

Selective breeding in a Korean strain of the rotifer *Brachionus rotundiformis*

Reza Malekzadeh Viayeh and Choon Bok Song

Laboratory of Fish genetics and Breeding, Department of Marine Biotechnology, College of Ocean Science, Cheju National University, Jeju city 690-756, Korea

Introduction

Ingestibility of food by larval or juvenile fish or shellfish is determined to a great extent by the size of food particle in relation to the mouth size of the predator (Shirdhankar and Thomas, 2003). Along with the worldwide development in aquaculture industries, providing live food with suitable characteristics such as size for larval stage of culturing fish has become a main challenge for aquaculturists. Rotifers are used as a live food in the larval rearing of more than 78 species of marine finfish and crustaceans and demand for them is still increasing (Fu et al., 1997). However, they have found to be too large for smaller-mouthed larvae of aquaculture candidate such as grouper, Family Serranidae and rabbit fish, Family Siganidae (Rodrigues and Hirayama, 1997). The most commonly used rotifer in aquaculture, genus *Brachionus* has a diverse size range over two main species of *B. plicatilis* and *B. rotundiformis* and several strains occur at different geographic areas. The objective of this study was to evaluate the response to a bi-directional selective breeding program for smaller and larger sizes through several generations of different parental lines in the rotifer *Brachionus rotundiformis*.

Materials and methods

Experimental rotifers and size measurement

Two colonial populations of a laboratory stock of the rotifer *B. rotundiformis* isolated from Jeju Island, Korea, were used in this experiment. Cultural condition for the rotifers was provided with autoclaved seawater in a salinity of 30‰, temperature of 28°C and light intensity of 2500Lux (18:6 L:D). A daily amount of 5.2×10^5 cell / ml of marine *Clorella* was fed to the rotifers. From the culture containers of each clone with a concentration of 400 rotifer/ml, a sample volume of 20ml was randomly taken and fixed in 5% formalin to allow the measurement of the average lorica length of each population using a stereo- microscope in 100X magnification. To estimate the size variation of each clone over its life span, a number of 30-40 rotifers from each of the times zero after hatch to the age of 72 hours in an interval of 6 hours were fixed and their size was measured.

Selection procedures

Batch selection by plankton net "Netting selection"

A preliminary attempt for selection of the rotifers using plankton nets in different mesh sizes of 80, 100 and 124 μm was practically inconclusive to get the expected size range and separated generations. This was basically because of the rotifer body shape, similarities in its size at different generations, effect of eggs on body volume and its unpredictable direction while passing through the net mesh.

Individual's selection

By using a 4X magnification microscope, the smallest and the largest egg bearing

females were picked up by pipetting from the rotifer media and transferred individually to 24-well tissue culture plates each supplied with 2 ml autoclaved seawater and standard cultural condition as mentioned above. For each direction of selection (small and large size selection) of each clone, a number of 10 parental individuals were monitored for 10 consecutive generations with a similar selection procedure for each generation. The size of parental individuals and part of produced population was measured to compare the effect of selection on size. The comparison of size values and analysis of the obtained data were performed by using t-test and analysis of variances (ANOVA).

Results and conclusions

The smallest rotifers chosen as the first parental individuals of different generations had the sizes of 121-135 μm in clone 1 and 89-134 μm in clone 2. The largest selected parents had the size ranges of 156-185 μm and 148-167 μm in clones 1 and 2, respectively. In small sized individuals of clone 1, the minimum and maximum size of parental populations (Mean \pm SD) were 130.8 \pm 9.7 μm and 142.1 \pm 14.2 μm , respectively, while those of their 10th generation were 137.1 \pm 12.2 μm and 148.1 \pm 11.7 μm , respectively. For the large sized individuals of clone 1 minimum and maximum body size of parental population were 131.4 \pm 14.5 μm and 145 \pm 8 μm , respectively, while after 10 generation, the least and the highest average body size of the population measured as 141.8 \pm 9.8 μm and 145.8 \pm 9.3 μm , respectively. In clone 2 and for the smallest selected parents, populations had a mean body size ranged between 139.1 \pm 11.9 μm and 149.9 \pm 10.3 μm and after 10 generation the rotifer populations showed a size ranged from 140.6 \pm 19.1 μm to 176.5 \pm 13 μm . Of the largest selected parents of clone 2, the minimum and maximum population size ranged from 131.4 \pm 14.5 μm to 145 \pm 8 μm , compared to the minimum and maximum size of their last generations, 141.8 \pm 9.8 μm and 145.8 \pm 9.3 μm , respectively. ANOVA didn't display considerable difference between size ranges of two studied clones (P=0.31). On the other hand, although t-test showed differences among the size of some successive generations in both clones and selected groups (P<0.05), these differences did not necessarily denote a similar pattern in size increase or decrease over successive generations. Further more, our results showed that in a colonial rotifer culture even with same age, at any sampling time we may have a variety of sizes and there is no correlation between body size of the rotifer and its produced parthenogenetic eggs. Consequently, a selected individual with a certain body size might be still on growing or produce eggs and eventually offspring with different sizes. In whole, our findings show that an artificial manipulation for size selection at certain generations of the rotifers, doesn't lead us to a directed size range.

References

- Fu, Y., A., Hada, T., Yamashita, Y., Yoshida and A. Hino. 1997. Development of a continuous culture system for stable mass production of the marine rotifer *Brachionus*. *Hydrobiol.*, 358, 145-151.
- Shirdhankar, M.M. and P.C. Thomas. 2003. Response to bidirectional selection for naupliar length in *Artemia franciscana*. *Aquacul. Res.*, 34, 535-541.
- Rodriguez, E.M. and K. Hirayama. 1997. Semi-mass culture of the dinoflagellate *Gymnodinium splendens* as a live food source for the initial feeding of marine finfish larvae. *Hydrobiol.*, 358, 231-235.

*Corresponding author: big24man@hanmail.net