J. Fish. Sci. Technol. 9(4), 160-166, 2006

JOURNAL OF
FISHERIES SCIENCE
AND TECHNOLOGY

Effects of Temperature, Salinity, and Diet on the Growth and Survival of the Freshwater Rotifer *Brachionus angularis*

Byeong Ho Kim, Hyung Seop Kim[1] and Soo-Gun Jo*
School of Marine Life Science, Kunsan National University, Kunsan 573-701, Korea
[1]*Kunsan Regional Maritime Affairs and Fisheries Office, Kunsan 573-030, Korea*

We explored the possibilities of using the freshwater rotifer *Brachionus angularis* as a live food for small fishes cultured in fresh- or brackish waters. *Brachionus angularis* were collected from a reservoir for isolation and laboratory culture. Length and width of the lorica were 102.3 μm and 76.6 μm, respectively, and those of amictic eggs were 64.4 μm and 47.9 μm, respectively. When their growth rates were examined at six different temperatures, i.e., 15, 20, 25, 30, 35, and 40°C, the highest daily growth rate of 0.801 was observed at 35°C, and growth was lower with decreasing temperature. Adaptation to salinity change was evaluated with two different modes of salinity increase: step-wise elevation lasting for short durations of 5 to 30 min or a long duration of 24 h. With the short duration modes, no individuals survived salinity higher than 10 psu, and the number of live individuals did not increase throughout the experiment. However, in the 24-h elevation, the number of individuals increased when salinity was elevated by 1 to 2 psu per day for the first 2 or 3 days, while no increase in number occurred at salinity increments higher than 3 psu per day. In addition, to assess the effect of different diets, four single-component diets (*Chlorella vulgaris*, *Nannochloris* sp., baker's yeast, or dry yeast) and three combination diets (*C. vulgaris* + *Nannochloris* sp. + baker's yeast + dry yeast; *C. vulgaris* 70% + baker's yeast 30%; *C. vulgaris* 30% + baker's yeast 70%) were used. The specific growth rates of *B. angularis* fed combination diets were higher than those of rotifers fed any single-component diet, with the highest rate of 0.648 in *B. angularis* fed a mixture of *C. vulgaris*, *Nannochloris* sp., baker's yeast, and dry yeast, and the lowest rate of 0.200 in those fed dry yeast only. Our results indicate that the freshwater rotifer *B. angularis* can be used for seedling production of both freshwater and brackish-water fishes that require small (less than about 120 μm) live food during their early stages.

Key words: *Brachionus angularis,* Rotifer, Growth, Survival, Fish feed

Introduction

Rotifers have widely been used as prey organisms for larval fishes and crustaceans (Hirayama and Ogawa, 1972; Lubzens, 1987; Lubzens et al., 1989). In particular, the brackish-water rotifer *Brachionus plicatilis* and the freshwater rotifer *B. calyciflorus* are preferably used as live food sources (Snell et al., 1983; Sugumar and Munuswamy, 2006).

The length of the lorica in *B. plicatilis* and *B. rotundiformis* ranges from 230 to 320 μm and 140 to 220 μm, respectively (Song et al., 1999), while that in a Thai strain of *B. calyciflorus* is about 126 μm (Hwang and Pyen, 1995). However, the production of fish and crustacean larvae that feed on prey less than about 120 μm requires much smaller rotifers than *B. plicatilis* or *B. calyciflorus*.

In this study, we chose the small freshwater rotifer *B. angularis*, which has a lorica length of about 100 μm, for possible use as a small live food organism. *Brachionus angularis* generally occurs in eutrophic lakes (Walz, 1987b; Walz and Gschloessl, 1988). Limited studies have been conducted on the population dynamics (Walz, 1987a; Gama-Flores et al., 2004) and ingestion and filtration rates (Walz and Gschloessl, 1988) of *B. angularis*. Walz (1987a) estimated the specific growth rate of this species under laboratory conditions, and Gama-Flores et al. (2004) examined population densities under labora-

*Corresponding author: sgjo@kunsan.ac.kr

tory conditions as part of a toxicity test. If this small freshwater rotifer can tolerate salinity changes, it can be used as a food source for the larvae of salt-water species.

The purpose of this study was to examine the growth and survival of *B. angularis* under different temperatures, salinity conditions, and diets in the laboratory.

Materials and Methods

Brachionus angularis was collected for isolation and laboratory culture from a reservoir in Jeonbuk, Korea, using a plankton net (mesh size, 30 μm). The rotifer was isolated under an optical microscope (Nikon, SMZ-U) for pure culture. Lorica length was measured in more than 100 individuals randomly selected from the cultured population, and measurements of egg size were performed on 40 amictic eggs.

To assess the effects of temperature on growth, the growth rate was measured at six different temperature regimes: 15, 20, 25, 30, 35, and 40°C, based on a preliminary experiment. *Brachionus angularis* was placed with *Chlorella vulgaris* (FC-12, Korea Marine Microalgae Culture Center) in 30-mL test tubes containing 10 indiv./mL. *Chlorella vulgaris* was cultured using f/2 medium (Guillard and Ryther, 1962). During the incubation period, the test tubes were shaken to ensure food suspension. The number of *B. angularis* was estimated by counting 1 mL of the rotifer culture.

To evaluate the salinity tolerance of *B. angularis*, an experiment was conducted to assess two modes of salinity increase: either a step-wise elevation for short durations of 5, 15, and 30 min, or for a long duration of 24 h. In the short-duration salinity elevation experiment, 30 *B. angularis* were placed in multi-well plates containing glass-fiber-filtered (GFF) seawater along with algal diets. Salinity was elevated by 1, 2, 3, 4, and 5 psu stepwise at 5-, 15-, and 30-min intervals. Total rotifer numbers in multi-well plates were checked just before salinity elevation. In the long-duration (24-h interval) salinity elevation experiment, *B. angularis* was placed in 30-mL test tubes containing 10 indiv./mL in GFF seawater. Salinity was elevated by 1, 2, 3, 4, and 5 psu at 24-h intervals. During the incubation period, distilled water was added to the tubes to replace water lost by evaporation.

To determine the effect of different diets on growth, rotifers were fed four single-component diets (*Chlorella vulgaris*, *Nannochloris* sp., baker's yeast, or dry yeast) and three combination diets (*C. vulgaris* + *Nannochloris* sp. + baker's yeast + dry yeast; *C. vulgaris* 70% + baker's yeast 30%; *C. vulgaris* 30% + baker's yeast 70%; Table 1). The diets used in this study were maintained at a density of 1.5×10^6 cells/mL under a 16-h light: 8-h dark regime. Experiments were performed in triplicate.

The specific growth rate (r) of *B. angularis* was determined at maximum density following the methods of Rico-Martinez and Dodson (1992). The relationships between the length and width of lorica or amictic eggs were analyzed by Pearson's correlation coefficient. To determine the differences in growth expressed as survival of the rotifers, one-way analysis of variance (ANOVA) and Tukey's HSD test were applied using the SPSS 10.1 statistical package (SPSS, 2001). Data are expressed as the mean (±SD) whenever possible. Statistical significance was set at $p<0.05$.

Results

Measurements of *B. angularis*

Measurements of the lorica and amictic eggs were made from 100 females of *B. angularis* and 40 amictic eggs (Tables 2, 3). The length of the lorica was 102.3±9.7 μm, ranging from 83.8 to 120.8 μm. The width of the lorica was 76.6±11.8 μm (50.4-98.8 μm).

The width to length ratio of the lorica was 0.75 (0.54-0.94). The regression equations for the relationships between length (y) and width (x) of the lorica (Fig. 1) and of the amictic eggs (Fig. 2), respectively, were $y=0.7705x-2.2783$ ($r^2=0.4039$, n=100) and $y=0.6415x+34.217$ ($r^2=0.0978$, n=40).

Table 1. Information on the different types of diets used for growth experiments in the present study

Food	Name code or brand name	Manufacturer	Storage (culture) temperature
Chlorella vulgaris	FC-01	KMCC[1]	20°C (in freshwater)
Nannochloris sp.	FC-42	KMCC[1]	20°C (in freshwater)
Baker's yeast	*Ottogi live yeast*	*Choheung*[2]	0-4°C (*at refrigerator*)
Dry yeast	Ottogi dry yeast	Choheung[2]	0-4°C (at refrigerator)

[1]KMCC: Korea Marine Microalgae Culture Center, Busan, Korea.
[2]Choheung: Food Additive Industry, Seoul, Korea.

Table 2. Lorica length and width of female *Brachionus angularis*

	Length (μm)	Width (μm)	Ratio (%, width/length)	Number of specimens
Range	83.8 - 120.8	50.4 - 98.8	54.2 - 93.2	100
Mean (±sd)	102.3 (±9.7)	76.6 (±11.8)	74.8 (±9.0)	

Table 3. Major and minor axes of amictic eggs in *Brachionus angularis*

	Major axis (μm)	Minor axis (μm)	Ratio (%, minor axis/major axis)	Number of specimens
Range	47.3 - 78.5	38.4 - 55.7	61.3 - 96.4	40
Mean (±sd)	64.4 (±7.7)	47.9 (±3.8)	74.3 (±9.0)	

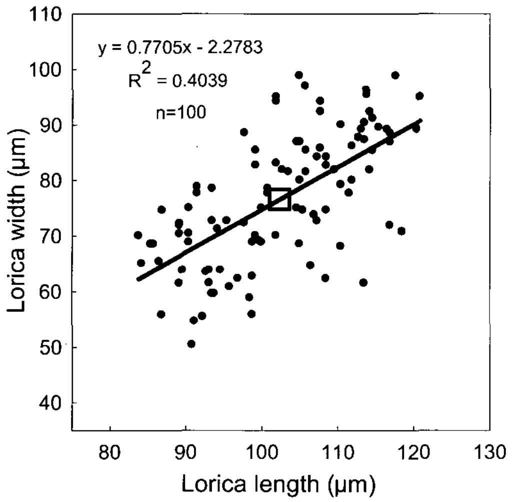

Fig. 1. Relationship between lorica width and lorica length in female *Brachionus angularis*. The large open square symbol in the middle denotes average value of the whole population.

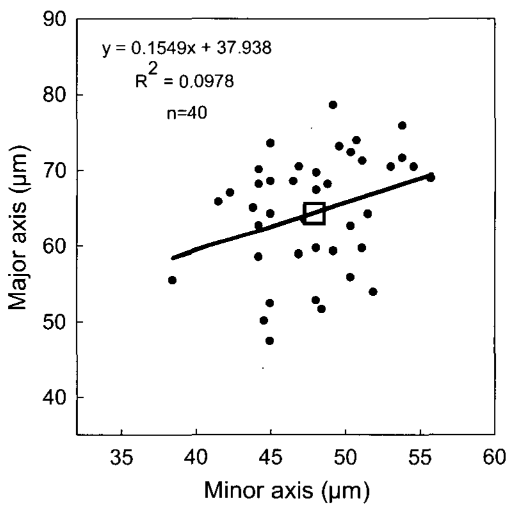

Fig. 2. Relationship between major and minor axes of amictic eggs in *Brachionus angularis*. The large open square symbol in the middle denotes average value of the whole population.

Effects of temperature on the growth of *B. angularis*

The growth of *B. angularis* cultured at different temperatures for 7 days are shown in Table 4 and Fig. 3. A significantly higher population growth was found at 20 and 25°C compared to that at any other temperatures ($p<0.05$), while the specific growth rates ($r=0.801$) at 30 and 35°C were significantly higher than those at other temperatures (Tukey's HSD test, $p<0.05$). The rotifer could not survive the highest temperature tested, 40°C, and its population density at 15°C remained at very low levels.

Effects of salinity on the survival of *B. angularis*

In the short-duration (5-30 min) salinity elevation experiment, the number of individuals decreased throughout the experiment regardless of salt levels. When the intervals of salinity elevation were shorter or salinity elevation magnitudes were greater, the number of individuals decreased more rapidly (Figs. 4, 5). Although the duration of survival of the rotifer varied from 40 to 250 min depending on elevation modes, they generally survived until the salinity reached 8 psu (Fig. 4). The number of rotifers that survived daily salinity increments of 1 to 3 psu increased initially, but then decreased when the salinity reached more than 3 or 6 psu (Fig. 5). No rotifers survived daily salinity increments exceeding 3 psu.

Growth of *B. angularis* fed different diets

Population growth of the rotifer fed different diets for 7 days are shown in Table 5 and Fig. 6. Of the four single-component diets, *Chlorella vulgaris* supported the highest maximum growth of 251.3 indiv./mL with a specific growth rate of 0.460. However, rotifers fed the mixed diet containing all four components (*C. vulgaris*, *Nannochloris* sp., baker's yeast, and dry yeast) showed significantly higher population densities and specific growth rates than those on any single-component diets ($p<0.05$). In fact, the four-component mix showed the highest maximum density of 487.7 indiv./mL and a specific growth rate of 0.648.

Table 4. Specific growth rate of female *Brachionus angularis* incubated at different temperatures for seven days

Temperature (°C)	Repetition			Mean (±sd)
	1st	2nd	3rd	
15	0.191	0.076	0.068	0.111[a] (0.069)
20	0.397	0.399	0.411	0.402[b] (0.008)
25	0.455	0.441	0.436	0.444[b] (0.010)
30	0.736	0.839	0.787	0.787[c] (0.052)
35	0.858	0.796	0.750	0.801[c] (0.054)
40	-	-	-	-

Mean values sharing a common letter are not significantly different (Tukey's HSD test, $p>0.05$).
- No individual survived.

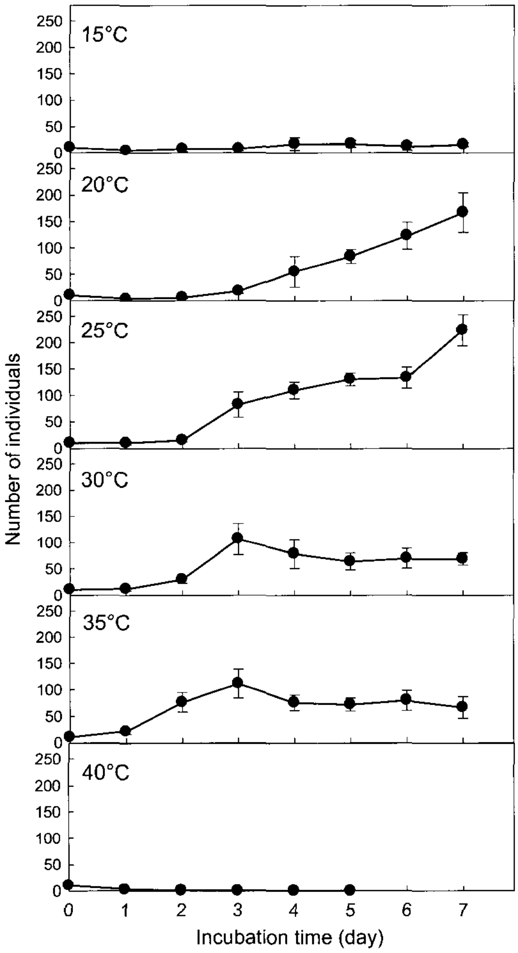

Fig. 3. Changes in the number of individuals in female *Brachionus angularis* cultured in freshwater at different temperatures for 7 days.

The rotifers fed only dry yeast showed the lowest density of 42 indiv./mL and a specific growth rate of 0.200.

Discussion

To use rotifers as food organisms for larval fish culture, the lorica size may be the most critical factor.

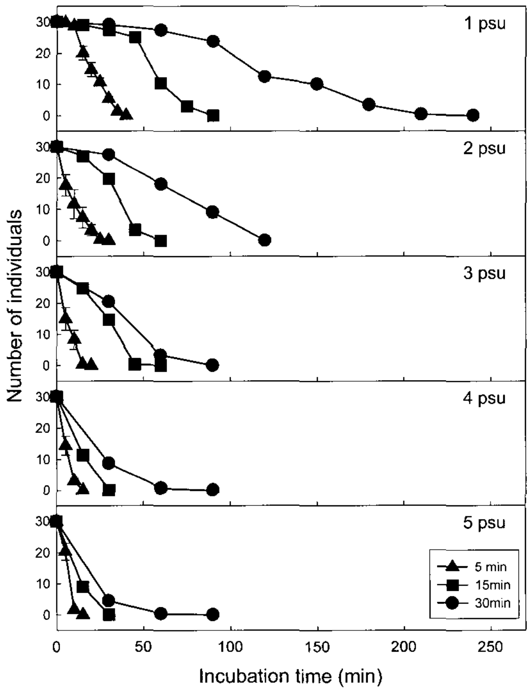

Fig. 4. Changes in the number of individuals in female *Brachionus angularis* cultured under different salinity elevation magnitudes (1-5 psu) and adaptation period (5-30 min) for each elevation step.

However, the sizes of rotifers tend to vary depending on their culture conditions (Kim et al., 1997; Song et al., 1999). In our study, the lorica length of *B. angularis* ranged from 83.8 to 120.8 μm, with a mean value of 102.3 μm. *Brachionus angularis* is much smaller than *B. plicatilis* and *B. rotundiformis*; lorica lengths in Korean strains of these species have been reported to be between 230 and 320 μm, and between 131 and 173 μm, respectively (Hur and Park, 1996; Song et al., 1999). *Brachionus angularis* is also smaller than the freshwater rotifer *B. calyciflorus*; the mean lorica length of a Korean strain of this species is 211.8-229.9 μm (Hur and Park, 1996). Considering the lorica length of rotifers as food organisms, *B. angularis* has high potential for the seedling production of saltwater fishes that require small live

Table 5. Maximum density and specific growth rate of female *Brachionus angularis* fed different diets at 25°C

Diet	Maximum density (inds./mL)	Specific growth rate (r)	Culture duration (days)
Chlorella vulgaris	251.3^{b} (19.5)	0.460^{b} (0.011)	7
Nannochloris sp.	210.2^{b} (4.5)	0.435^{bc} (0.003)	7
Baker's yeast	249.8^{b} (45.7)	0.458^{bc} (0.028)	7
Dry yeast	42.0^{a} (14.1)	0.200^{a} (0.048)	7
Mix. I[1]	487.7^{d} (14.0)	0.648^{c} (0.005)	6
Mix. II[2]	375.5^{c} (42.6)	0.517^{c} (0.016)	7
Mix. III[3]	382.8^{c} (34.7)	0.520^{d} (0.013)	7

[1]*Chlorella vulgaris* + *Nannochloris* + baker's yeast + dry yeast (each mixed at equal number).
[2]*Chlorella vulgaris* 70% + baker's yeast 30%.
[3]Baker's yeast 70% + *Chlorella vulgaris* 30%.
Maximum density and specific growth rate sharing a common letter are not significantly different (Tukey's HSD test, $p>0.05$).

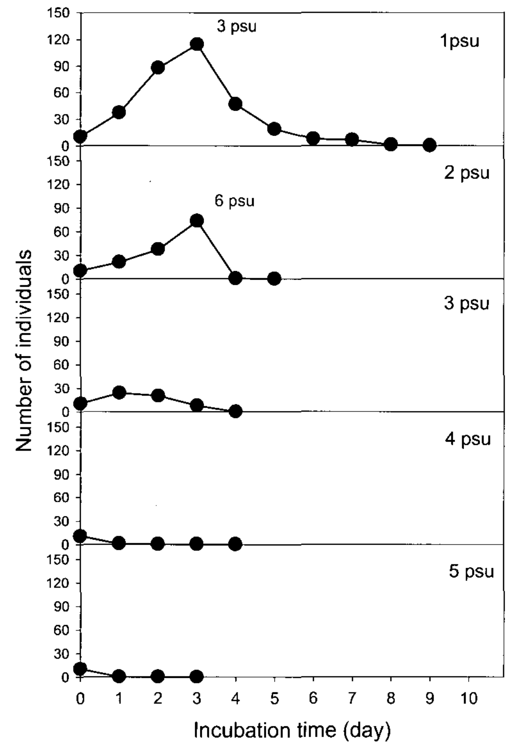

Fig. 5. Changes in the number of individuals of female *Brachionus angularis* under the culture condition of a daily step-wise increase in salinity (1-5 psu a day).

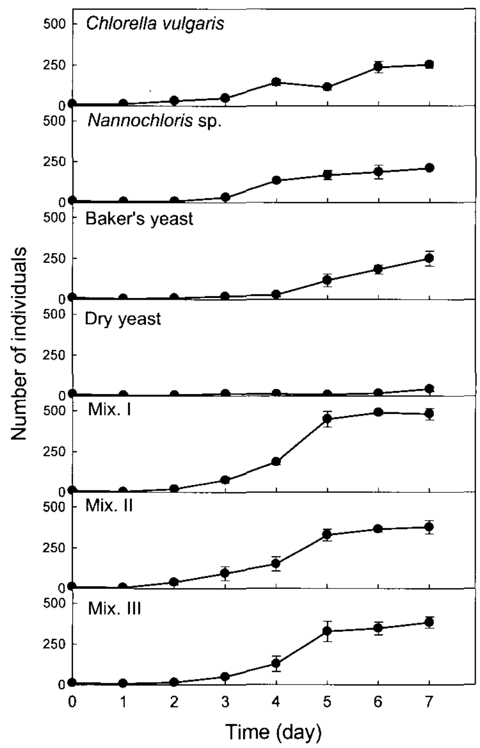

Fig. 6. Changes in the number of individuals in female *Brachionus angularis* fed different diets. Refer to Table 5 for details of Mix. I-III diets.

foods less than about 120 μm.

Brachionus angularis showed the highest population density of 487.7 indiv./mL with a specific growth rate of 0.648 at 25°C after 7 days of culture. However, for the same species, Gama-Flores et al. (2004) reported the highest population density as being only 200 indiv./mL in a similar culture to our experiment. Walz (1987a) reported that *B. angularis* cultured at 20°C showed its highest specific growth rate as being only 0.352. Thus, *B. angularis* in this study reached higher population densities or growth rates than those in the previous studies. This discrepancy in the highest population densities or growth rates may be related to the difference in the strains studied. *Brachionus angularis* in our study seemed to have a lower attainable population density than the two brackish-water rotifers *B. plicatilis* and *B. rotundiformis*, which can reach 1,420 indiv./mL at 36°C and 1669 indiv./mL at 34°C, respectively (Kim et al., 1997). However, population growth or specific growth rate may vary depending on culture conditions; thus, direct comparisons between different species should be made with care when comparing data obtained under different conditions. In the salinity-tolerance experiment, *B. angularis* survived until salinity was elevated up to 9 psu (by daily increments of 1 to 3 psu), and the number of individuals also increased, rather than decreased, up to a salinity of 3 or 6 psu. Lee et al. (2000) reported that the freshwater rotifer *B. calyciflorus* could adapt up to a salinity of 4 psu but not 8 psu. Schlüter and Groeneweg (1981) reported a similar result for *B. rubens*, which was able to survive up to 4 psu. Compared to the above-mentioned earlier findings, *B. angularis* is likely to have a similar salinity tolerance as other freshwater species. Therefore, it is possible that *B. angularis* can be used as a food organism for seedling production of some brackish-water fishes as well as freshwater species.

Of the algal foods used in rotifer culture, *Chlorella* is considered one of the best unialgal diets (Hirayama et al., 1979). When rotifers feed on *Chlorella* together with other algal species or with yeast, their reproductive rates are greatly enhanced (Hirayama and Watanabe, 1973; Snell et al., 1983; Lee et al., 2000). In this study, the rotifers fed *Chlorella vulgaris* showed a higher maximum density and specific growth rate than those fed any other single-component diet. Moreover, rotifers fed on mixed diets including *C. vulgaris* showed much better growth rates than those fed on mixed diets excluding *Chlorella*. The best growth rate was observed when *B. angularis* were fed a mixed diet composed of two green algae and two yeasts (Table 5). These results are in accord with reports on different rotifer species confirming the positive effect of mixed diets on the growth of rotifers (Snell et al., 1983; Lee et al., 2000).

In conclusion, the freshwater rotifer *B. angularis* has high potential as a prey organism for seedling production of either marine or freshwater fishes that require small-sized prey.

Acknowledgments

We wish to acknowledge the financial support of the Fisheries Science Institute of Kunsan National University made in the program year of 2005. We also thank two anonymous reviewers for improving the manuscript.

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(Received August 2006, Accepted December 2006)