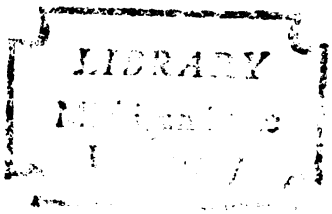
VERTEBRATE-MOSQUITO RELATIONSHIPS IN A MICHIGAN WATER QUALITY MANAGEMENT PROJECT

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
JOHN ALBERT WILDIE
1975



ABSTRACT

VERTEBRATE-MOSQUITO RELATIONSHIPS IN A MICHIGAN WATER QUALITY MANAGEMENT PROJECT

By

John Albert Wildie

This study was designed to obtain baseline data on California group virus activity in small indigenous mammals living in a sewage spray irrigation site prior to spraying. The mammals were trapped in collapsible and non-collapsible live traps, anesthetized and blood samples taken by cardiac puncture. The sera were sent to the Michigan Department of Public Health, Virology Laboratory, and tested for hemagglutination inhibition antibodies using the LaCrosse virus antigen and goose red blood cells. One hundred and sixty-two mammals, comprising 10 species, were trapped and 182 blood samples collected. Positive sera were obtained from 3 fox squirrels, Sciurus niger Linn.; 1 red squirrel, Tamiasciurus hudsonicus, Erxleben; and 1 chipmunk, Tamias striatus, Linn.

An attempt was made to obtain data on the host feeding preferences of the mosquito species present in the spray irrigation area by using mammal-baited mosquito

traps. Aedes triseriatus (Say), Aedes vexans (Meigen), Aedes sticticus (Meigen), Aedes fitchii-stimulans (Felt & Young), Culex pipiens Linn., Culex salinarius Coq., Aedes cinereus Meigen and Coquillettidia perturbans (Walk.) were collected but only in low numbers.

There was low level California group virus activity in Sciuridae (squirrels and chipmunks) in the spray irrigation area and at least one mosquito, Aedes triseriatus (Say), capable of transmitting the California encephalitis virus was in the study area.

VERTEBRATE-MOSQUITO RELATIONSHIPS IN A MICHIGAN WATER QUALITY MANAGEMENT PROJECT

By

John Albert Wildie

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1975

ACKNOWLEDGMENTS

I wish to thank Dr. Harold D. Newson for his invaluable guidance in the preparation of this manuscript and for his support both financial and moral during the course of this study.

I would like to extend my appreciation to Drs. Roland L. Fischer, James E. Bath, and Rollin H. Baker for serving on my guidance committee.

I would like to thank Dr. Maurice Becker and the personnel at the Michigan Department of Public Health, Virology Laboratory, for performing the necessary serological tests.

I would like to extend appreciation to Drs. Howard Tanner, Thomas Bahn, and the personnel of the Water Quality Management Project for allowing me to conduct my research in the project site.

I would like to extend a special thanks to Dr. John N. Stuht, Rose Lake Research Station, for assisting me with the trapping and bleeding of mammals and for just being there when I needed advice.

Finally a special note of appreciation to my wife, Suzanne, for her constant encouragement during my graduate studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

During the early part of 1971 the Institute of Water Research of Michigan State University designed and initiated a water quality management project. The project was designed to determine the level of water purification that could be obtained by passing sewage effluent through a series of oxidation ponds. The water upon reaching the final pond was to be transported to a second portion of the project where spray irrigation distributed it to a variety of field crops, old-fields and woodland environments to determine whether dissolved water pollutants would be filtered out as the effluent passed through the soil and entered the ground water table.

The study reported here was one of several conducted in the project area which included such diverse subjects as soil biology, ornithology, mammalogy and medical entomology. The medical entomology studies included a determination of the biting insects indigenous to the project site and the insect-borne disease potentials that existed in the project area before the start of any spray irrigation operations.

The human biting insect population studies were done by Tom Zorka, another graduate student, to determine

the indigenous insect populations that were in the area prior to the start of spray irrigation. The data obtained were to be used in the evaluation of any changes that could occur in the course of future spray operations. Thus, the effects of the spraying operation on the insect populations produced remain to be evaluated at some future time.

The part of the study reported here was divided into two parts: A serological survey to determine whether or not California encephalitis virus infections occurred in the indigenous small mammals and to determine the mammal host preferences of the mosquito species present in the spray irrigation area.

History of the California Encephalitis Group Virus

Arthropod transmitted viruses or arboviruses have been known to cause central nervous system diseases in humans in the United States since the 1930s. There are currently five epidemic or endemic forms of arboviral encephalitis of primary concern: Eastern equine (EEE), Western equine (WEE), Venezuelan equine (VEE), St. Louis (SLE), and California (CE) encelphalitis. The EEE, WEE, VEE, and SLE viruses are well documented in the literature and have received considerable study since they produce a relatively high mortality. CE virus infections, however, produce a more benign disease and have become a leading cause of human arboviral encephalitis in the United States.

The California encephalitis virus prototype was first isolated in 1943 and 1944 from Culex tarsalis Coq. and Aedes melanimon Dyar mosquitoes in Kern County, California, and was incriminated in three human cases of encephalitis that occurred in 1945 in California's San Joaquin Valley (Henderson & Coleman, 1971; and Hammon & Reeves, 1945). This virus was not reported again until Thompson and co-workers isolated a virus with antigenic properties similar to the CE prototype. This was obtained from frozen portions of brain tissue obtained from a 4-year-old girl who died shortly after being hospitalized in LaCrosse, Wisconsin, in 1959 with a meningencephalitis diagnosed illness (Thompson et al., 1965). It took five years to develop the techniques needed to isolate and identify this virus, subsequently named the LaCrosse strain (CE group), after the location where the girl died. The importance of Thompson's work was not merely the isolation and identification of the LaCrosse virus, but the development of the techniques needed to detect this virus. Of the 519 confirmed or presumptive human encephalitis cases attributed to the CE group that were reported between 1945 and 1970, the majority occurred prior to 1964, but were not diagnosed until after 1964 when the techniques sensitive enough to detect the virus had been developed (Sudia et al., 1971; and Vianna et al., 1971).

In 1964, the first reported CE epidemic occurred in Ripley County, Indiana, with 12 confirmed or presumptive

cases in children under the age of 16 (Beadle, 1966). In the period between 1967 and 1969, the number of CE cases reported in the United States outnumbered the combined total of EEE, WEE, SLE, and VEE cases. Approximately 90 percent of these CE cases occurred in the north-central region of the country. The increasing number of reported human CE infections during this time is not necessarily an indication of the activity of the virus. Sudia et al. (1971) suggested that the large number of reported cases from certain areas (i.e., the north-central region) could be related to the high interest level of the investigatory groups looking for the virus in that area. For example, prior to 1968, there were no reported cases from Michigan although numerous cases were reported from areas surrounding Michigan including Wisconsin, Ohio, Indiana, and Canada. Investigators in Michigan did not begin testing for CE until 1968, so it is not known whether human infections occurred there prior to that time. Human CE cases in the United States have been reported from 18 states with the majority occurring in Indiana, Ohio, Wisconsin, Minnesota, Iowa, and Michigan. Human CE infections have been reported as far south as North Carolina, indicating wide-spread occurrence of this virus.

One difficulty in the diagnosis of this virus is that there are apparently eight to ten types or subtypes. Several authors have suggested that this variation results from the lack of mobility of the small mammals that are

the vertebrate hosts of these subtypes which serves to isolate the virus in nature (Sudia et al., 1971; Johnson, 1970; Sather & Hammon, 1967; and Parkin, 1973). Johnson felt that these viruses are currently undergoing an evolutionary change which could explain the similiarities seen between the various strains in the CE group obtained from given locations at any one time. Of the types so far identified only four have been associated with human disease. These are CE prototype, LaCrosse subtype, Trivittatus subtype, and Jamestown Canyon subtype. The LaCrosse subtype appears to be responsible for most of the known human cases that have been reported in the United States.

Once man becomes infected with the virus, the sumptoms of the disease may or may not be expressed. Infections with the CE (LaCrosse subtype) generally product clinical symptoms in children under the age of 16, while adult infections typically are subclinical (Thompson et al., 1963; Thompson & Inhorn, 1967; and Johnson et al., 1968). The acute clinical symptoms seen in children are fever, headache, nausea, nuchal rigidity, convulsions and lethargy. Focal neurological signs that include paresis, paralysis and aphasia are also common. The typical illness usually runs its course in seven to ten days and is followed by complete recovery. However, there may be some post-encephalitic behavioral changes and impared scholastic abilities (Chun et al., 1968; and Matthews et al., 1968). The low mortality resulting from human CE infections

undoubtedly is why more research has not been conducted in many areas of the country.

The natural transmission cycle of CE does not normally involve humans. It is maintained in nature in a mosquito-small mammal-mosquito cycle with man becoming involved when he intrudes on the woodland environment where the virus exists. The primary vector(s) of the virus are mosquito species of the genus Aedes. Small mammals serve as reservoirs of the virus and maintain a readily available source of the virus to infect other mosquitoes. The role that a particular mosquito species plays in the transmission of the virus is partially based on its minimum field infection rate (MFIR), which is a reflection of the number of mosquitoes tested and the number of isolations obtained. Aedes species in general have higher MFIR's than do non-Aedes mosquitoes in the natural transmission cycle of the CE. This infection rate is a relative indication of the mosquito population infected and is not a direct relationship of the CE to human infection (Sudia et al., 1971).

The role of the vertebrate host in the transmission of CE is that of maintenance of a virus pool from which non-infected mosquitoes become infected. In this way a larger portion of the mosquito population can become infected and in turn infect more mammals. In nature this cycle would start as a small focus and keep building upon itself. Limiting factors that prevent or regulate this

build-up include: (1) Not all vertebrates serve as reservoirs; (2) not all mosquitoes serve as vectors; (3) environmental factors such as rain and snow that affect the population levels of suitable vector mosquitoes and; (4) the development of immune mammal populations as a result of the CE infections.

In order for the vertebrate to serve as a reservoir the virus must be transmitted from the arthropod vector to the vertebrate host. There are two possible mechanisms by which the vertebrate host can be infected, either by mechanical or biological transmission of the virus. CE viruses have been isolated from tabanoid species including Hybomitra lasiophthalma (Macq.) and Chrysops cincticornis (Walk.) incriminating them as possible vectors of CE in Wisconsin (Wright et al., 1970; and DeFoliart et al., 1969). This group of insects may be responsible for the transmission of the virus to the larger mammals such as deer, Odocoileus virginianus Zimmermann, and domestic animals with which these arthropods are usually associated. The role these arthropods play in CE transmission is still uncertain, for mechanical transmission of the virus via infected mouth parts is a possibility.

Mechanical transmission is also possible in the primary vector (mosquitoes), but biological transmission is considered more important. In this mechanism the virus multiplies within the tissues of the arthropod before it is transmitted to another vertebrate host. The detection of

the virus in the insect tissues is a good indication that the insect serves as a vector of the virus (Chernesky, 1967).

Once infected the mosquito must maintain the virus at a level high enough to infect the vertebrate host and for long enough to enable the mosquito to feed on another susceptable host. Watts et al. (1972) were able to infect Aedes triseriatus (Say) mosquitoes by feeding them on 8-day-old hamsters which had been previously innoculated subcutaneously with the LaCrosse virus subtype. The mosquitoes were able to transmit the virus (after a prepatent period of virus development of the virus of about 7 days) from day 7 to day 36 after feeding.

This relatively long capability of a mosquito to transmit the virus is an important factor in determining the importance of the mosquito as a vector of the virus. Besides having a long lasting transmission ability, Aedes triseriatus (Say) is able to pass the virus to the eggs by transovarial transmission (Watts et al., 1973). Transmission of the LaCrosse virus by this method is a possible mechanism for the survival of this arbovirus during the winter season in the north-central United States (Watts et al., 1974). As a result of the transovarial transmission of the virus to the eggs, it could be possible to detect the virus in a particular location by testing larval and egg samples for the virus.

Another criterion that the mosquito must exhibit before it can be incriminated as a potential vector is an association with the susceptable vertebrate host that serve as reservoirs of the virus. Much research has been conducted to determine these mosquito-host associations. Some mosquitoes such as Culex pipiens Linn. show a preference for avian hosts and Anopheles quadrimaculatus Say for mammalian hosts, while Culex salinarius Coq. and Coquillettidia perturbans (Walk.) feed freely on both types without showing any apparent preference (Murphy et al., 1967; Crans, 1963; Tempelis et al., 1967; Rempel et al., 1946; and Chamberlain et al., 1954). Although many mosquitoes show host preferences the availability of the host is an important factor in influencing the mosquitoes selection of its blood meal (Shemanchuk et al., 1963). Table 1 shows some of the mosquito-host associations of a variety of mosquito species occurring in Michigan. Rodents, raccoons, rabbits and squirrels (including chipmunks) are the hosts most frequently used by these mosquitoes. Those mosquito species that utilize a wide range of hosts have a greater potential to serve as vectors of the CE virus than do those that only use a limited number of hosts. In Table 1 Aedes canadensis (Theobald), Aedes cinereus Meigen, Aedes fitchii-stimulans (Felt & Young), Aedes sticticus (Meigen), Aedes triseriatus (Say), Aedes trivittatus (Coq.), Aedes vexans (Meigen), Culex pipiens Linn., Culex salinarius Coq. and Culiseta melanura (Coq.) were shown to

Table 1.--Known vertebrate hosts of mosquitoes occurring in Michigan.

Mosquito Species	Animal Hosts[a]										Literature Cited
	D	R	RA	RAC	FS	RS	C	S	W	O	
Aedes canadensis	X	X	X	X	X	X	X		X	X	27,36,68
Aedes cinereus	X	X	X	X	X	X	X		X	X	36,68
Aedes communis		X									19
Aedes dorsalis		X	X								21,49,55
Aedes excrucians		X	X								19,36
Aedes fitchii-stimulans	X		X	X	X	X	X		X	X	18,68
Aedes punctor	X	X		X					X		19,68
Aedes sticticus	X	X	X	X	X	X	X		X	X	55,68
Aedes trichurus		X	X	X							18,19
Aedes triseriatus			X	X	X	X	X		X	X	58,68
Aedes trivittatus		X	X	X	X	X	X		X	X	21,68
Aedes vexans	X	X	X	X	X	X	X		X	X	21,39,49,54,55,68
Anopheles punctipennis			X	X					X	X	54,68
Anopheles quadrimaculatis	X	X		X					X	X	15,39,54
Coquillettidia perturbans	X	X	X	X	X	X	X		X	X	15,18,27,36,39,54,68
Culex pipiens		X	X	X	X	X	X		X	X	27,36,39,68
Culex restuans		X	X	X	X	X	X		X		27,36,68
Culex salinarius	X	X	X	X	X	X	X		X	X	15,21,27,39,54,68
Culex tarsalis		X	X	X	X	X		X		X	49,54,55
Culex territans		X		X							15
Culiseta inornata		X	X	X	X	X					21,49,55,68
Culiseta melanura	X	X	X	X	X	X	X			X	15,27,32,36,38
Culiseta morsitans			X		X	X				X	27,68
Psorophora ferox			X	X					X	X	54,68

[a]D-Deer, R-Rodent, RA-Rabbit, RAC-Raccoon, FS-Fox Squireel, RS-Red Squirrel, C-Chipmunk, S-Skunk, W-Woodchuck, O-Opossum.

use the widest variety of animal hosts for blood meal sources and all but Aedes sticticus (Meigen) and Culex salinarius Coq. have been incriminated as potential or proven vectors of one or more types of the CE (Table 2).

Multiple feeding patterns of mosquitoes may be partially due to the host's activity. Edman and Downe (1964) found that those mosquitoes that had fed on multiple hosts had included a rodent as one of the hosts. The small rodent is able to brush away the mosquito feeding attempts. Once the mosquito is interrupted, it may move on to another host and possibly infecting several hosts before it receives a complete blood meal.

The vertebrate hosts in acting as reservoirs of the CE viruses may also serve to disperse the virus by their movements throughout the environment. This may be an important mechanism for dispersing the virus since some mosquitoes such as Aedes triseriatus (Say) have a limited flight range and usually do not venture more than a few hundred feet from their emergance site (Barr, 1958). There are probably other mosquito vectors that have greater flight capabilities but further research is needed to determine the mechanism of virus dissemination by the vertebrate's movement or mosquito dispersion.

A wide variety of mammal species including rabbits, hares and squirrels, are known to have natural infections with California group viruses (Parkin et al., 1972; Hammon & Reeves, 1945; and Gresikova et al., 1964). Hares

Table 2.--Potential vectors of the California encephalitis virus group in Michigan.[a]

Mosquito Species	Subtype[b]
Aedes canadensis	CAL,KEY,LAC,SH
Aedes cinereus	SH
Aedes communis	JC,LAC,SH,TVT
Aedes dorsalis	CAL,LAC
Aedes fitchii-stimulans	SH,JC
Aedes triseriatus	KEY,LAC
Aedes trivittatus	CAL,JC,LAC,TVT
Aedes vexans	CAL,JC,LAC,TVT,KEY,SH
Anopheles punctipennis	LAC
Culex pipiens	LAC,TVT
Culiseta inornata	CAL,JC,TVT,JS
Culiseta melanura	CAL
Psorophora ferox	CAL
Coquillettidia perturbans	TVT

[a]Sudia et al., 1971; Newhouse et al., 1963; Thompson et al., 1967; Thompson et al., 1972.

[b]CAL-California encephalitis virus (prototype), JC-Jamestown Canyon virus, KEY-Keystone virus, LAC-LaCrosse virus, SH-Snowshoe Hare virus, TVT-Trivittatus virus, JS-Jerry Slough virus.

appear to be associated with the Snowshoe Hare subtype, rabbits to the Keystone subtype and tree squirrels and chipmunks to the LaCrosse subtype (Burgdorfer et al., 1961; Newhouse et al., 1963; Bond et al., 1966; Moulton & Thompson, 1971; and Vianna et al., 1971). A number of other mammals tested in Michigan and elsewhere have had positive serological reactions to the hemagglutination inhibition test using the LaCrosse virus antigen, indicating that they have been infected with the virus (Table 3). The use of a single antigen test, however, may not be specific enough to detect only the antibodies of the virus strains suspected (Parkin, 1973; and Sather & Hammon, 1967). A positive hemagglutiation inhibition test using the LaCrosse antigen is not positive proof that the LaCrosse virus was the etiologic agent in the infection, only that one of the CE subtypes probably was involved.

Much is still unknown concerning the vertebrate-virus relationships and research is needed to determine which vertebrate hosts serve as reservoirs. Some species may only serve as indicators of the presence of the virus and may not be able to produce a level of viremia high enough to reinfect the arthropod vector (Cook et al., 1965; and Issel et al., 1972a,b). Another factor affecting the expression of the virus in the host is the possible interaction between the various CE types. Some of the types may be virulent and some non-virulent, and it may be possible to obtain an immunizing effect in the host in

Table 3.--Potential Michigan animal reservoirs of California encephalitis viruses.

Animal Species[a]	Virus Types[b]					
	CE	LAC	SH	JC	TVT	KEY
Chipmunk (*Tamias striatus*)	+	(+)	+	-	-	-
Squirrels						
Fox (*Sciurus niger*)	+	(+)	-	-	-	+
Grey (*Sciurus carolinensis*)	+	+	-	-	-	+
Red (*Tamiasciurus hudsonicus*)	-	(+)	-	-	-	+
Flying (*Glacomys volans*)	-	+	-	-	-	-
Cottontail Rabbit (*Sylvilagus floridanus*)	+	(+)	+	-	+	+
Snowshoe Hare (*Lepus americanus*)	(+)	-	+	-	-	-
Deer (*Odocoileus virginianus*)	-	(+)	+	+	+	-
Raccoon (*Procyon lotor*)	+	-	+	-	-	+
Mouse (*Peromyscus*)	+	+	+	-	-	-
Skunk (*Mephitis mephitis*)	+	-	-	-	-	-
Red Fox (*Vulpes fulva*)	+	-	-	-	-	-
Woodchuck (*Marmota monax*)	+	-	+	-	+	-
Opossum (*Didelphis marsupialis*)	+	-	-	-	+	+

[a]Animal species from which positive sera have been obtained.

[b]CE-California encephalitis virus (prototype), LAC-LaCrosse virus, SH-Snowshoe Hare virus, JC-Jamestown Canyon virus, TVT-Trivittatus virus, KEY-Keystone virus.

(+) Sera obtained from animals captured in Michigan (Becker, 1974-1975; Burdorfer *et al.*, 1961).

which the activity of the virulent type could be suppressed to a subclinical level by prior infections with the non-virulent (Henderson & Coleman, 1971). This could be a valuable tool in controlling those types able to produce human disease by introducing a non-virulent type into the mammal population to suppress the disease causing type.

The detection of the first human CE infection in Michigan was in 1968 when the LaCrosse antigen was used to diagnose an illness of a 4-month-old boy in St. Johns (Clinton County) who became ill during the summer of that year. Between 1968 and 1973, nine additional cases were confirmed using the hemagglutination inhibition test with the LaCrosse antigen. The data concerning human CE infections are still incomplete in Michigan and in many other states, partially due to the lack of interest or unawareness by physicians treating the children. There are probably many cases that are undetected (those that are subclinical) and undiagnosed (those termed "cause unknown" in which no blood sample was tested) (Gorton et al., 1975).

The status of CE in Michigan will remain incomplete for some time because the Michigan Department of Public Health (MDPH) lacks the facilities and resources needed to conduct the necessary research. The MDPH is currently attempting to increase the awareness of Michigan physicians as to the presence of CE and have them submit blood samples from children with meningitis-encephalitis type illness.

This is important in determining the statewide occurrence of the virus in humans. There also must be concurrent research to determine the location of the natural transmission cycles of the virus in nature, the mammal-pathogen assocations and the extent of the mammal involvement in maintaining the virus in nature, and the evaluation of the vertebrate-mosquito relationships to determine which mosquito species might serve as vectors of vertebrate and human infections.

Effects of Water Management on Mosquito Production

"Water management" may have a bad connotation to segments of the general public. They envision the destruction of waterfowl and wildlife species and habitats. Consequently, mosquito control groups in favor of some form of water management for reducing mosquito breeding sites have been on the defensive when proposing such programs (Brockway, 1960; and Springer, 1964). The use of proper water management of marsh lands does not necessarily mean the destruction of wildlife but often leads to better wildlife habitats. The successful use of a water management program is designed to reduce the number of favorable mosquito breeding sites (Rees, 1965). DuChanois and Alltop (1957) showed that there was a relationship between the water table level and potential mosquito production. There appears to be a minimum level below which normal areas of standing water remain dry, thus producing no mosquitoes.

If improper water management is used, a water level could be produced to create abundant mosquito breeding sites. The type of land use practice followed can also create conditions favorable for mosquito production. Hanson and Hanson (1970) evaluated a portion of rugged woodland that had been converted into a recreational area and found that these areas could produce potential health problems when humans intruded into areas with previously undetected natural enzootic virus transmission cycles.

The type of water management used will to some extent determine the mosquito abundance and diversity (Christopher & Bowden, 1957). There are a wide variety of water management programs being used; including impoundments for flood control, irrigation, hydroelectric power, recreational use and sewage treatment ponds. Irrigation is normally used in connection with farming practices to increase the crop yield of a given area. The soil type present must be considered when using irrigation but this was not done in the Milk River Valley region of Montana and "lakes" were formed, due to the clay in the soil which created prolific mosquito production sites (Davis, 1959). Mosquito breeding sites created as a result of irrigation can be eliminated by the construction of a drainage system, such as ditches to remove excess water, or installing drain tiles in the field like those used by turf growers to eliminate standing water. These practices increase the

amount of arable land and decrease the number of potential mosquito breeding sites (Stivers, 1957).

Besides irrigation, there is much interest in sewage lagoons as mosquito production sources. This is important as many sewage lagoons are constructed in close proximity to human habitation and recreational areas and are well within the flight ranges of most mosquitoes. Smith (1969) noted potential dangers to humans in resort, camping and suburban areas and attempted to determine the relationship between the sewage lagoon and the mosquito species produced. He found that mosquitoes of the genus Culex were the most common: including Culex pipiens Linn., Culex salinarius Coq., Culex tarsalis Coq. and Culex restuans Theobald. Smith indicated that there appeared to be an association between the low dissolved oxygen content due to the bacterial action and the attraction of ovipositioning female members of this genus.

The abundance and diversity of the species present will determine whether or not a specific mosquito population will become a health problem. Where high diversity and low abundance occur, no great health problems are likely to develop. Where low species diversity and high abundance occur, the potential for development of health problems is greater. Graham and Bradley (1969) found that a single species did not occur in abundance in those populations having a relatively high diversity which is probably due to the competition between the species

present. The type of habitat available in the sewage lagoon will determine to some extent the diversity of the species present. Smith's work indicates that the genus Culex is the group that commonly utilizes this habitat and this low diversity could produce abundant mosquito populations, thus creating potential health problems.

In 1955 a severe annoyance developed in the Chicago, Illinois, area due to Culex pipiens Linn. originating in sewage lagoons. People were reported to have killed as many as 70 mosquitoes in their bedrooms in a single night (Wray, 1959). Larval samples taken in the lagoons with a standard one pint dipper contained up to 500 larvae per dip. Mosquitoes being produced under this situation were controlled chemically using a mixture of DDT and fuel oil (Ibid.).

Schober (1966) also noted high larval counts in sewage lagoons in Suffolk County, New York, with over 1200 larvae per dip. He attempted control measures by manipulation of the breeding habitat. It was known that mosquito survival was lower in water subjected to wave and wind action so a sprinkling system was set up to create artificial waves on the lagoons and within 24 hours after the sprinkling was started, no larvae, pupae or egg rafts were found. Upon stopping the sprinkling, reinfestation occurred immediately. The wave action apparently prevented the females from ovipositing on the water surface. This sprinkling system appears to be a good method of mosquito

control in sewage lagoons without using chemicals that might be potentially dangerous to the environment.

The use of sewage lagoons in Michigan is not completely new; however, until recently there has not been much research into the effects of these lagoons on mosquito production. In many cases sewage treatment is coupled with spray irrigation so the potential of mosquito production is increased by the creation of additional breeding sites outside the lagoon area (Newson, 1975). Sewage treatment ponds and spray irrigation projects are situations where mosquito control may be necessary because they can create breeding sites favorable for mosquitoes that may serve as potential vectors of diseases of man and his domestic animals (Table 4) (Newson, 1975).

Two related studies are currently being conducted by investigators at Michigan State University. One of these, in Belding, Michigan, is to evaluate the insect production and insect-borne disease potentials resulting from spray irrigation using sewage effluent at this location. The other, south of the main campus of Michigan State University, is a water quality management project to determine the environmental effects of spray irrigation using sewage effluent. The Belding sewage project has been in operation for several years but the spray irrigation portion was not begun until 1973. Prior to the spraying operation Culex pipiens Linn. was the major species breeding in the oxidation ponds at Belding with sporadic

Table 4.--Potential mosquito-disease relationships in a sewage irrigation project in Belding, Michigan, 1972-1974.*

Mosquito Species	Eastern Equine Encephalitis	Western Equine Encephalitis	California Encephalitis	Dirofilaria immitis
Culex pipiens	-	a	a	c
Culex tarsalis	-	b	a	c
Culex territans	a	-	-	c
Aedes triseriatus	a	-	b	c
Aedes vexans	a	a	a	c
Anopheles quadrimaculatis	-	-	-	d
Anopheles punctipennis	-	-	a	c
Culiseta inornata	-	a	a	-
Culiseta melanura	b	a	a	-
Coquillettidia perturbans	a	-	a	c

*Newson, 1974.

a-Possible vector of encephalitis in which the virus has been isolated from the mosquito species.

b-Proven vector of encephalitis shown by experimental studies in which the mosquito transmitted the virus to a susceptable vertebrate host.

c-Possible vector of dog heartworn in which the infective stage of the larvae was obtained from the mosquito species.

d-Proven vector of dog heartworm shown by experimental studies in which the mosquito transmitted the helminth larvae to dogs.

collections of a few Culex territans Walk., Anopheles quadrimaculatus Say and Anopheles punctipennis (Say). After the spraying was started, the number and diversity of the mosquito species using the spray areas and oxidation ponds for breeding purposes dramatically increased. The irrigation spraying also created breeding sites in the spray area for the above species and three additional species: Culex restuans Theobald, Culex salinarius Coq. and Culiseta inornata (Will.), with all of the species using the oxidation ponds as breeding sites (Newson, 1975). The semipermanent water sites created in the spray area were also used as breeding sites by Aedes vexans (Meigen), and to a lesser degree by Aedes triseriatus (Say), Coquillettidia perturbans (Walk.), Culex erraticus (Dyar & Knab), Culex tarsalis Coq., Culiseta impatians (Walk.) and Culiseta melanura (Coq.).

In view of the widespread use of the spray area for breeding by these mosquitoes, there would seem to be a greater potential for health problems developing in those sewage treatment programs with associated spray irrigation sites, as opposed to those with sewage oxidation ponds. The spray irrigation system apparently increases the diversity of mosquito species in the ponds and thus increases the potential of disease transmission since disease transmission is dependent on the abundance of the species present (Graham & Bradley, 1969).

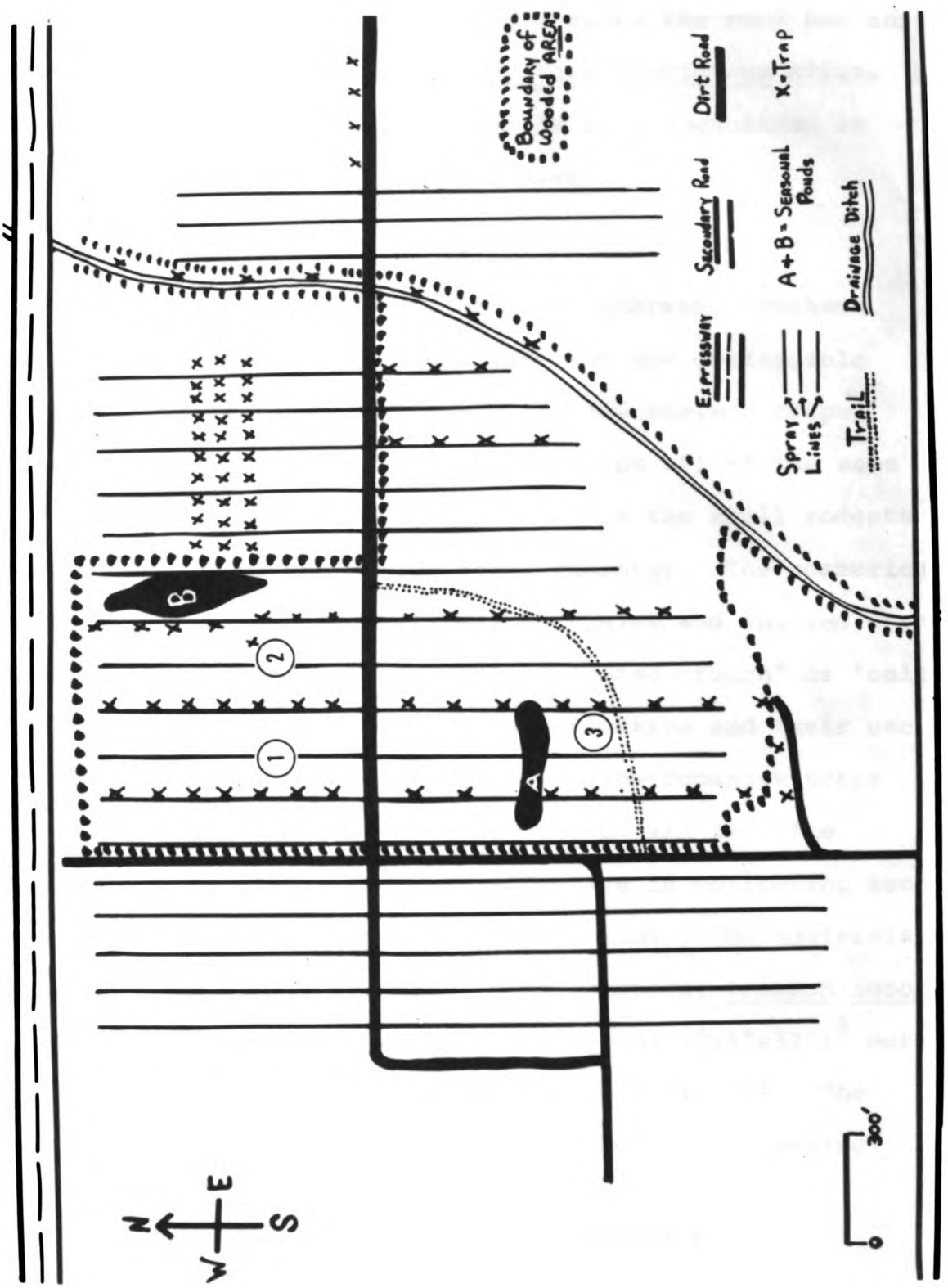
The Michigan State University project was developed to monitor the environmental effects of spray irrigation using sewage effluent. In 1973 the sewage oxidation ponds in the Michigan State University project were filled and in 1974 limited spraying was conducted to determine if the spray system was working properly. A full spraying schedule is not expected until sometime in the future so a complete assessment of its effects on mosquito production and diversity and possible disease transmission will have to be deferred until some future time.

MATERIALS AND METHODS

Description of Study Area

The study took place in the spray irrigation portion of a water quality management project developed by the Institute of Water Research of Michigan State University. The project is located in Ingham County on University property about three miles south of the main campus and contains both field and woodland habitats. The woodland portion of the irrigation site is a beech-maple-oak forest that contains several low areas in which water collects as result of spring rains and melting snow (map--Figure 1, locations A and B). These ponds were usually temporary and would dry up in the summer. An area of mixed oldfields and woodlands was trapped to determine the mammal species present and the presence of any CE activity. Mammal trapping was mainly concentrated in the woodland area because the natural transmission cycle of the CE (LaCrosse subtype) is known to occur in forested areas. Limited trapping was conducted in 1973 in the fallow fields east of the temporary pond "B" and along the road east of the ditch. The area east of the pond proved to have few small mammals with only a few deer mice, Peromyscus maniculatus

Figure 1. A map of the study area. Numbers 1, 2, and 3 are the location of the mosquito-bait traps.


Boundary of Wooded Area
Dirt Road
X-Trap
Secondary Road
A+B = Seasonal Ponds
Drainage Ditch
Expressway
Spray Lines
Trail
B
A
1
2
3
N
E
S
W
300'
0

Wagner, being collected in 1973 so trapping was discontinued there early in the study. The area along the road had an abundant cottontail rabbit, Sylvilagus floridanus Allen, population but trapping there had to be discontinued in 1974 due to construction in this area.

Materials

The mammals were collected in Sherman, Tomahawk, and Havahart live traps. The first two are collapsible while the third is non-collapsible. The Sherman traps (Figure 2A) were solid, sheet metal traps all of the same size (3"x3"x9")[1] and were used to sample the small rodents such as deer mice and meadow mice, Microtus. The commerical bait used consisted of a mixture of grains and shelled corn mixed with mollasses and was called "horse crunch" or "colt feed." The Sherman traps were not effective and their use was discontinued early in the study. The Tomahawk traps used were of three sizes (Figures 2B, C, and D). The smallest (5"x5"x16")[2] was very effective in collecting such rodents as chipmunks, Tamias striatus Linn.; fox squirrels, Sciurus niger Linn.; and very young raccoons, Procyon lotor Linn. The larger two sizes (6"x6"x24" and 9"x9"x32")[3] were used to collect mammals larger than fox squirrels. The smaller mammals were able to escape through the opening

---

[1]For metric conversion see Appendix C.

[2]Ibid.

[3]Ibid.

between the door and the side of the trap. The third trap type, Havahart, was a non-collapsible trap (Figure 3A and B). Due to its bulkiness and ineffective locking mechanism, it was not used after the first summer of collecting. The bait used in all of the Tomahawk and Havahart traps was dried ear corn.

Three modified Villavaso and Steelman (1970) animal-baited traps were constructed to collect the mosquitoes attracted to the mammals occurring in the study area and thus determine the host preferences of these mosquitoes. The traps were 36"(L) x 34"(W) x 20"(H)[1] and had two main components: (1) Two removeable side collecting boxes in which mosquitoes were collected (Figure 4); and (2) the center holding area (Figure 5) where the caged mammals were placed. A removeable screen barrier was placed in each collecting box to prevent the mosquitoes from feeding on the mammal used to attract the mosquitoes into the trap. The metal pan was used to prevent the mammal from scenting the trap with its wastes. It became necessary to attach metal legs on the traps in order to raise them off the ground and decrease the number of Harvestmen (Arachnida: Phalangida) from entering the trap (Figure 6). A very low mosquito population existed in the study area in 1974 resulting in very small mosquito collections (usually of 1 or 2 at a time). It was therefore felt that the Harvestmen, being

---

[1]For metric conversion see Appendix C.

Figure 2. Collapsible live traps.

	Type	Size
A.	Sherman	3" x 3" x 9"
B.	Tomahawk	5" x 5" x 16"
C.	Tomahawk	6" x 6" x 24"
D.	Tomahawk	9" x 9" x 32"

Figure 3. Non-collapsible live traps.

	Type	Size
A.	Havahart	5" x 5" x 18"
B.	Havahart	6" x 6" x 30"

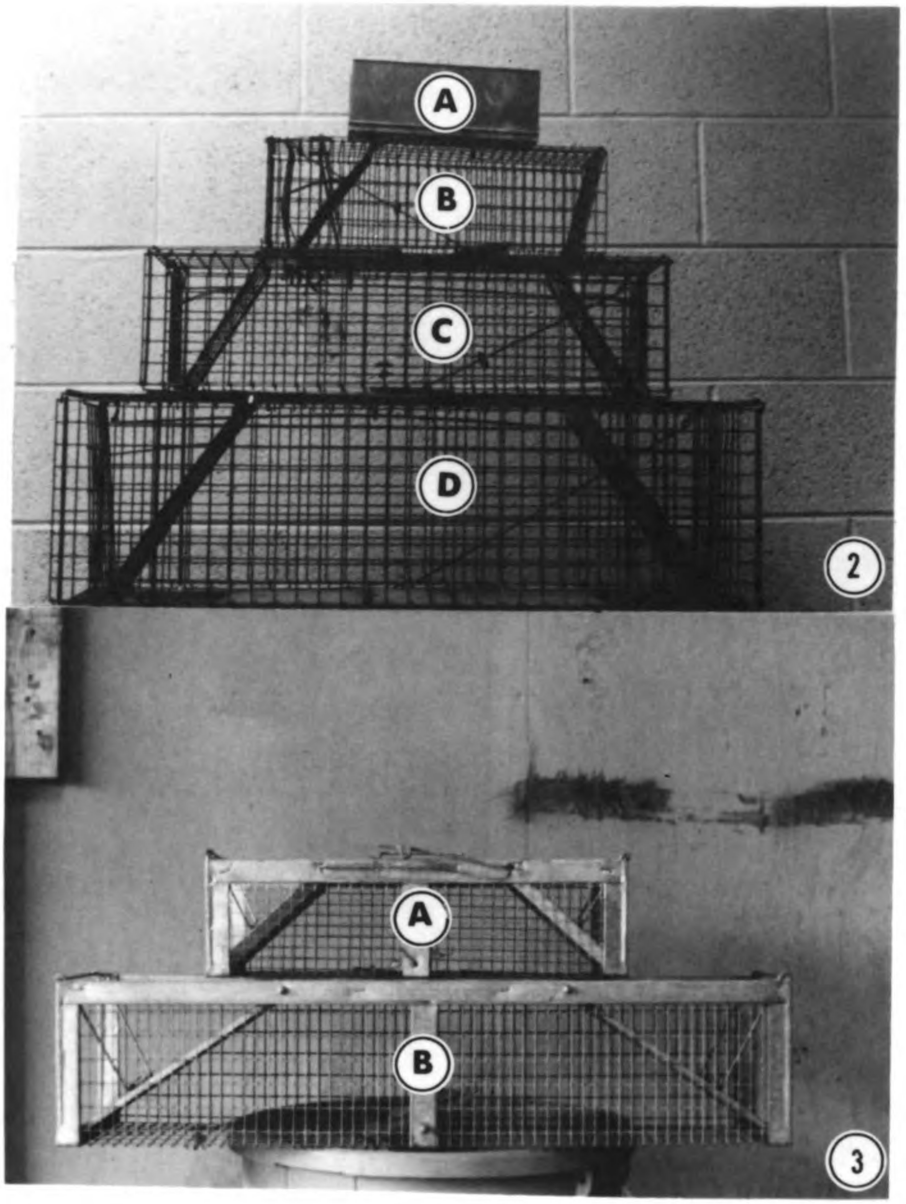
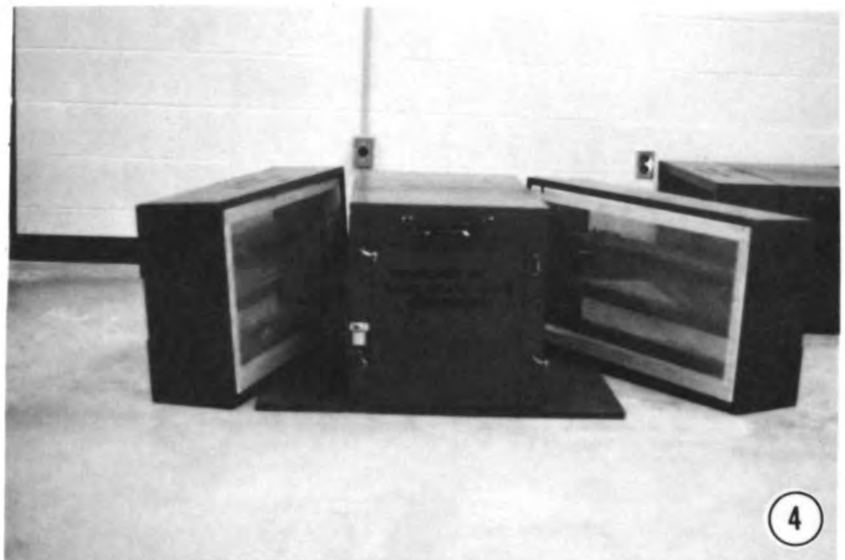
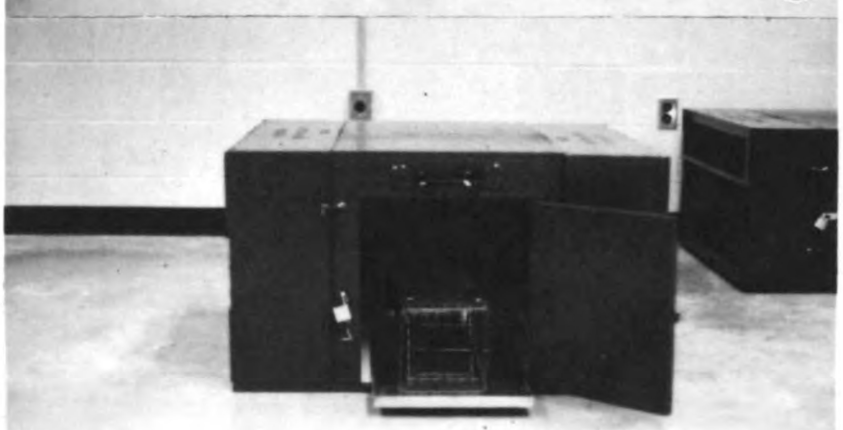

A
B
C
D
2
A
B
3

Figure 4. Mosquito bait trap with side collecting boxes detached.

Figure 5. Mosquito bait trap opened to show location of caged mammal.



4



5

Figure 6. Mosquito bait trap with metal legs.

Figure 7. A young raccoon anesthetized with Nembutal.


6


7

scavangers, could affect the collection results so their access into the traps had to be minimized.

The mosquito traps were all placed in the woodland area. Two of the traps were located in the north section (B) as it was in this section where the bulk of the mammals were trapped. All of the species trapped, except the deer mice, were used as bait in the mosquito traps. Because of the low mosquito populations present in 1974, it was often necessary to place several mammals of the same species in a trap to attract the mosquitoes.

Methods

The mammal traps were placed along three of the spray irrigation pipe lines at every fourth pipe joint to simplify the rechecking of the traps. Each trap was identified by a number-letter-number designation (e.g., 1-A-1) used later in determining the ranges of the mammals trapped and to see if any areas were used more than others. The first number was the pipe line designation (numbering from west to east), the letter indicated the section of woods in which the trap was located, and the second number was the location of the trap along the pipe line (numbering away from the dirt road in either direction). A dirt road running west-east through the spray area separated the irrigation site into a north and south section, B and A, respectively.

A blood sample was taken by cardiac puncture and a numbered ear tag was affixed to each mammal trapped. A blood sample of at least 1 cc was needed to insure enough serum for the California encephalitis antibody test. Mammals such as opossums, Didelphis marsupialis Linn.; cottontail rabbits; chipmunks; fox squirrels; red squirrels, Tamiasciurus hudsonicus Erxleben; and deer mice were anesthetized in the study area with chloroform and bled. Raccoons and woodchucks, Marmota monax Linn., were taken to the laboratory and partially anesthetized with chloroform and then given an injection (intramuscular-hip) of Nembutal (dosage determined by the mammal's weight, see Appendix A). When the mammal became semiconscious and could be handled safely (Figure 7), a blood sample was taken and a numbered ear tag affixed. After the mammal recovered from the Nembutal, it was released in the general area in which it had been trapped.

The blood samples were transferred to test tubes (13 x 100mm) and were allowed to clot at room temperature for several hours after which the clotted samples were placed in a refrigerator (at 4 to 5°C) overnight to allow the clot to retract. The blood samples were then centrifuged (at 1700 to 2000 rpms for 10 to 20 minutes) and the clear serum transferred with micro-pipettes into marked one-half dram vials and placed in a freezer (at -20 to -25°C). The samples were frozen until they could be tested for CEV antibodies at the Michigan Department of

Public Health, Virology Laboratory. The test used was a modified hemagglutination inhibition (HI) test using LaCrosse virus antigen in a suspension of goose red blood cells (Clarke & Casals, 1958).

While the animals were anesthetized the fleas (Siphonaptera) on them were collected. This survey was conducted to determine the species diversity associated with the animal species present.

RESULTS

Ten species of mammals were collected in the study area with chipmunks, fox squirrels, red squirrels, cottontail rabbits, raccoons, and opossums making up approximately 90 percent of the mammals trapped (Table 5). Two other species: deer, Odocoileus virginianus Zimmermann and a starnose mole, Condylura cristata Linn. were observed in the study area but no blood samples were obtained from these animals, or from a trapped muskrat, Ondatra zibethica Linn.; and skunk, Mephitis mephitis Schreber. Except for the rabbits and deer mice, all of the species were collected in the woodland habitat. The majority of the rabbits trapped were taken from the field east of the ditch and were collected during the first summer.

Table 6 shows the results of the hemagglutination inhibition tests on the 187 blood samples obtained from the 162 mammals trapped. Approximately 30 to 40 percent of the fox squirrels and 40 to 50 percent of the chipmunks trapped were probably resamples but their correct identification was not possible because their ear tags were torn out thus conversion data for these was not possible.

Table 5.--A survey of animals in the Michigan State University Water Quality Project site, 1973-1974.

Species	Number Collected
Chipmunk (*Tamias striatus*	28
Fox squirrel (*Sciurus niger*)	25
Red squirrel (*Tamiasciurus hudsonicus*)	20
Cottontail Rabbit (*Sylvilagus floridanus*)*	20
Raccoon (*Procyon lotor*)	30
Opossum (*Didelphis marsupialis*)	23
Woodchuck (*Marmota monax*)	8
Deer mouse (*Peromyscus maniculatus*)*	6
Muskrat (*Ondratra zibethica*)	1
Skunk (*Mephitis mephitis*)	1
Starnose mole (*Condylura cristata*)	1 (found dead)
Deer (*Odocoileus virginianus*)	**

*Taken in oldfield habitat, the other species taken in woodlands.

**A total of 5 were observed in study area.

Table 6.--Serological survey of indigenous small mammals in the Michigan State University Water Quality Project site, 1973-1974.

Animal Species	HI Titers to the LaCrosse subtype of California Encephalitis Virus*			
	<1:10	1:10	1:20	1:40
Chipmunk	29	7	1	0
Fox Squirrel	19	21	2	1
Red Squirrel	16	3	1	0
Cottontail Rabbit	9	11	0	0
Raccoon	21	9	0	0
Opossum	21	2	0	0
Woodchuck	6	2	0	0
Deer Mouse	5	1	0	0
Totals	126	56	4	1

*A titration $\leq$ 1:20 is considered positive.

Positive sera were obtained from 3 fox squirrels, 1 red squirrel and 1 chipmunk, all of which were trapped in the woodland habitat.

Three mosquito traps were constructed and used during the summer of 1974 to determine the host preferences of the mosquito species present. Mosquito populations observed during the summer of 1974 were dramatically lower than those observed in 1973, resulting in only 60 mosquitoes of 8 different species being attracted into these traps (Table 7): including Aedes cinereus Meigen, Aedes fitchii-stimulans (Felt & Young), Aedes sticticus (Meigen), Aedes triseriatus (Say), Aedes vexans (Meigen), Coquillettidia perturbans (Walk.), Culex pipiens Linn. and Culex salinarius Coq.

In conjunction with the serological sampling of the small indigenous mammals, a survey of the fleas (Siphonaptera) parasitizing these mammals was conducted (Table 8). Orchopeas howardii Baker made up 74.2 percent of the fleas collected. The remaining species consisted of Ctenophthalmus pseudagyrtes Baker, 17 percent; Cediopsylla simplex (Baker), 5.5 percent; Opiscrotis bruneri (Baker), 0.9 percent; Orchopeas leucopus (Baker), 0.9 percent; Epitedia faceta (Rothschild), 0.5 percent; Megabothris asio (Baker), 0.5 percent; and Oropsylla arctomys (Baker), 0.5 percent.

Birds frequently tripped the corn-baited mammal live traps used to sample the indigenous small mammals

Table 7.--1974 Mosquito bait trap collection results at the Michigan State University Water Quality Project site.

Mosquito Species	Animal Species				
	Racoon	Fox Squirrel	Red Squirrel	Woodchuck	Opossum
Aedes cinereus	+	+	+	-	-
Aedes fitchii-stimulans	+	+	+	-	-
Aedes sticticus	+	+	+	-	-
Aedes triseriatus	+	+	+	+	-
Aedes vexans	+	+	-	-	-
Coquillettidia perturbans	+	-	-	-	-
Culex pipiens	-	+	+	-	-
Culex salinarius	-	-	-	-	+

Table 8.--Most associations of fleas collected during 1973-1974 from Michigan State University Water Quality Project site.

Flea Species	Animal Hosts[a]							Totals per Flea Species	Percent of Total
	FS	RS	C	RAC	O	RAB	DM		
Family Pulicidae									
Cediopsylla simplex						12		12	5.5
Family Hystrichopsyllidae									
Epitedia facets				1				1	0.5
Family Dolichopsyllidae									
Orchopeas howardii	94	27	12	22	7			162	74.2
Orchopeas leucopus							2	2	0.9
Oropsylla arctomys						1		1	0.5
Opisocrostis bruneri				2				2	0.9
Megabothris asio					1			1	0.5
Ctenophthalmus pseudagyrtes			22		15			37	17.0
Total per animal species	94	27	34	25	23	13	2	218	100.0

[a]FS-Fox Squirrel, RS-Red Squirrel, C-Chipmunk, RAC-Raccoon, O-Opossum, RAB-Cottontail Rabbit, DM-Deer Mouse.

present. Seven species of birds were trapped during the course of this study. Blood samples were obtained from three species; including three grackles, Quisculus quiscula Vieill; two cardinals, Richmondena cardinal Linn.; and one purple finch, Carpodacus purpureus Gmel. No blood samples were obtained from the other four species trapped: including blue jays, Cyanocitta cristata Linn.; ruffed grouse, Bonasa umbellus Linn.; brown thrashers, Toxostoma rufum Linn.; and a rufous-sided towhee, Pipilo erythrophthalmus Linn.

DISCUSSION

The movements of the forest mammals (see Table 5) were determined by a capture-recapture method. It was found that raccoons and opossums showed the greatest degree of movement and dispersion while chipmunks and fox squirrels showed the least. Raccoons and opossums did not have any location preferences but squirrels and chipmunks tended to stay in the general area where they were first trapped. The majority of the mammals trapped appeared to be juveniles which would seem to indicate a good reproducing mammal population in the study area. The number of juvenile raccoons and opossums declined as the summer progressed.

Whenever possible the mammals were re-bled monthly to determine the virus activity by monitoring antibody conversion. In order to confirm the presence of a virus infection in the animal a paired sera is required. Due to the high frequency of lost ear tags in the fox squirrels and chipmunks, it was not possible to determine which of them may have undergone serological conversion. Unfortunately, 4 of the 5 positive mammals were from this group.

The antibody titers of the mammals bled ranged from less than 1:10 to 1:40 with titers of 1:10 or less considered negative and those of 1:20 or greater, positive. The reason for the relatively high percentage of reactions at the 1:10 dilution level is not known but may indicate the presence of heterologous CE antibodies. The infections in the mammals could involve strains of the CE other than the LaCrosse subtype that might cross react with the LaCrosse virus antigen used in the hemagglutination inhibition test. As mentioned earlier, the use of a single antigen test may not be specific enough to detect only one type of antibody. Sather and Hammon (1967) working in Wisconsin with antigenic patterns in the California encephalitis group found that there were apparent similiarities between the virus strains in the group, especially in those strains obtained from the same area at the same time. They also found that cross reactions would occur between the Snowshoe Hare antibodies and the LaCrosse antigen. Similiar cross reactions between other strains of the CE group were shown by Parkin (1973) with strains of the CE group found in domestic animals in Florida. This may explain in part the high proportion of 1:10 titers found in the animals tested in this study. The clarification of this will not be possible until the CE viruses present in this location have been isolated and typed.

No isolations of the CE viruses have yet been reported from fleas but research in this area has been

very limited. Fleas are known to serve as vectors of such disease agents as bacteria (plague), Rickettsia (typhus) and viruses (Myxomatosis) (James & Harwood, 1969). Due to the close association fleas have with their hosts, it would seem possible that they could become infected with the CE virus while feeding on an infected host. Whether fleas have a role in the transmission of the CE viruses is not known, but its blood-feeding habits might enable it to reinfect its host, thus keeping the virus active so that the primary vectors (mosquitoes) could subsequently be infected. Sudia et al. (1971) indicate that the apparent mechanism by which the virus is disseminated in nature is by the movements of the vertebrate reservoirs. It is not known exactly how long the viremia remains high enough to infect the primary vectors. It is also not known what possible immune reactions might occur as a result of the CE infection, thus preventing the vertebrate host from being reinfected for a period of time. During this period the host might move to an area where non-infected mosquitoes are present. If the fleas then could transmit the virus, they (fleas) could reinfect the host when its immunity subsided.

The number and abundance of possible vector species present in the study area will influence the virus transmission activity to the susceptable vertebrate hosts. Aedes cinereus Meigen, Aedes fitchii-stimulans (Felt & Young), Aedes triseriatus (Say), Aedes vexans (Meigen),

Coquillettidia perturbans (Walk.) and Culex pipiens were collected in the study area and have been incriminated elsewhere as either proven or potential vectors of one or more of the CE viruses (Tables 2 and 7). The abundance of these species is dependent upon the weather conditions (rain and snowfall, temperature, etc.) that make these sites favorable for mosquito production. There were numerous fallen trees with holes; artificial containers such as tin cans, glass jars and rubber tires; and several temporary ponds (locations A and B on map) that could serve as potential breeding sites in the study area. Larvae of Aedes fitchii-stimulans (Felt & Young) were collected in the temporary ponds and a few Aedes triseriatus (Say) were taken from some of the tree holes sampled in the study area (Zorka, 1975).

The adult mosquitoes collected during the summer of 1974 in mosquito bait traps gave an indication of the host preferences of these species. Only five mammal species attracted mosquitoes into the traps (Table 7) which, in part, may be due to the low mosquito populations occurring that year. These traps should be used again when the mosquito populations increase to obtain more complete host preference determinations. The host preferences are necessary to determine what mosquito species may serve as vectors of the CE in the study area.

Of the avian species occurring in the study area, blackbirds, cardinals, and grackles have been associated

with Eastern equine encephalitis (EEE) transmission elsewhere. Aedes triseriatus (Say), Aedes vexans (Meigen), and Coquillettidia perturbans (Walk.) have been incriminated as potential vectors of this virus (Williams et al., 1971). This would indicate that the potential for EEE transmission exists in the study area. The sera obtained from the birds sampled were not tested for EEE antibodies. Like CE, uncertainities will remain until virus isolations are done to determine which virus types are present in the bird populations in this location. The high mortality associated with the EEE infections could pose a greater threat to the humans living near this location than the CE, if the EEE is present. It is for this reason that further research is needed concerning arboviruses that may occur in the study area.

The natural transmission cycle of the CE virus involves a mosquito-small mammal-mosquito cycle and man does not become involved until he intrudes on the habitat where this "natural" cycle occurs. These "natural" cycles usually occur in undisturbed woodland habitats where abundant vertebrate hosts and arthropod vectors exist. However, when man disturbs this habitat by developing campgrounds, hiking and nature trails, and suburban housing areas, he displaces the vertebrate hosts used as blood meal sources by the arthropod vectors. These vectors then use man as a readily available blood meal source and thus may infect him with the virus causing California encephalitis.

SUMMARY

Hemagglutination inhibition tests on indigenous small mammals collected in a sewage spray irrigation area on the Michigan State University campus indicated the presence of California encephalitis virus activity at this location. Of 182 serum samples obtained from 162 mammals, 5 were positive: 3 fox squirrels, 1 red squirrel and 1 chipmunk. Although the data is limited, it suggests CE is maintained there in a mosquito-squirrel-mosquito cycle.

Eight mosquito species were collected in animal-baited mosquito traps: Aedes triseriatus (Say), Aedes cinereus (Meigen), Aedes fitchii-stimulans (Felt & Young), Aedes sticticus (Meigen), Aedes vexans (Meigen), Culex pipiens Linn., Culex salinarius Coq., Coquillettidia perturbans (Walk.). Of these Aedes triseriatus (Say), Aedes cinereus (Meigen), Aedes fitchii-stimulans (Felt & Young), Aedes vexans (Meigen), and Culex pipiens Linn. have been shown elsewhere to be capable of transmitting one or more of the CE group viruses.

A relatively large bird population was observed in the spray irrigation site, some of which may serve as

reservoirs of the EEE virus. A number of mosquito species present in the study area, including Aedes triseriatus (Say) and Aedes vexans (Meigen) are potential vectors of both EEE and CE viruses (James & Harwood, 1969; Newson, 1975; and Sudia et al., 1971). The potential for EEE transmission may exist in this location but additional studies will be required to determine whether or not the EEE virus is present.

Of major concern to the developers of the water quality management project is the possible effects of the proposed spray irrigation system on mosquito production and transmission of insect-borne diseases. Since the potential for disease transmission may increase if the spray irrigation procedures increase mosquito populations and species diversity in the spray site, it will be important to closely monitor the biting insect populations present and to continue research designed to assess the effects that the spraying operations will have on both the natural zoonotic transmission cycles.

LITERATURE CITED

LITERATURE CITED

1. Barr, A. R. 1958. The mosquitoes of Minnesota (Diptera:Culicidae:Culicine). Univ. Minn. Agr. Exp. Sta. Tech. Bull. 228. 154 pp.

2. Beadle, L. D. 1966. Epidemics of mosquito-borne encephalitis in the United States, 1960-1965. Mosq. News 26:483-486.

3. Becker, M. 1974. Personal communication.

4. Becker, M. 1975. Personal communication.

5. Bond, J. O., W. McD. Hammon, A. L. Lewis, G. E. Sather, and D. J. Taylor. 1966. California group arboviruses in Florida and report of a new strain, Keystone virus. Public Health Report 81:607-613.

6. Brockway, P. B., Jr. 1960. Water management in marsh areas for mosquito control and the conservation of wildlife. Mosq. News 20:235-237.

7. Burgdorfer, W., V. F. Newhouse, and L. A. Thomas. 1961. Isolation of California encephalitis virus from the blood of snowshoe hare (Lepus americanus) in Western Montana. Am. J. Hyg. 73:344-349.

8. Calisher, C. H., K. S. C. Maness, R. D. Lord, and P. H. Coleman. 1971. Identification of two South American strains of Eastern Equine Encephalitis virus from migrant birds captured on the Mississippi Delta. Am. J. Epidemiol. 94:172-178.

9. Chamberlain, R. W., R. K. Sikes, D. B. Nelson, and W. D. Sudia. 1954. Studies on the North American arthropod-borne encephalitides. Am. J. Hyg. 60:278-285.

10. Cherneshy, M. A. 1967. Transmission of California encephalitis virus by mosquitoes. Can. J. Micro. 14:19-23.

11. Christopher, G. S., and N. W. Bowden. 1957. Mosquito control in reservoirs by water level management. Mosq. News 17:273-277.

12. Chun, R. W. M., W. H. Thompson, J. D. Grabow, and C. G. Matthews. 1968. California arbovirus encephalitis in children. Neurology 18: 369-375.

13. Clarke, D. H., and J. Casals. 1958. Techniques for hemagglutination and hemagglutination inhibition with arthropod-borne viruses. Am. J. Trop. Med. & Hyg. 7:561-573.

14. Cook, R. S., D. O. Trainer, W. C. Glazener, and B. D. Nassif. 1965. A serological study of wild populations in south Texas. Trans. 13th. N. A. Wildl. Conf. 142-155.

15. Crans, W. J. 1963. Continued host preference studies with New Jersey mosquitoes. N. J. Mosq. Exterm. Assoc. 51:50-58.

16. Davis, S. 1959. Mosquito control problems of the irrigator. Mosq. News 19:68-69.

17. DeFoliart, G. R., R. O. Anslow, R. P. Hanson, C. D. Morris, O. Papadopoulos, and G. E. Sather. 1969. Isolation of Jamestown Canyon serotype of California encephalitis virus from naturally infected Aedes mosquitoes and Tabanids. Am. J. Trop. Med. & Hyg. 18:440-447.

18. Downe, A. E. R. 1960. Blood-meal sources and notes on host preferences of some Aedes mosquitoes (Diptera:Culicidae). Can. J. Zool. 38:689-699.

19. Downe, A. E. R. 1962. Some aspects of host selection by Mansonia perturbans (Walk.) (Diptera: Culicidae). Can. J. Zool. 40:725-732.

20. DuChanois, F. R., and L. B. Alltop. 1957. Progress report of an investigation of the relationship of water table level fluctuation to mosquito development and population density, 1954-1956. Mosq. News 17:186-189.

21. Edman, J. D., and A. E. R. Downe. 1964. Host-blood sources and multiple-feeding habits of mosquitoes in Kansas. Mosq. News 24:154-160.

22. Gorton, R. J., H. D. Newson, and R. Bail. 1975. California encephalitis in Michigan: Coincidence or an unrecognized health problem? Mich. Med. 74:75-79.

23. Graham, J. E., and I. E. Bradley. 1969. Mode of action of factors responsible for increase in Culex tarsalis Coq. populations in Utah. Mosq. News 29:678-687.

24. Gresikova, M., W. C. Reeves, and R. P. Scrivani. 1964. California encephalitis virus: An evaluation of its continued endemic status in Kern County, California. Am. J. Hyg. 80:229-234.

25. Hammon, W. McD., and W. C. Reeves. 1945. Recent advances in the epidemiology of arthropod-borne virus encephalitis. Am. J. Public Health. 35:994-1004.

26. Hanson, R. P., and M. G. Hanson. 1970. The effects of land use practices on the vector of California encephalitis (LaCrosse) in North Central United States. Mosq. News 30:215-221.

27. Hayes, R. O. 1961. Host preferences of Culiseta melanura and allied mosquitoes. Mosq. News 21:169-187.

28. Henderson, B. E., and P. H. Coleman. 1971. The growing importance of California arboviruses in the etiology of human disease. Prog. Med. Virol. 13:404-461.

29. Issel, C. J., D. O. Trainer, and W. H. Thompson. 1972a. Experimental studies with white-tailed deer and four California group arboviruses (LaCrosse, Trivittatus, Snowshoe Hare and Jamestown Canyon). Am. J. Trop. Med. & Hyg. 21:979-984.

30. Issel, C. J., D. O. Trainer, and W. H. Thompson. 1972b. Serological evidence of infections of white-tailed deer in Wisconsin with three California group arboviruses (LaCrosse, Trivittatus and Jamestown Canyon). Am. J. Trop. Med. & Hyg. 21:985-988.

31. James, M. T., and R. E. Harwood. 1969. Herms's Medical Entomology. 6th. Ed., Macmillan Co., New York. 484 pp.

32. Jobbins, D. M., P. P. Burbutis, and W. J. Crans. 1961. Mosquito blood-meal determinations. A cooperative project. N. J. Mosq. Exterm. Assoc. 48:211-215.

33. Johnson, K. P. 1970. California Encephalitis. Epidemiologic studies in Ohio using a quantitative metabolic-inhibition test. Am. J. Epidemiol. 92:203-209.

34. Johnson, K. P., M. L. Lepow, and R. T. Johnson. 1968. California Encephalitis. I. Clinical and epidemiological studies. Neurology 18: 250-254.

35. Matthews, C. G., R. W. M. Chun, J. D. Grabow, and W. H. Thompson. 1968. Psychological sequelae in children following California arbovirus encephalitis. Neurology 18:1023-1030.

36. Means, R. G. 1968. Host preferences of mosquitoes (Diptera:Culicidae) in Suffolk County, New York. Ann. Ent. Soc. Am. 61:116-120.

37. Moulton, D. W., and W. H. Thompson. 1971. California group virus infections in small forest-dwelling mammals of Wisconsin. Am. J. Trop. Med. & Hyg. 20:474-482.

38. Moussa, M. A., D. J. Gould, M. P. Nolan, Jr., and D. E. Hayes. 1966. Observations on Culiseta melanura (Coquillett) in relation to encephalitis in southern Maryland. Mosq. News 26: 385-393.

39. Murphy, F. J., P. P. Burbutis, and D. F. Bray. 1967. Bionomics of Culex salinarius Coquillett. II. Host acceptance and feeding by adult females of C. salinarius and other mosquito species. Mosq. News 27:366-367.

40. Newhouse, V. F., W. Burgdorfer, J. A. McKiel, and J. D. Gregson. 1963. California Encephalitis Virus. Serological survey of small wild mammals in Northern United States and Southern Canada and isolation of additional strains. Am. J. Hyg. 78:123-129.

41. Newhouse, V. F., W. Burgdorfer, and D. Corwin. 1971. Field and laboratory studies of the hosts and vectors of the Snowshoe Hare strain of California virus. Mosq. News 31:401-408.

42. Newson, H. D. 1975. Personal communication.

43. Parkin, W. E., W. McD, Hammon, and G. E. Sather. 1972. Review of current epidemiological literature on viruses of the California arbovirus group. Am. J. Trop. Med. & Hyg. 21:964-978.

44. Parking, W. E. 1973. The occurrence and effects of the local strains of the California encephalitis group of viruses in domestic mammals of Florida. Am. J. Trop. Med. & Hyg. 22:788-795.

45. Rees, D. M. 1965. Cooperative research on water management for wildlife, mosquito abatement and other benefical use. Mosq. News 25:91-93.

46. Rempel, J. G., W. A. Riddell, and E. M. McNelly. 1946. Multiple feeding habits of Saskatchewan mosquitoes. Can. J. Res. E. 24:71-78.

47. Sather, G. E., and W. McD. Hammon. 1967. Antigenic patterns within the California-Encephalitis-Virus group. Am. J. Trop. Med. & Hyg. 16: 548-557.

48. Schober, H. 1966. Agitation of water surfaces by sprinkling to prevent mosquito breeding. Mosq. News 26:144-149.

49. Schemanchuk, J. A., A. E. R. Downe, and L. Burgess. 1963. Hosts of mosquitoes (Diptera:Culicidae) from the irrigated area Alberta. Mosq. News 23:341-366.

50. Smith, L. W. 1969. The relationship of mosquitoes to oxidation lagoons in Columbia, Missouri. Mosq. News 29:556-563.

52. Stivers, J. O. 1957. Minimizing mosquito breeding in irrigated pastures. Mosq. News 17:268-272.

53. Sudia, W. D., V. F. Newhouse, C. H. Calisher, and R. W. Chamberlain. 1971. California group arboviruses: Isolations from mosquitoes in North America. Mosq. News 31:576-600.

54. Suyemoto, W., B. A. Schiefer, and B. F. Eldridge. 1973. Precipitin tests of blood-fed mosquitoes collected during the VEE surveillance survey in the southern United States in 1971. Mosq. News 33:392-395.

55. Tempelis, C. H., D. B. Francy, R. O. Hayes, and M. F. Lofy. 1967. Variation in feeding patterns of seven culicine mosquitoes on vertebrate hosts in Weld and Larimer Count, Colorado. Am. J. Trop. Med. & Hyg. 16:111-119.

56. Thompson, W. H., D. O. Trainer, V. Allen, and J. B. Hale. 1963. The exposure of wildlife workers in Wisconsin to ten zoonotic diseases. Trans. 234d. N. A. Wildl. Conf. 215-225.

57. Thompson, W. H., B. Kalfayan, and R. O. Anslow. 1965. Isolation of California encephalitis group virus from a fatal human illness. Am. J. Epidemiol. 81: 245-253.

58. Thompson, W. H., and S. L. Inhorn. 1967. Arthropod-borne California gorup viral encephalitis in Wisconsin. Wis. Med. J. 66:250-253.

59. Thompson, W. H., D. J. Engesfeth, J. O. Jackson, G. R. DeFoliart, and R. P. Hanson. 1967. California encephalitis-group virus in small mammals and insects in a decidious forest area of southern Wisconsin. Wild. Dis. Assoc. Bull. 3:92.

60. Thompson, W. H., R. O. Anslow, R. P. Hanson, and G. R. DeFoliart. 1972. LaCrosse virus isolations from mosquitoes in Wisconsin, 1964-1968. Am. J. Trop. Med. & Hyg. 21:90-96.

61. Vianna, N., E. Whitney, T. Bast, R. Deibel, J. Doll, and J. Culver. 1971. California encephalitis in New York State. Am. J. Epidemiol. 94: 50-55.

62. Villavaso, E. J., and C. D. Steelman. 1970. A collapsible dog-baited mosquito trap. Mosq. News 30:39-42.

63. Watts, D. M., C. D. Morris, R. E. Wright, G. R. DeFoliart, and R. P. Hanson. 1972. Transmission of LaCrosse virus (California encephalitis group) by the mosquito Aedes triseriatus. J. Med. Ent. 9:125-127.

64. Watts, D. M., S. Pantuwatana, G. R. DeFoliart, T. M. Yuill, and W. H. Thompson. 1973. Transovarial transmission of LaCrosse virus (California encephalitis group) in the mosquito Aedes triseriatus. Sci. 182:1140-1141.

65. Watts, D. M., W. H. Thompson, T. M. Yuill, G. R. DeFoliart, and R. P. Hanson. 1974. Over-wintering of LaCrosse virus in Aedes triseriatus. Am. J. Trop. Med. & Hyg. 23:694-700.

66. Williams, J. E., O. P. Young, D. M. Watts, and T. J. Reed. 1971. Wild birds as Eastern (EEE) and Western (WEE) Equine Encephalitis sentinels. J. Wild. Dis. 7:188-194.

67. Wray, F. C. 1959. Mosquito control in sludge lagoons. Mosq. News 19:81-85.

68. Wright, R. E., R. O. Anslow, W. H. Thompson, G. R. DeFoliart, G. Seawright, and R. P. Hanson. 1970. Isolations of LaCrosse virus of the California group from Tabanidae in Wisconsin. Mosq. News 30:600-603.

69. Zorka, T. 1975. Personal communication.

APPENDICES

APPENDIX A

NEMBUTAL DOSAGES BASED ON ANIMAL'S WEIGHT

APPENDIX A

NEMBUTAL DOSAGES BASED ON ANIMAL'S WEIGHT

Raccoons Wt. (Lbs)	Dosage (.75g/cc) Nembutal*
1.0	.4cc
2-2.5	.5cc-.75cc
3-3.5	.6cc-1.1cc
4.0	1.0cc-1.25cc
5.5	1.35cc
6.0	1.75cc
12-15.0	3.5cc
18.0	4.0cc

Woodchuck Wt. (Lbs)	Dosage (.75g/cc) Nembutal*
5-5.0	1.75cc-2.75cc
6.5	2.0cc
8.5	3.0cc
9.0	3.75cc
12.0	4.0cc

*Due to the variance between individuals, the amount of Nembutal needed to sedate the animal also varied.

APPENDIX B

HI TITER RESULTS OF INDIGENOUS SMALL MAMMALS IN A WATER QUALITY PROJECT SITE, 1973-1974

APPENDIX B

HI TITER RESULTS OF INDIGENOUS SMALL MAMMALS
IN A WATER QUALITY PROJECT SITE, 1973-1974

Fox Squirrel				Chipmunk			
Tag No.	Sex	Trapped	Result	Tag No.	Sex	Trapped	Result
44939	f	7-1-73	1:10	52441	m	7-1-73	
44926	f	7-6-73	1:10	died	f	7-1-73	
44927	f	7-6-73	1:10	52442	f	7-4-73	
52460	m	7-7-73	1:10	52450	m	7-4-73	
52461	f	7-7-73	1:20	52441	m	7-5-73	1:10
52499	m	7-7-73		52430	f	7-6-73	1:10
52500	m	7-7-73	1:10	52435	m	7-6-73	
52496	m	7-8-73		52475	f	7-13-73	
died	m	7-20-73		52469	m	7-14-73	
52468	m	7-23-73		52473	m	7-14-73	
52443	f	7-24-73		52450	m	7-21-73	1:20
52494	f	7-25-73		52487	f	7-22-73	
52461	f	8-10-73	1:10	52487	f	7-24-73	
5	f	8-10-73		52456	m	7-24-73	
6	f	8-10-73		52492	f	7-24-73	
52499	m	8-11-73		52444	m	7-24-73	
13	m	8-21-73		52446	m	7-24-73	
14	m	8-23-73		52435	m	8-10-73	
15	m	8-25-73	1:10	7	f	8-10-73	
16	m	8-25-73		8	m	8-10-73	
52496	m	8-26-73		52430	f	8-11-73	
101	f	6-23-74	1:10	died	f	8-12-73	
23	m	6-23-74	1:10	died	m	8-12-73	1:10
147	m	6-25-74	1:10	52444	m	8-12-73	
133	m	6-25-74	1:40	52492	f	8-20-73	1:10
29	f	7-9-74		52487	f	8-25-73	
26	m	7-11-74	1:10	52435	m	8-25-73	
38	f	7-12-74	1:10	untag	m	6-19-74	1:10
110	m	7-12-74	1:20	79	f	6-25-74	
52496	m	7-12-74	1:10	149	m	7-9-74	
146	f	7-13-74	1:10	25	m	7-11-74	
22	m	7-19-74	1:10	121	m	7-19-74	1:10
106	f	7-19-74		34	m	7-31-74	
129	f	7-19-74		48	f	8-31-74	
116	m	7-20-74		27	m	9-1-74	1:10
109	f	7-20-74		107	m	9-6-74	
148	f	7-22-74	1:10	140	f	9-6-74	
21	m	7-25-74	1:10				
20	m	7-26-74					

Fox Squirrel				Chipmunk			
Tag No.	Sex	Trapped	Result	Tag No.	Sex	Trapped	Result
101	f	8-2-74	1:10				
38	f	8-22-74	1:10				
29	f	8-24-74	1:10				
119	m	9-6-74					

Deleted titers were less than 1:10 (no reaction)
Titers of 1:20 or greater are positive

Red Squirrel				Cottontail Rabbit			
Tag No.	Sex	Trapped	Result	Tag No.	Sex	Trapped	Result
52437	f	7-5-73		52451	u	7-1-73	1:10
died	m	7-5-73		44915	u	7-20-73	1:10
52438	m	7-5-73	1:10	died	u	7-20-73	1:10
52440	f	7-5-73		44914	u	7-20-73	1:10
died	f	7-5-73		44908	u	7-21-73	1:10
52427	m	7-6-73		44907	u	7-22-73	
died	f	7-6-73		44906	u	7-22-73	1:10
52426	m	7-6-73	1:10	44911	m	7-23-73	1:10
52428	f	7-6-73		44912	m	7-23-73	
52429	m	7-6-73		52453	m	7-23-73	
52434	m	7-7-73	1:10	52458	f	7-23-73	1:10
52449	m	7-13-73		44922	f	7-24-73	
52464	f	7-21-73		44918	f	7-24-73	1:10
died	f	7-23-73		44924	m	7-25-73	
52485	f	7-25-73		44913	f	7-25-73	
52471	m	7-26-73		44920	f	7-26-73	
12	f	8-20-73		5	f	8-20-73	1:10
43	m	6-25-74		8	f	8-24-73	1:10
132	m	7-22-74	1:20	64	f	8-23-74	
105	f	7-27-74		70	f	8-31-74	

Woodchuck				Deer Mouse			
Tag No.	Sex	Trapped	Result	Tag No.	Sex	Trapped	Result
44937	m	7-1-73		killed	f	7-4-73	
44931	m	7-6-73		killed	f	7-15-73	
44921	m	7-22-73		killed	m	7-15-73	
3	m	8-10-73		killed	f	7-21-73	
4	f	8-10-73		killed	m	7-25-73	
7	f	8-23-73		killed	m	8-9-73	
79	f	8-25-74	1:10				
80	f	9-6-74	1:10				

Deleted titers were less than 1:10 (no reaction)
Titers of 1:20 or greater are positive
u--sex unknown

Raccoon				Opossum			
Tag No.	Sex	Trapped	Result	Tag No.	Sex	Trapped	Result
44934	f	6-29-73	1:10	44929	m	7-6-73	
44938	f	7-1-73		52452	f	7-7-73	1:10
44936	f	7-1-73	1:10	52498	m	7-8-73	
44935	f	7-1-73		52497	f	7-8-73	
44933	f	7-6-73		52432	f	7-13-73	
44940	m	7-7-73		52465	m	7-15-73	
44941	m	7-8-73		52447	m	7-20-73	
44942	m	7-8-73	1:10	52433	f	7-21-73	
44943	m	7-8-73		52448	f	7-23-73	
44904	f	7-13-73		52439	f	7-24-73	
44903	m	7-13-73		52495	m	7-25-73	
44902	f	7-13-73		52490	m	7-25-73	
44901	m	7-13-73		52482	f	7-25-73	
44905	f	7-14-73		52463	f	7-26-73	
44909	m	7-21-73		2	f	8-10-73	
44910	f	7-21-73		18	f	8-26-73	
44944	m	7-21-73		44	m	7-19-74	
2	m	8-10-73		118	m	7-19-74	
44938	f	6-20-74		128	f	7-22-74	
63	f	6-23-74	1:10	46	f	8-22-74	1:10
30	f	6-27-74	1:10	47	f	8-22-74	
31	f	6-27-74		134	m	9-1-74	
32	m	6-27-74		127	m	9-7-74	
69	m	6-27-74	1:10				
71	f	6-27-74	1:10				
54	m	6-30-74					
49	f	7-10-74					
77	f	7-30-74	1:10				
78	m	7-30-74					
27	f	9-8-74					

Deleted titers were less than 1:10 (no reaction)

APPENDIX C

METRIC CONVERSION TABLE

APPENDIX C

METRIC CONVERSION TABLE

English*	Metric**
3in x 3in x 9in	7.62cm x 7.62cm x 22.86cm
5in x 5in x 16in	12.7cm x 12.7cm x 40.64cm
5in x 5in x 18in	12.7cm x 12.7cm x 45.72cm
6in x 6in x 24in	15.24cm x 15.24cm x 60.96cm
6in x 6in x 30in	15.24cm x 15.24 cm x 76.2cm
9in x 9in x 32in	22.86cm x 22.86 cm x 81.28cm
34in x 20in x 36in	86.36cm x 50.8cm x 91.44cm

*in denotes inches.

**cm denotes centimeters.