

Effect of Storage at Low Temperatures on Development and Survival of Postparasitic Juveniles of *Romanomermis culicivorax* (Nematoda: Mermithidae)

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Abstract: Development of postparasites and adult females of *Romanomermis culicivorax* Ross and Smith, 1976 may be retarded by keeping cultures at 10 or 15 C for 15 wk. Storage at 5 C resulted in high mortality of postparasites. A storage temperature of 10 C is suitable if development of postparasites, young adults, and gravid females is to be greatly retarded. None of the females were gravid when postparasites (1–6 days old) were kept at 10 C for 15 wk. Development resumed after the cultures were returned to 26 C and no significant mortality occurred. A storage temperature of 15 C is suitable if development is to be only moderately retarded. After a 15-wk storage period, only a few weeks were required to return the cultures to full production status. **Key words:** *Romanomermis culicivorax*, low temperature storage, mosquito parasite.

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Romanomermis culicivorax Ross and Smith, 1976 is a promising biological control agent and can be readily mass produced (10,13). It has been used to control different species of mosquitoes in a variety of field experiments throughout the world (3,8,9, 11,16). Petersen and Chapman (7) reported 17 mosquito species as natural hosts of this mermithid and 33 species of mosquitoes exposed by field applications. If the mermithid becomes established it may continue to control mosquitoes for an indefinite period (12).

The use of *R. culicivorax* in mosquito control programs requires vast numbers of preparasites. This can be achieved by adjusting production of postparasites to precede a field release by 11–12 wk (13).

To date there has been no practical way to store the mermithid culture beyond the time required to complete the life cycle. Petersen (5) reported total yields of preparasites (up to six floodings at 3–4-wk intervals) to be highest when cultures were flooded for the first time at 8–10 wk of age. A negative regression in preparasite yield/week was observed with later floodings and no parasites were obtained from cultures 36 wk of age. Galloway (2) also reported a decreased first-flooding hatch after the cultures reached 10 wk of age; the hatch de-

creased rapidly thereafter to only 10% of the 10-wk level after 23 wk.

Storage of one or more stages of the mermithid culture, even for a few months, would greatly facilitate the application and commercial use of this organism in mosquito control. We report here the effect of constant temperatures (5, 10, 15, and 20 C) on postparasites and adult mermithids over a period of 15 wk.

MATERIALS AND METHODS

The *R. culicivorax* colony was originally obtained from Dr. J. J. Petersen, Gulf Coast Mosquito Research Laboratory, United States Department of Agriculture, Lake Charles, Louisiana, and maintained in our laboratory since 1973. Rearing methods were similar to those described by Petersen and Willis (10). About 1,800 preparasites were exposed to 300 one-day-old larvae of *Culex quinquefasciatus* Say (6:1 ratio) in a plastic pan, 33 × 23 × 6 cm, containing 1 liter of chlorine-free tap water. The larvae were reared at 27 ± 1 C and a photoperiod of 16 L:8 D. Larvae were fed liver powder daily. After 5–6 days, all mosquito larvae were placed in a nematode-collecting tray and emerged postparasites were removed at four selected intervals. Postparasites were sexed using the characters described by Tsai and Grundmann (15).

In order to test survival of postparasites and adult nematodes, and the gravid state of females, we placed postparasites at constant temperatures of 5, 10, 15, and 20 C for 15 wk.

Since postparasites take a minimum of

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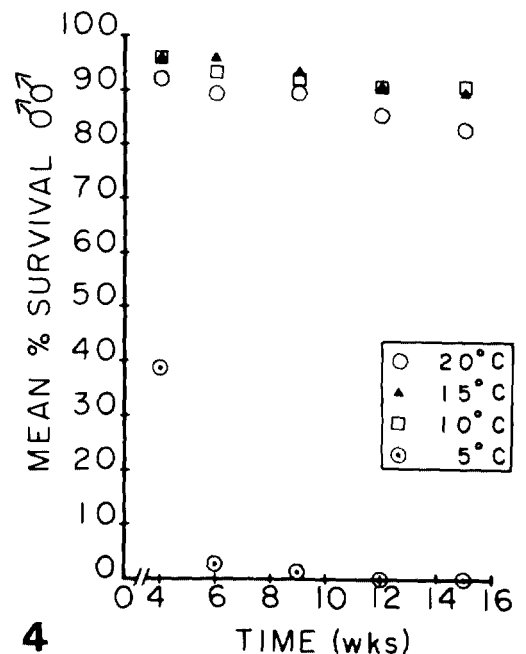
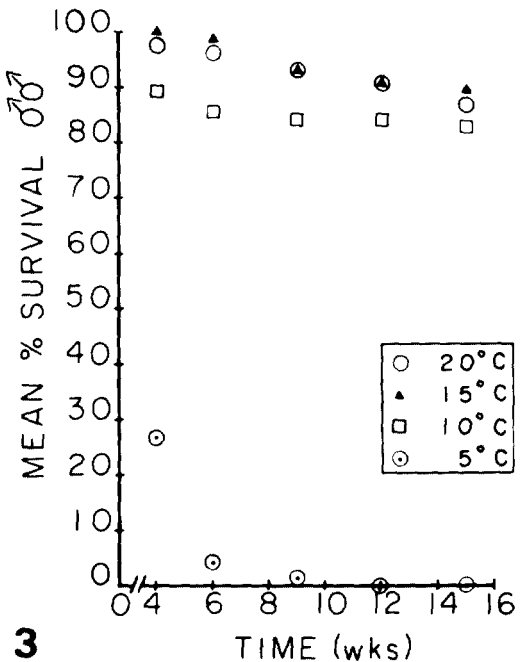
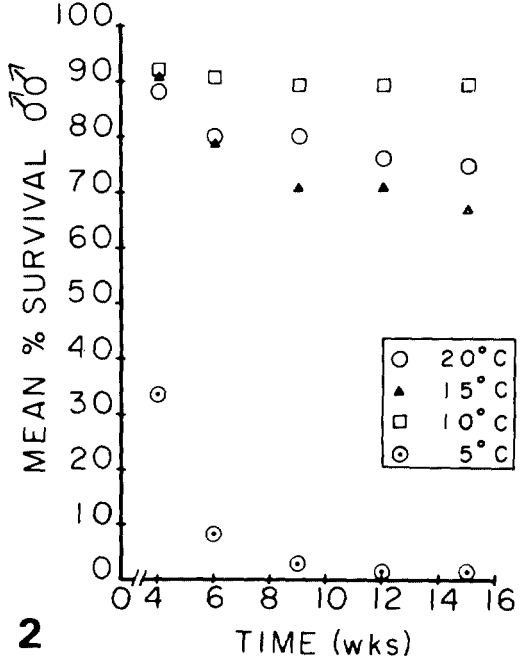
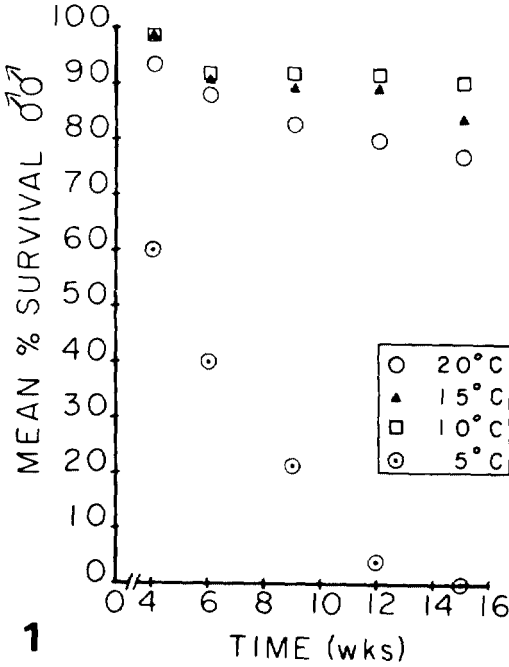
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8 days to reach the adult stage at 26 C, we divided the postparasitic stage into four age groups: 24 h (12–24 h), 48 h (36–48 h), 96 h (84–96 h), and 144 h (132–144 h) old at the beginning of the experiment (range in hours in parentheses). Five replicates of

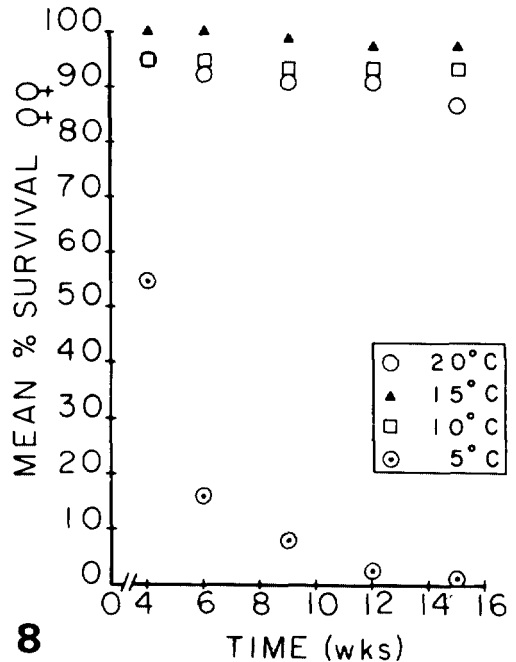
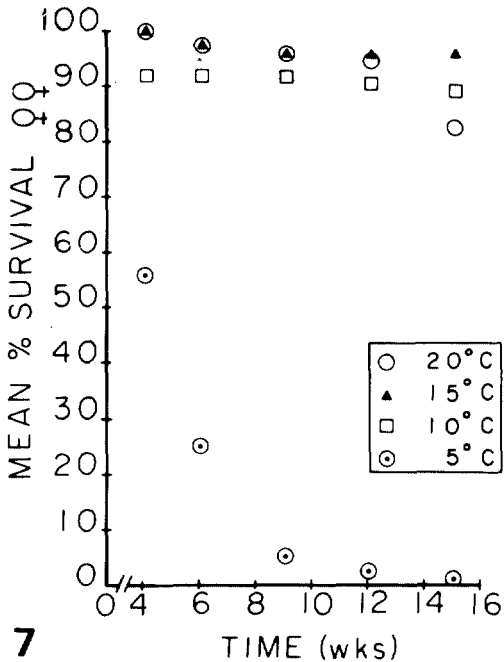
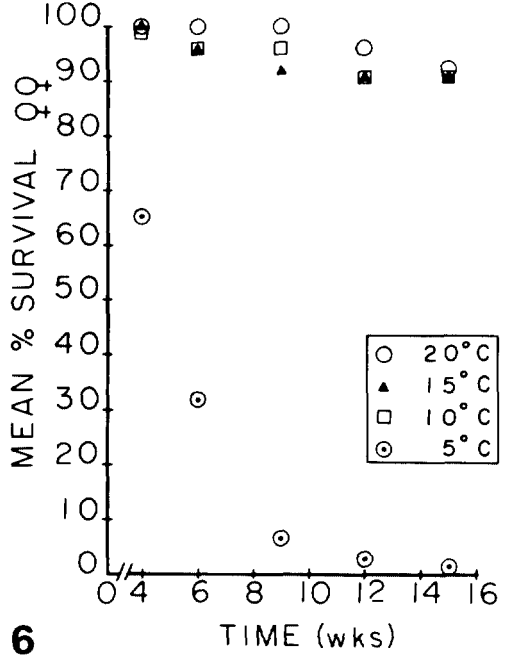
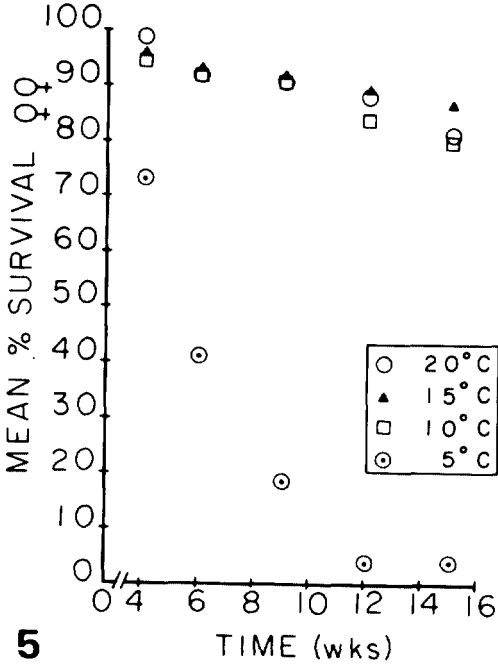
each age group were placed at each of four temperatures (5, 10, 15, and 20 C). Each replicate consisted of 25 male and 25 female postparasites in screw top glass jars, 50 × 55 mm, filled with 50 g of moist silica sand. Three of the five replicates per temperature



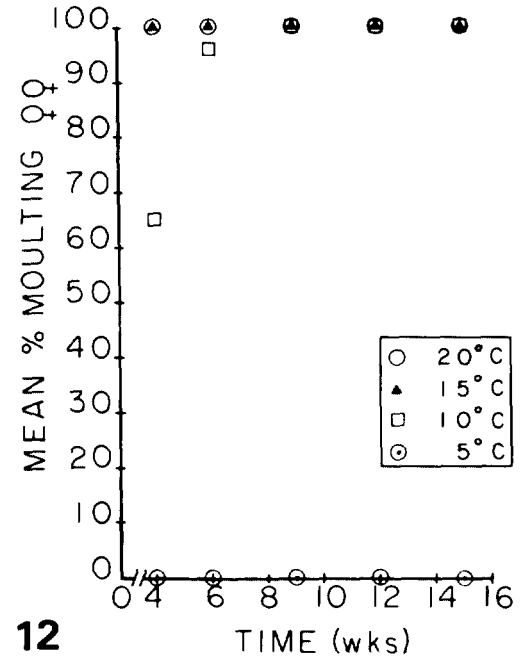
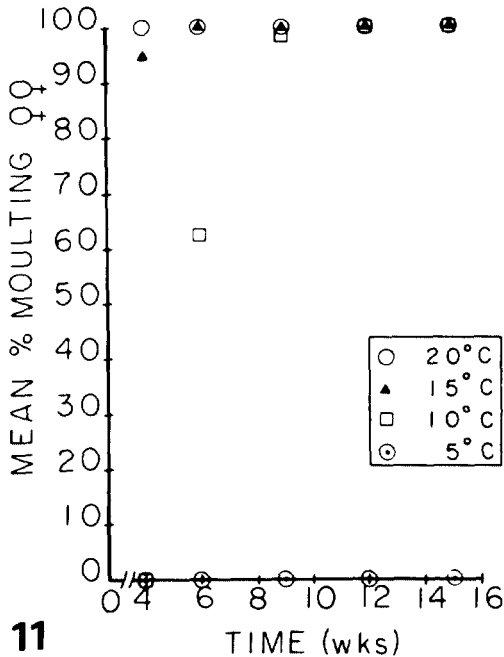
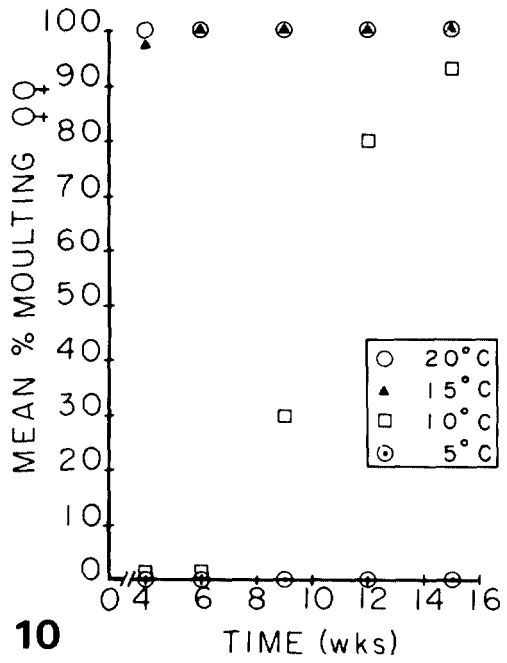
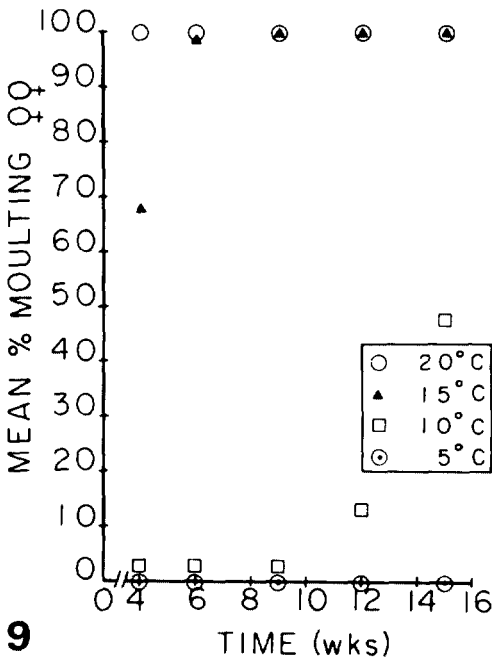
Figs. 1-4. Mean percent survival of four age groups of male *Romanomermis culicivoxax* postparasites exposed to four different temperatures for 15 wk. 1) 24 h old. 2) 48 h old. 3) 96 h old. 4) 144 h old.

and age group were examined after 4, 6, 9, 12, and 15 wk to determine the percent survival, and the numbers molted and gravid. Scoring was done at 23 C in the shortest possible time and each replicate was returned to its respective temperature. The

other two replicates per temperature and age group were examined only at the end of the experiment to determine the same parameters as above. All the nematodes from the first three replicates per temperature and age group were placed at 26 C for



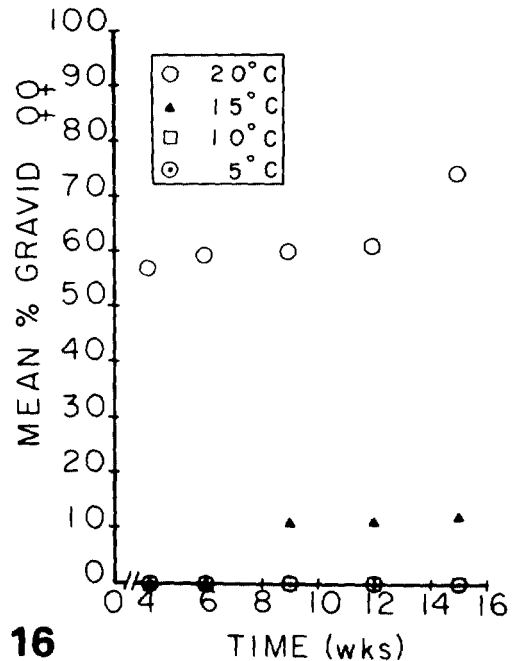
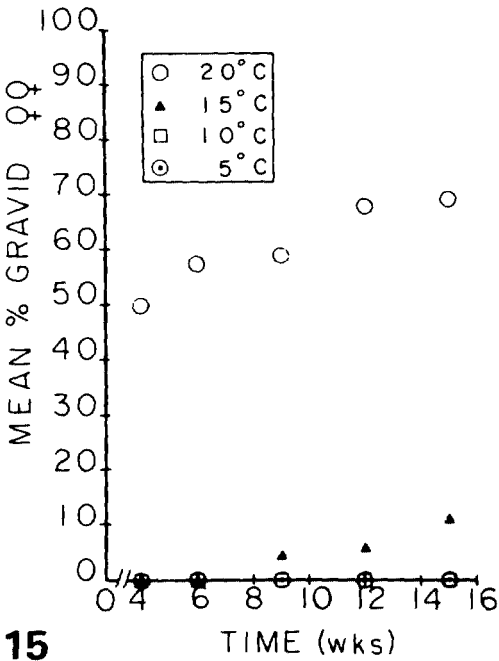
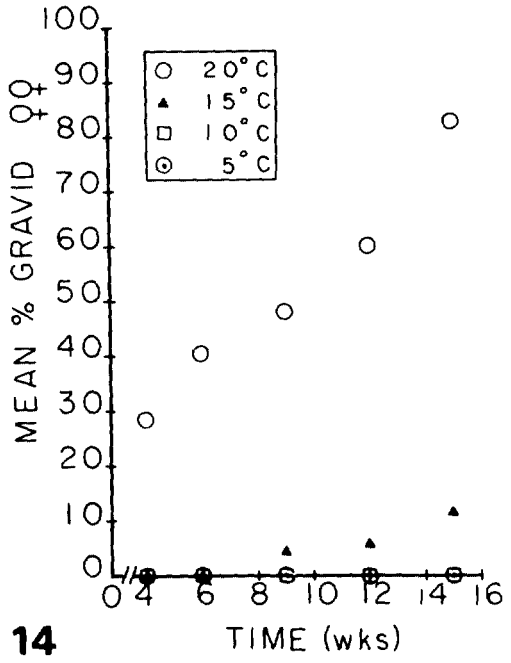
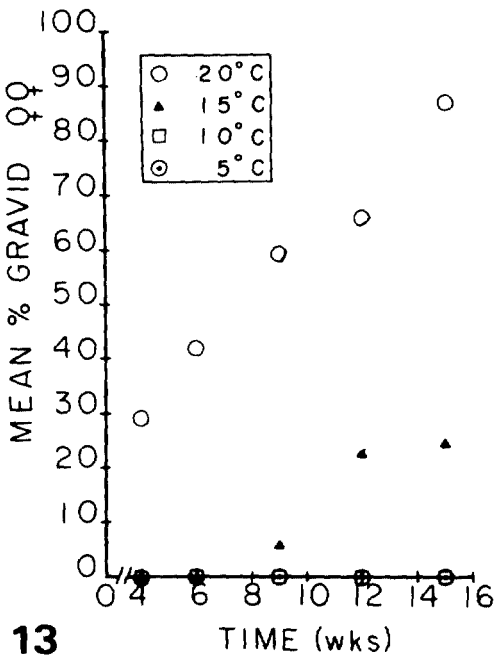
Figs. 5-8. Mean percent survival of four age groups of female *Romanomermis culcivorax* postparasites exposed to four different temperatures for 15 wk. 5) 24 h old. 6) 48 h old. 7) 96 h old. 8) 144 h old.



Figs. 9-12. Mean percent molting in four age groups of female *Romanomermis culicivorax* postparasites exposed to four different temperatures for 15 wk. 9) 24 h old. 10) 48 h old. 11) 96 h old. 12) 144 h old.

None of the adult females kept at 10 C became gravid during the 10 C exposure. However at 26 C, 95% of the 144-h females became gravid, 65% of the 96-h group, 32% of the 48-h group, and 25% of the 24-h group (Fig. 17). The percent gravid fe-

males, from postparasites exposed to 15 C for 15 wk and to 26 C for 3 wk, is shown in Figure 18. There was no difference in the percent gravid among the four age groups exposed to 15 C. Nearly all became gravid within 15 wk.



Figs. 13-16. Means percent female *Romanomermis culicivora* gravid in four age groups stored at four different temperatures for 15 wk. 13) 24 h old. 14) 48 h old. 15) 96 h old. 16) 144 h old.

An analysis of the variation between 10 C and 15 C for 15 wk, followed by 3 wk at 26 C, showed that the variation was significant ($P < 0.001$). The age groups differed ($P < 0.05$), and the interaction between temperature and age was significant

($P < 0.05$). When age groups at 10 C were compared, the variation was highly significant ($F = 41.8, P < 0.001, d.f. 1, 16$).

When the sand containing nematodes exposed to 10 and 15 C for 15 wk, followed by 26 C for 3 wk, was flooded with chlorine-

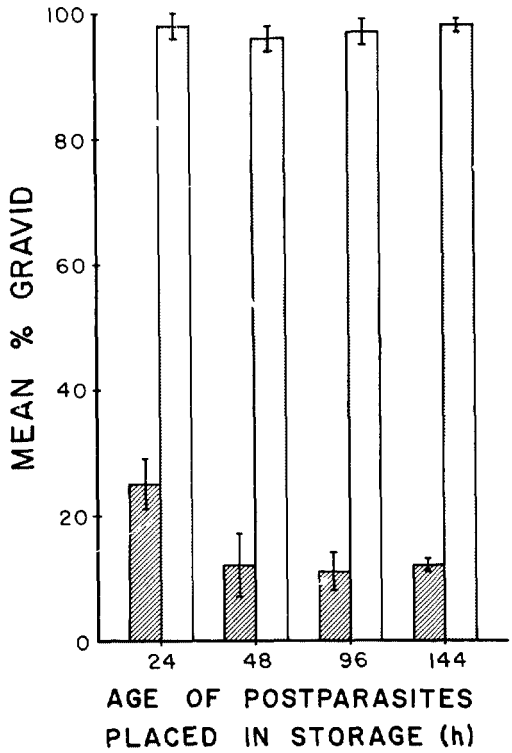
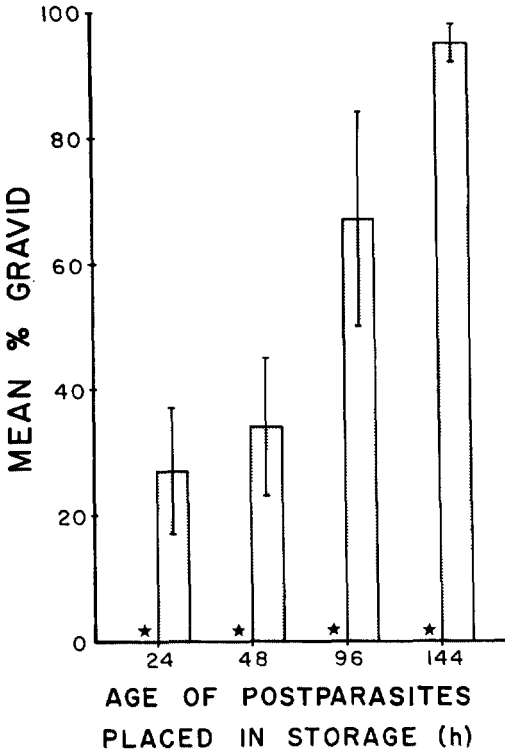


Fig. 17. Mean percent of female *Romanomermis culicivorax* gravid in four age groups stored for 15 wk at 10 C (stars represent 0%) and after 3 wk post-treatment at 26 C (stippled). Bars indicate one standard deviation.

Fig. 18. Mean percent of female *Romanomermis culicivorax* gravid in four age groups stored for 15 wk at 15 C (diagonal lines) and after 3 wk post-treatment at 26 C (stippled). Bars indicate one standard deviation.

free tap water for 24 h, preparasites were obtained from all four age groups from 15 C, but only from the 96-h and 144-h groups at 10 C.

After 15 wk, the two replicates of four different age groups and four temperatures were compared with the three replicates which had been checked at 4, 6, 9, 12, and 15 wk. We found no significant difference between the replicates handled five times and those handled only once. The percent survival, molting, and gravid were similar between the two groups.

DISCUSSION

Several criteria must be met for a particular set of conditions to be judged suitable for storing *R. culicivorax*: 1) Development should be greatly retarded. 2) Survival during and after storage must be high. 3) Following storage, development (i.e., molting), mating, female fecundity, hatching of eggs, and infectivity of preparasites should not be adversely affected.

Galloway (2) reported significant reduction in preparasite production by storing 10-wk-old cultures of *R. culicivorax* at 15 C for 7 wk. Prestorage production levels were attained by returning the culture to 27 C for 6 wk. At 23 wk of age, the culture in storage produced 10 times the number of preparasites per gram of sand as the culture kept continuously at 27 C, the standard method for colony maintenance (5,10,13). Petersen (6) examined storage of 4- and 8-wk-old cultures at 10, 15, and 20, and 26 C and found that culture longevity and preparasite production could be increased at 15 and 20 C. Petersen (6) concluded that storage of cultures at 10 C reduced overall preparasite production from those cultures. However his studies were conducted primarily with adults and eggs.

From our study, a storage temperature of 10 C appeared to be best if development of postparasites, adults, and gravid females is to be retarded for prolonged periods. None of the females were gravid after 15 wk, and

longer storage may be possible provided survival remains high. The 144-h postparasites appeared to have a slightly increased survival rate at 10 C as compared to the 24-h group. This could be important if longer storage at 10 C is attempted. It is not known if mating occurs at 10 C. If not, it is important that males survive as long as females.

A storage temperature of 15 C is best if development is to be only moderately retarded. It requires a shorter period (3-6 wk) after storage to prepare the culture for the production of preparasites (Fig. 18) (2).

A storage temperature of 20 C did not retard development significantly in any stage. Some females became gravid after 4 wk at 20 C, the same time as reported by Petersen (4) for cultures kept at 26 C.

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