Lifespan and Fecundity of Three Types of Rotifer, *Brachionus plicatilis* by an Individual Culture

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The lifespan and fecundity of three types(ultra small, small and large) rotifer, *Brachionus plicatilis*, were investigated. Generally, the lifespan and fecundity of three types rotifer were better at 25~27℃ than at 20~22℃, and this phenomenon was more distinct in the ultra small and the small type rotifers. With regard to salinity, while the ultra small and the large type rotifer preferred low salinity(16ppt) to high salinity(32ppt), fecundity of the small type rotifer was higher at high salinity(32ppt) than at low salinity(16ppt). Suitable food organisms were *Tetraselmis tetrathele* and *Chlorella ellipsoidea* for the three types rotifer. *Tetraselmis tetrathele* was more adequate for the ultra small and large type rotifer as live food. However, *Chlorella ellipsoidea* showed better dietary value for the small type rotifer.

Introduction

The rotifer *Brachionus plicatilis* has been divided into two types, according to its size, in small(S) type(ca. 170 μm) and large(L) type(ca. 220 μm) (Fu *et al.*, 1991a). Recently, an ultra small(Us) strain(ca. 140 μm) was isolated in Thailand and studied on life history(Kurokura *et al.*, 1991). The S and L types are considered genetically to be different from each other in morphology(Fu *et al.*, 1991 b; Rumengan *et al.*, 1991), including size(Fu *et al.*, 1991a), karyotypes(Rumengan *et al.*, 1991) and growth response to temperature(Ito *et al.*, 1981) and salinity(Mustahal and Hirata, 1991).

All three strains also exhibit differences in their life history characteristics(Kurokura *et al.*, 1991). Also, it has been demonstrated that different rotifer types have different nutritional(Yufera *et al.*, 1983) and environmental requirements(Hino and Hirano, 1988).

The common practice to culture rotifers has been using microalgae to feed them, since the general chemical composition of rotifers is closely related to that of the microalgae used as live food(Ben-Amotz *et al.*, 1987; James and Abu-Rezeq, 1988; Frolov *et al.*, 1991). As some of microalgae have been shown to have the nutritive elements, mainly highly unsaturated fatty acids(HUFA) and amino acids, that are required for fish and crustacean larvae(Watanabe *et al.*, 1983), and these nutrients can be transferred via rotifers to larvae(Fontaine and Revera, 1980).

The effect of different microalgae on the growth rate of rotifers has been studied(Hirayama *et al.*, 1973; 1979; Yufera *et al.*, 1983; Hirayama, 1985), since it has been demonstrated that different microalgae have influenced not only reproduction(Lubzens *et al.*, 1985) but also lifespan of rotifers(King and Miracle, 1980). Since two main objectives of culturing rotifers are to grow them rapidly and

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maintain them in high densities, it is important to determine which microalgae maximize their growth rate.

This study was carried out to understand the characteristics on lifespan and fecundity of rotifer by strain from an individual with different temperatures, salinities and microalgae.

Material and Methods

The microalgae used in the study as live food for the rotifer were *Chlorella ellipsoidea* Gerneck (NFUP -National Fisheries University of Pusan-27), *Tetraselmis tetrathele*(West) Butcher(NFUP 86), *Phaeodactylum tricornutum* Bohlin(NFUP 10), *Isochrysis galbana* Parke(NFUP 5), *Chaetoceros gracilis* Schutt(NFUP 6), *Thalassiosira weissflogii*(Grunow) Fryxell & Hasle(NFUP 65), *Pavlova lutheri* Droop(NFUP 15-1) and *Nannochloris oculata* Droop (NFUP 22). These microalgae were cultivated with f/2 medium(Guillard and Ryther, 1962), with aeration at 20℃(*N. oculata* at 26℃) and under 24 hr. illumination(ca. 5,000 lux) using a cool-white fluorescent lamp.

Three types of the rotifer, *Brachionus plicatilis* were used for the study. The average size of three types of rotifer is shown on Table 1. Laid amictic eggs of the three rotifer strains, L-type(Japan strain), S-type(Hawaii strain) and Us-type(Thailand strain), were collected from each mass culture systems and sieved with different mesh size nets(100 μm, 70 μm, 50 μm). They were cultured in the medium innoculated by *C. ellipsoidea* with mean density($2\sim3\times10^6$ cells/*ml*). These amictic females were used to determine some aspects of the reproductive biology of each type, as well as the effect of temperature, salinity and diet.

For all three strains, following an acclimatization period of 3~4 days in the respective environmental conditions and the diets to be tested, 12 amictic females were incubated individually in 1*ml* of filtered seawater in a tissue cell culture chamber. The microalgae food was supplied to the rotifer ad libitum and its concentration ranged from 1×10^6 cells/*ml* to 6×10^6 cells/*ml* according to the size of microalgae.

The rotifers were daily observed by a stereomicroscope. The offsprings and the laid eggs were removed and counted, and the adult rotifers were transferred to a new medium maintaining the same food concentration. The rotifers were cultured in two temperature regimes(20~22℃; 25~27℃) and in two salinity conditions(16ppt; 32ppt). The light period was 24:0(L:D) during the experiment.

For statistical analysis, the ANOVA test was used to compare the average rotifer lifespan and offspring production according to the factors studied for each strain.

Results

1. Ultra small type rotifer

The longest average lifespans and fecundity were recorded with *T. tetrathele* at 25~27℃ and both salinities tested. However, the range of lifespans as well as fecundity was highly variable with the diets tested. The longest individual lifespan was 14.5 days with *T. tetrathele.* The highest fecundity was 37 offsprings with *C. ellipsoidea.* The shortest average lifespan(2.79 days) and the lowest average fecundity(0.88) were recorded with *N. oculata,* although they were statistically not significant with

Table 1. Size of three types of *Brachionus plicatilis* cultured with *Chlorella ellipsoidea*

Rotifer Types	Source of strains	Microalgae	Size(μm)	
			Length	Width
Us-type	Thailand	*C. ellipsoidea*	144.75 ± 18.24	119.53 ± 17.4
S-type	Hawaii	*C. ellipsoidea*	163.39 ± 25.72	129.40 ± 23.0
L-type	Japan	*C. ellipsoidea*	195.19 ± 26.75	143.65 ± 20.3

other treatments. *P. tricornutum, T. weissflogii* and *P. lutheri* did not support rotifer growth for more than a 24h period(Table 2).

When *C. ellipsoidea* was used as a food, the lifespan and fecundity of the rotifer at 32ppt were higher at 25~27℃ than at 20~22℃. However, at a salinity of 16ppt the average lifespan was higher at 20~22℃, but the average fecundity was almost twice at 25~27℃.

Table 2. Effect of microalgae, temperature and salinity on the lifespan and fecundity of ultra small type rotifer, *Brachionus plicatilis*

Microalgae	Temperature (℃)	Salinity (ppt)	Lifespan(days)		Fecundity(ind./female)	
			Average	Range	Average	Range
Chlorella ellipsoidea	20~22	32	2.83 ± 1.95d	0.5~ 8.5	1.43 ± 1.37d	0~ 4
	20~22	16	5.59 ± 2.76b	0.5~10.5	6.18 ± 5.19b	0~16
	25~27	32	3.61 ± 2.10cd	0.5~ 8.5	3.22 ± 3.29c	0~19
	25~27	16	4.85 ± 2.41bc	0.5~11.5	12.10 ± 7.93a	1~37
Nannochloris oculata	20~22	32	2.79 ± 2.35d	0.5~ 9.5	0.88 ± 1.37d	0~ 4
	20~22	16	3.30 ± 2.40d	0.5~10.5	1.20 ± 1.28d	0~ 5
	25~27	32	3.35 ± 2.49d	0.5~ 8.5	1.42 ± 2.10d	0~ 8
	25~27	16	4.08 ± 2.93bcd	0.5~13.5	3.53 ± 3.73c	0~10
Tetraselmis tetrathele	20~22	32	3.14 ± 2.61d	0.5~ 8.5	3.21 ± 3.55c	0~13
	20~22	16	4.19 ± 3.04bcd	0.5~ 9.5	4.00 ± 3.66bc	0~13
	25~27	32	7.77 ± 4.88a	2.5~ 9.5	13.20 ± 9.47a	0~27
	25~27	16	8.00 ± 2.62a	4.5~14.5	13.80 ± 7.64a	4~31
Chaetoceros gracilis	25~27	32	4.83 ± 1.20b	3.5~ 7.5	5.00 ± 1.60bc	3~ 7
Phaeodactylum tricornutum	25~27	32	-	-	-	-
Thalassiosira weissflogii	25~27	32	-	-	-	-
Isochrysis galbana	25~27	32	4.75 ± 0.43b	4.5~ 5.5	3.00 ± 1.90c	0~ 6
Pavlova lutheri	25~27	32	-	-	-	-

Different superscripts indicate significance($p<0.05$).

2. Small type rotifer

The longest average lifespans were recorded with *C. gracilis* and *P. lutheri*(7.17 and 7.00 days respectively) and the highest average fecundity with *C. ellipsoidea* at 25~27℃ and both salinities tested(6.49, 6.50 individuals/female). However, the range of lifespans as well as fecundity was highly variable with the diets tested. The longest individual lifespan was 12.5 days with *C. ellipsoidea* and maintained at 25~27℃ and 32ppt. The highest individual fecundity was 25 offsprings with *T. tetrathele* at 25~27℃ and 32ppt. In all of these cases the longest lifespan matched with the highest fecundity within each food microalgae. The lifespan and fecundity were higher at 25~27℃ than at 20~22℃, when any microalgae were tested(Table 3).

P. tricornutum and *T. weissflogii* did not support rotifer growth for more than a 24h period as the result of ultra small type rotifer. In the trial with *N. oculata,* the individual growth was recorded continuously, observing the size of individuals several times a day. It was observed that size varied with the individuals. Some individuals grew from 120.6 ㎛ to 144.8 ㎛ in 3.5h, from that to 168.9 ㎛ in 3h, and from that to 193.0 ㎛ between 15~18h. Other ones needed a longer time span 1 or 2 days to grow to 193 ㎛ between these values in a longer time span: 1 or 2 days. In one case it was possible to record the egg growth from 72.4~48.3 ㎛ to 96.5~72.4 ㎛ in 2h.

Table 3. Effect of microalgae, temperature and salinity on the lifespan and fecundity of small type rotifer, *Brachionus plicatilis*

Microalgae	Temperature (℃)	Salinity (ppt)	Lifespan(days)		Fecundity(ind./female)	
			Average	Range	Average	Range
Chlorella ellipsoidea	20~22	32	3.60 ± 2.24cde	0.5~10.5	3.53 ± 3.18bc	0~14
	20~22	16	2.70 ± 1.37def	0.5~ 5.5	3.34 ± 3.14cd	0~15
	25~27	32	4.50 ± 3.35bcd	0.5~12.5	6.49 ± 5.81a	0~20
	25~27	16	3.00 ± 1.94def	0.5~ 5.5	6.50 ± 5.84ad	0~20
Nannochloris oculata	20~22	32	3.50 ± 1.69cde	0.5~ 5.5	2.00 ± 1.88def	0~ 6
	20~22	16	1.90 ± 1.32h	0.5~ 5.5	1.00 ± 1.87fg	0~ 8
	25~27	32	4.69 ± 3.04bc	0.5~11.5	3.52 ± 3.66bcd	0~15
	25~27	16	2.17 ± 1.40fh	0.5~ 4.5	2.13 ± 2.22bcd	0~ 7
Tetraselmis tetrathele	20~22	32	1.82 ± 1.63ghi	0.5~ 7.5	1.00 ± 1.76g	0~ 8
	20~22	16	1.63 ± 1.59hi	0.5~ 5.5	1.00 ± 1.83fg	0~ 7
	25~27	32	2.38 ± 2.35fgh	0.5~10.5	5.77 ± 3.70bcdef	0~25
	25~27	16	2.17 ± 2.12fgh	0.5~ 8.5	1.27 ± 2.98efg	0~12
Chaetoceros gracilis	25~27	32	7.17 ± 1.49a	4.5~ 8.5	5.33 ± 1.01a	3~ 6
Phaeodactylum tricornutum	25~27	32	-	-	-	-
Thalassiosira weissflogii	25~27	32	-	-	-	-
Isochrysis galbana	25~27	32	5.50 ± 2.32b	1.5~10.5	2.00 ± 1.44de	0~ 5
Pavlova lutheri	25~27	32	7.00 ± 0.67a	6.5~ 8.5	5.25 ± 1.34a	3~ 7

Different superscripts indicate significance($p<0.05$).

3. Large type rotifer

The longest average lifespan and the highest average fecundity were recorded with *T. tetrathele* at any temperatures and salinities tested, and the highest average fecundity was also recorded *C. ellipsoidea* at 25~27℃ and 16ppt(Table 4). However, the range of lifespans as well as fecundity was highly variable with the different microalgae tested. The longest individual lifespan was 16.5 days, using *T. tetrathele.* The highest individual fecundity was 35 and 36 offsprings, using *C. ellipsoidea,* at 20~22℃ and 25~27℃ respectively. The shortest average lifespan(2.72 days) was recorded with *C. ellipsoidea* at 25~27℃, and the lowest fecundity(1.92) with *N. oculata* at 25~27℃. *P. tricornutum* and *T. weissflogii* did not support rotifer growth for more than 24 h period.

When *C. ellipsoidea* was used as a food, and the salinity was 32ppt, the lifespan was higher at 20~22℃ than at 25~27℃. But, at 16ppt the average lifespan were similar at both temperatures tested. The fecundity was higher at 16ppt than at 32ppt, but the average fecundity was higher at 25~27℃. In the lifespan of rotifers when the other two microalgae were tested at different temperatures and salinities regimes, there were no significant differences($p<0.05$) within the treatments. When *N. oculata* was used, the fecundity was higher at 16ppt at both temperature regimes($p<0.05$).

No males, nor mictic females, could be detected in any one of the three strains. In all three strains when the survival rate and fecundity of offsprings from the same adult were examined, both parameters were different, nevertheless the offsprings were born on the same day or on successive days.

Discussion

According to the results, each strain of rotifers has its own favorable conditions. Us-type appeared to be more adapted to low salinities, and tempera-

Table 4. Effect of microalgae, temperature and salinity on the lifespan and fecundity of large type rotifer, *Brachionus plicatilis*

Microalgae	Temperature (℃)	Salinity (ppt)	Lifespan(days)		Fecundity(ind./female)	
			Average	Range	Average	Range
Chlorella ellipsoidea	20~22	32	4.28 ± 2.32cde	0.5~ 8.5	2.39 ± 2.36d	0~ 6
	20~22	16	4.76 ± 2.30c	0.5~11.5	6.70 ± 7.65b	0~36
	25~27	32	2.72 ± 2.13f	0.5~ 8.5	2.33 ± 2.36d	0~ 8
	25~27	16	4.67 ± 3.29cd	1.5~11.5	14.92 ± 14.20a	0~35
Nannochloris oculata	20~22	32	3.11 ± 2.14ef	0.5~ 6.5	2.33 ± 2.48d	0~ 8
	20~22	16	3.27 ± 2.64def	1.5~ 8.5	4.08 ± 2.87c	0~ 8
	25~27	32	3.50 ± 1.96def	0.5~10.5	1.92 ± 2.62d	0~10
	25~27	16	3.91 ± 2.83cdef	5.5~15.5	4.88 ± 3.77b	0~12
Tetraselmis tetrathele	20~22	32	11.43 ± 3.66a	5.5~15.5	13.40 ± 6.92a	2~30
	20~22	16	12.33 ± 3.28a	4.5~15.5	14.13 ± 8.26a	2~31
	25~27	32	10.50 ± 3.48a	4.5~16.5	12.33 ± 6.00a	2~22
	25~27	16	11.23 ± 3.68a	4.5~15.5	13.33 ± 5.92a	2~25
Chaetoceros gracilis	25~27	32	4.75 ± 0.75c	3.5~ 5.5	3.00 ± 2.09cd	0~ 6
Phaeodactylum tricornutum	25~27	32	-	-	-	-
Thalassiosira weissflogii	25~27	32	-	-	-	-
Isochrysis galbana	25~27	32	6.25 ± 2.14b	2.5~ 8.5	4.25 ± 3.05bc	1~10
Pavlova lutheri	25~27	32	4.83 ± 1.74c	2.5~ 7.5	2.67 ± 0.79d	2~ 4

Different superscripts indicate significance($p<0.05$).

tures over 25℃; S-type propagated better at high salinities and at temperatures over 25℃; and L-type appeared to be maintained in better conditions at low salinities and low temperatures. The rotifer, *B. plicatilis* is known to show a wide tolerance range of salinity(Fulks and Main, 1991; Mustahal and Hirata, 1991) and temperature(Ito *et al.*, 1981). Based on these findings the previous studies questioned the identity of the size groups *B. plicatilis*. Hagiwara *et al.*(1989) and Rumengan *et al.*(1991) identified the S-type as *B. plicatilis rotundiformis* and the L-type as *B. plicatilis typicus* and they separated them into genetically divergent groups. The Us-type is less known, and a few papers have dealt with it until now. On the other hand, within each type, differences in salinity and temperature adaptability, and also in the average size, have been recorded in the different countries(Fukusho and Okauchi, 1982; Fu *et al.*, 1991a; Mustahal and Hirata, 1991; Mustahal *et al.*, 1991) and also within the same country(Ito *et al.*, 1981).

The lifespan of rotifers was dependent on the strain(King and Miracle, 1980). The results of this experiment are similar to those obtained by King and Miracle(1980) in two American strains. Since these investigators could separate females and males and build two different survivorship curves, the average lifespan of females alone was a little superior to the results obtained in this experiment, where it was impossible to separate males or there were no males. Hagiwara and Hino(1990) recorded average lifespans from 8.08 days to 12.48 days, 2~4 times higher than that recorded with some diets and/or conditions tested in this experiment. Also the average number of offspring produced were higher. In another experiment the average fecundity was between 1.09 to 2.30 individuals(Hagiwara *et al.*, 1988), 3~4 times smaller than the results in the present experiment.

Mustahal and Hirata(1991) found differences in the average lifespan and offspring production among five stains and also among the five different salinities tested. Kurokura *et al.*(1991) detected variations in the individual life history characteristics of Us-type, S-type and L-type strains. The results obtained in the present experiment showed that not only salinity and temperature, but also the diet effects the life history of rotifers. However, since there are many intrinsic and extrinsic factors that can effect the rotifer lifespan as well as fecundity (King and Miracle, 1980; Yufera and Pascual, 1984; Snell, 1986), it is impossible to compare all these results.

One important thing is that in some of those experiments, every strain was able to reproduce either asexually or sexually(King and Miracle, 1980), and sexual reproduction occurs when rotifers have enough energy(Lubzens *et al.*, 1985; Snell, 1986). In the strains used in this experiment none were able to reproduce sexually, so that the lifespan and fecundity of the rotifer seemed to be also effected by the food nutritive quality.

Since no aeration was supplied in this experiment, some live food sunk at the bottom. It was not consumed by the rotifer(Esparcia and Serra, 1988), and affected the rotifers growth rate.

In rotifers, their size has been used as an important criterion to separate females and males(king and Miracle, 1980), while the present study revealed that the size was not an important parameter to determine the sex, particularly in case of S-type strain of *B. plicatilis*. On this aspect Hino and Hirano(1988) reported that the egg size had been used to determine a mictic or amictic female.

In this experiment it was not possible to detect resting eggs nor males. Although the sexual reproduction with males and resting eggs production has been shown in these types of rotifer(Hino and Hirano, 1985; Lubzens *et al.*, 1985; Snell, 1986), some strains, stocks or clones appear to do not to produce males and resting eggs(Hagiwara *et al.*, 1988), and only reproduce parthenogenetically.

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個體培養에 의한 3 Types 輪蟲(*Brachionus plicatilis*)의 壽命 및 繁殖力

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해산어류의 종묘생산시 초기 동물먹이생물로 널리 이용되는 미소형, 소형, 대형 윤충(*Brachionus plicatilis*)의 환경요인에 따른 수명과 번식력을 조사하였다.

전반적으로 온도는 25~27℃가 20~22℃보다 번식력과 수명이 높았으며 미소형과 소형 윤충은 이러한 현상이 더욱 뚜렷하였다. 염분은 미소형과 대형 윤충은 32ppt보다 16ppt에서 번식력이 더욱 높았던 반면 소형 윤충은 32ppt에서 더욱 높았다. 먹이생물은 미소형과 대형 윤충은 *Tetraselmis tetrathele*, 소형 윤충은 *Chlorella ellipsoidea*가 더 좋은 먹이효율을 보였다. 각 윤충의 먹이생물, 수온, 염분에 따른 최고의 번식력은 미소형 윤충은 *Tetraselmis tetrathele*, 25~27℃, 16ppt, 소형 윤충은 *Chlorella ellipsoidea*, 25~27℃, 32ppt, 대형 윤충은 *Chlorella ellipsoidea*, 25~27℃, 16ppt에서 나타났다.