# ANALYSIS OF THE ENVIRONMENTAL FACTORS AFFECTING THE LIFE OF THE BRACKISH POLYCHAETE, NEANTHES JAPONIOA (IZUKA) I. THE EFFECTS OF THE ENVIRONMENTAL FACTORS ON SURVIVAL AND GROWTH


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

## I. THE EFFECTS OF THE ENVIRONMENTAL FACTORS ON SURVIVAL AND GROWTH

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The effects of the environmental factors on the nereid *Neanthes japonica* were studied in the laboratory to help the analysis of effective factors in the natural habitat.

The nereid worms fed on a floc of activated sludge (composed mainly of micro-organisms and resembling detritus) discharged by sewage treatment plants. They took the sludge and egested well-formed fecal pellets. They could feed in the range of temperature from about 6 to 30°C, but could not survive at over 37°C. The optimum temperature for growth was about 20°C.

The nereids were able to survive in a wide range of chlorinity from 0.5 to 18.0 ‰ in non-polluted conditions, but difficult to live in concentrated saline water containing pollutants.

The amount of food intake of each individual, the food conversion efficiency and the rate of body weight gain declines with increasing population density.

Feeding activity was closely related with dissolved oxygen concentration, decreasing markedly unless the dissolved oxygen was maintained at more than 3 mg/*l*.

### INTRODUCTION

The nereid polychaete *Neanthes japonica* is a brackish-water benthic species which characteristically inhabits tidal flats. As these are frequently formed in estuaries, where sea water and fresh water are intermixed, environmental factors such as chlorinity, temperature, chemical substances, substratum, etc., tend to fluctuate widely with the periodic ebb and flow of the tide. TSUCHIYA and KURIHARA (1976) reported a close relationship between tidal flat benthos and environmental factors such as the particle size distribution, organic content, and oxidation-reduction potential of the substrate, and YOSHIDA (1970), and INAMORI and KURIHARA (1979a) made similar observations on *Perinereis nuntia* var. *vallata*. NAGOSHI (1972) cited, as factors which affect the growth of organisms, the inorganic

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environmental factors such as temperature, light, chemical substances, available space, etc., and biological factors such as the amount of available food.

The present paper reports several experimental findings on the reactions of *N. japonica* to changes in various environmental factors, in order to shed light on the ecology of adults of this species, which is one of the typical nereid of the tidal flat.

## METHODS

Rearing experiments were conducted according to the sand filtration method, by covering the bottom of a round acrylic resin container (3*l* in volume) with glass wool, by filling it to a depth of 10 cm with sea sand, into which the nereids were then introduced, and supplying a continuous flow of the diluted sea water from the upper part of the tank (Fig. 1). As a rule, population density was set at 20 individuals (of wet weight 1 g) per 150 $cm^2$ rearing vessel, and the experimental tanks were placed in constant temperature rooms to maintain water temperature

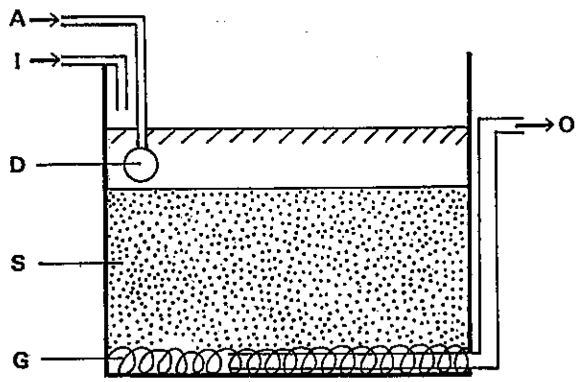


Fig. 1. Experimental apparatus. A: Air; I: Inflow; D: Diffuser; S: Sand; G. Glass wool; O: Outflow.

at 20°C. The diet consisted of activated sludge composed mainly of bacteria was produced in continuous culture tanks by aerating organic materials and inorganic salts. The sludge was placed carefully on the surface of the sand in which the nereids were kept, and the decrease in the amount of sludge present was taken to be the amount of food intake. The difference in individual body weights between the beginning and end of each experiment was divided by the number of days of that experimental period to obtain the body weight gain per day, and this was divided by the amount of food intake to derive the food conversion efficiency. To estimate the amount of food intake and body weights in terms of dry weight, the worms were dried at 110°C and weighed. The following relationship between wet weights and dry weights of worms at chlorinity of 4.5‰ was obtained:

$$\text{Dry weight} = 0.145 \times \text{wet weight}$$

This equation was used in principle in the following experiment.



Sea water collected from Sendai Bay was used after dilution with demineralized water. Chlorinity was determined by MOHR's method, COD by the $KMnO_4$ method, $NH_4^+$-N by the distillation method, and DO by the azide modification of the Winkler method (JAPAN SEWAGE WORKS ASSOCIATION 1967).

## RESULTS

### 1. *Effect of concentration of the sea water*

To investigate the effect of concentration of the sea water on growth, the sea water diluted with demineralized water to a chlorinity of 0.5, 1.0, 4.5, 9.0, 13.5 or 17.0‰ was passed into each tank at the rate of 1*l* per day, while activated sludge as much as the animals could consume was placed in each tank as diet, and their food intake, weight gain, and survival ratio were determined. The specimens about 1 g wet weight were placed, at a density of 20 individuals per 150 $cm^2$ vessel, and the experimental period was 14 days.

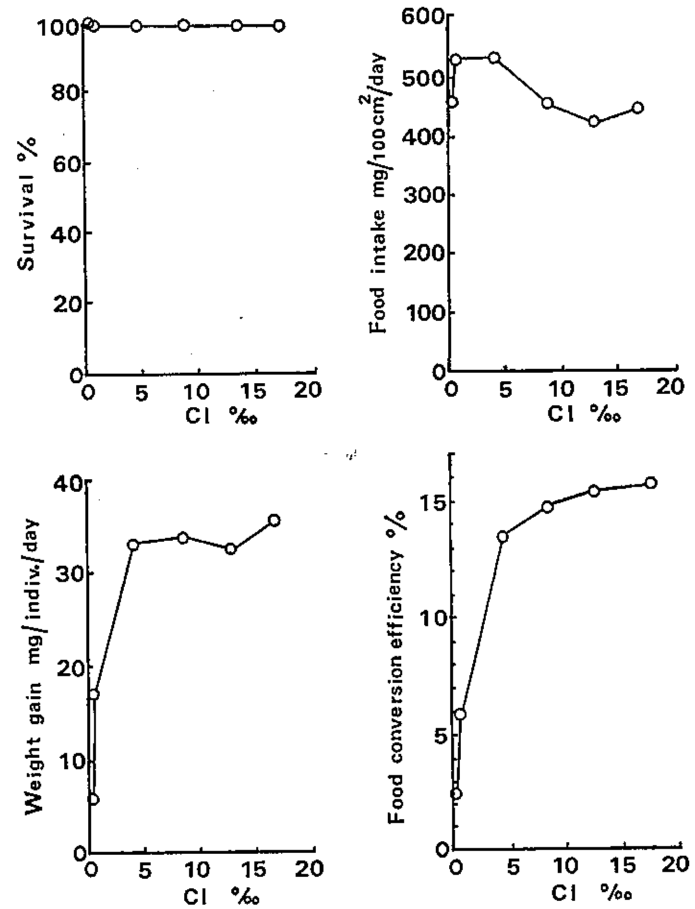


Fig. 2 Effect of concentration of the sea water (indicated by chlorinity).

Fig. 2 shows the food intake, the weight gain, the food conversion efficiency and the survival ratio of the nereids in sea water of different chlorinities. At each chlorinity all individuals survived and they took in 400–540 mg foods/100 cm²/day. Individual weight gain, however, was 30 mg/day or more at chlorinities of 4.5‰ and above, but only 6 and 17 mg/day at 0.5 and 1.0‰ respectively. Similarly, the food conversion efficiency was 12.1 percent or more at 4.5‰ and above, but decreased to 2.6 and 6 percent at 0.5 and 1.0‰, respectively. Thus, it was clear that growth was retarded in an environment of constantly low chlorinity and that adults of 1 g weight could survive adequately in the diluted sea water.

2. *Effect of water temperature*

The experimental tanks were placed in constant temperature rooms to maintain water temperature at 3.5, 6, 9.5, 20, 30, 35, 37, and 43°C.

Rearing was carried out with a supply of diluted sea water of chlorinity 4.5‰ and an adequate supply of activated sluege as diet. Twenty individuals of body wet weight about 1 g were placed in each tank, and cultured for 38 days at 3.5 and 9.5°C, and for 14 days at 20 and 30°C.

As shown in Fig. 3, adult nereids were able to survive adequately over a wide temperature range from 3.5 to 30°C, but ingestion of food ceased entirely at 3.5°C and these individuals therefore showed negative growth. Though feeding behavior was observed at 9.5°C, the weight gain decreased. At 20 to 30°C food intake showed only 10 percent decrease, but weight gain fell off by about 30 percent over this range. An actual increase in the frequency of peristaltic movements within the burrow was observed at 30°C. Thus the optimum water temperature for growth was found to be about 20°C for adults *N. japonica.*

The animals crawled out of their burrows and moved violently on the sand surface at 35°C. At 37°C they gradually weakened and died after 2 or 3 days. At 43°C they were observed to die either in the sand or on its surface after about 2 hours.

3. *Effect of population density*

To investigate the effect of population density on the growth of *N. japonica*, some experiments were carried out. The various size worms used for the experiment were collected at the intertidal flats of Gamô estuary, Miyagi Prefecture and collected at the rearing tanks in the laboratory.

First, three size groups, small size group with body wet weight of about 0.1 g, medium of about 0.4 g and large of about 1.0 g, were prepared in the containers at various densities. Diluted sea water of 4.5‰ Cl was passed through each container, and the water temperature was held at 20°C. The diet was supplied as much as the worms could consume. The experimental period was 14 days. The weight gain and the amount of food intake were measured on each case. The results are



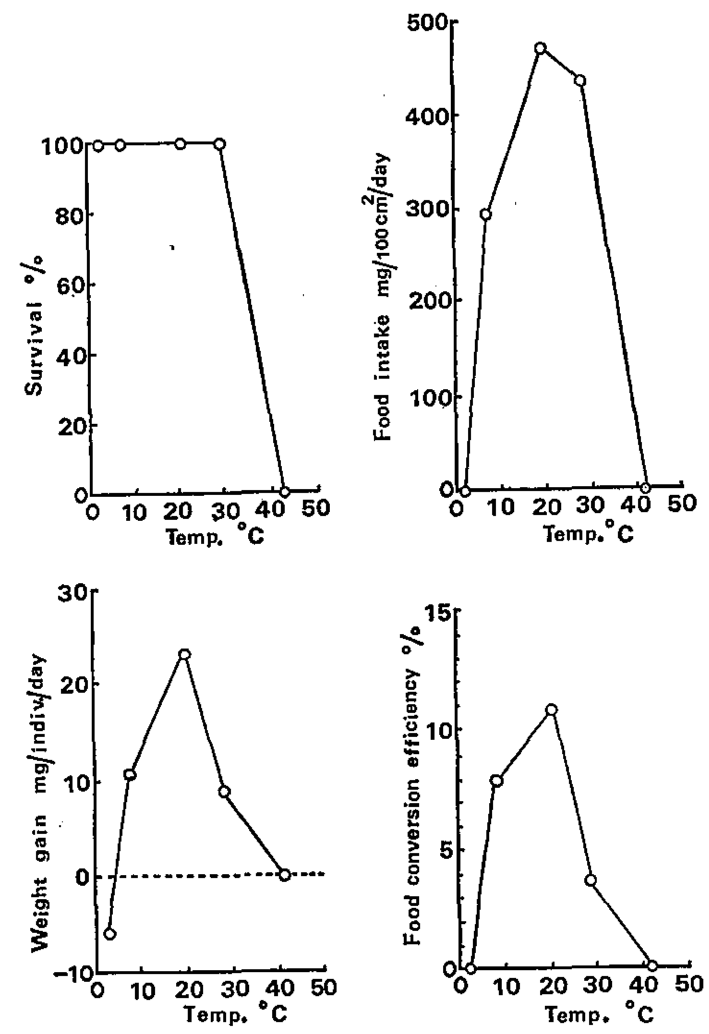

Fig. 3. Effect of water temperature.

shown in Fig. 4. In medium size group, at a density of three individuals per 100 cm², the amount of food intake was 77 mg per individual per day, compared to 33 mg at a density of 27 individuals per 100 cm². Similar findings were obtained in the small and large size groups. In the large size group, at densities of 3, 7, 13 and 28 individuals per 100 cm² the amount of food intake was 68.5, 59.8, 40.4 and 24.7 mg; the weight gain was 61, 60, 35 and 11 mg; and the food conversion efficiency was 12.8, 14, 12.6 and 6.7%, respectively. With increasing population density, the amount of food intake, weight gain and food conversion efficiency of each individual showed a sharp decline. When the amount of food intake per individual was converted to a unit area basis, however, the amount was found to increase

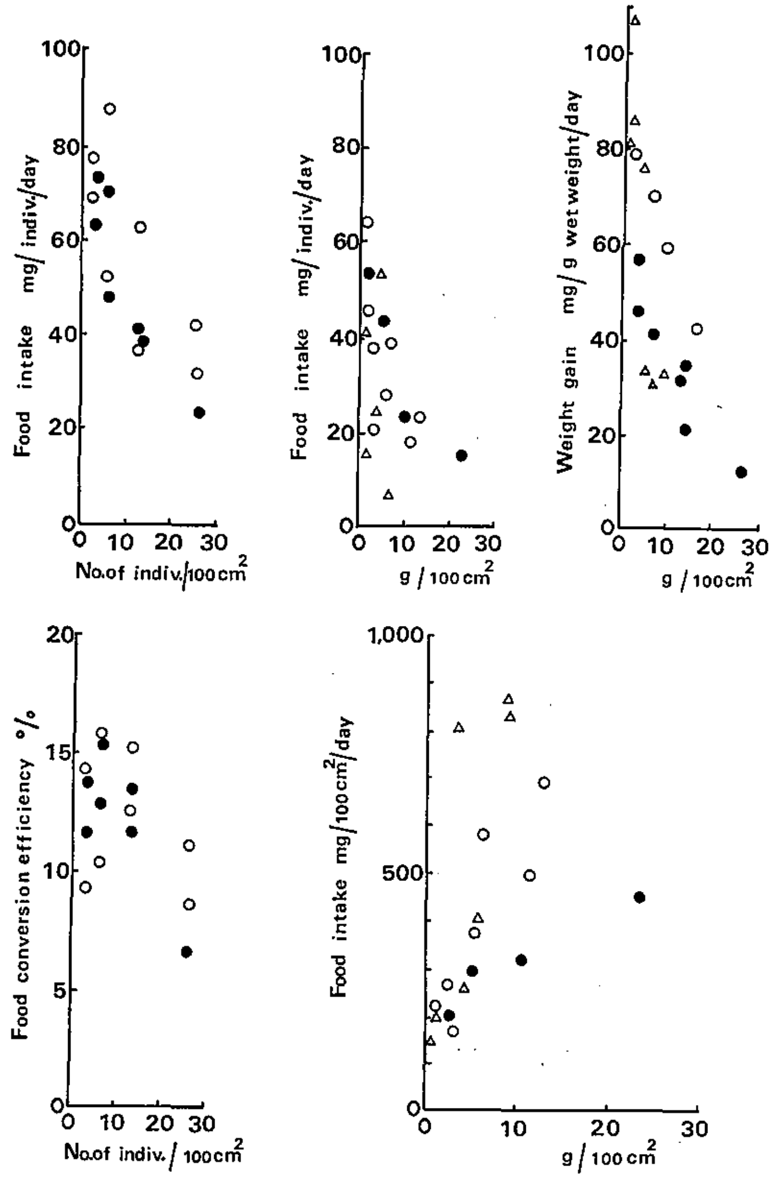


Fig. 4 Effect of population density. Initial wet weights of the individiduals are 0.1 g (△), 0.4 g (○) and 1.0 g (●). Population density is indicated in the abscissae with number of individuals or biomass.

together with density. The experiments as to other size groups were also carried out. These results are shown in Table 1.

Next experiment was conducted in the laboratory to determine the growth curves on high and low densities. Three hundred individuals of larvae with two setigerous



Table 1.
Relationships between population density and weight gain.
All weights are indicated with wet weight.

| Body weight (g/ind.) | | Experimental period (days) | Population density (No./100 cm²) | | Biomass (g/100 cm²) | | Weight gain | |
|---|---|---|---|---|---|---|---|---|
| initial | final | | initial | final | initial | final | mg/ind./day | mg/g*/day |
| 0.04 | 0.10 | 17 | 190 | 190 | 7.60 | 19.00 | 3.5 | 50.0 |
| 0.11 | 0.19 | 17 | 60 | 60 | 6.60 | 11.40 | 4.7 | 31.3 |
| 0.46 | 0.81 | 14 | 28 | 28 | 12.88 | 22.68 | 25.0 | 39.4 |
| 0.87 | 1.03 | 14 | 28 | 28 | 24.36 | 28.84 | 11.4 | 12.0 |
| 0.11 | 0.70 | 14 | 3.3 | 3.3 | 0.37 | 2.33 | 42.1 | 104.0 |
| 0.44 | 1.51 | 14 | 3.3 | 3.3 | 1.47 | 5.03 | 76.4 | 78.4 |
| 0.87 | 1.48 | 10 | 3.3 | 3.3 | 2.90 | 4.93 | 61.0 | 51.9 |

* (initial wt+final wt)/2
The worms collected from the field were classified to various size groups and then reared under various densities at water temperature of 20°C

segments on 6th day after artificial fertilization were placed into a 150 cm² rearing tank and supplied with activated sludge as diet. By 50th day, when feces were first detected, the worms had reached about 0.01 g in wet weight and decreased to about 100 individuals in number. At 130th day after fertilization, the number of worms was 45 individuals (30 individuals per 100 cm²), and the mean wet weight was about 0.8 g per individual. The population density varies during the long period of the continuous culture. The weight gain depends also on population density and worm size. The growth rate in the high and low density groups at different growth stages were calculated from the values shown in Table 1, being drawn in Fig. 5. At 20°C approximately 2 months were required for growing to be one gram adults under low density, 3.3 individuals per 100 cm², and approximately 5 months at high density, 30 individuals per 100 m². It is suggested that the growth rate varies according to population density and that the growth is inhibited as population density increases.

Next, the relationship between worm density and the number of burrows was observed (Fig. 6). The lower the number of animals, the higher the number of burrows per individual; there were 4.2, 4, 2 and 1.6 burrows per individual at densities of 3, 7, 13 and 27 individuals per 100 cm², respectively. In addition to this, it was observed that the burrows were arch-shaped at low density but U-shaped at high density (Fig. 7).

### 4. *Effect of organic pollution*

To investigate the effect of organic pollution of the sea water on survival with reference to the changes in chlorinity of the sea water, the effluent obtained by aerating artificial sewage (containing meat extract, peptone, urea, sodium chloride, potassium chloride, magnesium sulfate, and calcium chloride) was supplied to the

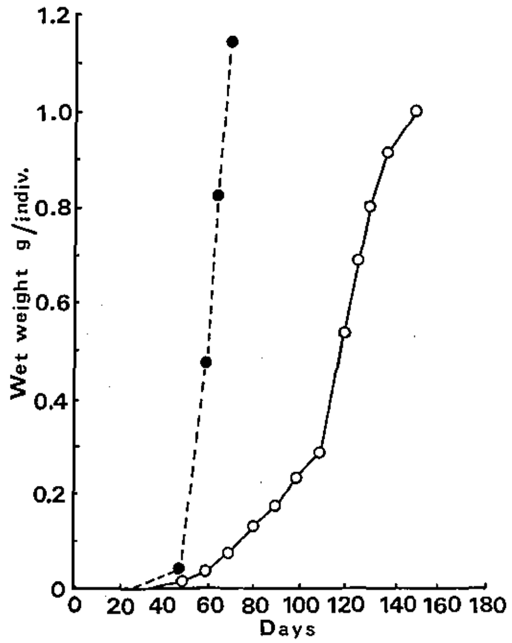


Fig. 5

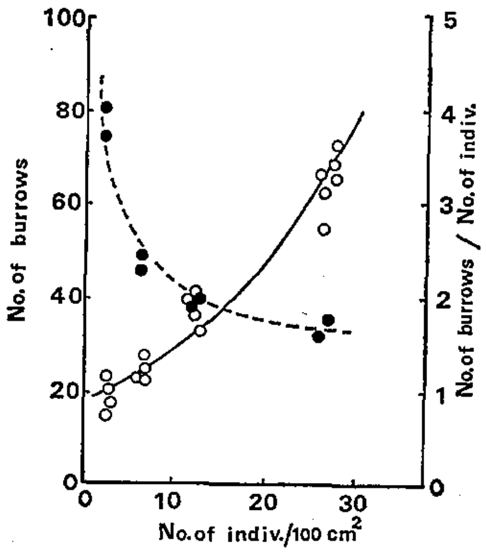


Fig. 6

Fig. 5 Growth curves in low population density (●) and in high population density (○).
Fig. 6 Relationship among population density, number of burrows (○) and number of burrows per individuals (●).

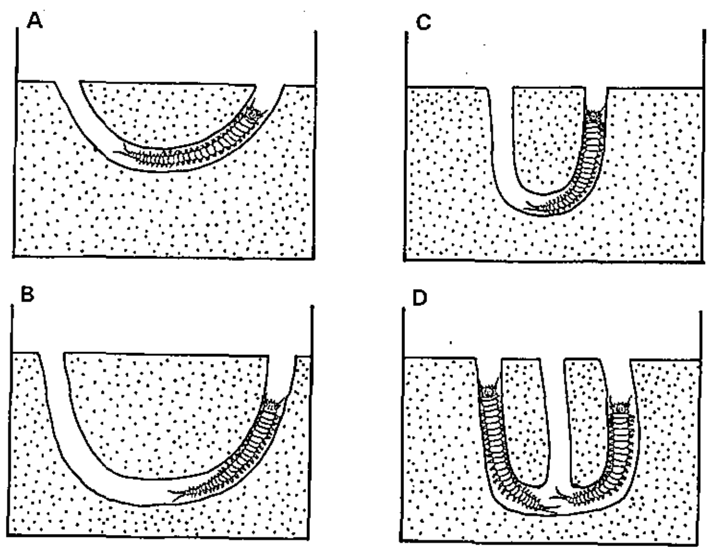


Fig. 7 Shapes of burrows in low population density (A and B) and high population density (C and D).



rearing tanks. In the sea water without pollutants the survival ratio at chlorinities of 0.5 to 18.0‰ is almost 100%. But, in the polluted sea water where the concentrations of $NH_4^+$-N ranged 3–13 mg/*l* and COD ranged 15–23 mg/*l* the survival ratio indicated 100% in chlorinites up to 9.0‰, but as the chlorinity approached that of full strength sea water, 18.0‰, the survival dropped to 2 percent (Fig. 8). This is thought to indicate that the nereids which live in the sea water with high chlorinity are more susceptible to the effects of pollutants than those living at low chlorinities.

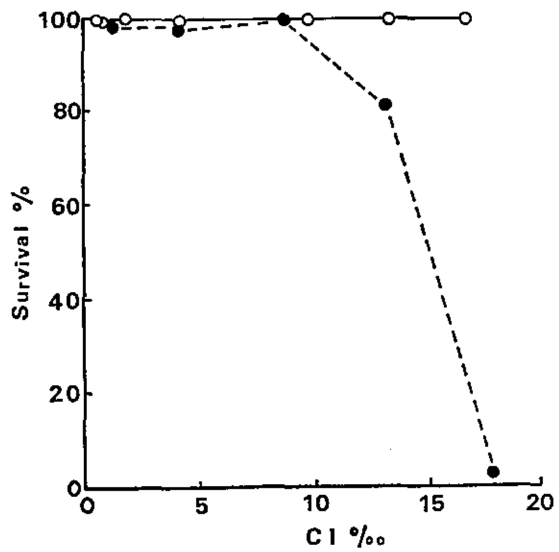


Fig. 8 Survivals in diluted sea water (○) and in diluted sea water with pollutant (●).

To investigate the effects of the COD and $NH_4^+$-N concentrations on the feeding behavior of the nereids, the sea water, containing different amounts of $NH_4^+$-N and organic carbon which were obtained by aeration of artificial sewage were introduced into the nereid culture tanks, and activated sludge was provided as diet. The results are shown in Fig. 9. No deleterious effect on feeding behavior was found up to $NH_4^+$-N concentration of 30 mg/*l* and COD of 18 mg/*l* at a chlorinity of 4.5‰, nor up to $NH_4^+$-N concentration of 5 mg/*l* and COD of 23 mg/*l* at a chlorinity of 9.0‰. Also, at a chlorinity of 13.5‰ normal feeding behavior was observed at a $NH_4^+$-N concentration of 7 mg/*l* and COD of 16 mg/*l*. Thus, the $NH_4^+$-N concentration and COD which affected feeding behavior varied according to chlorinity. Therefore, where chlorinity is variable as in the tidal flat environment, active feeding can be maintained in chlorinities ranging from 4.5 to 13.5‰, in $NH_4^+$-N below 5 mg/*l* and in COD below 18 mg/*l*.

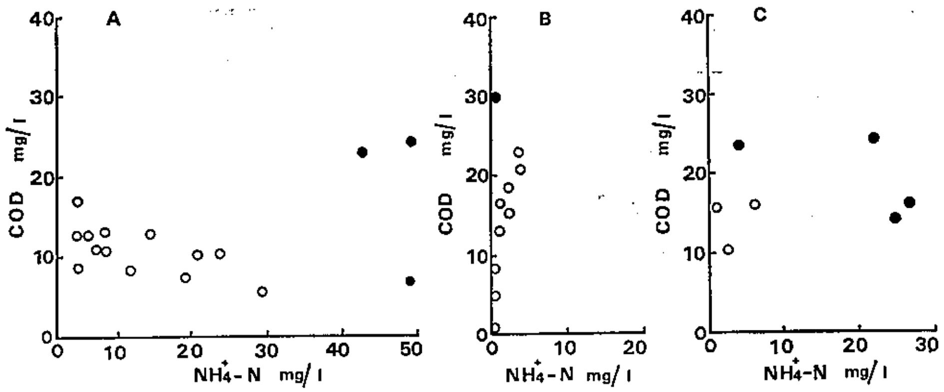


Fig. 9 Feeding activity under polluted conditions, active feeding (○) and inactive feeding (●) at chlorinities of 4.5‰ (A), 9.0‰ (B) and 13.5‰ (C).

## 5. *Effects of heavy metals*

In order to study the effects of heavy metal ions as water pollutants on the nereids, $K_2Cr_2O_7$, $CuCl_2$, or $ZnCl_2$ dissolved in the sea water diluted to 4.5‰ chlorinity were supplied to the rearing tanks and the survival ratio, weight gain and food intake at various concentrations of $Cr^{6+}$, $Cu^{2+}$, and $Zn^{2+}$ were determined over a period of 7 days (Table 2). In the case of chromium ions, no influence on survival was recognized up to a concentration of 1 mg/*l*, but the weight gain and food conversion efficiency began to be affected at 0.1 mg/*l*. In the case of cupric ions survival was satisfactory at 1 mg/*l*, but fell to zero at 3 mg/*l*, while the weight gain and food conversion efficiency declined at 0.5 mg/*l*. In the case of zinc ions survival was adequated up to 1 mg/*l*, but decreased to 30 percent at 5 mg/*l*, where no feeding was observed. In terms of survival ratio the toxicity of these heavy metals is in decreasing order: copper, chromium, zinc.

## 6. *Effcet of dissolved oxygen*

The Nereids whose body surfaces had been washed thoroughly with the diluted sea water were placed singly in 100 ml incubation bottles filled with diluted sea water (chlorinity 9.0‰) saturated with dissolved oxygen. The bottles were sealed and allowed to stand at 20°C for 2–4 hours, then the nereids were removed and the oxygen content of the diluted sea water was determined by the azide modification of the Winkler method (JAPAN SEWAGE WORKS ASSOCIATION 1967). The nereids used were 15 individuals of wet weights ranging from 0.4 to 1.2 g. The result is shown in Fig. 10. The average oxygen consumption per hour becomes 0.20 mg per gram wet weight.

To investigate the effect of dissolved oxygen concentration on food intake, the strength of aeration was varied, or in some cases air was supplied only by sprinkling.



Table 2.
Effects of heavy metals

| Heavy metal (mg/l) | | Survival (%) | Weight gain mg/individual/day | Food conversion efficiency (%) |
|---|---|---|---|---|
| 0 | Cr | 100 | 41.4 | 19.7 |
| | Zn | 100 | 41.4 | 19.7 |
| | Cu | 100 | 41.4 | 19.7 |
| 0.1 | Cr | 100 | 24.3 | 12.4 |
| | Zn | 100 | 35.7 | 16.5 |
| | Cu | 100 | — | — |
| 0.5 | Cr | 100 | 24.3 | 11.4 |
| | Zn | 100 | 30.0 | 14.4 |
| | Cu | 100 | 15.7 | 8.1 |
| 1.0 | Cr | 100 | 14.3 | 5.6 |
| | Zn | 100 | 28.6 | 13.5 |
| | Cu | 100 | 18.6 | 13.9 |
| 3.0 | Cr | 100 | 0 | 0 |
| | Zn | — | — | — |
| | Cu | 0 | 0 | 0 |
| 5.0 | Cr | 0 | 0 | 0 |
| | Zn | 30 | 0 | 0 |
| | Cu | 0 | 0 | 0 |

—: not determined

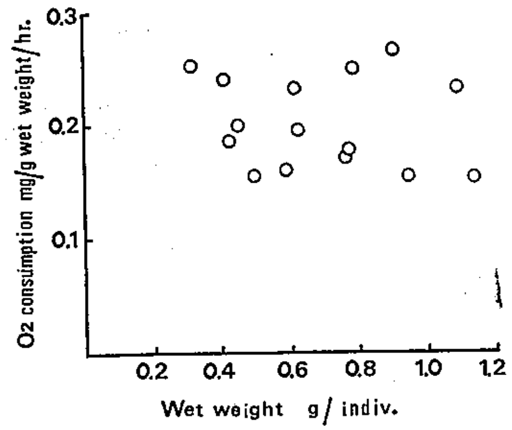


Fig. 10 Relationship between body weight and oxygen consumption.

In this way the food intake related to the dissolved oxygen concentration of the medium was determined. The experiment was carried out in a constant temperature room at 20°C, and 15 nereids (wet weight 1 g) per 100 cm² were used.

Fig. 11 shows the relationshop between dissolved oxygen and food intake. At a dissolved oxygen concentration of below 3 mg/l the food intake was extremely low, but increased steeply at 3.5 mg/*l* and above. The minimum dissolved oxygen required for feeding is considered to be 3 mg/*l*.

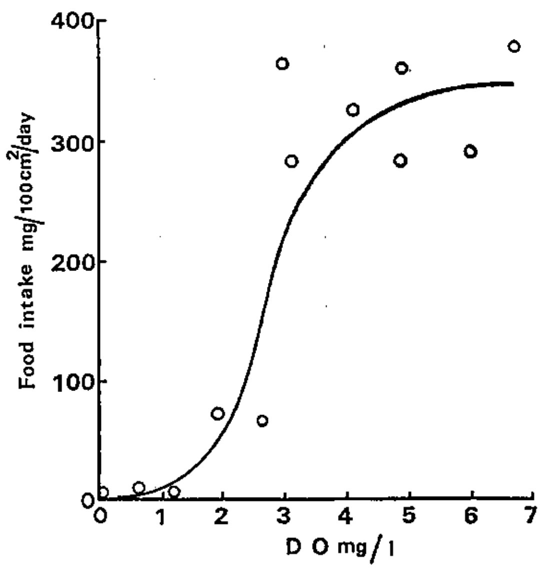


Fig. 11 Relationship between dissolved oxygen (DO) and food intake.

## DISCUSSION

The responses of *N. japonica* to changes of environmental factors were studied by a series of experiments in which a number of these factors were isolated. The diet used was a floc of activated sludge. Since the nereids which fed on this floc egested distinctive fecal pellets, actual feeding could be judged readily.

The nereids were able to survive over a wide range of chlorinity from a low level of 0.5‰ to a high level of 18.0‰, but growth rate seemed to decline at low chlorinity. At low chlorinity, however, their movement were more active within their burrows; i.e. the frequency of peristalsis per unit time increased.

The water in and over mud generally contained $NH_4^+$-N and organic carbon, but when the $NH_4^+$-N concentration and the COD rose to 3–13 mg/l and 15–23 mg/*l*, respectively, deleterious effects on the nereids were observed. These effects also depended on chlorinity. At a chlorinity of 18.0‰ death ocurred within 5 days, whereas at 4.5 to 13.5‰ virtually no harmful effects were observed. Thus the optimum chlorinity for this nereid seems to be at a low level. It can be said that in an area such as an estuary where either a high or a low chlorinity is constantly maintained and where pollution is liable to occur, a low chlorinity provides more suitable environments.



With regard to water temperature, it was found that at 3.5°C and at 34°C and above no feeding behavior occurred and negative growth was seen, while in the range of 9.5–30°C positive growth occurred; the optimal temperature was in the vicinity of 20°C. It is known that reproductive swarming of their species usually occurs in winter. The low water temperature of this season provides the worst environment in terms of feeding conditions, but their eggs can be fertilized and develop at lo temperatures (Dales 1950, Inamori and Kurihara 1979b). Therefore, it seems appropriate to suppose that energy stored in eggs or sperm is used for the development in this season in which the growth is inhibited extremely.

As population density increased the number of burrows per population density decreased, indicating an increase in sharing of burrows, or of U-shaped instead of arch-shaped burrows. At the optimum density, as far as they could be observed from the outside of the rearing tanks, all individuals had formed arch-shaped burrows, through which they maintained a current by peristaltic movements in order to replenish the oxygen supply. However, if the number of U-shaped burrows was to increase their efficiency of oxygen replenishment would be expected to decline and the number of peristalsis to increase. This is thought to be related to a decline in growth. Further, the feeding condition of the nereids varied widely with dissolved oxygen concentration, and no normal feeding was observed below 3 mg/*l*. Further, the presence of even a low concentration of heavy metals in the water prevented feeding and lowered the rate of weight gain.

The above findings showed that the growth of *N. japonica* was influenced to a large extent by variations of environmental factors such as chlorinity, water temperature, population density and presence of pollutants.

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