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Influence of Temperature on Development of Eggs and Larvae of *Gnathostoma nipponicum* Yamaguti, 1941

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Abstract

Influence of temperature on development of eggs and larvae of *Gnathostoma nipponicum* was examined under laboratory conditions. Eggs did not develop at 10°C, but they began to develop at 15°C or greater. As temperature rose, the period required for development of eggs to second-stage larvae shortened and was 6 to 8 days at 30°C. Second-stage larvae survived the longer as water temperature became the lower. Two species of copepods, *Cyclops vicinus* and *Thermocyclops hyalinus*, were infected with second-stage larvae, but larvae could not develop to early third-stage larvae in these copepods at temperatures below 15°C. Under laboratory conditions optical temperature for both egg and larval development in *T. hyalinus* and *C. vicinus* was 30°C. Possible life cycle of *G. nipponicum* in Ueno district, Mie, Japan was discussed.

Key words: *Gnathostoma nipponicum*, larvae, development, temperature, *Cyclops vicinus, Thermocyclops hyalinus*

Introduction

Gnathostoma nipponicium which was found in the esophagus of Japanese weasel, *Mustela sibirica itatsi*, in Osaka, was first identified as *G. spinigerum* (Yoshida, 1926). Later it was revised taxonomically and described as a new species, *G. nipponicum* (Yamaguti, 1941). So far, adult *G. nipponicum* has been found in weasels from mainly central and west regions of Japan. Its larval stage was recently found in snakes from Kagawa Prefecture (Koga and Ishii, 1981) and loaches in Mie Prefecture (Ando *et al.*, 1988a).

Larval development had been described for four gnathostomes; *G. spinigerum* (Prommas and Daengsvang, 1933, 1936), *G. doloresi* (Ishii, 1956), *G. procyonis* (Ash, 1962), and *G. nipponicum* (Arita, 1953 and Mabuchi, 1956, 1957). These studies have shown that first intermediate hosts are only cyclopoid copepods and that second intermediate hosts of the former three are various cold-blooded vertebrates. However, data on the influence of temperature on larval development are inadequate.

We report here the influence of temperature on the development of eggs and larvae of *G. nipponicum*.

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Materials and Methods

Eggs were obtained from the feces of two weasels infected with *G. nipponicum* which were maintained in our laboratory. The eggs were washed 5 times in water and incubated in a bottle containing a shallow layer (2 cm) of water at 10, 15, 20, 25, 30, and 35°C, respectively. Development of the eggs were observed at intervals of two days under the microscope and the hatchability was recorded. Water was renewed at each observation period.

The second-stage larvae hatched from the eggs were incubated in 24-well plate with water at various temperatures and the survival rate was recorded every two days.

Copepods, *Cyclops vicinus* and *Thermocyclops hyalinus*, ostracod, *Cypridopsis uenoi*,

and branchiopod, *Moina macrocopa* were collected from a pond in Tsu City. They were exposed to second-stage larvae in a bottle for 3 hr. The developmental stage of larvae were observed every day by dissecting them.

Results

Eggs in the feces were in the one-cell stage and had thin shells with a finely granulated surface. At one end of the egg, there was a transparent caplike thickening through which the second-stage larva escaped at hatching. The average measurements of 35 eggs were 73.7 ± 2.9 (Mean ± SD) × 42.0 ± 3.2 μm.

At 35 and 30°C, eggs of one-cell stage developed to morula on day 2 and embryonated eggs on day 4 post-incubation. Second-stage larvae began hatching on day 6 post-incubation (Fig. 1) and continued to do so for as long as 12 days. About 7% of eggs degenerated at 35°C. The first molt of larvae in eggs was difficult to observe. At lower temperatures, eggs developed more slowly than at 30 or 35°C and fewer eggs degenerated. At 15°C, larvae began hatching on day 50 post-incubation. At 10°C, eggs failed to develop during the observation period of 24-day (data not shown) but these eggs were alive because they began to develop normally when they were incubated at 25°C.

Second-stage larvae retained the sheath of the old cuticle which was shed at the first molt before hatching and swam actively. The average body length and width of 20 larvae were 280.3 × 12.9 μm (Fig. 3, day 0) and average sheath length was 343.8 μm. The anterior end of the larva shows a spinelike structure which probably is useful for both exsheathment and penetrating the wall of

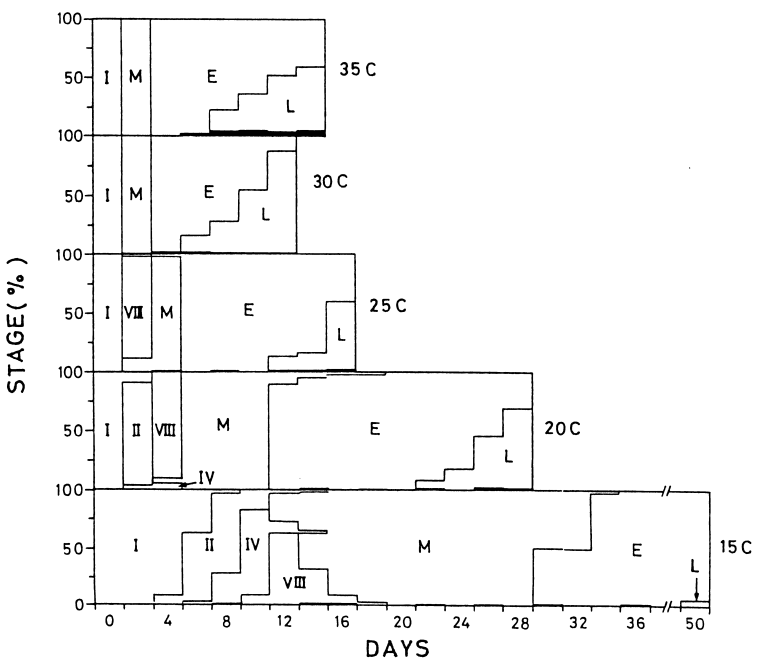


Fig. 1. Development of *Gnathostoma nipponicum* eggs at various temperatures.
I one-cell stage II two-cells stage
IV four-cells stage VIII eight-cells stage
M sixteen-cells to morula
E embryonated egg
L larva
■ degenerated

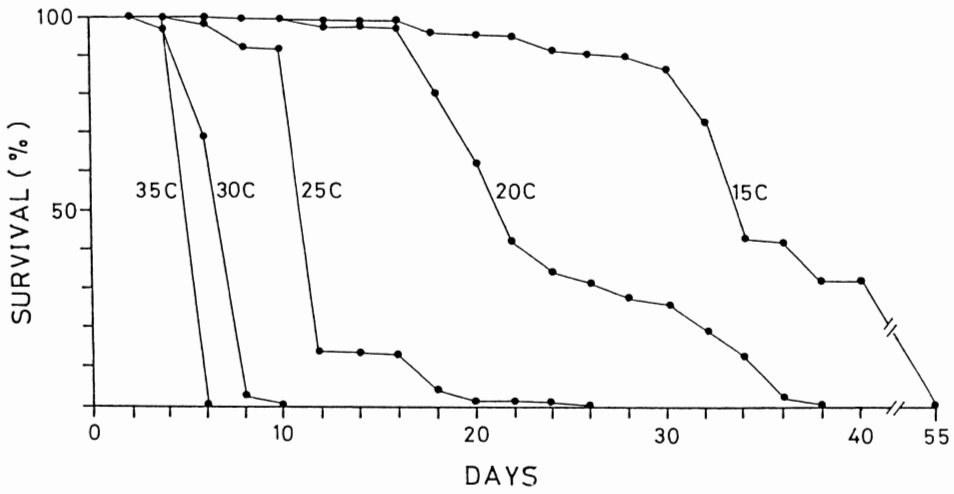


Fig. 2. Survival times of second-stage larval *Gnathostoma nipponicum* at various temperatures. About 50 larvae were incubated in each temperature.

the digestive tract of copepods (Fig. 4-a). The intestine of the larva is a narrow column containing coarse greenish granules. These second-stage larvae survived the longer as water temperature became the lower (Fig. 2). At 35, 30, and 25°C, 50% of the larvae changed to slow movement on day 2. At 20 and 15°C, 50% of larvae changed to slow movement on day 6. These feeble larvae did not develop to early third-stage larvae after ingestion by copepods. The second-stage larvae did not shed their cuticular sheaths in water.

Copepods, mainly copepodid stages, readily ingested second-stage larvae, but ostracod, *C. uenoi,* did not. Larvae ingested by branchiopod, *M. macrocopa*, did not survive. Two species of copepods were infected; *C. vicinus* (female 1.5—2.2 mm, male 1.0—1.3 mm) which lives in cold water, and *T. hyalinus* (female 0.75—0.95 mm, male 0.53—0.60 mm) which lives in warm water.

Detailed morphological change of larvae in *C. vicinus* was observed at 30°C. The number of larvae ingested by the copepod averaged 3.2 (range, 9-1). Almost immediately following ingestion larvae shed the cuticular sheaths in the intestine, penetrated immediately the wall of the intestine, and reached the body cavity of the copepods (Fig. 4-b). Their length became shorter but their width did wider on day 1 post-infection

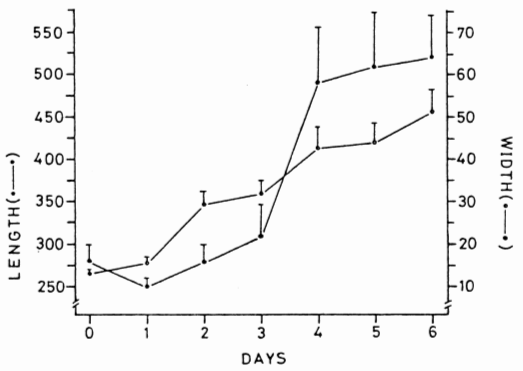
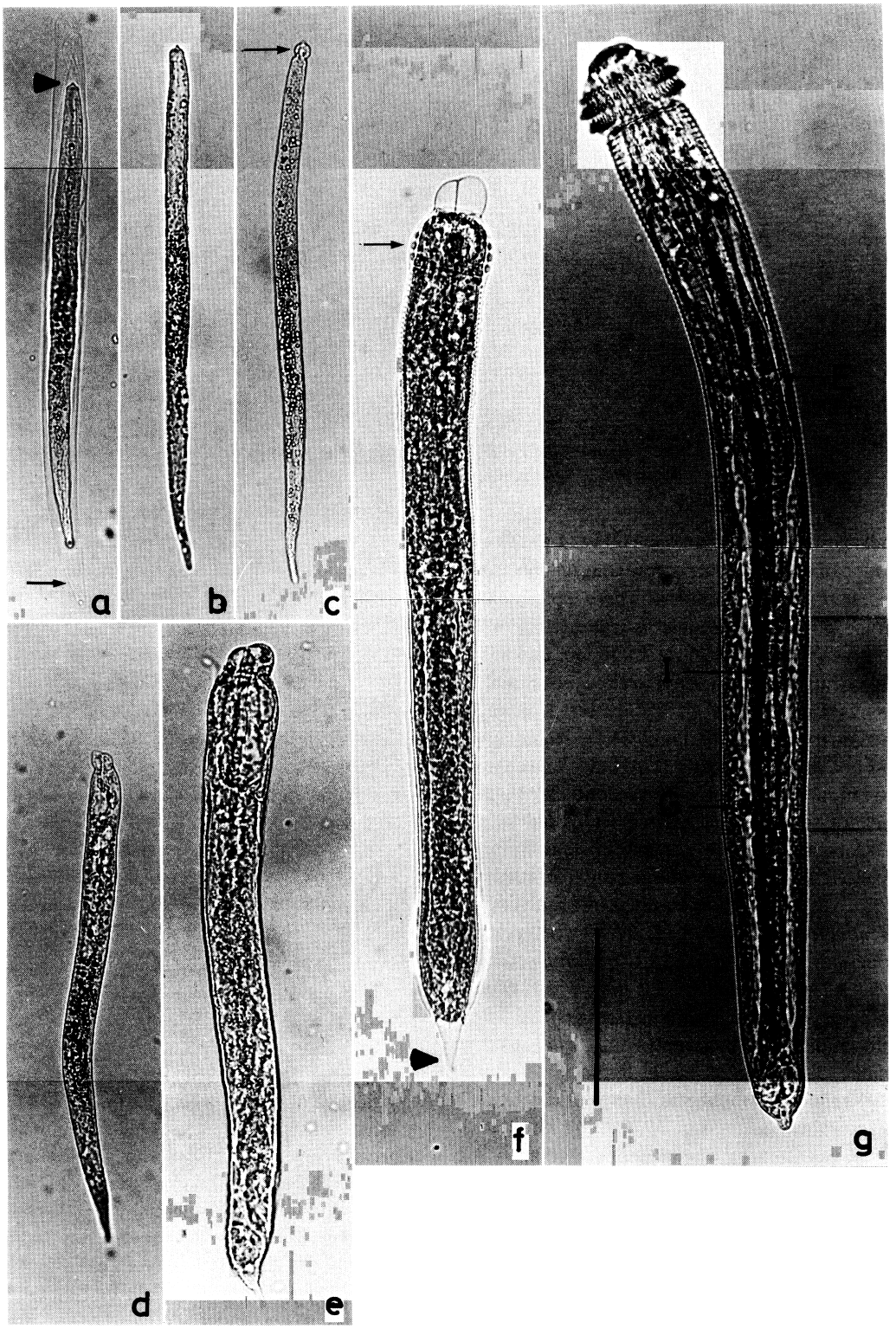


Fig. 3. Growth curve (μm) of larval *Gnathostoma nipponicum* in copepod, *Cyclops vicinus*, at 30°C (Mean ± SD). About 24 larvae were measured in each day.
Day 0; second-stage larvae hatched from eggs.
Day 6; early third-stage larvae.

(PI) (Fig. 3). The marked change in morphological characteristics occurred within 3 hr of infection; the spinelike structure at the anterior end became less conspicuous and lips became prominant (Fig. 4-c). Then larvae grew and the lips continued to expand unequally (Fig. 4-d,e). The larvae grew very rapidly between day 3 and 4 PI (Fig. 3). By day 5 PI, larvae had almost fully developed and the head bulb had minute rudimentary spines under the cuticle (Fig. 4-f). On


a
b
c
d
e
f
g

day 6 PI, the larvae developed to the late second-stage and molted to early third-stage larvae (Fig. 4-g). When fully developed they averaged 520.2 × 51.4 μm (Fig. 3, day 6) and the number of hooklets in the three rows (anterior to posterior) numbered 34.8, 36.1, and 39.7 on an average of ten larvae examined. The cervical sacs were fully developed and extended as tubular structures on either side along half the length of the esophagus. The genital primordium was clearly recognizable as a small group of cells attached to the mid-ventral wall (Fig. 4-g). The entire surface of the cuticle was covered with transverse rows of minute spines. The larvae were sluggish in the body cavity of the copepod.

The number of larvae ingested by *T. hyalinus* averaged 1.3 (range, 3-1). The second-stage larvae developed to early third-stage larvae in this species as well as in *C. vicinus*. Thus, the periods required for development of second-stage to early third-stage larvae were compared between two species of copepods at various temperatures (Table 1). At 35°C, larvae in *T. hyalinus* developed to early third-stage larvae on day 6, while *C. vicinus* died within 2 days PI. At 30°C, larvae in these species developed within the same period (6-8 days PI). At 25 and 20°C, the development of larvae in *T. hyalinus* was a bit faster than in *C. vicinus*. At 15°C, *C. vicinus* and *T. hyalinus* survived for 64 and 83 days, respectively, but the larvae in them developed only to the stage which the larvae in *C. vicinus* reached on day 3 at 30°C. They never developed to early third-stage larvae.

Table 1. Periods required for development of second-stage to third-stage larvae of *Gnathostoma nipponicum* in two species of copepods (days post-infection)*.

Temperature (C)	*Cyclops vicinus*	*Termocyclops hyalinus*
35	-†	6
30	6-8	6-8
25	13-17	9-11
20	20-23	15-17
15	-†	-†

* Twenty copepods in each group

† *C. vicinus* died within 2 days and 64 days at 35 and 15°C, respectively, and *T. hyalinus* died within 83 days post-incubation at 15°C.

Discussion

Adult *G. nipponicum,* reported only from Japan, is found in esophageal tumor of weasel. Its distribution includes Iwate, Miyagi, Gifu, Aichi, Mie, Shiga, Kyoto, Osaka, Nara, Wakayama, Hyogo, Tottori, Shimane, Okayama, Tokushima, Ehime, Kagawa, Fukuoka, Kumamoto, Saga, Oita, Nagasaki, and Kagoshima Prefectures with variation of infection rates (Ando *et al.*, 1988b). Recently, we reported two human cases of gnathostomiasis due to ingestion of raw native Japanese loaches, *Misgurnus anguillicaudatus*, from Ueno City, Mie Prefecture (Ando *et al.*, 1988a). Seven early third-stage larval *G. nipponicum* were recovered from 3098 loaches in this City. Eight out of 9 weasels captured in the same district were infected with adult worms of the same species. Clearly, the Ueno district in Mie Prefecture is on endemic area of *G. nipponicum*. As this district is cold

Fig. 4.(a-g) Morphological changes from second-stage to early third-stage larvae of *Gnathostoma nipponicum*. All photographs are the same magnification. Bar indicates 100 μm. Abbreviations used: E; Esophagus, I; Intestine, G; Genital primordium.

a. Free swimming second-stage larva. Note the cuticular sheath of the first molt surrounding the larva (small arrow) and the spinelike thicking at the anterior end (large arrow).
b. Second-stage larva at 0.5 hr PI in copepod.
c. Second-stage larva at 3 hr PI in copepod. Note the inflated anterior end (small arrow).
d. Second-stage larva on day 1 PI.
e. Second-stage larva on day 3 PI.
f. Second-stage larva on day 5 PI.
Note three rows of undeveloped hooklets (small arrow) and cuticle (large arrow) before second molt.
g. Early third-stage larva on day 6 PI.

during winter, it is important to know the influence of temperature on the development of *G. nipponicum* for the study of life cycle in nature.

Mabuchi (1956) conducted preliminary investigations on the influence of temperature on the development of eggs of *G. nipponicum* and reported that all eggs died within 20 and 10 days at 10 and 37°C, respectively. Arita (1953) revealed that eggs can hatch even 8 months after they were kept in refrigerator. Our and Arita's results show that eggs are resistant to low temperature.

Second-stage larvae survived for a long time at low temperature. Nevertheless, the free living period of larvae in nature is probably short because the larvae would be readily ingested by many copepods and branchiopods which live in ponds and rice fields.

Larval development in copepods also depended strikingly on temperature. At 15°C second-stage larvae could not develop to early third-stage larvae within the survival period of copepods.

Weasels usually defecate on stones or concrete bricks along small rivers and eggs in the feces may be dispersed into the river by rain fall. River water is frequently taken to rice fields when cultivation of rice fields begins. Water temperature of the Kizu river in Ueno City is usually over 15°C from May to October (Table 2). In this district, cultivation of rice fields begins at the beginning of May and ends in September. In rice fields, water is always present except for 1 week interruption in July. Since the water temperature during this periods is over 15°C in the river and much higher in the rice fields, it is considered likely that eggs and larvae develop mainly during this time.

Copepods which are reported to be susceptible to larval *G. nipponicum* under experimental conditions are *Mesocyclops leuckarti* (Arita, 1953 and Miyazaki, 1952), *T. hyalinus* (Koga and Ishii, 1981), *C. vicinus* (Koga and Ishii, 1981), *C. strenuus* (Mabuchi, 1956), and *Eucyclops serrulatus* (Arita, 1953, Mabuchi, 1956, and Miyazaki, 1952). *M. leuckarti* and *T. hyalinus* appear only in summer and *E. serrulatus* inhabits throughout the year. *C. vicinus* and *C. strenuus* appear mainly in winter, however, they are present throughout the year except for summer. For example, *C. vicinus* is present until the beginning of June (water temperature, 24°C) in Tsu City. *M. leuckarti, T. hyalinus,* and *C. vicinus* are widely distributed in Japan but *C. strenuus* lives in limited areas. Probably, all these species serve as first intermediate hosts in the field though nobody has discovered early third-stage larvae in cyclops from the field.

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Table 2. Water temperature (C) of Kizu River in Ueno City*

Year	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
1985	1.5	6.8	8.0	11.1	18.4	18.2	22.0	26.6	20.3	16.8	8.1	2.2
1986	4.5	3.0	6.2	12.1	18.6	20.4	24.3	25.8	19.1	15.2	9.2	7.8

* Temperature was measured about the middle of each month.

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