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Salinity Adaptability of Five Different Strains of the Rotifer *Brachionus plicatilis*

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In order to know the physiological adaptation of the rotifer, salinity adaptability of five strains of the rotifer *Brachionus plicatilis*, namely, YL, YS, MS, JS, and IL was observed under different salinities of 5, 15, 25, 35, and 45‰.

The number of offspring was used as an indicator of rotifer production rate and was observed on cultures acclimated to each salinity for 0, 15, 35, 55, and 75 days. The index of adaptation was deduced from the slope of the linear regression equation obtained by plotting data on the number of offspring vs. acclimation periods.

The YL and YS strains showed high adaptability to all salinities, showing a highly euryhaline characteristic. MS exhibited high tolerance to low salinity with high production rate. However, at high salinities, the tolerance was low with low production rate inspite of acclimation for 75 days. JS and IL were both more euryhaline than MS, but not as euryhaline as YL and YS.

The rotifer *Brachionus plicatilis* is used throughout the world as a main live food source for fish seedling production. Japanese workers pioneered the culture of this species and introduced it to many other countries. Recently, almost every country with a developing fish aquaculture has its own strain of rotifer.[1,2] Some workers[3-5] have been studying the growth characteristics of various strains at various temperatures using various feeds. Fu *et al.*[6] studied the genetic variation of rotifer strains from different locations. As a result, different growth characters and genetic variations were observed among the strains.

In this study, adaptability to salinity was examined on five strains of rotifer to know the specific physiological adaptation of each strain to various salinity conditions.

Materials and Methods

The five strains of the rotifer *Brachiouns plicatilis typicus*,[7] which is called L-type by usage among fish culturists, from Yashima (YL) and Israel (IL); and *Brachionus plicatilis rotundiformis*,[7] which is called S-type, from Yashima (YS), Malaysia (MS), Indonesia (JS) were used as materials for this study. YL and YS were obtained from stock culture in our laboratory (initially isolated by Seto Inland Sea Farming Fisheries Association, Yashima Station in 1964).[8] MS was obtained from the *Macrobrachium* culture pond of University Pertanian Malaysia in 1987. JS was isolated from the brackish water pond in the National Institute of Oceanography in Jakarta, Indonesia in 1980[9] and IL, from the Israel Oceanographic and Limnological Research Ltd. Eilat, Israel.

For culture preparation, the 45‰ salinity medium was produced by adding crude salt to natural seawater. For the lower salinity medium, the natural seawater was diluted with distilled water. Culture media were autoclaved at 120°C for 30 min before use.

All strains of the rotifer used in this study had been maintained in stock cultures in our laboratory for at least 3 months before the experiment at a salinity of 20‰, fed with alga *Nannochloropsis* sp.[10] at a concentration of $5–8 \times 10^6$ cells/ml. All experiments were conducted in an incubator under a light intensity of 1,000 lx with 15L: 9D photoperiod, temperature of 25°C and pH 8 ± 0.5.

For preliminary culture, healthy individuals of amictic egg-bearing females were isolated individually into 3 m*l* chambers of microplate which contained modified seawater of 5, 15, 25, 35, and 45‰ salinity. Each chamber was filled with 1 m*l* of algal suspension of the same concentration as maintaining the stock culture. After 5 to 7 days, 3 chambers of the well grown rotifers were selected from each different salinity and strain,

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and all individuals in the respective chambers were transferred into 3 different bottles of 50 m*l*. Every two days, the medium in each bottle was was renewed by filtering the rotifer through 55 μm mesh of plankton net. One bottle of the best grown rotifers were selected from the 3 bottles and the culture was continued for 75 days.

For experimental culture, 48–72 healthy amictic egg-bearing females were transferred individually from respective bottles of preliminary culture, on 15, 35, 55, and 75th day, into each chamber of microplates with 1 m*l* medium. The alga, *Nannochloropsis* sp was supplied as food at 2.5–3.0 $\times$ 10^6 cells/m*l*. Salinity was adjusted to that of the above 5 salinities. For 0 day acclimation, the rotifers were directly transferred from the stock culture of 20‰ salinity to each salinity treatment.

Observation commenced for newly hatched neonates and continued until their death. Data on surviving individuals and offsprings produced were recorded daily under a binocular dissecting microscope. Surviving adults and their eggs were transferred into new culture media for further observation until the adult rotifer's death. The average number of offspring produced by a rotifer during its life time was used for analysis as a practical indicator of the production rate.

Results and Discussion

The number of offspring produced by the rotifer of all strains increased in all salinities as the adaptation period increased during 75 days. Linear regression analysis[11] was applied between the number of offspring produced and the adaptation periods. The relationship was described by the following equation:

$$Y=aX+b$$

where Y is the number of offspring produced and X, the adaptation period. All data of each series of experimental observation was applied for determination of the regression line. The slope, a, of each regression line as shown in Fig. 1 was considered to be the adaptability of the strain, while, the Y axis intercept, b, which indicated the number of offsprings produced at 0 day of adaptation period, can be considered as the latent adaptability of strain. In the following discussion, the slope of the regression line was used as the index of adaptation (Table 1), since latent adaptability, b, reflected the 20‰ salinity of the stock culture.

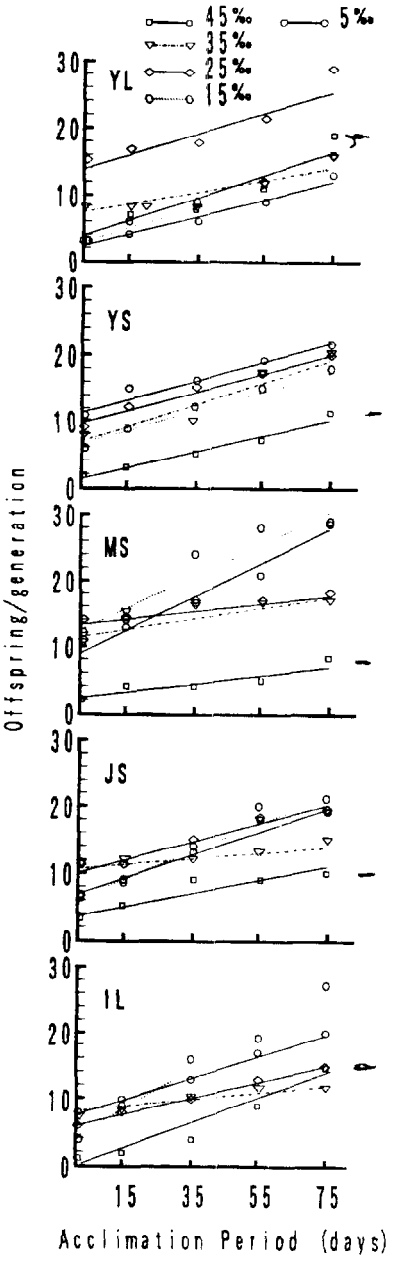

Fig. 1. Regression correlation of offspring numbers of the rotifer *Brachionus plicatilis* in various salinities and acclimation period.

YL and YS showed evenly high adaptabilities in all salinities, showing a highly euryhaline characteristic (Fig. 2). On the other hand, MS exhibited higher adaptability only at salinity of 5 and 15‰ as indicated by high production rate. However, at higher salinities it showed low production rate even though it was acclimated for 75 days. JS and IL showed the highest adaptability

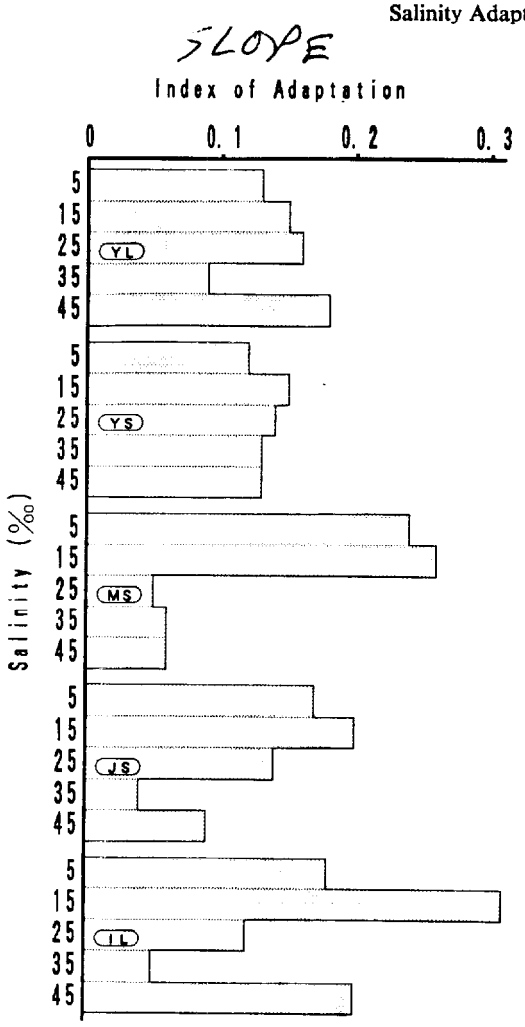

Fig. 2. Index of adaptation, indicate the adaptability of the rotifer *Brachionus plicatilis* in various salinities.

at 15% salinity. In addition, at high salinity levels, they showed more euryhaline than MS, but not as euryhaline as YL and YS.

The rotifer *Brachionus plicatilis* has already been well known as a euryhaline species,[12] having a wide geographical distribution. It is able to adapt to a wide range of salinities tested in all strains, although the strength of adaptability depended on salinity and strain. According to the previous report of Ito,[13] the rotifer *Brachionus plicatilis* was able to survive in the high medium salinity of above 50‰ to low salinity of 2‰. This may account for their ability to adapt to wider ranges of salinity.

In addition, the adaptability is also affected by former culture conditions. Recently cultured strains have a tendency of high adaptability within

Table 1. Values of slopes, intercepts, and correlation coefficients obtained from the regression equation ($Y=aX+b$) from the lines of number of offsprings in each salinity as shown in Fig. 1

Rotifer strain	Salinity (‰)	Slopes (a)	Intercepts (b)	Correlation coefficients (r)
YL	5	0.13	2.2	0.98
	15	0.15	3.3	0.99
	25	0.16	13.8	0.93
	35	0.09	6.7	0.90
	45	0.18	2.7	0.95
YS	5	0.12	11.8	0.97
	15	0.15	6.3	0.99
	25	0.14	9.5	0.98
	35	0.11	7.4	0.92
	45	0.11	1.4	0.96
MS	5	0.23	9.8	0.98
	15	0.25	12.3	0.97
	25	0.05	13.6	0.98
	35	0.06	12.6	0.94
	45	0.06	2.1	0.92
JS	5	0.17	7.1	0.98
	15	0.20	6.5	0.95
	25	0.14	10.0	0.90
	35	0.04	10.8	0.95
	45	0.09	3.8	0.85
IL	5	0.16	7.7	0.99
	15	0.29	4.4	0.99
	25	0.12	6.0	0.99
	35	0.05	2.1	0.97
	45	0.18	−0.5	0.96

the salinity range close to that of the former culture salinity. YL and YS used to be cultured for about 15 years in a salinity of about 35‰ and after that, they were cultured at 20‰ for 10 years. This may explain their high adaptabilities to wider salinity ranges. IL had been cultured for about 10 years in the salinity above 35‰,[14] then cultured in salinity of about 20‰ in our laboratory for 3 years. So, IL showed a high adaptability at 15 and 45‰ salinity (Table 1). On the other hand, MS was isolated only one year before this experiment from its natural habitat at salinity of below 15‰. This may explain for its low adaptabilities to high salinity condition.

Furthermore, genetic differences were recognized between both S and L type rotifers which were collected from different geographical areas.[6] It can be considered, that adaptability to salinity is also partly affected by genetic differences among strains.

From an aquacultural stand point, recognition of the adaptability to salinity allows more efficient use of strains of rotifers.

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References

1) K. Fukusho and M. Okauchi: *Bull. Natl. Res. Inst. Aquaculture*, **4**, 107–108 (1982).
2) E. Lubzens, A. Tandler, and G. Minkoff: *Hydrobiologia*, **186/187**, 387–400 (1989).
3) S. Ito, M. Sakamoto, M. Hori, and K. Hirayama: *Bull. Fac. Fish. Nagasaki Univ.*, **51**, 9–16 (1981).
4) M. Serra and M. Miracle: *Hydrobiologia*, **147**, 83–89, (1987).
5) J. Korstad, Y. Olsen, and O. Vadstein: *Hydrobiologia*, **186/187**, 43–50. (1987).
6) Y. Fu, K. Hirayama, and Y. Natsukari: The Second Asian Fish. Forum (ed. by R. Hirano and I. Hanyu), Asian Fish. Soc., Manila, 1990, pp. 37–40.
7) K. Hirayama: *Coll. Fr-Japon Oceanogr.*, **8**, 50 (1985).
8) H. Hirata: *Yoshoku*, **1** (11), 34–37 (1964).
9) K. Fukusho and M. Okauchi: *Bull. Nat. Res. Inst. Aquaculture*, **3**, 135–138 (1983).
10) I. Maruyama, T. Nakamura, T. Matsubayashi, Y. Ando, and T. Maeda: *Jap. J. Phycol.*, **34**, 319–325 (1986).
11) W. E. Ricker: *J. Fish. Res. Board Can.*, **30**, 409–434 (1973).
12) T. Ito: *Rep. Fac. Fish. Pref. Univ. Mie*, **2**, 317–324 (1956).
13) T. Ito: *Rep. Fac. Fish. Pref. Univ. Mie*, **3**, 708–739 (1960).
14) E. Lubzens: *Hydrobiologia*, **73**, 55–58 (1980).

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