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Fundamental Studies on Physiology of Rotifer for its Mass Culture—III Influence of Phytoplankton Density on Population Growth*

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The population growth of the rotifer, *Brachionus plicatilis* cultured with a marine species of *Chlorella* suspended at various densities was investigated.

Experiments were performed by culturing many individuals in many test tubes each containing two individuals in the experimental medium. From daily counts of eggs laid and surviving individuals, time intervals from hatching to 50% survival and to peak of fecundity, net reproduction rate, intrinsic rate of population increase and mean generation time were estimated.

The results obtained are summarized as follows.

1. The values of the indices concerning population growth of the rotifer indicate that for its mass production the marine *Chlorella* should be kept always at the density of 85–213 $\times 10^4$ cells/m*l*, where 150×10^4 cells/m*l* may be the most desirable because of the highest values of intrinsic rate of population increase and net reproduction rate.

2. Population growth of the rotifer can be restrained in *Chlorella* suspension even at the density (less than 213×10^4 cells/m*l*) where the rotifer can not reach a satiated condition.

3. The weight and volume of the *Chlorella* were estimated at 7.1×10^{-6} μg and 1×10^{-8} mm^3 per average cell, respectively. Therefore, the most suitable density for population growth of the rotifer is estimated as 10.7 μg/m*l* or 1.5×10^{-2} mm^3/m*l*.

The previous investigation[1] on the filter feeding of the rotifer *Brachionus plicatilis*, 'Shiomizutsubowamushi' in Japanese, with a marin species of *Chlorella* suggested that for mass production of the rotifer, the *Chlorella* should be kept at the density more than 213×10^4 cells/m*l*. This suggestion was based on the assumption that the rotifer should grow most rapidly in population while being always maintained under a satiated condition.

In the present study, the population growth of the rotifer cultured with the *Chlorella* suspended at various densities was investigated. In order to isolate only the influence of phytoplankton density from that of many factors affecting the population growth of the rotifer, experiments were performed by culturing many individuals separately in many test tubes with the *Chlorella* suspension at a definite density.

Materials and Methods

The rotifer, *Brachionus plicatilis*, and the *Chlorella* used as food plankton were derived from the same clones as those in the preceding investigation on the effect of temperature. The parthenogenetic eggs offered to experiments were collected by the method

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described in the preceding report.[2] They were the first-laid eggs just before hatching of amictic females of the group which had been actively increasing under the experimental condition at least 10 days before experiment. The number of individuals offered to each experiment ranged from 17 to 47. They were cultured in many test tubes each containing two individuals in 5.0 m*l* of experimental medium. These culture media were renewed daily and at that time the number of eggs laid and of surviving individuals in all tubes was counted. From these daily counts, time intervals to 50% survival (M) and to peak of fecundity (P) from hatching were obtained and net reproduction rate (R_0), intrinsic rate (r) of population increase and mean generation time (T) at each food density were estimated on the basis of BIRCH's computational methods.[3]

More details of experimental procedure and of explanation of these indices were referred to the description in the preceding report.[2]

The most desirable density of the *Chlorella* for mass production of the rotifer was estimated from the values of these indices obtained at various food densities.

The culture medium used for the experiments on the *Chlorella* density was Miquel sea water at Cl of about 12.8‰, at the temperature on 22°C±0.1°C except in the experiment without *Chlorella* (0 cells/m*l*) where it was 25°C and at pH ranging 7.4–8.2.

Results

The experiments were performed at 9 intervals of density ranging 0–852×10⁴ cells/m*l*. *Chlorella* density was estimated from optical density of culture medium which is proportional to the cell density.

The survivorship and fecundity curves were constructed from age-specific survival and fecundity data at daily observations. Several examples of them are shown in Figs.

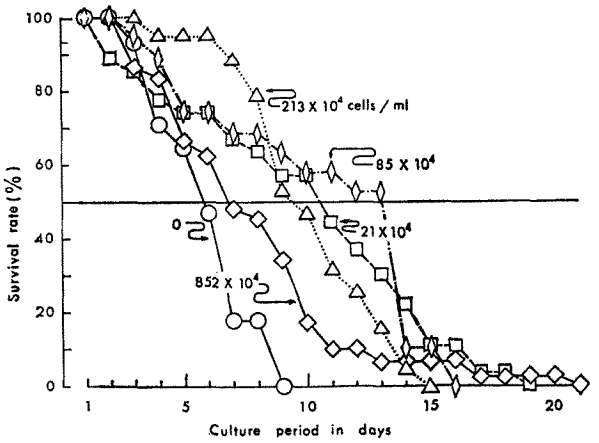

Fig. 1. Survivorship of the rotifer cultured with *Chlorella* suspended at various densities.

1 and 2, respectively. Values of the above-mentioned indices at various densities are shown in Fig. 3.

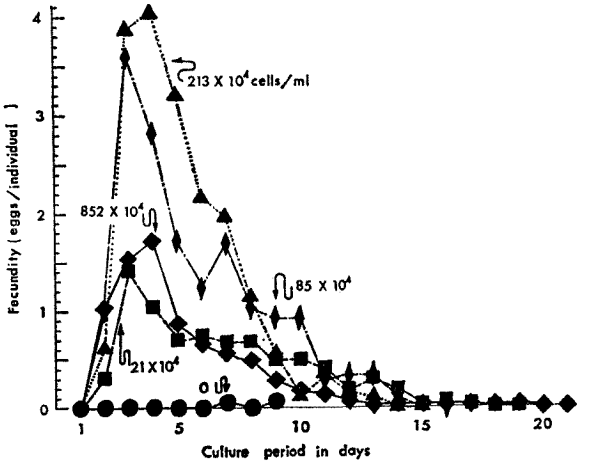

Fig. 2. Fecundity of the rotifer cultured with the *Chlorella* suspended at various densities.

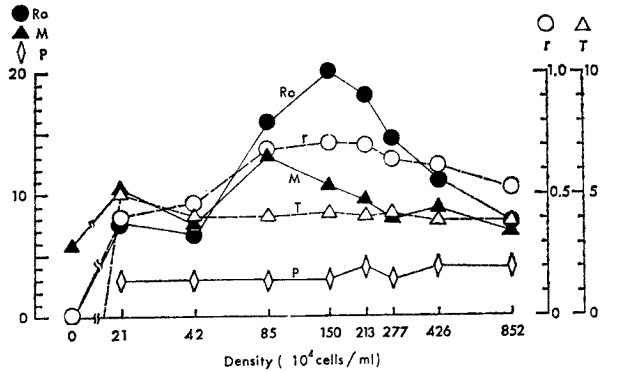

Fig. 3. Days to 50% survival (M) and to peak of Fecundity (P), net reproduction rate (R_0), intrinsic rate (r) of increase and mean generation time (T) of the rotifer cultured with the *Chlorella* suspended at various densities.

Time (M) from start of experiment to 50% survival is the longest at 85×10^4 cells/m*l* being 13.1 days, and tends to shorten slightly with the further rise of density, e.g., to 6.9 days at 852×10^4 cells/m*l*, while time (P) from start of experiment to peak of fecundity stays at almost the same value of 3 or 4 days in every density.

Net reproduction rate (the average number of eggs laid by a female in her life time) (R_0) has the highest value of 20.1 at 150×10^4 cells/m*l*, and gradually decreases to 7.7 when *Chlorella* increases to 852×10^4 cells/m*l* or decreases to 21×10^4 cells/m*l*. Mean genera-

tion time (T) shows a little variance ranging 3.9–4.2 days at every examined density except for 5.0 days at 21×10^4 cells/m*l*.

Reflecting these facts, intrinsic rate (r) of population increase rises with increase of the density from 0.41 at 21×10^4 cells/m*l* to the highest value of 0.71 at 150×10^4 cells/m*l* as shown in Fig. 3. The further increase of the density to 852×10^4 cells/m*l* decreases the value of intrinsic rate (r) to 0.53.

Discussion and conclusion

There are some reports that, in dense phytoplankton suspension, ingestion rate of zooplankters stays at almost constant level or sometimes decreases with further increase of food density. For example, *Artemia, Calanus* and *Daphnia* in Crustacea were studied by NIMURA[4] and REEVE[5], MULLIN[6] and RYTHER[7], respectively. Some kinds of rotifer were investigated by EDMONDSON[8] and KING[9], who made a brief reference to the relation between filter feeding and population growth, but scarecely discussed in detail this phenomenon in connection with full-stomach.

The values of indices concerning population growth of the rotifer, *Brachionus plicatilis*, may indicate that for its mass production the marine *Chlorella* should be always kept at the density of $85\sim213\times10^4$ cells/m*l*, where 150×10^4 cells/m*l* may be the most desirable because of the highest values of intrinsic rate of population increase and net reproduction rate. On the other hand, the previous investigation on filter feeding of the rotifer clarified that it can not reach a satiated condition until the *Chlorella* density increases to 213×10^4 cells/m*l*[1]. From the fact that population growth of the rotifer can be restrained in *Chlorella* suspension even at the density where the rotifer can not reach a satiated condition, it is clarified that the dense *Chlorella* suspension not only brings the rotifer under a satiated condition, but also inhibits population growth of the rotifer to some extent. This conclusion disagrees with the assumption in the previous report on filter feeding of the rotifer that the population should grow most rapidly under constantly satiated condition.

The weight and volume of the *Chlorella* were estimated at 7.1×10^{-6} μg and 1×10^{-8} mm^3 per average cell, respectively. The former was obtained in the following procedure. *Chlorella* suspension was filtered with a millipore filter (H.A. 0.45 μ) and, after drying at 50°C for 1 hour, the filter together with the *Chlorella* and other materials caught was weighed. Filter weight being known, it was subtracted to give the weight of the *Chlorella* and suspended materials. Then culture medium without the *Chlorella* (control) was filtered with another filter and was processed in the same manner. The difference between these two values was given as the net weight of the *Chlorella*. The volume was obtained by gathering the *Chlorella* from the suspension with centrifuge (3000 rpm, 15 min.). As an average individual should take about 200 cells of the *Chlo-*

rella per minute under a satiated condition[1], ingestion rate of the rotifer cultured with the *Chlorella* suspended at the density more than about 15.1 μg/m*l* or 2.1×10^{-2} mm^3/m*l* is estimated as about 1.4×10^{-3} μg/min/individual or 2.0×10^{-6} mm^3/min/individual. The most suitable density(150×10^4 cells/m*l*) of the *Chlorella* for population growth of the rotifer is estimated as 10.7 μg/m*l* or 1.5×10^{-2} mm^3/m*l*.

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* Other reports on feeding habits of zooplankters are omitted.