Development and Fecundity of *Reesimermis nielseni*, a Nematode Parasite of Mosquitoes[1]

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Abstract: Maturation of the mermithid nematode *Reesimermis nielseni* to the adult stage began by the tenth day after emergence of the nematodes from their hosts at ambient temperatures (24-27 C). Most postparasitic males and females reached the adult stage after 50 and 70 days, respectively. The first females exhibiting egg development and oviposition were observed 25-30 days after emergence, but some oviposition was still taking place 150 days later. *Reesimermis nielseni* laid an average 2,480 eggs per female over an 18-day oviposition period. A majority of the mature eggs hatched within 7 h after the cultures were flooded. The preparasites are short-lived, and only a few were able to infect exposed hosts after 72 h. *Key Words:* Biological control, oviposition, postparasites, life cycle, hatching.

Reesimermis nielseni Tsai and Grundmann, a promising biological control agent of larval mosquitoes, has been reproduced in vivo in the laboratory (3) and successfully used for mosquito control in the field (2, 4, 5). Although host-parasite relationships and factors influencing parasitism have been studied extensively, little work has been done on the biology of this parasite. These studies were conducted to determine the time required for maturation of postparasites and adults, the hatching time, the survival and infectivity of preparasites, and the duration of oviposition and fecundity of *R. nielseni* mass-reared in the laboratory.

MATERIALS AND METHODS

The maturation time of the postparasite and adult stages was measured by microscopically examining random samples of 100 male and 100 female nematodes of *R. nielseni* from newly established cultures every 10 days. These cultures were grown in aluminum cake pans (22 × 33 × 5 cm) containing 15 g (wet weight) of newly emerged postparasitic larvae in moist sterile sand (3). Every 10 days the stage of development of the larvae were determined under a dissecting microscope. The males were then segregated into postparasitic juveniles, mature adults, and spent adults; the females were segregated into postparasitic juveniles, mature adults, adults exhibiting egg development and oviposition, and spent adults. At 9 wk, the cultures were flooded and counts made of hatched preparasites. They were reflooded and counts were made every 3 wk thereafter in an attempt to correlate the number of preparasites with nematode maturation and oviposition determined by the sampling procedure.

Fecundity and duration of oviposition were determined by placing individual gravid females in cells of spot plates containing water. If a female laid eggs within 24 h after isolation, it was discarded on the chance that oviposition had commenced before isolation. The remaining females were transferred every 24 h to clean cells, and the eggs that were recovered from the used cell were counted under a dissecting microscope. This procedure was continued until the females died or became spent; since mermithids do not feed after emergence from a host, a female was determined to be spent when the trophosome (stored food reserves) was consumed in developing eggs. Females were discarded if they died before completion of oviposition unless oviposition was essentially complete at the time of death as determined by the amount of trophosome remaining.

The hatching rate of preparasites was measured by flooding a sand culture of *R. nielseni* that contained unhatched eggs with 1 liter of chlorine-free water. The numbers of hatched preparasites were then counted by removing samples from the culture 0.5, 1-7, and 24 h later. Counts were made of diluted samples by procedures described earlier (3), and the mean for three such counts was used as the number of parasites that hatched within the specific time period. Hatch rates were replicated 10 times with cultures that were 11-19 wk old and were flooded for the first time.

Survival and infectivity of the preparasitic stage was determined by decanting about 800 ml of water containing newly hatched preparasites from sand cultures of *R. nielseni* [22 × 33 × 5 cm aluminum cake pans containing 15 g (wet wt) of nematodes] and allowing the water to stand for 30 min to

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permit separation of the free-swimming preparasites from any suspended eggs by allowing the eggs to settle out. Then a volume of about 500-600 ml of water was carefully aspirated off. This procedure was run twice to reduce the chances of leaving unhatched eggs in the water. The number of preparasites per ml of water was determined. Aliquants containing approximately 1,500 8-h-old nematodes were then added to each of three beakers containing 100 first-instar *Culex pipiens quinquefasciatus* Say in 50 ml of water. This procedure was repeated for 24- and 48-h-old preparasites. Aliquants were doubled for the 72-h-old preparasitic nematodes and increased 10-fold for 96- and 120-h-old nematodes. Host larvae were transferred to rearing containers 24 h after exposure to each of the six age groups of preparasites and held until the extent of parasitism was determined by microscopic examination for the developing nematodes. The incidence of parasitism was determined from the mean of the three exposures. Counts were made of the number of actively swimming preparasites in the original source of inoculum at 24-h intervals during the test. The preparasitic survival test was replicated three times. All four tests were conducted in the laboratory at ambient temperatures (24-27 C).

RESULTS AND DISCUSSION

Maturation of postparasites and adults: Previously, maturation time of *R. nielseni* in mass culture had been determined indirectly by flooding cultures at various intervals and measuring increases and decreases in egg hatch. Such cultures generally produced some hatches after 7-8 wk, but the maximum hatches occurred after the cultures were 11-16 wk old. Also, substantial hatches were obtained from cultures up to 20 wk, and small hatches occurred in some after 34 wk, and after six floodings (3).

In the present study, developmental periods varied somewhat from culture to culture, but molting of both sexes to the adult stage generally started before the tenth day after emergence of the nematodes from their hosts. Males matured more rapidly as a group than females with about 10 percent of the males reaching the adult stage after 10 days and essentially the entire population reaching the adult stage after 50 days (Fig. 1). Also, the males persisted for a protracted period since 70-75 percent were still active 180 days after emergence. Females of *R. nielseni* generally lagged about 10-14 days behind males, but essentially all reached the adult stage after 70 days. The first females which exhibited egg development and oviposition were observed about 25-30 days after emergence. Thereafter, maturation to this stage continued at a steady rate of about 1 percent of the population per day. After about 140 days, most females had begun developing eggs; however, 10-20 percent still had not started to oviposit after 180 days. About 75 percent of the total hatch occurred during the three floodings between the 12th and 18th wk after emergence of the nematodes. However, other cultures not used in the study, but handled in a similar manner during the same period, produced substantial hatches as early as 8 wk and as late as 30 wk following emergence from hosts.

Duration of oviposition and fecundity of R. nielseni: In the papers that include discussions of the life cycle of aquatic mermithids, little mention is made of fecundity or duration of oviposition. For example, Petersen et al. (1) reported that *Perutilimermis culicis* (*Agamomermis culicis*) laid thousands of eggs in a 2- to 4-day period, and Petersen and Willis (3) reported indirectly that *R. nielseni* laid about 2,500 eggs.

The fecundity of 62 *R. nielseni* was observed in three trials; however, 14 of the females were discarded because of early oviposition or mortality (Fig. 2). Individual females laid from 1,388 to 4,431 eggs and averaged 2,480 eggs over an 18-(8-27) day period. Peak oviposition generally occurred the fourth or fifth day and averaged 312 (135-582) eggs; however, with some females it occurred as early as the first day and as late as the tenth day. After about the fifth day, the number of eggs laid declined steadily until oviposition ceased on about the eighteenth day. Thus, 50 percent of the eggs were laid in the first 6 days of oviposition, and 90 percent in the first 12 days.

Hatching rate of preparasites: In previous studies, cultures were generally flooded 16-24 h before the preparasites were needed so there would be sufficient time for a majority of the mature eggs to hatch (3). The present study was made to measure the rate of hatch and the time required for a majority of the mature eggs in a mass culture to hatch.

The 10 cultures used produced an average

2.96 (0.5 to 6.2) $\times 10^6$ preparasites. Maximum hatch generally required about 7 h; however, on one occasion the maximum hatch occurred after 6 h and on two occasions 24 h after flooding (Fig. 3). The hatching rate was essentially linear for the first 3-4 h with the number of preparasites increasing by about 22 percent per hour. Thus, about 90 percent of the hatch had occurred after 5 h, and essentially 100 percent had occurred after 7 h. Twenty-four hours after flooding the number of living parasites had declined about 15 percent. Therefore, 5 or more hours should be allowed between the flooding of a culture and the expected use of the preparasites if maximum use is to be made of the cultures.

Survival of preparasites: Although a number of authors have reported that the preparasitic stage of aquatic mermithids is short, generally 1-3 days (6, 7, 8, 9), little quantitative data concerning the duration of this stage are available. Thus, tests were made to measure both the rate of loss of infectivity and the increase in mortality of the preparasitic stage of *R. nielseni*.

Figure 4 shows that for the first 24 h after hatching both the number of active nematodes and infectivity of the preparasite decreased at a similar rate, about 15 percent. However, after 24 h, infectivity decreased more rapidly than the numbers of swimming preparasites. Thus infectivity of *R. nielseni* held at ambient temperature appears to decline very rapidly after the first 24 h. Little infectivity remains after 72 h, although nearly 50 percent of the preparasite population may be still actively swimming after 72 h. Apparently, they had weakened and lost their infectivity.

The information obtained in the study

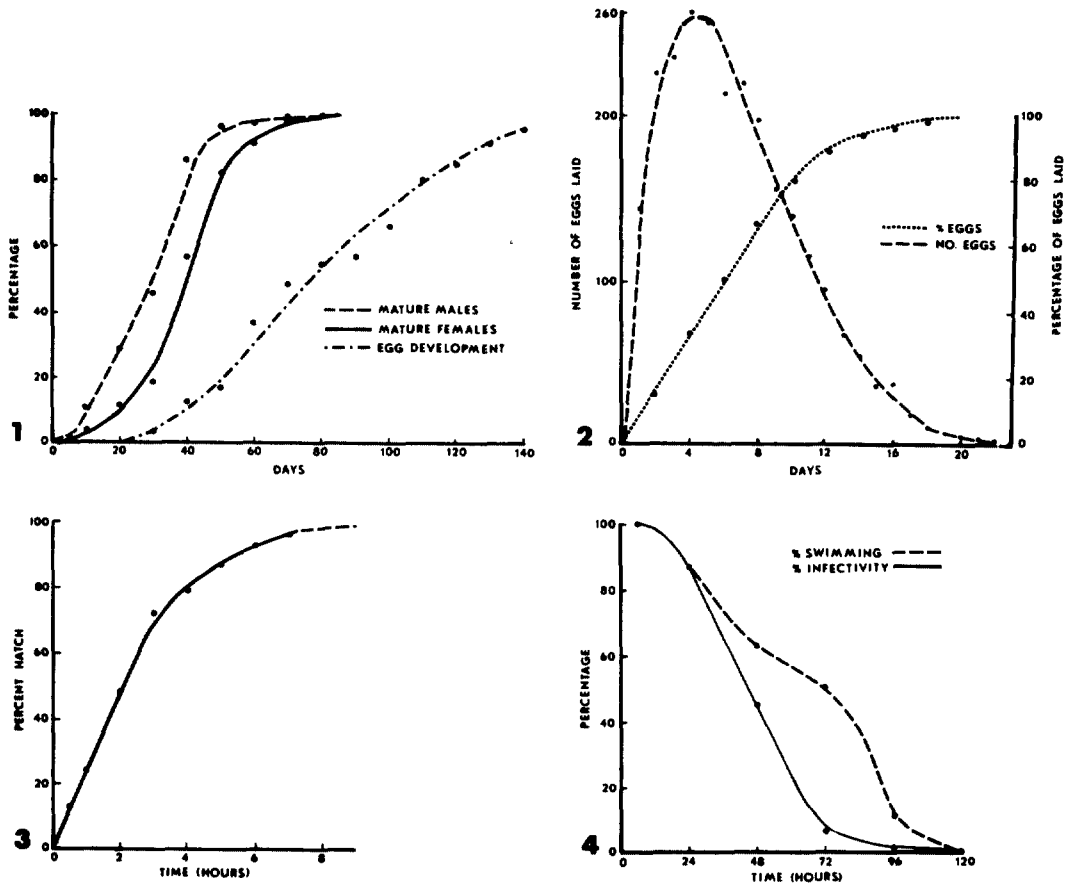

FIG. 1-4. Development and fecundity of *Reesimermis nielseni* at ambient temperatures. 1) Time required for postparasites to mature to the adult stage, for the development of eggs, and for oviposition to begin. 2) Mean daily pattern of oviposition and cumulative oviposition. 3) Mean time required to hatch eggs. 4) Duration of infectivity (parasitism of exposed hosts) and survival time of preparasites.

provides a basis for future studies of the influence of temperature on culturing procedures. A better understanding of the effects of time and temperature should result in more efficient production and longer survival of the nematodes and their eggs. Also, data are now available for a species of aquatic mermithid so other species can be compared.

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