ANALYSIS OF THE ENVIRONMENTAL FACTORS AFFECTING THE LIFE OF THE BRACKISH POLYCHAETE, NEANTHES JAPONICA (IZUKA) II. THERMAL AND SALTY CONDITIONS REQUIRED FOR DEVELOPMENT AND GROWTH

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ANALYSIS OF THE ENVIRONMENTAL FACTORS AFFECTING THE LIFE OF THE BRACKISH POLYCHAETE, *NEANTHES JAPONICA* (Izuka)

II. THERMAL AND SALTY CONDITIONS REQUIRED FOR DEVELOPMENT AND GROWTH

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Some laboratory experiments were conducted concerning the effects of water temperature and chlorinity on the development and growth of *Neanthes japonica*. Both development and growth proceeded normally at water temperatures between 10 to about 30°C and the optimum temperature was around 20°C. The chlorinity range in which developmental changes proceeded normally was found to be 5.2–19.3‰ in the fertilization process, 10.5–17.6‰ in the cleavage stage and 5.7–17.6‰ in the metatrochophore stage at 20°C. In low water temperatures ranging from 6 to 10°C, development accelerated with increasing chlorinity from 10.0 to 17.6‰. The developmental rate of the fertilized egg to the cleavage stage at water temperatures of 4 and 6°C was markedly slower than at 10°C and over. When the water temperature was raised during culture, however, development was accelerated. These results suggest that the natural occurrence of reproductive swarming under the conditions of low temperature and high chlorinity in winter is suitable for the development of the present worms.

INTRODUCTION

The polychaetous annelid *Neanthes japonica* inhabits characteristically in muddy-sand flats in brackish waters where environmental factors such as water temperature and chlorinity are usually variable. It is known that this organism floats out from the substrate and swarms during spring tides in winter. Kagawa (1955) reported that swarming occurred under high chlorinity in the winter seasons, and that the metamorphosed worms migrated upstream after acquiring tolerance to low chlorinities. To know environmental conditions for development and growth, the effects of water temperature and chlorinity on the development after fertilization were studied in the laboratory.

METHODS

Adults *N. japonica* were collected in early winter from the muddy-sand flats at Gamô estuary, Miyagi Prefecture, and reared in sea sand in glass containers

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(length, 68 cm; depth, 22 cm; width, 41 cm) supplied continuously with dilute sea water of 7.0–12‰ chlorinity. Hereafter the concentration of sea water is described as chlorinity (Cl). The stock culture was maintained at 20°C and supplied with activated sludge as diet, which was a floc of bacteria and protozoa. Fertilized eggs obtained by mixing sperm and eggs taken from mature individuals, which floated out from the submerged sand in the stock cultures, were used for the present experiments. All experiments were repeated 8 times, and the mean value was used to minimize variations due to individual differences.

RESULTS

1. *Effects of water temperature and chlorinity on early development*

N. japonica is known to reproduce under the environmental conditions of low water temperatures and high chlorinity (Izuka 1908). Taking water temperature and chlorinity as parameters, an experiment was conducted to find the effects of chlorinity and water temperature on development.

The glass vessels containing sea water diluted to chlorinities of 2.0, 5.2, 7.2, 10.5, 14.0, 17.6 and 19.3‰ were placed for about 2 hours in constant temperature rooms at 3.5, 10, 18, 30 and 40°C. After the water temperature reached the set value, the eggs were inseminated in these vessels, and thereafter fertilization, cleavage, larval formation and metamorphosis were observed intermittently. Fertilization was estimated by the formation of a fertilization membrane. 'Cleavage' means arriving at the morula or early gastrula stage in the present paper. The trochophore stage was estimsted by the number of individuals which formed apical tuft of cilia and prototrochs (Fig. 1 g-i). The nectochaete and segmented larva stage (hereafter called mainly metatrochophore) was estimated by the number of individuals which formed chaetal sacs in the trunk (Fig. 1 k) and formed setigerous segments (Fig. 1 l-n).

(1) *Fertilization* (Fig. 2)

The formation of the fertilization membrane was observed as a criterion of fertilization 2 hours after insemination. Although 90 percent of the eggs were fertilized in the chlorinity range of 5.2–19.3‰ and temperature range of 4–30°C, the eggs swelled and ruptured within a few hours at 2.0‰ Cl. At the extremely high

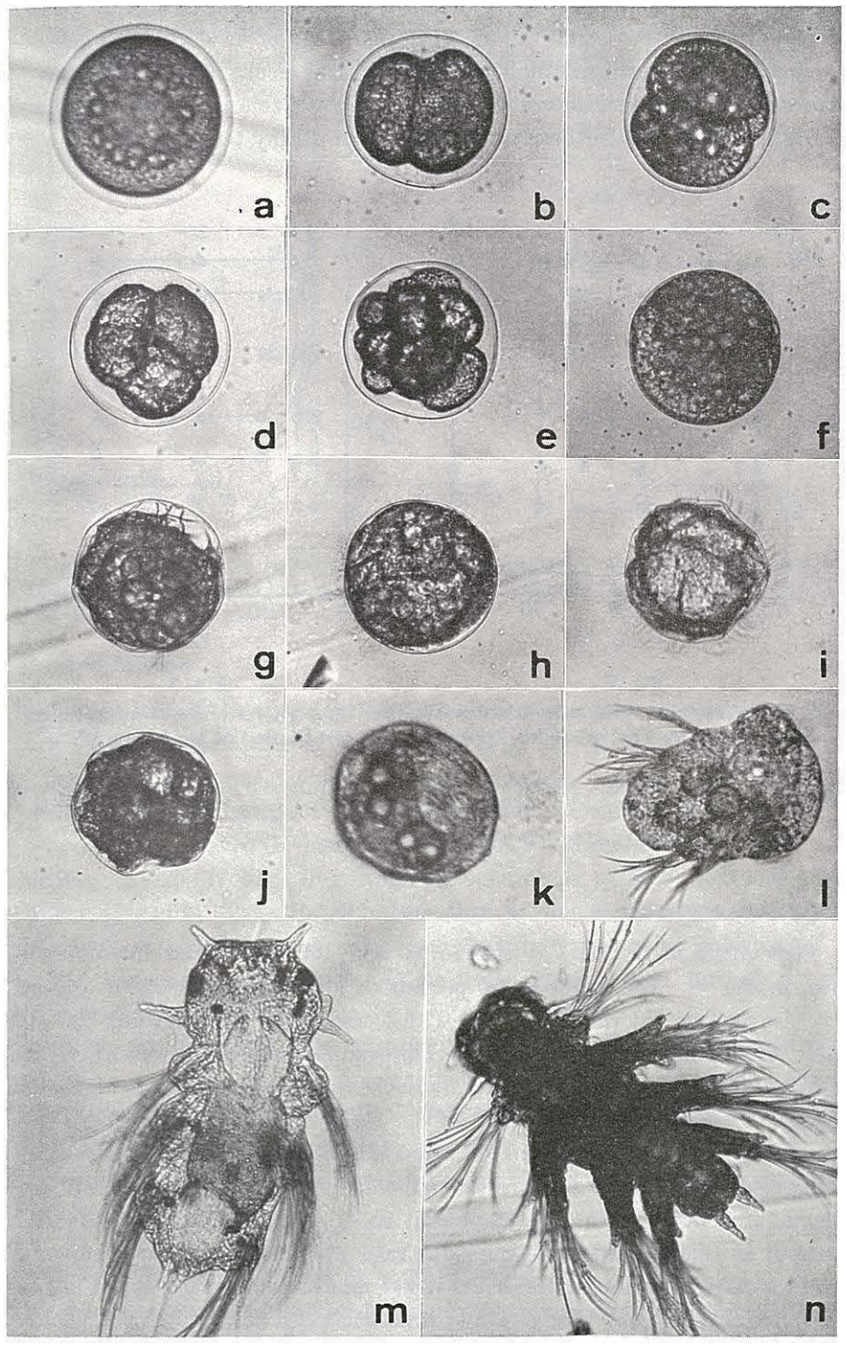

Fig. 1 Developmental stages of *N. japonica*. a: Fertilization, 20 minutes after insemination at 18°C. b: Two cell stage, 3 hours after insemination at 18°C. c: Four cell stage. d: Eight cell stage. e: 4 hours and 30 minutes after insemination at 18°C. f: Cleavage stage. g: Trochophore stage, 18 hours and 30 minutes after insemination at 18°C. h: Trochophore stage. i: Trochophore stage. j: Trochophore stage. k: Stage on changing from trochophore to metatrochophore, 20 hours and 30 minutes after insemination at 18°C. l: Larva with two setigerous segments, 51 hours after insemination at 18°C. m: Larva with three setigerous segments, 72 hours after insemination at 18°C. n: Larva with four setigerous segments, 120 hours after insemination at 18°C. × 100.

temperature of 40°C fertilization did not take place irrespectively of chlorinity. The spermatozoa were highly motile over the range of 4–30°C, but weakened in movement and lost fertilizing ability at 40°C. These results indicate that at a water temperature of 40°C fertilization will not occur at any chlorinity, and at chlorinities of 2.0‰ or less normal fertilization will not occur at any temperature.

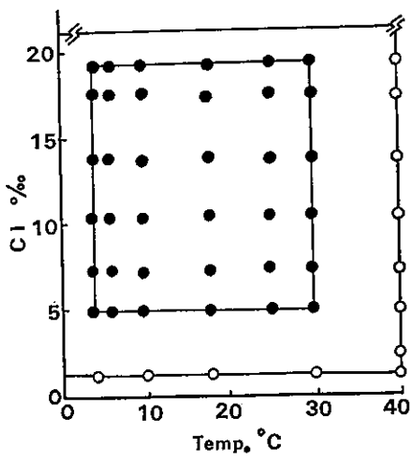

Fig. 2

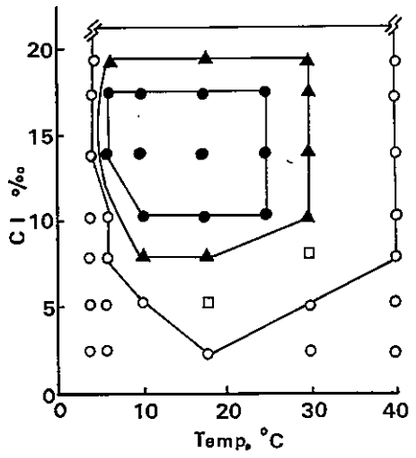

Fig. 3

Fig. 2 Fertilization ratio as a function of water temperature and concentration of sea water (indicated by chlorinity). (●): percent fertilization of more than 90, (○): no fertilization.

Fig. 3 Cleavage ratio as a function of water temperature and concentration of sea water (indicated by chlorinity). Cleavage was scored 100 hours after insemination. (●): more than 90%, (◆): 60–85%, (□): 20–50%, (○): 0%

(2) *Cleavage*

Percentages of the cleaved eggs 25, 55 and 100 hours after insemination are shown in Fig. 4. The majority (90 percent or more) of the fertilized eggs underwent cleavage at chlorinities of 10.5–17.6‰ and water temperatures of 10 and 18°C. At chlorinities of 5.2 and 7.2‰, however, cleavage at a water temperature of 18°C became 20 and 70 percent, respectively. Cleavage was retarded further at water temperature lower than 10°C. At a chlorinity of 19.3‰, cleavage occurred irregularly irrespective of temperature (Fig. 3). This indicates that development seems to be impaired at high chlorinities. At 6°C, the cleavage ratio 25 hours after insemination was 60 percent at 17.6‰ Cl, 3 percent at 14.0‰, and 0 percent at 10.5‰. After 55 hours, the rato was 100 percent at 17.6‰ and 82 percent at 14.0‰, and after 100 hours it was almost 100 percent at all chlorinities except 10.5‰ (Fig. 4). The cleavage ratio increased with increasing chlorinity. At 4°C the eggs did not cleave at all chlorinities and showed no morphological

changes even after 100 hours. At 30°C there was greatly abnormal cleavage resulting in malformed embryos irrespective of chlorinities. This indicates that temperatures of 30°C and over are unfavorable for cleavage.

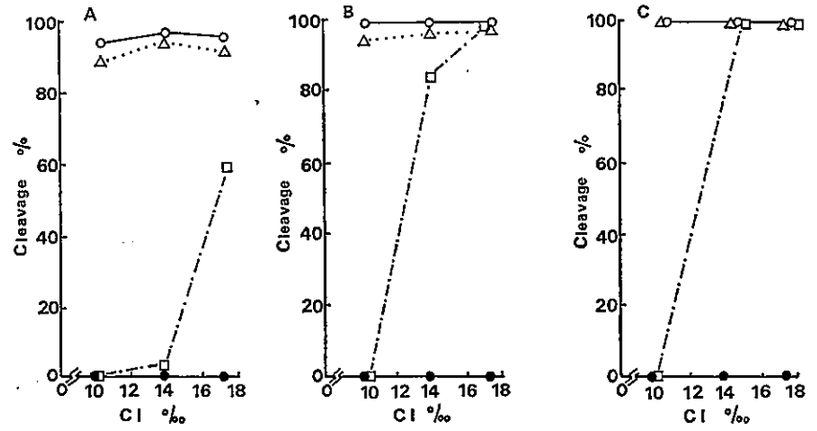

Fig. 4 Cleavage related to temperature and concentration of sea water (indicated by chlorinity). Cleavage at 18 (○), 10 (△), 6 (□) and 4°C (●) 25 hours (A), 55 hours (B) and 100 hours after insemination (C).

(3) *Trochophore*

More than half of the eggs developed into trochophores at Chlorinities of 10.5–17.6‰ and water temperatures of 10 and 18°C (Fig. 5). The rate of development varied depending on water temperature and chlorinity. At 18°C all individuals entered the trochophore stage within 30 hours after insemination and swam by ciliary movement. At 10°C, however, trochophore formation 100 hours after insemination was 25, 77 and 75 percent at chlorinities of 10.5, 14.0 and 17.6‰ respectively (Fig. 6). These results show that at this temperature trochophore formation was not inhibited but rather accelerated by increasing chlorinity. At further low temperatures of 4 and 6°C, no trochophores were formed at any chlorinity even after 150 hours.

When the fertilized eggs were cultured at 30°C for 12 hours and thereafter at 18°C for 12 hours, many malformed individuals were formed. When the normally developed trochophores cultured at 18°C were transferred to 30°C before formation of the setigerous segment, all individuals died 40 hours after transference. These findings indicate that early development is adversely affected by higher temperatures of 30°C and over.

2. *Effect of temperature shock on early development*

The results mentioned above reveal that development is unfavorably affected by chlorinities of less than 2.0‰ or more than 19.3‰ irrespective of water temperature,

and by water temperatures of more than 30°C irrespective of chlorinity. This seems to show that the optimal chlorinity for development is 10.5–17.6‰ in the region of 20°C (Figs. 2–5). It is, therefore, difficult to account for winter swarming in terms of temperature; however, it is an interesting fact that at low temperatures development proceeded more rapidly with increasing chlorinity (Figs. 4, 6). Thus, some attempts were made to determine how development was affected by thermal changes after fertilization following spawning at low temperatures.

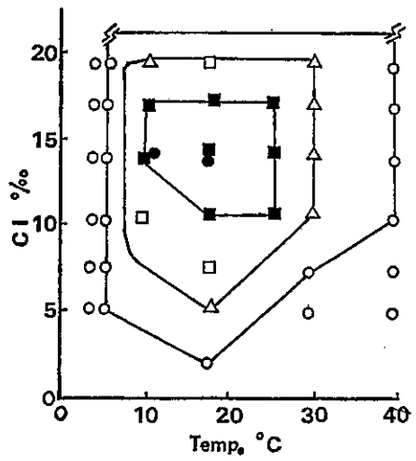

Fig. 5

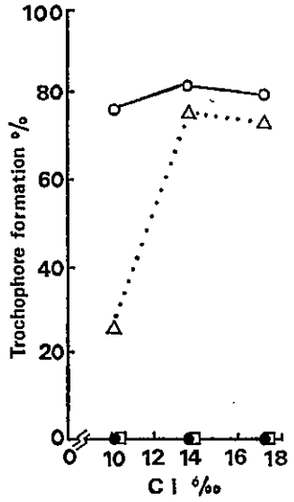

Fig. 6

Fig. 5 Trochophore formation as a function of water temperature and concentration of sea water (indicated by chlorinity). Scored 100 hours ofter insemination. (●): more than 90%, (■): 50–70%, (□): 20–50%, (△): 5–15%, (○): 0%

Fig. 6 Trochophore formation related to temperature and concentration of sea water (indicated by chlorinity). Trochophore formation at 18 (○), 10 (△), 6 (□) and 4°C (●) 100 hours after insemination.

At a constant chlorinity of 14.0‰, which was optimal in the preceding experiment, fertilized eggs were cultured at water temperatures of 4, 6, 10 and 18°C. At 18°C trochophore formation was completed 30 hours after insemination, whereas at 10°C the eggs developed to the gastrula stage 20 hours after insemination and to the trochophore stage after 85 hours. At 6°C the development proceeded to near the gastrula stage in 100 hours after insemination but no trochophores were formed even after 150 hours, while at 4°C neither cleavage nor trochophore formation occurred during this time (Fig. 7).

The fertilized eggs were cultured at 4 and 6°C for 45 and 150 hours, after which temperature was raised to 18°C. As shown in Table 1, cleavage proceeded almost normally after the thermal rise. The ciliary movements and the motility of

the trochophores were as active as those in control cultures at 18°C only. These results show that the eggs retain their developmental ability after exposure to low temperature though their development is apparently retarded during the cooling treatment. Few eggs could develop into trochophores when the fertilized eggs cultured at 4°C for 30 days were transferred to 18°C. This suggests that developmental ability gradually disappears with the passing time even if they are cultured at low water temperature.

To investigate the effect of temperature on the metamorphosis from trochophores to metatrochophores, the eggs were fertilized and allowed to develop until the

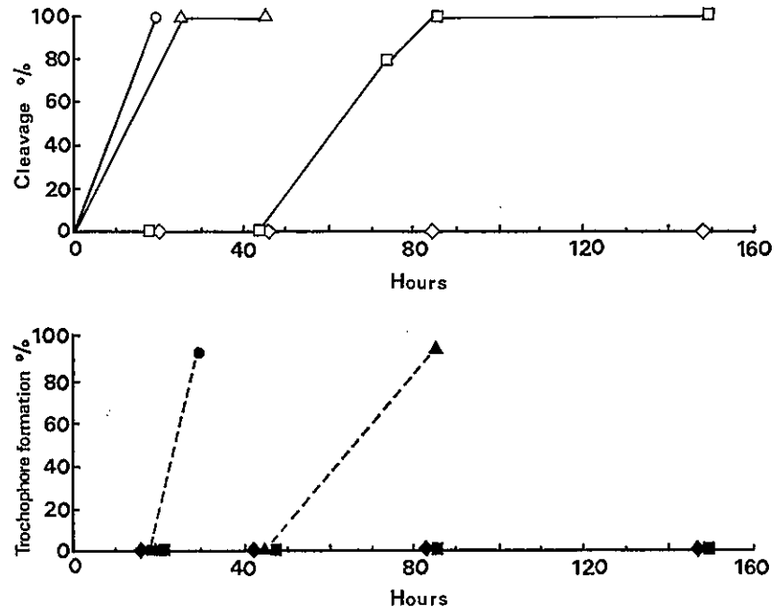

Fig. 7 Cleavage (open) and trochophore formation (closed) related to temperature at 18°C (○, ●), 10°C (△, ▲), 6°C (□, ■) and 4°C (◇, ◆).

Table 1.
Cleavage and trochophore formation under different culture conditions

Culture condition	Cleavage (%)	Trochophore formation (%)
4°C for 45 hrs.→18° for 45 hrs.	96	55
4°C for 150 hrs.→18°C for 45 hrs.	98	10
6°C for 45 hrs.→18°C for 45 hrs.	100	75
6°C for 150 hrs.→18°C for 45 hrs.	100	90
18°C for 30 hrs.	100	95

trochophore stage at 18°C. Then they were transferred to other culture vessels maintained at 4, 6, 10 and 18°C (Tables 1 and 2, and Fig. 1 k-n). The morphological changes of the trochophores were observed at various intervals of time after the transference.

Table 2.
Effect of water temperature on the metatrochophore formation

Water temperature (°C)	4	6	10	18
Time from pretrochophore to metatrochophore (hrs.)	∞*	120	90	27

* No morphological changes were observed even after 200 hours.

At 4°C, no morphological changes were observed even 200 hours after the transference. At 6°C, the trochophores changed into nectochaetes after 120 hours, but setigerous segments were not formed even after 200 hours. At 10°C metamorphosis from trochophores to nectochaetes occurred after 90 hours; after 140 hours the nectochaetes had two setigerous segments and eye-spots, but the yolk persisted in the endopermal tissues even after 200 hours. At 18°C the trochophores changed into the nectochaetes after 27 hours and formed two setigerous segments after 51 hours and a third segment after 72 hours, while at the same time the yolk disappeared and the digestive system became recognizable. When these metamorphosed worms were provided with a diet of *Navicula* sp. and *Chlorella* sp., they developed a fourth setigerous segment after 120 hours and a fifth after 200 hous. Development from the trochophores to the metamorphosed worms with three setigerous segments was seen to be highly susceptible to environmental temperature, retarding in low water temperatures.

The above experiment, in which the water temperature was changed at various stages from the fertilization to the nectochaete stage, showed the development was inhibited to a considerable extent at low temperatures (4 and 6°C), but accelerated rapidly with rising temperature (Table 1, 2). Thus, the embryos and larvae of *N. japonica* are thought to be relatively tolerant to low water temperatures.

3. *Effects of water temperature and chlorinity on post-larval development*

The above experiments mainly dealt with the relationship of chlorinity and water temperature responsible for development from the fertilization to the trochophore stage. The present experiment was conducted to examine the effects of these two factors on post-trochophore development. Thirty larvae with three setigerous segments were cultured in 100 m*l* beakers containing 20 m*l* of dilute sea water with and without aeration at different temperatures of 3.5, 10 and 20°C and chlorinities ranging from 2.0 to 17.6‰ (Fig. 8). In the cultures at 3.5°C and at

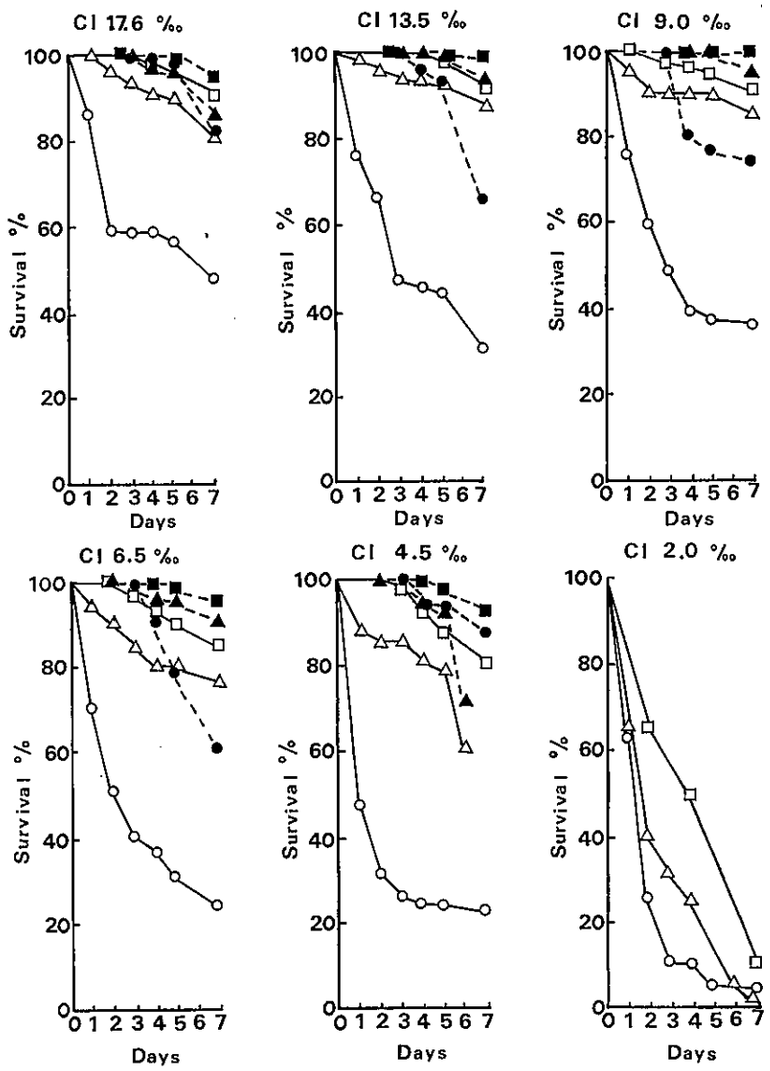

Fig. 8 Survival of metatrochophores related to temperature and concentration of sea water (indicated by chlorinity) with (closed) and without (open) aeration at 20°C (○, ●), 10°C (△, ▲), and 3.5°C (□, ■).

chlorinities of 4.5‰, the majority of the metatrochophores survived although the movements were sluggish. At 2.0‰ chlorinity, however, many metatrochophores swelled and ruptured due to hypotonicity at all temperatures, and the mortality was 90 percent or more. At 10°C and chlorinity of 4.5‰ or over, the survival was 80–90 percent in the cultures without aeration and 90–100 percent in the cultures with aerations. The metatrochophores moved more actively than at 3.5°C. At 20°C

and chlorinity of 4.5‰ or over, the survival in aerated cultures was 80–95 percent, but mortality increased with decreasing chlorinity in the cultures without aeration. This was attributed to the adverse effects not only of the decrease in chlorinity but of the decrease in dissolved oxygen concentration accompanying temperature rise.

To know the effect of water temperature on the metatrochophores thirty larvae with three setigerous segments were placed in diluted sea water of chlorinity 17.6‰, which was optimal for the survival, and cultured at various temperatures with and without aeration (Fig. 9). In aerated cultures little difference was seen in the survival over the range of 3.5–20°C, but at 30°C the movements of setigerous segments became sluggish and the animals remained almost still. At 35°C this trend became more marked and a moribund condition persisted until death occurred on the fifth day. In the cultures without aeration the temperature rise decreased the survival in the order of 35, 30, 20, 15 and 10°C.

These results show that the metatrochophores are able to survive in well-oxygenated media of chlorinity exceeding 4.5‰ under the temperature of up to 30°C, but the temperatures of 10–20°C are more suitable for growth and survival.

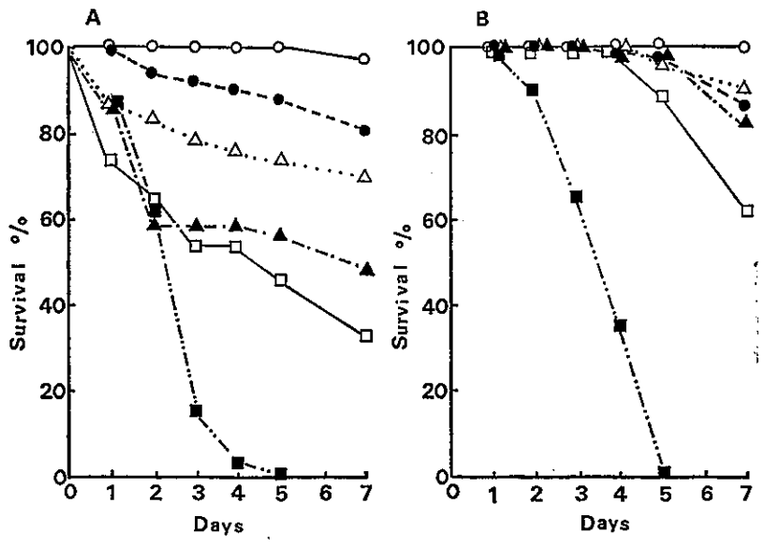

Fig. 9 Changes of survival in metatrochophores under different water temperatures with (B) and without (A) aeration at 3.5 (○), 10 (●), 15 (△), 20 (▲), 30 (□) and 35°C (■).

4. *Effect of water temperature on growth of the young worm*

A number of experiments were conducted in constant temperature rooms of 3.5, 10 and 20°C, using plastic containers (length, 13 cm; depth, 4 cm; width, 8 cm) containing sea sand supplied with dilute sea water of 5.0‰ chlorinity, and activated sludge was supplied as diet. The young worms 30 days after insemination at 20°C were transferred to the culture vessels. They were classified into two groups of 0.01 and 0.05 g in mean wet weight. In each group seventy larvae were cultured in each vessel for 20 days, and then their wet weight was measured (Table 3-a, b). At 3.5°C the worms remained motionless in the sand, showing no feeding behavior and almost no growth (negative growth in some cases). At 10°C feeding behavior and growth were observed; the body weight increased to 0.1–0.2 g in 36.4 percent of the worms which originated from the group having 0.05 g initial weight, and to 0.01–0.05 g in 79.6 percent of the 0.01 g group. At 20°C the animals fed actively and increased remarkable in body weight. These findings indicate young worms require at temperature of at least about 10°C for their growth.

Table 3.
Distribution of body weight of young worm related to water temperature

a. Initial wet weight 0.01 g

Water temperature (°C)	Wet weight (g) 0.01 ~ 0.05	0.051 ~ 0.100	0.101 ~ 0.150	0.151 ~ 0.200	0.201 ~ 0.250
3.5	100 %	0 %	0 %	0 %	0 %
10	79.6	20.4	0	0	0
20	40.0	30.0	6.7	20.0	3.3

b. Initial wet weight 0.05 g

Water temperature (°C)	Wet weight (g) 0.01 ~ 0.05	0.051 ~ 0.100	0.101 ~ 0.150	0.151 ~ 0.200	0.201 ~ 0.400	0.401 ~ 0.600
3.5	58.3 %	41.7 %	0 %	0 %	0 %	0 %
10	27.2	36.4	18.2	18.2	0	0
20	0	0	0	0	81.8	18.2

DISCUSSION

According to our observation at Gamô estuary, the breeding of *N. japonica* may occur during spring tides in winter. KATSUTANI *et al.* (1971) have reported that *N. japonica* swarms from late December to early January, and that in this period the temperatures of the water and of the mud substrate are 7–11°C and 8–9°C in December, and 5–8°C and 7–8°C in January respectively. The experimental results obtained in this study show that water temperature and chlorinity were the important environmental factors which control the developmental rate. Although development was almost inhibited at 4°C irrespective of chlorinity, the developmental rate was increased at 6°C with increasing chlorinity from 10.5‰ to 17.6‰. At 18°C the change in the developmental rate was scarcely recognizable even though

chlorinity was changed. Thus, this reveals that the temperature below 4°C is undesirable as an environmental condition for development, and a temperature of more than 6°C is necessary to maintain development which is accelerated with increasing chlorinity at low water temperatures. When the eggs placed at a temperature lower than 5°C were transferred to higher temperature conditions, they were able to initiate normal development. The young worms also responded vigorously to food and grew rapidly at a temperature of more than 10°C.

Thus, the occurrence of breeding during spring tides in winter may be explained by the results that the environmental conditions with high chlorinity and low temperature are more preferable to fertilization, cleavage and survival of larvae. The growth during the season from winter to spring may be accelerated by the rising in the water temperature in this season.

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