ISSN 0970 - 6011

Volume 57, Number 1, 2010

Indian Journal of Fisheries

Indian Council of Agricultural Research

Published by
The Director
Central Marine Fisheries Research Institute
Cochin - 682 018, Kerala, India
www.cmfri.org.in

Indian J. Fish., 57(1) : 31-37, 2010

Reproductive potential of the rotifer, *Brachionus rotundiformis* Tschugunoff in relation to salinity, feed type and feed concentration

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ABSTRACT

The rotifer, *Brachionus rotundiformis* was isolated from Cochin backwaters, off the Vypeen Island. Pure cultures were developed and experiments were conducted to evaluate the reproductive potential, using different salinities, feed types and feed concentrations as variables. The studies indicated that, these three variables exert significant influence on reproductive potential of this rotifer. For the four different feed types tested, the r_{max} values were found to decrease in the order, *Nannochloropsis oculata* → *Chlorella marina* → *Isochrysis galbana* → Baker's yeast. For all the 4 feed types tested, the r_{max} values were maximum at the highest feed concentrations used in the experiment. The influence of salinity, feed type and feed concentration, individually as well as in combination, on the reproductive potential of the species is presented.

Keywords: *Brachionus*, Cochin backwaters, Feed, Reproductive potential, Rotifer, Salinity

Introduction

The reproductive potential is a measure of the inherent ability of an organism to reproduce, which is symbolized by 'r'. It summarizes all life table parameters, as it combines survival, fecundity, timing of development and reproduction. As aquaculture of finfish is gaining momentum in recent years, it is essential to have a regular supply of seed of food fishes. Since the rotifer, *Brachionus rotundiformis* is considered to be an indispensable and widely accepted livefeed for the larval stages of fin fishes, studies on reproductive potential of the species is important and useful in hatchery operations. Even though studies have been carried out on different aspects of culture of rotifers, especially *Brachionus plicatilis* in India as well as in other parts of the world, only limited work has been carried out to investigate the reproductive potential of *B. rotundiformis*. In fact, Segers (1995) recognized that the S type of *B. plicatilis* is *B. rotundiformis* and henceforth that name has been accepted all over the world. Hagiwara *et al*. (1995) dealt with the morphology, reproduction, genetics and mating behavior of small tropical marine *Brachionus* strains. The influence of dilution rate on the population dynamics of rotifers, *B. plicatilis* and *B. rotundiformis* in semi-continuous culture, fed on freeze-dried microalgae was studied by Navarro and Yufera (1998). Rumengan *et al*. (1998) observed the morphology and resting egg production of the tropical ultra-minute rotifer, *B. rotundiformis,* fed on different algae. Gopakumar and Jayaprakas (2004) discussed the life table parameters of *B. plicatilis* and *B. rotundiformis* in relation to salinity and temperature. The influence of salinity and reproductive potential of S and SS strains of *B. rotundiformis* were studied by Hagiwara *et al*. (1995). Anitha (2003) studied the 'r' values of 6 species of *Brachionus viz*., *B. angularis, B. caudatus, B. calyciflorus, B. plicatilis, B. murray* and *B. rotundiformis* in the samples collected from southern part of Kerala and noticed that the reproductive potential of a particular species is different from another species, so also for different strains of the same species. Published reports on the reproductive potential of the species are scarce and there is no previous report on the reproductive potential of rotifers from the central part of Kerala. In the present study, the impact of salinity, feed type and feed concentrations on the reproductive potential of *B. rotundiformis* collected from Cochin backwaters was investigated. Studies were also conducted to see whether there is any variation in 'r' values, when rotifers with and without eggs were initially employed for the experiments.

Materials and methods

The rotifer, *B. rotundiformis* was isolated from the Cochin backwaters off the Vypeen Island. Pure cultures were then developed and experiments were conducted to find out the reproductive potential at different salinities using different feed types and different feed concentrations. Salinities selected were 35, 21, 14 and 7 ppt, as most of the food fishes require feed of these salinities. Feed types used were *Nannochloropsis oculata, Chlorella marina, Isochrysis galbana* and baker's yeast, *Saccharomyces cerevisiae*. The microalgal cultures maintained in the laboratory of Central Marine Fisheries Research Institute, Kochi, were used for the experiments. Before starting the experiments, the rotifer cultures were acclimatized to the particular feed types and salinities for one month. As the size of *I. galbana* and *S. cerevisiae* are bigger than that of *N. oculata* and *C. marina*, different feed concentrations were selected for different feed types. Feed concentrations selected were 8, 4, 2 and 1 million cells per ml in the case of *N. oculata* and *C. marina*. With regard to *I. galbana* and *S. cerevisiae*, 4, 2, 1 and 0.5 million cells per ml were selected. The different feed concentrations were prepared by centrifuging the algal cultures and serial dilutions were made by adding water of that particular salinity. The algal cell counts were estimated using a haemocytometer.

The experimental design was as follows. One millilitre of each of the feed concentrations were taken in 5 ml glass tubes; 10 tubes for each concentration, each salinity and for each feed type were taken. In order to study the variations, if any, between the reproductive potential of rotifers with and without egg, the experiments were conducted in two sets of 320 tubes each. In set I, rotifers without egg were used for the experiment and in set II, rotifers with 1 egg were used. To each tube containing 1 ml of feed, one rotifer each was transferred with the help of a micropipette. All the tubes were kept under illumination for 9 h a day and after 3 days, they were fixed using 4% formaldehyde solution. The rotifer counts in each tube was taken and recorded. The reproductive potential was calculated using the formula, r = lnNt – lnNo/t where, Nt = Number of rotifers after time t; N_o = Number of rotifers initially present and t = time taken in days.

Three-way ANOVA was done using SYSTAT version 7.0.1, SPSS INC, to compare the influence of salinity, set of experiment and feed concentration separately as well as their interactions in different combinations on reproductive potential values of each feed type. ANOVA test was also performed to study the influence of these variables at different levels on the r values, with respect to the four feed types separately.

Results and discussion

The reproductive potential in relation to salinity and feed concentration in the two sets of experiments using the four feed types *viz., N. oculata, C. marina, I. galbana* and baker's yeast, are presented.

Nannochloropsis oculata

The mean numbers, mean reproductive potentials along with their standard deviations when rotifers without as well as with 1 egg was used for the experiments, are given in Tables 1a and 1b.

In all the feed concentrations selected, as well as in the four salinities adopted for the study, the 'r' values were slightly higher when rotifers with 1 egg was used for the experiment, compared to that with rotifers without egg. Also, the reproductive potential was found to increase with feed concentrations, and maximum was noticed at the highest feed concentration of 8 million cells per ml used. Among the four salinities adopted for the experiments, the lowest 'r' value of 0.792 was noticed at 35 ppt salinity and the highest of 1.756 was observed at 14 ppt salinity.

Table 1. Reproductive potential of *Brachionus rotundiformis* in different salinities and feed concentrations of *Nannochloropsis oculata*

a) When rotifer without egg was used for the experiment

Conc.of feed (Cells ml^{-1})	Salinity 7 ppt		14 ppt		21 ppt		35 ppt	
	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD
1x10^6	26±4	1.08±0.05	38±4	1.21±0.04	26±3	1.09±0.03	16±3	0.92±0.07
2 x10^6	31±16	1.08±0.24	63±4	1.38±0.03	49±6	1.29±0.04	12±5	0.79±0.16
4 x10^6	37±21	1.10±0.31	96±9	1.52±0.03	75±14	1.44±0.06	16±7	0.88±0.18
8 x10^6	53±20	1.28±0.17	196±31	1.76±0.05	86±21	1.48±0.07	24±11	0.99±0.27

b) When rotifer with 1 egg was used for the experiment

Conc.of feed (Cells ml^{-1})	Salinity 7 ppt		14 ppt		21 ppt		35 ppt	
	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD
1x10^6	26 ± 12	1.04±0.19	42±3	1.24±0.03	30±2	1.13±0.03	17±1	0.94±0.03
2 x10^6	39 ± 10	1.21±0.09	68±8	1.40±0.04	50±4	1.30±0.03	25±5	1.06±0.08
4 x10^6	55 ± 20	1.31±0.13	106±9	1.56±0.03	80±13	1.45±0.06	21±6	1.00±0.09
8 x10^6	43 ± 12	1.23± 0.12	181±16	1.73±0.03	100±37	1.51±0.14	28±9	1.09±0.14

Three-way ANOVA showed that the influence of salinity and feed concentration on reproductive potential in two sets of experiments were significant (p<0.01). The variations between salinities at four levels *viz*., 35 ppt, 21 ppt, 14 ppt and 7 ppt in all combinations were found to influence the 'r' values. In the case of feed concentrations, the variations in 'r' values of $1x10^6$ cells ml^{-1} with those of $2x10^6$ cells ml^{-1}, $4x10^6$ cells ml^{-1} and $8x10^6$ cells ml^{-1}, were significant (p<0.01). The variations between 2 million and 4 million cells per ml with that of 8 million cells per ml were also found to influence the 'r' values, significantly (p<0.01).

The salinity and feed concentration in two sets of experiments independently influence the 'r' values significantly. The interactions of feed concentration + salinity on 'r' value were found to be significant (p<0.01). But, the interactions of the set of experiment + feed concentration, set of experiment + salinity and set of experiment + feed concentration + salinity on 'r' values were not significant.

Chlorella marina

The mean numbers, mean reproductive potentials along with their standard deviations, when rotifers without as well as with 1 egg used for the experiment, are given in Tables 2a and 2b.

The reproductive potentials showed a gradual increase, along with the increase in feed concentrations, in all the salinities used for this experiment, except at feed concentration of 8 million cells per ml at 35 ppt salinity. This observation was true when rotifers without and with 1 egg were used for the study. During the experiment, the overall variation in reproductive potential was between 0.858 at 35 ppt salinity and 1.573 at 21 ppt salinity. At 14 ppt, the 'r' value observed was 1.563, which was only slightly lower than the maximum. The minimum was noticed at feed concentration of 1 million cells per ml and maximum at 8 million cells per ml.

The influence of salinity and feed concentration on 'r' values were found to be significant (p<0.01). Detailed studies indicated that, in the case of salinity, the variations of 'r' values between that of 35 ppt with other 3 levels of salinities *viz*., 21 ppt, 14 ppt and 7 ppt were found to be significant and other variations were not significant. The variations of 'r' values between all the levels of feed concentrations, *viz*., 1 million, 2 million, 4 million and 8 million cells per ml were found to be significant and the 'r' values were not influenced by sets of the experiments *viz*., rotifers without egg or with 1 egg.

The interactions of salinity + feed concentration on r values were significant (p<0.01). The interactions of set of experiment + feed concentration, set of experiment + salinity and set of experiment + feed concentration + salinity on 'r' values were not significant.

Isochrysis galbana

The mean numbers, mean reproductive potentials along with their standard deviations, when rotifers without as well as with 1 egg was used for the study are given in Table 3a and 3b.

Table 2. Reproductive potential of *Brachionus rotundiformis* in different salinities and feed concentrations of *Chlorella marina*

a) When rotifer without egg was used for the experiment

Conc.of feed (Cells ml^{-1})	Salinity 7 ppt Mean nos. ± SD	Mean r ± SD	14 ppt Mean nos. ± SD	Mean r ± SD	21 ppt Mean nos. ± SD	Mean r ± SD	35 ppt Mean nos. ± SD	Mean r ± SD
$1x10^6$	15±2	0.90±0.05	21±5	1.01±0.07	16±4	0.91±0.09	15±8	0.86±0.17
$2 x10^6$	25±9	1.04±0.15	35±5	1.18±0.06	28±9	1.09±0.12	25±15	1.02±0.18
$4 x10^6$	50±4	1.30±0.03	64±4	1.38±0.02	52±7	1.31±0.05	42±5	1.25±0.04
$8 x10^6$	93±16	1.50±0.06	109±2	1.56±0.00	112±13	1.57±0.03	31±16	1.10±0.16

b) When rotifer with 1egg was used for the experiment

Conc.of feed (Cells ml^{-1})	Salinity 7 ppt Mean nos. ± SD	Mean r ± SD	14 ppt Mean nos. ± SD	Mean r ± SD	21 ppt Mean nos. ± SD	Mean r ± SD	35 ppt Mean nos. ± SD	Mean r ± SD
$1x10^6$	23±2	1.04±0.03	15±2	0.91±0.04	16±5	0.92±0.09	25±8	1.05±0.11
$2 x10^6$	24±9	1.04±0.11	29±3	1.13±0.03	29±6	1.12±0.06	34±13	1.15±0.12
$4 x10^6$	41±4	1.24±0.03	67±6	1.40±0.03	56±9	1.34±0.05	56±32	1.28±0.21
$8 x10^6$	96±9	1.52±0.03	108±4	1.56±0.01	95±10	1.52±0.04	24±11	1.03±0.13

Table 3. Reproductive potential of *Brachionus rotundiformis* in different salinities and feed concentrations of *Isochrysis galbana*

a) When rotifer without egg was used for the experiment

Conc.of feed (Cells ml^{-1})	Salinity 7 ppt		14 ppt		21 ppt		35 ppt	
	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD
0.5x10^6	36±13	1.16±0.17	24±16	0.94±0.31	51±13	1.30±0.09	26±2	1.08±0.02
1 x10^6	50±16	1.28±0.12	39±17	1.20±0.12	70±25	1.39±0.16	15±9	0.82±0.25
2 x10^6	63±29	1.34±0.18	44±25	1.19±0.26	71±23	1.40±0.12	24±12	1.01±0.18
4 x10^6	73±24	1.41±0.11	33±2	1.16±0.02	46±26	1.20±0.24	22±10	0.96±0.24

b) When rotifer with 1egg was used for the experiment

Conc.of feed (Cells ml^{-1})	Salinity 7 ppt		14 ppt		21 ppt		35 ppt	
	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD
0.5x10^6	38±8	1.21±0.07	26±21	1.00±0.24	57±10	1.35±0.06	40±10	1.22±0.08
1 x10^6	48±8	1.29±0.06	29±15	1.05±0.24	41±30	1.14±0.26	30±11	1.12±0.10
2 x10^6	75±28	1.42±0.11	49±28	1.23±0.22	42±40	1.11±0.28	16±3	0.91±0.06
4 x10^6	105±46	1.52±0.15	99±72	1.41±0.30	42±33	1.14±0.25	37±18	1.13±0.28

The increase in reproductive potential values was associated with increase in feed concentrations at salinities 14 ppt and 7 ppt. But, the ‘r’ values were found to fluctuate at salinities of 35 ppt and 21 ppt. The overall variation of reproductive potential was between 0.82 at feed concentration of 1 million cells per ml and 1.518 at feed concentration of 4 million cells per ml. The minimum was observed at 35 ppt and maximum at 7 ppt salinity.

The influence of salinity on ‘r’ values were found to be significant ($p<0.01$). Between salinities, the variations were significant in all combinations, except between 21 ppt and 7 ppt. The combined interactions of feed concentration + salinity on ‘r’ values were found to be significant ($p<0.05$). In this feed, the influence of feed concentration, set of experiment + feed concentration, set of experiment + salinity and set of experiment + feed concentration + salinity on ‘r’ values were not significant.

Baker’s yeast

The mean numbers, mean reproductive potentials along with their standard deviations when rotifers without as well as with 1 egg used for the experiments, are given in Table 4a and 4b.

Table 4. Reproductive potential of *Brachionus rotundiformis* in different salinities and feed concentrations of Baker’s yeast

a) When rotifer without egg was used for the experiment

Conc.of feed (Cells ml^{-1})	Salinity 7 ppt		14 ppt		21 ppt		35 ppt	
	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD
0.5x10^6	4±2	0.44±0.17	5±2	0.48±0.17	3±1	0.32±0.19	5±2	0.52±0.18
1 x10^6	11±7	0.67±0.31	6±4	0.52±0.21	4±2	0.41±0.22	5±2	0.51±0.14
2 x10^6	15±7	0.87±0.18	8±5	0.59±0.25	6±5	0.47±0.29	7±2	0.66±0.08
4 x10^6	21±8	0.98±0.18	11±8	0.71±0.23	8±8	0.58±0.26	3±1	0.35±0.09

b) When rotifer with 1egg was used for the experiment

Conc.of feed (Cells ml^{-1})	Salinity 7 ppt		14 ppt		21 ppt		35 ppt	
	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD	Mean nos. ± SD	Mean r ± SD
0.5x10^6	5±2	0.53±0.13	5±2	0.48±0.16	2±0	0.27±0.06	4±1	0.49±0.08
1 x10^6	7±5	0.55±0.23	7±4	0.59±0.21	2±0	0.27±0.06	9±1	0.73±0.03
2 x10^6	14±7	0.83±0.15	7±4	0.59±0.19	2±4	0.63±0.23	7±3	0.61±0.14
4 x10^6	19±9	0.92±0.20	12±6	0.78±0.16	3±5	0.61±0.34	14±4	0.86±0.11

There was no considerable variation between 'r' values when rotifers without as well as with 1 egg was taken for the study. In majority of cases, the 'r' values were found to increase along with the increase in feed concentrations. During the experiment with baker's yeast, the overall variation in reproductive potential was from 0.265 at 21 ppt salinity to 0.978 at 7 ppt salinity.

The 'r' values were significantly influenced by feed concentrations and salinity ($p<0.01$). Indepth studies showed that the variations of 'r' values between salinities were significant except that between 35 ppt and 14 ppt. In the case of feed concentrations, the variations of 'r' values between feed concentrations were significant except in two instances *viz*., (1) between that of 1 million and 2 million cells per ml and (2) between that of 4 million and 8 million cells per ml. The interactions of set of the experiment, set of experiment + feed concentration, set of experiment + salinity, feed concentration + salinity and set of experiment + feed concentration + salinity on 'r' values were not significant.

The above observations indicated that the different variables *viz*., salinity, feed type, feed concentration and set of experiment influence the reproductive potential of *B. rotundiformis* in varying magnitudes.

Of the four types of feeds tested, *viz*., *N. oculata, C. marina, I. galbana* and baker's yeast; *N. oculata* gave maximum 'r' value of 1.756 at a feed concentration of 8 million cells per ml at 14 ppt salinity. The r_{max} values decreased in the order, *N. oculata* → *C. marina* → *I. galbana* → Baker's yeast. The 'r' values were very low in baker's yeast as compared to algae. The present observation is in agreement with the findings of Gopakumar (1998) who reported high values of 'r' in microalgae when compared to their combinations with baker's yeast. Also, Hagiwara *et al*. (1995) suggested that *N. oculata* is the most suitable diet for optimum reproductive potential of *B. rotundiformis.*

Salinity was found to influence the 'r' values in all the 4 feed types tested in the present work. Ito (1960), Ruttner-Kolisko (1972) and Lubzens *et al*. (1985) also pointed out that, the reproductive rates of rotifers are strongly influenced by the salinity of the culture medium. In the present study, the r_{max} values of 1.756 for *N. oculata* and 1.573 for *C. marina* were recorded at salinities 14 ppt and 21 ppt respectively. In the case of *C. marina*, 'r' value of 1.563 was obtained at 14 ppt salinity which was very close to 1.573. So, the optimum salinity for r_{max} in *N. oculata* and *C. marina* was 14 ppt. This is in agreement with the finding of Hagiwara *et al*. (1995), who observed the optimum salinity for the best 'r' value for *B. rotundiformis* as 11 ppt. Anitha (2003) and Anitha and Rani (2006) recorded the highest 'r' value for *B. rotundiformis* at 15 ppt salinity which is more close to 14 ppt, reported during the present study. When the feed type employed was *I. galbana*, r_{max} was noticed at 7 ppt salinity. So, the optimum salinity at which r_{max} was observed in the 3 types of algae tested was between 7 and 14 ppt. Above or below this salinity, the 'r' values were found to decrease. When baker's yeast was used as feed, the r_{max} was only 0.978 which was much lower than that obtained, when algae were employed. However, the optimum 'r' value was at 7 ppt salinity when baker's yeast was used as feed. The 'r' values were found to decline above this salinity. The 'r' values were the least at 35 ppt in the 3 types of feeds – *N. oculata, C. marina* and *I. galbana*. In baker's yeast, the minimum 'r' value was observed at 21 ppt. James and Abu-Rezeq (1990) summarized that the productivity of *B. rotundiformis* depends on the salinity of the culture medium used and on the rotifer strain cultured.

The r_{max} values in all the 4 feed types employed were observed at the highest feed concentration used for the study which were 8 x 10^6 cells ml^{-1} in *N. oculata* and *C. marina* and 4 x 10^6 cells ml^{-1} in *I. galbana* and baker's yeast. James and Abu- Rezeq (1988) observed that rotifer fed with *Chlorella* sp. showed an increase in population density, production and growth rate upto a feed concentration of 10x10^6 cells ml^{-1}. The reproductive rate and survival of *B. plicatilis* depends on the concentration of food in the culture medium (Hirayama *et al*., 1979; Lubzens, 1981; Snell *et al*., 1983; Yamasaki *et al*., 1984). Yufera *et al*. (1983) observed an optimum concentration of the algae, *Nannochloropsis* sp. as high as 70 x 10^6 cells ml^{-1} for an increase in density of rotifer, *B. plicatilis* in culture. They also reported a linear relationship between rotifer population growth rate and cell densities of *Chlorella,* and according to them the increase in rotifer growth rate between 5 and 15x10^6 cells ml^{-1} algal concentrations was highly significant ($p<0.5$). A significant increase in the production of *B. plicatilis* was achieved at a density of 50 x 10^6cells ml^{-1} of *Chlorogibba trochisciaeformis* (Rafiuddin and Neelakantan, 1990). Again, Gopakumar (1998) reported the optimum 'r' value for S strain of *B.plicatilis* when *C. marina* was used, at a feed concentration of 4x10^6 cells ml^{-1}, while, Anitha (2003) recorded the highest 'r' value when *I. galbana* was used at a feed concentration of 2 x 10^6 cells ml^{-1}. The above works indicate that the feed concentration of algae required to have the r_{max} for rotifers, vary for different species/strains, and this explains the difference in the feed concentration at which r_{max} was obtained in the present work. During the present study, the minimum 'r' values were observed at 2 x 10^6 cells ml^{-1} in *N. oculata*, 1 x 10^6 cells ml^{-1} in *C. marina* and 1 x 10^6 cells ml^{-1} in *I. galbana*. In baker's yeast, the 'r' value was the least, in feed concentrations of both 0.5 x 10^6 and 1 x 10^6 cells ml^{-1}. These low values can be due to insufficient feeding.

The results of the present work points out that the reproductive potential of *B. rotundiformis* is influenced by salinity, feed type and feed concentrations, at a magnitude higher than that of sets of experiments. Among the interactions, the salinity and feed concentrations together influence the 'r' values significantly with respect to all the three microalgae tested in the present study. Hirayama and Ogawa (1972) also showed that filtration rates of *B. plicatilis* change with salinity and food concentration. Compared to that of baker's yeast, the reproductive potentials were higher in all the 3 algal feeds tested during this experiment. The maximum 'r' values were noticed between 7 and 14 ppt salinity in these 3 algal feeds. Carmona *et al.* (1995) observed that *B. rotundiformis* is euryhaline. This observation is true for the present experimental study also. Among the four feeds tested, *N. oculata* gave maximum 'r' value for *B. rotundiformis*. In another study, James and Al-Khars (1990) noticed that the total ω 3 HUFA and the essential fatty acid eicosapentaenoic acid (EPA) content were significantly higher in *Nannochloropsis* sp. compared to *Chlorella* sp., showing that the former is more suitable for aquacultural purposes since EPA is mandatory for the feeding of marine fish larvae. In a similar study, James and Abu-Rezeq (1989) also indicated that the rotifers produced using *Nannochloropsis* sp. contain adequate quantities of the essential fatty acids required for feeding marine fish larvae and, therefore no further nutritional enrichment of rotifers is required which could save space and manpower utilization in a marine fish hatchery. The information on reproductive potential of rotifers, influence of variables like salinity, feed type and feed concentrations along with their combined interactions on 'r' values will be helpful in culture activities of rotifers. As *B. rotundiformis* cultures are widely being used as an excellent live feed organism in the successful larval rearing operations of marine finfishes, the results of this study can be effectively used in aquaculture practices.

Acknowledgements

The authors are thankful to the Director, CMFRI, for providing facilities to carry out this work. The first author is grateful to Dr. G. Gopakumar, Scientist-in-Charge, Mandapam Regional Centre of CMFRI and to Dr. Rani Mary George, Scientist-in-Charge, Vizhinjam Research Centre of CMFRI, for their timely help and encouragements.

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Date of Receipt : 07/03/2008
Date of Acceptance : 04/07/2009

INDIAN JOURNAL OF FISHERIES
Volume 57 Number 1 (2010)

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Indexed/Abstracted in: Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Fish and Fisheries Worldwide (NISC), Science Citation Index Expanded, SCOPUS and Zoological Records