Rotifer Culture in *Nannochloropsis* sp. at Different Salinity

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Abstract: Larval fish have very specific food requirements for the first meals following the resorption of their yolk before growing to a size where they can be fed on *Artemia* nauplii or even weaned onto an artificial diet. This is applicable especially to marine fish larvae which have very small mouth opening at first feeding stage. Rotifer is a very important live feed in controlling the early larval stages of many farmed aquatic animals. In order to maintain the population of the small size rotifer, *Nannochloropsis* sp. has been chosen as a feed. This experiment was carried out to determine the best salinity for culturing rotifer in *Nannochloropsis* sp. medium and to see whether it can maintain its size using this alga as feed. The experiment was conducted for 14 days at four different salinities i.e., 15, 20, 25 and 30 ppt. The temperature was controlled at 29°C with pH recorded daily. The rotifer abundance was maintained at 300 ind. ml^{-1} from day 4 to the end of the experiment in 15 ppt salinity as compared with the control 30, 20 and 25 ppt. Maximum densities of rotifer recorded for each salinity were 394 ind. l^{-1}, 345 ind. l^{-1}, 344 ind. l^{-1} and 221 ind. l^{-1} in 15, 20, 25 and 30 ppt respectively. Average density of rotifer calculated when it reached the first peak (day 4 onwards) was 321 ind. l^{-1}, 259 ind. l^{-1}, 254 ind. l^{-1} and 145 ind. l^{-1} in 15, 20, 25 and 30 ppt respectively. There was no significant difference in the size of rotifer, which read 136 ± 6.00 µm, 136 ± 6.27 µm, 136 ± 3.98 µm and 139 ± 5.90 µm for lorica length (LL) and 98 ± 3.69 µm, 97 ± 2.92 µm, 96 ± 2.46 µm and 98 ± 3.08 µm for lorica width (LW) in 15, 20, 25 and 30 ppt respectively. Hence it is suggested that the size of rotifer can be maintained using *Nannochloropsis* sp. as feed and the best salinity for culturing rotifers with *Nannochloropsis* sp. is 15 ppt.

Keywords: rotifer, *nannochloropsis*, culture salinity

Abstrak: Larva ikan mempunyai keperluan makanan yang spesifik sebagai makanan permulaan selepas kehabisan yolka sebelum ia boleh membesar sehingga boleh memakan naupli *Artemia* atau diasuh memakan makanan rumusan. Hal ini penting terutamanya bagi larva ikan marin yang mempunyai saiz mulut kecil pada peringkat pertama ia mula makan. Rotifer merupakan makanan hidup yang penting dalam mengawal pertumbuhan peringkat awal kebanyakan haiwan ternakan akuatik. Dalam mengekalkan saiz dan populasi rotifer, alga *Nannochloropsis* sp. telah dipilih sebagai makanan. Eksperimen ini dijalankan untuk mencari kemasinan terbaik untuk menternak rotifer di dalam medium *Nannochloropsis* sp. dan untuk melihat sama ada saiz rotifer boleh dikekalkan dengan menggunakan alga sebagai makanan. Eksperimen ini dijalankan selama 14 hari pada empat saliniti berlainan iaitu 15, 20, 25 and 30 ppt. Suhu dikawal pada 29°C dan pH diukur setiap hari. Kepadatan rotifer berada pada paras 300 ind. ml^{-1} dari hari keempat sehingga tamat eksperimen pada saliniti 15 ppt berbanding kawalan 30, 20 dan 25 ppt. Kepadatan maksimum yang direkodkan adalah 394 ind. l^{-1}, 345 ind.l^{-1}, 344 ind. l^{-1} dan 221 ind. l^{-1} dalam 15, 20, 25 dan 30 ppt masing-masing. Purata rotifer dikira bermula dari puncak pertama (dari hari keempat) ialah 321 ind. l^{-1}, 259 ind. l^{-1}, 254 ind. l^{-1} dan 145 ind. l^{-1} dalam 15, 20, 25 dan 30 ppt masing-masing. Tidak terdapat sebarang perbezaan yang nyata dalam saiz rotifer iaitu 136 ± 6.00 µm, 136 ± 6.27 µm, 136 ± 3.98 µm dan 139 ± 5.90 µm untuk panjang lorika (LL) dan 98 ± 3.69 µm, 97 ± 2.92 µm, 96 ± 2.46 µm dan 98 ± 3.08 µm untuk lebar lorika (LW) dalam 15, 20, 25 dan 30 ppt masing-masing. Oleh itu, *Nannochloropsis* sp. didapati mampu mengekalkan saiz rotifer dan saliniti terbaik untuk ternakan rotifer di dalam *Nannochloropsis* sp. ialah pada 15 ppt.

Introduction

The rearing of many marine fish larvae requires the use of live food. The rotifer, *Brachionus plicatilis* and the crustacean, *Artemia salina*, are the two organisms most extensively used as food (Carić *et* al., 1993) for a large variety of finfish and crustacean larvae (Walford and Lam, 1992; Su *et al.*, 1994a). *B. plicatilis* is a small rotifer first developed as larval fish food in Japan in 1950s. Since then, many methods of culturing it have been developed around the world (Treece and Davis, 2000) and it has been actively

cultured in South Asian countries for a whole year (Fukusho and Okauchi, 1982) with water temperature of 25-30°C. *B. plicatilis* has been widely used due to its ideal size, quick reproductive rate and ability to feed on different unicellular algae and baker's yeast (Carić *et al.*, 1993). It is an euryhaline species, small and slow-swimming, with good nutritional value. It is well suited to mass culture because it is prolific and tolerates a wide variety of environmental conditions. Rotifer may tolerate 1 to 97 ppt salinity, but optimum reproduction occurs below 35 ppt. The optimum temperature for most strains is 28 to 32°C (Treece and Davis, 2000). The size of *B. plicatilis* ranges between 100-400 μm. It can be separated into three strains, i.e. the L-type (190-320 μm), the S-type (140-220 μm) and the SS-type (100-160 μm).

The nutritional quality of live food is very important for the survival of fish larvae. Therefore, along with the production of sufficient quantities of rotifers, it is also necessary to ensure proper nutritional quality to satisfy the needs of larvae (Carić *et al.*, 1993). Researchers have determined that highly unsaturated fatty acids (HUFAs) are essential for the survival and growth of marine finfish larvae. Rotifer feeds containing docosahexaenoic acid (DHA:22:6n-3) and eicosapentaenoic acid (EPA: 20:5n-3), can be valuable, with DHA the more essential for marine fish larvae. Depending upon their food source, rotifers contain about 52-59% protein, up to 13% fat, and 3.1% *n*-3 HUFA (Treece and Davis, 2000).

A major problem in relation to feeding fish larvae is that rotifers cultured with baker's yeast alone have low levels of n-3 HUFA, especially 20:5n-3 and 22:6n-3. Although these fatty acids can be synthesized in yeast-fed rotifers either de novo or through elongation and desaturation of precursor molecules, the process is slow and the amounts of fatty acids accumulated are insufficient (Walford and Lam, 1992).

The chemical composition of rotifers is similar to that of the algae upon which they feed (after Carić *et al.*, 1993). Few reports have been made regarding the use of *Nannochloropsis* as food for rotifers. Growth rate, doubling time, lipid, carbohydrate and protein content of rotifers fed either *Nannochloropsis* sp. or *Phaeodactylum tricornutum* monoculture indicated that these are the most suitable diets for the rotifer and are expected to satisfy the nutritional demand of fish larvae (Carić *et al.*,1993). The range of LL and LW of rotifer cultured in *Nannochloropsis* are also smaller in size compared with the work done by Fukusho and Okauchi (1982) using Baker's yeast and other algae such as *Tetraselmis* sp. and *Chlorella*. Therefore, in this study *Nannochloropsis* sp. was chosen as feed for the rotifer in order to fulfil the nutrient requirement of the fish larvae as well as to maintain the small size of rotifer to be used in the rearing of grouper larvae at our Centre.

Materials and Methods

Source of Rotifer

Small marine Brachionus strain from single clone have been isolated and maintained in the test tubes and fed with *Nannochloropsis* sp. The strain originally collected in Lembaga Kemajuan Ikan Malaysia (LKIM) pond nearby National Prawn Fry Production and Research Centre (NAPFRE) and was maintained in the laboratory for a month prior to experiment. It was maintained at 25°C temperature, 30 ppt salinity and fed with *Nannochloropsis* sp. daily at a density of $4x10^6$ cells/ml. The density was observed daily and the size measured every three days to ensure that there was no mixed stock with L and S type rotifer.

Source of Nannochloropsis sp.

Algae *Nannochloropsis* sp. was acquired from the Algae Laboratory in NAPFRE and cultured in seawater at four different salinities i.e. 15, 20, 25 and 30 ppt. It was maintained using Conway medium.

Culture Maintenance

In this experiment, 4 individual rotifers were stocked in 1-L beaker as seeds. Initial water volume was 500 ml and increased to 750 ml from second day onwards. Culture temperature was maintained at 29°C using water bath and fed with *Nannochloropsis* sp. cultured at four different salinities i.e. 15, 20, 25 and 30 ppt. The alga was supplied to rotifer once a day during water exchanged at a density of 3.0 x 10^6cells/ml. It was done by filtering out culture water and topped up with *Nannochloropsis* sp. cultured in respective salinity.

Sampling and data collection

Sampling included collecting water for determination of rotifer density and size measurement, algae density and pH determination. Algae density was calculated before and after water exchange to ensure adequate supply of food for the rotifer. 10 ml sample was collected daily, fixed with 5% formalin and measured under ocular micrometer using Sedgewick Rafter Counter. For each treatment, at least 60 (max. 100) individuals were measured for lorica width and length. The data was analysed using One Way ANOVA and Duncan Multiple Test. Reproduction rate of *Brachionus* sp. was calculated using the formula below (after Su *et al.*, 1994a):

$$\text{Reproduction rate, } r = \frac{\ln N_t \ \ln N_0}{t}$$

where N_t = maximum density,
N_0 = initial density,
t = day of culture when reached maximum density

Results

Results for pH measurement and algal density throughout the experiment are shown in Table 1. There is no significant difference ($p>0.05$) in pH and algal density of each treatment which means that rotifer cultured in *Nannochloropsis* sp. can maintain pH from falling as long as water quality is taken care of by changing water and siphoning the debris every day.

Table 1: pH readings and *Nannochloropsis* sp. density at different salinity

Salinity (ppt)	pH measurement (mean ± SD)	Algae density (mil. Cells/ml) (Ave ± SD)
15	7.9 ± 0.13	2.92 ± 1.53
20	7.9 ± 0.10	2.87 ± 0.10
25	7.9 ± 0.09	3.22 ± 1.68
30	8.0 ± 0.06	3.02 ± 1.93
mean ± SD	7.9 ± 0.07	3.04 ± 1.53

From Fig.1, the numbers of rotifers increased rapidly in all treatments from day 2 to day 4 which read 373, 345, 260 and 148 ind. l^{-1} in 15, 20, 25 and 30 ppt respectively. However on day 5, the reading decreased in all treatments and went on fluctuating and dropped in all salinities except 15 ppt. Although the numbers increased again in day 14, the maximum number noted was in 15 ppt salinity. Numbers of rotifers carrying eggs and total number of eggs in each treatment also gave the same pattern i.e. fluctuation in each salinity but 15 ppt salinity still produced the highest number (Fig. 2 & 3).

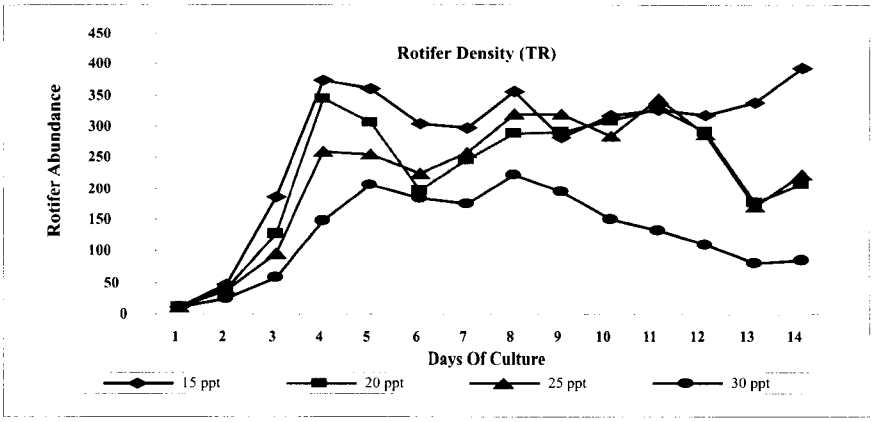

Figure1: Rotifer density in different salinity fed with *Nannochloropsis* sp

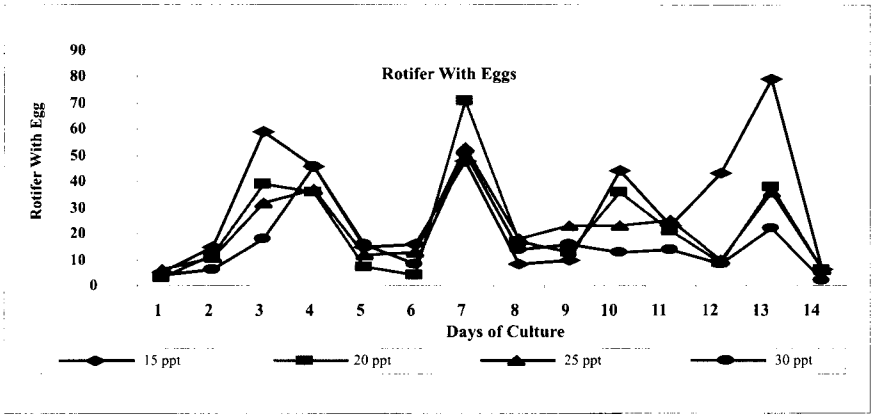

Figure 2: Number of rotifers carrying eggs during the experiment

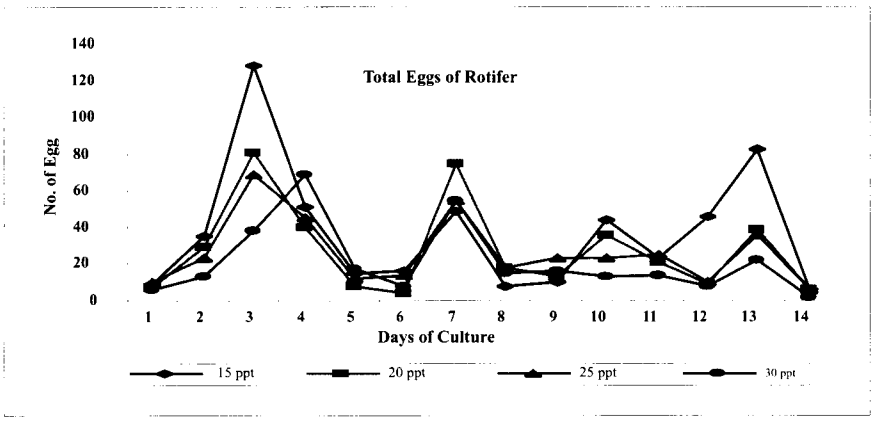

Figure 3: Total number of eggs in each culture tank during the experiment

Table 2 shows that there is no significant different in reproductive rates between 15, 20 and 25 ppt. However, rotifers cultured in *Nannochloropsis* at 30 ppt salinity gave the lowest reproductive rates. This is true according to Su *et al.* (1994a) that rotifer were distinguished according to their size where the optimum culture conditions for SS-type were at salinity 10-20 ppt with suitable feed of *Tetraselmis chui, Nannochloropsis oculata* and *Isochrysis galbana*.

Table 2: Reproductive rate of *Brachionus* sp. at the end of the 14 days experiment with *Nannochloropsis* sp.

Salinity (ppt)	Ini. density	Max. density	Peak day	Reproductive rate
15	4	373	4	1.133821[a]
20	4	345	4	1.114313[a]
25	4	260	4	1.043597[a]
30	4	205	5	0.787343[b]

Measurement values of length and width with standard deviation of rotifer cultured in this experiment are shown in Table 3. Size of rotifers cultured in different salinities ranged from: lorica length (LL) of 126-153 μm and lorica width (LW) of 88-103 μm in 15 ppt salinity; LL of 129-152 μm and LW of 92-102 μm in 20 ppt; LW of 132-146 μm and LW of 92-101 μm in 25 ppt salinity and LL of 132-157 μm and LW of 90-102 μm in 30 ppt respectively. All lorica length and width were nearly the same ($p>0.05$).

Table 3: Size distribution of the rotifer fed with *Nannochloropsis* sp. in different salinity

Salinity (ppt)	Size distribution (μm)	
	Lorica length (LL) ± SD	Lorica width (LW) ± SD
15	136 ± 6.00 (126-153)	98 ± 3.69 (88-103)
20	136 ± 6.27 (129-152)	97 ± 2.92 (92-102)
25	136 ± 3.98 (132-146)	96 ± 2.46 (92-101)
30	139 ± 5.90 (132-157)	98 ± 3.08 (90-102)

Discussion

Several techniques of culturing rotifer have been developed in NAPFRE since 1990s. They were used as feed for rearing prawn and crabs larvae. Started with freshwater *Chlorella* sp. as feed, the culture practice continued using baker's yeast to overcome the shortage of algae supply. Since 1997, when the focus changed to rearing marine fish larvae, other techniques of culturing rotifer have to be developed, especially when dealing with grouper larvae. Then the culture of rotifer using *Nannochloropsis* sp. began.

Groupers of serranid genus *Epinephelus* are one of the important food fishes of coastal aquaculture in East and Southeast Asia countries. Nevertheless, fry culture trials have encountered many difficulties particularly in early larval rearing. This difficulty is attributed to their small mouth size at the onset of feeding which resulted in poor feeding ability. This is in contrast to sea bass larvae which can start feeding on rotifers and survive at high rate. As alternative food organisms, researchers have tried such food

organisms as oyster larvae, screened small-size rotifers, SS-strain rotifers and artificial diets in combination with rotifers (Ali *et al.*, 1998).

In tropical aquaculture, the SS-type rotifers (super small rotifers) are preferred as the first feeding of fish larvae with small mouth openings (rabbitfishs, groupers and other fishes with mouth openings at the start of feeding of less than 100 μm). Those rotifers are smaller than common S-strains but are not genetically isolated from S-strains. Some reports indicated that mainly small size *B. plicatilis* were ingested by early grouper larvae (Fukusho and Okauchi, 1982).

Many marine animals, including peanaeid prawns and fish larvae need ω-3 HUFA for growth and survival. However, rotifers generally synthesize only small amounts of ω-3 HUFA. Therefore, the ω-3 HUFA must be provided to the rotifers via their food to meet the possible needs of marine fish larvae (Su *et al.*, 1994a). An important problem in relation to feeding fish larvae is that rotifers cultured with baker's yeast alone have low level of ω-3 HUFA, especially 20:5n-3 and 22:6n-3 which are essential fatty acids for marine fish larvae. Although these fatty acids can be synthesized in yeast-fed rotifers either *de novo* or through elongation and desaturation of precursor molecules, the process is slow and the amounts of fatty acids accumulated are insufficient (Walford and Lam, 1992).

According to Su *et al.* (1994), rotifers enriched with *Nannochloropsis* alone contained higher level of EPA and DHA. Incorporation of EPA (20:5n-3) and DHA (22:6n-3) occurred in microalgae-fed rotifers with *Nannochloropsis* gives highest content of this fatty acid, followed by other algae such as *Chaetoceros*, *Imantonia*, *Tetraselmis*, *Isochrysis*, *Dunaliella* and *Chlorella*. Furthermore, salinity appeared to have some effect on the incorporation of fatty acids by rotifers; lower salinity generally gave higher levels of incorporation. Levels of incorporation was higher at 15 ppt than at 33 ppt. This may be due to higher food conversion rate of rotifers at lower salinities.

From the results of the experiment it has been shown that the rotifers were able to maintain their sizes when cultured in *Nannochloropsis* sp. at 15-30 ppt and the best salinity for culturing rotifer in *Nannochloropsis* sp. is at 15 ppt. Su *et al.* (1994b) also found that the optimum culture conditions for SS-type were at salinity of 10-20 ppt and temperature of 30-33°C; with suitable feeds such as *Tetraselmis chui*, *Nannochloropsis oculata* and *Isochrysis galbana* and there was no significant difference in size of SS-type cultured at different temperatures and salinities, and by different algal items. Another problem of rotifers fed on baker's yeast which are usually larger than those fed on live algae (Fukusho and Okauchi, 1982) can be overcome since the size of rotifer can be maintained in *Nannochloropsis* sp. Therefore, it is hoped that the results of this study will be useful in helping the industry to increase marine fish fry production for future demand.

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