

Determination of the Optimal Salinity and Algal Diet for Culture of the Rotifer, *Brachionus rotundiformis* for Rearing of Larval Fin Fish in Iran

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Abstract: Rotifer is one of the most important live foods used for feeding larviculture of fish. The optimal salinity may increase the reproductions of rotifers. In this project, the rotifers were cultured at different salinities (15, 20, 25, 30 and 35). To feed the rotifers, we used two types of microgae, *Nannochloropsis* and *chlorella*. *Brachionus rotundiformis*, at salinity 25 ppt and when using *Nannochloropsis* rather than *Chlorella* for feeding could significantly increase its densities in the experimental tanks.

Key words: Salinity • Algal diet • Rotifer • Fin fish

INTRODUCTION

To feed newly hatched and undeveloped marine fish larvae, rotifer is an excellent food. *Nannochloropsis* serves as initial feed for the rotifer as soon as they are fed with yeast until the rotifers would be harvested on day 5 [1].

B. Rotundiformis, called S-type, is commonly cultured; making it a good fit as live food for selection in different larval stages, thus improving hatchery production [2].

Batch culture and semi continuous culture are two methods for rotifer culture. Due to reduce space, labor requirements and in order to improve the quality of rotifers, the hatcheries are trying to use high-density culture and continuous culture [3]. European hatcheries are still rearing rotifers in batch systems with little change in water quality [4].

A review of Fielders *et al.* [2] showed that the rotifers have an optimal reproduction at any salinity within the range 4-35‰, but they are generally cultured at salinities between 10 and 20‰.

Yoshimura *et al.* [5] investigated the major factors of inhibiting or limiting the culture of rotifers such as feed concentration, dissolved oxygen and toxicity of undissociated ammonia. Some studies on the improvement of nutritional aspects and culture techniques of rotifers have been done on a commercial scale [6].

In Iran, the method that we are used to culture the rotifers, called three days culture system is mentioned in the following section. This project has tried to determine the optimal salinity that would improve the rotifers densities in Culture system in Iran.

MATERIALS AND METHODS

This project was conducted during a one-month period in the Station for Research at Bandar-e emam, Khuzestan province, Iran. To achieve these objectives, five different water salinity treatments (15, 20, 25, 30 and 35) were tested along with two types of microscopic algae (*Chlorella* and *Nannochloropsis*). Meanwhile, rotifer culture was carried out for a duration of 3 days. Each treatment was tested in triplicate, in experimental tanks of volumes holding 900 liters.

On day 1, the tanks were filled with chlorine-sterilized water (of course neutralized) and seawater at a different salinity. One type (*Chlorella* or *Nannochloropsis*) of microscopic algae at a concentration of 10 million/ml and rotifer at a density of 40-50/ml, respectively, were added. About six hours after stocking rotifers, these were fed with yeast at a concentration of 1g per 4 million rotifers.

On day 2, the rest of the 400 liters left, were filled with sterilized water and microscopic algae at a concentration of 10 million/ml. Six hours later, they were fed with yeast at a concentration of 1g per 4 million rotifers.

On day 3, rotifers were collected for the finfish hatchery. For each experimental tank, three 1-ml subsamples were taken.

Rotifer density was estimated for each sample, using a counting cell under a microscope at 10x magnification.

The densities of rotifers were daily determined. The rotifers were carefully siphoned from the tanks into a 400-500 μm sieve placed in a bowl, then filled with water so that rotifers would not be squashed onto the mesh. The collected data were analyzed with Kruskal-Wallis one-way ANOVA on ranks and Mann-Whitney rank sum test using SPSS media to determine significant differences among treatments.

RESULTS

Rotifer Culture with *Nannochloropsis*: The results on day 1 demonstrated that there was a significant difference among the densities of rotifers in treatments (Kruskal-Wallis one-way anova on ranks, $H = 13.00$, $df = 4$, $p = 0.01$). On day 2 and 3 there were also a significant difference among the density of rotifers in treatments (Kruskal-

allis one-way anova on ranks for day 2, $H = 13.50$, $df = 4$, $p = 0.09$, Kruskal-Wallis one-way anova on ranks for day 3, $H = 13.54$, $df = 4$, $p = 0.09$, respectively). The overall rotifer densities for all five treatments over a period of three days are shown in Fig. 1. Observation of the average of the densities of rotifers in different treatments indicates that the rotifers could increase their number and their densities more than the rest of salinity when the salinity was focused at 25 ppt in experimental tanks.

Rotifer Culture with *Chlorella*: The results on day 1 demonstrated that there was a significant difference among the densities of rotifers in treatments (Kruskal-Wallis one-way anova on ranks, $H = 9.55$, $df = 4$, $p = 0.04$). On days 2 and 3 there was also a significant difference among the densities of rotifers in treatments (Kruskal-Wallis one-way anova on ranks for day 2, $H = 13.01$, $df = 4$, $p = 0.01$, Kruskal-Wallis one-way anova on ranks for day 3, $H = 13.17$, $df = 4$, $p = 0.01$, respectively). The overall rotifer densities for all five treatments over a period of three days are shown in Fig. 2. Observation of the averages of the densities of rotifers in different treatments

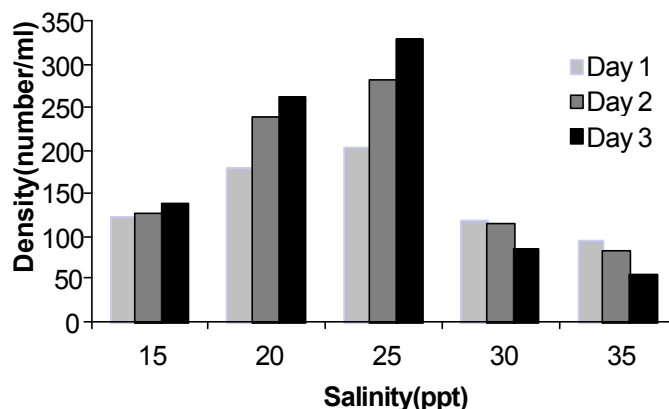


Fig. 1: Average of the Densities of Rotifers in Different Treatment with Feeding Nannochloropsis

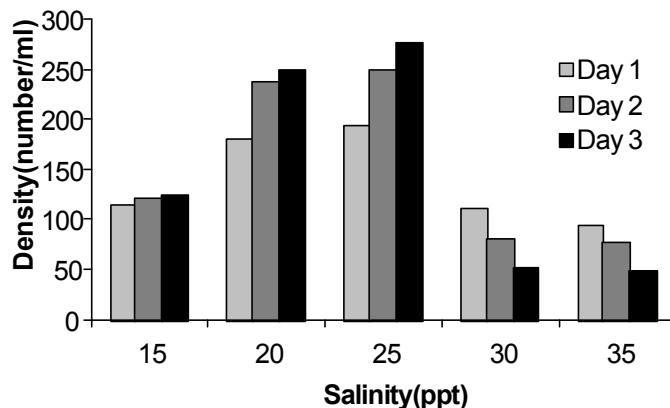


Fig. 2: Average of the Densities of Rotifers in Different Treatment with Feeding Chlorella

indicates that the rotifers could increase the number and their densities more than the rest of salinity when the salinity was focused at 25 ppt in experimental tanks.

When *Nannochloropsis* fed the rotifers, they could significantly increase densities more than *Chlorella* in the experimental tanks (Mann-Whitney rank sum test, $T=19$, $p=0.06$)

DISCUSSION

Because rotifers are osmo-conformers, they can tolerate salinities ranging from 1 to 97‰ [2, 7], although they are generally cultured at a particular salinity.

Brachionus plicatilis in Japan was cultured (combination feeding of *Nannochloropsis* and freshwater *Chlorella*) in a continuous culture system at salinity of 25-27 ppt [3]. In Thailand, to produce microalgae and rotifers, *B. plicatilis* operated in a closed-recirculating, continuous culture system at salinity of 25 ‰ for larval fish culture [8].

Grouper is one of the finfish cultured in Iran. We are rearing the larval grouper at a salinity of 20 ppt in culture tanks. Our results demonstrate that *B. rotundiformis* was significantly able to increase the density at 25 ppt more than 15, 20, 30 and 35 ppt.

Rapid changes in salinity cause shock, such as affecting swimming activity and oxygen consumption of the rotifer, *B. plicatilis*, whereas that causes the rotifers sink to bottom and adhere to the side of the vessels with their sticky feet [2]. Research indicated that even with the effect of initial transfer salinity shock on rotifer availability by Fiedler *et al.* [2], the availability of rotifers in a water column was not affected by transfer from 23°C to 28°C. We believe that according to these results by Fiedler *et al.* [2], in larval culture in Iran, when the rotifers are introduced from 25 to 20 °C to a fin fish culture tank, may have some effect on the availability of rotifers.

Our results indicated that we should use salinity of 25 ppt to amass culture in the hatcheries of Iran. We should also work on rapid changes in salinity that may cause shock in the rotifers, *B. rotundiformis*.

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