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An Attempt to Apply an Experimental Microcosm for the Mass Culture of Marine Rotifer, *Brachionus plicatilis* MÜLLER

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Abstract

The experiments were carried out preliminarily in order to know how to introduce an experimental microcosm to the water for mass culture of marine rotifer, *Brachionus plicatilis*.

Round polycarbonate tanks (Pan-Light) with 30 liters were employed in the rearing experiments. About 10 liters of gravels crushed were set on the bottom as a filter. The water was recirculated by an air bubbling through a pipe-like an air lift pump located around the center of the tank. The air volume supplied was increased gradually from 3 to 13 or 15 liters per minute in process of yeast feeding.

Marine type of *Chlorella* was innoculated at the beginning with 6.0 *g* of KNO_3, 0.6 *g* of KH_2PO_4, 0.9 *g* of Clewat-32 and 0.1*g* of Clewat-Ca in the tank. When the *Chlorella* grew up to the stationary phase, a small number of rotifer were transfered into the water. Frozen marine yeast were fed to the rotifer, after the majority of *Chlorella* cultured were taken up. The yeast were supplied at 6-hour intervals keeping with a certain amount of feed levels between 100 $\times$ and 2,000 $\times 10^4$ cells per ml of yeast. The experiment was repeated three times; Exp. I, II and III, during February and May in a sunny exterior of the Laboratory.

Each experiment showed a similar result on the population growth of the rotifer. The maximum density of rotifer cultured in Exp. I, II and III were 1,450, 1,170 and 1,270 individuals per *ml*, respectively. Higher feeding ratio, 140%, was found in lower temperature, 21.1°C. On the contrary, the lower ratio, 43%, was obtained when it was 27.6°C, the highest temperature through the experiments. It was clear that the feeding ratio, thus, was affected by the water temperature within those ranges. Furthermore, a negative relationships between feeding ratio and population density were also found in each experiment. Typical example on this relationship was obtained in Exp. II. Higher feeding ratio, 400%, was indicated in the cases of lower density, 10 or 25 individuals per *ml*. However, when the rotifer population grew up to 1,000 per ml, the feeding ratio decreased to 120%.

Interesting results, concerning the growth of *Chlorella* cells in the tank, were observed in the final experiment, Exp. III. The *Chlorella* cells, which have been taken up once by the rotifer at the middle part of the experiment, grew again in the tank at the end of the rearing. This might be due to the reproduction mechanisms of phytoplankton in the closed-system like an experimental microcosm.

Introduction

In 1967, HIRATA and MORI reported on the outdoor culture of marine rotifer, *Brachionus plicatilis*, fed the mixture of *Chlorella* and bread yeast in 500 liters tanks. It may safely be said that their experiments were worthy of the dawning in the mass culture of marine rotifer, since they succeeded on the rearing of ear-

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ly larval stage of yellow tail, *Seriola quinqueradiata*, fed with the marine rotifer alone (SETO-INLAND SEA FARMING FISHERIS ASSOCIATION: 1971). Thus, the importance of the rotifer as a larval food became so increasing. Many fish biologists, in the field of seed productions, investigated the technique for mass culture of marine rotifer, and the procedure of culture has been rapidly improved (FURUKAWA and HIDAKA; 1972, HIRATA, KANAZAWA, YAMAMIDORI and YASUDA; 1973, HIRAYAMA and KUSANO; 1972, HIRAYAMA and OGAWA; 1972, NOZAWA, OOHARA, KITAMURA and NAKAGAWA; 1972).

Up to date, almost no problem is remained in the lower density of rotifer culture. There are, however, still two big problems to solve in the case of higher density. Those are their feedings and excretions. It was estimated roughly that a rotifera eats 60,000~156,000 cells per day under the temperature around 25°C (MORI and HIRATA; 1968, WATANABE and HIRATA; 1968). And it may be considered that they excrete the faeces more ten times than their body weight.

The present experiments were prepared preliminarily in order to know how to solve those two problems in the same tank at once. A recirculating culture system was, then, devised for keeping with an ecological balance in the water by introducing an idea of experimental microcosm (KURIHARA; 1969). The methods employed here were more simpler than that of the culture system for larvae of the American lobster reported by SERFLING, VAN OLST and FORD (1974). Possibility of the higher density cultivation in marine rotifer are discussed here.

Materials and Methods

The experiments were carried out in a sunny exterior of our Laboratory during February to May, 1972. The observation was repeated three times, Exp. I, II and III, by the same method under natural light and temperature conditions.

Round polycarbonate tanks (Pan-Light) with 30 liters were employed for rearing of *Chlorella* and rotifer. About 10 liters of gravels crushed were set on the bottom of the tank as a filter. Average volume of the gravels was approximately 7.0 cm^3, and the rotifer could pass through an interstice among the gravels. The water was recirculated by an air bubbling through a pipe-like an air lift pump located around the center of the tank as shown in Fig. 1. The bubbling was made continuously by a rotary compressor (Hitachi-RC 200). The air volume supplied was increased gradually from 3 to 13 or 15 liters per minute in process of yeast feeding.

Marine type of *Chlorella* was innoculated in the tank at the beginning for a few days with 6.0 *g* of KNO_3, 0.6 *g* of KH_2PO_4, 0.9 *g* of Clewat-32 and 0.1 *g* of Clewat-Ca, by modifying the medium reported by HIRATA (1972). A small number of rotifer as the seeds were transfered into the water, when the *Chlorella* have grown up to the stationary phase with the density of 20 $\times$ or 30 $\times$ 10^6 cells per *ml.* Frozen marine yeast were fed to the rotifer (FURUKAWA and HIDKAKA; 1972, OOSAWA and KAWANO; 1971), after the majority of *Chlorella* cultured were taken

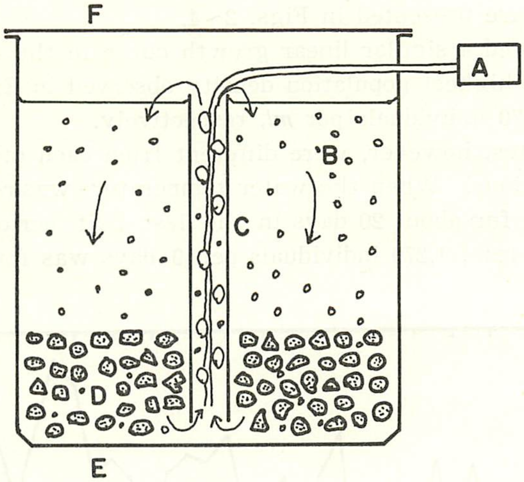

Fig. 1. A schematic view of the culture system. The medium with rotifer was re-circulated as shown as the direction of the arrow. Here, A ; aerating pump, B ; marine rotifer in the medium, C ; vinyl pipe with 25 cm in diameter as an air lift pump, D ; crushed gravels with about 7 cm^3 as a re-circulating filter, E ; round polycarbonate (Pan-Light) tank with 30 liters, and F ; glass cover.

up. The yeast were supplied at 6-hour intervals keeping with a certain amount of feed levels 100 $\times$ to 2,000 $\times$ 10^4 cells per *ml*. Number of the cells remained in the tank were measured at each feeding time with haemocytometer. Approximately 3 *g* of marine yeasts were fed a day during the first half period, and 10 to 40 *g* of foods were supplied during the last half period.

Microscopic observations were conducted with the numbers of *Chlorella* cells and the rotifer per *ml* once a day. Special caution was taken upon an appearance of another single celled phytoplankton which was growing spontaneously in the tank during Exp. III. Daily variations of water temperature, pH and oxydation-reduction potential (ORP) were measured at around 10 o'clock in each day.

Calculations of the feeding ratio and eggs percent were conducted as follows:

$$\text{Feeding ratio} = \frac{\text{amount of yeast supplied } (g) \times 100}{\text{total body weight of rotifer in tank } (g)}$$

$$\text{Eggs percent} = \frac{\text{number of rotifer with eggs} \times 100}{\text{total number of rotifer}}$$

Here, weight of rotifera was estimated to 0.02 mg each, refering from NOZAWA, OOHARA, KITAMURA and NAKAGAWA (1972).

Results and Discussion

1. Population growth and environmental factors

The population growth of rotifer with some environmental factors observed daily in Exps. I~III are presented in Figs. 2~4.

Each result showed a similar linear growth curve in the last half period of the experiments. The highest population density observed in Exp. I, II and III were 1,450, 1,170 and 1,270 individuals per *ml*, respectively.

Daily growth rates, however, were different from each other depending on the temperature conditions. When the water temperature was relatively lower, 25.3°C in average, it took for about 20 days in the last half period of Exp. I. On the contrary, the best rate, 1,270 individuals per 9 days was found in Exp. III, when

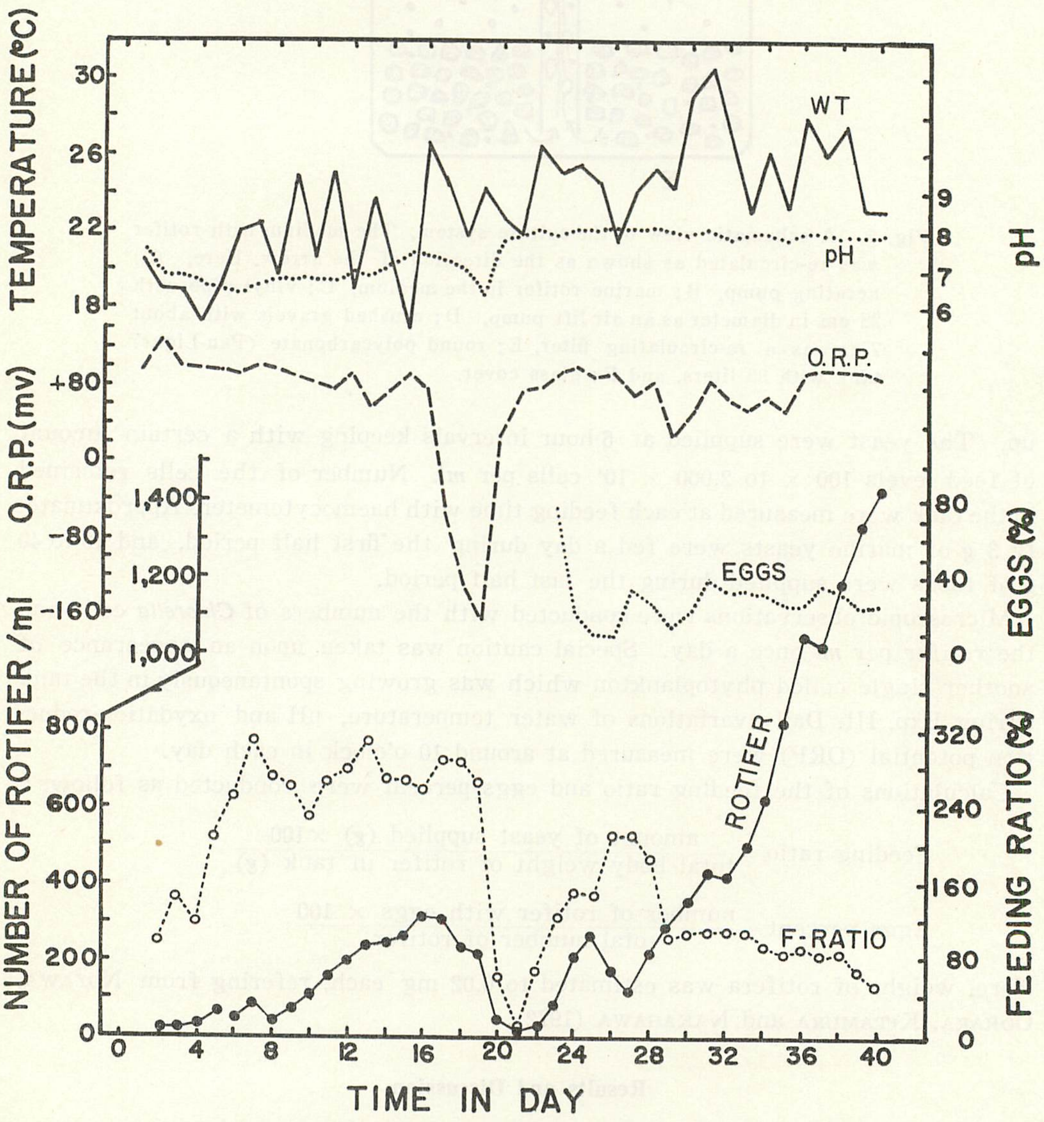

Fig 2. The population growth of marine rotifer, *Brachionus plicatilis*, with the feeding ratios and some environmental factors observed in Exp. I. Here, O.R.P ; oxydation-reduction potential.

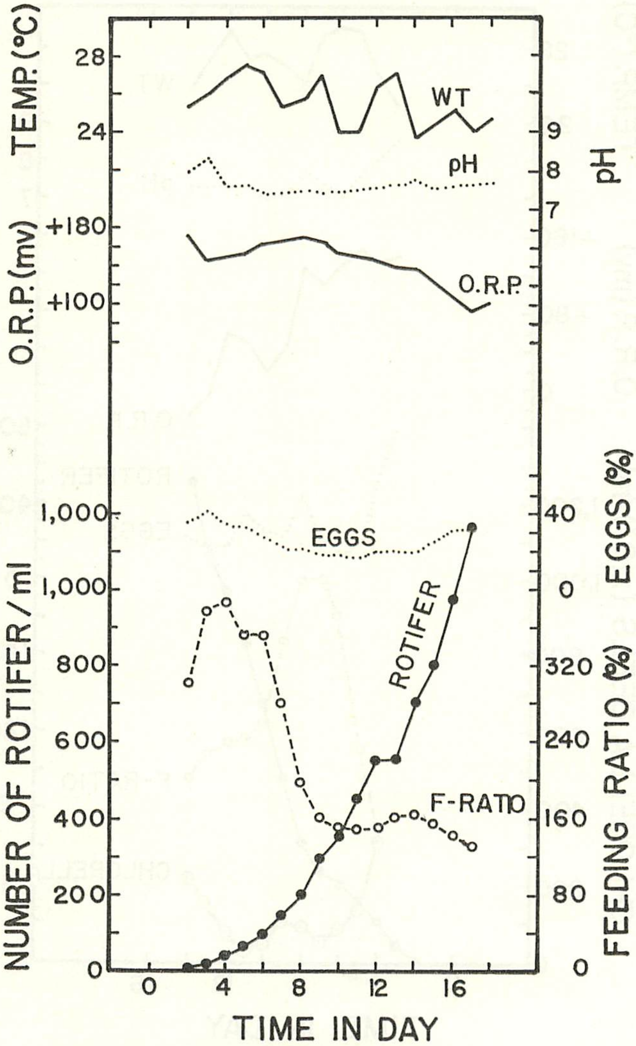

Fig. 3. The population growth of marine rotifer, *Brachionus plicatilis*, with the feeding ratios and some environmental factors observed in Exp. II. Here ; O.R.P. ; oxydation-reduction potential.

the average temperature, 27.6°C, was the highest. Intermediate growth rate, 1,170 individuals per 15 days, was found in Exp. II with 25.9°C in medial. From those results it may concluded that the growth rates were influenced by the water temperature, and they grew faster when it was higher temperature. Furthermore, feeding ratios as shown in Fig. 5 were also affected clearly by the temperature within those ranges.

Concerning about pH variation, a linear growth of the population was found in the last half period of the experiment while no pH fluctuation was measured. A little fluctuation of pH with feeble growth was observed during the first half period,

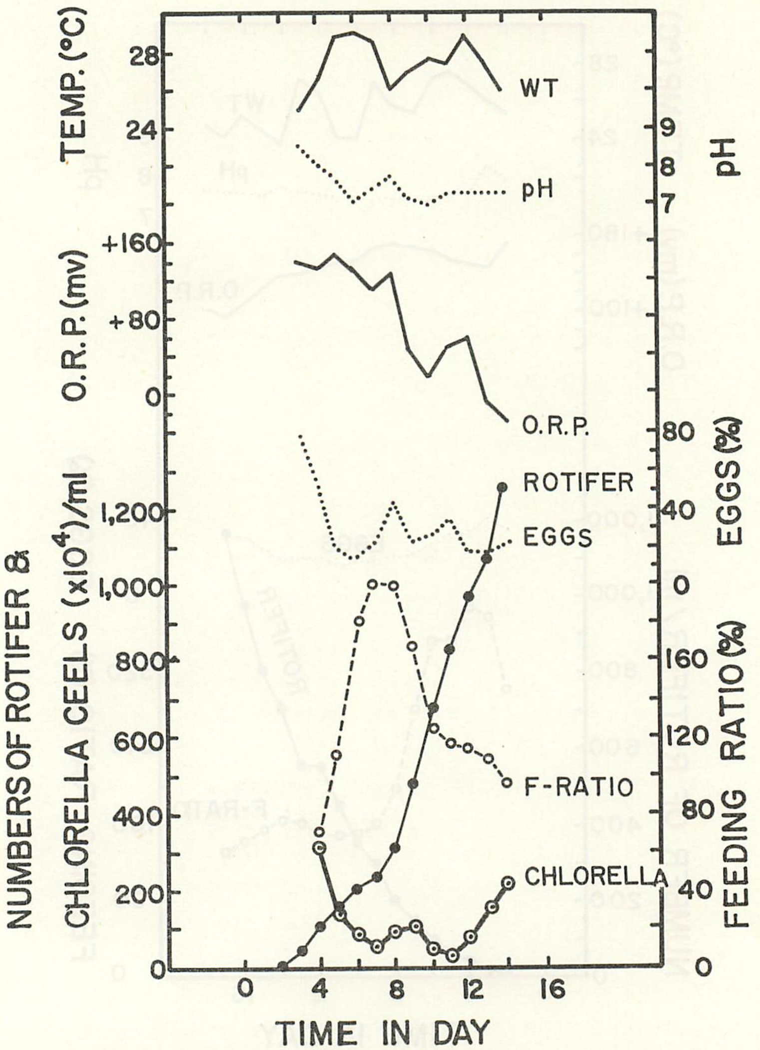

Fig. 4. The population growth of marine rotifer, *Brachionus plicatilis*, with the feeding ratios and some environmental factors observed in Exp. III. Here, O.R.P. ; oxydation-reduction potential.

especially in the case of Exp. I. It might be considered that the rotifer could grow well under the stable conditions of pH value, even the value was corresponded to the optimum conditions (Furukawa and Hidaka; 1972).

Clear depression of ORP was found during the 15th to the 22nd day in Exp. I. The population of rotifer was also decreasing on 2 or 3 days after the ORP depression. Those variations might be due to over-feeding, since feeding ratios during 6th to 19th days were over 250 %. ORP showed more larger variation than that of pH fluctuation in those three experiments. Therefore, it is possible to suggest that ORP is one of a suitable indicator of water quality in rotifer feeding.

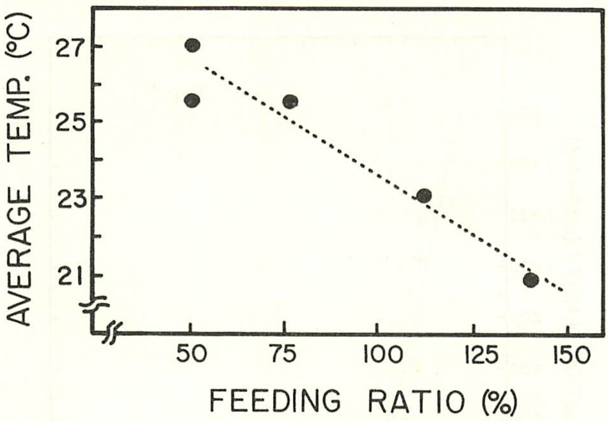

Fig. 5. Relationship between the average temperature and feeding ratio in those three experiments except in the first half period of Exp. I when it was considered to be over feeding. Under the condition of those temperature ranges, the negative relationship was found.

2. Population density and feeding ratio

Correlation between population density and feeding ratios in Exp. I~III are indicated in Figs. 6~8.

The results taken from all together showed negative correlation between populatoin density and feeding ratio. This means that rotifer in lower density need more food than that of higher population. It is more interesting that the lines showed a hyperbola rather than a strait. Typical example of hyperbolic correlation was found in Exp. II which is presented in Fig. 7. Until the population grew up to 200 individuals per *ml* the feeding ratio decreased rapidly from about 400 to 200 %. The population then grew so fast from 200 to 1,170 individuals per *ml*, while feeding ratio decreased only 50 or 60 %.

Why did hyperbolic correlation occurr? In this connection, the following two reasons are considerable at present; viz. 1) reproduction of phytoplankton which were grown spontaneously in the tank, and 2) decreasing of the body weight by the passage of rearing time.

The number of single celled organisms, including *Chlorella* cells, were increasing day by day during the last half period of Exp. III as shown in Fig. 8. (No observation was done in Exp. I and II). The gravel-filter, which was set on the bottom of the tank with strong bubbling, might serve ecologically as the ground of transformation from excretives to some nutrients for phytoplankton. Thus, the *Chlorella* cells were increased gradually at the end of the experiments. It seems that the results mentioned above showed a possibility of introduction of a microcosm into the rearing water (Kurihara; 1969).

The other subject on the hyperbolic correlation between population density and feeding ratio is size variation of rotifer. Ichikawa and Hirata (1973) have examined preliminarily on a daily variation of the size distribution in

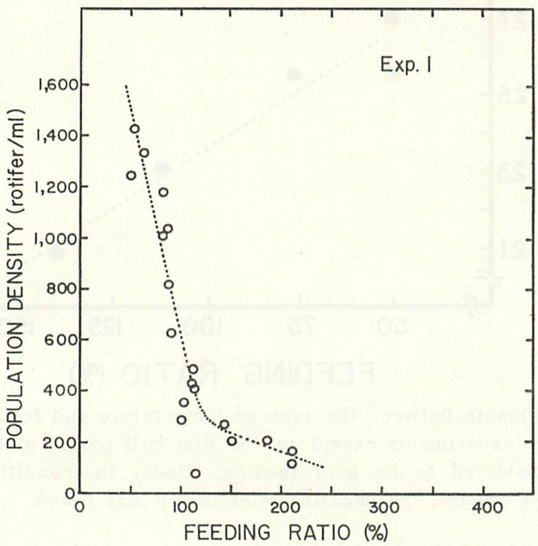

Fig. 6. Relationship between the population density of the rotifer, *Brachionus plicatilis*, and their feeding ratio which was made a calculation of "amount of yeast supplied per total rotifer's weight" with per centage. The results were obtained from Exp. I. A negative relationship, like a hyperbola, was found in this experiment.

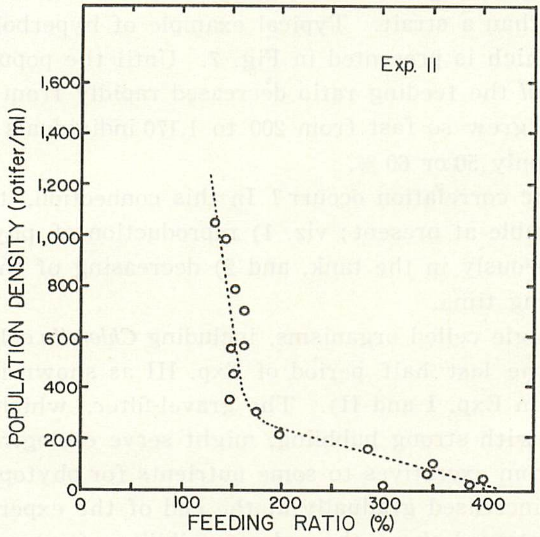

Fig. 7. Relationship between the population density of rotifer, *Brachionus plicatilis*, and their feeding ratio. The results were obtained from Exp. II. Typical example of the negative relationship, like a hyperbolic curve, was found in this experiment. The relationship was discussed in the text.

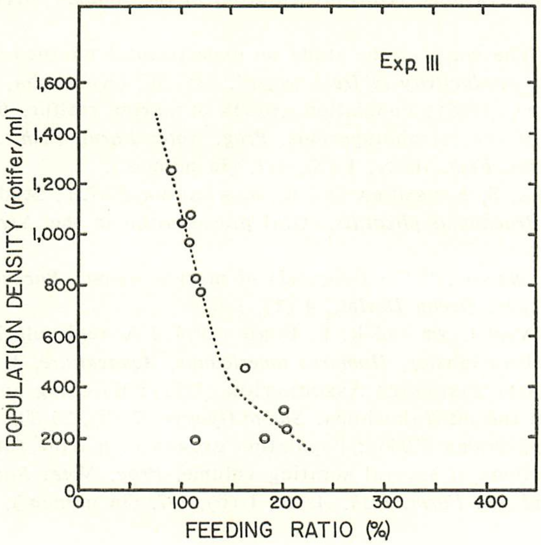

Fig. 8. Relationship between the population density of the marine type of rotifer, *Brachionus plicatilis*, and their feeding ratio. The results were obtained from Exp. III. A hyperbolic curve was not clear, but a negative relationship was found as seen in Figs. 5 and 6.

culture, and they observed that average body depth of the population became smaller by the passage of rearing. So, the decreasing of body depth should be considered for understanding of the correlation mentioned above. We will discuss more in details on this matter after finishing the size examinations.

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