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Development of *Camallanus adamsi* Bashirullah, 1974 (Nematoda: Camallanidae) in cyclopoid copepods

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The larval development of *Camallanus adamsi* Bashirullah, 1974 was followed in intermediate hosts, *Mesocyclops leuckarti* (Claus) and *Thermocyclops crassus* (Fischer), which were kept at 24 °C and 27 °C (average). The nematode molted twice in the haemocoel of copepods. The first molt occurred 117 h after infection at 24 °C and the second molt after 249 h. At 27 °C, the first and the second molts occurred 72 and 168 h respectively after the infection. Three larval stages are described.

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On a suivi le developpement larvaire de *Camallanus adamsi* Bashirullah, 1974 chez les hOtes intermediaires *Mesocyclops leuckarti* (Claus) et *Thermocyclops crassus* (Fischer) gardes a 24 et 27 °C (en moyenne). Le nematode mue deux fois dans l'hemocele des copepodes. La premiere mue se produit 117 h apres l'infection a 24 °C, et la seconde mue, 249 h apres l'infection. A 27 °C, les mues se produisent au bout de 72 h et 168 h respectivement. On decrit trois stades larvaires.

[Traduit par le journal]

Introduction

Camallanidae usually have copepod intermediate hosts. The life cycles of several species of *Camallanus* have been worked out using copepods (Moorthy 1938a; Kupriianova 1954; Moravec 1969, 1971 ; Stromberg and Crites 1974a). In the present study, *Mesocyclops leuckarti* (Claus) and *Thermocyclops crassus* (Fischer) were successfully used as intermediate hosts for *Camallanus adamsi* Bashirullah under laboratory conditions.

Materials and Methods

Two separate experiments were done at different times of the year, but differed only in the temperature at which larvae were reared.

Gravid *Camallanus adamsi* were collected from the naturally infected *Channa (Ophiocephalus) striatus* (Bloch) in the river Buriganga and its tributaries near Dacca, Bangladesh, where fish are frequently infected. Larvae were obtained by placing female *C. adamsi* in lake water filtered through No. 10 bolting silk and allowing them to burst. Tissues of burst worms were removed and the larvae were held temporarily in the refrigerator at 15 °C before inoculating the culture on the same day.

Copepods *Mesocyclops leuckarti* (Claus) and *Thermocyclops crassus* (Fischer) were collected from Dacca University pond, kept at room temperature in 2-gal (1 gal = 4.546 1) glass jars, and fed a culture of mixed Protozoa three times a week. One hundred copepods of each species were dissected before experimental use and none were found to be infected with any worms.

About 1000 copepods from the stock culture were concentrated with a small plankton bucket and placed in two 4-in. (1 in. = 2.54 cm) finger bowls in a minimal volume of water. An average of five larvae per copepod were added and left for 24 h at an average of 27 °C (26-28 `C) in the experiment of March 1972, and at 24 °C in the experiment of January 1973. After the 24 h exposure the infected copepods were transferred to 1-gal jars set in running water and kept at the constant temperatures mentioned above. The percentage of infection in the copepods was determined by examining 20 copepods on a slide under a low-power microscope.

The course of development of the larvae was followed by removing about 10 infected copepods at 3-h intervals after the 24-h infection period. Larvae were dissected from the haemocoel, killed, and preserved in hot glycerine alcohol. Preserved larvae were stained in 0.0004% lactophenol — cotton blue and drawings were made with the aid of a drawing tube. Descriptions of larval stages are based upon examination of 10 worms of each stage.

In the March 1973 experiment, a third culture was usedto study the ingestion of larvae by copepods. Two hundred and fifty copepods were exposed to 1000 larvae and a sample of five copepods withdrawn every 30 min for the next 6 h.

Results

First-stage larvae survived in pond water for 17 days at 27 °C and 28 days at 24 °C. The infectivity of larvae at this age was not studied.

The larvae in the dish lashed their bodies vigorously and that seemed to attract the copepods. Larvae generally tended to stay at the

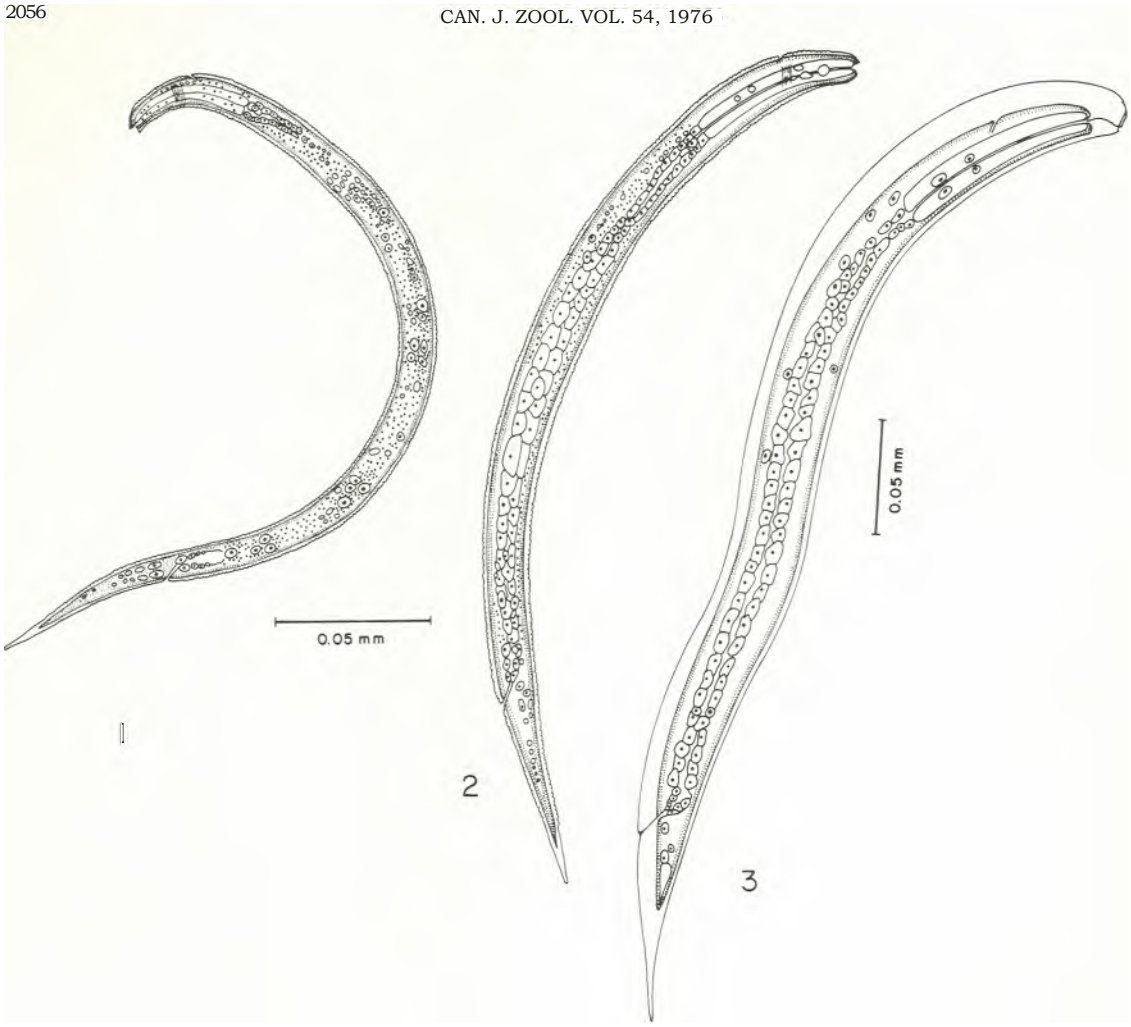


FIG. 1. Lateral view of first-stage larva of *Camallanus adamsi* (ex utero). FIG. 2. Lateral view of first-stage larva from copepod. FIG. 3. Sheathed first-stage larva.

bottom, only coming up to the surface when disturbed, then returning slowly to the bottom. Copepods ingested the larvae from the beginning of the 2nd h and most of the ingested larvae migrated into the haemocoel by the 3rd h after the exposure. Male copepods were more susceptible to the infection than the females. Early copepodite stages could not be infected.

The percentage of infection of copepods was 90 % in both the experiments of March 1972 and January 1973 with an average intensity of four larvae per copepod. The range of larvae in copepods was 3-7, with an exception of one copepod that had 47 larvae. The development of larvae in this copepod was not uniform. When autopsied 72 h after infection, only 4 out of 47 larvae were observed to have normal development.

The infected copepods survived up to 47 days at 27 °C and 65 days at 24 °C. Molting times Were determined for the two different culture temperatures. At 27 °C, the first molt occurred at 72-87 h and the second at 135-150 h; at 24 °C the two molts occurred at 117-144 and 190-222 h respectively.

Description

First-stage Larvae Ex Utero (Fig. 1)

The body is colourless, slender, 275 (264-286) (measurements are in micrometres and given as the average of 10, followed by the range in parentheses). The cuticle possesses dense trans-

verse striations, beginning directly posterior to the cephalic end. A large dentate process is present on the dorsal side of the head. The buccal cavity is short and tubelike. The oesophagus is cylindrical, muscular throughout, slightly dilated at its posterior end, 37 (35-39) in length. The nerve ring is distinct at 12 (11-13) from the anterior end. The excretory pore is situated at the middle of oesophageal length. The intestine is composed of a group of cells containing pigmented granules, followed by a prominent saclike rectum composed of glandular cells. The anus opens through the cuticle. The genital primordium is not visible. The tail is attenuated, 55 (52-57) long and terminates without spikes or mucrones.

First-stage Larvae in Copepods (Fig. 2)

The first-stage larvae from copepods closely resemble those from the uterus, but are slightly larger (332 (227-387)) in total length. The oesophagus is 55 (50-61) long and undifferentiated. The intestine possesses a series of compact cells carrying a large number of dark granules, connecting posteriorly with glandular cells of the rectum. The dentate cuticular process is still present. The nerve ring and the excretory pore are situated at 15 (13-17) and 55 (50-61) respectively from the anterior end.

Sheathed First Stage (Fig. 3)

The first-stage larvae are found to molt in the copepods after 72 h and 80% of the larvae completed their first molt by 87 h at 27 °C. Molting and molted larvae were found in the haemocoel of the same copepod. Generally, the old cuticle loosened at the posterior and the anterior ends of larvae before exsheathing. The body inside the old cuticle is slender, 378 (365-391) long. The anterior end of the larvae becomes rounded and lacks the dorsal denticular process. The nerve ring and the excretory pore are located at 26 (24-28) and 28 (26-30) respectively from the anterior end. The oesophagus is 74 (61-88) long and possesses two large nuclei in its posterior region. The intestinal cells are arranged in longitudinal rows, surrounding the lumen. The tail becomes thick and pointed, 55 (52-57) in length.

Second Stage (Fig. 4)

The body is stumpy, 431 (365-497) long. The cuticle is thin, with indistinct transverse striations. Dorsal dentate process is absent. Colour of the intestine becomes orange. The rectum is swollen with three pairs of glandular cells and a narrow lumen. The oesophagus is not differentiated, 89 (79-99) long. The genital primordium is composed of three uninucleated cells, 242 (220-264) from the anterior end. The tail is pointed, 41 (39-44) long.

Sheathed Second Stage (Fig. 5)

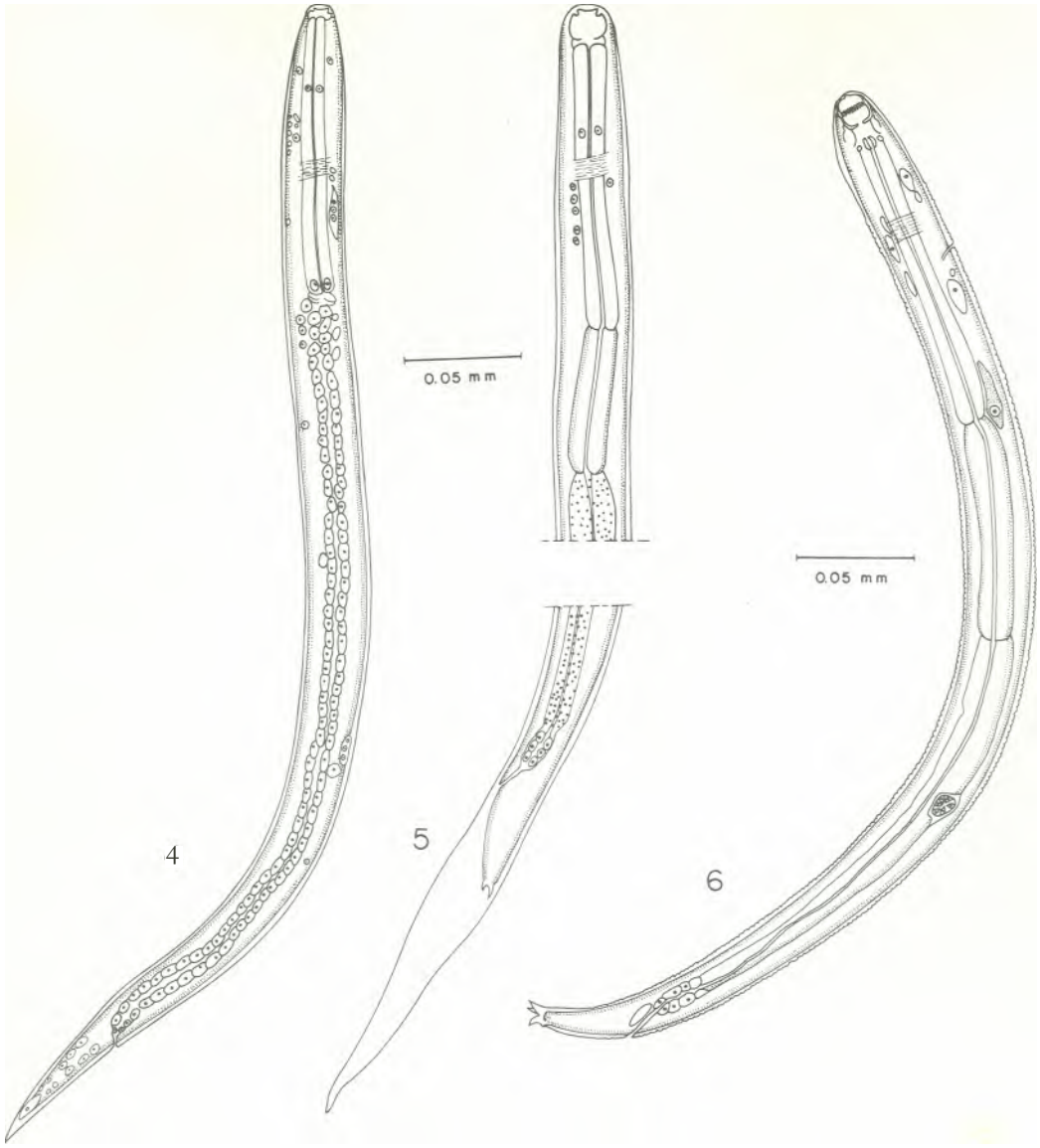
The larvae become less motile during the process of molting. The body inside the sheath is 523 (506-541) long. The buccal cavity inflates laterally forming the buccal valves and gradually becomes bell shaped with the anterior part narrow and thick walled while the posterior portion is wider and thin walled. The oesophagus differentiates into the muscular and the glandular parts, 107 (105-112) and 74 (70-79) long respectively. The intestinal wall is thick, heavily granulated, and orange in colour. The nerve ring is located at 66 (64-69) from the anterior end. The anus opens upon the surface through a long, straight tube. The multicellular genital primordium is distinct, at 319 (308-330) from the anterior end. The tail is 39 (37-41) long and terminates in three well developed mucrones.

Third Stage (Fig. 6)

The third-stage larvae become rich orange in colour. The body is fairly plump, widest at the posterior end of the buccal region, 574 (541-606) in length. The buccal capsule is fully formed and composed of partially sclerotized lateral valves, yellowish brown in colour. It is divided into two chambers by a row of sclerotized cross-shaped bars. The oesophagus consists of two distinct parts : anterior muscular, 116 (110-123) and the posterior glandular, 87 (79-96) long. The intestine is thick walled, filled with a dense granular material, and opens into the tubular rectum. Large glandular rectal cells are present around the anus. The nerve ring is located at 68 (66-70) and the excretory pore is at 76 (70-83) from the anterior end. The tail is thick, 44 (44-46) long, and terminates in three distinct mucrones.

Effect of Temperature

The data, accumulated during 3-h intervals, is presented as 24-h averages in Fig. 7. At 24 °C, worms molted for the first time after 117 h and reached third stage after 190 h, but 249 h were needed for 100% of the larvae to reach third stage. At 27 °C, the first molt occurred in a few larvae after 72 h and 100% larvae reached the third stage after 168 h. More than 90% of the larvae reached the second stage by 114 h at 27 °C,



FIGS. 4-6. Larval stages of *Camallanus adamsi*. FIG. 4. Lateral view of second-stage larva. FIG. 5. Lateral view of sheathed second-stage larva. Fla. 6. Lateral view of third-stage larva.

whereas the same percentage of larvae did not show any molting at 24 °C during the same length of time. Similar variability in rate of development is observed at other stages.

Discussion

The development of larval nematodes in copepod intermediate hosts may be accelerated by increasing the temperature. An increase of 5 °C increases the rate of development in *Philonema oncorhynchi* by two times (Ko and Adams 1969). With an increase of 3 °C, there was an increase of rate of development in *Camallanus adamsi* by 1+ times. Stromberg and Crites (1974a) encountered almost the same rate of development in *C. oxycephalus* in copepod, when increasing the temperature by 5 °C.

Thomas (1929) observed that the copepods are attracted to the wriggling movements of the larvae. Similar observations were made in the case of the first-stage larvae of C. *adamsi* and the larval activities were high initially but gradually

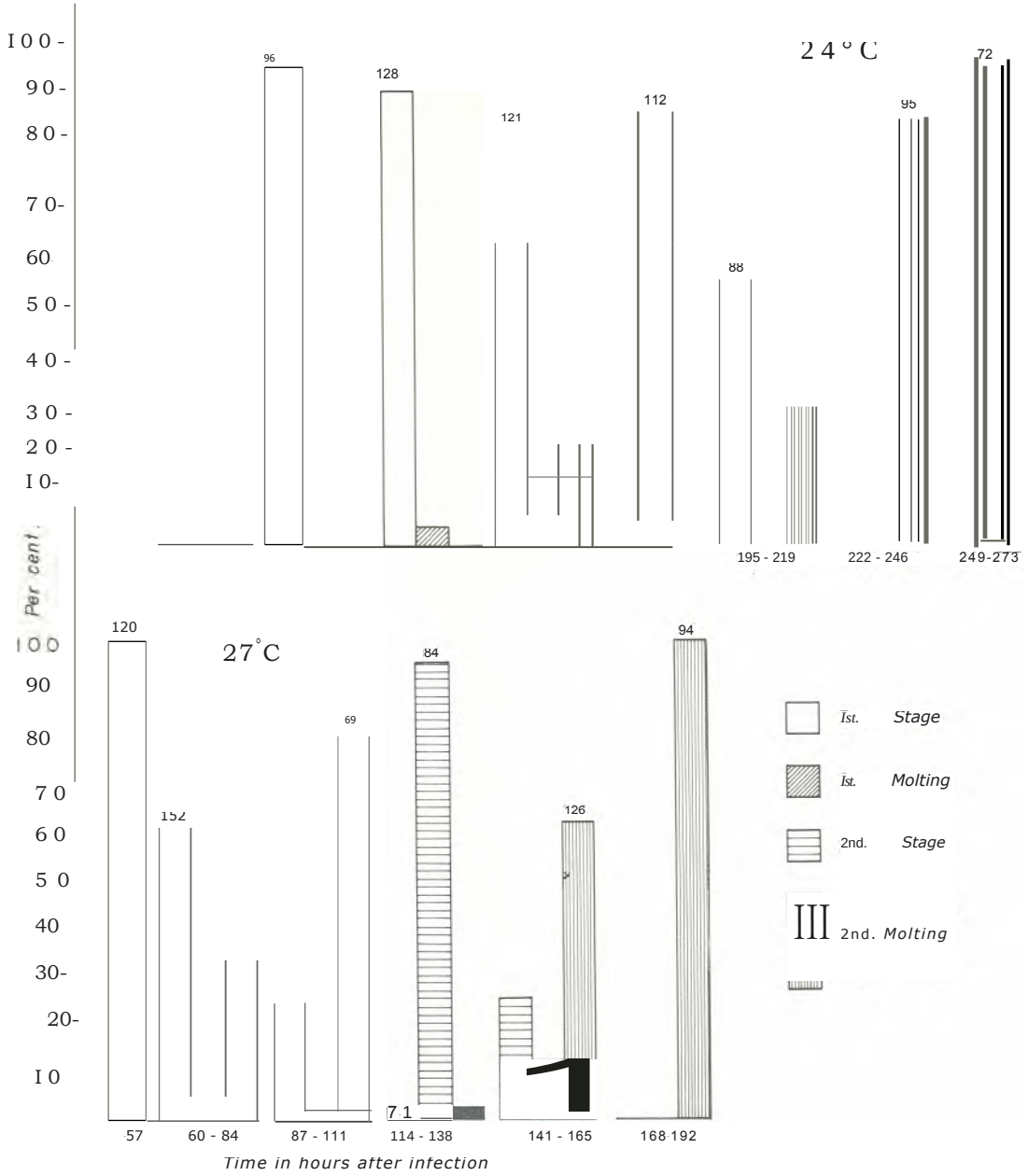


FIG. 7. Frequency distribution of larval stages of *Camallanus adamsi* in copepods at two different temperatures. Accumulated 3-h data are presented in 24-h average. Numbers above the columns indicate total number of larvae examined in each 24-h group.

slowed down with age. Stromberg and Crites (1974b) showed that the number of larvae eaten by copepods was related to the rate of undulation for *C. oxycephalus.* The same authors reported that the penetration efficiency decreased logarithmically with age of larvae. Muller (1971) noted the considerable fall in infection of copepods by larvae of *Dracunculus medinensis* stored for 3 days, and no infection at all when stored for 6 days.

The first-stage larvae of *C. adamsi* needed 1 h to migrate to the haemocoel after ingestion.

Moorthy (1938a) observed penetration in 1 h for *C. sweeti,* while Stromberg and Crites (1974a) cite 2 h for *C. oxycephalus.*

The oesophageal differentiation was distinct in the third-stage larvae of C. *adamsi.* This is similar to the findings of Campana-Rouget (1961) in *C. lacustris* and Stromberg and Crites (1974a) in *C. oxycephalus,* whereas Moravec (1969) for C. *lacustris* and Moorthy (1938b) for C. *sweeti* noted this differentiation during the second stage. Moravec (1969) located the genital primordium in *C. lacustris* in third stage, while Stromberg and Crites (1974a) observed it in the first stage of *C. oxycephalus,* and we found it in the second stage of *C. adamsi.* Moravec (1969) and Campana-Rouget (1961) noted the transformation of cross-shaped bars of the buccal capsule of the third-stage larvae of C. *truncatus* and C. *lacustris* to longitudinal ridges in the fourth stage larvae. The authors could not trace the transformation in C. *adamsi.*

Three mucrones appeared on the tail during the second molt in C. *adamsi* and became very prominent in the third stage. Stromberg and Crites (1974a) observed the same in C. *oxycephalus* but reported their disappearance during the final molt in the final host. The same result could be expected in the case of *C. adamsi,* as the adult worm does not possess this character (Bashirullah 1974).

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