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Activity, Aging and Penetration of First-Stage Larvae of *Procamallanus heteropneustus*

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Abstract - Observations on the activity, survival and penetration of first-stage larvae of the nematode *Procamallanus heteropneustes* to the gut wall of the copepod intermediate host were made. Survival was influenced by temperature. Larvae survived 9 d at summer (32 ± 1°C) and 14 d at winter (23 ± 1°C) temperatures. Penetration decreased with the age of larvae and was more rapid at winter temperature. Larval activity rates also decreased with age. Activity was found to be the most important factor for penetration of the copepod gut wall.

The nematode *Procamallanus heteropneustus* Ali, 1957 (Nematoda: Camallanidae) is a frequent gastrointestinal parasite of the stinging catfish, *Heteropneustes fossilis*. It is an ovoviviparous nematode, very small in size, yel-lowish to reddish in color, frequently found attached to the stomach and the intestine.

Information on the life cycle and early larval development of *P. heteropneustus* is rather scarce. However, the results of systematic studies are available from various workers (Ali 1957; Sood 1967; Soota 1983). In Bangladesh, Rahman and Ali (1968) reported this worm from two catfishes (*Clarias batrachus* and *H. fossilis*) and recorded its prevalence. In natural waters, the dispersal of the first-stage larva and its capacity to infect the intermediate host (a copepod) may be influenced by its interaction with the environment. This active larva, which is released from the gravid female worm by uterine prolapse, is a dispersal stage. This paper therefore, describes observa-tions on the ability of these larvae to remain active, survive and penetrate the copepod gut wall.

Host fish were collected from experimental ponds of the Department of Fisheries Biology and Limnology, Bangladesh Agricultural University, Mymensingh, Bangladesh. First-stage larvae were obtained by placing gravid female worms in distilled water, causing rupture of the body wall and uterus. Larvae were separated from the females with a pipette and stored in a petri dish. Experiments were conducted in the laboratory in summer at 32 ±1°C and in winter at 23 ± 1°C. These temperatures fall within the normal range of summer and winter temperatures of the experimental pond.

Survival was determined by isolating five lots of 100 larvae for each temperature in small petri dishes (50 mm diameter) containing filtered pond water and counting the number of dead larvae at 24 h intervals.

To measure the ability of the larvae to remain active, larvae were placed in a petri dish containing 25 ml filtered pond water and left undisturbed for 1 h. Upon settling, the larvae attached to the substrate by their tails and started undulations. This activity (undulation) was continuous, easily observed and could be readily measured. The larvae were not disturbed with any object for excitation nor was the water in the petri dish changed during the experiment. Activity was therefore measured as the mean rate of undulation per minute of 15 individuals maintained at summer (32 ± 1°C) and at winter (23 ± 1°C) temperatures.

Penetration was measured by exposing copepods, *Diaptomus* sp, individually to 10 larvae in 2 ml of filtered pond water for 24 h. Number of larvae penetrated was determined by counting the larvae remaining in the petri dish after the copepod was removed and by dissecting each copepod and counting the number of larvae within its hemocoel. Five replicates of this procedure were set up every 24 h to determine the rate of penetration for each age class of larvae. Penetration rate (Pe) was calculated as indicated by Stromberg and Crites (1974):

$$Pe = \frac{\text{No. of larvae in hemocoel}}{\text{No. of larvae eaten}}$$

Survival curves for each experiment were plotted from life tables constructed using the method of Deevey (1947). Statistical analysis correlation coefficients and linear regression were calculated to estimate relations between variables in this experiment (Ali 1973; Clarke 1994).

The survival of first-stage larvae at winter (23 ±1°C) and summer (32 ± 1°C) temperatures is illustrated in Fig. 1. Larvae survived a maximum of 14 d in winter and 9 d in summer. Half of the larvae died within 8 d in winter and within 6 d in summer. At the lower temperature, larvae survived longer, and the decline in survival was also more gradual than at the higher temperature.

Although larvae survived up to 14 d at winter temperature in filtered pond water, their ability to successfully penetrate the copepod gut wall was lost long before the larvae died (Fig. 2). No larvae penetrated the gut wall after 7 d in winter; while in summer, penetration continued for up to 8 d. Penetration decreased with age of larvae, the decrease being more rapid at lower temperature. For every day, the larval penetration rate decreased by 0.72 and 0.082 at winter and summer temperatures, respectively. The rate of penetration (regression coefficient) was also significant (t = 13.458, d.f. = 5; and t = 10.351, d.f = 6, for winter and summer temperatures, respectively) at 0.01 level of significance for both the winter and summer temperatures. However, the difference in penetration rate between winter and summer temperatures was not significant (t = 0.984, d.f. = 11).

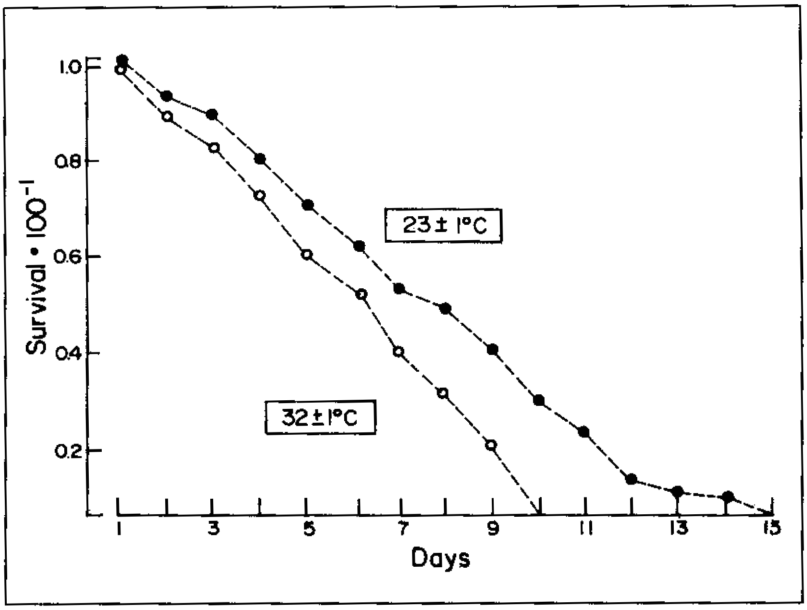

Fig. 1. Survival of first stage larvae of *Procamallanus heteropneustus* at 23 ± 1°C (●) and 32 ± 1°C (○).

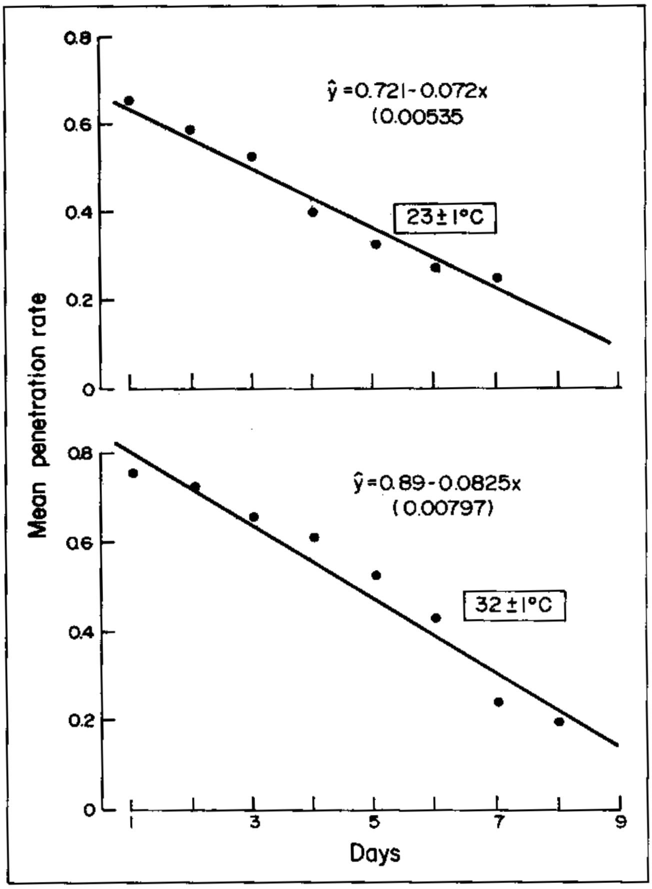

Fig. 2. Relationship between mean penetration rate and age of *Procamallanus heteropneustus* larvae at 23 ± 1°C (upper graph) and 32 ± 1°C (lower graph).

The initial rate of larval activity was high. Larvae exhibited a mean rate of 32 undulations·min^{-1} at the winter temperature and 42.2 undulations·min^{-1} at the summer temperature. Larval activity was significantly related to age ($P < 0.01$) and the decline in activity was linear. The slopes of the lines were -2.43 and -3.48 at winter and at summer temperatures, respectively (Fig. 3), and were significantly different ($t = 4.458$, d.f. = 19). Larvae also continued spontaneous activity for up to 10 d at winter and 8 d at summer temperatures, respectively.

The relationship between the mean activity rate of nematode larvae and the number of larvae eaten by copepods at both winter and summer temperatures was tested, and a positive correlation observed (Fig. 4) ($r = 0.95$; $P < 0.01$).

The ability of the larvae of *P. heteropneustes* to survive in the external environment is related to temperature. First-stage larvae of *Neocamallanus ophiocephali* (Pearse 1993) survived in pond water for 17 d at 27°C and 28 d at 24°C (Bashirullah and Ahmed 1976). According to Moravec (1969), first-stage larva of *Camallanus lacustris* (Zoega 1776 ; cited in Moravec 1969) can live 12 d at 22°C and 80 d at 7°C in the laboratory. Stormberg and Crites (1974) observed that *C. oxycephalus* Ward and Magath, 1916 could survive for 24 and 39 d at temperatures of 25 and 20°C, respectively, but lost penetration ability before death at both temperatures. In the case of *P. heteropneustus*, larvae also lost penetration ability prior to death at both summer and winter temperatures. The rapid decrease of penetration ability at winter temperature suggests that the ability to infect the copepod is not related completely to survival. Croll and Matthews (1973) pointed out that the free-living larvae of certain nematodes have a lipid reserve which is their only energy source. They correlated the rate

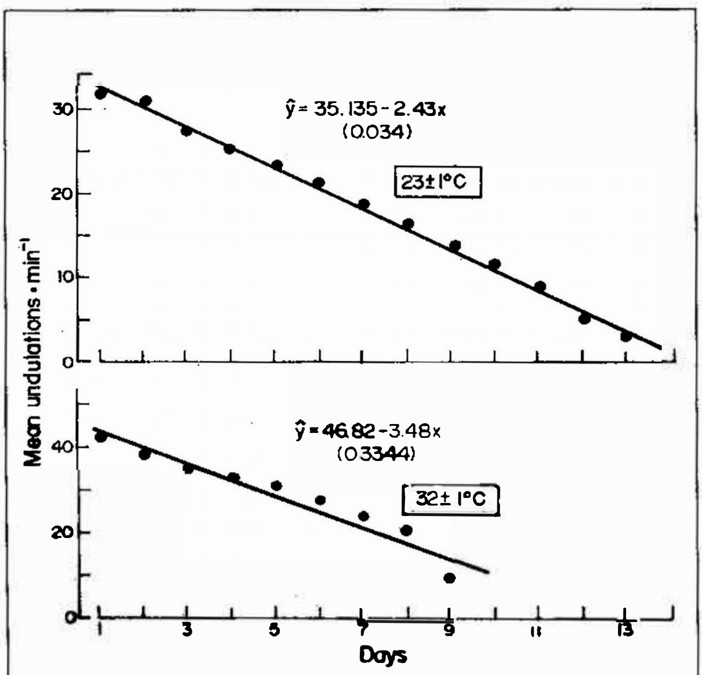

Fig. 3. Relationship between mean rate of activity and age of first-stage larvae of *Procamallanus heteropneustus* at 23 ± 1°C (upper graph) and 32 ± 1°C (lower graph).

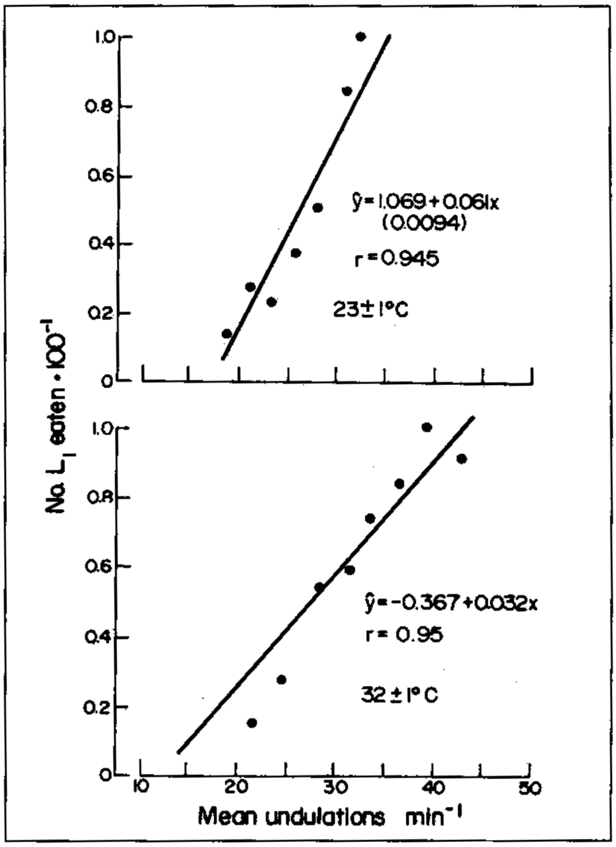

Fig. 4. Relationship between the mean activity rate of first-stage larvae of *Procamallanus heteropneustus* and ingestion by copepods at 23 ± 1°C (upper graph) and 32 ± 1°C (lower graph).

of activity and penetration ability of *Ancylostoma tubaeforme* (Zeder 1880; cited in Stromberg and Crites 1974) with the amount of neutral lipid. In the case of *P. heteropneustus*, the rapid decline and cessation of penetration by larvae at winter temperature suggests a lowered metabolic energy which is insufficient to permit penetration of the copepod gut wall.

Stromberg and Crites (1974) observed that larval activity was important in the penetration of the copepod gut wall by *C. oxycephalus*. This is also evident for *P. heteropneustus*. Rogers (1939) noted a logarithmic decline in the activity rate of *Ancylostoma tubaeforme* larvae maintained at 37 and 7°C, and that larvae remained infective for 5.5 and 10.5 weeks, respectively. Log transformation of the activity and penetration data at both winter and summer temperatures revealed a significant positive correlation between these variables (Fig. 5), indicating that larval activity is definitely the most important factor in the penetration of copepod gut wall.

The present investigation supports the findings of Stromberg and Crites (1974) that very active first-stage nematode larvae are more likely to be eaten by copepods than are sluggish larvae. Thus a high rate of larval undulation appears to attract copepods and significantly increases the probability of host contact. Young larvae, which are more active and which have higher penetration ability, are thus brought into contact with the intermediate host.

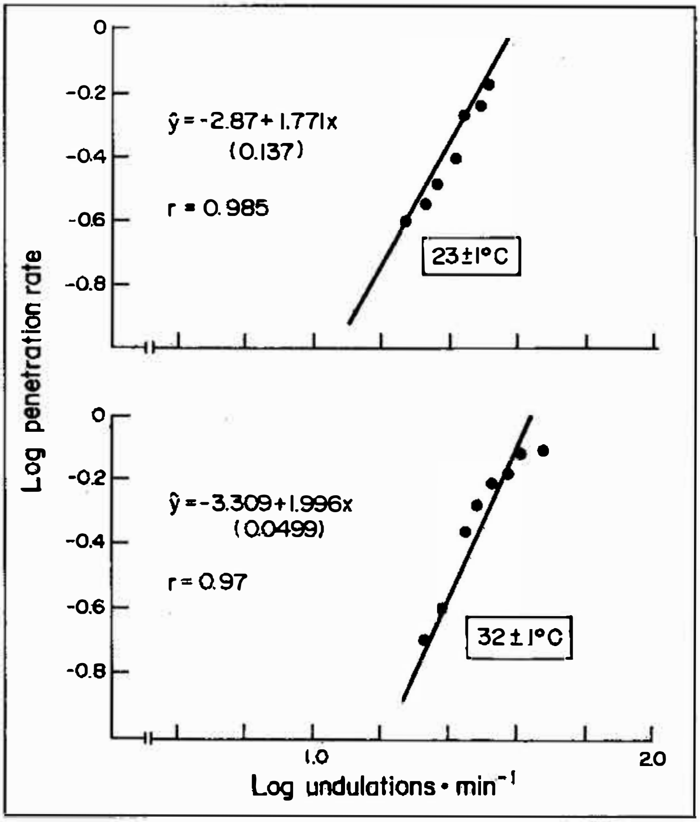

Fig., 5. Relationship between the log penetration rate and the log activity rate of first stage larvae of *Procamallanus heteropneustus* at 23 ± 1°C(upper graph) and 32 ± 1°C (lower graph).

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References

Ali, M.A. 1973. Theory of statistics (Volume II). Dacca Book Mart, Banglabazar, Dacca. 546 pp.

Ali, S.M. 1957. Studies on the nematode parasites of fishes and birds found in Hyderabad State. Indian Journal of Helminthology 8: 1-83.

Bashirullah, A.K.M. and B. Ahmed. 1976. Development of *Camallanus adamsi* Bashirullah, 1974 (Nematoda : Camallanidae) in cyclopoid copepods. Canadian Journal of Zoology 54: 2055-2060.

Clarke, G.M. 1994. Statistics and experimental design. An introduction for biologists and biochemists. (Third edition). Edward Arnold, A division of Hodder Headline Plc. Eudton Road, London. 208 pp.

Croll, N.A. and B.E. Matthews. 1973. Activity, aging, and penetration of hookworm larvae. Parasitology 66: 279-289.

Deevey, E.S. 1947. Life tables for natural populations of animals. Quarterly Review of Biology 22: 283-314.

Matthews, B.E. 1972. Invasion of skin by larvae of cat hookworm, *Ancylostoma tubaeforme*. Parasitology 66: 457-468.

Moravec, F. 1969. Observations on the development of *Camallanus lacustris* (Zoega, 1776) (Nematoda : Camallanidae). Vestnik Ceskoslovenske Spolecnosti Zoologicke 33: 15-33.

Rahman, A.K.A. and M.Y. Ali. 1968. The incidence of nematode *Procamallanus heteropneustus* in the stomach of *Heteropneustes fossilis*. Pakistan Journal of Scientific and Industrial Research 11: 112-113.

Rogers, W.P. 1939. The physiology of aging of ancylostome larvae. Journal of Helminthology 17: 195-202.

Sood, M.L. 1967. Two nematode parasites from freshwater fishes of Lucknow. Indian Journal of Helminthology 18: 181-187.

Soota, T.D. 1983. Studies on nematode parasites of Indian vertebrates. 1. Fishes. Record of Zoological Survey of India, Miscellaneous Publication, Occasional Paper No. 54. 352 pp.

Stromberg, P.C. and J.L. Crites. 1974. Survival, activity and penetration of the first stage larvae of *Camallanus oxycephalus* Ward and Magath, 1916. International Journal of Parasitology 4: 417-421.

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