STUDIES ON THE LIFE HISTORY AND NATURAL TRANSMISSION OF **PLASMODIUM CIRCUMFLEXUM** KIKUTH, 1931

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STUDIES ON THE LIFE HISTORY AND NATURAL TRANSMISSION OF *PLASMODIUM CIRCUMFLEXUM* KIKUTH, 1931

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by

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ABSTRACT

A two year study was undertaken in 1972 to investigate various aspects of the epizootiology of *Plasmodium circumflexum* and other avian malarias in waterfowl on the managed wetlands of the Tantramar Marshes, New Brunswick.

Mosquito studies provided information on 14 species of potential vectors, many of which were eliminated from consideration as vectors of avian malaria on the basis of their bionomics. *Anopheles earlei, Culex restuans, C. territans, Aedes dorsalis, Ae. cinereus, Ae. fitchii, Ae. aurifer, Ae. excrucians, Ae. c. canadensis* and *Ae. punctor* were captured only a few times in the baited traps and are not considered as potential vectors of avian malaria. *Culiseta morsitans, Mansonia perturbans, Anopheles walkeri* and *Aedes cantator* engorged frequently on bait birds and were considered as potential vectors.

The strains of *P. circumflexum* and *P. polare* used in these experiments were isolated from *Aix sponsa* and *Anas discors* respectively. Thereafter they were maintained by blood transfers in hatchery-bred white Pekin ducklings *(Anas boschas)*. Traps baited with infected ducks were set on the shore of Front Lake, Long Lake and in adjacent wooded areas in the late afternoon; mosquitoes were collected the following morning. In 1973, 7,235 female mosquitoes of which approximately 30 percent were engorged were collected in the traps. Overall mosquito abundance was noted to reach a peak in early July; *Mansonia perturbans* occurred in very large numbers.

Dissection of mosquitoes from trap collections indicated a natural sporozoite rate of about 9 percent (7 of 75 dissected) in *Culiseta morsitans*. No natural infections were noted in other mosquito species dissected.

Dissection of *Anopheles walkeri* and *Aedes cantator* which had experimentally fed on *P. circumflexum* and *P. polare* infected ducks show that these mosquitoes are refractory. Sporogony of both *P. circumflexum* and *P. polare* was observed in *Mansonia perturbans* but the one attempt to transmit *P. polare* via this mosquito was unsuccessful. *Culiseta morsitans* was shown to be susceptible to *P. polare*. Sporogony and transmission of *P. circumflexum* to waterfowl was achieved via *Culiseta morsitans*. Three of 10 attempts to transmit *P. circumflexum* via intraperitoneal inoculation of sporozoites from *C. morsitans* were successful. Prepatent periods of 14 and 19 days in ducklings and 31 days in a gosling were recorded.

The course of *P. circumflexum* sporogony in *C. morsitans* was studied. Growth of the oocysts at an ambient temperature is fairly regular with sporozoites appearing in the salivary glands on the 8th post infection day.

Laboratory study of the asexual cycle of *P. circumflexum* in a white China gosling indicates no periodicity is present and only slight variations in the numbers of different stage parasites are observed. A short term of infection and low parasitemia appears characteristic for *P. circumflexum*.

The evidence indicates that *Culiseta morsitans* is a natural vector of *P. circumflexum* in the Tantramar area and while *Mansonia*

perturbans may be a useful experimental host, there is no evidence it is a natural vector.

ACKNOWLEDGMENTS

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I am grateful to the National Rifle Association of America for providing the funds that made this study possible and to the Canadian Wildlife Service for providing required equipment and research facilities. Special acknowledgment is given to members of the Sackville office of the Canadian Wildlife Service, particularly Dr. William R. Whitman and Al Smith for their assistance in obtaining malaria strains during night waterfowl banding operations.

Other people are acknowledged for their assistance in diverse ways. My sister, Dr. Susan S. Meyer Durst, University of Mannheim, provided translations on very short notice. Jim Livingstone and Richard Blacquierre, students at Mount Allison University, and Mel Walsh provided help in caring for ducks. Several additional graduate students and friends contributed support and assistance for which I am grateful.

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INTRODUCTION

Organisms of the genus *Plasmodium* are best known as the etiological agents of the diseases collectively known as malaria. Although malaria in humans has been recorded as a serious disease since antiquity, it was not until the late nineteenth century that Ronald Ross (1898) succeeded in establishing the mosquito transmission of malaria, using birds as experimental hosts. Bignami's* successful transmission of human malaria by the bite of mosquitoes in 1898, was no doubt indebted in part, to Ross's earlier work with avian malaria.

Research on avian malaria has played an important role in the development of our knowledge of malaria parasites in general. In fact, an underlying impetus of early research in avian malaria was the hope that many of the discoveries could be directly applied to human malaria, thereby expanding the existing knowledge on the morphology and physiology of human malaria parasites (Hewitt, 1940). While it is true that much of the experimental work which has been done with birds can be repeated with human malaria parasites, the avian plasmodia differ in many respects from mammalian malaria parasites. Still, the comparatively low costs of maintaining facilities for experimental work on birds, the intrinsic interest of bird malaria, and the opportunity of investigating aspects of cellular immunity (requiring sacrifice of the experimental hosts) which is difficult when using human hosts, continues to make investigations on avian malaria both practical and desirable.

While the epidemiology of human malaria is one of the best understood and most extensively studied aspects of the disease, the

* Seen in Hewitt (1940).

epizootiology of avian malaria, especially transmission under natural conditions, remains an area where little work has been done, indicating the need for further research in this direction.

Studies by Drs. Gordon Bennett and Carlton Herman on the prevalence and significance of avian hematozoa on the managed wetlands on the New Brunswick-Nova Scotia border, indicated a relatively high incidence of *Plasmodium* in the passeriformes and anatids of that area (pers. comm.). Since no detailed studies on the natural transmission of avian malaria had been carried out in Canada, and in view of the acquisition of wetland areas for waterfowl management in Atlantic Canada, it seemed desirable to engage on a project designed to determine the natural vector or vectors of avian malaria in waterfowl of that area.

This thesis specifically details studies on the transmission of *Plasmodium circumflexum* to waterfowl through a natural vector, and sporogony in the vector, *Culiseta morsitans*. Concurrent mosquito studies contribute data concerning fourteen species of potential vectors.

REVIEW OF THE LITERATURE

Many studies have dealt with the transmission of avian malaria by mosquitoes, perhaps the most famous work being the demonstration by Ronald Ross (1898) of the mosquito transmission of *Proteosoma* (= *Plasmodium*) to birds; experiments immediately followed on human malaria which confirmed the discovery. Other early work on the transmission of avian malaria was reported by Daniels (1899), but in both of these reports it was not possible to identify either the parasite or the mosquito.

Much of the research done upon the transmission of avian malaria since that time has been reviewed and discussed by Huff (1932, 1954, 1963, 1965), Garnham (1966), Vargas (1949), and Hewitt (1940).

While there has been some transmission of avian *Plasmodium* in laboratory studies, little proof exists that in a given locality a species of mosquito exists as the principal vector. Rosen and Reeves (1954) and Reeves *et al.*, (1954) in field studies have shown certain mosquitoes to have a high vector potential for avian malaria.

The species of *Plasmodium* infecting birds are classified into the subgenera *Haemamoeba*, *Giovannolaia*, *Novyella* and *Huffia*, following the classification scheme of Corradetti *et al.*, (1963). Relatively few studies have dealt with the transmission of the parasites of the subgenus *Giovannolaia*.

Proven culicine vectors of *P. circumflexum* have only been rarely reported in the literature. Herman (1938a) transmitted two strains isolated from the red-wing blackbird (*Agelaius p. phoeniceus*)

and cowbirds (*Molothrus a. ater*) via *Culiseta melaneura* on Cape Cod, Massachusetts. Niles, Fernando and Dissanaike (1965) transmitted a *Plasmodium* resembling *P. lophurae* [later identified by Garnham (1966) as the Ceylon strain of *P. circumflexum*] by *Mansonia crassipes*.

A number of workers by demonstrating sporogony or through epizootiological evidence, have implicated various mosquitoes as potential natural vectors of *Plasmodium circumflexum*. Mosquitoes previously reported susceptible are *Culex tarsalis* (Huff, 1965) and two species of the genus *Culiseta* (*Theobaldia*) (Reichenow, 1932, and Corradetti *et al.*, 1964). Reichenow (1932) demonstrated the susceptibility of *Culiseta annulata* to a strain of *P. circumflexum* isolated from a thrush in Germany. Corradetti *et al.*, (1964) obtained sporozoites in both *Culiseta annulata* and *Culiseta longiareolata* of an Italian strain of *P. circumflexum* (isolated from *Turdus iliacus*).

Attempts by Herman (1938a) to transmit two strains of *P. circumflexum* by *Aedes sollicitans, A. c. canadensis, A. cantator, A. vexans, Culex pipiens* and *C. apicalis* were unsuccessful. Huff (1965) reports that *Aedes albopictus* is refractile to infection with *P. circumflexum*.

Attempts by Corradetti and Scanga (1963) to infect *Culex pipiens* with a strain of *Plasmodium polare* isolated from *Falco tinnunculus tinnunculus* were made without success, but they were successful in transmitting the same strain of *P. polare* in the laboratory with *Culiseta longiareolata* (Corradetti and Scanga, 1965).

The natural mosquito host of *Plasmodium fallax* is not known, though Huff *et al.*, (1950) succeeded in infecting *Aedes albopictus*,

Ae. atropalpus, *Ae. aegypti*, *Ae. triseriatus*, *Culex tarsalis*, *C. quinquefasciatus*, and *Anopheles quadrimaculatus* by feeding these mosquitoes upon infected pigeons. *Culex p. pipiens* appeared refractile to infection by *P. fallax*.

Garnham (1966) reports that the natural vector of *Plasmodium lophurae* is unknown, but that several mosquitoes were susceptible to the parasite under laboratory conditions. Mosquitoes found susceptible were: *Aedes atropalpus*, and *Culex restuans* (Laird, 1941), and *Culex pipiens* (Coggeshall, 1940). Transmission of *P. lophurae* was obtained with *Anopheles quadrimaculatus* (Hurlbut and Hewitt, 1942), *Aedes albopictus* (Laird, 1941), and *Aedes aegypti* (Jefferey, 1944).

Nothing is known of the sporogonic stages of *Plasmodium gundersi*, *P. durae*, *P.pinottii*, *P.anasum*, *and P. formosanum* (Garnham, 1966).

The natural vector of *Plasmodium garnhami* is not known but Garnham (1966) reports that Hoogstraal and Ezzat have found a laboratory colony of *Culex pipiens molestus* to be susceptible to the parasite.

Few investigators have published work on the transmission of avian malaria under natural conditions (Herman, 1938b; Herman *et al.*, 1954; Janovy, 1966). Herman's (1938b) epizootiological investigation of *P. circumflexum* and *P. cathemerium* in red-wing blackbirds is a classic work in this field. By taking blood smears of nestlings before they left their nests and further smears when the birds were recaptured after leaving the nests, he showed that the birds generally acquired infections after leaving the nest. Transmission experiments were conducted with mosquitoes reared from wild-caught larvae from

the study area. Janovy's (1966) study of *Plasmodium hexamerium* in the meadowlark and starling determined the seasonal incidence as well as the probable mosquito vector of the parasite. Janovy showed that the birds become infected at a very early age, and that the epizootiology of avian malaria tends to be specific with respect to the species of bird host.

Any discussion of the epizootiology of avian plasmodia must include the works describing the ecology of avian malaria. Foremost among these is a model proposed by Beaudoin *et al.*, (1971) which describes a spring relapse of infected birds and summer and fall transmission of infections to susceptible birds. Applegate (1971) was able to show conclusively that the high spring prevalence of *Plasmodium* in birds was the result of an elevation of circulating parasite populations in established infections. Applegate, Beaudoin, and Seeley's (1971) work involving *Plasmodium relictum* in English sparrows, showed that the increase in parasitemia is associated with an increased infectivity to mosquitoes. The results of these studies support the hypothesis of Beaudoin *et al.*, (1971) that relapse is involved in the reintroduction of avian *Plasmodium* into the temperate zone transmission cycle.

THE STUDY AREA

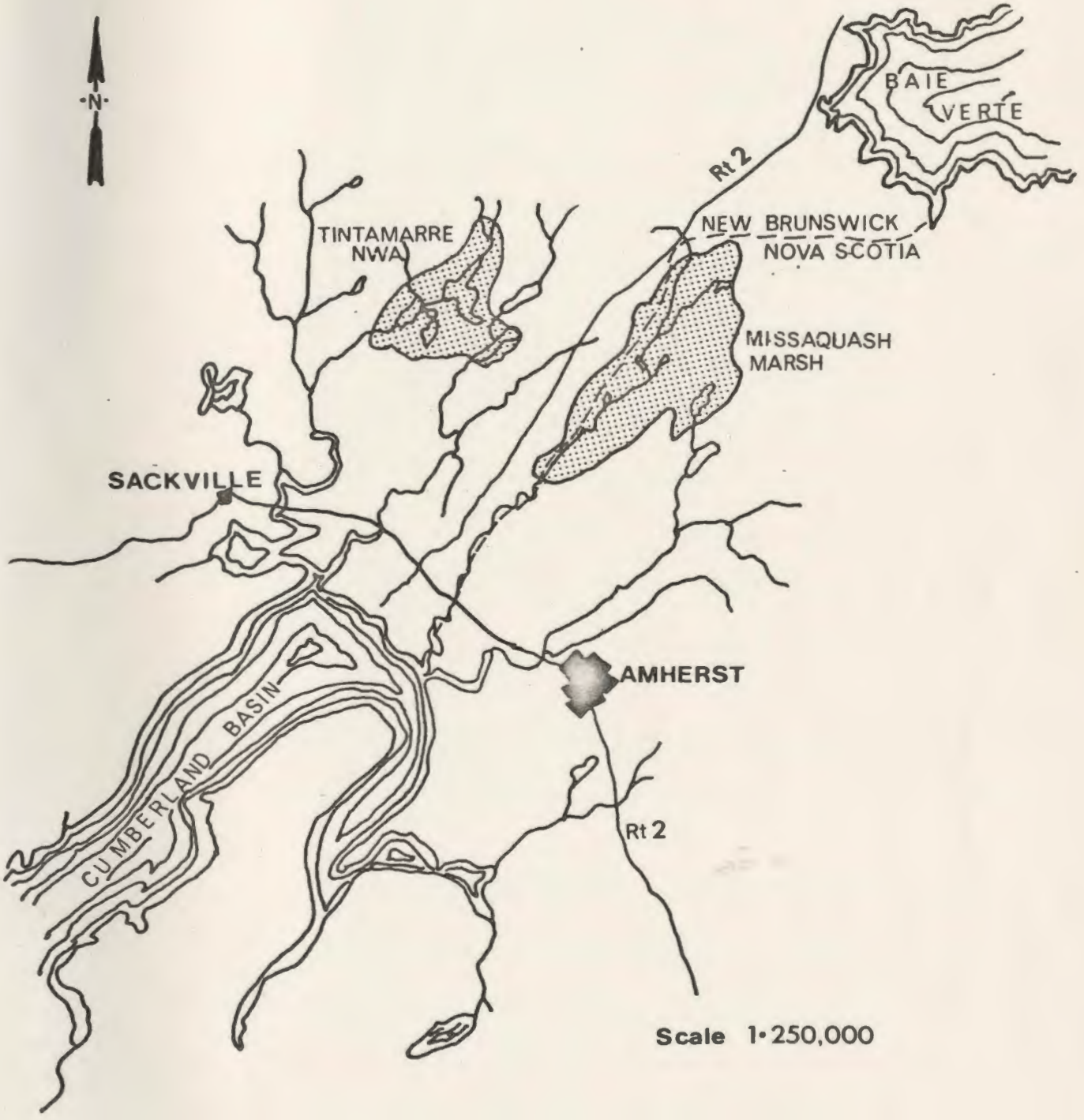
The Tantramar Marshes located in the New Brunswick - Nova Scotia border area are the locale of a concerted effort being made by federal and provincial government agencies and private concerns to preserve existing waterfowl production areas and develop new ones in eastern Canada. Wetland areas include the Tintamarre, John Lusby, Amherst Point National Wildlife Areas (NWA), and the Missaquash Marsh. Breeding and migration habitat has been improved and/or restored through the installation of dikes and water control structures.

The Tintamarre NWA is located at the head of the Bay of Fundy, approximately 7 miles northeast of the town of Sackville, New Brunswick (Fig. 1). The Missaquash Marsh is in the same area, approximately 3 miles to the east. The general area contains several natural lake basins, upland forests and fields, and semi-drained and drained marshland which were originally used for agriculture.

The study area (Fig. 2a), which is in the Tintamarre NWA, consists of a natural lake basin (Long Lake) and a 275 acre natural marsh (Front Lake), and borders on seven man-made impoundments with a total area of about 300 acres. The adjacent areas are spruce and larch forests and upland fields. Cattails, sedge, blue-joint, and fresh-water cord grass are abundant, as well as cinquiefoil, spiraea, smartweed and some other submerged and floating-leaf aquatics. The invertebrate populations contain a wide variety of taxa, many of which are undoubtedly attractive as waterfowl foods.

FIGURE 1

Location of the Tintamarre, N.W.A. and Missaquash Marsh.


N
BAIE VERTE
Rt 2
NEW BRUNSWICK
NOVA SCOTIA
TINTAMARRE NWA
MISSAQUASH MARSH
SACKVILLE
AMHERST
CUMBERLAND BASIN
Rt 2
Scale 1•250,000

METHODS AND MATERIALS

Strains and Species of *Plasmodium*

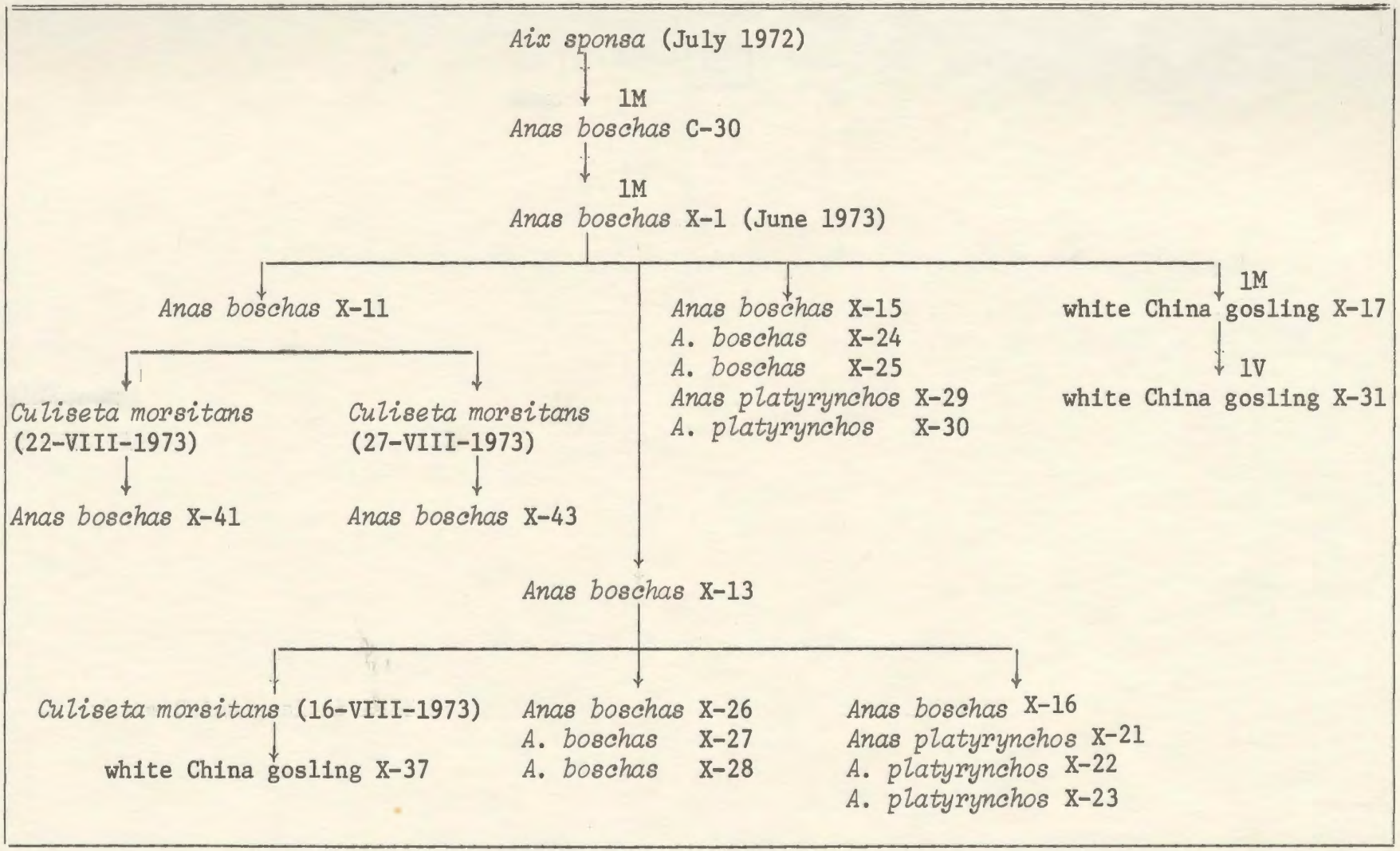
The strain of *Plasmodium circumflexum* and *Plasmodium polare* studied were isolated from waterfowl captured during banding operations in 1972 on the Missaquash Marsh and Tintamarre National Wildlife Area. Blood (0.45 ml.) was removed from the femoral or brachial vein of a wild bird and injected intramuscularly into the leg of a young duckling. Heparin was used as the anticoagulant for all blood transfers. The strain of *P. circumflexum* was isolated from a wood duck *(Aix sponsa)* on the evening of 31 July 1972. The strain of *P. polare* was isolated in the evening 8 August 1972. Both strains were isolated by blood transfer to hatchery-bred domestic ducklings (White Pekin), *Anas boschas*. Strains were maintained by blood transfers in *Anas boschas;* mallard ducks, *Anas platyrynchos*; and white China goslings. Tables 1 and 2 illustrate the history of the strains since they were isolated in the wild, and the ways they were handled throughout the experiments.

Vertebrate Hosts

Vertebrate hosts used throughout this study were various species of Anatidae. For experimental purposes, domestic ducklings (*Anas boschas*) and white China goslings were obtained from a commercial hatchery on Prince Edward Island. These birds were parasite-free on purchase (1 - 5 days old) and were maintained in vector-proof cages until used experimentally. Domestic ducklings provided the great majority of the

Table 1

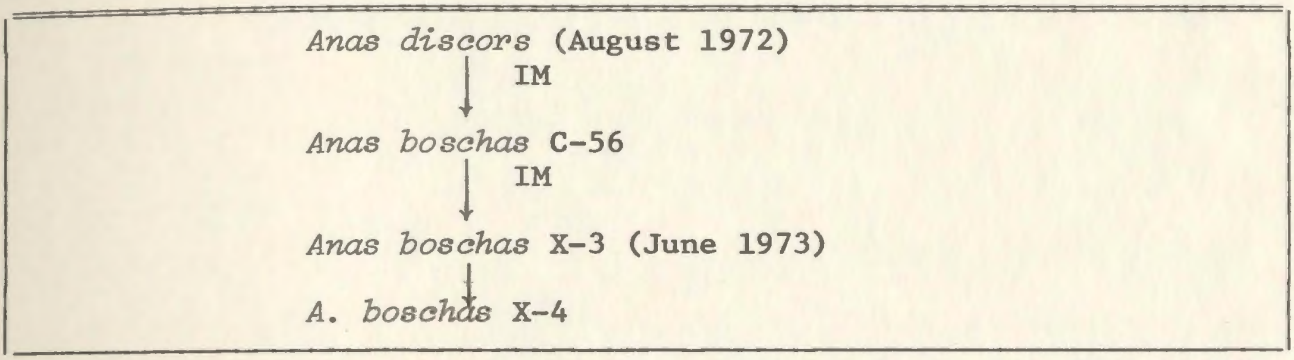
History of *Plasmodium circumflexum* *



* Isolated and used herein.

Table 2

History of *Plasmodium polare*.*



* Isolated and used herein.

experimental hosts. One brood of black ducks (*Anas rubripes)* and Mallard ducks (*Anas platyrynchos*) were obtained locally. These birds were maintained for 2 weeks in vector-proof cages until their parasite-free status was confirmed.

Over 200 sentinel domestic ducks were exposed continuously from June to September 1972 and 1973 at Front Lake and Missaquash Marsh. These birds failed to become infected with any species of *Plasmodium* (Bennett and Herman, unpublished data).

The ducklings used in these studies were removed directly from the hatching incubator and placed in mosquito-proof cages; nevertheless a thin blood film was examined for blood parasites prior to their use.

Invertebrate Hosts

Mosquito Trapping

Sites

Funnel traps (Fig. 3) baited with ducks or goslings were used extensively in the study area, for the purpose of capturing blood-engorged adult mosquitoes, during June - August 1972 and May - September 1973. The trapping sites are described below and their location can be seen in Figure 2b.

1. The shore of Front Lake at the bottom of a gentle hill running down from the research facility. The site was unprotected from the wind.

1A. An open field about 50 yards south of the research facility. This site was unprotected from the wind.

2. The east shore of Long Lake about 100 yards north of the dirt road. The site was protected from the wind, in the middle of a line of alders (6 - 30 ft. in width) bordering the east shore of Long Lake.

FIGURE 2a

The Study Area.

FIGURE 2b

Detailed Sites of Collection Traps

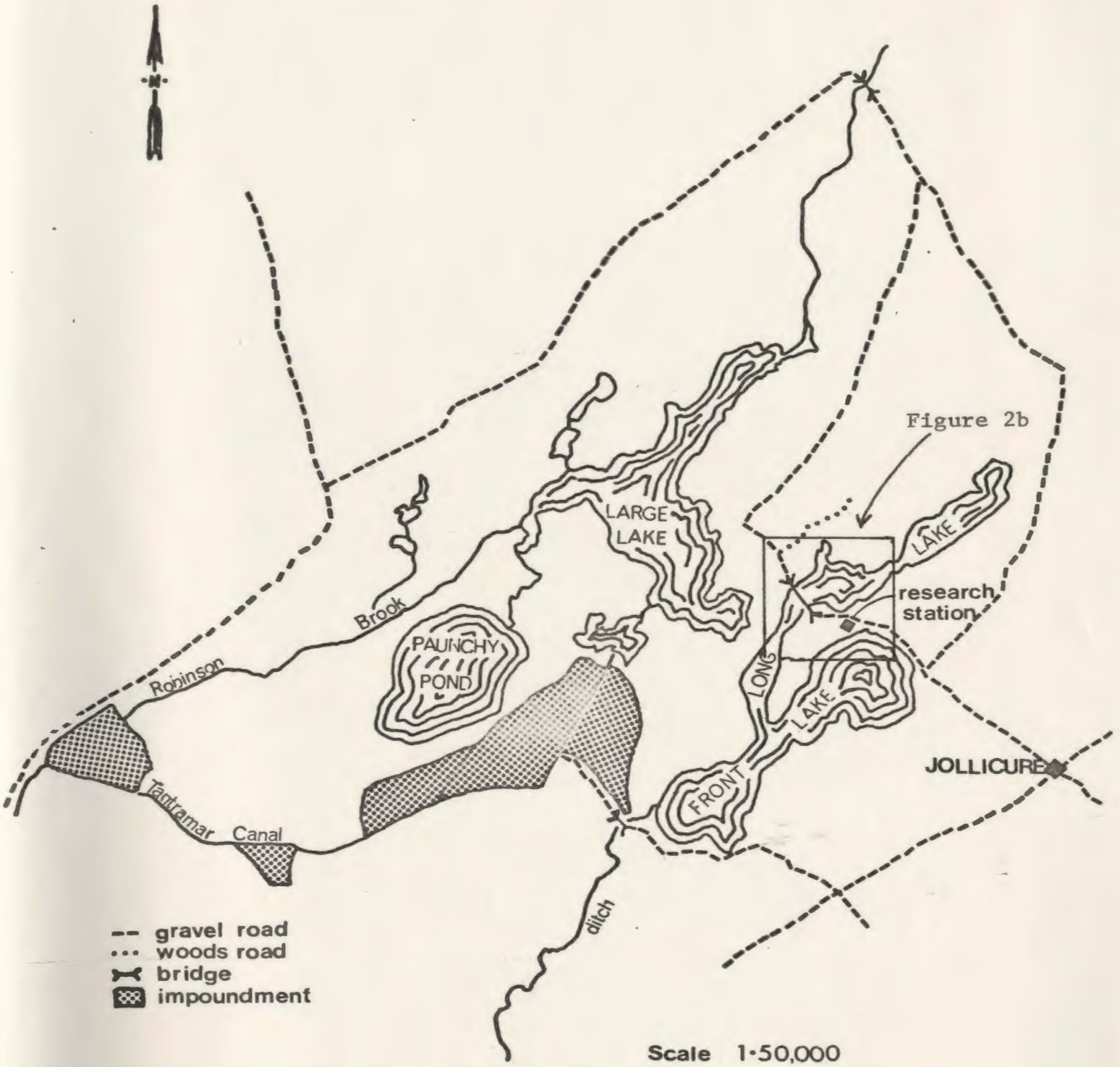


Figure 2b
LARGE LAKE
LAKE
research station
Brook
Robinson
PAUNCHY POND
LONG
LAKE
JOLLICURE
FRONT
Tantramar Canal
ditch
gravel road
woods road
bridge
impoundment
Scale 1·50,000

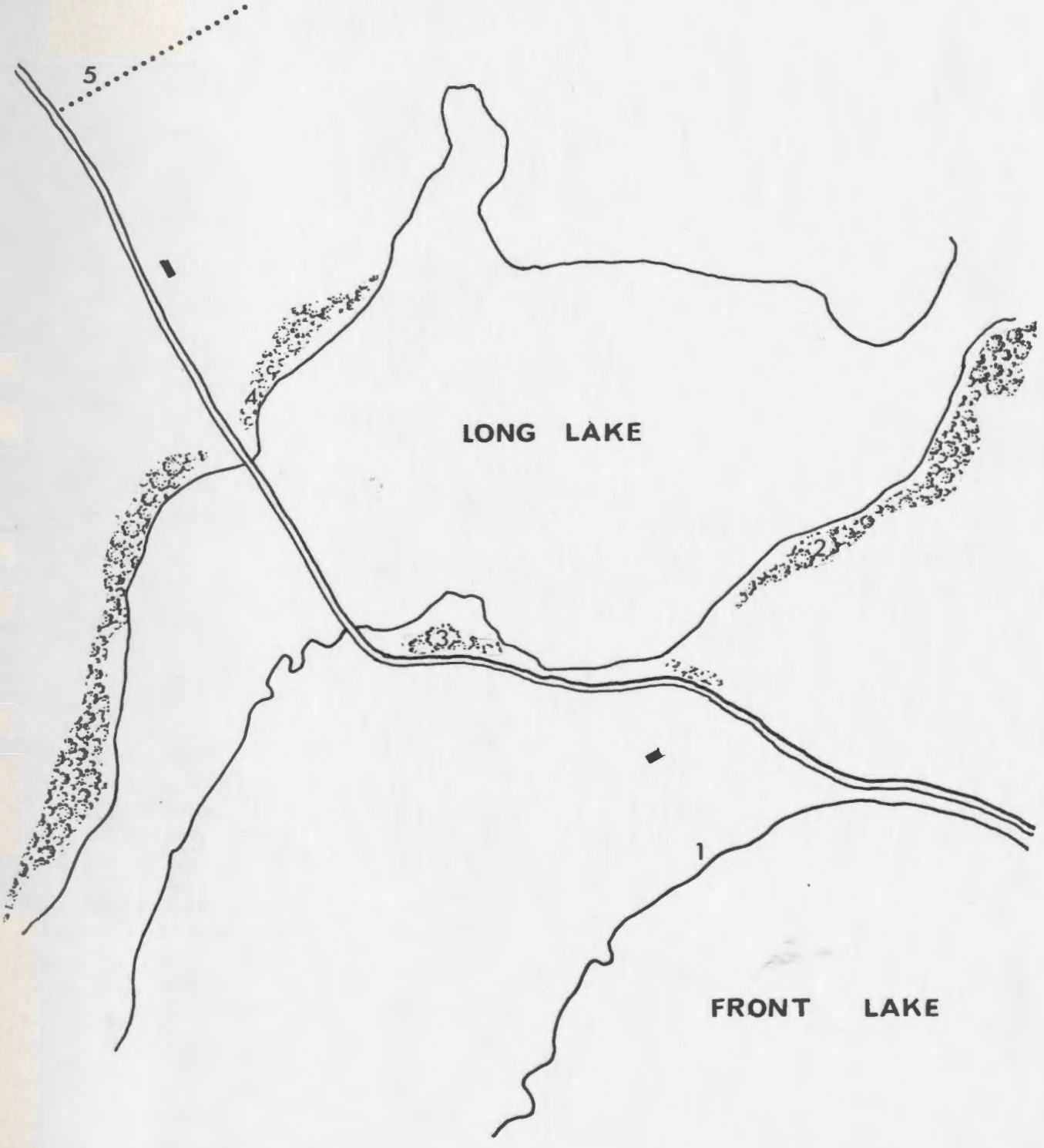
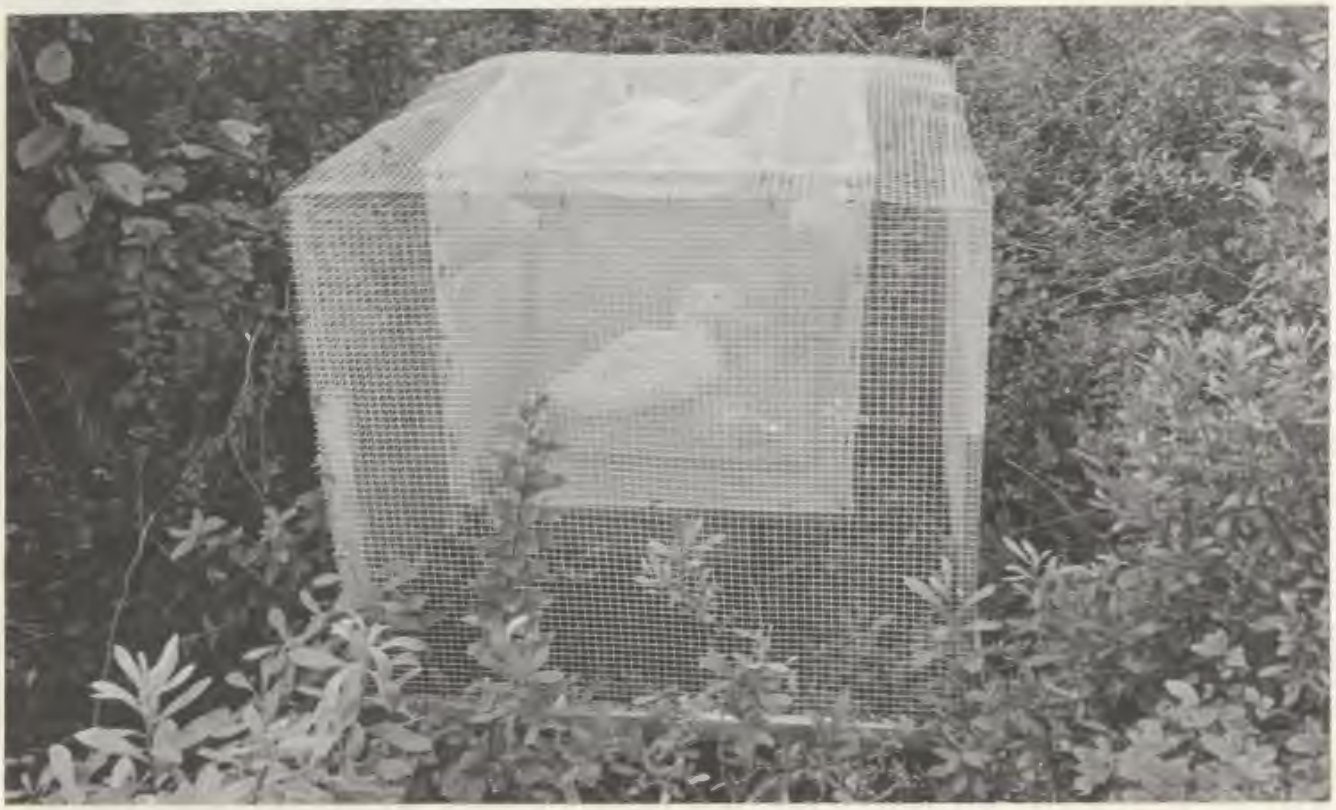
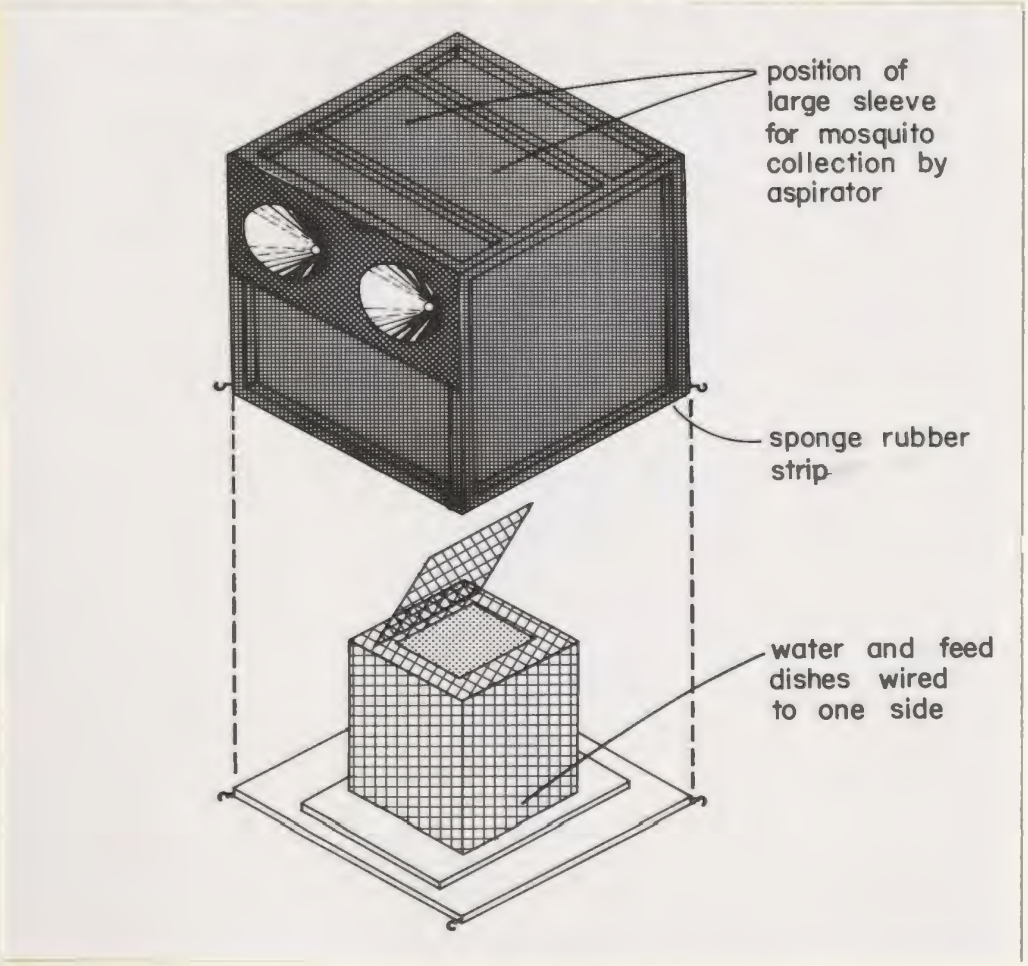

5
LONG LAKE
4
2
3
1
FRONT LAKE

FIGURE 3

The mosquito funnel trap.




position of large sleeve for mosquito collection by aspirator
sponge rubber strip
water and feed dishes wired to one side

The ground here was firm and dry.

3. The east shore of Long Lake (directly west of the research station) in an extremely dense patch of alders. This site was well protected from the wind and intermittently covered with up to 15 in. of water during wet periods (Fig. 3).

3A. Same as above.

4. The west shore of Long Lake about 15 yd. north from the dirt road. The trap was in a transition zone between the alders and field, about 20 yd. from the water.

5. A mixed hardwood-softwood forest about 30 yd. from the dirt road and on an old logging road.

6. Same as above.

Traps baited with ducks were set up at the trapping sites in the late afternoon, and mosquitoes were collected as early as possible the following morning.

The Trap

The trap was composed of essentially three portions: (1) the exposure or holding cage for the bait birds, (2) the collecting cage for the biting flies and (3) a larger cage to keep out predators.

Bait birds were confined in exposure cages measuring 12 X 11 1/2 X 14 3/4 inches. The sides and top of the cage were cut and bent out of a piece of 1/2 in. mesh hardware cloth measuring 36 inches square. The edges of the cage were wired together, an 8 1/2 X 7 inch hole cut in the top to allow removal of the bait bird and was covered with an 8 X 10 1/2 inch hardware cloth flap hinged with 3 hog rings. The whole assembly was centered and nailed to a 15 1/2 X 19 3/4 X 3/4 inch piece of plywood.

The exposure cages containing the birds were placed on 2 ft. squares of plywood at the trapping sites. The collecting cage consisted of a wood frame (2 X 2 X 2 ft.) covered with nylon mesh (60 meshes to the inch) on five sides, leaving the bottom open. A tight seal, preventing escape of trapped flies, was insured by glueing a strip of rubber sponge along the bottom of the frame. Four polyethylene opaque plastic funnels (obtained at a local hardware store) were attached to 12 X 24 in. panels on 2 sides of the trap. The funnels, 8 inches in diameter and tapering to approximately 2 inches, allowed mosquitoes attracted to the bait to enter and engorge, but made exit difficult. The funnels incorporated in the trap were modifications of those obtained in the hardware store. Funnels were glued to the panels in previously cut holes (Fig. 3).

Mosquitoes were collected in aspirators through a sleeve at the top of the cage. Birds remained relatively motionless in the exposure cages and it was not found necessary to hood them as described by Bennett (1960). The same bird was seldom used on 2 successive days to avoid placing undue stress on the bird.

The Infective Feed

Mosquitoes collected coming to bait birds were stored in either wooden holding cages or ice cream cartons modified according to Collins and Jeffery (1962), and were carried back to the laboratory where the mosquitoes were allowed the opportunity to engorge on the bait bird for an additional hour.

The donor bird (with a known infection) was placed on its back,

immobilized on a wooden board with large rubber bands which were stretched between nails lining the edges of the board, and its abdomem was shaved. The wood board with the trussed bird was turned over and placed on two wood blocks. The nylon mesh of the mosquito-holding cage was pressed against the shaved abdomen of the bird, and the mosquitoes were allowed to feed for one hour (Figure 4).

Mosquitoes which had not fed after this period were removed: male mosquitoes were discarded; female mosquitoes were pinned and identified. The fed mosquitoes were then placed in an insectory, at an ambient temperature (average 22°C) and high relative humidity until dissections to determine oocyst and sporozoite infections were made. Wet paper toweling was kept on the floor of the holding cage and a cube of sugar was put on top of the cage. In the high humidity, the sugar cube quickly turned to a syrup which formed a pool on the nylon mesh. Water was added to the paper toweling daily to keep it moist and maintain a high humidity.

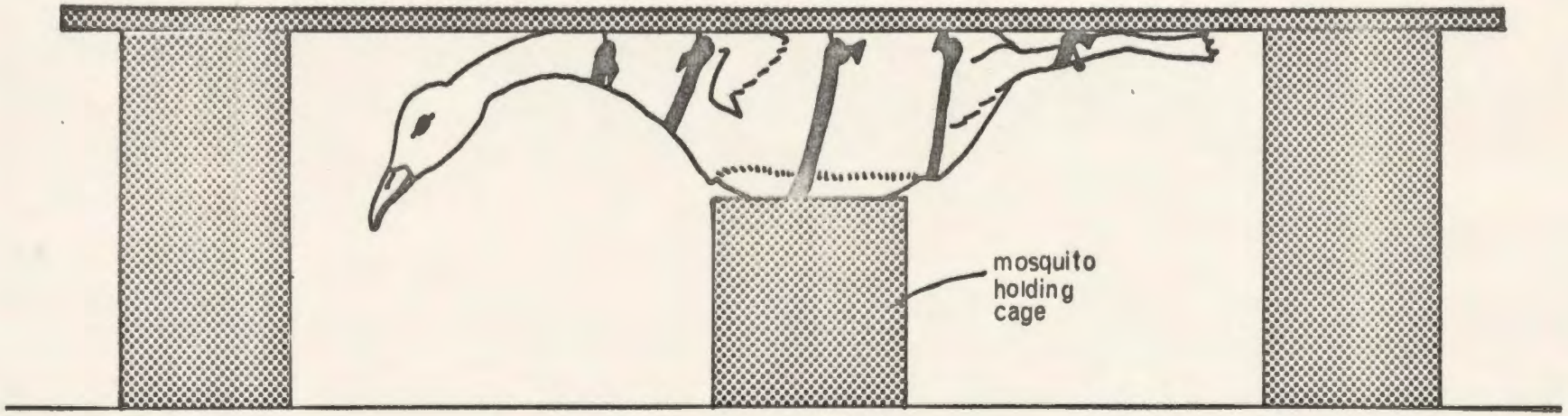
Dissections of mosquitoes' midguts were carried out from the third day following the infective blood meal obtained in the laboratory (Fig. 4). The infection rate, the number of oocysts per midgut, the regularity of their growth, the presence of free sporozoites around the gut, and the presence of sporozoites in the salivary glands, were determined for *Culiseta morsitans* infected with *P. circumflexum*.

Harvesting Sporozoites and Injection to a New Host

Various methods exist for injecting sporozoites into a vertebrate host: (1) By the natural bite of mosquitoes, which is

FIGURE 4

Method of feeding mosquitoes on infected waterfowl.


mosquito
holding
cage

lacking in precision for experimental purposes as it is impossible to know how many sporozoites have been injected, (2) By crushing the bodies or the separated thoraxes in a grinder (Shortt, Garnham and Malamos, 1948). The resulting suspension must be centrifuged and the supernatant containing the sporozoites is then injected. A considerable loss of sporozoites which stick to the chitinous debris is a result of these manipulations (Garnham, 1966). (3) Dissection of the salivary glands of each infected mosquito into a drop of saline/nutrient medium. Although this method is time-consuming it is by far the most efficient method (Garnham, 1966) and was used throughout this work.

The suspending media used in the present work was a physiological saline solution (0.85% NaCl). Mosquitoes were killed individually, using as little ethyl acetate vapor as possible. The legs and wings were removed using jeweler's forceps. Such prepared mosquitoes were then dissected one at a time on a slide, and the salivary glands were moved by the dissecting needle into a drop of saline, which had been placed to one end on the slide. When the pair of glands were in the drop of saline, a coverslip was placed on the slide and the glands were viewed under a microscope for the presence or absence of sporozoites. When transmission was attempted the glands were crushed between the slide and coverslip by administering light pressure. Sporozoites were rinsed from the slide and coverslip with additional saline, and inoculated intravenously or intraperitoneally into malaria-free ducklings or white China goslings.

Staining Techniques

Blood films were taken from the femoral vein, allowed to dry, and fixed by dipping in 100% methanol. They were then placed face up on a staining rack and stained with Giemsa at pH 7.2 for 40 to 50 minutes.

To facilitate recognition of oocysts, stomachs of mosquitoes were stained in 2% mercurochrome solution (in 0.85% NaCl solution) after the protocols of Eyles (1950); mature cysts (Fig. 5) were readily recognized.

Whole permanent mounts of midguts containing oocysts were prepared with modifications as described below (Shute and Maryon, 1966). Dissected mosquito stomachs were stored in 70 percent alcohol until mounts were to be made (storage periods of over one year does not appear to affect the preparation). The midguts were fixed in Bles' fixative for 12 - 24 hours and processed as follows:

Wash in 70 percent alcohol for at least two hours.

Replace the 70 percent by water and then stain with Erlich's hematoxylin, diluted 1/5 in distilled water, for ten minutes.

"Blue" the midguts in alkaline tap water for at least twenty minutes.

Differentiate in 1 percent acid alcohol for a few seconds.

"Blue" in alkaline tap water for twenty minutes or more.

Dehydrate in absolute alcohol for fifteen minutes.

Clear in clove oil for twenty minutes.

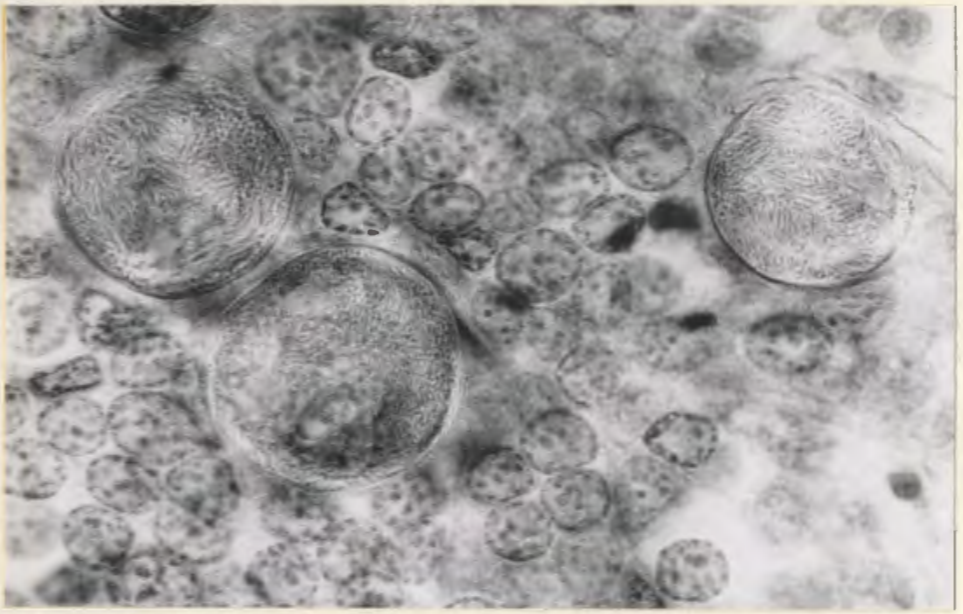
Remove clove oil with Xylene (ten minutes) and mount in Canada balsam.

Preparations of sporozoites were made by dissecting a salivary gland in saline (0.85% NaCl) on a slide, putting on a coverslip and

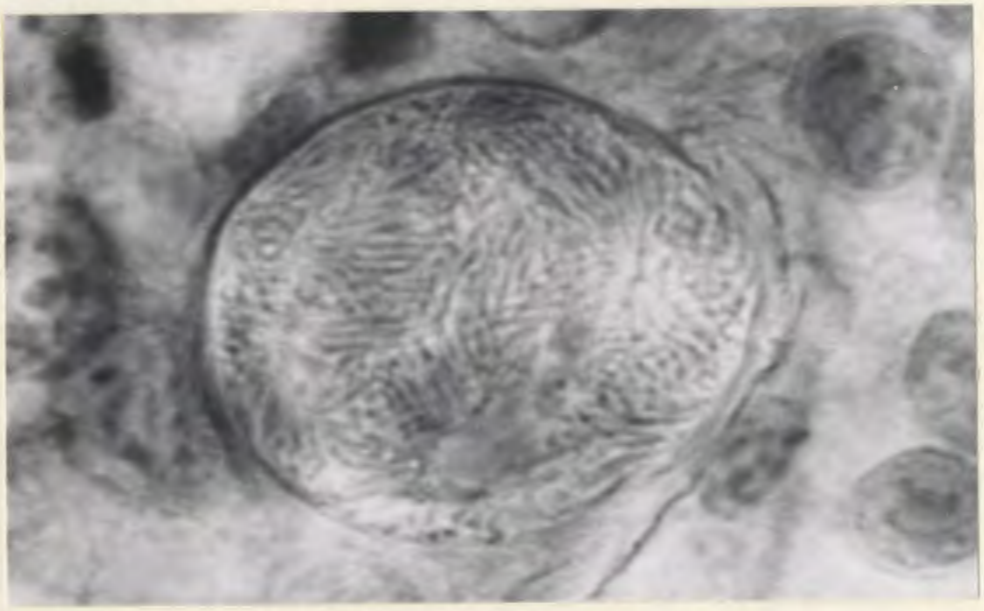
FIGURE 5

Photomicrographs of fully differentiated oocysts from the guts of *Culiseta morsitans*, preserved dissections.
A. Differentiated oocysts, X2500.
B. Differentiated oocysts, X6250.

A



B



examining under a microscope. If sporozoites were seen, the glands were crushed by light pressure on the coverslip, which was then removed and the smear was allowed to dry. It was then fixed with 100% methanol and stained with Giemsa as described for blood smears. Other techniques are cited in context.

RESULTS

Mosquito Survey

Over the two year period, 14 species of culicids were collected in the Tantramar Marshes using waterfowl baited funnel traps. The baited trap collections during 1973 yielded 7,235 females during the 37 trap nights between June 4 and September 11. Overall mosquito abundance during 1973 was noted to reach a peak in early July (Fig. 6 and Table 3). Extensive baited trap data is not available for 1972. The following eleven species: *Anopheles earlei* Vargas 1943, *Aedes fitchii* (Felt and Young) 1904, *Aedes canadensis canadensis* (Theobald) 1901, *Aedes cinereus* Meigen 1818, *Aedes aurifer* (Coquillett) 1903, *Aedes punctor* (Kirby) 1837, *Aedes excrucians* (Walker) 1856, *Aedes dorsalis* (Meigen) 1830, *Culex territans* (Walker) 1856, and *Culex restuans* Theobald 1901, were captured only a few times and were not considered numerous enough to warrant consideration as potential vectors of avian malaria in waterfowl (Table 4). *Mansonia perturbans* (Walker) 1856, was collected throughout the mosquito season and in large numbers during early July (Fig. 7). *Culiseta morsitans* (Theobald) 1901, *Aedes cantator* (Coquillett) 1903, and *Anopheles walkeri* Theobald 1901, were collected throughout the summer season but did not exhibit the July peak in numbers shown by *Mansonia perturbans* (Fig. 8). The biology of the principal species trapped in the Tantramar Marshes is discussed below.

Mansonia perturbans (Walker) 1856:

Adults were collected in the baited traps as early as June 23

Table 3

Average nightly catch per trap based on a week's collection

(June - September 1973)

WEEK	TRAP NUMBER							
	1	1A	2	3	3A	4	5	6
June 2 - 9	0.5		1.5	3.0		0		
9 - 16	10.0		5.5	6.5		0		
16 - 23	5.5		4.5	17.5		1		
23 - 30			2.0	128.0		25.0		
June 30 - July 7				1,445.0				
July 7 - 14				542.0				
14 - 21				473.5				
21 - 28	61.0	76.0	37.0	219.0			14.0	
July 28 - Aug. 4	1.0	2.0	*	50.0			6.0	*
Aug. 4 - 11	2.5		13.6	27.5	10.0		1.8	0
11 - 18			0	27.0	13.0		0	
18 - 25			13.0	3.3	19.0		2.0	

Table 3 (continued)

WEEK	TRAP NUMBER							
	1	1A	2	3	3A	4	5	6
Aug. 25 - Sept. 1				28.0	60.0		3.0	
Sept. 1 - 8				55.0	38.0			
8 - 15				1.0			0	

*Rain prevented mosquito collection from traps

FIGURE 6

Weekly fluctuations in overall mosquito abundance in 1973 at Trap 3 (average nightly catch based on a week's collection).

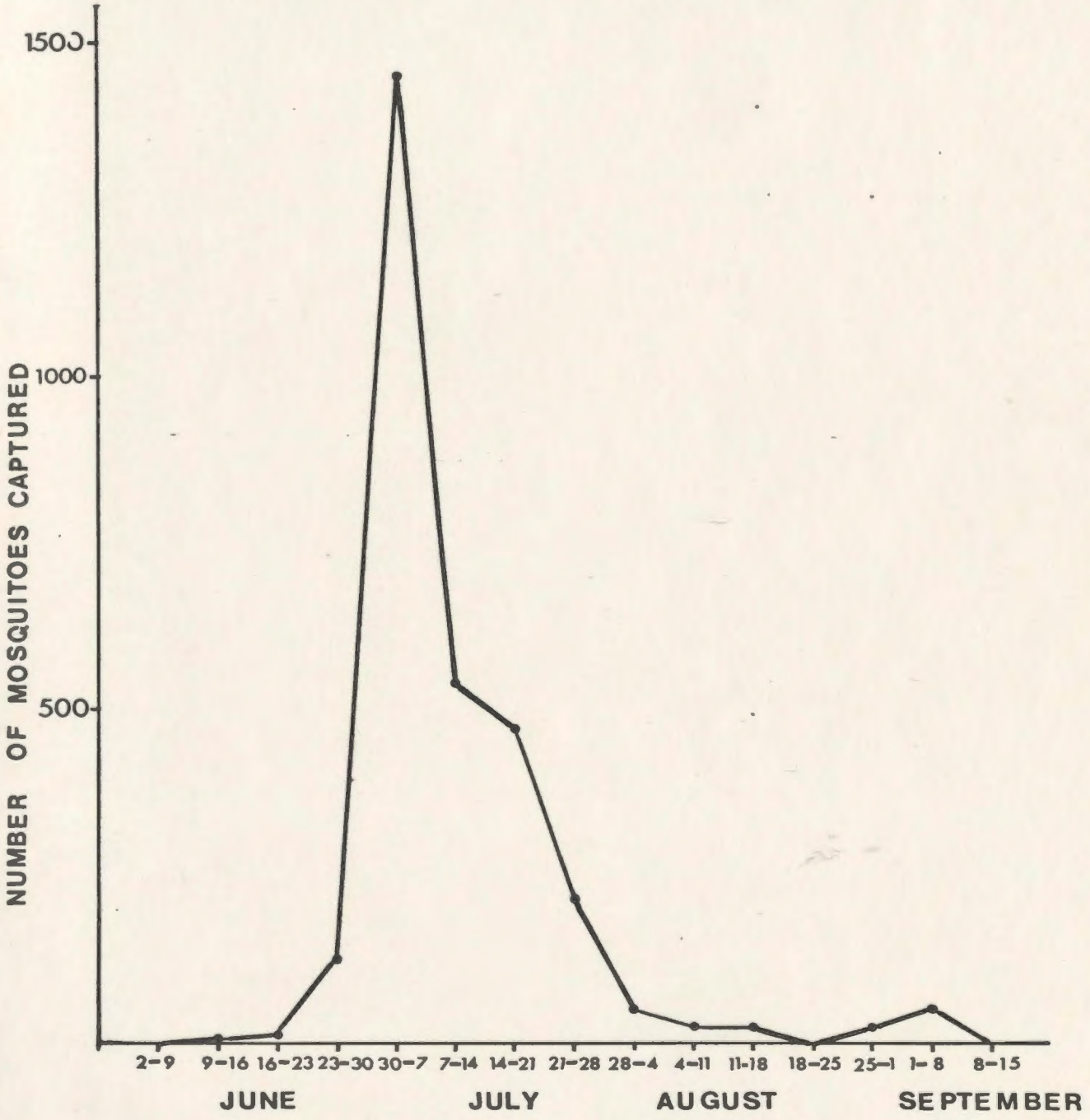

1500
1000
500
NUMBER OF MOSQUITOES CAPTURED
2-9
9-16
16-23
23-30
30-7
7-14
14-21
21-28
28-4
4-11
11-18
18-25
25-1
1-8
8-15
JUNE
JULY
AUGUST
SEPTEMBER

Table 4

Total number of mosquitoes trapped in bird baited traps (1973)

Mosquito species	Total collected
Aedes	
A. cantator	132
A.c. canadensis	25
A. aurifer	5
A. excrucians	1
A. cinereus	1
A. fitchii	1
A. punctor	55
Aedes sp.	18
Anopheles	
An. walkeri	330
An. earlei	13
Anopheles sp.	63
Mansonia	
M. perturbans	6,333
Culiseta	
C. morsitans	257
Culex	
C. territans	1
Totals	7,235

N.B. Only a single specimen of each of A. dorsalis and C. restuans were captured in 1972; no experimental feedings were attempted.

FIGURE 7

Weekly fluctuations of numbers of *Mansonia perturbans* in 1973 at Trap 3 (average nightly catch based on a week's collection).

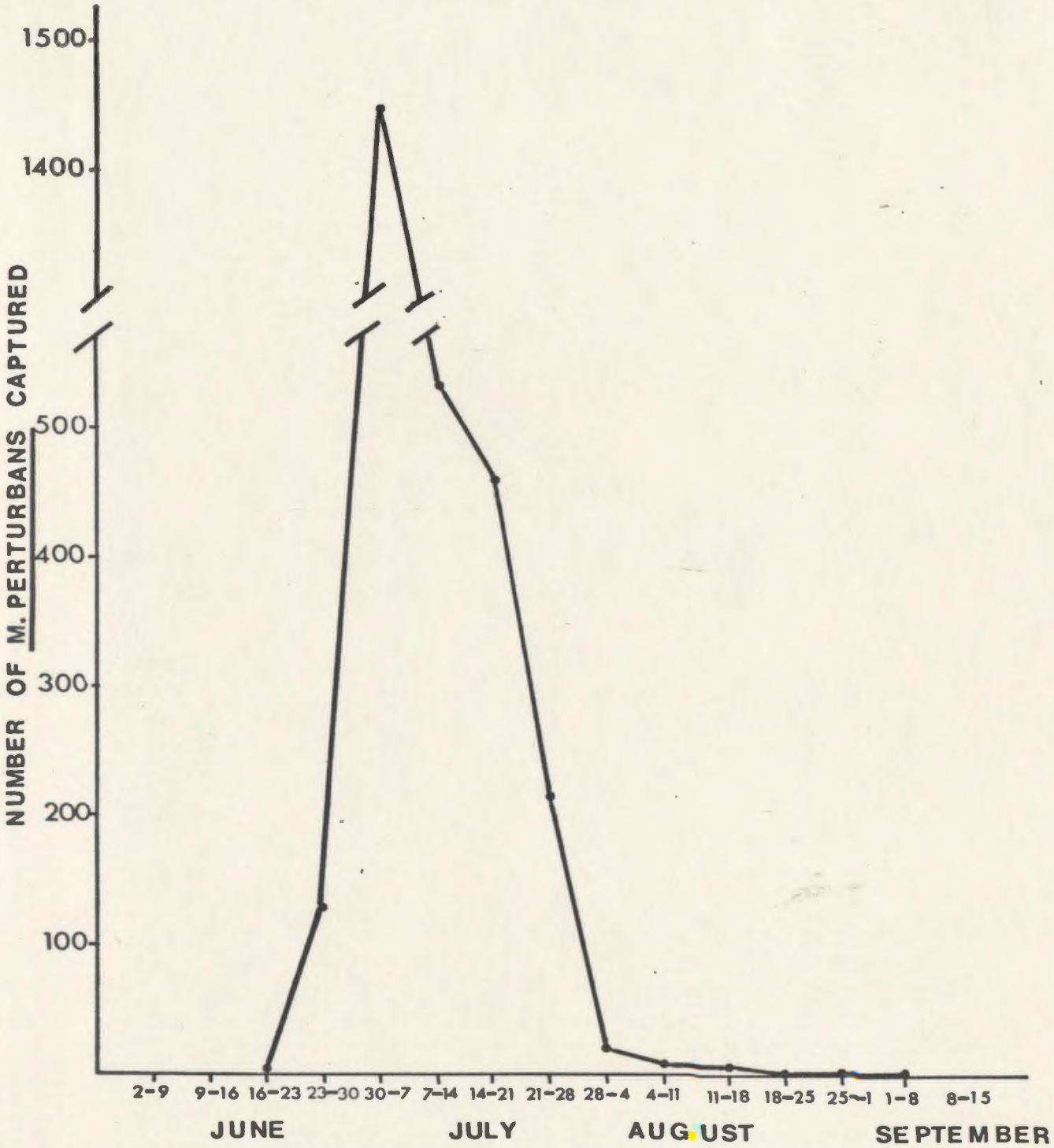
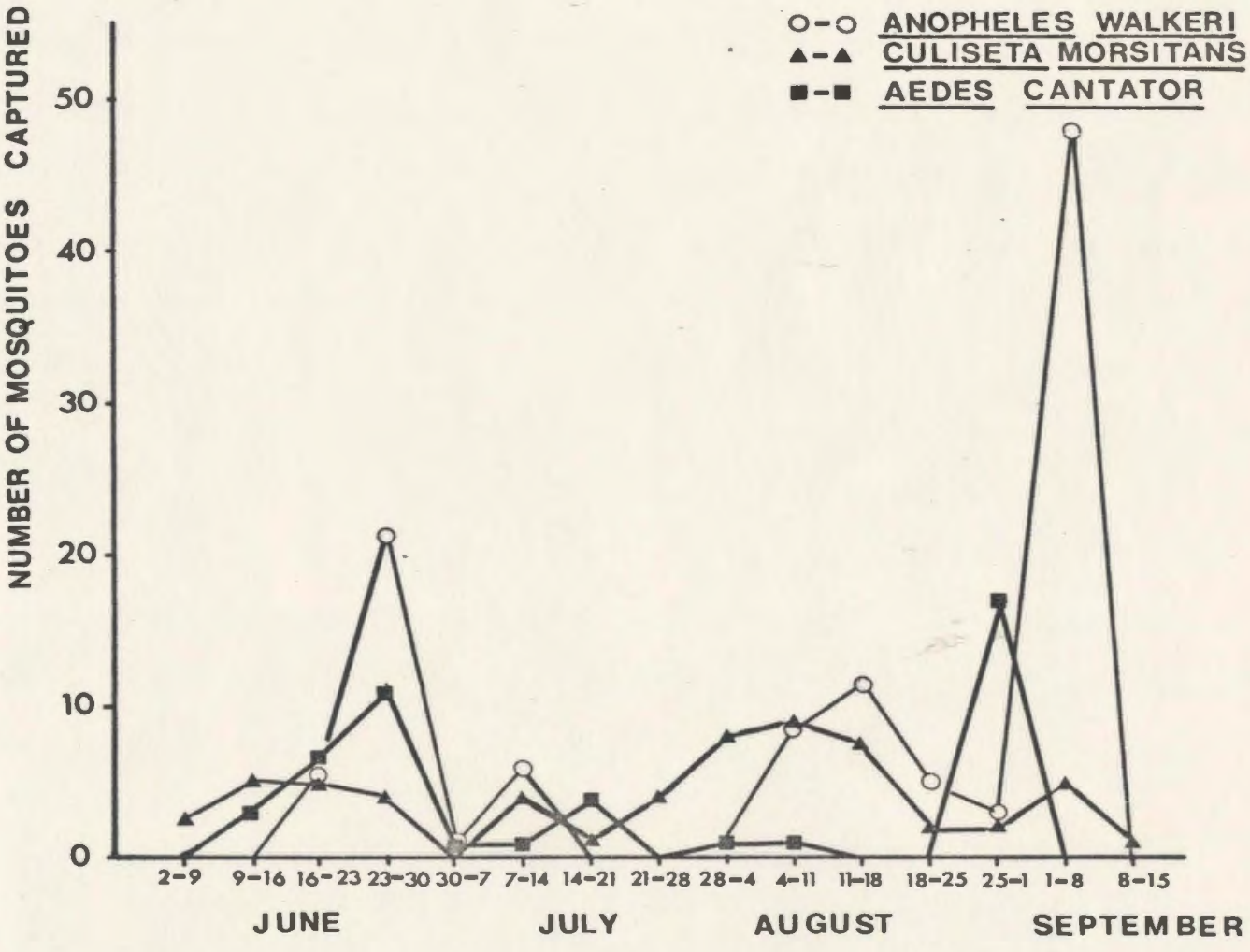

NUMBER OF M. PERTURBANS CAPTURED
1500
1400
500
400
300
200
100
2-9
9-16
16-23
23-30
30-7
7-14
14-21
21-28
28-4
4-11
11-18
18-25
25-1
1-8
8-15
JUNE
JULY
AUGUST
SEPTEMBER

FIGURE 8

Weekly fluctuations of numbers of *Anopheles walkeri*, *Culiseta morsitans* and *Aedes cantator* in 1973 at Trap 3 (average nightly catch based on a week's collection).


O-O ANOPHELES WALKERI
▲-▲ CULISETA MORSITANS
■-■ AEDES CANTATOR
NUMBER OF MOSQUITOES CAPTURED
0
10
20
30
40
50
2-9
9-16
16-23
23-30
30-7
7-14
14-21
21-28
28-4
4-11
11-18
18-25
25-1
1-8
8-15
JUNE
JULY
AUGUST
SEPTEMBER

and as late as September 2, 1973. This species was the most abundant mosquito, representing up to 87.5% of the population. Adults exhibited a peak in early July and decreased sharply till the first week in August (Fig. 7).

Peak abundances of adult females occurred between July 5 and July 10, of which 3,783 were female *Mansonia perturbans*. *Mansonia perturbans* must be considered the major species numerically. The greatest number taken on one night was 1,934 female mosquitoes (July 7) from trap 3.

The females were noted to be principally active at night and in the evening and activity appeared to be highest during calm humid nights, especially if there was a fog. Thick swarms surrounded both the baited traps and men in the march during early July. The largest population of *M. perturbans* present in July required long collection times (3 hours) from the baited traps; therefore, all but one trap were closed during July. The adults are noted to be important pests in communities near shallow lakes which are partly overgrown with emergent aquatic vegetation (Carpenter and LaCasse, 1955). This would explain their abundance in the study area.

Difficulties encountered by others in laboratory rearing of the larvae (Hagmann, 1952, 1953) and collection of larvae and pupae (Dorer, Carter and Bickley, 1950) discouraged attempts to sample larvae.

Culiseta morsitans (Theobald) 1901:

Adults were collected in the baited traps as early as June 5 and as late as September 11, 1973. Larvae were collected in the permanent water along the shore of Front Lake on July 17. While there is little known of the biology of *C. mortisans*, Carpenter and LaCasse (1955) report

that "the females rarely, if ever, feed on man" and indicate that *C. morsitans* probably feeds on birds. During the total of three summers in the Tantramar marshes, at no time were *C. morsitans* noted to feed on man by either the author or other workers (Eveleigh, pers. comm.). Sweep net collections of mosquitoes around men in the marshes were negative for *C. morsitans* during 1973 (Lewis, pers. comm.) though other species were numerous.

A total of 257 adult females were trapped in 1973 making this species the third most abundant trapped. *Culiseta morsitans* was collected many times during the mosquito season but never in large numbers (Fig. 8).

Anopheles walkeri Theobald 1901:

Adult females were collected in the baited traps from June 22 to September 2, 1973. Larvae were collected on July 17 and 20 at site 3. Three hundred and thirty adult females were captured making this species the second most abundant trapped. Adults fed readily on humans as well as the bait birds.

Anopheles earlei Vargas 1943:

Thirteen females were captured in 1973 between August 6 and 18.

Culex territans (Walker) 1856:

One unengorged female was captured in a bait trap at site 3 on August 2, 1973. None of this species was captured in 1972. Larvae were collected on July 20 at site 3 and reared to the adult stage. Attempts to induced starved female *Culex territans* to feed on immobilized ducks were unsuccessful.

Aedes cantator (Coquillett) 1903:

Adults were collected in the baited traps from June 5 to September 2, 1973. Larvae of this species were collected July 3 in a ditch along the north shore of Front Lake.

Aedes cinereus Meigen 1818:

One female was captured in a bait trap at site 2 on June 23, 1973. Larvae were collected in the ditch along Front Lake on July 3 and 4.

Aedes fitchii (Felt and Young) 1904:

One female specimen was captured in a bait trap at site 5, on July 24, 1973.

Aedes aurifer (Coquillett) 1903:

Five female specimens were collected in 1973 between June 27 and September 2.

Aedes canadensis canadensis (Theobald) 1901:

Twenty-five females were captured in 1973 between July 24 and August 11.

Aedes punctor (Kirby) 1837:

Twenty-five females were collected in 1973 between June 6 and August 11.

Local feeding habits of the various species were not investigated in detail; a project investigating this aspect is currently being carried out by another student.

Although the funnel traps did not capture large numbers of

individuals--with the exception of *Mansonia perturbans*, there were no indications that other species were in great abundance. Trapping of mosquitoes with a boxtrap (Bennett, 1960), modified boxtrap, and aspiration of blood-fed mosquitoes off the inside walls of a tent placed over the bait bird, was neither as successful as the funnel trap in capturing larger quantities of mosquitoes, nor revealed the presence of any species which were not obtained using the trap described herein. Conversely, the funnel trap used here, while excellent for mosquitoes, did not yield many simuliiids or ceratopogonids, whose presence in the area was verified by the abundant *Leucocytozoon* and *Haemoproteus* (*Parahaemoproteus*) infections in wild anatid and passeriform populations (Bennett and Cameron, 1974 - in press).

Operation of baited funnel traps appears to be the most efficient method of determining the number and species of mosquitoes acquiring blood meals from waterfowl. The total number of species feeding on waterfowl, as determined by baited funnel trap results in 1972 and 1973, was checked by the use of other types of baited traps in 1972 and the results appear to be real and not an artifact of the trapping methods.

Infectivity Studies

Large numbers of mosquitoes trapped in the waterfowl baited traps were engorged when collected in the morning. Of 7,235 adult female mosquitoes trapped in 1973, approximately 30 percent obtained blood meals while in the baited funnel trap. The total number of mosquitoes engorging while in the baited trap and those feeding in the laboratory

on the trussed bird was 4,968 (68.7 percent of the total collected in 1973). For the purposes of this thesis, the "infective blood meal" was considered to have been obtained from the trussed bird in the laboratory and the exogenous malarial parasites began development at this time.

No attempt was made to infect *Aedes fitchii*, *A. aurifer*, *A. excrucians*, *A. dorsalis* and *Culex restuans*. Attempts to feed *Culex territans* and *Aedes cinerus* on immobilized birds were not successful. One dissection was made of a blood fed *Aedes punctor*. Attempts to infect the remaining species of mosquitoes are discussed below.

Mansonia perturbans:

At most collections from bait birds, a sample of mosquitoes was dissected to determine the natural sporozoite rate in the mosquito population for that species. No sporozoite or oocyst infections were seen in the 144 wild caught *M. perturbans* examined.

Oocysts were noted in 10 of 27 *M. perturbans* (27.0%), 5 - 16 days following engorgement on ducks infected with *P. circumflexum*. Three of 20 mosquitoes dissected 8 - 16 days following the infective meal had sporozoites in the salivary glands on the 16th day. No attempts to transmit *P. circumflexum* to bird hosts were made with these infected mosquitoes.

Oocysts were noted in 3 of 12 *M. perturbans*, 6 - 11 days following engorgement on ducks infected with *P. polare*. Sporozoites were noted in the salivary gland of one of these mosquitoes on the 11th day. The transmission attempt using glands from this mosquito was unsuccessful.

Culiseta mortisans:

Sporozoites and/or oocytes were noted in 7 of 75 (9.3%) wild-caught *Culiseta morsitans* examined. Two attempts to identify the parasite by inoculation of sporozoites into young ducklings were unsuccessful.

Two of 4 *Culiseta morsitans* which had engorged on ducks infected with *Plasmodium polare* were noted to have oocysts (average = 98 oocysts) on the midgut with a range of 10.0 - 22.5 μ (median = 17.5 μ) on the fifth day following the infective blood meal.

Culiseta morsitans is very susceptible to infection with *Plasmodium circumflexum*. A complete description of the sporogony of *P. circumflexum* in *C. morsitans* is located in the discussion under that heading.

Anopheles walkeri:

No sporozoite or oocyst infections were seen in the 28 wild-caught *An. walkeri* examined. Fifteen mosquitoes dissected 3 - 16 days following engorgement on ducks infected with *P. circumflexum* were refractory. One mosquito dissected on the 6th day following engorgement on a duck infected with *P. polare* was also refractory.

Anopheles earlei:

No sporozoite or oocyst infections were seen in the 4 wild-caught *An. earlei* examined. Two mosquitoes examined on the 10th and 11th day following engorgement on ducks infected with *P. circumflexum* were refractory.

Aedes cantator:

Thirteen adult females were examined for sporozoites and oocysts

4 - 12 days following engorgement on ducks infected with *P. circumflexum;* they were refractory. Four *A. cantator* examined 6 - 11 days following engorgement on ducks infected with *P. polare* were also refractory.

Aedes canadensis canadensis:

Seven adult female mosquitoes dissected 4 - 12 days following engorgement on ducks infected with *P. circumflexum* were refractory.

Aedes punctor:

One adult female *A. punctor* dissected on the 6th day following engorgement on a duck infected with *P. polare* was refractory.

Sporogony of *Plasmodium circumflexum* in *Culiseta morsitans*

Dissections of midguts in *Culiseta morsitans* to study the development of the parasite on the midgut wall, were commenced the 3rd day following the infective blood meal obtained in the laboratory, and concluded the 16th day following the infective blood meal. A minimum of 25 oocysts per mosquito per day were measured unless gut infections of this magnitude were not available. The number of mosquitoes examined daily was dependent on the number available by the method of baited funnel trap collections. Sporogony studies were initiated in August 1973, following the successful transmission of *P. circumflexum* by *Culiseta morsitans*. Dissections were limited by the low population of *Culiseta morsitans* present at the time.

The following data was recorded for this study: (1) the median Q_1 to Q_3 (median 50 percentile range) and the range of diameters of the oocysts, (2) total number of oocysts in mosquitoes examined and number

of oocysts measured, (3) number of mosquitoes examined, together with the number of gut and/or salivary gland infections.

The diameter of the oocysts was measured by means of an ocular micrometer. To arrive at the number of oocysts on any midgut, the oocysts were counted on one side of the midgut and the result multiplied by two.

In the present experiments, at an ambient temperature (average 22.3°C) the oocysts reached maturity (diameter 37.5 μm) and free sporozoites were seen in the dissection fluid around the gut on the 7th day. Growth of the oocysts was fairly regular (Fig. 9), the number of oocysts varying from 10 to more than 400 (average 123) on each midgut. Dissection of midguts in *C. morsitans* 3 - 16 days following engorgement on ducks infected with *Plasmodium circumflexum* indicated a high index of infection (96.8%) (Table 5).

Recognizable oocysts were seen 72 hr. after the infective meal at which time the median diameter was 10 μm. The diameter of the oocysts increased daily thereafter and reached a maximum median diameter of 40 μm on the 10th post infection day. The largest oocysts measured were (57.7 μm) seen on the 10th and 14th days. The Q_1 to Q_3 range remained small till after the 10th post infection day, indicating time was the controlling factor at this stage (Bennett, Warren and Cheong, 1966c) and differentiation of smaller oocysts was occurring following the 10th day.

The sporozoites were seen in the salivary glands from the 8th day following the laboratory infective blood meal. Intensity of sporozoite infections in the salivary glands was variable possibly

FIGURE 9

Growth of *Plasmodium circumflexum* in *Culiseta morsitans*.

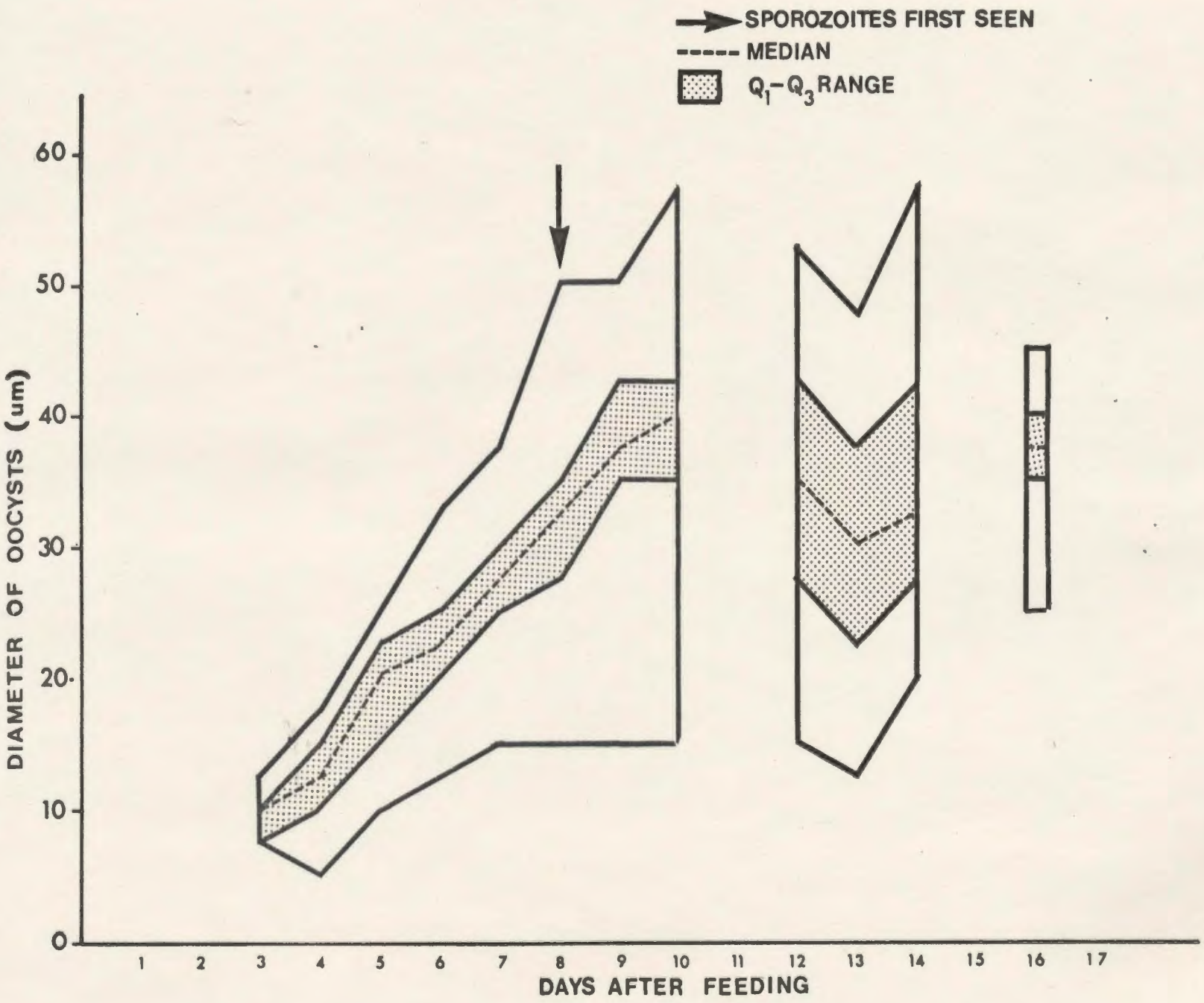

SPOROZOITES FIRST SEEN
MEDIAN
Q_1–Q_3 RANGE
DIAMETER OF OOCYSTS (um)
0
10
20
30
40
50
60
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
DAYS AFTER FEEDING

Table 5

Sporogony of *Plasmodium circumflexum* in *Culiseta morsitans*

Days after feeding	Infected guts/ Total examined	Average no. oocysts/Infected gut	Infected glands/ Total examined
3	1/1	400	0/1
4	2/2	125	0/3
5	1/1	134	0/1
6	3/3	100	0/4
7	4/4	147	0/3
8	2/2	195	3/3
9	1/1	50	1/1
10	3/3	48	2/3
11	1/1		1/1
12	6/6	127	6/6
13	2/3	118	2/3
14	2/2	103	2/2
15	1/1	8	1/1
16	2/2	20	2/2

reflecting the variable intensity of midgut infections. Twenty of 22 (90.9%) *C. morsitans* dissected 8 - 16 days following the infective meal had sporozoites in the salivary glands (Table 5).

Transmission of *Plasmodium* sp. by Mosquitoes

A total of 12 white Pekin ducklings and 1 white China gosling were injected with infected salivary glands. The results following injection of infected salivary glands into uninfected waterfowl are given in Table 6. Under "Days in Mosquito" is given the length of a period, timed from the infective blood meal obtained on the trussed bird in the laboratory to the date of the mosquito dissections and subsequent syringe injection of infected glands. The figures represent the minimal time for the development of sporozoites; since the baited funnel traps were set the previous evening it is possible that some infections were nearly 20 hours older than recorded. Mosquitoes marked by an asterisk were used for transmission attempts but the glands were not examined for sporozoites; in these cases oocysts were observed on the midgut and salivary glands were injected without crushing them first on a slide.

Transmission of *P. circumflexum* to uninfected ducklings and a gosling was obtained with *Culiseta morsitans*. Two of 9 *Anas boschas* ducklings and one white China gosling contracted *P. circumflexum* following intraperitoneal inoculation of sporozoites from the salivary glands of *Culiseta morsitans*. Prepatent periods of 14 and 19 days in the ducklings, and 31 days in the gosling were recorded.

Infected glands of 2 wild caught *Culiseta morsitans* were

Table 6

Transmission of *Plasmodium* sp. by mosquitoes

Mosquito Species	*Plasmodium* Species	Recipient Animal	Age at Inoc. (days)	Route of Inoc.	Prepatent Period	(Pairs) Salivary Glands Injected	Intensity of Gland Infection	Days in Mosquito	Trans-mission
C. morsitans 2818	?	white Pekin 32	20	SC		1	++	?	-
C. morsitans 2826	?	white Pekin 33	20	IV		1	+	?	-
C. morsitans 2856	*P. circumflexum*	white Pekin 34	21	IP		1	+	8 d	-
C. morsitans 2941	*P. circumflexum*	white Pekin 35	23	IP		1	++	9 d	-
C. morsitans 2993	*P. circumflexum*	white Pekin 36	27	IP		1	+	8 d	-
C. morsitans 3016	*P. circumflexum*	gosling 37	28	IP	31 d	1	+++	10 d	+
C. morsitans 3037	*P. circumflexum*	white Pekin 38	29	IP		1	+	10 d	-
C. morsitans 3069 *3070 *3071 *3073 *3074 *3076	*P. circumflexum*	white Pekin 39	32	IP		6	++	12 d	-

Table 6 (continued)

Mosquito Species	*Plasmodium* Species	Recipient Animal	Age at Inoc. (days)	Route of Inoc.	Prepatent Period	(Pairs) Salivary Glands Injected	Intensity of Gland Infection	Days in Mosquito	Trans-mission
M. perturbans 3100	*P. polare*	white Pekin 40	33	1P		1	++	11d	-
C. morsitans *3106	*P. circumflexum*	white Pekin 41	34	1P	19d	1		11d	+
C. morsitans 3118	*P. circumflexum*	white Pekin 42	34	1P		1	+++	8d	-
C. morsitans *3204 *3206	*P. circumflexum*	white Pekin 43	39	1P	14d	2		16d	+
C. morsitans *3269 *3271 *3306	*P. circumflexum*	white Pekin 44	40	1P		3		14d	-

*No examination of salivary glands. Oocysts were observed on midgut of mosquito

injected, one pair subcutaneously (2818) and the other pair (2826) intravenously into 2 uninfected *Anas boschas* ducklings. No infections were noted inthe ducklings based on examination of blood smears for more than 66 days following injections.

The infected glands of one *Mansonia perturbans* which had fed on a *P. polare* infected duck 11 days previous were injected into an uninfected duckling. No infection was noted in the injected duckling following examination of blood smears for 83 days.

The Asexual Cycle of *Plasmodium circumflexum*

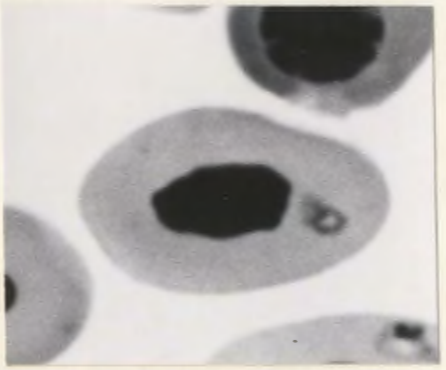
The progress of the asexual cycle of *Plasmodium circumflexum* was followed by counting the total number of parasites per 10^3 erythrocytes and the percentage of the different stages. Two thin blood films were taken at 8 hr intervals. The stages recognized for the purpose of counting (Fig. 10) were similar to those used by Hawking, Worms and Gammage (1968) but have been adapted for use with *P. circumflexum* viz.: Stage 1, length of the parasite less than half the long diameter of the nucleus of the erythrocyte. Stage 2, area of the parasite less than that of the nucleus. Stage 3, parasite greater in area than nucleus but chromatin not divided. Stage 4, 2 to 3 pieces of chromatin. Stage 5, more than 3 pieces of chromatin. Stage 6, more than 10 pieces of chromatin.

The infection discussed was produced by intramuscular blood inoculation (white China gosling X-17). A series of slides at 8 hr intervals was taken from gosling X-17 beginning the 6th day of the patent period and continuing for 7 days. No attempt was made to increase

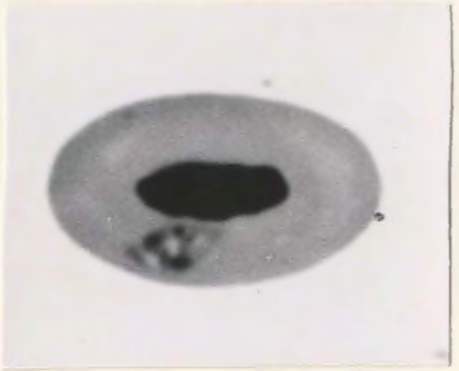
FIGURE 10

Stages of *Plasmodium circumflexum*, wood duck strain: (A) Stage 1; (B) Stage 2; (C) Stage 3; (D) Stage 4; (E) Stage 5; (F) Stage 6; X8750.

A

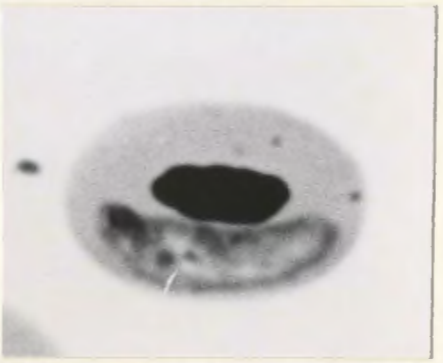


B

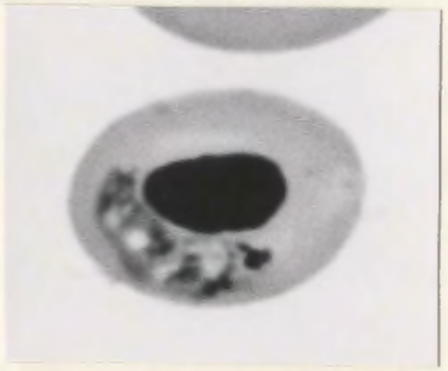


D

C



E



F

the synchronicity artificially and the birds were kept under natural light conditions.

A short term of infection and low parasitemia appears characteristic of *P. circumflexum*, (Wolfson, 1936) making any study of the periodicity of its cycle difficult. Gosling X-17, however, showed parasites in its peripheral blood for 36 days after the infection became patent and conclusions are based on counts of those smears. The infections in gosling X-37 and in ducklings X-41 and X-43 failed to reached a level of parasitemia sufficient to determine the periodicity of their cycle. A series of slides was prepared early in the patent period from these birds.

In gosling X-17 there is an indication of cyclic variations in Stage 1 parasites (Fig. 11) though the variation is not marked and the length of the period, if it is real, cannot be established. That the variations persist with Stage 2 and 3 parasites, indicates that the variations are real rather than apparent ones due to probable errors in counting. The presence of periodicity in X-17 cannot be firmly established and the variations present show no constant relation to the 24 hour light-dark cycle. Attempts to achieve a more sensitive indication of the time of schizogony (Hawking and Gammage, 1970) by plotting the total number of Stage 1 parasites (percentage of Stage 1 parasites X total number of parasites) was unsuccessful (Fig. 13). Fig. 13 includes a plot of the parasitemia in 10^3 erythrocytes from the 6th through the 12th day of patency.

FIGURE 11

Proportions of stages 1, 2 and 3 of *P. circumflexum* found in the circulating blood of gosling X-17 (The horizontal scale shows the time, hours of the clock on successive days of the patent period).

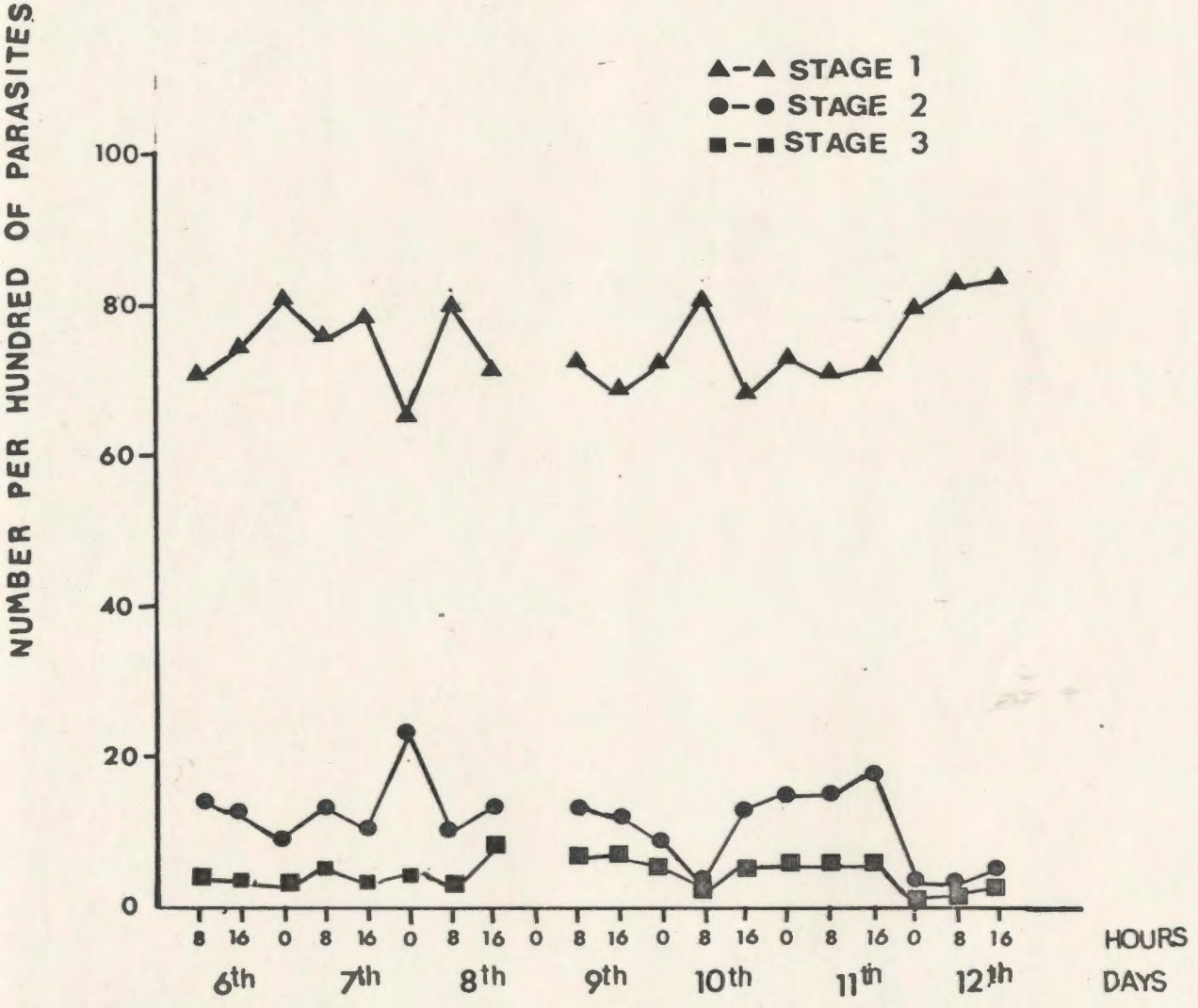

▲–▲ STAGE 1
●–● STAGE 2
■–■ STAGE 3
NUMBER PER HUNDRED OF PARASITES
100
80
60
40
20
0
8 16 0 8 16 0 8 16 0 8 16 0 8 16 0 8 16 0 8 16
HOURS
6th 7th 8th 9th 10th 11th 12th
DAYS

FIGURE 12

Proportions of stage 4, 5, 6 and gametocytes of *P. circumflexum* found in the circulating blood of gosling X-17 (The horizontal scale shows the time, hours of the clock on successive days of the patent period).

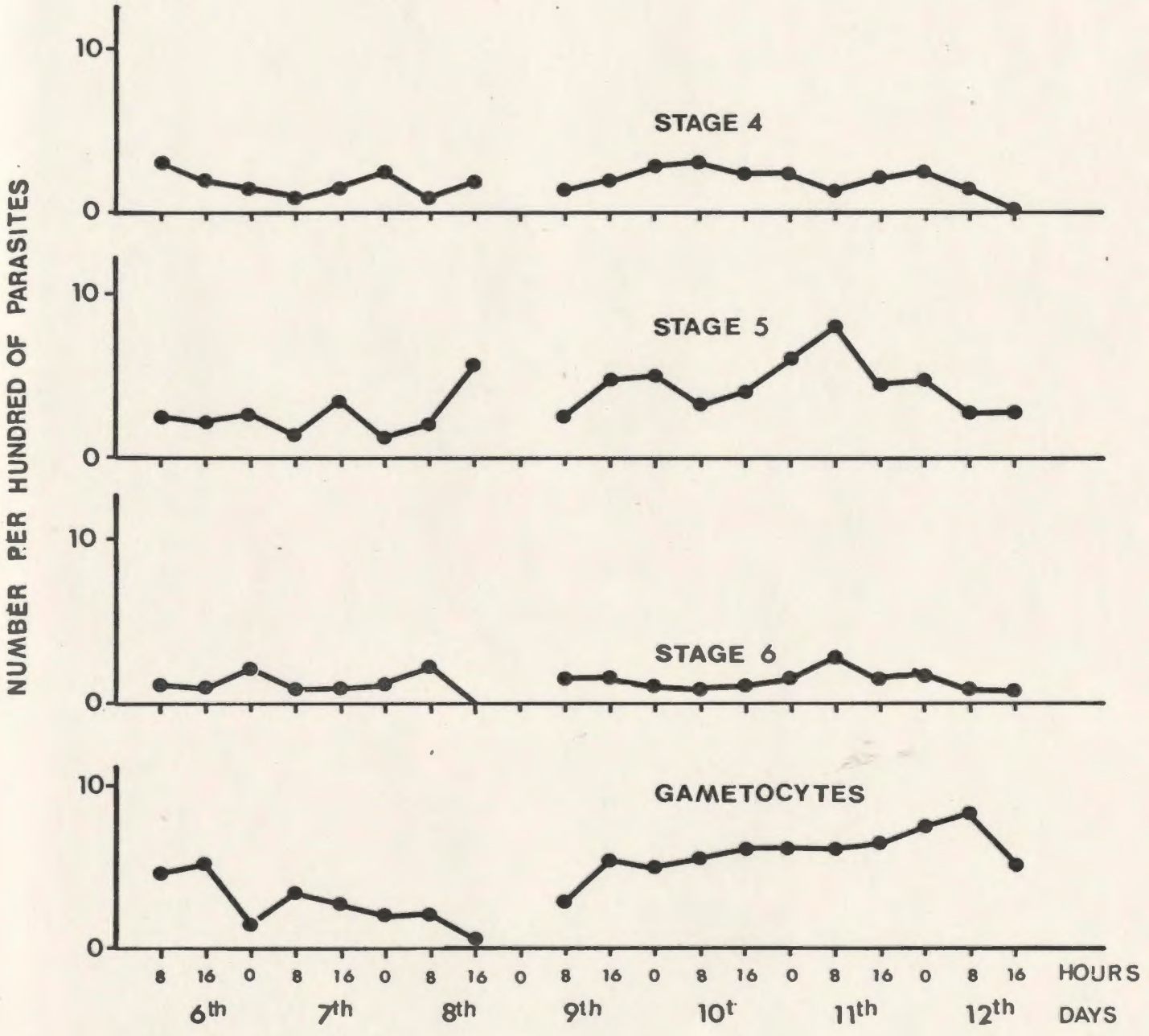
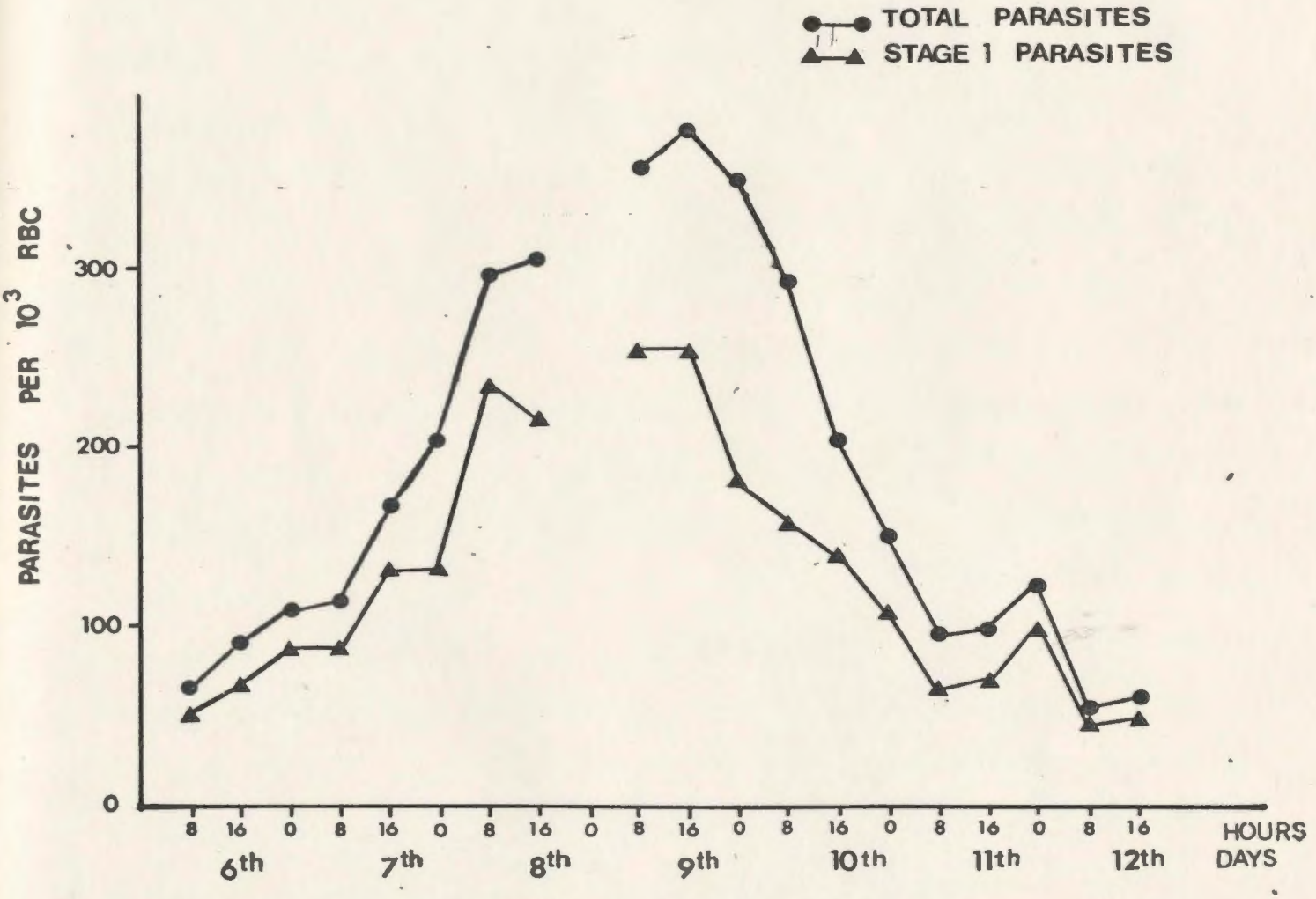

NUMBER PER HUNDRED OF PARASITES
10
0
STAGE 4
STAGE 5
STAGE 6
GAMETOCYTES
8 16 0 8 16 0 8 16 0 8 16 0 8 16 0 8 16 0 8 16 0 8 16
HOURS
6th 7th 8th 9th 10t 11th 12th
DAYS

FIGURE 13

The cycle of *P. circumflexum* in gosling X-17. (The horizontal scale shows the time, hours of the clock on successive days of the patent period. The vertical scale shows the total numbers of parasites and numbers of stage I parasites per 10^3 RBC).


TOTAL PARASITES
STAGE 1 PARASITES
PARASITES PER 10^3 RBC
300
200
100
0
8 16 0 8 16 0 8 16 0 8 16 0 8 16 0 8 16 0 8 16 0 8 16
HOURS
6th 7th 8th 9th 10th 11th 12th
DAYS

DISCUSSION

The Asexual Cycle of *Plasmodium circumflexum*

The erythrocytic schizogony of *P. circumflexum* was studied in several ducklings and goslings to determine its synchronicity and periodicity in waterfowl. Previous studies have indicated this to be a highly variable character (Garnham, 1966) and the results of efforts in this study to investigate the phenomenon in waterfowl confirm this observation. The earliest studies to determine the length of the asexual cycle of *P. circumflexum* were performed by Manwell and Wolfson (1934) utilizing six canaries over a period of 72 hours. Comparisons of the mean areas of the parasites and counts of the number of segmenting stages in blood smears taken at 4 hour intervals were inconclusive in elaborating any periodic cycle. Repeating these investigations, Wolfson (1936) observed definite synchronicity and periodicity in one canary infected with *P. circumflexum* but in other canaries the infection was short and of too low parasitemia to give satisfactory evidence. The cycle of schizogony in the blood appeared to last 48 hours, since there was a 48 hour periodicity in the prevalence of any growth stage. The degree of synchronicity was relatively high; at times there was a complete lack of some growth stages and a large predominance of others. Paraense (1952), using a strain of *P. circumflexum* isolated from *Gnorimopsar chopi chopi* (Viellot, 1819), observed a high synchronicity and a high periodicity in three canaries inoculated intravenously. The asexual cycle was observed to be 24 hours in length with the period of sporulation occurring about 12 a.m. In the present study there is

an indication of cyclic variations in the asexual cycle of *P. circumflexum* (Fig. 11) although the range of stage I parasites (83.0 - 64.8) is low, not great enough to establish the periodicity. Unlike the results of Paraense (1952) and Wolfson (1936), the cyclic variations appeared with no constant relation to the 24 hr. light-dark cycle and the apparent synchronicity of schizogony was very low.

Hawking and Gammage (1970) note that a more sensitive indication of the time of schizogony may be provided by the curve of the total number of stage 1 parasites (percentage of stage 1 X total number of parasites). Using this method to examine the development of *P. circumflexum* in the blood of gosling X-17, segmentation appeared to be asynchronous (Fig. 13).

Sporogony of *Plasmodium circumflexum*

The course of sporogony of *Plasmodium (= Proteosoma) circumflexum* in *Culiseta (= Theobaldia) annulata* has been described by Reichenow (1932) and Corradetti *et al.*, (1964) has observed the presence of oocysts and sporozoites of *P. circumflexum* in both *Culiseta annulata* and *Culiseta longeareolata.*

Reichenow's investigations utilized the original strain of *P. circumflexum* which was transmitted from a juniper thrush *(Turdus pilaris)* to canaries (Kikuth, 1931) and provided him by Dr. Kikuth (Reichenow, 1932). Reichenow's work is noteworthy for it expanded the knowledge of the development of malaria parasites, particularly the exogenous stages of *P. circumflexum* in *C. annulata.*

Reichenow noted the index of infection in *C. annulata* to be

near 100 percent (20 specimens were dissected and all displayed oocysts and/or sporozoites); he stated that *C. annulata* could therefore be considered a good carrier for *P. circumflexum*. The index of infection of *P. circumflexum* for *C. morsitans* obtained in this study (97.0%) compares closely with Reichenow's value and we can assume a high susceptibility of each species of mosquito for the parasite.

While many factors are noted to be influential in producing infections of a given parasite in a species of mosquito, Reichenow noticed that in *C. annulata* the digestive process in the midgut was slow to start, fully intact erythrocytes and totally normal zygotes being found three days after the infective meal at a development temperature of 22-23°C. Garnham (1966) notes that 3 days is "extraordinarily prolonged"* though if this is the case it would allow young gametocytes to mature and sporogony after a single meal could commence at once or 3 days later. Results of Reichenow offer supportive value for he noticed 3 days after the infective meal many ookinetes among the contents of the midgut, although the oldest oocysts in the intestinal wall were already 4-8 "cellular".

According to Stohler (1957) a different interpretation is indicated. In the sporogonic development of *P. gallinaceum* in *Aedes aegypti*, the peritrophic membrane grows more and more solid after the 30th hour following the infective meal; ookinetes still in the midgut find themselves captured in the peritrophic sac and eventually die.

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*(Bennett, Warren and Cheong, 1966c) - Blood meals in *Culex sitiens* did not clear until the 5th postinfection day.

Thus, the peritrophic membrane of *Aedes* seems to have a regulative function on the infection by the malarial parasite. The peritrophic membrane in *C. annulata* was noticed by Reichenow, but he apparently failed to recognize the interpretation of James (1931), who suspected that an individual permeability of this membrane was responsible for limiting the number of oocysts which could develop.

A large range in the size of oocysts was attributed by Reichenow to the retardation of blood meal digestion by *C. annulata*, but in the present study, where no excessive lag in digestion was noted, slightly greater discrepancy in oocyst size occurred (Fig. 9). On the seventh post-feeding day in *C. annulata*, a diameter of oocysts varying between 13 and 28 μm* was found and after 12 days the oldest oocysts were already mature (60 μm diameter); yet some of them were so retarded in their development that cell division had not yet begun. On the seventh post-feeding day in *C. morsitans* the oocyst diameters varied between 15 and 37.5 μm, but a diameter of 57.5 μm was the largest oocyst noted at any time in sporogony and "juvenile" oocysts were not seen concurrently. While discrepancies are apparent between the two different host-parasite relationships, the most obvious character being the difference in maturation periods of the oocysts and time of sporozoite appearance in the salivary glands, the ranges of oocyst diameters during sporogony are similar enough to infer a close genetic relationship

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*Garnham (1966) is apparently mistaken in this regard for he notes that "Four or 5 days after infection, the oocyst is about 13 μm in diameter; by 7 days it is 28 μm and at maturity on the twelfth day it may attain a diameter of 60 μm."

of the *P. circumflexum* (*Turdus pilaris* strain) - *C. annulata* union and the *P. circumflexum* (*Aix sponsa* strain) - *C. morsitans* union. While many individuals have shown that the susceptibility of species, varieties and strains of mosquitoes can vary widely to the same species of parasite, the susceptibility of *C. annulata* and *C. morsitans* to the 2 strains of *P. circumflexum* appears contrary to the influences of geographic and other environmental influences and apparently reflects their close systematic relationship. A better understanding of this relationship could undoubtedly be achieved by a more extensive study of sporogonic stages of *P. circumflexum* in *C. annulata*, but Reichenow's data is limited and the prospects of repeating his investigations do not appear promising.

It is unfortunate that almost all records relative to the susceptibility of mosquitoes to avian malaria are the culmination of laboratory investigations involving cultured mosquito strains rather than considerations of susceptible mosquitoes influenced by a specific natural environment. In other words, the studies are often not oriented to the ecology of a natural situation rather than a laboratory situation. It is a generally accepted belief that the "feeding preferences of mosquitoes are determined by several factors such as the kinds and numbers of vertebrates available, location of these animals, the ecological influences which bring them into proximity, and genetic changes within the mosquitoes" (Huff, 1965). The establishment of laboratory colonies of mosquitoes is an almost indispensable tool in some studies for such colonies provide a continuous supply of material for experimental purposes, but it seems to me that transmission studies

concerned with factors governing vector efficiency are most readily studied by field orientated research and techniques. Weitz (1960) has also noted that food preferences are not truly indicated by blood meals collected under artificial conditions, for this view neglects the all important difference between the natural host and artificial ones.

Vectors

A great deal of attention has been given, particularly in the case of the epidemiology of human malaria, to the associations of the vectors, parasites and hosts, which are responsible for the occurrence of malaria in a particular locale. The primate and avian malarias on the other hand, have been used extensively for studies of the factors governing mosquito susceptibility, so that any synthesis of our knowledge of mosquito-plasmodia-host relationships would currently require information on all three groups of parasites.

The factors which are generally considered to govern vector efficiency of mosquitoes are: a close association of the vector with the vertebrate host, with the mosquito feeding readily and often on the host; and the susceptibility of mosquitoes to the given species or strain of plasmodium (Bates, 1949; Herms and James, 1961; Bennett *et al.*, 1966a, b; Boyd, 1940; Russell, 1959). A functional vector is generally considered to fulfill these requirements. Although our knowledge of the factors governing vector efficiency have been developed from our work on human plasmodia, these are applicable to the epizootiology of avian malaria. The same factors can be directly

applied to the present study and their consideration is important in studying the natural transmission of *Plasmodium circumflexum*.

Aedes cinereus, Aedes fitchii, Aedes aurifer, Aedes excrucians, Aedes c. canadensis, Culex territans and *Anopheles earlei* were in low abundance in the study area, as compared to other species (Table 4). Of the above mosquito species, only *Culex territans* has been found capable of harboring an avian plasmodia; in these cases only oocysts were found (Huff, 1927, 1932).

The single specimen captured during the two summers in which the baited funnel traps were utilized and the inability in the present study to obtain feeding by *Culex territans* (laboratory reared) upon ducklings makes it improbable that this species serves as a vector of avian malaria in the study area. *Culex restuans* has also been shown to be susceptible to avian plasmodia (Laird, 1941) but the absence of this species in the 1973 baited trap collections and the presence of only one specimen in the 1972 baited trap collections makes it improbable that this species serves as a vector of avian plasmodia in the study area.

Of the remaining species, *Aedes cantator* has been found capable of transmitting *P. gallinaceum* in the laboratory (Cantrell and Jordon, 1945). However, work done during the present study found *Aedes cantator* refractory to both *P. circumflexum* and *P. polare*. *Anopheles walkeri* can be infected with human plasmodia (Kitchen, Bradley, 1936; Matheson, Boyd and Stratman-Thomas, 1933) and has been found infected in nature (Bang, Quinby and Simpson, 1940). Various *Anopheles* have been tested for susceptibility to avian malaria and Huff (1965)

indicates a high percentage of positive results. In the current study dissections of *Anopheles walkeri* following engorgement on ducks infected with *P. circumflexum* and *P. polare* indicated it was refractory to these malarial parasites.

Because of the relative abundance of *Mansonia perturbans* in the study area and its appearance in the baited traps (Fig. 7), this mosquito was suspected early in the study to be a probable local vector of some avian malaria. However, no infections were noticed in the 144 wild-caught *M. perturbans* examined for oocyst and sporozoite infections during 1973; continued studies by Bennett and Eveleigh during the summer 1974 which involved the dissection of approximately 700 wild-caught *M. perturbans*, resulted in no natural infections (Bennett, pers. comm.). A relatively low index of infection (37.0%: 10 of 27 midguts dissected 5 - 16 days post infection showed oocysts) of *M. perturbans* for *P. circumflexum* was noted. Sporozoites were seen on the 16th post infection day in 3 of 20 dissections for salivary glands performed 8 - 16 days following feeding on ducks infected with *P. circumflexum*.

A low index of infection (25.0%) was also noted of *M. perturbans* for *Plasmodium polare* (3 of 12 midguts dissected 6 - 11 days post infection displayed oocysts). Sporozoites were seen in 1 of 3 dissections for salivary glands, all performed on the 11th day. The one attempt to transmit *P. polare* by syringe passage of sporozoites from *M. perturbans* was unsuccessful.

On the basis of the low apparent susceptibility of *M. perturbans* for *P. circumflexum* and *P. polare*, *M. perturbans* appears less than ideal as a vector for these avian malarias in the Tantramar Marsh area.

In any case, the general inclination of *M. perturbans* to feed on most any available animal (Downe, 1962; Schaefer *et al.*, 1969), combined with the low susceptibility to *P. circumflexum* and *P. polare*, seems to indicate that transmission of these plasmodia by *M. perturbans* may be considered negligible.

M. perturbans has been reported to be susceptible to *Plasmodium gallinaceum* (Cantrell and Jordon, 1945) with oocysts being observed in a single specimen. This appears to be the only report on the susceptibility of *M. perturbans* to avian malaria, but at least one other member of the genus has been incriminated in the transmission of malaria to birds. Both *P. circumflexum* and *P. gallinaceum* have been transmitted by *Mansonia crassipes* (Niles *et al.*, 1965). While this may reflect the relative susceptibility of the genus to avian plasmodia, the lack of information i.e., the transmission of avian plasmodia by other members of the genus *Mansonia* leaves such an assumption with little basis.

Culiseta morsitans is demonstrated in this study to be the natural vector of *P. circumflexum* in the Tantramar Marshes, with an experimental infection rate of 96.8% for oocyst infections and 90.9% for sporozoite infections using wild-caught specimens. The wild population of *C. morsitans* was noted to have a natural infection level of 9.3% for avian malaria. Two ducklings and one gosling contracted *P. circumflexum* following intraperitoneal inoculation of sporozoites. Although *C. morsitans* is not a particularly abundant species, it was the third most abundant species trapped during 1973 (Table 4). It exhibits ornithophily in its feeding habits and meets the functional

requirements of a good natural vector. Collection results from the waterfowl baited traps coupled with its apparent absence around human baits (Lewis, pers. comm.) indicates that *Culiseta morsitans* in the study area is strongly ornithophilic in its feeding habits. The accuracy of these results is supported by the laboratory identification of the contents of the gut of bloodfed *C. morsitans* based on the recognition of chracteristic avian blood cell morphology. The presence of nucleated red blood cells is a reliable cytological criterion for establishing an index of birds or reptiles (or possibly amphibians or fishes) as distinguished from mammals, whose red blood cells are non-nucleated (Weitz, 1960).

Little is known of the feeding habits of most of the species of the genus *Culiseta*, but *C. melaneura* has been the subject of intensive research. The use of host baits caged in various kinds of traps indicates the preference of *C.melaneura* for birds is very marked (Haynes, 1961; Means, 1968) and the results of precipitin tests indicates *C. melaneura* is almost exclusively an avian feeder (Jobbins, Burbutis and Crans, 1961; Crans, 1964; Schober and Collins, 1966; Joseph and Bickley, 1969).

It appears that members of the genus *Culiseta* may display a marked susceptibility to the avian plasmodia that has not hitherfore been appreciated. The apparent similarities in the feeding preferences of *C. morsitans* and *C. melaneura* and the incrimination of both mosquitoes in the transmission of *P. circumflexum* (Herman, 1938a) (Meyer, Bennett and Herman, 1974) and *C. melaneura* in the transmission of *P. cathemerium* (Herman, 1938a) is impressive. These studies comprise

two of only a small number of epizootiological field investigations of the transmission of avian malaria which report in their results, proven Culicine vectors (Niles *et al.*, 1965; Bennett *et al.*, 1966c; Herman, 1938a; Meyer *et al.*, 1974).

In laboratory studies Ed. and Et. Sergent (1918) were successful in determining *Culiseta (= Theobaldia) longeareolata* to be susceptible to *Proteosoma praecox (= Plasmodium relictum)* and Reichenow (1932) observed the development of this parasite in *Culiseta annulata*. In his investigations of the susceptibility of various mosquito species to several avian plasmodia, Reichenow (1932) found that *Culiseta annulata* permitted development of *P. circumflexum* with an index of infection in the mosquito host near 100%. His comment is interesting:

> *Theobaldia annulata* ist für *Proteosoma circumflexum* ein ebenso guter Überträger wie *Culex pipiens* für *P. praecox*.*

While Reichenow had obtained a 93% index of infection in *Culex pipiens* for *P. relictum*, he was unable to obtain development of *P. circumflexum* or *P. elongatum* in *Culex pipiens*. This was apparently a surprise to Reichenow since experiments by Huff (1927) had left him with the impression, at least originally, that *Culex pipiens* was a suitable vector for all avian plasmodia (Reichenow, 1932).

Corradetti *et al.*, (1964) were successful in obtaining mature oocysts and sporozoites of *P. circumflexum* in both *Culiseta annulata* and *Culiseta longeareolata* but transmission attempts (5 canaries injected) were not successful. Corradetti and Scanga (1965, 1968) later

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*Translation: *Theobaldia annulata* can therefore be considered as good a carrier for *Proteosoma circumflexum* as *Culex pipiens* is for *P. praecox*.

achieved success in transmitting *P. polare* with *Culiseta longeareolata* and obtained a 45.6% (30/64) index of infection for oocysts and/or sporozoites (Corradetti and Scanga, 1968). *Plasmodium polare* was also found to infect *Culiseta morsitans* in the present study (2 of 4 midguts dissected on the 5th post infection day had oocysts), all of which appears to indicate two things: 1) the close relationship of *P. circumflexum* and *P. polare*, and 2) the apparent susceptibility of species of *Culiseta* to plasmodia of the subgenus *Giovannolaia*. If more knowledge were available on the natural vectors of this group, it appears probable that we could reach a better understanding of the host-parasite association as it affects the natural transmission of the Plasmodidae by the Culicidae.

Janovy's (1965) study of the epidemiology of avian malaria on a Kansas waterfowl management area eliminated *Culiseta inornata*, though present and feeding on birds, from consideration as a probable vector of avian malaria on the basis of bionomics. Unfortunately no dissections for salivary gland infections were made on the 560 *Culiseta inornata* collected, though this species has been shown to be susceptible to *P. gallinaceum* (Cantrell and Jordon, 1945).

Whereas mosquitoes of the genus *Culex* have formerly been associated with transmission of avian malaria, in much the same way "anophelism" has been associated with the transmission of human plasmodia, it is apparent from the review literature that such an assumption is outdated. The mosquito species known to be susceptible to avian malaria are distributed over 6 of the 20 genera containing species which suck blood (Huff, 1965). Among the 6 genera with

susceptible species, the genus *Culiseta* appears to have one of the highest indexes of susceptibility to avian malaria, indicated by the relation of the number of species of mosquitoes susceptible to the total number of species tested (Huff, 1965). Inclusion of data acquired since 1965 would raise that index even higher.

Attempts to elucidate the factors which permit or stop the development of sporogony in the mosquito have drawn much attention. Bennett *et al.*, (1966a) have classified the susceptibility of mosquitoes to strains of *P. cynomolgi* in 3 major categories: 1) Failure of the parasite to grow in the species; 2) development of oocysts but failure of the sporozoites to reach the salivary glands, and 3) complete development with viable sporozoites in the salivary glands. Huff (1941) suggests that failure or success of the ookinete to penetrate the midgut wall is the most important incident responsible for infection or escape from infection. The same author (Huff, 1934) observed that *P. cathemerium* can develop as far as the ookinete stage in insusceptible mosquitoes. Bennett *et al.*, (1966b) observed that under controlled conditions of temperature and humidity such factors as minimum time required for oocyst differentiation, the rate of growth and maximum oocyst size were similar for *P. cynomolgi* in 6 different susceptible mosquito species. They concluded that these features of sporogonic development appeared to be biological constants within the parasite. Aspects other than the growth of the oocyst, viz. oocyst degeneration before maturity and ability of sporozoites to reach the salivary glands appeared to vary with the species of mosquito. Similar constants in the development of malaria parasites were observed by Huff and Marchbank

(1955) who concluded that oocyst size does not vary with fluctuations in degree of parasitemia nor with the number of oocysts per mosquito.

The innate or genetic resistance of mosquitoes is not the only factor affecting sporogony. Insufficient supplies of food, unsuitable incubation temperatures and competitive action of other microorganisms can effectively stop gametocyte development in the mosquito midgut.

It would appear that growing oocysts need food or certain nutritional requirements before reaching maturity and producing thousands of sporozoites. Some workers have observed that the growth of oocysts is quicker and more regular when the mosquito is given blood meals, than when it receives only glucose (Ghosh and Ray, 1957). Others (Ball, 1964; Clark and Ball, 1952) have suggested that differences in amino acid distribution between species and genera of mosquitoes, may be important in explaining why some species of mosquitoes are easily infected with *Plasmodium*, some species are infected with difficulty, and some are totally refractory.

Competition was proposed to explain the fact that microsporidia actively multiplying in the mosquito interfered with the growth of oocysts (Garnham, 1956; Bray, 1958). By adding chloramphenicol and streptomycin to the diet of mosquitoes, Micks and Ferguson (1961) were able to double the number of oocysts on the midgut. They concluded that having killed the bacterial flora of the midgut, they had suppressed the competition between microbes and oocysts. Terzian, Stahler and Miller (1953) observed that penicillin and thyrothricin increased the susceptibility of *Aedes aegypti* to *P. gallinaceum*.

Effects of temperature stresses on exogenous stages have been

investigated by Ball (1964) and Ball and Chao (1964) who note that the period of exflagellation is marked by limited resistance to low temperatures - more so than at later stages. Time of sporogonic cycles also appears to be extended by lowering the temperature.

When the sporozoites reach the salivary glands after a single blood meal, they remain there until the death of the mosquito. In the first few days after their arrival in the glands the parasites are the most infective. Porter, Laird and Dusseau (1954) have shown that sporozoites of *P. gallinaceum* in the glands of *Aedes ægypti* should be harvested in the first 2 weeks after their arrival. Viability is reduced 1/4 between the 2nd and 3rd week, to 1/40 in the course of the 4th week and to 1/400 in the course of the 5th week, even in perfectly adapted mosquitoes.

Despite the knowledge concerning mosquito susceptibility to the *Plasmodium* organisms, the relatively low success experienced in transmission attempts during this study remain unexplained. Sporozoites within the salivary glands appeared viable at the time of dissection, indicating that causative factors in the suppression of transmission interacted at a subsequent period.

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