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Development of rotifer strains with useful traits for rearing fish larvae

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Abstract

The euryhaline rotifer *Brachionus plicatilis* is a species complex, which is commonly used for rearing marine fish larvae. Providing cultures with an appropriate size of rotifers facilitates size dependent selectivity of the feeding larvae and results in larvae with higher survival, growth and stability. It is also important to obtain rotifers with higher growth rate and tolerance against environmental stress and better nutritional quality after enrichment. This paper reviews the importance of feeding rotifers with appropriate size for fish larvae, as well as how much variation of rotifer traits can be expected from natural population of rotifers, as well as from artificial manipulation. These artificial manipulations include (1) manipulation of culture history to establish descendant rotifer clones with different reproductive traits, (2) manipulation of culture environmental conditions and chemical treatments on rotifer traits, such as size and reproduction, (3) cross-mating and a molecular approach for obtaining rotifer strains with useful traits in clones of their descendants. Recent progress on the production of rotifer strains with ideal characteristics in terms of size, population growth, tolerance against external conditions and resting egg formation is discussed.

Keywords: Live food; Rotifer; Size; Reproduction; Selection; Environmental control; Genetic approach

1. Introduction

The euryhaline rotifer *Brachionus plicatilis* has been used as an indispensable source of initial live food for mass-rearing of marine fish larvae. *B. plicatilis* is a species complex, which includes different morphotypes; culturists describe them based on lorica size as L (large), S (small), and SS (super small) types, and biological traits were compared among morphotypes by Hagiwara et al. (1995b). Based on information on morphology (Fu et al., 1991a), allozyme pattern (Fu et al., 1991b) and karyotypes (Rumengan et al., 1991), Segers (1995) classified L-type as *B. plicatilis* and others as *B. rotundiformis*. Recent studies indicate that the so-called S- and SS-type rotifers can be classified as different species; the former as *B. ibericus* and the latter as *B. rotundiformis* (Ciros-Perez et al., 2001; Kotani et al., 2005). It should be mentioned, however, that despite the strong species boundary observed between *B. plicatilis* and others, a weak species boundary was observed between *B. ibericus* and *B. rotundiformis* based on the male mating behavior when challenged with females of different species (Kotani et al., 1997). Recent studies in molecular phylogeny using ITS1 and COI (see Section

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6) indicate that *B. plicatilis* sp. complex may include at least 9 species (Gomez et al., 2002). Their results indicate that so-called L-, S- and SS-type rotifers include 4, 4 and 1 species, respectively. But their morphological differences among species have not been clarified and species names have not been given. It is important to continue and further confirm the molecular phylogenic results as the current analyses are based on only two partial DNA sequences. As the current results suggest that the taxonomy of *B. plicatilis* sp. complex is not yet clear, and in this paper, we use in this publication the terms of L-, S- and SS-type, that are commonly used terms among scientists and technologists in the area of aquaculture biology.

In order to provide cultured rotifers to fish larvae, it is important to produce sufficient large number of rotifers, not only as a cost effective food, but also for increasing survival, growth and viability of fish larvae, resulting in better quality and quantity of larvae (Hagiwara et al., 2001b). For this purpose, it is important to provide the appropriate size of rotifers to the larvae (Hagiwara et al., 2001a), in parallel to enhancing the stability of rotifer cultures to meet the demand of fish larvae consistently. Providing cultures with an appropriate size of rotifers facilitates size dependent selectivity of the feeding larvae and results in larvae with higher survival, growth and stability. For example, selection of smaller and larger sized rotifers is essential for rearing fishes with smaller and larger mouths (Oozeki et al., 1992; Fernandez-Diaz et al., 1994; Olsen et al., 2000; Tanaka et al., 2005). To conduct larval rearing of cold water fish species, it is strongly expected to have rotifer strains with higher tolerance of transfer to cold temperatures.

In this paper, we review recent progress of this area of research, especially those studies that were conducted after the last review publication by the same research group (Hagiwara et al., 2001b). We start with a description of importance of selection of appropriate size of rotifers for successful rearing fish larvae. Second, we discuss the degree of size variation among genetically different rotifer strains and how rotifer size can be artificially manipulated. Third, we review the difference in rotifer reproduction, i.e. population growth and resting egg formation among strains, as well as the resistance against environmental stress (such as an increase in ammonia concentration, protozoa contamination and the effect of increased viscosity). These topics highlight the significance of strain selection for conducting stable rotifer cultures. In this section, we also reviewed the benefit of application of chemical compounds for recovering the health status of rotifers. Fourth, we indicated the significance of cross-mating trials resulting in hybrid strains, which have useful characteristics in terms of size, reproduction and stress resistance. Finally, we describe the initial stage of our work on molecular tools for analyzing specific characteristics of rotifers, which could lead to improve our understanding on the mechanisms involved in the regulation of the rotifer life cycle, and their application in the future, for the development of ideal rotifer strains. In our research project, we employ rotifer strains from the culture collection maintained in our laboratory. These were originally collected from geologically different natural sites and aquaculture farms and cloned before they were added to the culture collection.

2. Importance of rotifer size for rearing fish larvae

Prey size is important in foraging behavior of fish larvae (Ivlev, 1965; Shirota, 1970). In order to compare the effect of feeding of rotifers with different size, yellowtail *Seriola quinqueradiata*, spotted halibut *Verasper variegatus* and *Platycephalus* sp. were fed from day 0 to day 15–20 posthatch (Hagiwara et al., 2001a). Fish larvae reared in 2000 l tank were transferred to 30 l tank, and rotifers were fed to larvae at 5–30 ind./ml. After 30 min, 20 larvae were sampled and their gut was dissected to determine the number and lorica length of fed rotifers. Measured rotifers were divided into three groups with different lorica size. Food size electivity of fish larvae was evaluated using Ivlev's electivity index as follows (Ivlev, 1965).

$$\mathrm{Ei} = (\mathrm{ri} - \mathrm{ni})/(\mathrm{ri} + \mathrm{ni})$$

where, Ei is the Ivlev's electivity index of the food type *i*, ri is the percentage of food type *i* in larval gut, ni is the percentage of the food type *i* in the environment. This index varies from −1.0 to +1.0, with positive values indicating preference.

The mouth size (upper jaw length times $2^{0.5}$, Shirota, 1970) of three fish larvae at the onset of feeding was 510, 280 and 260 μm for *V. variegatus*, *S. quinqueradiata* and *Platycephalus* sp., respectively. The mouth size increased respectively to the growth of the larvae (Table 1). For all fish species, larger (more developed) larvae selectively ingested large sized rotifers, according to their growth. But the trend was strongest with *Platycephalus* sp. (Fig. 1), which has the smallest mouth size among the three species. It should be noted that the feeding selectivity of larvae against different sized rotifers is not only dependent on mouth size of larvae, but on species specific characteristics. Fish larvae show better growth, survival and viability through rearing them by feeding rotifers with size of higher selectivity