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

III. THE EFFECTS OF THE ENVIRONMENTAL FACTORS ON FERTILIZATION, CLEAVAGE AND POST-LARVAL DEVELOPMENT

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The effects of the environmental factors on the development of the polychaete *Neanthes japonica* were studied. The population density of the worms in nature reduced remarkably in January because of the floating for reproductive swarming. The percentage of the mature individuals produced from the worms collected from May to September was as low as 0–6.4%, while it increased to 43% in early winter. When the unfertilized eggs were pre-incubated at 4°C, their fertilization and cleavage ability at 18°C increased by 3–4 times those of the eggs kept at 18°C. At a water temperature of around 10°C protozoans such as those of *Trachelophyllum* type, a predator of eggs, did not appear, but at 20°C the metamorphosis from the trochophore to the nectochaete reduced to less than 3% probably due to the appearance of the numerous protozoans. The development in the early stage from fertilization to cleavage stage was normal at COD 50 mg/l, while as flocculation was accelerated with the increase of bacteria, the trochophore died even at COD 30 mg/l because of floc that entangled their cilia and inhibited dissolved oxygen uptake.

INTRODUCTION

The polychaetous annelid *Neanthes japonica* is a characteristic inhabitant of intertidal muddy-sand flats in brackish waters. As IZUKA (1908) has reported, its breeding season is winter; around mid December the adults of which body cavities are filled with gametes, emerge from the bottom up into the water to swarm. INAMORI and KURIHARA (1979) found that the development proceeded more rapidly with the increasing chlorinity at low water temperatures. To elucidate the reasons for such winter swarming, the effects of the environmental factors on individuals developing until the completion of normal growth need clarification. In the present study, the field observations were made to confirm the swarming season, and some experiments were conducted to see the effects of the environmental factors on fertilization, cleavage and development from the trochophore to the metatrochophore.

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METHODS

Field survey on the population dynamics of *N. japonica* was done at the tidal flats of the Gamô estuary, Miyagi Prefecture. The adults were collected from 50 stations of the tidal flat by digging out a 50 cm square area to a depth of 30 cm during the period from May 21 to December 31 in 1975, and after counting population densities of the adults they were reared in tanks for two months with a density of 10–27 individuals/100 cm^2 to determine the occurrence of reproduction. In rearing experiments, the sand filtration method was used to supply sea water diluted to a chlorinity of 7.0–12.0‰ into the tanks containing clean sea sand. The diet used was activated sludge which consisted of a mixture of bacteria and protozoans, cultured by aerating a substrate of organic matter and inorganic salts. The emergence of young and reproductive worms was observed every day during the periods of experiments from May 21 in 1975 to February 20 in 1976. Additionally, the effects of the environmental factors on the development and growth were examined by exposuring the gametes obtained from the reproductive worms into the different cultural conditions. The experiments were repeated several times using eggs and sperms from different parents to minimize variation due to individual difference, and all the data were given by the mean values of the results.

RESULTS

1. *Seasonal changes in the occurrence of the reproductive worms.*

The seasonal variation of population size in the field was investigated by sampling *N. japonica* at intervals of 2 and 3 months. The population number of *N. japonica* in natural environment varied seasonally as follows: 1,200/m^2 in June; 1,000/m^2 in September; 1,100/m^2 in December; 2/m^2 in January. They were abundant from June to December while most of them disappeared in January.

The adults collected from the tidal flats at different seasons were reared in experimental tanks for two months, and the numbers of the adult, reproductive worms and young worms are shown in Table 1. A few individuals emerged from the samples collected in the data except December 31 and their ratio to the initial worm number was extremely low in the range of 0–6.4 precent. The samples collected on June 15, July 10 and August 30 showed the occurrence of a large number of young worms bred during rearing. The gametes obtained from the mature worms during the periods from May to September were not fertilizable. However, a large number of mature worms emerged from speciments collected on December 31, and the ratio of mature individuals to the initial worm number attained to 43 percent. The reproductive worms obtained during this period had fertilizable gametes. Number and sex ratio of individuals emerged from the December 31 – samples are shown in Table 2. Both males and females were observed to emerge in large numbers from 9 to 12 P.M., although there were some daily fluctua-

Table 1.
Maturation of the individuals collected from the field in different seasons

Date	Initial number of adults /container	Final number of young worms /container	Final number of reproductive worms /container			Maturation*
			Male	Female	Total	
May 21	175	0	1	1	2	1.1
June 15	70	225	1	8	9	5.1
July 10	15,000	300,000	—	—	—	—
July 17	140	0	7	2	9	6.4
August 13	320	0	0	0	0	0
August 30	100	200	1	1	2	2.0
September 5	421	0	0	0	0	0
December 31	1,370	—	303	289	592	43.0

$$* \frac{\text{Number of reproductive worms at final}}{\text{Number of adults at initial}} \times 100$$

Table 2.
Number of emerged mature individuals

Days		Male	Female
January	22	2	3
〃	23	1	1
〃	24	3	1
〃	25	6	10
〃	26	16	14
〃	27	25	21
〃	28	8	9
〃	29	14	10
〃	30	7	6
〃	31	0	1
February	6	7	7
〃	7	17	23
〃	8	12	17
〃	9	9	9
〃	10	12	14
〃	11	17	17
〃	12	17	11
〃	13	15	16
〃	14	13	14
〃	15	20	26
〃	16	25	15
〃	17	26	20
〃	18	23	18
〃	20	8	6
Total individuals		303	289

Note: Reproductive worms emerged from samples collected on December 31. The sex ratio (male/female) was 1.05.

tions. The emergence of mature individuals is said to be related to the age of the moon in the field (IZUKA, 1908). In this experiment, however, the emergence occurred every day from January 22 to February 20 irrespective of the age of the moon.

From the results as above, it was proved that *N. japonica* had the possibility of reproduction throughout the year but the gamete maturation tended to be synchronized with winter when low water temperature prevailed. Therefore swarming period seems to be apparently limited to winter.

2. *Effect of water temperature on the developmental ability of eggs.*

The gametes obtained from the mature worms from the December 31–sample were used to examine the thermal effects on the maintenance of the developmental ability of the eggs. The unfertilized eggs were immersed in diluted sea water (Cl 13.5 and 17.0‰) contained in the petri dishes, 10 cm in diameter, which were placed in the constant temperature rooms at either 4 or 18°C. After being exposed to 4 or 18°C for 10–100 hours, they were inseminated with the actively motile sperm and allowed to develop at 18°C. Resulting fertilization (fertilization membranes formation) was observed 10–120 minutes after insemination and cleavage (early gastrula formation) after about 20 hours.

As shown in Fig. 1, when the eggs were kept at 18°C and 13.5‰ Cl, their fertilizability were maintained at a high level of more than 90 percent fertilization for the first 40 hours of the pre-treatment, but they became infertilizable in the more prolonged treatment. The cleavage proceeded normally for the first 25 hours of the pre-treatment, but were arrested or blocked in the longer treatment. At

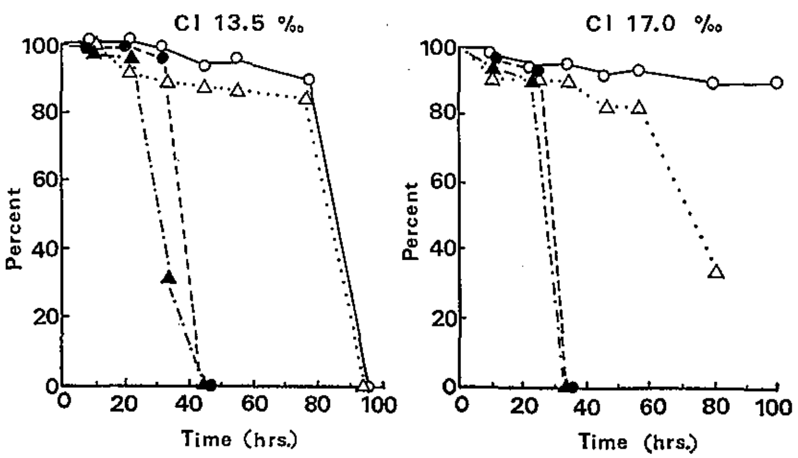

Fig. 1 Fertilization and cleavage in the eggs kept at 4°C and 18°C prior to insemination. ○: Fertilization at 4°C; ●: Fertilization at 18°C; △: Cleavage at 4°C; ▲: Cleavage at 18°C. The oridinates indicate fertilization and cleavage in percent and the abscissas times of preincubation in hours.

17.0‰ Cl, both ratios of fertilization and cleavage were high for the first 25 hours and then declined rapidly. In comparison, when the eggs were kept at 4°C both fertilization and cleavage at 13.5‰ Cl remained at 90 percent over for 80 hours, but fell to zero after 100 hours. At 17.0‰ Cl fertilization was possible for 100 hours but cleavage declined to about 30 percent after 80 hours.

Thus, it was found that newly released eggs kept at 4°C maintained their developmental ability for three or four times longer than those at 18°C. This shows that at low water temperatures the developmental ability of the eggs is maintained over a long period. This suggests that in the natural habitat the probability of fertilization is increased in winter.

3. *Effect of the presence of protozoan on development*

When the fertilized eggs were placed for 24 hours at 20°C. The protozoans were observed to multiply in the culture vessels, although no artificial inoculation was performed with protozoans. Almost all of the protozoans which appeared in cultures were of the free swimming *Trachelophyllum* type (Holotricha, 40 μ length), the reptant *Oxytricha* sp. (Hypotricha, 40 μ length), or *Euplotes* sp. (Hypotricha, 80 μ length). Metatrochophore formation under the contamination of the protozoans was shown in Table 3. The metatrochophore formation seems to be little affected by the presences of *Oxytricha* sp. and *Euplotes* sp. On the other hand, the metatrochophore formation varied negatively with the number of *Trachelophyllum* type. In the vesseles which contained 1,510–7,170 individuals/ml of *Trachelophyllum* type, the metatrochophore formation was completely suppressed. The protozoans seemed to attack actively and prey on embryos and on trochophores. In culture at water temperatures of less than 10°C neither *Trachelophyllum* type, *Oxytricha* sp., nor *Euplotes* sp. appeared, and only a few small flagellates were observed. Thus, harmful effect of protozoan on the development is thought to be markedly reduced at low water temperatures.

Table 3.
Effect of protozoans on metatrochophore formation

Number of protozoans (/ml)			Metatrochophore formation (%)
Euplotes sp.	*Oxytricha* sp.	*Trachelophyllum* type	
30	290	7,170	0
20	210	7.800	0
30	200	1,510	3
40	250	20	43

4. *Effect of organic pollution on development*

Since bacterial reproduction was also known to be inhibited at low temperatures, an experiment was conducted to determine how development was affected by bacterial reproduction and the underlying factors of culture media.

Organic pollution progresses accompanying with multiplication of bacteria which eventually aggregate to form floc-like bodies (called "floc" hereafter). When bacteria are active the survival ratio of *N. japonica* during the developmental process may drop, perhaps because of a decrease in dissolved oxygen concentration (DO) in and around the floc, in which the cilia or setigerous segments of trochophores and nectochaetes were entangled. Samples of 200 eggs were placed in the rearing vesseles to which diluted sea water (Cl 14.0‰) containing corn-steep liquor with chemical oxygen demand (COD) determined by the $KMnO_4$ method (JAPAN SEWAGE WORKS ASSOCIATION 1967) ranging from 0 to 60 mg/l were supplied continuously. The eggs were inseminated in these solutions at 20°C and allowed to develop to the swimming larvae. At COD of up to 50 mg/l the spermatozoa were active and the eggs were fertilizable in almost 100 percent (Fig. 2). There was also very little effect on cleavage: 90 percent eggs cleavaged at 50 mg/l COD. However, trochophore formation was inhibited at COD of 20 mg/l and above. Until trochophore stage, 35 hours after fertilization, the bacteria multiplied to form

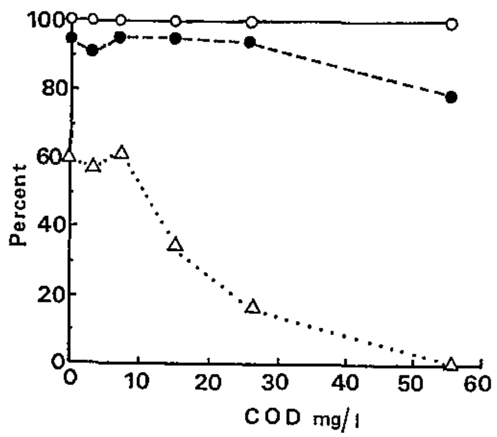

Fig. 2 Effect of COD in the culture-media on development.
○: Fertilization; ●: Cleavage; △: Trochophore formation.

a floc in the sea water with COD from 20 to 50 mg/l, and the cilia of trochophores were entangled with floc. These results show that the early development from fertilization to gastrula tolerates a fairly high concentration of organic pollution, but in the swimming larvae bacterial floc becomes a harmful factor. As an example to the inhibitory action of floc, 50 and 2,000 mg of activated sludge washed well with saline water by centrifugation were added to two tanks (10 cm in length and breadth, 20 cm in depth) containing a 10 cm layer of sea sand. In each of these tanks 200 nectochaetes immediately after changing from trochophores were introduced and reared at 20°C in diluted sea water (Cl 14.0‰). After one month,

no survivors were found in the tank with 2,000 mg of sludge, while in the tank with 50 mg of sludge about 100 juveniles (as estimsted by the number of burrows) were still alive.

5. *Vertical distribution of fertilized eggs and metatrochophores with different stages.*

The vertical distribution of individuals from the fertilized egg to the metatrochophore stage was investigated using 100 ml graduated cylinders, 17 cm in depth, containing diluted sea water (Cl 14.0‰) and an adequate dissolved oxygen content. About 200–300 individuals at various developmental stages were introduced into these cylinders. After shaking vertically several times the cylinders were allowed to stand for 1 hour, and then the number of individuals

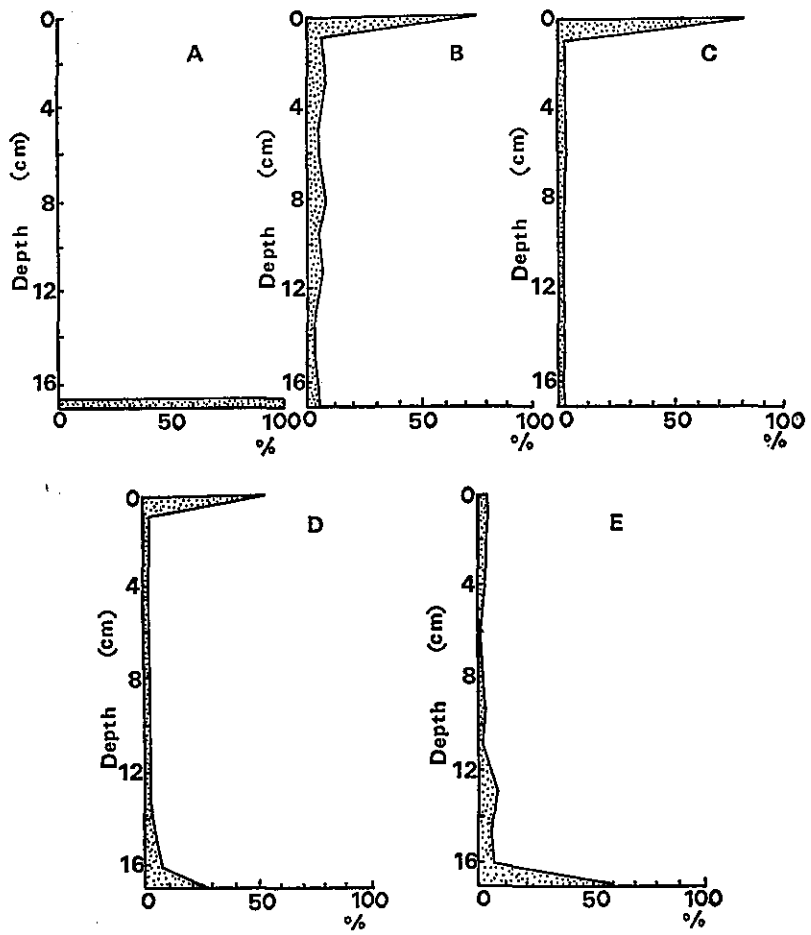

Fig. 3 Vertical distribution of different developmental stages. A: Pre-trochophores, B: Metatrochophores without setigerous segments, C: Metatrochophores with 2 setigerous segments, D: Metatrochophores with 3 setigerous segments, E: Metatrochophores with 3–4 setigerous segments.

distributed at each water depth was counted with the naked eye. The observation was made after 1 hour at 20°C because a distinctive distribution pattern did not appear until 1 hour after standing. As shown in Fig. 3 the unfertilized eggs were on the bottom, but a majority of the larvae before formation of setigerous segments and of the 2-day-old metatrochophores with two setigerous segments were swimming at the surface. On the fourth day after changing from trochophores to metatrochophores, the larvae with three setigerous segments were distributed mainly at the surface (54 percent) and at the bottom (30 percent), whereas on the tenth day, when the fourth setigerous segments had begun to appear, less than 10 percent larvae were at the surface while 60 percent were at the bottom. The habitat shifted gradually toward the bottom as time passed.

From the above results and those reported previously (INAMORI and KURIHARA 1979) that the metamorphosis from nectochaetes with 0–2 setigerous segments to more than 3 setigerous segments was inhibited at low water temperature (4–10°C), it can be deduced that at low temperature the planktonic stage of the life cycle is lengthend. Thus, under the low-temperature environment of winter the time during which the nectochaetes dispersed over the tidal flats and the entire river mounth area by the tidal movement may be extended with a consequent enlargement of their habitat. These nectochaetes then grow and settle in the same locality, but some are thought to migrate upstream after they have developed larvae with six pairs of setigerous segments and acquired tolerance to low chlorinity as reported by KAGAWA (1955).

Larvae with three or four setigerous segments were cultured in tanks containing sea sand supplied with diluted sea water of 14.0‰ chlorinity. No individuals were found floating or swimming in the water before the formation of burrows. This shows that when there is little or no change in chlorinity *N. japonica* may enter benthic life even at the three or four setigerous segments stage.

DISCUSSION

When the adults of *N. japonica* collected during the periods from Spring to Autumn were reared in the vessels at a temperature of 20°C, the reproductive worms developed were very few, whereas the worms collected in winter produced a large number of reproductive worm. These results coincided with the field observations that the population size of *N. japonica* fell remarkable in winter and only a few matured individuals were collected, indicating that reproduction occurred little in the seasons except winter. From the observations that the worms disappeared suddenly from the substrate in winter and the production of the reproductive worms was extremely low in seasons other than winter, it is an indisputable fact that the reproductive swarming occurs in winter. The present experiments revealed the following points. 1) Since the fertilizable and developmental abilities are retained for a longer time at low water temperatures, the chance of fertilization is increased.

2) Ciliate protozoa, *Trachelophyllum* type, seems to attack the eggs after fertilization and during cleavage, reducing the proportion of individuals which complete the metatrochophore formation. 3) Bacterial flocs entangle the cilia or the chaetal bundles of trochophores or nectochaetes and inhibit their development. This seems to be attributed both to the activity of the bacteria in the floc and to the accompanying oxygen decrease in the region of the floc. 4) Metamorphosis from the trochophores to the metatrochophores needs a long time at low temperature. Hence the planktonic period is prolonged and the distribution is meanwhile disperesed over a wider area. 5) Since eggs are often present at high densities after the winter swarming, in the experimental tanks as well as in nature local oxygen deficiency is expected to occur due to oxygen consumption by the eggs.

Thus, it is evident that the initial stage of development and growth are affected by various factors such as DO, water temperature, chlorinity, protozoal and bacterial activity, floc and organic pollution. All the above factors are considered to act nagatively to the early development at high water temperature. Further, it is thought that the winter period does not provide a favorable living environment for *N. japonica*, as the feeding behavior of the adult becomes sluggish. Therefore, it is considered that *N. japonica* was adapted to grow in the period from spring to autumn when warm water temperatures are maintained, and to mature and breed in winter during which growth ceases. The growth of the new individuals were accelerated by the rise in water temperature during the period from winter to spring, and thus the life cycle of *N. japonica* is established.

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