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Effects of Microalgae and Salinity on the Growth of Three Types of the Rotifer *Brachionus plicatilis*

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We investigated the effects of salinity and three food species of microalgae on the growth of three types of the rotifer *Brachionus plicatilis*, with the aim of improving mass culture of rotifers in hatcheries. Three types (large, small, and ultra-small) of the rotifer were cultured at 16 ppt and 32 ppt salinity with the green algae *Chlorella ellipsoidea*, *Nannochloris oculata*, or *Tetraselmis tetrathele*. The maximum density and specific growth rate were compared for each rotifer type. Ultra-small rotifers grew significantly faster at 16 ppt salinity than at 32 ppt, and *C. ellipsoidea* and *T. tetrathele* promoted significantly higher growth than did *N. oculata*. However, small rotifers grew significantly better at 32 ppt salinity than at 16 ppt, and small rotifers fed on *N. oculata* achieved the highest density at 1,185 individuals/ml. Large rotifers grew faster at 16 ppt salinity than at 32 ppt, with a diet of *T. tetrathele* resulting in the fastest growth. Each type of rotifer thrived under different regimens of microalgae and salinity.

Key words: Rotifer, *Brachionus plicatilis*, Microalgae, Salinity

Introduction

The rotifer *Brachionus plicatilis* is used in aquaculture as a live food source for marine fishes and crustacean larvae. Ito (1960) demonstrated the limits of salinity tolerance of this rotifer and discussed its reproductive biology. Since then, many researchers have studied this organism, which is considered to be the best live food in aquaculture due to its ease of culture (Gilberto and Mazzola, 1981), high density (Gilberto and Mazzola, 1981; Fulks and Main 1991), small size and slow motility (Kinne, 1977; Hoff and Snell, 1989; Fulks and Main 1991), high nutritive value (Gilberto and Mazzola, 1981; Watanabe et al., 1983), and potential for bioenrichment (Fontaine and Revera, 1980).

Three size-class types of *B. plicatilis* have been identified: a large type; a small type, known as *B. rotundiformis*; and an ultra-small type isolated from tropical areas. Types used in hatcheries are based on the mouth size of the animals reared in the hatchery (Cabrera and Hur, 2001).

Since two objectives of rotifer culture are rapid growth and maintenance of high densities, it is important to understand the optimum foods and environmental factors, such as temperature and salinity, for culturing rotifers. However, different rotifer strains have disparate nutritional (Yufera et al., 1983) and environmental requirements (Hino and Hirano, 1988).

Microalgae are commonly used as food for rotifer cultures, due to the high concentration of nutritive elements, mainly highly unsaturated fatty acids and amino acids, that are essential in the larval culture of fishes and crustaceans (Watanabe et al., 1983). These nutrients can be also transferred via rotifers to the larvae by the enrichment technique (Fontaine and Revera, 1980). The general proximate chemistry composition of a rotifer is closely related to that of its food (Ben-Amotz et al., 1987; James and Abu-Rezeq, 1988; Frolov et al., 1991).

Many researchers have studied the effect of various microalgae on the growth of rotifers (Hirayama et al., 1973, 1979; Yúfera et al., 1983; Hirayama, 1985). Different microalgae have been demonstrated to influence both the type of reproduction (Lubzen et al., 1985) and the lifespan of the rotifers (King and Miracle, 1980).

Although many species of microalgae can be used as live food for rotifers, the most popular for mass

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culture are *Chlorella* (*Nannochloropsis*), *Nannochloris*, and *Tetraselmis* because of their high growth rates and nutritive merit.

Rotifers isolated from different habitats have dissimilar adaptability to salinity concentrations (Cabrera et al., 1993). In this study, we examined the effects of the three most widely used microalgae and two different salinity concentrations on the growth of three strains of the rotifer *B. plicatilis*.

Materials and Methods

We used the microalgae *Chlorella ellipsoidea* Gerneck (KMCC C-20), *Tetraselmis tetrathele* (West) Butcher (KMCC P-2), and *Nannochloris oculata* Droop (KMCC C-31), all obtained from the Korea Marine Microalgae Culture Center. Microalgae were cultured using f/2 media (Guillard and Ryther, 1962) in 20-L aerated carboys at 20°C (*N. oculata* at 26°C) and continuous illumination (ca. 5,000 lux) with a cool-white fluorescent lamp.

The rotifers were obtained from the same *Brachionus plicatilis* strain used by Cabrera et al. (1993). Three rotifer types, ultrasmall (mean length 145 μm), small (mean length 163 μm), and large (mean length 195 μm), were used. Amictic eggs laid by each type were collected from the culture tanks and kept in *C. ellipsoidea* (3×10^6 cells/mL) tanks until they hatched.

Amictic females were used to determine the nutritional value of the microalgae species. Individuals of each rotifer type were incubated in 1 mL of filtered seawater in a tissue cell chamber (26°C for the ultrasmall and small rotifers and 21°C for the large rotifer) under continuous illumination (ca. 3,000 lux). Salinity was maintained at either 16 ppt or 32 ppt. The media were renewed daily to maintain a food density of 4×10^6 cells/mL of *C. ellipsoidea*, 9×10^6 cells/mL of *N. oculata*, and 1×10^6 cells/mL of *T. tetrathele*. The rotifer population in each well was counted daily using a stereomicroscope and a pasteur pipette. The specific growth rate (r) of the rotifer types was determined at maximum density using the following formula:

$$r = (\text{Ln } Nt - \text{Ln } No)/t$$

where Nt: population at time t; No: initial population; t: time (days)

The growth rates of the rotifer types were analyzed statistically using Statix 4.0 software (St. Paul, MN, USA). Differences among the treatments were compared by one-way ANOVA, $p < 0.05$.

Results

The daily population growth and the specific growth rate at maximum density of the three rotifer types fed on three species of microalgae are shown in Fig. 1 and Table 1. For all three species of microalgae, populations of the ultra-small-type rotifer grew faster in 16 ppt salinity than in 32 ppt. At 16 ppt, the *C. ellipsoidea* diet yielded the highest density with 925 inds./mL; *T. tetrathele* produced a density of 814 inds./mL, and a diet of *N. oculata* resulted in 235 inds./mL. At 32 ppt, *T. tetrathele* yielded a significantly higher maximum density of rotifers than did *C. ellipsoidea* while *N. oculata* resulted in a maximum density of only 20 inds./mL (Table 1). Thus, for growing ultra-small rotifers, 16 ppt salinity was significantly better than 32 ppt, and *C. ellipsoidea* and *T. tetrathele* yielded much higher growth than *N. oculata* did.

On the other hand, populations of the small-type rotifer grew about 13 times faster in 32 ppt salinity than in 16 ppt. At 32 ppt salinity, the maximum density of rotifers was high and differed significantly among the three microalgae species, ranging from 479 to 1,185 inds./mL. However, at 16 ppt, there was no significant difference among the microalgae, with maximum densities ranging from 54 to 97 inds./mL (Table 1).

The large-type rotifer achieved higher densities at 16 ppt salinity, growing 2.5 times faster than at 32 ppt, and *T. tetrathele* yielded the highest density at both 16 ppt and 32 ppt salinity (Table 1).

Since the culture periods from inoculation to maximum density differed among the experiments, the specific growth rates for each experiment could not be compared directly. However, they tended to be correlated with the maximum density results reported above.

Discussion

Rotifer growth is influenced by diet (Hirayama et al., 1979; Yúfera and Pascual, 1984). In this study, the microalgae *N. oculata* and *T. tetrathele* were the optimal food for maximal growth of the small- and large-type rotifers. This result agrees with other studies that have reported better growth of rotifers cultured with these microalgae (Yúfera et al., 1983), particularly when the culture temperature is around 25°C (Hur et al., 1989). *Tetraselmis tetrathele* has a high food value for rotifers and is considered a good substitute for marine *Chlorella* (Fukusho et al., 1984). In our study, the relative low growth of rotifers fed on

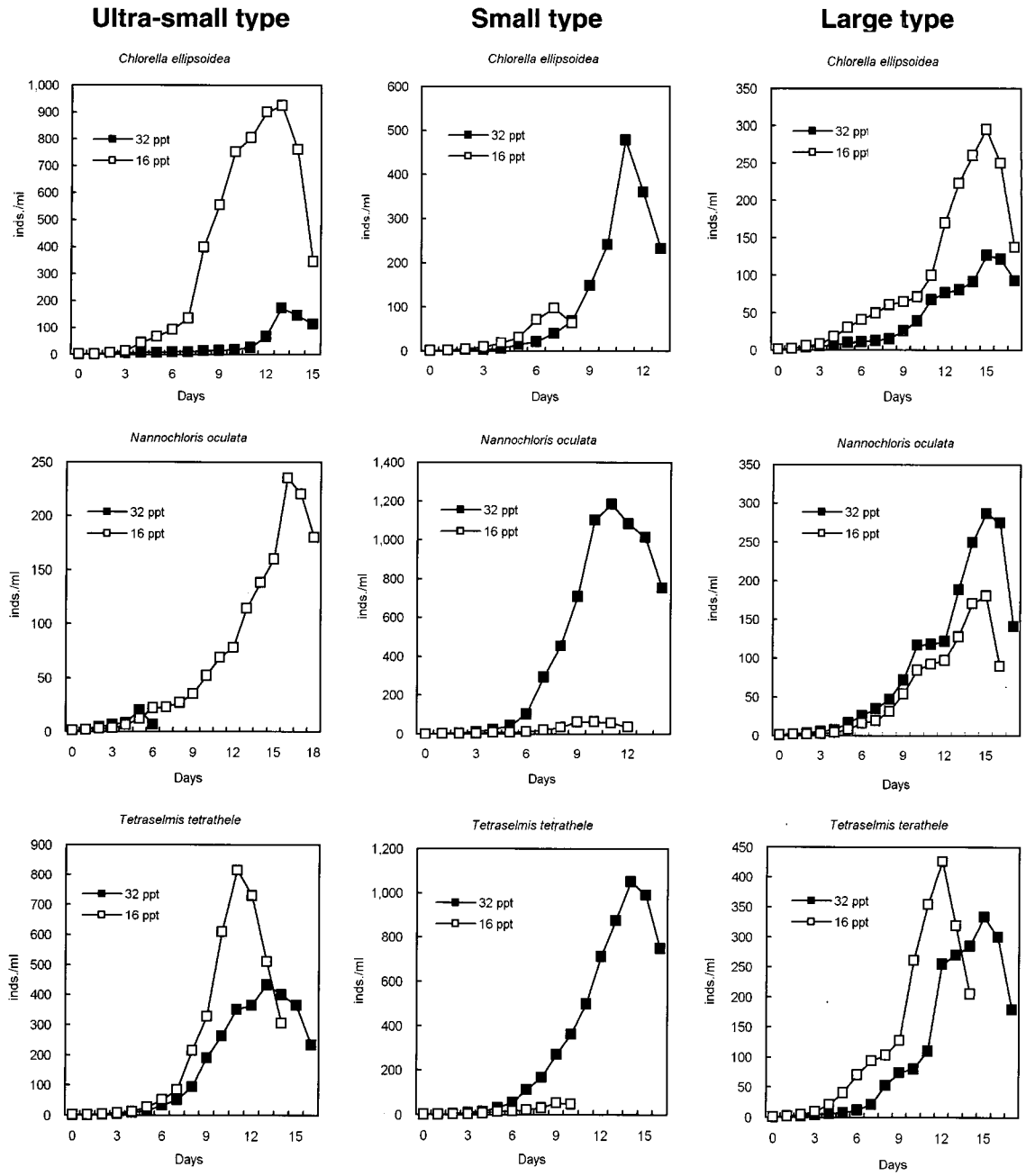

Fig. 1. Daily growth of populations of ultra-small, small- and large-type *Brachionus plicatilis* cultured at 16 ppt and 32 ppt salinity with *Chlorella ellipsoidea*, *Nannochloris oculata*, or *Tetraselmis tetrathele*.

C. ellipsoidea can be explained by the lack of vitamin B_{12} in this species (Maruyama et al., 1989), which is an essential nutrient for rotifers (Satuito and Hirayama, 1989; Yu et al., 1989). However, for the ultra-small-type rotifers cultured at 16 ppt salinity, *C. ellipsoidea* appeared to be a much more suitable food than *N. oculata* when compared to feed results for the small and large rotifers.

The specific growth rates (r) of rotifer types vary widely based on the microalgae diet (Hirayama, 1985; Satuito and Hirayama, 1990). The r-values reported from rotifers cultured with *N. oculata* (r = 0.34 -0.64; Yufera et al., 1983) and *T. tetrathele* (r >1.0; Hirayama, 1985) were higher than those obtained here. However, the r-values we found were similar or in some cases higher than those from studies using the microalgae *Isochrysis*, *Phaeodactylum*, and *Chaetoceros* (Hirayama and Kusano, 1972;

Table 1. Comparison of the maximum density and specific growth rate (means ± S.D.) of three types of the rotifer *Brachionus plicatilis*

Type	℃	Microalgae	Salinity ‰	Density (inds./mL)	Specific growth rate	Days
Ultra-small	26	*Chlorella ellipsoidea*	32	172.5±27.58^{e}	0.40±0.012^{de}	13
			16	925.0±21.21^{a}	0.53±0.002^{bc}	13
		Nannochloris oculata	32	20.0±7.07^{f}	0.59±0.072^{ab}	5
			16	235.0±21.21^{d}	0.34±0.006^{e}	16
		Tetraselmis tetrathele	32	433.5±2.12^{c}	0.47±0.0004^{cd}	13
			16	814.0±19.80^{b}	0.61±0.002^{a}	11
Small	26	*Chlorella ellipsoidea*	32	479.0±15.56^{c}	0.56±0.003^{b}	11
			16	97.0±9.90^{d}	0.65±0.015^{a}	7
		Nannochloris oculata	32	1185.0±18.38^{a}	0.64±0.001^{a}	11
			16	65.0±1.41^{d}	0.42±0.002^{e}	10
		Tetraselmis tetrathele	32	1050.0±70.71^{b}	0.50±0.005^{c}	14
			16	54.0±8.49^{d}	0.44±0.018^{d}	9
Large	21	*Chlorella ellipsoidea*	32	127.0±4.24^{c}	0.32±0.002^{d}	15
			16	295.0±7.07^{b}	0.38±0.002^{b}	15
		Nannochloris oculata	32	287.0±39.60^{b}	0.38±0.009^{b}	15
			16	181.0±36.77^{c}	0.35±0.014^{c}	15
		Tetraselmis tetrathele	32	333.0±9.90^{b}	0.39±0.002^{b}	15
			16	426.0±41.01^{a}	0.50±0.008^{a}	12

Density values and specific growth rates for rotifer types with different superscripts are significantly different at $p < 0.05$.

Hirayama and Watanabe, 1973; James and Abu-Rezeq, 1988). The difference in r-values when the same species were used may be explained by the chemical composition and nutritional quality of the microalgae, which depends on the culture medium (Borowitzka, 1988) and the growth stage of the microalgae at harvesting time (Phatarpekar et al., 2000), as well as the culture system (James and Abu-Rezeq, 1988). Misidentification of the microalgae is also a possibility (James and Abu-Rezeq, 1988; Whyte and Nagata, 1990).

The r-values in this study varied according to the rotifer type. *Nannochloris oculata* appeared optimal for the large- and small-type rotifers, but it did not maintain the ultra-small type. Some species of microalgae have a growth-inhibiting effect on rotifers (Yúfera and Pascual, 1980; Trotta, 1983). Since rotifers exhibit genetic variability among strains (Hagiwara et al., 1989), it is possible that *N. oculata* does not have the nutritive value required by the ultra-small rotifer.

In this study, the growth rate of rotifers cultured at low salinity (16 ppt) was higher than that at seawater salinity, which has been also found by other researchers (Hino and Hirano, 1988; Hagiwara et al., 1989). Since the large-type rotifers, classified as *B. plicatilis typicus* and found in eel culture ponds with a chlorinity of 2-3 ppt, exhibited a high rate of parthenogenetic reproduction (Hino and Hirano, 1988), we expected that their population growth would be higher at low salinity. However, populations of the small rotifers grew faster at 32 ppt salinity. Since the small rotifers originate from coastal waters (Hino and Hirano, 1988), their growth rate was expected to be greater in the high salinity cultures.

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