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Effect of Temperature and Salinity on Production of Resting Egg in Korean Rotifer, *Brachionus plicatilis* (L and S-type)*

Heum Gi Park and Sung Bum Hur

Department of Aquaculture, Pukyong National University, Pusan 608-737, Korea

Production of resting egg from the Korean rotifer, *Brachionus plicatilis* (L and S-type) was investigated at different temperatures (L-type ; 20, 24, 28℃, S-type ; 28 32, 36℃) and salinities (10, 20, 30 ppt). The rotifer was cultured in 25 ml test tube and fed on *Nannochloris oculata.*

With regard to mixis rate, L-type rotifer showed higher rate at lower temperature, and the highest rate was observed at 20 ppt of salinity at each temperature of the experiment. However, for S-type rotifer, the optimum temperature and salinity were 28~32℃ and 20 ppt, respectively. The highest number of resting egg was 173 eggs/ml in 16 days at 24℃, 10 ppt for L-type rotifer and 410 eggs/ml in 14 days at 28℃, 10 ppt for S-type rotifer. The maximum number of resting egg produced per 10,000 rotifers was 8,122 eggs at 20℃, 20 ppt for L-type rotifer and 8,700 eggs at 28℃, 20 ppt for S-type rotifer. The maximum number of resting egg produced 10^8 cells of *N. oculata* was 50.7 eggs for L-type rotifer (24℃, 20 ppt) and 79.6 eggs for S-type rotifer (32℃, 10 ppt). The number of resting egg produced per day was 1~11 eggs/ml for L-type rotifers and 21~35 eggs/ml for S-type rotifer in 9 combination experiments.

In this study, S-type rotifer is better than L-type rotifer in resting egg production, and the optimum temperature and salinity for resting egg production were 20℃, 20 ppt for L-type rotifer and 28℃, 20 ppt for S-type rotifer. This result shows the difference of Korean rotifer in the optimum condition for resting egg production from other rotifers reported earlier.

Key words : Rotifer, *Brachionus plicatilis,* Resting egg, Temperature, Salinity

Introduction

The marine rotifer, *Brachionus plicatilis* has been used as a live food by aquaculturist. Major constraints to use the rotifer in aquaculture are the requirements of considerable amounts of manpower and extensive facilities for culturing phytoplankton as rotifer food. To minimize these constraints, resting egg production of rotifer by bisexual reproduction has been suggested (Hagiwara and Hirayama, 1993). The eggs can be used as seed of rotifer mass culture for the following season, as well as a direct diet for fish larvae after hatching, similar to Artemia cyst (Hagiwara et al., 1993b). Pourriot and Snell (1983) and Hagiwara and Hirayama (1993) reported that resting egg production was influenced by both internal and external factors. Temperature and salinity among external factors play important regulatory roles in the marine *B. plicatilis* (Hino and Hirano, 1984, 1988 ; Lubzen et al., 1985 ; Snell, 1986 ; Hagiwara et al., 1988a, 1989 ; Hagiwara and Lee, 1991).

Mixis rate and resting egg production

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of L-type rotifer (Hino and Hirano, 1984, 1988 ; Lubzen et al., 1985 ; Hagiwara et al., 1988a) were high at low temperatures (15~20℃) and salinity levels (8~16 ppt). However, for the S-type rotifer (Snell, 1986 ; Hagiwara et al., 1989), mixis rate and resting egg production were observed at high temperature (28~30℃) and salinity (25~30 ppt). Thus, Hagiwara et al. (1989) and Hagiwara and Lee (1991) concluded that resting egg production between L and S-type rotifers were inversely affected by the temperatures and salinities.

In this study, the effects of temperature and salinity on resting egg production of the Korean rotifer, *B. plicatilis* (L and S-type) isolated from salt ponds in Korea were investigated and the results were compared with those of previous researchers.

Materials and Methods

B. plicatilis, L-type rotifer (O-L strain) and S-type rotifer (C-S strain) were originally isolated on 19 June 1994 from Okku salt pond and on 28 September 1993 from Chaedu salt pond, respectively, in Korea (Hur and Park, 1996). These two strains were the highest resting egg producing strains among 20 rotifer strains collected different areas.

Each strains were cultured at 15ppt, 20℃ for L-type and at 15ppt, 28℃ for S-type, and resting eggs produced at these conditions were stored at 5℃, 15 ppt for 4 months in a refrigerator. From each eggs, 15 clones were chosen to produce the clone with the highest rate of resting egg production. To select clone with the highest resting egg, culture environment conditions for this test were 15ppt and 20℃ for L-type and 15 ppt and 28℃ for S-type, in 15 ml test tube. The L and S-type clones were fed on *Nannochloris oculata* (KMMCC-C-31) received from Korea Marine Microalgae Culture Center for 20 days and 15 days, respectively. *N. oculata* was cultured at 20℃, 30 ppt and 3,000 lux in 20 ℓ culture carboy containing f/2 medium (Guillard and Ryther, 1962).

Prior to the experiment, selected clone of L and S-type rotifers with the highest resting egg production was acclimated to the experiment temperatures and salinities for 5 days. Temperatures were adjusted at 20, 24, 28℃ for L-type and 28, 32, 36℃ for S-type. Salinities were adjusted at 10, 20, 30 ppt by diluting natural sea water with distilled water. From each culture, young female before laying egg were innoculated at a density of one per ml in 25 ml test tube and algae were centrifuged and fed to rotifers. Triplicate cultures of three temperatures and salinities were used for resting egg production.

Rotifer and resting egg densities were monitored every day after culture medium was mixed by shaking. The types of female and male were observed according to Hagiwara et al. (1988a). Unknown females were cultured in a 3 ml multi culture chamber and later identified by the shape of eggs produced. Mixis rate (percentage of male-producing mictic females plus resting egg-producing mictic female among total female) and fertilization rate (percent resting egg-producing mictic female among total mictic female) were estimated according to Hagiwara et al. (1989). Specific growth rate (r) was estimated from : $r = 1/T \ln(N_T/N_0)$, where, T is culture days that rotifer density was the highest, and N_0 and N_T are the initial and rotifer density, respectively. The number of resting eggs produced by 10,000 rotifers (D) and 10^8 *Nannochloris* cells (N) were calculated : D=(resting egg production/maximum rotifer density)×10,000 and N=(resting egg production/total amount of *Nannochloris* cells)×10^8 *Nannochloris* cells, respectively.

Duncan's multiple range test (Duncan, 1955) was performed to evaluate the effect of temperature and salinity using SPSS for window program.

Results

The growth and sexual reproduction of L-type and S-type rotifers in various condi-

Table 1. Growth and sexual reproduction of the Korean rotifer, *Brachionus plicatilis* L-type at different temperatures and salinities

Temperature (℃)	Salinity (ppt)	Maximum density (inds./ml)	Specific growth rate (r)	Mixis rate (%)	Fertilization (%)
	10	230 ± 30.6^{a}	0.239 ± 0.0058^{a}	42.6 ± 1.01^{de}	58.6 ± 3.33^{c}
20	20	208 ± 17.4^{a}	0.231 ± 0.0035^{a}	54.1 ± 2.14^{f}	60.5 ± 0.89^{c}
	30	221 ± 21.1^{a}	0.237 ± 0.0048^{a}	41.3 ± 1.29^{de}	47.8 ± 7.94^{bc}
	10	557 ± 78.4^{c}	0.402 ± 0.0047^{d}	39.1 ± 1.54^{cd}	44.0 ± 2.01^{bc}
24	20	369 ± 35.8^{b}	0.369 ± 0.0059^{c}	46.0 ± 2.98^{e}	45.3 ± 6.16^{bc}
	30	250 ± 15.3^{a}	0.345 ± 0.0036^{b}	35.2 ± 2.17^{c}	43.4 ± 2.83^{bc}
	10	220 ± 12.8^{a}	0.490 ± 0.0051^{f}	15.7 ± 1.69^{a}	35.9 ± 7.98^{b}
28	20	175 ± 26.0^{a}	0.468 ± 0.0129^{e}	23.5 ± 1.95^{b}	36.0 ± 2.86^{b}
	30	221 ± 20.2^{a}	0.450 ± 0.0123^{e}	12.8 ± 1.04^{a}	13.3 ± 7.56^{a}

Values in the same column within the same letter are not different ($P<0.05$).

Table 2. Growth and sexual reproduction of the Korean rotifer, *Brachionus plicatilis* S-type at different temperatures and salinities

Temperature (℃)	Salinity (ppt)	Maximum density (inds./ml)	Specific growth rate (r)	Mixis rate (%)	Fertilization (%)
	10	850 ± 17.3^{de}	0.482 ± 0.0015^{a}	11.8 ± 1.35^{a}	83.4 ± 4.65^{a}
28	20	360 ± 27.3^{a}	0.420 ± 0.0175^{a}	38.1 ± 2.17^{c}	76.4 ± 6.63^{a}
	30	520 ± 11.5^{abc}	0.481 ± 0.0017^{a}	28.3 ± 1.77^{b}	72.8 ± 3.21^{a}
	10	$1{,}010\pm126.7^{e}$	0.769 ± 0.0364^{c}	10.5 ± 2.67^{a}	81.0 ± 1.01^{a}
32	20	330 ± 51.4^{a}	0.644 ± 0.0096^{b}	39.7 ± 4.59^{c}	78.8 ± 5.26^{a}
	30	393 ± 22.5^{ab}	0.664 ± 0.0497^{c}	29.1 ± 3.44^{b}	75.1 ± 3.49^{a}
	10	906 ± 141.0^{de}	0.851 ± 0.0257^{d}	11.3 ± 1.57^{a}	80.0 ± 2.48^{a}
36	20	626 ± 23.3^{bc}	0.805 ± 0.0033^{d}	26.8 ± 2.42^{d}	66.5 ± 2.55^{a}
	30	716 ± 69.4^{cd}	0.822 ± 0.0068^{d}	14.6 ± 2.81^{a}	66.7 ± 9.83^{a}

Values in the same column within the same letter are not different ($P<0.05$).

tions are summarized in Table 1 and 2. The highest density of L-type rotifer was 557 inds./ml at 24℃ and 10 ppt, but not nfluenced significantly by the salinity levels at 20℃ and 28℃ except at 24℃. For S-type rotifer, the density was the highest at low salinity in each temperature. The highest rotifer density was observed at 32℃ and 10 ppt with the density of 1,010 inds./ml.

The specific growth rate indicated that each rotifer tended to grow higher at high temperature, and L-type rotifer was higher at 10 ppt than other salinities at 24℃ and 28℃, but the growth rate of S-type rotifer was not affected with salinity except at 32℃.

The highest mixis rates in L-type rotifer were 23.5%, 46.0%, 54.1% in 28, 24, 20℃ with 20 ppt, respectively. When temperature decreased, mixis rate was increased, but fertilization was not affected by the salinity and temperature between 20℃ and 24℃ except at 28℃. For S-type rotifer, the highest mixis rate was 38.1%, 39.7%, 26.8% in 28, 32, 36℃ with 20 ppt, respectively and low rates were observed at 10 and 30 ppt in each temperatures. However, fertilization was not affected by salinity and temperature.

The number of resting egg of L and S-type rotifer with culture day was shown on Table 3. In L-type rotifer, the production of resting egg in 28, 24, 20℃ occurred until 11, 16, 23 days of culture, repectively. The periods of resting egg production was longer

Table 3. Production of resting eggs per ml of the Korean rotifer, *Brachionus plicatilis* L and S-type with culture day at different temperatures and salinities

L-type					S-type				
Temp. (℃)	Salinity (ppt)	Culture days			Temp. (℃)	Salinity (ppt)	Culture days		
		11	16	23			8	10	14
20	10	10± 3.8[a]	47± 4.4[b]	155±18.0[b]	28	10	103±27.4[abc]	335±49.1[b]	410±52.0[a]
	20	6± 0.3[a]	54± 3.0[b]	167± 4.4[b]		20	68±13.0[ab]	151±24.6[a]	340±25.2[a]
	30	0± 0.0[a]	2± 1.7[a]	68± 8.8[a]		30	40±13.2[a]	127±42.5[a]	331±41.5[a]
24	10	56±12.3[cd]	173±21.9[c]		32	10	248±45.7[d]	346±31.8[b]	
	20	69± 9.6[d]	135± 7.6[c]			20	156±14.5[bcd]	283±23.3[b]	
	30	5± 0.0[a]	63±16.9[b]			30	148±24.6[abcd]	263± 8.8[b]	
28	10	35± 2.5[b]			36	10	166±17.6[bcd]		
	20	41±11.7[bc]				20	216±78.6[bcd]		
	30	5± 0.0[a]				30	180±28.9[bcd]		

Values in the same column within the same letter are not different (P〈0.05).

Table 4. Production of resting eggs of the Korean rotifer, *Brachionus plicatilis* L-type at different temperatures and salinities

Temperature (℃)	Salinity (ppt)	Number of resting eggs/ml/day	Number of resting eggs/10^3 rotifer	Number of resting eggs/10^8 *Nannochloris* cells
20	10	6.7±0.79[c]	7,128±1596.8[c]	42.1±6.09[c]
	20	7.2±0.18[c]	8,122± 750.7[c]	50.6±2.21[c]
	30	3.0±0.38[b]	3,080± 103.2[b]	22.3±3.13[b]
24	10	10.8±1.38[d]	3,257± 702.5[b]	50.7±7.02[c]
	20	8.4±0.49[c]	3,719± 412.3[b]	47.3±5.49[c]
	30	4.0±1.05[b]	2,488± 592.6[ab]	23.8±5.98[b]
28	10	3.2±0.20[b]	1,604± 160.5[ab]	9.7±0.70[ab]
	20	3.7±1.09[b]	2,296± 456.5[ab]	11.3±3.24[ab]
	30	0.5±0.00[a]	357± 44.3[a]	1.4±0.00[a]

Values in the same column within the same letter are not different (P〈0.05).

at lower temperature. However, in S-type rotifer, the period was shorter at higher temperature. The highest resting egg production of L-type rotifer on 11 day was 69 eggs/ml at 24℃ and 20 ppt, but, on the 16 day, it was 173 eggs/ml at 24℃ and 10ppt. On 23 day, it was 167 eggs/ml at 20℃, 20 ppt.

The highest resting egg production of S-type rotifer on 8 day was 248 eggs/ml at 32℃, 10ppt, but not influenced significantly by the salinity levels in each temperature. On 10 day, it was 335 and 346 eggs/ml at 28℃ and 32℃, 10 ppt, respectively. The highest resting egg production on 14 day was 410 eggs/ml at 28℃, 10 ppt.

The number of resting egg of L and S-type rotifer produced by a day, 10,000 rotifers and 10^8 *Nannochloris* cells were shown on Table 4 and 5. The number of resting egg produced per day for L-type rotifer at 24℃, 10ppt was the highest (10.8 eggs/ml /day). The number of resting egg produced per day for S-type rotifer was 20.8~34.6 eggs/ml/day in all experiments, but not affected by temperature and salinity levels.

The number of resting egg produced by 10,000 rotifers increased with decreased temperature from 28℃ to 20℃ for L-type rotifer and from 36℃ to 28℃ for S-type rotifer. It showed the highest at 20 ppt in every temperature tested for L and S-type rotifer.

The highest number of resting egg produced by 10^8 *Nannochloris* cells for L-type rotifer was 50.7 eggs at 24℃, 10 ppt and 20℃,

Table 5. Production of resting eggs of the Korean rotifer, *Brachionus plicatilis* S-type at different temperatures and salinities

Temperature (℃)	Salinity (ppt)	Number of resting eggs/ml/day	Number of resting eggs/10^3 rotifer	Number of resting eggs/10^8 *Nannochloris* cells
	10	29.3±3.76^{a}	4,866±693.4^{bcd}	76.3±9.27^{c}
28	20	24.3±2.33^{a}	7,779±385.8^{e}	63.8±4.98^{abc}
	30	23.7±3.05^{a}	6,391±690.2^{de}	62.6±8.00^{abc}
	10	34.6±3.18^{a}	3,835±297.1^{abc}	79.6±8.04^{c}
32	20	28.3±2.33^{a}	7,779±385.8^{e}	65.9±5.42^{bc}
	30	26.3±0.88^{a}	5,365±176.5^{cd}	60.4±2.74^{abc}
	10	20.8±2.33^{a}	1,877±108.3^{a}	42.7±4.53^{ab}
36	20	33.7±7.22^{a}	3,518±951.3^{abc}	56.3±9.53^{abc}
	30	22.5±3.46^{a}	2,595±325.7^{ab}	46.2±7.42^{a}

Values in the same column within the same letter are not different (P<0.05).

Table 6. Statistical analysis for the Korean rotifer, *Brachionus plicatilis* L and S-type at different temperatures and salinities

	Two-way ANOVA			
	L-type		S-type	
	Temperature	Salinity	Temperature	Salinity
Mixis rate	P<0.001	P<0.001	P<0.001	P<0.001
Fertilization	P<0.001	P<0.005	NS	NS
Number of resting egg/10,000 rotifers	P<0.001	NS	P<0.001	P<0.001

NS : no significant.

20 ppt, and the numbers indicates that higher salinity decreased the effect of food. The highest number of resting egg produced by 10^8 *Nannochloris* cells for S-type rotifer was 79.6 eggs at 32℃, 10 ppt, but not affected significantly at other conditions except the low numbers in 36℃, 10 and 30ppt.

Table 6 shows that temperature and salinity affect mixis rate and fertilization of L-type rotifer. However, these didn't affect fertilization of S-type rotifer. The effective numbers of resting egg produced by 10,000 rotifers for L-type rotifer showed the influence of temperature. However, S-type rotifer on temperature and salinity didn't show significant relationship.

Disscussion

Resting egg production of rotifer, *B. plicatilis,* was influenced by mixis rate and fertilization, and these values are important indicators of reproductivity (Hagiwara et al., 1989). Several authors (Hino and Hirano, 1984, 1985, 1988 ; Lubzen et al., 1985 ; Hagiwara et al., 1988a ; Hagiwara and Lee, 1991) indicated that mixis rate and number of resting egg produced by 10,000 rotifers of L-type rotifer increased with decreasing temperature (15~30℃) and salinity (8~16 ppt). For S-type rotifer, those increased when the temperature and salinity increased from 20 to 30℃ and from 5 to 30 ppt (Snell, 1986 ; Higiwara et al., 1989 ; Hagiwara and Lee, 1991), but decreased at higher than 30℃ and 30 ppt (Snell, 1986). Resting egg production between L and S-type rotifers are inversely affected by the same temperatures and salinities (Hagiwara et al., 1989 ; Hagiwara and Lee, 1991 ; Hagiwara and Hirayama, 1993).

In our results, the effect of mixis rate and resting egg production on temperature for L-type and S-type rotifer was consistent with previous reports, but the effect of salinity was a little different from those reports because of higher mixis rate and resting egg produced by 10,000 rotifers at 20 ppt in this study. Hino and Hirano (1988) indicated that the effect of salinity on mixis induction was influenced by culture salinity during formation of resting egg of the ancestral strain. Hagiwara et al. (1995) reported that this variation might be caused by differences in the environment of ancestral strains. The results of the present study might be also explained with the same reason mentioned above. Another reason for this result, it was reported that mixis rate and resting egg production differs among strains (Hagiwara et al., 1988b ; Hagiwara 1994 ; Park and Hur, 1996).

Hamada et al. (1993) reported that the growth rate of Hawaiian S-type rotifer was declined by the high toxicity of ammonia at the high temperature and salinity in which resting egg production was high. Thus, L-type rotifer was better than S-type rotifer for the mass production of resting egg. To solve the problem, Hamada et al. (1993) indicated S-type rotifer strain with bisexual reproductive characteristics smiliar to the L-type rotifer might be good for the mass production of resting egg.

In this study, we selected strains which showed the highest resting egg production among 2 L-type and 18 S-type rotifer strains. From each strain, clone with the highest resting egg production was used in this experiment. The highest number of resting egg produced by 10,000 rotifer for S-type rotifer was similar to that of the L-type rotifer, and the highest number of resting egg per day for S-type rotifer was 34.6 eggs/ml/day which are 3.2 times higher than that of the L-type rotifer. In the Korean rotifer, *B. plicatilis,* the mass production of resting egg of S-type is higher than that of L-type in mass culture (Park and Hur, In press).

In this study, we could conclude that these variations observed between the current study and previous study (Hamada et al., 1993) might be caused by the strains and salinity conditions.

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