

ASSESSMENT OF THE GROWTH POTENTIAL OF THE ROTIFER *BRACHIONUS PLICATILIS* BY EVALUATING BIOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS

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ABSTRACT

To anticipate the changes that occur during continuous mass culture of the rotifer *Brachionus plicatilis*, biological characteristics such as extent of digestive organs in the body, frequency distribution of lorica length, and physiological tolerance to a hyper-saline environment were examined. The extent of digestive organs in the body was measured as the proportion of digestive-organ area to the body (PDA) of non-egg-bearing females fed on freshwater *Chlorella*. Tolerance to hyper-saline environment was evaluated as the percentage of individual rotifers still swimming after exposure to 70 ppt saline water for 3 h. The PDA and size frequency distribution of lorica length of feeding individuals changed only after culture conditions deteriorated sharply. The non-feeding individuals in the size range of 210-240 μm increased from d 7 during culture under unfavorable conditions. Hyper-saline tolerance suddenly dropped when rotifer growth was close to peak level and this was more evident in egg-bearing than non-egg-bearing females. From those results, the size frequency distribution (210-240 μm) of non-feeding individuals seemed to be useful in forecasting changes during mass culture. Hyper-saline tolerance (70 ppt for 3 h) was also evaluated and found to be a suitable index of rotifer activity and both criteria can be used to assess the growth potential of rotifers under mass culture conditions.

INTRODUCTION

Marine rotifers are widely used as a live food in the early stages of marine fish larval rearing. Mass rotifer production incurs problems, however, such as a sudden decrease of rotifer density and decline of population growth. If these problems can be anticipated, countermeasures can be taken to prevent or curtail a total collapse of the culture. Swimming speed (Snell et al. 1987; Korstad et al. 1995), egg ratio (Korstad et al. 1995), and tolerance to chemical toxicity (Juchelka and Snell 1994; Janssen et al. 1994) have been good indices to monitor the population growth of rotifers. These indices require intensive effort, however, and accordingly they are not practical at the rotifer production site. Therefore, it is important to determine simple yet accurate indices for monitoring the changing conditions when rotifers are under mass culture. To understand these changing conditions that occur under high-density rotifer culture conditions, biological characteristics, such as the extent of the digestive organs in the body, size frequency distribution of lorica length, and physiological

tolerance to a hyper-saline environment were examined in individual *Brachionus plicatilis*.

MATERIALS AND METHODS

The Kinki L-type strain of *B. plicatilis* was used in all experiments. This strain has been cultured and used for larval rearing at the Amami Station of the Japan Sea-Farming Association since 1994. Three experimental batch cultures were performed (culture I, II and III) using 500-L polycarbonate (PC) tanks for 12 d. Filtered sea water was used as the culture medium, and temperature was regulated at 26 C. Commercially available concentrated freshwater *Chlorella*, (Nisshin Science Co. Ltd; average cell density, 150×10^8 cell ml^{-1}) was used as food for the rotifers. This algae suspension (0.4 -1.0 L) was added twice/d to each culture tank. The number of rotifers in 0.5 ml of culture medium was counted three times in order to estimate the rotifer density.

Proportion of Digestive-organ Area to the Body (PDA)

A green-colored area, corresponding to the digestive organs, appeared in the rotifer body when fed *Chlorella*. The proportion of this green-colored area to the body (PDA) was used as an index of feeding activity. The PDA was measured on d 1, 4, 7 and 10 in cultures I and II using the following procedure. Rotifers were transferred from the experimental culture to a 1-L flask containing algae suspension with a density of 10×10^5 cells ml^{-1} . PDAs of 30 non-egg-bearing individuals were then measured at 1, 5, 10, and 15 min using a microscope with a picture-analysis system (Olympus Co. Ltd; RS-3100). Data were statistically analyzed using the *t*-test.

Size Frequency Distribution of Lorica Length

The size frequency distribution of lorica length of both feeding and non-feeding rotifers was evaluated on d 1, 3, 5, 7, 9 and 11 in culture III. One sample containing 100 feeding and 50 non-feeding individual rotifers was measured under the microscope described previously.

Tolerance to Hyper-saline Environment

The hyper-saline challenge test was performed by calculating the percentage of swimming rotifers when exposed to high salinity. At first, to determine the adequate experimental conditions, the changes in the percentage of swimming rotifers were evaluated at various salinity and exposure times. Salinity was adjusted from 34 to 80 ppt (at 6 ppt increments) by adding sodium chloride (NaCl) to sea water. Exposure time was 0.5, 1, 2, 3, 5, 10 and 20 h. From the results of those preliminary experiments, the hyper-saline challenge test was designed as follows: rotifers were transferred into wells of a Multiwell Plate (Iwaki Glass Co. Ltd; 6-well type) containing 70 ppt saline water. After 3 h, the percentages of swimming rotifers were calculated. The hyper-saline challenge test was performed every day during culture I.

RESULTS AND DISCUSSION

Proportion of Digestive-organ Area to the Body (PDA)

Culture I resulted in good growth during which rotifer density attained 825 ind./ml^{-1} on d 8. Conversely, the rotifers in culture II did not obtain desirable growth and the peak was not clear (Fig.1). The digestive organs of egg-bearing rotifers could not be clearly distinguished from the developing ovary area. Therefore, only the change in PDA of non-egg-bearing rotifers is

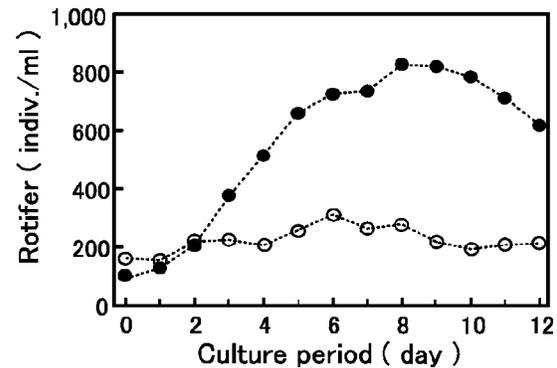


Figure 1. Growth of rotifer in cultures I and II. ●, culture I; ○, culture II.

shown in Fig. 2. PDAs in both culture I and II were not different from d 1 to d 7. On d 10, however, when the growth peak passed, the PDA of rotifers in culture II was significantly ($P < 0.01$) less than that of rotifers in culture I. These results mean that PDA did not fully reflect the changing

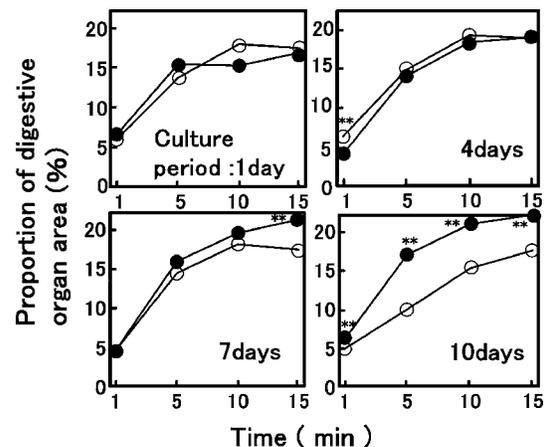


Figure 2. Changes in proportion of digestive-organ area of rotifer in cultures I and II. ●, culture I; ○, culture II; Significantly different between culture I and culture II (**: $P < 0.01$).

conditions of the culture. One explanation is that reproductive activity may be affected by declining environmental conditions prior to feeding activity. Therefore, the PDA index is apparently not useful as an index to readily monitor rotifer population growth.

Size Frequency Distribution of Lorica Length

Culture III showed good growth, with 839 ind./ml⁻¹ at the peak of rotifer density on d 7 (Fig. 3). The size frequency distribution of feeding rotifers did not change from d 1 to d 9. But on d 11, when the growth peak passed, production of offspring decreased and the proportion of rotifers over 250 μm increased (Fig. 4). The size frequency distribution of lorica length of feeding

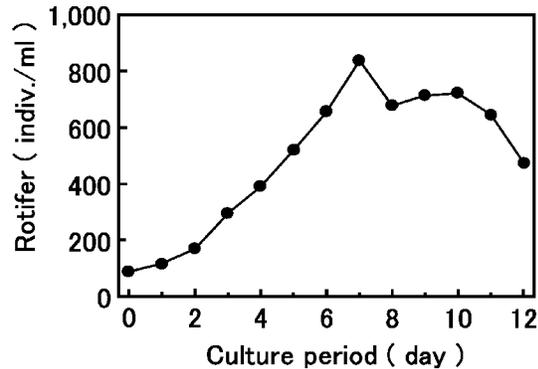


Figure 3. Growth of rotifer in culture III.

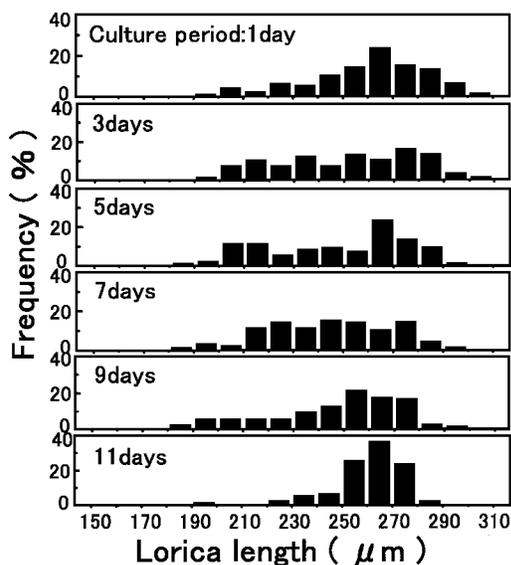


Figure 4. Changes in frequency distribution of lorica length of feeding rotifer in culture III.

rotifers was not suitable as an index to monitor population growth, since it changed only when culture conditions deteriorated sharply. On the other hand, in the non-feeding rotifers (Fig. 5), the small size group of 160-200 μm and the large size group of 250-300 μm were observed mainly from d 1 to d 5. The former are offspring, while the latter may be old and weak adults which can not feed. The intermediate group between 210 and 240 μm appeared from d 7, and increased over the course of culture. As first spawning size of this rotifer is about 240 μm , this intermediate group appeared young and active. It was concluded that the appearance of these young and non-feeding rotifers give an early warning of an impending collapse of the culture. Therefore, this result indicates that the size frequency distribution of non-feeding rotifer seems to be useful as an index to monitor rotifer population growth.

Tolerance to Hyper-saline Environment

The percentage of swimming rotifers at various salinity and exposure times is shown in Fig. 6 where changes are reflected when salinity ranged between 60 and 80 ppt. The midpoint (70 ppt) was therefore chosen for the hyper-saline challenge test. The percentage of swimming rotifers in 70 ppt was observed to be stable from 3 to 5 h, which indicated a suitable incubation time. Therefore the challenge test of 70 ppt salinity

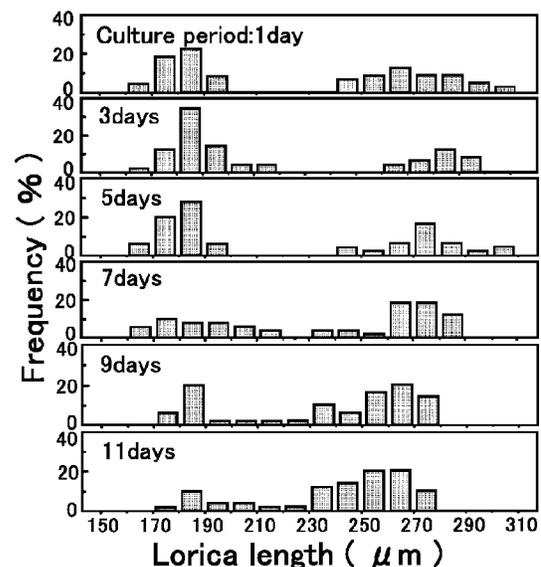


Figure 5. Changes in frequency distribution of lorica length of non-feeding rotifer in culture III.

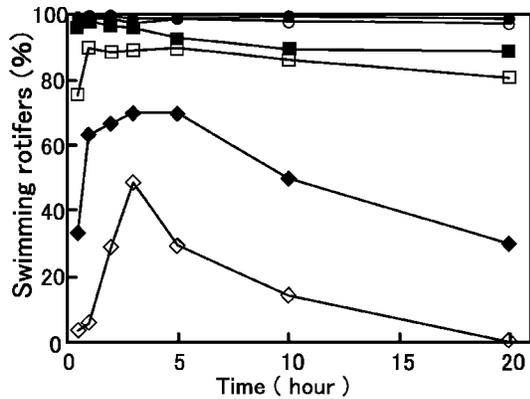


Figure 6. Changes in percentage of swimming rotifers exposed to the hyper-saline water. ●, 34 ppt; ○, 40 ppt; ■, 50 ppt; □, 60 ppt; ◆, 70ppt; ◇, 80 ppt.

for 3 h was adopted. The relationship between the percentage of swimming rotifers and population growth in culture I is shown in Fig.7. The percentages were nearly constant at 90% from d 1 to d 6. But in egg-bearing rotifers, a sudden drop to 68% was observed on d 7 when growth was near peak level. Therefore, tolerance of the egg-bearing rotifers to a hyper-saline environment was concluded to be a suitable index to measure rotifer activity, since this index changes prior to the peak of population growth.

If we can use this index during the mass culture of rotifers, it may be possible to forecast the impending collapse of a culture in a relatively short amount of time and also select vigorous rotifers that are in a rapid growth phase. Consequently, mass rotifer production can be designed to be more stable and efficient.

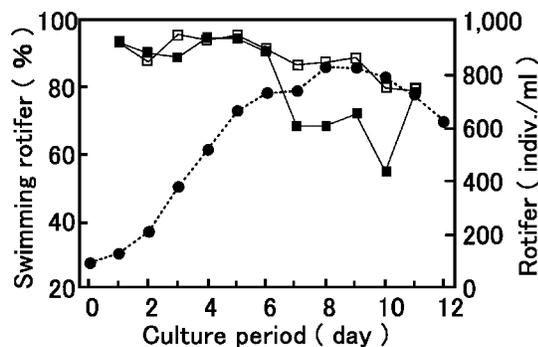


Figure 7. Population growth (●, rotifer density) of rotifer in culture-I and changes in the percentage of swimming rotifer (■, egg-bearing; □, non-egg-bearing rotifer) in hyper-saline challenge test (70 ppt).

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LITERATURE CITED

- Janssen, C.R., M.D. Ferrando and G. Persoone. 1994. Ecotoxicological Studies with the Freshwater Rotifer *Brachionus calyciflorus*. *Ecotoxicology Environmental Safety*. 28: 244-255.
- Juchelka, C.M. and T.W. Snell. 1994. Rapid toxicity assessment using rotifer ingestion rate. *Arch. Environ. Contam. Toxicol.* 26: 549-554.
- Korstad, J., A. Neyts, T. Danielsen, I. Overrein and Y.Olsen. 1995. Use of swimming speed and egg ratio as predictors of the status of rotifer cultures in aquaculture. *Hydrobiologia*. 313/314: 395-398.
- Snell, T.W., M.J. Childress, E.M. Boyer and F.H. Hoff. 1987. Assessing the status of rotifer mass culture. *Journal of the World Aquaculture Society*. 18: 270-277.