the hyperendemic foci of the interior to all parts of the Republic of Vietnam and beyond and that wherever suitable vectors occur, malaria has increased as new reservoirs are established.

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THE RELATIONSHIP OF MOSQUITOES TO OXIDATION LAGOONS IN COLUMBIA, MISSOURI

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INTRODUCTION

Species of mosquitoes reported from sewage lagoons include *Aedes campestris* (Dyar and Knab) from North Dakota, *Aedes dorsalis* (Meigen) from North and South Dakota, *Aedes vexans* (Meigen) from Arizona and Nebraska, *Anopheles quadrimaculatus* Say from Missouri and Tennessee, *Culex erraticus* (Dyar and Knab) from Mississippi and Tennessee, *Culex restuans* (Theobald) from Nebraska and North Dakota, *Culex salinarius* (Coquillett) from Georgia and Texas, *Culex thriambus* (Dyar) from Texas, *Culiseta inornata* (Williston), from Nebraska, Missouri, and Oklahoma, *Psorophora confinnis* (Lynch-Arribálzaga) from Arizona, *Culex tarsalis* Coquillett from the midwest, and the *Culex pipiens* complex from the south-west (Eads and Mengies 1956, Beadle and Harmston 1958, Rapp 1960, Beadle and Rowe 1960, Rapp 1961, Myklebust and Harmston 1962, Rapp and Emil 1965, Smith and Enns 1967, 1968, Smith 1969a, and Smith 1969b).

Sewage lagoons constructed near cities which are well within the flight range of most mosquitoes may create a potential danger for the residents of trailer courts, outdoor camps, resort motels, and other suburban dwellings that use lagoons for sewage disposal.

This investigation was undertaken to study the relationship of mosquitoes to sewage lagoons and to acquire basic biological data concerning the principal species of mosquitoes in the lagoon environment. During the summers of 1965–1966, a survey was made of the mosquitoes of Columbia, Missouri. The objectives were to determine which species of mosquitoes were present and to relate this information to mosquito production in the lagoon environment.

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MATERIALS AND METHODS

Mosquito populations were sampled by utilizing a combination of the following methods.

EGG RAFT SAMPLING METHOD. Since female mosquitoes oviposit in such objects as floating inner tubes, tin cans, and cinder block openings, it seemed reasonable that a floating device might be used to study the oviposition habits of certain mosquito species in the field. Artificial oviposition blocks were made from polystyrene plastic by cutting each block to measure 18″ x 18″ x 3″. Four openings, each measuring 6″ x 6″ x 3″, were cut in each block and a 2-inch margin was left between the outer edges and between the openings. The total sampling area was 1 square foot. Two 4½-inch eye-bolts were pushed through the plastic, one on each side of the block. A 2-foot length of nylon cord was fastened to the eye-bolt while the other end was tied to an eye-bolt embedded in a ½ lb. cement anchor. The completed oviposition blocks were distributed among the study lagoons during the summers of 1965–1966.

LARVAL SAMPLING METHODS. Fewer techniques and methods exist for collecting mosquito larvae than for adults. Since larvae cannot be enticed into a trap they must be searched for and captured in their natural environment. Larvae were collected from their natural habitats and sewage lagoons with a long-handled dipper. The numbers of larvae per dip were counted, or estimated if the numbers were large. Mosquito larvae retained for identification were placed in vials containing 70 percent ethyl alcohol or cellosolve, and some were collected alive for rearing purposes.

ADULT SAMPLING METHODS. Two kinds of light traps were employed to collect adult mosquitoes: (1) the standard New Jersey light trap and (2) the USPHS CDC light trap. Both light traps were used for general survey work. Chloroform tubes and aspirators were also used to collect biting mosquitoes.

DESCRIPTION OF STUDY LAGOONS. In Columbia, two municipal, two residential, and two animal waste lagoons were studied. Lagoons A and B were municipal lagoons, and were operated in series. They were built in the spring of 1963 in the west end of Columbia near old highway 40. Cell A, the primary lagoon, was 4.5 acres in size and received sewage which created a load of approximately 20 pounds of biochemical oxygen demand (B.O.D.) per acre per day. The secondary Cell, lagoon B, was 4.4 acres and had a daily load of approximately 20.6 pounds B.O.D. per acre. Cells C and D located at the University of Missouri Swine Farm were primary and secondary lagoons respectively.

During the summer of 1966 there was a negligible sewage input until August. The shorelines were kept free of overhanging and emergent vegetation during the experimental field studies. Since lagoon C received the highest sewage input it was obvious that this lagoon had a higher B.O.D. than lagoon D. Study lagoon E was located between South Drive and Fleetwood Drive. This 0.5 acre lagoon served 26 residences that produced a daily B.O.D. of 34.0 pounds. The average depth was 42 inches, and the steep banks prevented adequate control of the surrounding vegetation. Therefore, overhanging and emergent vegetation was abundant. Lagoon F was a trailer park lagoon which was located on old Highway 63, south of Columbia. This heavily loaded lagoon produced 64.0 pounds B.O.D. per acre per day. The average depth was 38 inches. The lagoon was poorly located in that leaves dropped into the lagoon from nearby trees and silt was brought in by surface runoff water, thus creating an additional B.O.D. on the lagoon system. Shoreline vegetation was abundant but emergent aquatic vegetation was sparse.

RESULTS AND DISCUSSION

Approximately 1,600 collections were examined during the 2-year period. These collections comprised approximately 7,000 specimens distributed among 22 species and 8 genera. The relative numerical importance of certain species recorded for 1965–1966 is summarized in Table 1.

From the 1965 light trap data, it will be seen that *A. vexans* and the *C. pipiens* complex made up 75.8 percent of the total collections. The third most abundant species was *C. tarsalis*. The 1966 light trap data also revealed that the *C. pipiens* complex and *A. vexans* made up the largest percentage (84.44 percent) of the total collections. Fewer mosquitoes (2,720) were collected during 1966 compared to the large number (4,218) collected from light traps in 1965. *C. tarsalis* were found infrequently in light trap collections throughout the summer and fall of 1966. The seasonal occurrence of adult mosquitoes collected by light traps and bite counts is summarized in Table 2.

Of the 22 mosquito species that were collected, only the following 10 species might occur in sewage lagoons: *A. punctipennis, A. quadrimaculatus, C. territans, C. erraticus, C. pipiens* complex, *C. restuans, C. salinarius, C. tarsalis, C. inornata,* and *U. sapphirina*. For the most part, the breeding habits of these mosquitoes are well known, but various data appearing in later sections of this report will show that certain species, although occurring in the lagoon environment, do not breed in the sewage lagoons.

TABLE 1.—Relative numerical importance of mosquitoes collected in light traps at Columbia, Missouri, 1965–1966.[1]

Species	Total Collected		Percent	
	1965	1966	1965	1966
Aedes vexans	2,308	1,041	54.71	38.27
Culex pipiens complex	868	1,256	20.57	46.17
Culex tarsalis	270	25	6.40	.91
Anopheles punctipennis	177	87	4.19	3.19
Culex erraticus	174	64	4.12	2.35
Anopheles quadrimaculatus	141	166	3.34	6.10
Psorophora confinnis	132	20	3.12	.73
Uranotaenia sapphirina	63	23	1.49	.84
Aedes triseriatus	28	19	.63	.69
Psorophora ciliata	23	1	.54	.03
Aedes nigromaculis	8	2	.18	.07
Orthopodomyia signifera	7	9	.16	.33
Aedes trivittatus	6	1	.14	.03
Mansonia perturbans	6	2	.14	.07
Anopheles barberi	4	3	.09	.11
Psorophora cyanescens	2	0	.04	0
Psorophora ferox	1	1	.02	.03

[1] Unidentified species are not included in this table.

SPECIES COMPOSITION OF WEST LAGOON (B- MUNICIPAL). This lagoon was exposed to a rather extensive mosquito fauna because of its location near heavily wooded areas and an adjacent stream which became quite low during mid-summer and produced large numbers of mosquitoes. The mosquito species recorded by bite counts, stream sampling, and light-trapping are presented in Table 3.

Of the 11 species collected in the lagoon environs, 7 have been recorded from sewage lagoons. In order to ascertain the number and kind of species occurring within the lagoon habitat, ten oviposition blocks were placed around the lagoon shoreline, and five larval dipping stations were selected around the periphery of the lagoon including the overflow drain device.

Although few egg rafts were recovered

TABLE 2.—Seasonal occurrence of adult mosquitoes in Columbia, Missouri, 1965–1966.

Species	May	June	July	Aug.	Sept.	Oct.	Nov.
Aedes dorsalis	x	x	x	x	x		
Aedes nigromaculis				x	x		
Aedes triseriatus	x	x	x	x	x	x	x
Aedes trivittatus		x	x	x	x		
Aedes vexans		x	x	x	x	x	x
Anopheles barberi			x	x	x		
Anopheles punctipennis		x	x	x	x	x	
Anopheles quadrimaculatus			x	x	x	x	
Culex territans		x	x	x	x	x	
Culex erraticus		x	x	x	x	x	
Culex pipiens complex	x	x	x	x	x	x	x
Culex restuans	x	x	x	x	x	x	
Culex salinarius		x	x	x	x	x	
Culex tarsalis				x	x	x	x
Culiseta inornata	x	x			x	x	
Mansonia perturbans	x	x	x	x	x	x	x
Orthopodomyia signifera		x	x	x	x	x	x
Psorophora ciliata		x	x	x	x	x	
Psorophora confinnis		x	x	x	x	x	
Psorophora cyanescens			x	x	x	x	
Psorophora ferox			x	x	x	x	
Uranotaenia sapphirina			x	x	x	x	

from the oviposition blocks during this study, numerous rafts were found routinely in the overflow drain device. Since both objects, i.e., the drain device and the oviposition blocks, offered a stable air-water interface suitable for oviposition, the reasons for the avoidance by night-flying mosquitoes of the oviposition blocks remains unknown. An important reason might be that the drain device, while rigidly anchored within the lagoon waters, offered a protected and more stable air-water interface than the slightly mobile oviposition blocks whose chamber walls were considerably lower than those of the drain device. The oviposition block used in other lagoons seemed to provide suitable sites for egg-laying, thus suggesting

TABLE 3.—List of mosquito species collected from the environs of oxidation lagoons, Columbia, Missouri during 1965–1966.

Species	Lagoon Designation [2]				
	B	C	D	E	F
Aedes canadensis	+	−	−	−	+
Aedes triseriatus	+	−	−	−	+
Aedes trivittatus	+	−	−	−	+
Aedes vexans	+	−	−	−	+
Anopheles punctipennis	+	+	+	+	+
Anopheles quadrimaculatus	+	−	−	−	−
Culex erraticus	+	+	+	+	−
Culex pipiens complex	+	+	+	+	+
Culex salinarius	+	+	+	+	+
Culex tarsalis	+	+	+	+	−
Culiseta inornata	+	+	+	+	+
Psorophora ferox	+	−	−	−	−

[2] B— Municipal lagoon.
C and D— Animal wastes lagoons.
E and F— Residential lagoons.

that the difference in the oviposition response might be caused by sampling error. Sampling error may have occurred because of the small size of the sampling unit in relation to the size of the potential breeding area.

A single oviposition block provides a 1-square foot sampling area or oviposition site. The theoretical area for egg-laying was that part of the lagoon from the shoreline to a distance of 2 feet from the shore extending around the periphery of lagoon B from May through August. Of the egg rafts collected, 27 were *C. tarsalis* and the remaining 216 were identified as those of the *C. pipiens* complex. The peak of oviposition occurred during June and declined thereafter.

The number of mosquito larvae collected from the drain device is presented in Table 5.

The oviposition blocks were less efficient than in the other lagoons, presumably because of certain physical attributes of the

TABLE 4.—Number of egg rafts collected from oxidation lagoons during 1966 at Columbia, Missouri.

Lagoon	Number of Egg Rafts Per Month					
	April	May	June	July	August	Total
B—Municipal[3]	NS[4]	15	173	15	40	243
C—Animal Wastes	35	43	3,580	1,444	1,033	6,135
D—Animal Wastes	0	0	128	213	697	1,038
E—Residential	4	6	41	14	21	86
F—Residential	8	25	51	53	79	216

[3] Egg rafts collected from drain device.
[4] NS=Not sampled.

the lagoon. Thus, an obvious relationship would be a ratio expressed in square feet between the sampling unit and the potential egg-laying area. Failure to consider this apparent relationship may account for the small number of egg rafts obtained from the oviposition blocks from April until the end of August. Egg rafts and mosquito larvae were collected routinely from the drain device during this period. The data obtained from this habitat are shown in Table 4.

Two hundred and forty-three egg rafts were recovered from the drain device in blocks, and sampling error. Of the 11 species found in the lagoon environs, only 2, the *C. pipiens* complex and *C. tarsalis* were breeding in the lagoon and then only in the drain device habitat.

SPECIES COMPOSITION OF THE UNIVERSITY SWINE FARM LAGOON (C- ANIMAL WASTES). This lagoon together with its secondary cell (D- lagoon) produced more mosquitoes than any of the other study lagoons. The mosquito species recorded by bite counts and light traps are presented in Table 3.

The *C. pipiens* complex were the most

TABLE 5.—Number of mosquito larvae sampled from oxidation lagoons at Columbia, Missouri during June–August, 1966.

Lagoon Designation	Number of Larvae					
	Total per Month			Average/Station/Month		
	June	July	August	June	July	August
B	149	52	82	29.8	10.4	16.4
C	176	19,223	42,356	35.2	3844.6	8471.2
D	9	1,419	33,267	1.8	283.8	6653.4
E	142	180	375	28.4	36.0	75.0
F	37	175	421	7.4	35.0	84.2

abundant mosquitoes taken by bite counts and in the light trap from the environs of this lagoon. *C. inornata,* the first species to appear in and around the sewage lagoons, was virtually absent from the lagoon environment during July and August, and occurred in small numbers during September and October.

Although *C. tarsalis* was not taken near the lagoons or nearby swine sheds, several specimens were collected from the adjacent University Poultry Farm. In order to determine which species were breeding in the lagoon, ten oviposition blocks were placed on the water surface close to the shoreline, and five larval dipping stations were selected. The number of egg rafts collected is presented in Table 4 and the number of larvae in Table 5.

A total of 6,135 egg rafts were collected from lagoon C from April until the end of August. Oviposition activity reached its peak during the month of June and gradually declined toward the end of the summer. All of the egg rafts, i.e., 35, collected during April were those of *C. inornata* while the rest of the egg rafts were those of the *C. pipiens* complex. No *C. tarsalis* egg rafts were recovered from this lagoon.

Of the six mosquito species found in the lagoon environs only four, *C. pipiens* complex, *C. salinarius, C. inornata,* and *A. punctipennis* were found in the lagoon. The most abundant species was the *C. pipiens* complex. *C. inornata* was present during late spring and early summer but was virtually absent from mid July through August. *C. salinarius* larvae were infrequently encountered and *A. punctipennis* larvae were collected only once at a single dipping station during July. *C. tarsalis* was not found in the lagoon. Maximum egg raft density occurred during June and larval density was highest during the last half of August.

SPECIES COMPOSITION OF THE UNIVERSITY SWINE FARM LAGOON (D- ANIMAL WASTES). This sewage lagoon was considerably less productive than C- lagoon. The same number of oviposition blocks and larval sampling stations was utilized for ascertaining which species were breeding in the lagoon. The number of egg rafts recovered is presented in Table 4 and the number of larvae in Table 5.

A total of 1,038 egg rafts were collected from April until the end of August. Oviposition activity did not reach a peak until August. The egg rafts were identified as those of the *C. pipiens* complex. No *C. tarsalis* egg rafts were recovered from this lagoon.

Two mosquito species were found in this lagoon, *C. pipiens* complex and *C. inornata.* The *C. pipiens* complex was the dominant species, as in C-lagoon during April, May and June. Egg raft and larval density was highest during the month of August. No sewage input was received by this lagoon until the latter part of July and during the month of August.

SPECIES COMPOSITION OF THE SOUTH DRIVE LAGOON (E-RESIDENTIAL LAGOON). In order to determine what species of mosquitoes were present in the environs of this lagoon, bite counts, larval dipping in the effluent area, and light trapping methods were used. The mosquito species collected are presented in Table 3.

The *C. pipens complex* was the most abundant species collected. *C. inornata* was collected in late spring and early summer and again in late August and early September with no specimens taken during June and July. *C. erraticus* was the second most abundant species collected. This species was taken more often during bite counts than in the light trap. A few *C. tarsalis* adults were collected during August and September.

Ten oviposition blocks were placed close to the shoreline around the periphery of the lagoon so that the oviposition activity of certain species might be studied. The number of egg rafts collected is presented in Table 4.

A total of 86 egg rafts were collected from April until August. Eight egg rafts were *C. inornata* while the remaining 78 were the *C. pipiens* complex. No *C. tar-*

salis egg rafts were recovered from this lagoon.

Five larval dipping stations were selected and the number of mosquito larvae collected is presented in Table 5.

Of the six species found in the lagoon environs only two, the *C. pipiens* complex and *C. inornata,* were found in the lagoon. Egg raft density was highest in June and declined thereafter. Larval density was maximum during the latter part of August and September. No *C. tarsalis* were recovered.

SPECIES COMPOSITION OF WALNUT HILLS TRAILER COURT LAGOON (E-RESIDENTIAL LAGOON). In order to ascertain what mosquito species were present in the lagoon environs, bite counts and light trapping methods were used. The mosquito species collected are presented in Table 3.

The *Aedes* mosquitoes were far more abundant than the *C. pipiens* complex in the environs of this lagoon. *A. canadensis* and *C. inornata* were collected early in May and June. *A. trivittatus* were omnipresent from June through August. No *C. tarsalis* were collected.

Ten oviposition blocks were placed around the edge of the lagoon and five larval dipping stations were selected to determine which species were occurring in the lagoon. The number of egg rafts collected is presented in Table 4 and the number of larvae in Table 5.

A total of 216 egg rafts were collected from April until the end of August. Oviposition activity was erratic. Twenty-three egg rafts were *C. inornata* and the remainder were the *C. pipiens* complex.

Although eight mosquito species were found in the environs of this lagoon, only two, the *C. pipiens* complex and *C. inornata,* were found in the lagoon. Egg raft and larval density was highest during August and September, respectively.

SUMMARY

Of the 22 mosquito species known to occur in Columbia, 12 were found in the lagoon environs. Although 7 of these 12 species were capable of laying their eggs directly on water, only 4 were recovered from the sewage lagoons.

The most abundant species were the *Culex pipiens* complex. This group of mosquitoes was collected consistently from all lagoons from April until September. *Culiseta inornata,* with its characteristic bivoltine cycle, was present in all lagoons except B lagoon. *Culex tarsalis* was infrequently encountered and was recovered only from the drain device of B lagoon during August and September. Additional data showed that this species was never abundant in the lagoon environment, and when encountered, it was not found until the end of summer.

Data obtained from the University Swine Farm Lagoons indicated an apparent biological relationship between the type and amount of organic wastes received by a lagoon and the resulting increase in the density of mosquito egg rafts and larvae. The secondary lagoon, in which this significant change was observed, was unattractive to ovipositing female mosquitoes during April and May. However, with the advent of periodic sewage flow from the primary lagoon, egg raft density increased from 8.5 rafts per day in June to 11.8 per day in July and 49.8 rafts per day in August. Sewage flow into the secondary lagoon was greatest during the latter month. During the months of April, May and part of June, the secondary lagoon received little or no wastes and maintained a high dissolved oxygen content in contrast to the low dissolved oxygen content of the primary lagoon for the same period of time. These data tend to support the theory that water low in dissolved oxygen content is more attractive to ovipositing female mosquitoes than water with high dissolved oxygen content. This theory may be applicable only to the *Culex pipiens* complex, *Culiseta inornata, Culex salinarius, C. tarsalis,* and possibly *C. restuans.*

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AN AGAR GEL DIFFUSION METHOD FOR THE IDENTIFICATION OF MOSQUITO BLOOD-MEALS [1]

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Serological techniques based on the precipitin reaction have been widely used since Nuttall established the importance of the antigen-antibody system for the identification of unknown bloods. Arthropod blood-meals are routinely identified by subjecting a saline extract of the gut contents of an engorged insect to multiple tests with suitable antisera. When used for the identification of mosquito blood-meals, the system is generally conducted either in small diameter test tubes as reviewed by Weitz (1956) or in capillary tubes according to the method described by Tempelis and Lofy (1963). A precipitate formed at the interface between antigen and antiserum constitutes a positive reaction.

The agar gel diffusion technique described by Ouchterlony (1948) involves the diffusion of antigen and antibody through agar gel so that precipitate reactions leave a distinct precipitate band in the gel which is easily read even many days after the test has been conducted. The method is routinely used in systematic serology but until recently has not been applied to the identification of arthropod blood-meals. Chamberlain and Sudia (1967) describe an agar gel method applicable to the identification of mosquito blood-meals and cite some of the advantages of the technique.

The method described herein differs in many respects from techniques used by other workers. This system has been used to identify more than 10,000 mosquito blood-meals in our laboratory and has proven most satisfactory for this purpose.

MATERIALS AND METHODS. The agar preparation used in these tests consists of

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers—The State University of New Jersey, New Brunswick, N. J. 08903.