most species are not equally distributed throughout the forest but tend to be more concentrated in flyways associated with the roads; (3) in studies of potential attractants, traps should be at least 0.3 mile apart to prevent undue trap competition; (4) collection periods of at least 6 hrs. appear feasible for studies of tabanid attractants.

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A TWO-YEAR SURVEY TO DETERMINE THE INCIDENCE OF A MERMITHID NEMATODE IN MOSQUITOES IN LOUISIANA

J. J. PETERSEN AND O. R. WILLIS [1]

Entomology Research Division, Agr. Res. Serv., U. S. Department of Agriculture
Lake Charles, Louisiana 70601

ABSTRACT. When a 27-month survey was made to determine the activity of the mermithid *Reesimermis nielseni* Tsai and Grundmann in mosquito larvae in Louisiana, 13 of the 19 species of mosquitoes present proved to be hosts, and 52 percent of the *Anopheles crucians* Wiedemann, 37 percent of the *Culex erraticus* (Dyar and Knab), and 30 percent of the *Uranotaenia sapphirina* (Osten-Sacken) were parasitized. In southwestern Louisiana, the parasite was active from April to November when mean water temperatures were above 65° F. Low densities of host, loss of water from the habitats, and physical and chemical changes in the habitats were factors reducing the incidence of parasitism. Thus, the number of mosquitoes of any species captured and the level of infection varied greatly, both for a given species and between species.

Numerous authors have reported the incidence of mermithid nematodes in mosquitoes, but most of these reports have been limited to one or at most to a very few observations. These studies generally indicated that when mermithids were present the incidence of parasitism was high, but no long-term observations have been made to determine fluctuations in parasite activity or in the long-term incidence of parasitism.

An earlier study of the bionomics of *Reesimermis nielseni* Tsai and Grundmann in Louisiana included periodic observations of the incidence of parasitism in several species of mosquitoes and reported natural parasitism in 13 species (Petersen *et al.*, 1968). Also, studies with *R. nielseni* were made in the laboratory to determine the potential of various species of mosquitoes as hosts (Petersen *et al.*, 1969) and the effects of the ratio of nematodes to hosts, volume and salinity of the water, and age of the larvae on the incidence of parasitism (Petersen and Willis, 1970).

The present study was made at the Gulf Coast Marsh and Rice Field Mosquito Investigations Laboratory, Lake Charles, Louisiana from October 1968 to January 1971 to determine the effects of environmental factors on the incidence of parasitism by *R. nielseni* in mosquitoes; to measure the yearly fluctuations in parasite activity; and the effectiveness of species of mosquitoes as natural hosts of this parasite. This mermithid (previously reported as an undescribed species of *Romanomermis*) has been tentatively identified as

[1] In cooperation with McNeese State University, Lake Charles, Louisiana 70601.

Reesimermis nielseni by Dr. William R. Nickle, Plant Science Research Division, ARS, U. S. Department of Agriculture, Beltsville, Maryland and was originally described from Wyoming mosquitoes (Tsai and Grundmann 1969).

MATERIALS AND METHODS. Five habitats known to produce mosquitoes parasitized by *R. nielseni* were surveyed once a week for 27 months. Larval populations were sampled by standard dipping procedures, and the identifications and incidence of parasitism were determined by examination in the laboratory. Maximum-minimum temperatures, depth and conductivity of the water, and rainfall were also recorded.

HABITATS. The C-1 site is a shallow, grassy, semipermanent pond (about 15 x 20 ft.). Cover consists of a mixture of pasture grasses, and aquatic vegetation. The pond remains flooded throughout the winter and sometimes well into the summer and may hold water for several weeks after a summer rain. The salinity of the water was low and averaged 47 (25–80) micromhos per cm. Water temperature ranged from 38° to 101° F. Although the site is in a semiwooded area consisting mostly of pine trees, it is exposed to direct sunlight. Large numbers of aquatic predators of mosquito larvae were generally present in the pond.

The C-2 site, which is about 4 miles from the C-1 site, is similar to C-1 in most respects but is larger (25 x 35 ft.), holds water for longer periods, and has a heavy growth of aquatic vegetation. Temperatures ranged from 39° to 96° F., and the salinity of the water averaged 54 (25–88) micromhos per cm.

The HM-1 site is also located in a wooded area of predominantly pines and is a circular depression about 25 feet in diameter, which is semipermanent; it dries up only after prolonged summer drought. The depth of the water varies, but the maximum is about 4 feet in depth; the salinity averaged 113 (52–320) micromhos per cm. The pond is exposed to direct sunlight, and water temperatures ranged from 40° to 100° F.

The HM-2 site is about 200 yards from site HM-1 and is a shallow pond (about 75 x 200 ft.) that is heavily vegetated and semipermanent. The salinity averaged 52 (20–111) micromhos per cm, and temperatures ranged from 40° to 98° F. Throughout most of the study, the site contained a large population of *Gambusia affinnis* (Baird and Girard) and other minnows.

Site HM-3 is about 1.5 miles from HM-1, is heavily wooded, and is part of a larger swampy area. The salinity varied widely during the study because of nearby oil drilling operations; the average was 453 (132–1800) micromhos per cm. This site dried up periodically during the summer, and sampling was further hampered by fish, construction, and leaking oil pipes. Temperatures varied similarly with the other sites, and ranged from 40° to 96° F.

RESULTS. MOSQUITO SPECIES: *Anopheles* species—*Anopheles crucians* Wiedemann was the second most abundant species collected and occurred all year round in all five sites (sampled 545 times). A total of 29 percent of 4481 *A. crucians* contained parasites but mean parasite activity (incidence of parasitism) at the individual sites ranged from 8 percent in HM-1 to 42 percent in C-1 and C-2. The highest monthly mean incidence (80 percent) occurred in October 1969 and 1970; the lowest incidence occurred in February and March 1969 and 1970: only one of 820 larvae was parasitized (Fig. 1). The activity of *R. nielseni* peaked twice in both 1969 and 1970; both years the first peak occurred during April and May and the second during October.

Anopheles quadrimaculatus Say was collected from all five sites and during all seasons but was much less abundant than *A. crucians*. Also, only 5 percent of the 764 larvae collected were infected. Parasitism ranged from 1 percent in HM-3 to 12 percent in C-1. Many of the infected *A. quadrimaculatus* exhibited host resistance to the parasite (melanized parasites), and this resistance probably accounted for the low incidence of parasitism.

Anopheles punctipennis (Say) was the

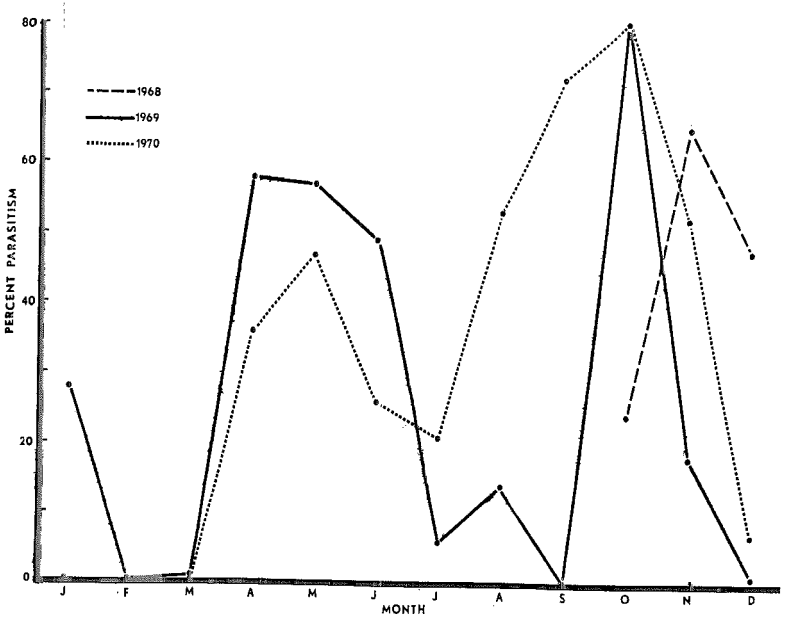

FIG. 1.—Mean monthly incidence of *Reesimermis nielseni* in all larval collections of *Anopheles crucians* (October 1968–January 1971).

only other anopheline species collected, but the very low numbers (3) prevented any meaningful conclusions except that it was susceptible to *R. nielseni*.

Aedes species—Six *Aedes* species, *A. atlanticus* Dyar and Knab, *A. canadensis* (Theobald), *A. mitchellae* (Dyar), *A. sollicitans* (Walker), *A. taeniorhynchus* (Wiedemann) and *A. vexans* (Meigen) were collected, but only *A. atlanticus* and *A. vexans* in large enough numbers to permit comparisons. *Aedes atlanticus* was collected from four of the five sites, but 90 percent of the 654 larvae were collected from C-2. Parasitism ranged from zero percent in HM-2 to 20 percent in C-2; the mean for all *A. atlanticus* was 19 percent. *Aedes vexans* was collected in good numbers from all five sites, but parasitism was low: 1 percent were infected in C-1, C-2, and HM-2 and none in HM-1 and HM-3. Thus only 0.4 percent of the 1206 *A. vexans* contained *R. nielseni*. A majority of the *A. vexans* were collected during the winter months when parasite activity was generally lowest, but sufficient numbers were collected during periods when activity was high to indicate that in nature this host species is somewhat resistant to the parasite. However, host-resistance in the form of the melanized parasites seen in *Anopheles quadrimaculatus* was not observed in *Aedes vexans*.

Seventeen or fewer larvae of *A. canadensis, A. mitchellae,* and *A. taeniorhynchus* were collected, and none were parasitized. However, 2 of 53 *A. sollicitans* were infected, the first record of *R. nielseni* infecting *A. sollicitans* in nature, though an earlier laboratory study showed this species to be susceptible (Petersen *et al.,* 1969).

Culiseta species—*Culiseta inornata* (Williston) was collected 569 times from four sites; 0.7 percent were infected with *R. nielseni*. This low incidence of parasitism

can undoubtedly be attributed to the fact that *C. inornata* occurred only during the periods of low parasite activity.

Culex species—*Culex erraticus* (Dyar and Knab) had the second highest incidence of parasitism of the 19 species collected: 37 percent of 218 larvae from three sites were infected, and parasitism ranged from 28 percent in HM-1 to 100 percent (one larva only) in C-2. *Culex restuans* Theobald averaged 0.3 percent parasitism but, like *Culiseta inornata,* was found only during the periods of low parasite activity. *Culex salinarius* Coquillett and *C. territans* Walker were parasite free. The majority of *C. salinarius* were collected from HM-3 during the later part of the study when parasite activity was low at that site; however, the species does appear to be resistant to *R. nielseni. Culex territans* was the most abundant of the 19 species and occurred commonly at all five sites throughout all seasons. However, none of the 4619 larvae were parasitized by the nematode. In laboratory tests, we also failed to produce parasitism by *R. nielseni* in *C. territans* (Petersen *et al.,* 1969).

Psorophora species—*Psorophora ciliata* (F.) occurred in low numbers at all five sites, but infected larvae were collected only from C-2 (7 percent parasitism). *Psorophora confinnis* (Lynch-Arribálzaga) were collected in adequate numbers, and infected larvae, though only a few, were taken from all five sites. Parasitism ranged from 1 percent in HM-3 to 27 percent in HM-2 and averaged 7 percent for the 1638 larvae collected. Only eight larvae of *P. ferox* (Humboldt) were collected, and none were infected with *R. nielseni;* this host was found to be resistant to the parasite in the laboratory (Petersen *et al.,* 1969).

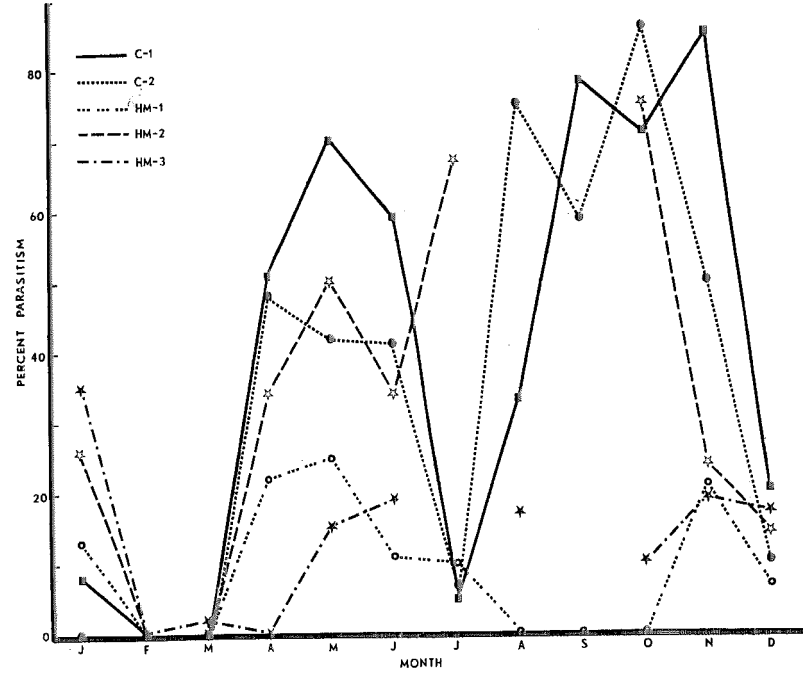

FIG. 2.—Comparison of the mean monthly incidence of *Reesimermis nielseni* in larval collections of *Anopheles crucians* for five sites (October 1968–January 1971).

Uranotaenia species—Two of the only three *U. lowii* Theobald collected (from C-1) contained *R. nielseni*. *Uranotaenia sapphirina* (Osten-Sacken) was the third most abundant species collected (2175) during the study and was found in large numbers at all sites except HM-2. However, parasitism was low in HM-3, an average of 7 percent compared with a mean of 45 percent for C-1 and C-2 and with 30 percent for all *U. sapphirina*.

TEMPERATURE. Both *Uranotaenia sapphirina* and *Anopheles crucians* were collected in large numbers and had a high incidence of parasitism, but only *Anopheles crucians* occurred throughout the winter. As a result, the effects of temperature on parasite activity could be determined only for *Anopheles crucians*. Figure 2 shows the mean monthly parasitism by *Anopheles crucians* from each of the five sites. Infected larvae were collected from four sites during January, but no activity was observed in any sites during February, and only one of 301 larvae contained nematodes in March. With the onset of warm weather during April, four sites showed renewed activity of *R. nielseni;* the fifth, HM-3, showed no activity, but only 9 *Anopheles crucians* were collected at HM-3 during April of both years. Parasite activity generally increased at all sites during May, decreased sharply during June and July, and trends could not be determined during August, September, and October at HM-1, HM-2, and HM-3 because of insufficient hosts; however, the percentage parasitized increased sharply during August at C-1, and C-2 and remained high there through November. In December, activity decreased at all sites.

The mean monthly parasitism of *Anopheles crucians* for October–December 1968 and for all of 1969 and 1970 is compared in Figures 3 and 4 for sites C-1 and C-2, respectively. At C-1 the trend was similar during the first 6 months of both years, activity commenced during April, peaked in May, and dropped sharply in July. No comparison could be made for August through November because C-1 lacked water in 1969. At C-2 the activity of *R. nielseni* (Fig. 4) began during April, remained high through June (the first peak of activity occurred in June 1969 and in May 1970), and then dropped sharply during July as in C-1, increased sharply during August 1970, and remained high through October. However, in 1969 larvae were not collected during August, and only two were collected during September; then as mosquito breeding increased in October, 80 percent were parasitized. Thereafter the activity of *R. nielseni* decreased steadily during November and December at both sites with the onset of cool weather. Thus, temperature appeared to be the major factor causing *R. nielseni* to resume activity in the spring in southwestern Louisiana.

Maximum and minimum water temperatures were recorded once a week at the five sites during the spring of 1969 and 1970, but 7 of the 10 series of readings could not be compared with the resumption of parasite activity because of the lack of larvae at critical times. When sufficient numbers of *Anopheles crucians* were present, the weekly minimum and maximum water temperatures did not exceed 57° or 78° F., respectively, at any time prior to the onset of parasite activity (Table 1). During the week of first parasite activity, the low water temperature was 61° F., and the high temperature ranged from 76° to 84° F. When minimum temperatures remained above 60° F., parasite activity resumed and this temperature appears to be the approximate threshold. However, one infected larva was collected in C-2 when weekly temperatures ranged from 49° to 81° F., and one was collected from HM-3 during early March when the weekly temperatures ranged from 46° to 63° F. Therefore, prolonged water temperatures above about 65° F. may be needed rather than specific low or high temperatures to stimulate the resumption of activity of the nematode.

The mean daily air temperatures for the 3 weeks prior to the resumption of nematode activity during 1969 were 53°, 61°, and 59° F.; the air temperature was 69° F.

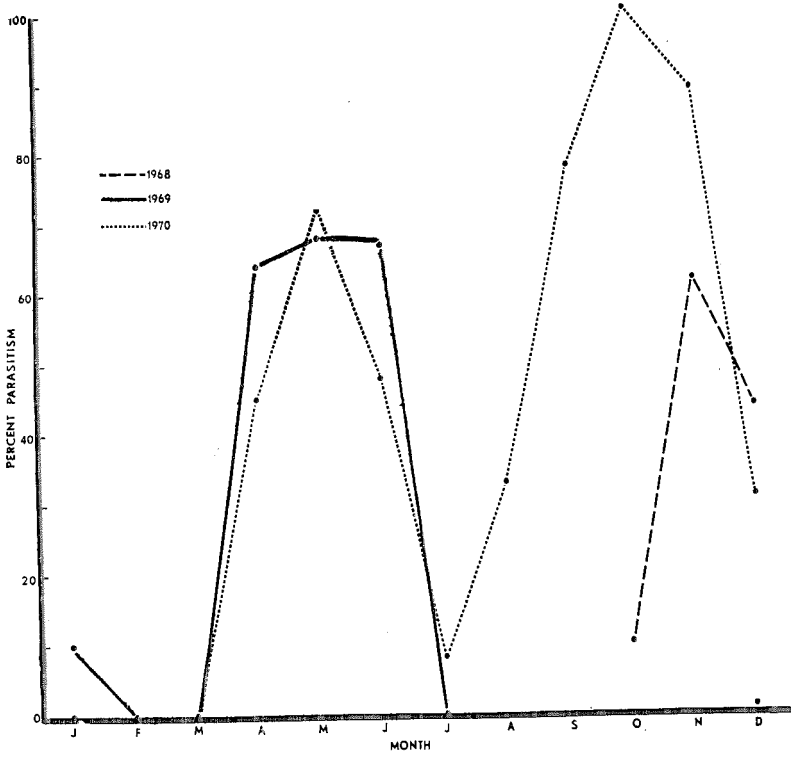

FIG. 3.—Comparison of the incidence of *Reesimermis nielseni* in larval populations of *Anopheles crucians* for 1968 (October–December), 1969, and 1970 at the C-1 site.

during the week of first parasite activity at sites C-1 and HM-1. The relationship between the mean monthly air temperature (U. S. Weather Bureau, Lake Charles, Louisiana) and the average monthly percentage parasitism of *Anopheles crucians* is shown in Figure 5. Parasite activity resumed when the air temperature was above about 60° F. and decreased rapidly when temperatures dropped below about 55° F. Thus, the 28 percent parasitism shown for January 1969 (Fig. 1) is misleading; it was an actual decrease from the 47 percent parasitism during December 1968. Moreover, the parasitized larvae collected in late December 1968 and January 1969 appeared to have been infected earlier, and they persisted in the environment because of the cold temperatures. Parasitized larvae were not collected during January 1970.

Since the activity of *R. nielseni* is curtailed during cold weather, the incidence of parasitism in *Anopheles crucians* was much higher than the yearly average during the months of parasite activity: from April to November, for 1969 and 1970 parasitism ranged from 16 percent in HM-3 to 70 percent in C-1 and averaged 52 percent at all five sites. Also, 43 percent of the *Culiseta inornata,* which is a winter species in southwestern Louisiana, that were collected before December were infected compared with only 0.7 percent of all *Culiseta inornata* collected. The other species were either active only in

TABLE 1.—The relationship between minimum and maximum water temperatures and the beginning of the spring activity of *Reesimermis nielseni* in *Anopheles crucians*.

	Weekly temperatures (° F.) at								
	Site C-1 (1969)			Site HM-1 (1969)			Site C-1 (1970)		
Week[1]	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum
−3	54	57	60	50	57	64	47	56.5	76
−2	53	60.5	68	54	63.5	73	49	63.5	78
−1	49	61	73	57	65	73	54	66	78
0	61	68.5	76	66	72	78	67	75.5	84
+1	64	70	84	66	69.5	73	56	71	86
+2	60	68	76	63	67.5	72	65	77.5	88

[1] — and + denote the number of weeks before or after the resumption of *R. nielseni* activity at the site.

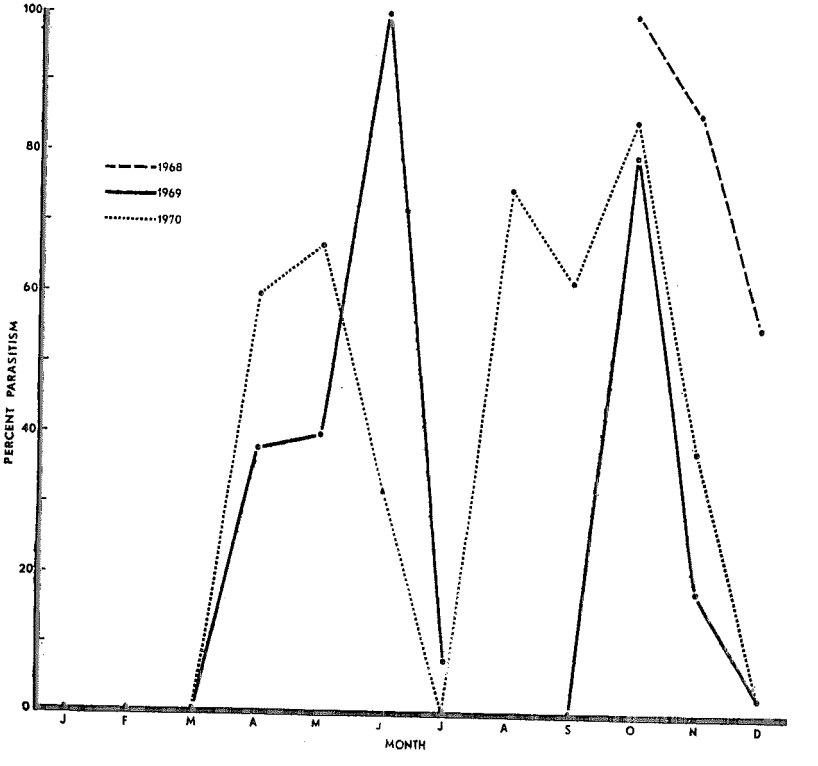

FIG. 4.—Comparison of incidence of *Reesimermis nielseni* in larval populations of *Anopheles crucians* for 1968 (October–December), 1969, and 1970 at the C-2 site.

summer or winter or were collected in numbers too low to show any significant difference in parasitism by season.

HOST DENSITY AND WATER LEVEL FLUCTUATIONS. The reduction in the activity of *R. nielseni* between June and August (Fig. 1–5) appeared to be related to host densities. The lowest monthly collections of *Anopheles crucians* (62) occurred during July (1969 and 1970), the same period that saw the lowest monthly incidence of parasitism by *R. nielseni* for the summer (Fig. 5). Also, the monthly collections during the summer in habitats that yielded fewer than 100 *Anopheles crucians* had a mean incidence of parasitism of 30 percent compared with 37 percent and 56 percent for sites producing 100–200 and 200 or more *Anopheles crucians,* respectively. However, this mid-summer reduction in parasite activity may also have resulted, wholly or in part, from the availability of water in the sites since volume of water directly affected the numbers of *Anopheles crucians.*

During the study, all five sites dried at least once during the summer; then, on several occasions, the water persisted several weeks after reflooding, which permitted a buildup of *Anopheles crucians.* On these occasions, the sites generally produced *Psorophora confinnis* when they reflooded, and some parasite activity was always recorded in this species. However, only 8 percent of the 611 *P. confinnis* collected contained *R. nielseni.* Then the following week, a few *Anopheles crucians* reappeared in the sites though none contained nematodes. The mean incidence of parasitism for these sites increased to 23 percent by the third week and 79 percent by the fourth week. Thus the parasites appear to need time to build up the number of hatching preparasitic juveniles

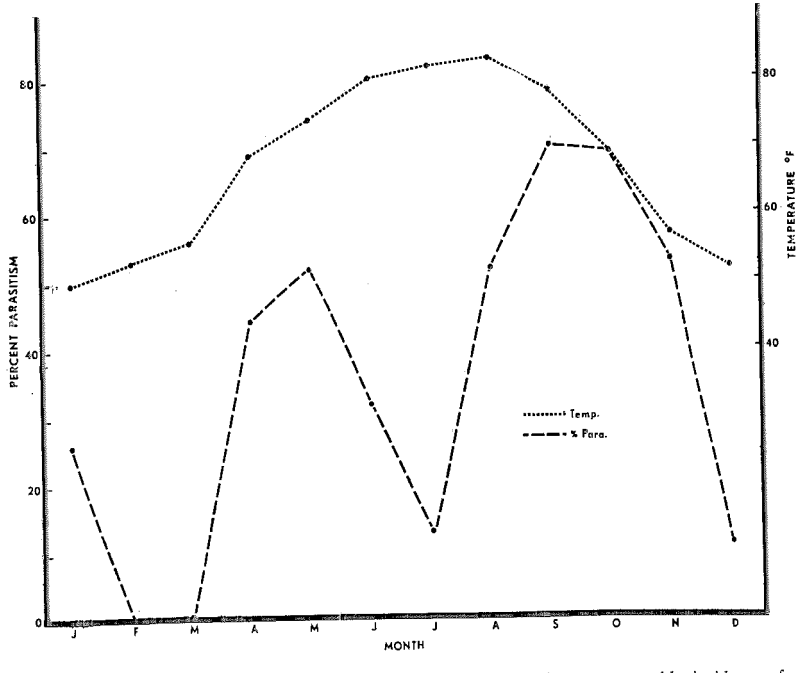

FIG. 5.—Comparison of the mean monthly air temperature and the mean monthly incidence of *Reesimermis nielseni* in larval populations of *Anopheles crucians* during 1969 and 1970.

after a period of drought, which would partly explain the low incidence of parasitism observed when populations of *Anopheles crucians* were low.

Weekly changes in the depth of the water in each site were recorded, but no correlation could be found between changes in the level of water (except for complete drying) and the activity of *R. nielseni.*

DISCUSSION. The data suggest that temperature, periods of drought, and host densities influence the activity of *R. nielseni.* Also, predation of the host and physical and chemical changes in the environment undoubtedly influence the extent of parasitism. The influence of predation is difficult to measure as all five sites had an abundance of predacious aquatic insects and three of the sites contained G. *affinnis* and other top minnows from time to time. However, these fish did not appear to influence the incidence of parasitism by *R. nielseni* in *Anopheles crucians:* although the fish were present in C-2 during August–October 1970, 76 percent of the *Anopheles crucians* contained nematodes; also, HM-1, the site with the lowest overall incidence of parasitism (8 percent) was devoid of fish (top minnows did prevent the sampling of many of the other mosquito species). In addition, physical and chemical factors influenced parasitism markedly at the HM-3 site: parasitism was relatively high during the fall of 1968 (50 percent in December), but just before parasite activity resumed in April 1969, leaking oil pipes, coupled with drought, reconstruction of a road, and the placement of a utility pole in the site upset the ecology and reduced the incidence of parasitism in *Anopheles crucians* to 3 percent for 1969 and 1970.

Reesimermis nielseni is therefore effective in reducing populations of mosquitoes, especially the permanent water breeders (*Anopheles, Uranotaenia,* and some *Culex*). During April–November, 52 percent of all *Anopheles crucians* and 70 percent of those from one site contained *R. nielseni.* This parasite appears to be well adapted to sites that dry up periodically and to those that are more permanently flooded. Also, this nematode tolerates a wide range of ecological changes. Once *R. nielseni* is established in a new habitat, it will apparently persist for long periods and give continuous partial control of many mosquito species.

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Course Announcement

Center for Disease Control, Atlanta, Georgia 30333
announces course in

COLLECTING, CULTURING AND CLASSIFYING MOSQUITOES

to be given March 6–17, 1971 at the Center for Disease Control in Atlanta;
a course in mosquito identification with instruction and practice in collecting and culturing.