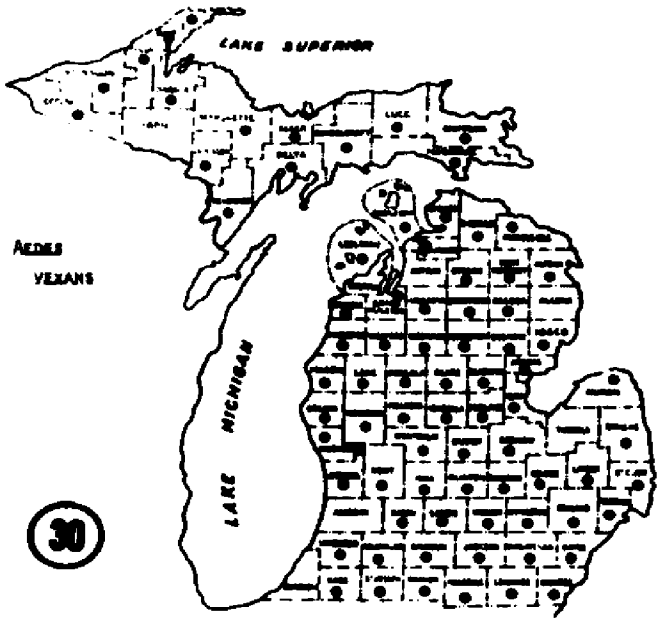
29


LAKE SUPERIOR
LAKE MICHIGAN
30

blood meal and with remarkably low mortality to the mosquitoes. Keegan et al. (1968) found this species ideal for laboratory studies of heartworm especially since it readily fed on dogs. Intermill (1973) also found that A. triseriatus readily fed on dogs but noted that this mosquito was not generally found in large numbers in the field. He reported no histological damage to the Malpighian tubules from the migration of larvae but did observe some encapsulation in those excretory organs. Infective larvae were recovered from the labium in as little as 13 days after the infective blood meal. Similarly Kaska (personal communication) obtained infective larvae in the labium of a Michigan strain of A. triseriatus in only 12 days development time and also noted low mosquito mortality. At least under laboratory conditions A. triseriatus appears to be an ideal vector of D. immitis. Field studies by Morris and DeFoliart (1971) indicate that 35.4% of the females of this species seek more than one blood meal. It may be that this species has adequate longevity to support development of D. immitis under natural conditions. Trap collections indicate that this species is only locally abundant (Tables 1 and 2) although it is widely distributed in Michigan (Figure 28). At Site 5 (Table 2) this species was the fifth most abundant mosquito collected in CDC miniature light traps and was collected consistently throughout the season (Figure 13). A. triseriatus appears to be an excellent host and potential vector of D. immitis. Because of its local distribution it may have only secondary importance in the natural maintenance of this disease.

As shown in Figure 14, Aedes trivittatus (Coquillett) can, at times, be an abundant pest. Occasionally it was collected in the dog-baited traps used in this study (Table 1). Morris and DeFoliart (1971)

estimated that 39.9% of the females in a Wisconsin population sought more than one blood meal. This may indicate sufficient longevity to support development of heartworm under natural conditions. Yen (1938) found that none of 16 A. trivittatus feeding on an infected dog harbored infective larvae and concluded that this species was entirely refractory as a host for D. immitis. On the other hand, Christensen and Andrews (1976) concluded that A. trivittatus is the principal vector of D. immitis in central Iowa. Yen (1938) may have based his conclusions on too little data. Although Cristensen and Andrews (1976) collected for only a two week period, their finding of infective larvae, possibly D. immitis, indicates that this mosquito must be considered to have at least secondary importance as a potential vector of dog heartworm in Michigan. It has been reported from many parts of the state (Figure 29).

Aedes vexans (Meigen) was, by far, the mosquito collected most frequently during this study. Larvae, presumably D. immitis, were extracted from pooled mosquitoes on 3 occasions. In Maryland, Bickley et al. (1976) also isolated possible D. immitis larvae from field-captured specimens and Bemrick and Sandholm (1966) reported 5 isolations of Dirofilaria larvae from field-captured A. vexans. As discussed earlier, laboratory studies indicate that this species is a good host for D. immitis larvae. A. vexans is abundant and present throughout Michigan (Figure 30). It is among the best potential vectors of dog heartworm in this state.

Anopheles

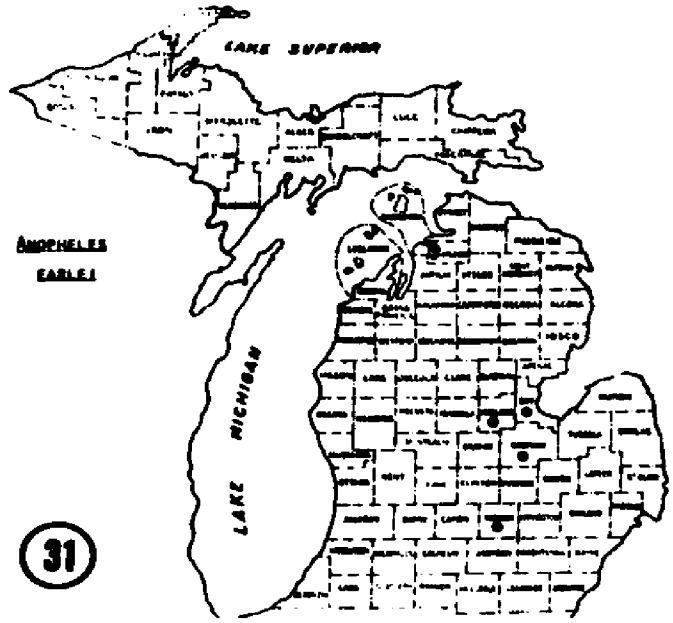
Anopheles spp. typically prefer mammalian hosts (Tempelis, 1975). It will be assumed that all Anopheles species discussed would take a blood-meal from an available dog and satisfy Barnett's criterion 1.

Anopheles earlei Vargas was captured only at Site 5 (Table 2) during this study and is reported from only 4 Michigan counties (Figure 31). Although Bemrick and Sandholm reported third stage larvae of D. immitis in this mosquito, migration to the labium has not yet been demonstrated. For this reason A. earlei must be considered, at best, to have only minor importance as a potential vector of dog heartworm and its ability to support complete development of D. immitis larvae must still be proven.

Anopheles punctipennis (Say) was captured in low numbers (Table 2) at all sites in the Lansing, Michigan area. This species, however, is known from nearly every county in Michigan (Figure 32). Hu (1931) found this species to tolerate high numbers of developing larvae. Yen (1938) agreed with Hu in finding 100% infectivity of mosquitoes taking an infective blood meal. Yen found larvae in the labium in as little as 12 postprandial days. Phillips (1939) likewise found this species an excellent host for D. immitis although he reported some encapsulation. Bickley et al. (1976) have isolated filarid larvae from field-captured specimens of A. punctipennis. This species must be considered a potential vector of dog heartworm in Michigan but because of its low incidence, at least in the Lansing, Michigan area, probably has only minor importance.

Anopheles quadrimaculatus Say was collected at all sites in the study area and was commonly collected in the dog-baited traps (Tables 1

Figs. 31-36. Reported distribution of *Anopheles earlei*, *A. punctipennis*, *A. quadrimaculatus*, *A. walkeri*, *Culex pipiens*, and *C. restuans*, respectively, in Michigan.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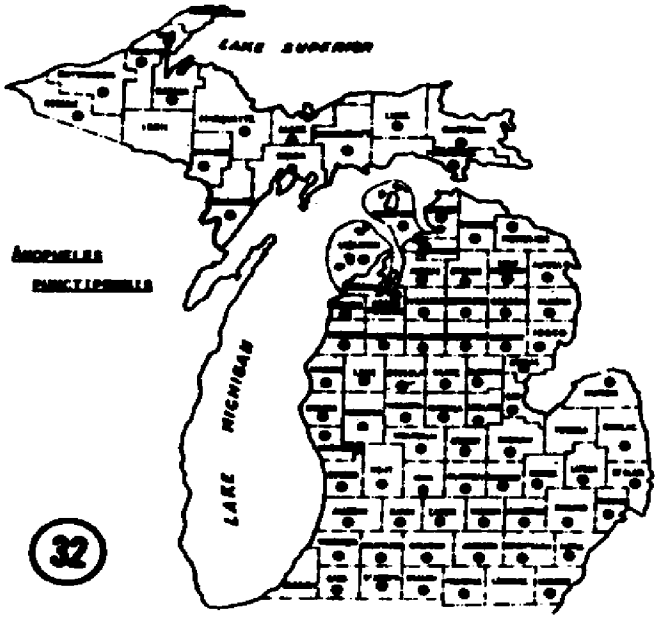
LAKE MICHIGAN
31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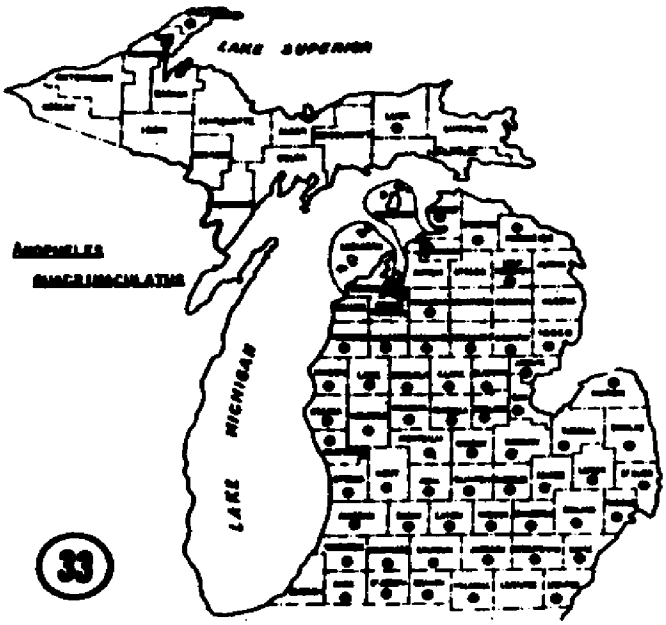
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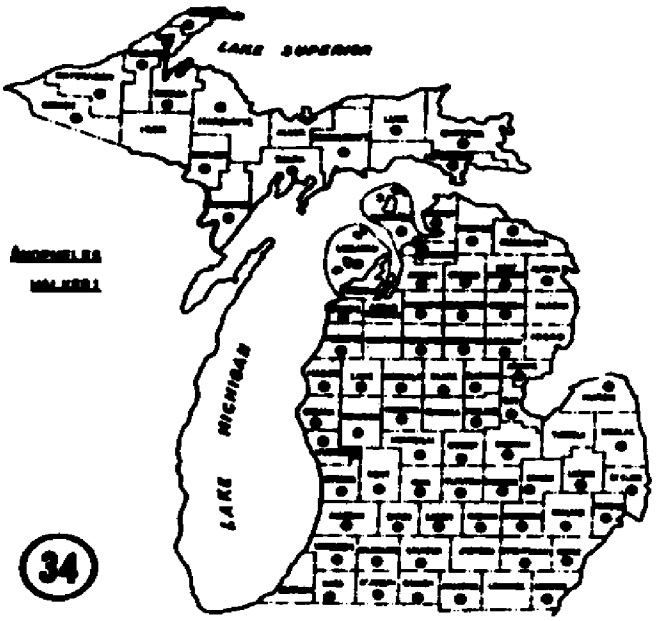
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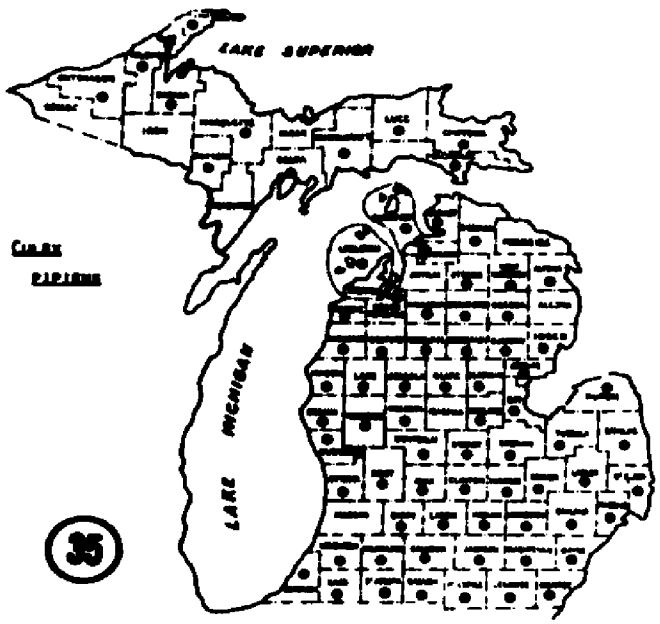
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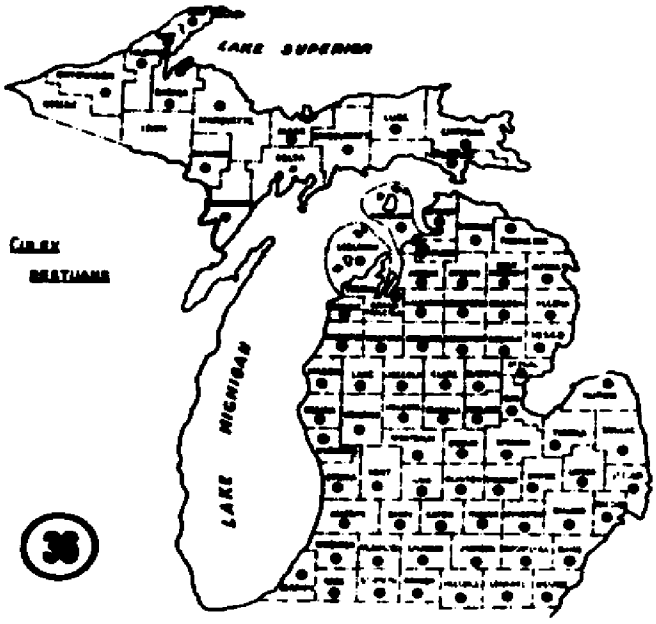
35


LAKE SUPERIOR
LAKE MICHIGAN
36

and 2). At Site 1 fully engorged females were often observed on the walls of the kennels. This species is readily attracted to dogs and is willing to enter buildings to obtain a blood meal. Laboratory studies as well as the finding of filarid larvae in field-captured specimens have already been discussed. These findings along with the widespread distribution of this species in Michigan (Figure 33) supports the hypothesis that this species plays an important role in the natural maintenance of dog heartworm infections.

Anopheles walkeri Theobald was collected at all sites in the Lansing, Michigan area (Tables 1 and 2). This species was collected more frequently than A. quadrimaculatus in one of the dog-baited traps at Site 1, the dog-baited trap at Site 5 and in the CDC miniature light traps set at all of the sites. No D. immitis larvae were isolated from field-captured specimens. Several unsuccessful attempts were made to colonize this mosquito in the laboratory. Bemrick and Sandholm (1966) showed A. walkeri capable of supporting complete development of D. immitis larvae in the laboratory. This mosquito appears to be very abundant in the Lansing area. It is known from nearly every county in Michigan (Figure 34). A. walkeri should be considered a primary potential vector of dog heartworm in Michigan.

Culex

The feeding habits of Culex species are more varied and will be discussed individually.

Culex pipiens Linnaeus, present throughout Michigan (Figure 35) is generally very abundant. Laboratory studies discussed earlier have shown it to be an inefficient host for D. immitis larvae. Additionally,

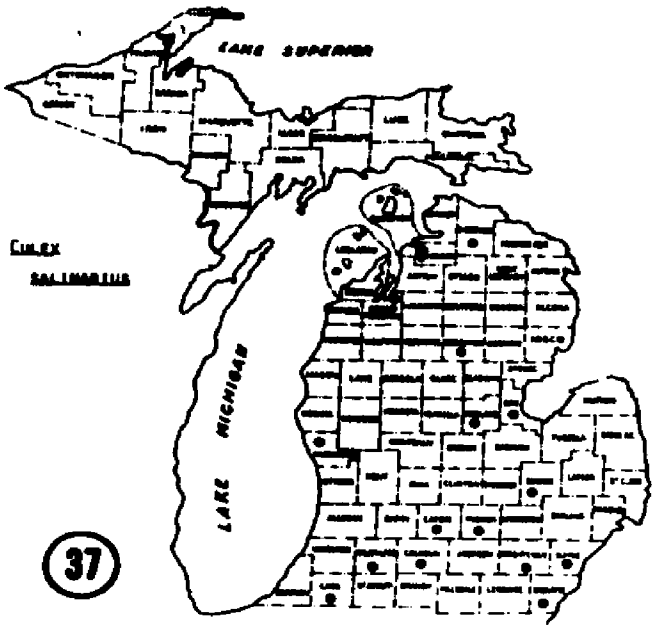
Tempelis (1975) reported that it feeds mainly on birds. Thus, C. pipiens is not likely to be an important vector of dog heartworm in Michigan but it must still be considered a potential vector of D. immitis.

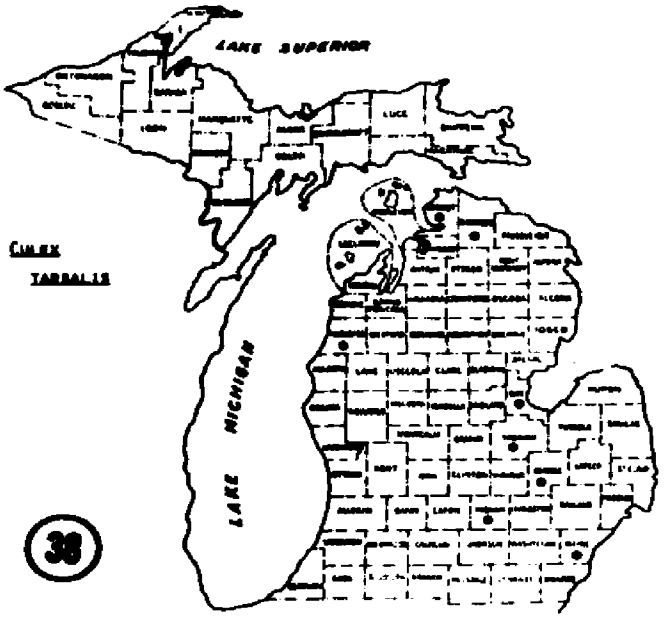
Culex restuans Theobald occurs throughout Michigan (Figure 36) but was rarely collected in the study area (Table 2). Bemrick and Sandholm (1966) concluded that this species was a poor host for dog heartworm but they did observe infective larvae in the head of one mosquito. Because complete development is possible, C. restuans must be considered a potential vector of D. immitis even though this mosquito feeds primarily on birds (Tempelis, 1975).

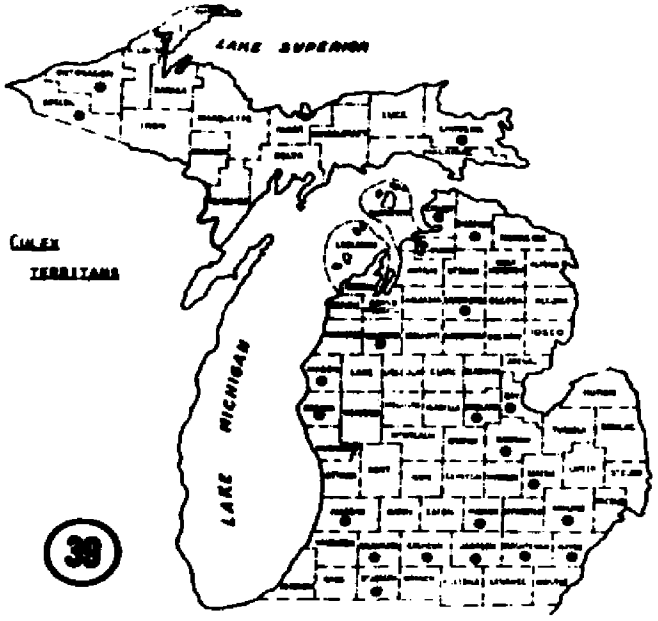
Likewise, Culex salinarius, known from scattered areas in Michigan (Figure 37) was rarely collected during this study. Reports by Crans (1973) and Tempelis (1975) indicate that this species has a wide variety of hosts. Hu (1931) observed only partial development of D. immitis larvae in this species and Summers (1943) did not observe development beyond the sausage stage and reported some encapsulation of larvae. Seeley and Bickley (1974), however, reported complete development of D. immitis in one of three United States' strains of C. salinarius. Bickley et al. (1976) found filarid nematodes believed to be D. immitis in field-captured C. salinarius. Until further information is obtained, this mosquito must be considered to have at least minor importance as a potential vector of dog heartworm in Michigan.

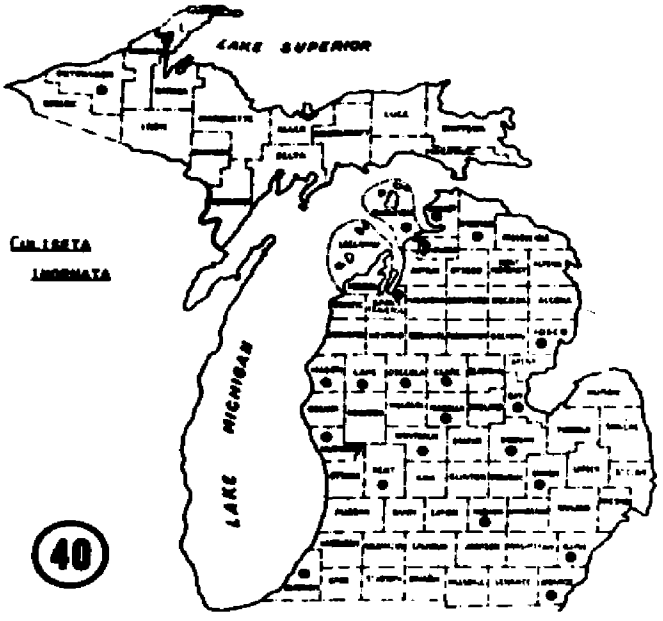
Culex tarsalis Coquillett was collected on only one occasion at Site 2 (Table 2) and to date has been reported from only a few counties in Michigan (Figure 38). Yen (1938) reported that most D. immitis

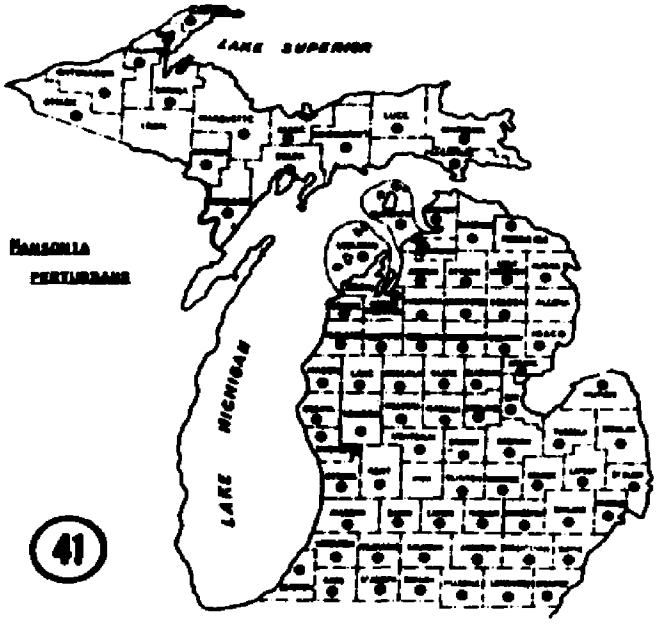
Figs. 37-42. Reported distribution of *Culex salinarius*, *C. tarsalis*, *C. territans*, *Culiseta inornata*, *Mansonia perturbans*, and *Psorophora ferox*, respectively, in Michigan.

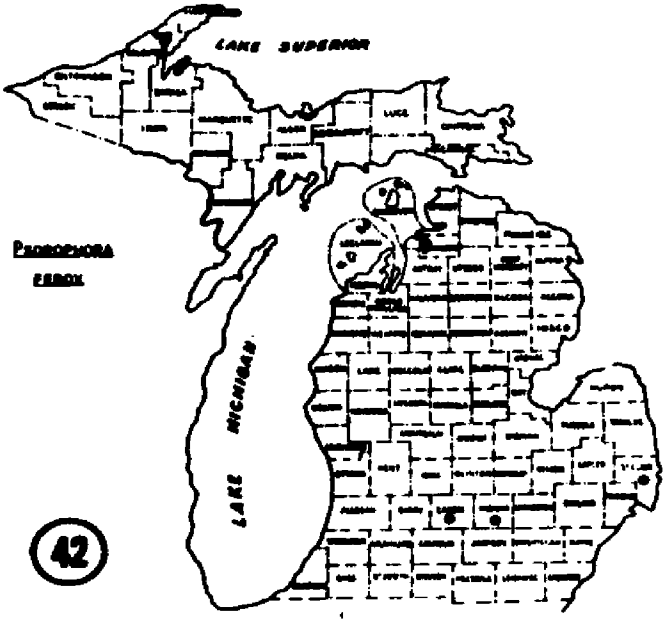

LAKE SUPERIOR
LAKE MICHIGAN
37


LAKE SUPERIOR
LAKE MICHIGAN
38


LAKE SUPERIOR
LAKE MICHIGAN
39


LAKE SUPERIOR
LAKE MICHIGAN
40


LAKE SUPERIOR
LAKE MICHIGAN
41


LAKE SUPERIOR
LAKE MICHIGAN
42

larvae did not become established in the Malpighian tubules of this mosquito.

Never was more than one infective larvae seen in the labium of a mosquito at any one time. Bemrick and Sandholm (1966) had results similar to Yen. In their study 9 of 94 mosquitoes feeding on an infected dog retained D. immitis larvae and all of these were found to have infective larvae in their heads. Tempelis (1965) found that C. tarsalis fed primarily on birds but more readily fed on mammals during the summer. C. tarsalis must be considered a minor potential vector of dog heartworm in Michigan.

Culex territans Walker was trapped on only one occasion at Site 3-B (Table 2) and more commonly at Site 5 (Table 1 and 2). Although Summers (1943) noted some encapsulation of developing larvae in this species, Hu (1931) and Yen (1938) as well as Summers did report complete development of D. immitis to the infective stage. C. territans is known from scattered areas of Michigan (Figure 39) and feeds primarily on amphibians (Crans, 1970). It must be considered at least a minor potential vector of dog heartworm in this state.

Culiseta

Culiseta inornata (Williston) was captured only at Site 3-A Table 2) and is known only from a few counties in Michigan (Figure 40). This species is thought to prefer larger mammals as a blood meal source (Edman et al., 1972). Yen (1938) noted encapsulation and arrested development of D. immitis larvae in this mosquito. Keegan et al. (1968) reported that D. immitis larvae did not develop in C. inornata. It

seems very doubtful that this mosquito is a vector of dog heartworm in Michigan.

Mansonia

Mansonia perturbans (Walker) was collected very frequently in the study area (Tables 1 and 2) and has been reported throughout Michigan (Figure 41). It was captured more than any other mosquito in dog-baited traps at Site 1 (Table 1). For these reasons it was selected for laboratory studies. Results of this study (discussed earlier), in agreement with Yen (1938) have shown that this species is an unacceptable host for D. immitis larvae. It cannot be considered a vector of dog heartworm in Michigan.

Psorophora

Psorophora ferox (Humboldt) was captured only at Site 5 and then only one specimen was trapped (Table 2). This species has been reported from only three Michigan counties (Figure 42) Edman (1971) reports that it prefers mammalian hosts as a blood-meal source. In 1954 Steuben (Steuben, 1954) reported the recovery of infective larvae from this mosquito but details of his findings are incomplete. Because of this report P. ferox must be considered a potential vector of dog heartworm in Michigan but its apparent low density indicates that at best it has only minor importance as a potential vector in this state.

## SUMMARY AND CONCLUSIONS

1. Mosquitoes were collected in dog-baited and CDC miniature light traps at 7 sites in the Lansing, Michigan area. These traps worked well together to determine which mosquitoes were attracted to dogs in the study area and to indicate their local abundance.
2. Trapping results showed that Aedes cinereus, A. fitchii, A. sticticus, A. stimulans, A. triseriatus, A. vexans, Anopheles quadrimaculatus, A. walkeri, Culex pipiens, and Mansonia perturbans were the most abundant species attracted to dogs in the study area.
3. Mosquitoes were brought to the laboratory to be identified and a pooling technique was used to isolate filarid larvae from field-captured mosquitoes.
4. Suspected D. immitis larvae were extracted from field-captured specimens of Aedes vexans, Anopheles quadrimaculatus and Culex pipiens.
5. A histochemical stain developed by Chalifaux and Hunt (1971) was used to try to differentiate Dirofilaria immitis and D. tenuis.
6. The results of the histochemical stain showed that D. immitis and D. tenuis could not be differentiated after being treated by this method. Thus, filarid larvae isolated from field-captured mosquitoes could not be positively identified.
7. Based on field collection results, Aedes stimulans, A. vexans, Anopheles quadrimaculatus, Culex pipiens, and Mansonia perturbans

were selected for D. immitis developmental studies in the laboratory. Aedes vexans, A. stimulans and Anopheles quadrimaculatus also were used in transmission attempts.

8. It was postulated that the ideal vector of dog heartworm, at least in the Lansing, Michigan area, should have a life-span of a minimum of 30 days under natural conditions.

9. Developmental studies showed Anopheles quadrimaculatus to be an excellent host for D. immitis larvae. All specimens taking an infective blood meal became infected and there was no observed encapsulation of developing larvae. Additionally, A. quadrimaculatus seemed to tolerate a heavier parasite load than Aedes vexans. Aedes vexans was also an efficient host but some encapsulation was observed and not all individuals taking an infective blood meal became infected. Culex pipiens supported development of D. immitis larvae but is an extremely inefficient host. Aedes stimulans and Mansonia perturbans did not support complete development of D. immitis larvae.

10. D. immitis developmental studies indicate that the larvae isolated from field-captured Culex pipiens were not D. immitis larvae.

11. Efficiency of a mosquito as a host for D. immitis larvae must be determined locally.

12. Twenty four potential mosquito vectors are known to occur in Michigan. Their suspected importance as vectors of dog heartworm in this state are given in Table 7. Anopheles quadrimaculatus and Aedes vexans appear to be the mosquito species most likely involved in the natural maintenance of D. immitis in Michigan. Anopheles walkeri may be equally important.

Table 7

Hypothesized Importance of Various Mosquitoes as Vectors of Dog Heartworm in the Lansing, Michigan Area

| Species | Collected During Study | Importance as a Vector | | | |
|---|---|---|---|---|---|
| | | Primary | Secondary | Minor | Doubtful |
| Aedes | | | | | |
| atropalpus | - | | | | X |
| canadensis | + | | X | | |
| cinereus | + | | X | | |
| fitchii | + | | | | X |
| excrucians | - | | | X | |
| punctor | - | | | | X |
| sollicitans | + | | | X | |
| sticticus | + | | | | X |
| stimulans | + | | | | X |
| triseriatus | + | | X | | |
| trivittatus | + | | X | | |
| vexans | + | X | | | |
| Anopheles | | | | | |
| earlei | + | | | X | |
| punctipennis | + | | | X | |
| quadrimaculatus | + | X | | | |
| walkeri | + | X | | | |
| Culex | | | | | |
| pipiens | + | | | X | |
| restuans | + | | | X | |
| salinarius | + | | | X | |
| tarsalis | + | | | X | |
| territans | + | | | X | |
| Culiseta | | | | | |
| inornata | + | | | | X |
| Mansonia | | | | | |
| perturbans | + | | | | X |
| Psorphora | | | | | |
| ferox | + | | | X | |

# APPENDICES

## APPENDIX A

Mosquitoes Collected in a Dog-baited Trap at Site #1 in a Woodland Area, 1974

| Species Month/Day | 6/13 | 6/18 | 6/20 | 7/1 | 7/3 | 7/7 | 7/10 | 7/16 | 7/18 | 7/24 | 7/30 | 7/31 | 8/5 | 8/27 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aedes canadensis | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | 1 |
| communis complex[a] | 2 | 1 | 4 | - | - | - | - | - | - | - | - | - | - | - | 7 |
| fitchii-stimulans | 7 | 14 | 23 | 1 | - | 1 | 1 | 1 | 2 | 1 | - | - | - | - | 51 |
| triseriatus | - | - | - | - | - | 1 | - | - | - | - | - | 7 | 3 | - | 11 |
| vexans | 1 | 16 | 11 | 2 | - | 1 | - | - | - | 1 | - | - | - | 2 | 34 |
| Anopheles quadrimaculatus | - | - | - | - | - | - | - | - | 2 | - | - | 2 | 1 | - | 5 |
| walkeri | - | - | - | - | - | - | - | | - | - | 1 | - | - | - | 1 |
| Culex pipiens | - | - | - | - | - | 8 | 13 | 2 | 1 | 12 | 38 | 29 | 41 | 40 | 184 |
| Mansonia perturbans | 1 | 5 | 17 | 51 | 22 | 42 | 44 | 48 | 21 | 22 | 66 | 40 | 42 | 2 | 423 |
| TOTAL | 11 | 36 | 55 | 55 | 22 | 53 | 58 | 51 | 26 | 36 | 105 | 78 | 87 | 44 | 717 |

[a]Includes *Aedes cinereus* and excludes *A. aurifer* and *A. sticticus*.

## APPENDIX B

Mosquitoes Collected in a Dog-baited Trap at Site #1 in an Open Area, 1974

| Species Month/Day | 6/4 | 6/18 | 6/20 | 7/1 | 7/3 | 7/10 | 7/16 | 7/18 | 7/24 | 7/30 | 7/31 | 8/5 | 8/26 | 8/27 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aedes | | | | | | | | | | | | | | | |
| canadensis | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 |
| communis complex[a] | - | - | 2 | 2 | - | - | - | - | - | - | - | - | - | - | 4 |
| fitchii-stimulans | 6 | - | 1 | - | - | - | 1 | - | - | 1 | - | - | - | - | 9 |
| triseriatus | 13 | 5 | 1 | - | - | - | - | - | - | - | - | - | - | - | 19 |
| vexans | 71 | 33 | 19 | 3 | 7 | - | 1 | - | 4 | - | - | - | 10 | 1 | 149 |
| Anopheles | | | | | | | | | | | | | | | |
| quadrimaculatus | - | - | - | - | - | - | 2 | 44 | 1 | 24 | 11 | 8 | 1 | 1 | 92 |
| walkeri | 60 | 1 | 3 | 29 | 5 | 4 | 1 | - | 9 | - | - | - | - | - | 112 |
| Culex | | | | | | | | | | | | | | | |
| pipiens | - | - | - | 5 | 1 | 22 | 6 | 18 | 2 | 20 | 11 | 16 | 29 | 28 | 168 |
| Mansonia | | | | | | | | | | | | | | | |
| perturbans | - | 2 | 4 | 120 | 37 | 132 | 83 | 4 | 39 | 11 | 10 | 15 | 2 | 2 | 461 |
| Total | 152 | 41 | 30 | 159 | 50 | 158 | 94 | 66 | 55 | 56 | 32 | 39 | 52 | 32 | 1,016 |

[a]Includes Aedes cinereus and excludes A. aurifer and A. sticticus.

APPENDIX C

CDC Trap Collections at Site #2, 1975

| Species Month/Day | 6/8 | 6/15 | 6/22 | 6/29 | 7/6 | 7/13 | 7/20 | 7/27 | 8/3 | 8/10 | 8/17 | 8/24 | 8/31 | 9/7 | 9/14 | 9/21 | 9/28 | 10/5 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aedes | | | | | | | | | | | | | | | | | | | |
| canadensis | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| communis complex[a] | 7 | - | 6 | - | - | 1 | 1 | - | 9 | 3 | 1 | - | - | 7 | 1 | - | - | 1 | 37 |
| fitchii-stimulans | - | 4 | 27 | 3 | 3 | 2 | 2 | - | 2 | 1 | - | - | - | - | - | - | - | - | 44 |
| flavescens | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| sticticus | - | 7 | 18 | 2 | 1 | 4 | 1 | 5 | 19 | 5 | - | - | - | 72 | 31 | 1 | 26 | - | 192 |
| triseriatus | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | 1 |
| trivittatus | - | - | 8 | - | 2 | 3 | 1 | - | 22 | 12 | 4 | 1 | - | 982 | 25 | 1 | 33 | 8 | 1,102 |
| vexans | 2 | 17 | 443 | 28 | 215 | 219 | 375 | 238 | 1,012 | 1,043 | 203 | 276 | 783 | 13,895 | 626 | 50 | 143 | 202 | 19,770 |
| Anopheles | | | | | | | | | | | | | | | | | | | |
| punctipennis | - | - | - | 1 | - | - | 2 | - | 2 | 10 | 1 | 8 | 1 | 9 | - | - | - | - | 34 |
| quadrimaculatus | - | - | 1 | - | 4 | 7 | - | - | 10 | 10 | 12 | - | - | - | 1 | - | - | - | 45 |
| walkeri | - | 1 | 1 | 3 | - | - | - | - | 5 | 5 | 5 | 5 | - | 29 | 1 | - | - | - | 55 |
| Culex | | | | | | | | | | | | | | | | | | | |
| pipiens | - | - | 2 | - | 3 | 5 | 5 | - | 12 | 8 | 4 | 11 | 23 | 127 | 21 | 1 | - | 5 | 227 |
| salinarius | - | - | - | - | - | 1 | - | - | - | - | - | - | - | 7 | - | - | - | - | 8 |
| tarsalis | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | 1 |
| Mansonia | | | | | | | | | | | | | | | | | | | |
| perturbans | - | - | 36 | 19 | 147 | 71 | 18 | 59 | 14 | 8 | - | 1 | - | 2 | - | - | - | - | 375 |
| Orthopodomyia | | | | | | | | | | | | | | | | | | | |
| spp. | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | 1 |
| Psorophora | | | | | | | | | | | | | | | | | | | |
| ciliata | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 | - | - | - | - | 2 |
| TOTAL | 9 | 29 | 543 | 57 | 375 | 313 | 405 | 302 | 1,107 | 1,105 | 231 | 303 | 807 | 15,133 | 706 | 53 | 202 | 216 | 21,896 |

[a]Includes Aedes cinereus and excludes A. aurifer and A. sticticus.

APPENDIX D

CDC Trap Collections at Site #3 - A, 1975

| Species | 6/8 | 6/15 | 6/22 | 6/29 | 7/6 | 7/13 | 7/20 | 7/27 | 8/3 | 8/10 | 8/17 | 8/24 | 8/31 | 9/7 | 9/14 | 9/21 | 9/28 | 10/5 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Aedes** | | | | | | | | | | | | | | | | | | | |
| canadensis | - | - | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 |
| cinereus | - | - | - | - | - | - | - | - | - | - | - | - | - | 7 | 6 | 4 | 15 | - | 32 |
| communis complex[a] | 10 | - | 2 | 1 | - | - | - | - | 3 | 1 | - | 1 | - | - | - | - | - | - | 18 |
| dorsalis | - | - | 1 | - | - | - | - | - | - | - | - | - | - | 1 | 9 | - | - | - | 11 |
| fitchii-stimulans | 24 | 9 | 33 | 12 | 6 | 1 | - | 3 | - | - | - | - | - | - | - | - | - | - | 88 |
| flavescens | 2 | 4 | 35 | 8 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 49 |
| sollicitans | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | 1 |
| sticticus | 6 | - | 5 | - | - | 1 | 1 | - | 1 | 3 | - | - | 1 | 22 | 42 | - | 13 | 1 | 96 |
| triseriatus | - | - | - | - | - | - | - | - | 1 | - | 1 | - | - | - | - | - | - | - | 2 |
| trivittatus | - | - | - | - | 2 | 2 | 2 | 1 | 3 | 1 | - | - | 8 | 228 | 42 | - | 38 | - | 327 |
| vexans | 8 | 21 | 185 | 129 | 612 | 97 | 133 | 128 | 390 | 1,037 | 27 | 67 | 873 | 6,281 | 1,557 | 416 | 680 | 64 | 12,705 |
| **Anopheles** | | | | | | | | | | | | | | | | | | | |
| punctipennis | 1 | - | - | 1 | 1 | 2 | 1 | - | - | 5 | - | - | 3 | 2 | 1 | - | - | 1 | 18 |
| quadrimaculatus | - | - | - | 1 | 3 | 5 | 2 | - | - | 5 | - | - | 1 | 1 | 1 | - | - | - | 19 |
| walkeri | - | 20 | 23 | 34 | 22 | 13 | - | 19 | 3 | 26 | 1 | 9 | 37 | 63 | 3 | 15 | 11 | 1 | 300 |
| **Culex** | | | | | | | | | | | | | | | | | | | |
| pipiens | 3 | 2 | 4 | 7 | 23 | 57 | 28 | 14 | 34 | 7 | 1 | 1 | 63 | 25 | 22 | 1 | - | 1 | 293 |
| salinarius | - | - | - | - | - | - | - | - | - | - | - | - | 9 | 6 | - | - | - | - | 15 |
| **Culiseta** | | | | | | | | | | | | | | | | | | | |
| inornata | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| **Mansonia** | | | | | | | | | | | | | | | | | | | |
| perturbans | - | 1 | 11 | 8 | 33 | 14 | 3 | 6 | 3 | 3 | - | 1 | 2 | 2 | - | - | - | - | 87 |
| TOTAL | 54 | 57 | 302 | 201 | 702 | 192 | 170 | 171 | 438 | 1,088 | 30 | 79 | 998 | 6,638 | 1,683 | 436 | 757 | 68 | 14,064 |

[a]Includes Aedes cinereus and excludes A. aurifer and A. sticticus.

APPENDIX E

CDC Trap Collections at Site #3 - B, 1975

| Species Month/Day | 6/15 | 6/22 | 6/29 | 7/6 | 7/13 | 7/20 | 7/27 | 8/3 | 8/10 | 8/17 | 8/24 | 8/31 | 9/7 | 9/14 | 9/21 | 9/28 | 10/5 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aedes | | | | | | | | | | | | | | | | | | |
| canadensis | - | 1 | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 3 |
| cinereus | - | 8 | - | 1 | - | - | - | - | - | - | - | - | 5 | 3 | 3 | 18 | - | 38 |
| communis complex[a] | - | - | 1 | - | - | - | - | 3 | 3 | - | - | - | - | - | - | - | - | 7 |
| dorsalis | - | - | - | - | - | - | - | - | - | - | - | - | - | 11 | - | 1 | - | 12 |
| fitchii-stimulans | 4 | 61 | 105 | 27 | 8 | 3 | - | 4 | 2 | - | - | - | - | - | - | - | - | 214 |
| flavescens | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | 1 |
| sticticus | 1 | 1 | 2 | - | - | 1 | 3 | - | 1 | - | 1 | 2 | 9 | 13 | - | 12 | - | 46 |
| triseriatus | - | 1 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 |
| trivittatus | - | 1 | 1 | 2 | - | 2 | 2 | - | 6 | 1 | 1 | - | 247 | 40 | - | 38 | 3 | 344 |
| vexans | 2 | 105 | 123 | 822 | 194 | 235 | 211 | 88 | 447 | 18 | 200 | 409 | 6,877 | 336 | 68 | 184 | 157 | 10,476 |
| Anopheles | | | | | | | | | | | | | | | | | | |
| punctipennis | - | 2 | 2 | 2 | 3 | 6 | - | 1 | 1 | - | - | 6 | 12 | 1 | - | - | - | 36 |
| quadrimaculatus | - | - | - | 3 | - | - | 1 | 3 | - | 1 | - | - | - | - | - | - | - | 8 |
| walkeri | - | 3 | 3 | 1 | - | - | 7 | - | 4 | - | 8 | 6 | 16 | - | - | 4 | 3 | 55 |
| Culex | | | | | | | | | | | | | | | | | | |
| pipiens | - | - | 5 | 3 | 25 | 19 | 4 | 3 | 2 | 1 | 1 | 57 | 28 | 25 | - | 1 | 6 | 180 |
| restuans | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 1 |
| salinarius | - | - | - | 1 | - | - | - | - | - | - | - | 3 | - | - | - | - | - | 4 |
| territans | - | - | - | - | - | - | - | - | - | - | - | - | 2 | - | - | - | - | 2 |
| Mansonia | | | | | | | | | | | | | | | | | | |
| perturbans | - | 5 | - | 18 | 10 | 11 | 3 | 1 | 2 | - | - | - | 3 | - | - | - | - | 53 |
| TOTAL | 7 | 188 | 245 | 880 | 240 | 277 | 232 | 103 | 468 | 21 | 211 | 483 | 7,199 | 429 | 71 | 258 | 170 | 11,482 |

[a] Includes Aedes cinereus and excludes A. aurifer and A. sticticus.

APPENDIX F

Mosquitoes Collected in a Dog-baited Trap at Site #4, 1975

| Species | Month/Day | 6/24 | 6/26 | 7/1 | 7/3 | 7/8 | 7/10 | 7/15 | 7/17 | 7/24 | 7/29 | 7/31 | 8/5 | 8/7 | 8/12 | 8/14 | 8/19 | 8/21 | 8/26 | 9/2 | 9/10 | 9/11 | 9/16 | 9/18 | 9/23 | 9/25 | 10/2 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aedes | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| canadensis | | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| fitchii-stimulans | | - | - | - | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 2 |
| triseriatus | | - | - | - | 1 | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 3 |
| vexans | | - | - | - | - | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 3 |
| Anopheles | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| quadrimaculatus | | - | - | - | - | 2 | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 4 |
| Culex | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| pipiens | | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| Mansonia | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| perturbans | | - | - | - | 2 | 1 | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 5 |
| TOTAL | | 0 | 0 | 0 | 5 | 9 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19 |

APPENDIX C

CDC Trap Collections at Site #4, 1975

| Species | Month/Day | 6/10 | 6/19 | 6/24 | 6/26 | 7/1 | 7/3 | 7/8 | 7/10 | 7/15 | 7/17 | 7/22 | 7/24 | 7/29 | 7/31 | 8/5 | 8/7 | 8/12 | 8/14 | 8/19 | 8/21 | 8/26 | 8/28 | 9/2 | 9/4 | 9/9 | 9/11 | 9/16 | 9/18 | 9/23 | 9/25 | 10/3 | 10/7 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aedes | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| aurifer | | - | 2 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 3 |
| canadensis | | 25 | 20 | 7 | 15 | 12 | 16 | 2 | - | 1 | 4 | - | 6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 108 |
| cinereus | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 19 | 10 | 6 | 2 | - | 2 | 39 |
| communis complex[a] | | 8 | 6 | 2 | 8 | 5 | 3 | 3 | - | 2 | - | - | - | - | - | 7 | 5 | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | 50 |
| dorsalis | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | 2 | - | - | - | - | - | - | - | 3 |
| fitchii-stimulans | | 45 | 129 | 109 | 119 | 66 | 68 | 17 | 11 | 8 | 5 | 7 | 6 | - | - | 1 | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 593 |
| sticticus | | 24 | 24 | 7 | 4 | 6 | 5 | 5 | 2 | 1 | - | - | 3 | 1 | - | - | - | - | - | - | - | - | 1 | - | 8 | 11 | - | 3 | 5 | 7 | - | - | - | 117 |
| triseriatus | | - | 3 | 3 | 12 | 15 | 26 | 5 | 2 | 5 | 3 | 7 | 3 | 4 | 5 | 5 | 3 | 9 | 10 | 7 | 2 | 34 | 24 | 14 | 9 | 13 | - | 5 | 5 | 2 | - | - | - | 236 |
| trivittatus | | - | - | 2 | 6 | 7 | 15 | 9 | 3 | 6 | 3 | 34 | 25 | 14 | 7 | 27 | 24 | 10 | 5 | 2 | 2 | 8 | 1 | 30 | 120 | 110 | 13 | 56 | 123 | 73 | 2 | - | 64 | 801 |
| vexans | | 135 | 141 | 64 | 239 | 42 | 239 | 177 | 51 | 48 | 70 | 65 | 30 | 38 | 26 | 85 | 50 | 17 | 40 | 28 | 8 | 118 | 62 | 423 | 1,831 | 248 | 275 | 655 | 456 | 289 | 27 | - | 66 | 6,044 |
| Anopheles | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| punctipennis | | 2 | - | 3 | 12 | 3 | 3 | 1 | - | 1 | 1 | 1 | 1 | 2 | - | 4 | 1 | 2 | 6 | - | 2 | 15 | 3 | - | 1 | - | - | - | - | - | - | - | 1 | 65 |
| quadrimaculatus | | 3 | 2 | 7 | 4 | 11 | 26 | 10 | 10 | 33 | 47 | 24 | 21 | 41 | 23 | 9 | 1 | 38 | 27 | 4 | 13 | 9 | 25 | 2 | 1 | 2 | - | - | 2 | 1 | - | - | - | 396 |
| walkeri | | - | - | - | 3 | 4 | 1 | 1 | - | 1 | - | 115 | 1 | 1 | 1 | - | - | - | - | - | - | 1 | - | - | 1 | - | - | 3 | 1 | - | - | - | - | 134 |
| Culex | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| pipiens | | 3 | 2 | 3 | 9 | 2 | 2 | 1 | - | 6 | 8 | 4 | 5 | 2 | - | 2 | 2 | 4 | 9 | 1 | 4 | 7 | 7 | 2 | 4 | 6 | 6 | 3 | 4 | 1 | - | 1 | - | 110 |
| restuans | | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| salinarius | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | 1 |
| Culiseta | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| inpatiens | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | 1 |
| Mansonia | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| perturbans | | 5 | 11 | 2 | 6 | 3 | 28 | 7 | 6 | 11 | 12 | 21 | 8 | 2 | 2 | - | - | 1 | 1 | 1 | 1 | 4 | 5 | - | - | - | - | - | - | - | - | - | - | 137 |
| Uranotaenia | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| sapphirini | | - | - | 1 | - | - | - | - | - | - | - | - | - | - | 4 | - | - | 1 | 2 | - | 5 | - | 1 | - | - | - | - | - | - | - | - | - | - | 14 |
| TOTAL | | 251 | 340 | 211 | 437 | 176 | 432 | 238 | 85 | 123 | 153 | 278 | 109 | 105 | 68 | 143 | 86 | 83 | 100 | 43 | 37 | 196 | 130 | 471 | 1,975 | 393 | 294 | 745 | 606 | 379 | 32 | 1 | 133 | 8,853 |

[a]Includes Aedes cinereus and excludes A. aurifer and A. sticticus.

## APPENDIX H

Mosquitoes Collected in a Dog-baited Trap at Site #5, 1975

| Species | Month/Day | 6/25 | 6/30 | 7/2 | 7/7 | 7/9 | 7/14 | 7/16 | 7/21 | 7/23 | 7/28 | 8/4 | 8/6 | 8/11 | 8/18 | 8/20 | 8/25 | 8/27 | 9/1 | 9/3 | 9/8 | 9/15 | 9/17 | 9/24 | 9/29 | 10/6 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aedes | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| canadensis | | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| cinereus | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 30 | 16 | - | - | - | 46 |
| communis complex[a] | | 2 | - | - | 2 | - | 1 | - | - | - | - | 3 | - | 1 | - | - | - | - | - | 2 | - | - | - | - | - | - | 11 |
| fitchii-stimulans | | - | 3 | 3 | 1 | 1 | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 9 |
| sticticus | | - | - | - | - | - | - | - | - | - | - | 2 | - | - | - | - | - | - | - | - | - | 1 | 1 | - | - | - | 4 |
| trivittatus | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | 6 | - | - | - | 7 |
| vexans | | - | - | - | - | - | 2 | 1 | - | - | - | 5 | - | 1 | - | - | - | - | - | 50 | - | 11 | 4 | - | - | - | 74 |
| Anopheles | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| quadrimaculatus | | 6 | 7 | 16 | 3 | 2 | 16 | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 51 |
| walkeri | | 31 | 2 | 17 | 5 | 2 | 5 | 10 | 1 | 11 | - | 1 | - | 2 | - | - | - | - | - | - | - | 11 | 33 | - | - | - | 131 |
| Culex | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| pipiens | | 1 | - | 1 | 2 | - | 1 | 1 | - | 1 | - | - | - | - | - | - | - | - | - | 2 | - | - | - | - | - | - | 9 |
| territans | | - | - | - | - | 2 | 2 | 16 | - | 8 | - | - | - | - | - | - | - | - | - | 3 | - | - | - | - | - | - | 31 |
| Culiseta | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| impatiens | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 2 | - | - | - | 3 |
| Mansonia | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| perturbans | | 2 | 10 | 2 | 4 | 5 | 19 | 4 | 3 | 9 | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 59 |
| TOTAL | | 42 | 22 | 39 | 17 | 12 | 46 | 32 | 6 | 29 | 0 | 13 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 58 | 0 | 54 | 62 | 0 | 0 | 0 | 436 |

[a] Includes *Aedes cinereus* and excludes *A. aurifer* and *A. sticticus*.

APPENDIX I

CDC Trap Collections at Site #5, 1975

| Species Month/Day | 6/9 | 6/11 | 6/18 | 6/23 | 6/25 | 6/30 | 7/2 | 7/7 | 7/9 | 7/14 | 7/16 | 7/21 | 7/23 | 7/28 | 7/30 | 8/4 | 8/6 | 8/11 | 8/14 | 8/18 | 8/20 | 8/25 | 8/27 | 9/1 | 9/3 | 9/8 | 9/15 | 9/17 | 9/24 | 9/29 | 10/6 | 10/13 | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Aedes** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *aurifer* | - | 1 | 5 | 3 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 10 |
| *canadensis* | 12 | 10 | 6 | 2 | 2 | 1 | 1 | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 36 |
| *cinereus* | - | - | - | - | - | - | 1 | 2 | - | - | - | - | - | - | - | - | - | - | 7 | - | - | - | - | 5 | 10 | 30 | 1,302 | 599 | 1 | 35 | 14 | 34 | 2,040 |
| *communis complex*[a] | 150 | 11 | 62 | 18 | 8 | 7 | 1 | 4 | 4 | 5 | 1 | - | 1 | 1 | 2 | 43 | 35 | 9 | - | 1 | - | 1 | 3 | 12 | - | 2 | - | - | - | - | - | - | 381 |
| *dorsalis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 3 | - | 1 | - | - | - | - | 4 |
| *fitchii-stimulans* | 124 | 49 | 136 | 131 | 47 | 34 | 32 | 21 | 20 | 18 | 1 | 3 | 5 | 1 | - | 1 | 5 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 628 |
| *sticticus* | - | 80 | 47 | 12 | 7 | - | 2 | 8 | 14 | 4 | - | - | - | 2 | 19 | 85 | 30 | 8 | 9 | 1 | 2 | 2 | 1 | - | 1 | 493 | 240 | 127 | - | 8 | 23 | 6 | 1,231 |
| *trisseriatus* | - | - | - | 3 | - | 2 | 2 | 1 | - | - | - | - | - | - | - | - | - | 2 | 6 | - | - | - | 2 | 2 | 1 | 1 | - | - | - | - | - | - | 22 |
| *trivittatus* | - | - | - | 2 | 2 | 2 | 5 | 1 | 3 | - | 1 | - | - | 1 | 4 | 26 | 23 | 2 | 12 | 3 | 3 | 2 | 4 | 11 | 50 | 318 | 186 | 85 | 1 | 19 | 18 | 3 | 787 |
| *vexans* | 11 | 18 | 120 | 112 | 48 | 23 | 15 | 503 | 93 | 102 | 100 | 24 | 137 | 129 | 1,430 | 735 | 133 | 225 | 601 | 313 | 230 | 323 | 86 | 1,452 | 5,955 | 1,706 | 1,805 | 3,527 | - | 202 | 68 | 211 | 20,437 |
| **Anopheles** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *earlei* | - | - | - | 2 | 4 | - | 2 | 2 | 1 | 2 | 2 | - | 11 | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 27 |
| *punctipennis* | - | - | 2 | 2 | 1 | - | 3 | 3 | - | 1 | 1 | - | 2 | - | 2 | 2 | - | - | 3 | 3 | 3 | 2 | 3 | 4 | - | - | - | - | - | - | - | - | 37 |
| *quadrimaculatus* | 20 | 7 | 19 | 75 | 26 | 118 | 131 | 331 | 246 | 255 | 30 | 6 | 184 | 61 | 157 | 149 | 24 | 50 | 100 | 140 | 59 | 11 | 12 | 45 | 5 | 6 | 1 | 1 | - | 1 | - | 2 | 2,282 |
| *walkeri* | 10 | 45 | 78 | 465 | 377 | 50 | 446 | 295 | 107 | 53 | 260 | 27 | 261 | 13 | 47 | 138 | 6 | 118 | 329 | 28 | 317 | 57 | 1 | 53 | 173 | 30 | 318 | 1,064 | - | 159 | 11 | 45 | 5,391 |
| **Culex** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *erraticus* | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | 6 | - | 2 | - | - | - | - | - | - | - | - | - | - | - | 9 |
| *pipiens* | 3 | 10 | 10 | - | 8 | 14 | 7 | 18 | 7 | 17 | 3 | 1 | 49 | 14 | 14 | 27 | 26 | 4 | 34 | 14 | 39 | 6 | 49 | 72 | 96 | 42 | 59 | 36 | - | 2 | 4 | 6 | 691 |
| *restuans* | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| *salinarius* | - | - | - | - | - | - | 1 | - | - | 3 | - | - | - | - | - | 1 | - | - | 1 | 2 | - | - | - | 1 | 2 | 2 | 1 | 5 | - | - | - | - | 19 |
| *territans* | - | 1 | - | 1 | - | - | 1 | 3 | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | 11 | 19 |
| **Culiseta** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *morsitans* | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | 1 | - | 4 | 6 | 5 | - | 4 | - | - | - | - | - | - | - | - | - | 21 |
| **Mansonia** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *perturbans* | 3 | 3 | 11 | 38 | 65 | 60 | 153 | 210 | 88 | 106 | 71 | 18 | 99 | 14 | 26 | 22 | 6 | 2 | 9 | 8 | 3 | 4 | 1 | 1 | 3 | - | - | - | - | - | - | - | 1,024 |
| **Orthopodomyia** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *spp.* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | 1 | - | - | - | - | 2 |
| **Psorophora** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *ciliata* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | 1 |
| *ferox* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| **Uranotaenia** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *sapphirini* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | 1 | - | - | 1 | - | - | - | - | - | 3 |
| TOTAL | 333 | 235 | 496 | 866 | 596 | 311 | 803 | 1,405 | 583 | 588 | 470 | 79 | 749 | 236 | 1,701 | 1,231 | 289 | 421 | 1,122 | 519 | 663 | 409 | 167 | 1,659 | 6,297 | 2,633 | 3,913 | 5,446 | 2 | 426 | 138 | 318 | 35,104 |

[a]Includes *Aedes cinereus* and excludes *A. aurifer* and *A. sticticus*.

# APPENDIX J

Representative Study Area Weather Data - Capital City Airport
Environmental Data Service Ashville, North Carolina

| Date | May 1974 Maximum °F | May 1974 Minimum °F | May 1974 Precipitation (inches) | May 1975 Maximum °F | May 1975 Minimum °F | May 1975 Precipitation (inches) |
|---|---|---|---|---|---|---|
| 1 | 62 | 36 | | 61 | 41 | .01 |
| 2 | 69 | 41 | | 70 | 40 | T |
| 3 | 57 | 33 | .06 | 71 | 42 | .04 |
| 4 | 61 | 32 | | 57 | 45 | .11 |
| 5 | 53 | 32 | .16 | 67 | 42 | .01 |
| 6 | 50 | 28 | .09 | 63 | 43 | T |
| 7 | 54 | 22 | | 70 | 39 | |
| 8 | 43 | 37 | .29 | 72 | 39 | |
| 9 | 51 | 38 | T | 74 | 40 | |
| 10 | 63 | 30 | | 77 | 37 | |
| 11 | 66 | 46 | .38 | 76 | 43 | .03 |
| 12 | 63 | 45 | .02 | 56 | 41 | .75 |
| 13 | 50 | 36 | .02 | 70 | 36 | |
| 14 | 75 | 48 | .42 | 70 | 45 | T |
| 15 | 62 | 45 | T | 65 | 47 | .02 |
| 16 | 70 | 46 | 1.73 | 66 | 42 | |
| 17 | 68 | 52 | .18 | 76 | 40 | |
| 18 | 66 | 46 | .01 | 81 | 44 | |
| 19 | 70 | 60 | T | 88 | 57 | |
| 20 | 75 | 43 | | 89 | 64 | .12 |
| 21 | 84 | 53 | | 86 | 62 | .83 |
| 22 | 73 | 62 | T | 77 | 60 | |
| 23 | 72 | 54 | | 83 | 56 | |
| 24 | 61 | 46 | | 88 | 63 | |
| 25 | 63 | 41 | | 86 | 64 | .21 |
| 26 | 65 | 39 | | 77 | 64 | |
| 27 | 62 | 35 | T | 76 | 52 | |
| 28 | 66 | 42 | .32 | 77 | 44 | |
| 29 | 73 | 59 | .39 | 79 | 57 | .02 |
| 30 | 76 | 60 | | 79 | 60 | .81 |
| 31 | 73 | 50 | T | 69 | 55 | T |

# APPENDIX K

Representative Study Area Weather Data - Capital City Airport
Environmental Data Service Ashville, North Carolina

| Date | June 1974 | | | June 1975 | | |
|---|---|---|---|---|---|---|
| | Maximum °F | Minimum °F | Precipitation (inches) | Maximum °F | Minimum °F | Precipitation (inches) |
| 1 | 73 | 44 | | 72 | 45 | .25 |
| 2 | 73 | 46 | | 70 | 42 | |
| 3 | 75 | 45 | | 72 | 42 | |
| 4 | 84 | 61 | | 74 | 50 | .24 |
| 5 | 84 | 63 | | 76 | 59 | .38 |
| 6 | 82 | 66 | .04 | 68 | 54 | |
| 7 | 81 | 67 | .88 | 60 | 51 | |
| 8 | 80 | 68 | .03 | 66 | 44 | |
| 9 | 86 | 69 | .07 | 76 | 40 | |
| 10 | 76 | 51 | .51 | 78 | 52 | |
| 11 | 65 | 46 | .27 | 70 | 58 | .50 |
| 12 | 72 | 42 | | 76 | 60 | .02 |
| 13 | 75 | 46 | T | 80 | 60 | T |
| 14 | 78 | 49 | | 81 | 59 | .25 |
| 15 | 75 | 53 | .11 | 76 | 60 | .50 |
| 16 | 57 | 49 | .01 | 75 | 56 | T |
| 17 | 57 | 46 | .16 | 83 | 66 | .11 |
| 18 | 73 | 48 | .07 | 85 | 68 | T |
| 19 | 79 | 59 | .31 | 88 | 71 | .01 |
| 20 | 85 | 54 | .02 | 85 | 68 | T |
| 21 | 82 | 65 | T | 88 | 57 | |
| 22 | 71 | 54 | | 92 | 69 | |
| 23 | 64 | 49 | | 86 | 73 | |
| 24 | 68 | 43 | | 78 | 65 | .67 |
| 25 | 75 | 44 | | 77 | 61 | |
| 26 | 78 | 51 | | 82 | 57 | |
| 27 | 80 | 48 | | 86 | 62 | |
| 28 | 79 | 50 | | 88 | 57 | |
| 29 | 81 | 53 | | 87 | 60 | |
| 30 | 79 | 57 | .33 | 86 | 53 | |

## APPENDIX L

Representative Study Area Weather Data - Capital City Airport
Environmental Data Service Ashville, North Carolina

| Date | July 1974 Maximum °F | July 1974 Minimum °F | July 1974 Precipitation (inches) | July 1975 Maximum °F | July 1975 Minimum °F | July 1975 Precipitation (inches) |
|---|---|---|---|---|---|---|
| 1 | 86 | 52 | | 87 | 59 | |
| 2 | 90 | 67 | .55 | 87 | 60 | |
| 3 | 92 | 71 | | 87 | 62 | T |
| 4 | 85 | 64 | .44 | 84 | 56 | |
| 5 | 76 | 51 | | 87 | 58 | |
| 6 | 83 | 50 | | 85 | 65 | .58 |
| 7 | 88 | 54 | | 85 | 61 | T |
| 8 | 93 | 62 | | 90 | 60 | T |
| 9 | 94 | 67 | | 80 | 48 | |
| 10 | 82 | 61 | .01 | 75 | 54 | T |
| 11 | 80 | 53 | | 74 | 45 | |
| 12 | 84 | 43 | | 75 | 50 | .01 |
| 13 | 94 | 55 | | 78 | 50 | .65 |
| 14 | 98 | 72 | | 70 | 57 | .03 |
| 15 | 84 | 60 | | 82 | 62 | |
| 16 | 81 | 49 | | 88 | 50 | |
| 17 | 87 | 55 | T | 88 | 65 | |
| 18 | 91 | 73 | T | 83 | 66 | .78 |
| 19 | 91 | 63 | | 85 | 59 | .38 |
| 20 | 78 | 51 | | 82 | 52 | T |
| 21 | 83 | 38 | | 80 | 57 | |
| 22 | 68 | 61 | .12 | 87 | 54 | |
| 23 | 76 | 54 | T | 86 | 63 | .02 |
| 24 | 84 | 54 | | 80 | 58 | .01 |
| 25 | 81 | 65 | .07 | 78 | 51 | |
| 26 | 87 | 62 | .02 | 82 | 45 | |
| 27 | 89 | 54 | | 84 | 55 | .01 |
| 28 | 92 | 50 | | 86 | 53 | |
| 29 | 82 | 58 | T | 91 | 53 | |
| 30 | 80 | 55 | | 93 | 61 | |
| 31 | 81 | 52 | | 95 | 62 | |

# APPENDIX M

Representative Study Area Weather Data - Capital City Airport
Environmental Data Service Ashville, North Carolina

| Date | August 1974 Maximum °F | August 1974 Minimum °F | August 1974 Precipitation (inches) | August 1975 Maximum °F | August 1975 Minimum °F | August 1975 Precipitation (inches) |
|---|---|---|---|---|---|---|
| 1 | 82 | 48 | .02 | 97 | 65 | |
| 2 | 82 | 61 | .55 | 79 | 68 | .73 |
| 3 | 84 | 54 | T | 84 | 67 | .39 |
| 4 | 72 | 50 | .01 | 87 | 59 | |
| 5 | 78 | 50 | | 74 | 58 | .08 |
| 6 | 84 | 51 | | 72 | 52 | T |
| 7 | 84 | 48 | | 77 | 44 | |
| 8 | 83 | 63 | .09 | 82 | 46 | |
| 9 | 83 | 59 | | 88 | 58 | |
| 10 | 83 | 56 | | 88 | 67 | .06 |
| 11 | 86 | 69 | .03 | 87 | 65 | T |
| 12 | 87 | 53 | | 88 | 57 | |
| 13 | 81 | 60 | 1.22 | 84 | 60 | .55 |
| 14 | 78 | 51 | | 80 | 50 | T |
| 15 | 83 | 53 | | 70 | 57 | T |
| 16 | 87 | 65 | .25 | 82 | 49 | |
| 17 | 80 | 60 | | 83 | 52 | T |
| 18 | 83 | 49 | | 73 | 50 | |
| 19 | 87 | 54 | | 75 | 53 | T |
| 20 | 90 | 57 | | 79 | 55 | .20 |
| 21 | 88 | 61 | | 79 | 59 | 3.08 |
| 22 | 88 | 66 | | 71 | 61 | .83 |
| 23 | 82 | 59 | | 83 | 61 | .37 |
| 24 | 72 | 48 | | 86 | 71 | .14 |
| 25 | 81 | 43 | | 87 | 67 | T |
| 26 | 92 | 61 | | 79 | 60 | .07 |
| 27 | 80 | 56 | .40 | 79 | 53 | |
| 28 | 74 | 46 | | 83 | 54 | |
| 29 | 77 | 47 | | 78 | 67 | 1.34 |
| 30 | 81 | 53 | T | 69 | 64 | .35 |
| 31 | 73 | 44 | .10 | 78 | 64 | 1.62 |

## APPENDIX N

Representative Study Area Weather Data - Capital City Airport
Environmental Data Service Ashville, North Carolina

| Date | September 1974 Maximum °F | September 1974 Minimum °F | September 1974 Precipitation (inches) | September 1975 Maximum °F | September 1975 Minimum °F | September 1975 Precipitation (inches) |
|---|---|---|---|---|---|---|
| 1 | 70 | 46 | | 75 | 63 | T |
| 2 | 57 | 45 | .61 | 76 | 55 | |
| 3 | 66 | 40 | | 69 | 53 | .51 |
| 4 | 67 | 35 | | 72 | 55 | |
| 5 | 72 | 36 | | 64 | 53 | .48 |
| 6 | 74 | 38 | | 71 | 49 | |
| 7 | 75 | 40 | | 75 | 44 | |
| 8 | 80 | 50 | | 65 | 42 | |
| 9 | 82 | 55 | | 64 | 37 | |
| 10 | 81 | 61 | | 77 | 43 | |
| 11 | 87 | 67 | T | 77 | 51 | .10 |
| 12 | 81 | 68 | .40 | 60 | 36 | T |
| 13 | 69 | 49 | .04 | 59 | 35 | T |
| 14 | 64 | 38 | | 65 | 31 | |
| 15 | 72 | 43 | | 68 | 46 | |
| 16 | 71 | 35 | | 69 | 55 | |
| 17 | 80 | 44 | T | 71 | 45 | |
| 18 | 68 | 42 | | 67 | 52 | .01 |
| 19 | 84 | 43 | | 71 | 51 | .17 |
| 20 | 61 | 42 | .02 | 65 | 48 | T |
| 21 | 64 | 41 | .03 | 58 | 49 | .13 |
| 22 | 52 | 31 | T | 60 | 41 | |
| 23 | 59 | 28 | | 64 | 37 | |
| 24 | 64 | 45 | T | 63 | 43 | |
| 25 | 67 | 36 | T | 53 | 45 | T |
| 26 | 82 | 38 | | 57 | 43 | T |
| 27 | 78 | 53 | .51 | 67 | 35 | |
| 28 | 75 | 62 | .23 | 70 | 34 | |
| 29 | 65 | 41 | .76 | 70 | 36 | .25 |
| 30 | 49 | 41 | T | 70 | 48 | |

# APPENDIX O

Representative Study Area Weather Data - Capital City Airport
Environmental Data Service Ashville, North Carolina

October 1975

| Date | Maximum °F | Minimum °F | Precipitation (inches) |
|---|---|---|---|
| 1 | 60 | 41 | .02 |
| 2 | 51 | 32 | |
| 3 | 66 | 36 | |
| 4 | 73 | 45 | |
| 5 | 72 | 43 | |
| 6 | 70 | 42 | |
| 7 | 69 | 33 | |
| 8 | 68 | 44 | T |
| 9 | 59 | 47 | .21 |
| 10 | 71 | 41 | |
| 11 | 57 | 40 | |
| 12 | 67 | 32 | |
| 13 | 83 | 53 | |
| 14 | 82 | 62 | |
| 15 | 68 | 41 | T |
| 16 | 57 | 34 | |
| 17 | 51 | 35 | |
| 18 | 46 | 42 | .08 |
| 19 | 49 | 45 | .23 |
| 20 | 64 | 45 | .07 |
| 21 | 70 | 43 | |
| 22 | 73 | 36 | |
| 23 | 75 | 55 | |
| 24 | 78 | 53 | |
| 25 | 71 | 33 | .34 |
| 26 | 56 | 29 | |
| 27 | 64 | 35 | |
| 28 | 60 | 46 | |
| 29 | 51 | 31 | |
| 30 | 46 | 24 | |
| 31 | 57 | 33 | |

## APPENDIX P

### Developmental Trial

### *Aedes stimulans* Trial #1

| Postprandial Day | # Alive | # Dead | Observed Stage Development | Comments |
|---|---|---|---|---|
| 0 | 57 | - | - | |
| 2 | 55 | 2 | $L_1$ | |
| 5 | 32 | 23 | $L_1$ | |
| 7 | 28 | 4 | - | |
| 8 | 25 | 3 | $L_1$ | |
| 10 | 21 | 4 | $L_1$ | |
| 12 | 20 | 1 | - | 1 mosquito with no larvae |
| 14 | 16 | 4 | - | 3 mosquitoes with no larvae |
| 15 | 15 | 1 | - | 1 mosquito with no larvae |
| 16 | 14 | 1 | - | 1 mosquito with no larvae |
| 17 | 9 | 5 | $L_1$ | 4 mosquitoes with no larvae |
| 18 | 6 | 3 | $L_1$, $L_3$ in M.T. | 2 mosquitoes with no larvae; encapsulation of $L_1$ and all $L_3$ in one mosquito |
| 19 | 5 | 1 | - | 1 mosquito with no larvae |
| 20 | 5 | 0 | | |
| 21 | 4 | 1 | $L_2$ | Encapsulation of $L_2$ |
| 22 | 4 | 0 | | |
| 23 | 4 | 0 | | |
| 24 | 3 | 1 | - | 1 mosquito with no larvae |
| 25 | 2 | 1 | - | 1 mosquito with no larvae |
| 26 | 0 | 2 | $L_2$, $L_3$ in M.T. | 1 mosquito with no larvae; about 16 encapsulated $L_2$ and $L_3$ in one mosquito |

# APPENDIX Q

## Developmental Trial

### *Mansonia perturbans* Trial #1

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 0 | 35 | 0 | | |
| 1 | 31 | 4 | $L_1$ | |
| 2 | 23 | 8 | - | |
| 3 | 22 | 1 | - | |
| 4 | 21 | 1 | $L_1$ | |
| 7 | 16 | 5 | | |
| 8 | 14 | 2 | $L_1$ | 1 mosquito with no larvae |
| 9 | 13 | 1 | - | |
| 10 | 6 | 7 | | |
| 11 | 4 | 2 | - | 2 mosquitoes with no larvae |
| 17 | 0 | 4 | - | 1 mosquito with no larvae |

## APPENDIX R

### Developmental Trial

### *Mansonia perturbans* Trial #2

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 0 | 54 | | | |
| 3 | 20 | 34 | $L_1$ | 1 mosquito with no larvae<br>1 mosquito with complete encapsulation of all $L_1$. |
| 4 | 16 | 4 | $L_1$ | Encapsulation of some $L_1$ in 1 mosquito. |
| 5 | 2 | 14 | $L_1$ | Encapsulation of some $L_1$ in 1 mosquito. |
| 6 | 0 | 2 | | |

# APPENDIX S

## Developmental Trial

### *Mansonia perturbans* Trial #3

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 0 | 84 | | | |
| 3 | 74 | 10 | | |
| 6 | 52 | 22 | $L_1$ | |
| 7 | 48 | 4 | $L_1$ | 1 mosquito with no larvae |
| 8 | 40 | 8 | $L_1$ | 5 mosquitoes with no larvae |
| 9 | 31 | 9 | $L_1$ | 7 mosquitoes with no larvae, 1 mosquito with encapsulation of $L_1$. |
| 10 | 23 | 8 | $L_1$ | |
| 11 | 20 | 3 | - | 1 mosquito with no larvae |
| 12 | 11 | 9 | - | 4 mosquitoes with no larvae |
| 13 | 5 | 6 | - | 2 mosquitoes with no larvae |
| 14 | 0 | 5 | - | 2 mosquitoes with no larvae |

## APPENDIX T

### Developmental Trial

### *Mansonia perturbans* Trial #4

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 1 | 71 | 0 | | |
| 2 | 63 | 8 | $L_1$ | |
| 3 | 61 | 2 | $L_1$ | |
| 4 | 58 | 3 | $L_1$ | 1 mosquito with no larvae |
| 5 | 49 | 9 | $L_1$ | 4 mosquitoes with no larvae |
| 6 | 35 | 14 | $L_1$ | 6 mosquitoes with no larvae |
| 7 | 17 | 18 | $L_1$ | 11 mosquitoes with no larvae |
| 8 | 3 | 14 | $L_1$, $L_2$ | 8 mosquitoes with no larvae |
| 9 | 0 | 3 | $L_1$ | |

APPENDIX U

Developmental Trial

*Aedes vexans* Trial #1

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 1 | 45 | 0 | | |
| 2 | 29 | 16 | $L_1$ | |
| 3 | 26 | 3 | $L_1$ | |
| 4 | 24 | 2 | $L_1$ | |
| 5 | 20 | 4 | $L_1$ | |
| 6 | 19 | 1 | - | 1 mosquito with no larvae |
| 7 | 19 | 0 | | |
| 8 | 19 | 0 | | |
| 9 | 19 | 0 | | |
| 10 | 17 | 2 | $L_1$, $L_2$ | 1 mosquito with encapsulation of $L_1$, 1 mosquito with $L_2$ |
| 11 | 16 | 1 | - | 1 mosquito with no larvae |
| 12 | 14 | 2 | $L_1$, $L_2$, $L_3$ | 1 mosquito with no larvae; 1 mosquito with partially encapsulated $L_1$ (2), and 3 $L_3$ in head, 1 $L_3$ in abdomen and 8 $L_3$ in M.T. |
| 13 | 11 | 3 | | |
| 14 | 8 | 3 | $L_2$, $L_3$ | 1 mosquito with 1 $L_3$ in the head and 1 in the thorax; 1 mosquito with 4 $L_3$ in the Malpighian tubules. |
| 15 | 7 | 1 | $L_2$, $L_3$ | 1 mosquito with 1 $L_3$ in the proboscis, 3 $L_3$ in the head, 1 $L_3$ in the thorax and 1 partially encapsulated $L_3$ in the Malpighian tubules. |
| 16 | 4 | 3 | $L_3$ | 2 mosquitoes with no larvae; 1 mosquito with 3 $L_3$ in the head and 1 $L_3$ in the Malpighian tubules. |
| 17 | 3 | 1 | - | 1 mosquito with no larvae. |
| 18 | 2 | 0 | | |
| 19 | 2 | 0 | | 2 mosquitoes placed in cage with *A. vexans* from trial #3. |

# APPENDIX V

## Developmental Trial

## *Aedes vexans* Trial #2

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 0 | 14 | | | |
| 1 | 12 | 2 | $L_1$ | |
| 2 | 3 | 9 | $L_1$ | |
| 3 | 2 | 1 | | |
| 4 | 1 | 1 | | |
| 5 | 1 | 0 | | |
| 6 | 1 | 0 | | |
| 7 | 0 | 1 | | |

# APPENDIX W

## Developmental Trial

## *Aedes vexans* Trial #3

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 1 | 210 | | | |
| 2 | 152 | 58 | $L_1$ | |
| 3 | 91 | 91 | $L_1$ | |
| 4 | 73 | 18 | $L_1$ | |
| 5 | 53 | 20 | $L_1$ | |
| 6 | 51 | 2 | | |
| 7 | 41 | 10 | $L_1$, $L_2$ | |
| 8 | 35 | 6 | | |
| 9 | 27 | 8 | $L_2$, $L_3$ | 1 mosquito with 13 $L_3$ in the Malpighian tubules |
| 10 | 21 | 6 | $L_2$, $L_3$ | 1 mosquito with 2 $L_3$ in the Malpighian tubules; 1 mosquito with 1 $L_3$ in the Malpighian tubules. |
| 11 | 12 | 9 | $L_1$, $L_3$ | 2 mosquitoes with no larvae; 1 mosquito with 2 $L_3$ in the Malpighian tubules and 2 encapsulated $L_1$. |
| 12 | 3 | 9 | $L_3$ | 1 mosquito with no larvae; 1 mosquito with 4 $L_3$ in the head. |

2 mosquitoes from Trial #1 placed in cage with the 3 mosquitoes from this trial for transmission trials.

| | | | | |
|---|---|---|---|---|
| 20/13 | 4 | 1 | $L_1$ | 1 mosquito with encapsulated $L_1$. |
| 21/14 | 4 | 0 | - | |
| 22/15 | 2 | 2 | $L_2$ | |
| 23/16 | 2 | 0 | | |
| 24/17 | 1 | 1 | $L_3$ | 1 mosquito with 1 $L_3$ in the head and 3 $L_3$ in the thorax. |
| 25/18 | 0 | 1 | $L_2$ | |

# APPENDIX X

## Developmental Trial

### *Aedes vexans* Trial #4

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 0 | 212 | | | |
| 1 | 167 | 45 | | |
| 2 | 149 | 18 | | |
| 3 | 141 | 8 | $L_1$ | |
| 4 | 125 | 16 | $L_1$ | |
| 5 | 106 | 19 | $L_1$ | |
| 6 | 93 | 13 | $L_1$ | |
| 7 | 65 | 28 | $L_1$, $L_2$ | 1 mosquito with some encapsulated $L_1$. |
| 8 | 51 | 14 | $L_2$ | |
| 9 | 35 | 16 | $L_1$, $L_2$ | 1 mosquito with some encapsulated $L_1$. |
| 10 | 20 | 15 | $L_2$, $L_3$ | 1 mosquito with 3 $L_3$ in the Malpighian tubules. |
| 11 | 12 | 8 | $L_1$, $L_2$ | |
| 12 | 5 | 7 | $L_3$ | 1 mosquito with no larvae; 1 mosquito with 5 $L_3$ in the head, 1 $L_3$ in the hemocoel, and 1 $L_3$ in the Malpighian tubules. |
| 13 | 4 | 1 | $L_1$ | 1 mosquito with encapsulated $L_1$. |
| 14 | 2 | 2 | | |
| 15 | 2 | 0 | | |
| 16 | 0 | 2 | - | 1 mosquito with no larvae but Malpighian tubules were damaged. |

# APPENDIX Y

## Developmental Trial

### *Anopheles quadrimaculatus* Trial #1

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 1 | 10 | 0 | | |
| 2 | 10 | 0 | | |
| 3 | 7 | 3 | $L_1$ | |
| 4 | 7 | 0 | | |
| 5 | 7 | 0 | | |
| 6 | 7 | 0 | | |
| 7 | 7 | 0 | | |
| 8 | 7 | 0 | | |
| 9 | 5 | 2 | $L_2$ | |
| 10 | 4 | 2 | $L_2$ | |
| 11 | 3 | 1 | $L_2$, $L_3$ | Malpighian tubules packed with $L_2$ and $L_3$. |
| 12 | 3 | 0 | | |
| 13 | 0 | 3 | $L_3$ | 1 mosquito with 6 $L_3$ in the thorax and many $L_3$ in the Malpighian tubules; 1 mosquito with 3 $L_3$ in the proboscis, 7 $L_3$ in the head, 11 $L_3$ in the thorax. |

## APPENDIX Z

### Developmental Trial

### Anopheles quadrimaculatus Trial #2

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 1 | 37 | | | |
| 2 | 33 | 4 | | |
| 3 | 31 | 2 | | |
| 4 | 28 | 3 | $L_1$ | |
| 5 | 24 | 4 | $L_1$ | |
| 6 | 24 | 0 | | |
| 7 | 20 | 4 | | |
| 8 | 20 | 0 | | |
| 10 | 14 | 6 | $L_3$ | 1 mosquito with $L_3$ in the Malpighian tubules. |
| 11 | 12 | 2 | $L_2$, $L_3$ | 1 mosquito with 9 $L_3$ in the Malpighian tubules; 1 mosquito with $L_3$ and $L_2$ molting. |
| 12 | 9 | 3 | $L_2$, $L_3$ | 1 mosquito with $L_3$ in the Malpighian tubules. |
| 13 | 5 | 4 | | |
| 14 | 4 | 1 | $L_3$ | 1 mosquito with 37 $L_3$ removed from its body. 4 $L_3$ found in the proboscis, 4 $L_3$ in the head, 15 $L_3$ in the thorax, 2 $L_3$ in the abdomen, and 4 $L_3$ in the Malpighian tubules. |
| 15 | 4 | 0 | | |
| 16 | 2 | 2 | | |
| 17 | 0 | 2 | $L_3$ | 1 mosquito with 4 $L_3$ in the proboscis, and 5 $L_3$ in the head. |

# APPENDIX AA

## Developmental Trial

## *Anopheles quadrimaculatus* Trial #3

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 1 | 15 | 0 | | |
| 2 | 10 | 5 | | |
| 4 | 8 | 2 | $L_1$ | |
| 6 | 1 | 7 | $L_1$ | |
| 7 | 1 | 0 | | |
| 8 | 1 | 0 | | |
| 9 | 1 | 0 | | |
| 10 | 0 | 1 | | |

## APPENDIX BB

### Developmental Trial

### *Anopheles quadrimaculatus* Trial #4

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 1 | 15 | 0 | | |
| 2 | 10 | 5 | | |
| 4 | 8 | 2 | $L_1$ | |
| 6 | 1 | 7 | $L_1$ | Advanced sausage larvae |
| 7 | 1 | 0 | | |
| 8 | 1 | 0 | | |
| 9 | 1 | 0 | | |
| 10 | 0 | 1 | | |

## APPENDIX CC

### Developmental Trial

### *Anopheles quadrimaculatus* Trial #5

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 0 | 6 | 0 | | |
| 1 | 5 | 1 | | |
| 2 | 3 | 2 | | |
| 3 | 2 | 1 | $L_1$ | "sausage" larvae |
| 4 | 1 | 1 | $L_1$ | |
| 5 | 1 | 0 | | |
| 6 | 1 | 0 | | |
| 7 | 1 | 0 | | |
| 8 | 1 | 0 | | |
| 9 | 0 | 1 | $L_2$ | |

## APPENDIX DD

### Developmental Trial

### *Culex pipiens* Trial #1

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 1 | 10 | 0 | | |
| 4 | 6 | 4 | | |
| 5 | 6 | 0 | | |
| 6 | 6 | 0 | | |
| 7 | 6 | 0 | | |
| 8 | 6 | 0 | | |
| 9 | 4 | 2 | | |
| 10 | 4 | 0 | | |
| 11 | 4 | 0 | - | All 4 mosquitoes dissected. No larvae were seen and the Malpighian tubules were not damaged. |

## APPENDIX EE

### Developmental Trial

### Culex pipiens Trial #2

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 0 | 84 | 0 | | |
| 1 | 75 | 9 | | 6 mosquitoes with no larvae; 1 mosquito with 1 microfilaria in the Malpighian tubules. |
| 2 | 62 | 13 | | 1 mosquito with no larvae. |
| 3 | 56 | 6 | | 2 mosquitoes with no larvae. |
| 4 | 51 | 5 | $L_1$ | 1 mosquito with no larvae; 1 mosquito with 1 "sausage" larvae. |
| 5 | 49 | 2 | | |
| 6 | 42 | 7 | $L_1$ | 2 mosquitoes with no larvae. |
| 7 | 39 | 3 | | 2 mosquitoes with no larvae. |
| 8 | 36 | 3 | | 3 mosquitoes with no larvae. |
| 9 | 35 | 1 | $L_2$ | 1 $L_2$ in Malpighian tubules. |
| 10 | 35 | 0 | | |
| 11 | 33 | 2 | | 1 mosquito with no larvae. |
| 12 | 30 | 3 | | 1 mosquito with no larvae. |
| 13 | 29 | 1 | $L_2$ | 1 $L_2$ in Malpighian tubules. |
| 14 | 26 | 3 | $L_3$ | 1 mosquito with no larvae; 1 mosquito with 1 $L_3$ in Malpighian tubules. |
| 15 | 24 | 2 | | 1 mosquito with no larvae. |
| 16 | 0 | 24 | $L_3$ | All mosquitoes sacrificed; 1 mosquito with 1 $L_3$ in Malpighian tubules; 19 mosquitoes with no larvae. |

## APPENDIX FF

### Developmental Trial

### *Culex pipiens* Trial #3

| Postprandial Day | # Alive | # Dead | Observed Stage of Development | Comments |
|---|---|---|---|---|
| 0 | 27 | 0 | | |
| 1 | 27 | 0 | | |
| 2 | 27 | 0 | | |
| 3 | 26 | 1 | | |
| 4 | 22 | 4 | $L_1$ | 1 mosquito with 1 early "sausage" larva, 3 mosquitoes with no larvae. |
| 5 | 21 | 1 | | |
| 6 | 20 | 1 | | |
| 7 | 20 | 0 | | |
| 8 | 20 | 0 | | |
| 9 | 20 | 0 | | |
| 10 | 20 | 0 | | |
| 11 | 20 | 0 | | |
| 12 | 20 | 0 | | |
| 13 | 20 | 0 | | |
| 14 | 19 | 1 | $L_2$ | |
| 15 | 0 | 19 | $L_3$ | All mosquitoes sacrificed; 16 mosquitoes with no larvae; 2 mosquitoes with 1 $L_3$ in the Malpighian tubules; 1 mosquito with 1 $L_3$ in the proboscis. |

## APPENDIX GG

### Concentration of Microfilaria at the Time of the Infective Blood Meal

**Aedes stimulans**

| Sample # | Microfilariae/20ul sample | |
|---|---|---|
| Trial # 1 - Feeding occurred on an infected dog | | |
| 1 | 294 | |
| 2 | 210 | |
| 3 | 192 | |
| 4 | 135 | |
| 5 | 179 | |
| 6 | 163 | |
| 7 | 225 | |
| 8 | 216 | |
| 9 | 122 | |
| 10 | 270 | $\bar{X}$ = 201.5 |

**Coquillettidia perturbans**

| Sample # | Microfilariae/20ul sample | |
|---|---|---|
| Trial # 1 - Feeding occurred on an infected dog | | |
| 1 | 841 | |
| 2 | 627 | |
| 3 | 400 | |
| 4 | 504 | |
| 5 | 1050 | |
| 6 | 855 | |
| 7 | 1091 | |
| 8 | 546 | |
| 9 | 771 | |
| 10 | 1387 | $\bar{X}$ = 807.3 |
| Trial # 2 - Feeding occurred on an infected dog | | |
| 1 | 1550 | |
| 2 | 1111 | |
| 3 | 1203 | |
| 4 | 937 | $\bar{X}$ = 1200 |
| Trial # 3 - Feeding occurred on an infected dog | | |
| 1 | 1171 | |
| 2 | 1012 | |
| 3 | 1238 | |
| 4 | 1261 | |
| 5 | 1204 | $\bar{X}$ = 1177 |

APPENDIX GG CONT'D

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Coquillettidia perturbans

| Sample # 3 | Microfilariae/20ul sample | |
|---|---|---|
| Trial # 4 - Feeding occurred through a membrane | | |
| 1 | 262 | |
| 2 | 239 | |
| 3 | 233 | |
| 4 | 260 | |
| 5 | 263 | $\bar{X}$ = 251.4 |

Aedes vexans

| Sample # | Microfilariae/20ul sample | |
|---|---|---|
| Trial # 1 - Feeding occurred through a membrane | | |
| 1 | 382 | |
| 2 | 392 | |
| 3 | 481 | |
| 4 | 402 | |
| 5 | 345 | |

Trial # 2 - Feeding occurred on an infected dog. Microfilaremia was not determined.

| Trial # 3 - Feeding occurred through a membrane | | |
|---|---|---|
| 1 | 111 | |
| 2 | 137 | |
| 3 | 105 | |
| 4 | 151 | |
| 5 | 121 | $\bar{X}$ = 125 |

| Trial # 4 - Feeding occurred through a membrane | | |
|---|---|---|
| 1 | 150 | |
| 2 | 173 | |
| 3 | 183 | |
| 4 | 213 | |
| 5 | 181 | $\bar{X}$ = 180 |

Anopheles quadrimaculatus

| Sample # | Microfilariae/20ul sample | |
|---|---|---|
| Trial # 1 - Feeding occurred on an infected dog | | |
| 1 | 1372 | |
| 2 | 1156 | |
| 3 | 906 | $\bar{X}$ = 1144.6 |

APPENDIX GG CONT'D

## Anopheles quadrimaculatus

| Sample # | Microfilariae/20ul sample | |
|---|---|---|
| **Trial # 2 - Feeding occurred on an infected dog** | | |
| 1 | 680 | |
| 2 | 914 | |
| 3 | 708 | |
| 4 | 878 | $\bar{X}$ = 795 |
| **Trial # 3 - Feeding occurred on an infected dog** | | |
| 1 | 888 | |
| 2 | 880 | |
| 3 | 881 | $\bar{X}$ = 883 |
| **Trial # 4 - Feeding occurred on an infected dog** | | |
| 1 | 776 | |
| 2 | 748 | |
| 3 | 748 | |
| 4 | 821 | |
| 5 | 856 | $\bar{X}$ = 790 |
| **Trial # 5 - Feeding occurred on an infected dog** | | |
| 1 | 981 | |
| 2 | 954 | |
| 3 | 779 | |
| 4 | 966 | $\bar{X}$ = 920 |

## Culex pipiens

| Sample # | Microfilariae/20ul sample | |
|---|---|---|
| **Trial # 1 - Feeding occurred through a membrane** | | |
| 1 | 204 | |
| 2 | 189 | |
| 3 | 231 | |
| 4 | 217 | $\bar{X}$ = 210 |
| **Trial # 2 - Feeding occurred through a membrane** | | |
| 1 | 264 | |
| 2 | 287 | |
| 3 | 285 | |
| 4 | 282 | |
| 5 | 249 | $\bar{X}$ = 273 |

APPENDIX GG CONT'D

| Sample # | Microfilariae/20ul sample | |
|---|---|---|
| | Culex pipiens | |
| Trial # 3 - Feeding occurred through a membrane | | |
| 1 | 351 | |
| 2 | 307 | |
| 3 | 272 | |
| 4 | 289 | $\bar{X}$ = 305 |

## Appendix HH

### Transmission Trial

*Aedes stimulans* Trial #1 Dog MW 75

| Postprandial Day | # Alive | Time Offered | Comments |
|---|---|---|---|
| 16 | 15 | 4:00 - 5:00 P. M. | Most mosquitoes fed |
| 17 | 14 | 3:00 - 3:30 P. M. | All remaining mosquitoes fed |
| 18 | 9 | 3:15 - 3:45 P. M. | No feeding |
| 19 | 6 | 3:15 - 3:45 P. M. | No feeding |
| 20 | 5 | 3:15 - 3:45 P. M. | No feeding |
| 21 | 5 | 3:15 - 3:45 P. M. | No feeding |
| 22 | 4 | 3:15 - 3:45 P. M. | No feeding |
| 23 | 4 | 3:15 - 3:45 P. M. | No feeding |
| 24 | 4 | 3:00 - 3:25 P. M. | No feeding |
| 25 | 3 | 3:15 - 3L45 P. M. | No feeding |
| 26 | 2 | No feeding trial | |
| 27 | 0 | | |

# APPENDIX II

## Transmission Trial

### *Aedes vexans* Trial #1 Dog ER 54

| Postprandial Day | # Alive | Time Offered | Comments |
|---|---|---|---|
| 13 | 11 | 2:30 - 3:00 P. M. | One mosquito fed |
| | | 7:15 - 7:30 P. M. | No feeding |
| 14 | 8 | 8:30 - 8:45 A. M. | No feeding |
| | | 11:15 - 11:30 A. M. | No feeding |
| | | 3:20 - 3:35 P. M. | No feeding |
| | | 6:15 - 6:45 P. M. | No feeding |
| 15 | 7 | 8:45 - 9:15 A. M. | No feeding |
| | | 12:15 - 12:30 P. M. | No feeding |
| | | 3:15 - 3:35 P. M. | No feeding |
| 16 | 4 | 8:00 - 8:10 A. M. | No feeding |
| | | 5:05 - 5:15 P. M. | No feeding |
| 17 | 3 | 8:30 - 8:45 A. M. | No feeding |
| | | 5:45 - 5:50 P. M. | No feeding |
| 18 | 2 | 8:45 - 8:55 A. M. | No feeding |
| | | 8:00 - 8:15 P. M. | No feeding |

2 mosquitoes remaining alive from Trial combined with those from Trial 3

### Trial 1 - 3

| | | | |
|---|---|---|---|
| 12/19 | 5 | 9:55 - 10:10 A. M. | No feeding |
| 13/20 | 4 | 9:45 - 10:05 A. M. | No feeding |
| 15/22 | 2 | 9:45 - 10:00 A. M. | No feeding |
| 16/23 | 1 | No feeding trial | |
| 17/24 | 0 | | |

### Trial #4

| | | | |
|---|---|---|---|
| 14 | 2 | 8:25 - 8:35 A. M. | No feeding |
| 15 | 2 | No feeding trial | |
| 16 | 0 | | |

## APPENDIX JJ

### Transmission Trials

### Anopheles quadrimaculatus Dog HT 05

Trial # 1

| Postprandial Day | # Alive | Time Offered | Comments |
|---|---|---|---|
| 13 | 2 | 2:00 - 2:15 P. M. | No feeding |
| | | 7:45 - 8:00 P. M. | No feeding |
| 14 | 0 | | |

Trial # 2

| | | | |
|---|---|---|---|
| 13 | 5 | 8:50 - 9:15 A. M. | Several mosquitoes landed on dog and appeared to probe. No mosquitoes took a blood meal. |
| 14 | 4 | 1:50 - 2:15 P. M. | Several mosquitoes landed on dog and appeared to probe. No mosquitoes took a blood meal. |
| 15 | 4 | 8:40 - 8:55 A. M. | No probing or feeding. |
| 16 | 2 | 9:15 - 9:25 A. M. | No probing or feeding. |
| 17 | 0 | | |

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