FAS http://e-fas.org
Fisheries and Aquatic Sciences

Original Article
Fish Aquat Sci 18(3), 287-296, 2015

No Response to Bidirectional Size-Based Selection in the Rotifer *Brachionus rotundiformis*

Reza Malekzadeh-Viayeh[1] **and Choon Bok Song**[2]*

[1]Artemia and Aquatic Research Institute, Urmia University, Urmia 57153, Iran
[2]Department of Aquatic Biomedical Sciences, Jeju National University, Jeju 63243, Korea

Abstract

Although rotifers have been considered the best feeding option for several species of fishes in aquaculture, they are sometimes larger than appropriate for the early larval stage of some marine fishes. Thus, we aimed to determine whether size-based selection of the parents could affect the average body size of their progeny in two clonal populations of the rotifer *Brachionus rotundiformis*. From each of the clones, 20 individuals were bi-directionally selected toward both smaller and larger sizes and each individual-based selection was conducted for 10 consecutive generations. The results showed that although there were sometimes differences in mean body size between parents and their progeny, no directional trend was observed in all selected lines of both clones. We demonstrated that artificial selection in a rotifer stock cannot lead to an expected size range although they appear to exhibit a large degree of body size polymorphism.

Key words: *Brachionus rotundiformis*, Clones, Generations, Size-based selection

Introduction

Size-based selection of food in fish (Ghan and Sprules, 1993; Deudero and Morales-Nin, 2001; Shaw et al., 2003) and crustaceans (Harvey and Epifanio, 1997) has been demonstrated in both natural and cultural environments. Shirdhankar and Thomas (2003) reported that the food digestibility of larval or juvenile fish was determined to a great extent by the size of food particles in relation to the mouth size of the predator, and Shaw et al. (2003) indicated that the relationship between prey size and mouth size was the primary determinant of prey selection. With the worldwide development of aquaculture industries, a main challenge for aquaculturists has been to provide live food with suitable characteristics, including the proper size, during the larval stage of fish development. This has been a critical challenge with some groups of fish. For instance, a high mortality rate in grouper larvae was related to their sensitivity to prey size (Kohno et al., 1997).

Rotifers are used as live food in the larval rearing of more than 78 species of marine finfish and crustaceans. The demand for rotifers is still increasing (Fu et al., 1997). However, even small strains of rotifers have sometimes been found to be too large for smaller-mouthed larvae of aquaculture candidates such as grouper and rabbit fish (Rodriguez and Hirayama, 1997). In order to supply cultured rotifer as a cost-effective feed for fish larvae, it is important to provide the appropriate size of rotifers to the larvae (Hagiwara et al., 2001). A preliminary approach for this is the selection of rotifer strains with suitable size ranges (Kotani and Hagiwara, 2003). Several investigators have studied the effect of various environmental conditions on rotifer body size. Fukusho and Iwamoto (1981) examined the influence of various feeds on the size and shape of L-type rotifers and reported that body size increased when rotifers were fed with ω-yeast or a combination

---

Received 23 September 2014; **Revised** 8 July 2015
Accepted 10 July 2015

***Corresponding Author**
E-mail: cbsong@jejunu.ac.kr

of baker's yeast and formula feed for prawns. In contrast, Yufera (1982) concluded that rotifer body size was primarily determined by genetics and not greatly influenced by environmental conditions, e.g., dietary manipulation. Snell and Carrillo (1984) conducted a more intensive investigation of body size variability to determine the effects of salinity, temperature, and rotifer strain on lorica length. They concluded that while lorica size was largely determined by the genetics, small modifications of lorica size were possible by environmental manipulation, although an independent effect of either temperature or salinity was not statistically significant. Glavic et al. (2000) proposed that it was possible to produce rotifers with required lorica size by changing environmental conditions such as temperature and salinity.

Although rotifers have been used as live food in aquaculture for nearly 50 years, there is an apparent lack of information on its quantitative genetic aspects. Selective breeding, as used in farm animals and plants, is a time-consuming genetic manipulation technique that can play a major role in the development of lines having required traits. However, quantitative analyses such as monitoring the selection pressure and tracing the genetic pathways and heritability in rotifers seem to be limited by life history characteristics such as microscopic size, short life span, parthenogenetic reproduction, short generation length, and short time to maturity. Different types and strains of the genus *Brachionus* have body sizes ranging from 90 to 340 μm (Hagiwara et al., 2001). The Jeju Island (Korea) strain of *B. rotundiformis* has been introduced as a unique strain with a relatively small size range (Song et al., 1999). In our previous work on its demographic characteristics at different salinities (Malekzadeh-Vaiyeh and Song, 2004), we suggested that this strain could be a valuable candidate for both marine and fresh water aquaculture. According to size-based categories (Snell and Carrillo, 1984; Yoshimura et al., 1997; Sue et al., 1997; Rumengan et al., 1998; Hagiwara et al., 2001), this strain is an intermediate between S-type and ultra-minute type (SS-type) *Brachionus* rotifer. However, it may still have some limitations when being fed to certain small-mouthed marine fish such as red grouper.

The objective of this study was to evaluate the response of *Brachionus* rotifer to a bi-directional selection for smaller and larger body sizes through 10 generations with 10 different parental lines.

Materials and Methods

Source and maintenance of rotifers

Two colonial populations of a laboratory stock of the rotifer *B. rotundiformis*, isolated from Jeju Island in Korea, were used in this experiment. The rotifers were cultured in autoclaved seawater at a salinity of 30 psu, temperature of 28ºC, and a light intensity of 2,500 Lux (L:D=18:6). A daily amount of 5.2 × 10^5 cells/mL of marine *Chlorella* was fed to the rotifers.

Measurement of body size and estimating age-dependent size variation

From cultures of each clone containing 400 rotifers/mL, a volume of 20 mL was randomly sampled and fixed with 5% formalin to allow the measurement of mean lorica length of each population using a stereo-microscope at 100X magnification. The sizes of two experimental clones at different measuring times are shown in Table 1. To estimate size increments during early life, 30-40 rotifers were sampled and fixed with 5% formalin at 6-h intervals until the age of 72 h after hatching.

Selection procedures

According to the size range of the rotifers (Table 1), and in a preliminary attempt to select rotifers with small lorica length, plankton nets of different mesh sizes (80, 100 and 124 μm) were used to select for neonate rotifers of similar size and of the same generation. Several trials of batch filtration failed to collect rotifers at the expected size ranges. This was due to the characteristic inequality of the length and width of the rotifer

Table 1. Size values of two rotifer *Brachionus rotundiformis* clones chosen for size-based selection at different times

Measuring date	Clone No.	Lorica length (μm)			Sample size (n)
		Maximum	Minimum	Mean ± SD	
May 24	1	181	123	150.3 ± 12.0	45
	2	165	103	135.3 ± 12.3	63
Jul. 5	1	166	107	141.0 ± 14.5	53
	2	160	103	139.3 ± 13.4	49
Aug. 22	1	169	105	135.5 ± 13.6	91
	2	152	92	128.7 ± 13.1	119
Sep. 13	1	161	105	141.1 ± 11.3	65
	2	169	120	139.1 ± 9.7	63

body and its unpredictable orientation while passing through the mesh, the soft and flexible body texture, the presence of attached eggs, and difference in body size even at the same age. An additional disadvantage of this method is that, because of the short generation time to maturity, several generations of rotifer were present at any given time, and prevented the isolation of individuals of the same age, tracing of descendancy, and eventual calculation of genetic factors such as heritability.

An alternative method of individual selection was adopted to minimize the aforementioned disadvantages. Isolated individuals of an appropriate size range were used to examine selection effects in successive generations. A brief description of this method is as follows: When good cultural conditions were maintained, many egg-bearing female rotifers were observed under the microscope at 40X magnification. Parts of each colonial culture of rotifer were transferred to a Petri dish for microscopic viewing. With careful observation, the smallest and largest females carrying eggs were removed by pipetting them from the culture medium and transferring them individually to single wells of a 24-well tissue culture plate. Each well was supplied with 2 mL of autoclaved seawater and the culture conditions and feeding rate were the same as that used in the initial culture. For each selective direction (small and large body size) of each clone, 10 parental individuals were monitored for 10 generations. The first parental females (P) selected for both small and large size were checked for progeny (F1), and the second selection was conducted in the same manner with F1 individuals. After the F1 selection, the parental individuals were fixed in formalin and their sizes were measured. The remaining progeny of each parent were left to grow and make their own population. When a large enough number of rotifers were grown in each generation, an aliquot was fixed for size measurement.

Statistical analysis

The collected data were analyzed with the SPSS software (Ver 14.0 SPSS Inc.). Both analysis of variance (ANOVA) and Student's t-tests were performed to examine the bi-directional size-based selection effect on the rotifers. As the latter test turned out to be more robust than the former, most of the statistical analyses consisted of Student's t-tests. Simple correlation analyses were performed to understand the relationship between some morphological traits by calculating Pearson correlation coefficient (r). Intra- and inter-colonial size variations were compared using two statistical factors such as the coefficient of variance (CV) and ANOVA.

Results

The changes in body size of the two clones were examined until 72 h post-hatching, and the results are shown in Fig. 1. Growth curves indicate that the rotifers grew faster during the first 12 to 18 h after hatching than any other point in their life span, and that their body size increased even after adulthood. The first eggs are usually produced 12–18 h post hatching and hatched rotifers reach their maximum size after about three days. Changes in lorica length was correlated with body width in both clones (Fig. 2), so that body width increased linearly with length (r = 0.843 and 0.919 for clones 1 and 2, respectively). Conversely, lorica length of the parental rotifers was not likely correlated with egg length because the correlation coefficient for these two properties were r = 0.080 and 0.184 for clones 1 and 2, respectively (Fig. 3). In order to determine the effect of size-based selection, mean body sizes and standard deviations (SD) were estimated for 10 consecutive generations. Each generation selected for either small or large body size produced populations using two clones. The results are presented in Tables 2 to 5. The sizes of the smallest rotifers chosen as the first parental individuals were 121–135 μm for clone 1 and 89–134 μm for clone 2, while the sizes of the parents selected for large size ranged from 156–185 μm and 148–167 μm for clones 1 and 2, respectively. In clone 1, the mean lorica length of the small-sized parental population ranged from 130.8 ± 9.7 μm to 142.1 ± 14.2 μm, while those at the 10th generation were between 137.1 ± 12.2 μm and 148.1 ± 11.7 μm (Table 2). The large-sized parental population from clone 1 ranged from 131.4 ± 14.5 μm to 145.0 ± 8.0 μm, while those of the 10th generation were between 141.8 ± 9.8 μm and 145.8 ± 9.3 μm (Table 3). In clone 2, the mean lorica length

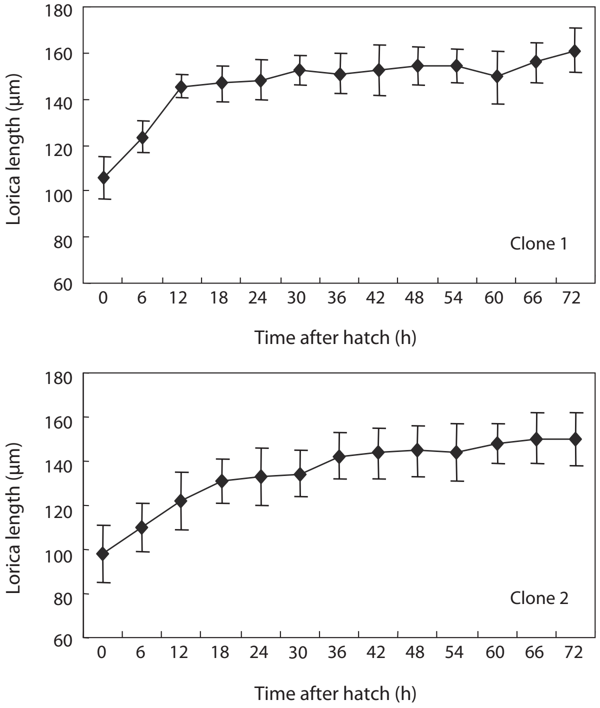

Fig. 1. Changes in body size of *Brachionus rotundiformis* over its life span.

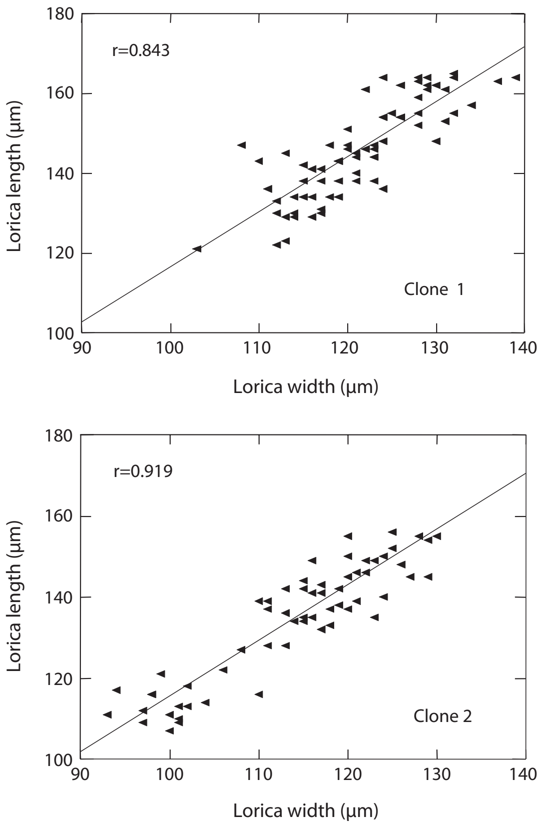

Fig. 2. Regression graphs showing the degree of correlation between lorica length and lorica width in two clones of *Brachionus rotundiformis*.

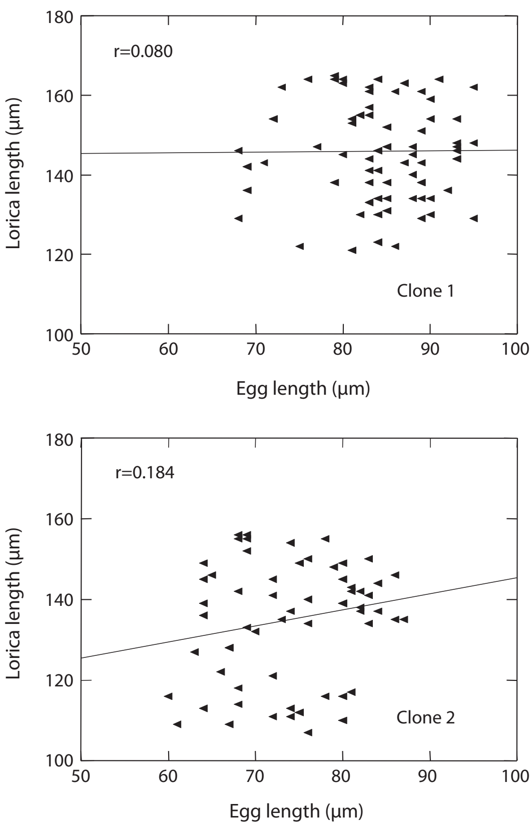

Fig. 3. Correlations between lorica length of adult rotifers and the length of their parthenogenetic eggs in the two clones of *Brachionus rotundiformis*.

of the small-sized parental populations ranged from 139.1 ± 11.9 µm to 149.0 ± 8.3 µm, while those at the 10th generation were between 140.6 ± 19.1 µm and 176.5 ± 13.0 µm (Table 4). However, the largest mean lorica length (176.5 ± 13.0 µm) of the 10th generation seemed to be unusual since it was observed only in some generations of the selected colonial line No. 7. The mean lorica length of the large-sized parental population of clone 2 ranged from 130.2 ± 12.1 µm to 148.2 ± 8.2 µm, while those at the 10th generation were between 134.2 ± 12.0 µm to 151.4 ± 9.9 µm (Table 5). In Tables 2 through 5, the mean body size of each generation having the same designation was not statistically different from that of the first parental population ($P > 0.05$), while the others differed significantly from their original parents ($P < 0.05$). However, this difference does not necessarily denote a pattern of increasing or decreasing size over successive generations. The size variation of selected individuals and their progeny for 10 generations is shown in Fig. 4. There was no certain pattern of size changes resulting from size-based selection. This implies that the mean body sizes of progenies were not influenced by the sizes of their selected parents. Conversely, when an ANOVA was conducted with the pooled data of all parental samples and those of their 10th generation, there were no significant differences observed ($P > 0.05$). The results of these statistical analyses reveal similarities as well as differences in rotifer size through the generations of each parental line. When colonial size variations were examined, the CVs of selected individuals of clone 1 for small and large size were 9.0% and 7.3%, and those for clone 2 were 8.9% and 9.2%, respectively. According to these values, body size along both directions of selection in the two clones was similar.

Discussion

The body size of rotifers is considered a critical characteristic and determines the adequacy of rotifers as food for young larvae (Rumengan et al., 1998). Rotifer body size was found to be primarily determined by genetics and the influence of environmental conditions was negligible (Yufera, 1982; Snell and Carrillo, 1984). Song et al. (1999) found that the lorica length of the rotifer *B. rotundiformis* was largely affected by colonial differences. In this study, we explored the effect of serial selection for sizes smaller and larger than that of the population mean on the size of the 10 successive generations. Comparisons between the average body size of each genera-

Table 2. The lorica length (mean ± standard deviation) of small-sized parental populations (clone 1) and their descendants

Generation	Colonial Line No.									
	1	2	3	4	5	6	7	8	9	10
P	141.2 ± 11.1 A N=36 (131)	130.8 ± 9.7 a N=41 (132)	141.6 ± 14.4 A N=20 (131)	138.9 ± 9.1 a N=41 (134)	134.0 ± 13.5 A N=45 (121)	139.7 ± 12.9 a N=36 (128)	137.5 ± 12.3 A N=42 (135)	137.2 ± 12.8 a N=42 (131)	142.1 ± 14.2 A N=42 (129)	138.5 ± 12.1 a N=53 (135)
F1	137.0 ± 12.7 A N=33 (133)	143.8 ± 9.7 b N=53 (131)	141.5 ± 9.4 A N=46 (135	138.5 ± 14.3 a N=37 (124)	145.6 ± 12.6 B N=45 (129)	135.9 ± 12.0 a N=41 (116)	137.2 ± 11.7 A N=41 (128)	138.3 ± 13.2 a N=44 (123)	141.7 ± 11.9 A N=35 (125)	140.2 ± 9.9 a N=41 (130)
F2	128.7 ± 11.8 B N=39 (129)	144.7 ± 10.1 b N=32 (131)	127.6 ± 10.3 B N=47 (118)	142.2 ± 12.8 a N=42 (131)	140.4 ± 12.8 B N=42 (136)	143.6 ± 11.1 a N=44 (134)	142.6 ± 11.1 A N=33 (132)	143.6 ± 11.4 b N=39 (120)	141.0 ± 12.2 A N=43 (128)	143.6 ± 11.8 b N=42 (134)
F3	139.8 ± 10.6 A N=47 (125)	134.2 ± 13.0 a N=34 (124)	136.9 ± 10.2 A N=25 (128)	142.2 ± 12.3 a N=40 (133)	139.2 ± 14.1 B N=39 (135)	146.4 ± 13.5 b N=40 (134)	143.2 ± 13.7 A N=37 (131)	136.9 ± 12.5 a N=40 (133)	136.4 ± 12.3 A N=39 (130)	144.0 ± 11.9 b N=47 (132)
F4	141.6 ± 11.4 A N=38 (131)	138.2 ± 13.0 b N=37 (131)	146.2 ± 9.7 A N=37 (133)	141.6 ± 11.0 a N=36 (124)	134.9 ± 14.6 A N=37 (128)	136.2 ± 13.3 a N=42 (123)	139.2 ± 12.4 A N=44 (135)	136.7 ± 12.2 a N=43 (121)	143.1 ± 12.0 A N=44 (129)	140.1 ± 11.7 a N=40 (128)
F5	140.1 ± 13.0 A N=41 (119)	140.0 ± 12.8 b N=38 (109)	139.6 ± 14.1 A N=34 (132)	149.6 ± 9.8 b N=23 (127)	135.7 ± 11.3 A N=55 (131)	136.8 ± 11.8 a N=38 (134)	143.2 ± 11.1 A N=33 (126)	141.2 ± 14.0 a N=46 (123)	135.0 ± 13.5 B N=47 (131)	141.8 ± 12.9 a N=44 (133)
F6	141.4 ± 13.5 A N=34 (130)	140.9 ± 13.4 b N=40 (122)	140.9 ± 11.8 A N=45 (118)	146.9 ± 7.3 b N=34 (132)	138.9 ± 13.1A N=41 (131)	140.0 ± 11.0 a N=43 (135)	148.0 ± 10.0 B N=48 (113)	138.5 ± 13.9 a N=46 (124)	137.4 ± 12.2 A N=42 (132)	141.9 ± 12.4 a N=41 (130)
F7	141.9 ± 12.4 A N=39 (117)	143 ± 10.9 b N=45 (134)	135.1 ± 13.0 A N=31 (131)	142.0 ± 13.0 a N=40 (123)	136.3 ± 12.6 A N=40 (121)	138.8 ± 13.0 a N=46 (131)	143.9 ± 11.1 B N=39 (129)	136.7 ± 14.6 a N=40 (134)	139.2 ± 10.9 A N=44 (115)	141.5 ± 11.0 a N=38 (127)
F8	140.6 ± 10.8 A N=24 (132)	148.4 ± 9.4 b N=45 (131)	140.1 ± 12.9 A N=41 (122)	143.3 ± 9.4 b N=37 (115)	145.2 ± 13.5 B N=58 (119)	140.9 ± 13.4 a N=39 (132)	136.6 ± 11.2 A N=40 (125)	142.4 ± 12.5 a N=38 (121)	141.2 ± 12.4 A N=46 (134)	138.1 ± 12.4 a N=40 (129)
F9	153.2 ± 9.6 B N=45 (118)	148.5 ± 9.9 b N=46 (127)	147.3 ± 8.5 A N=34 (124)	143.7 ± 10.9 b N=31 (132)	142.6 ± 12.0 B N=48 (115)	138.7 ± 13.0 a N=43 (136)	132.1 ± 12.2 B N=44 (131)	141.1 ± 12.1 a N=42 (122)	141.7 ± 10.8 A N=45 (124)	137.7 ± 13.0 a N=37 (134)
F10	139.7 ± 11.8 A N=28 (135)	145.0 ± 10.9 b N=40 (132)	148.1 ± 11.7 A N=39 (121)	144.6 ± 14.5 b N=35 (110)	139.3 ± 11.5 B N=44 (123)	141.9 ± 14.1 a N=34 (128)	142.3 ± 12.3 A N=41 (131)	139.9 ± 9.4 a N=43 (133)	143.6 ± 11.8 A N=47 (122)	137.1 ± 12.2 a N=40 (119)

The value in parentheses reveals the size of a selected individual that was proliferated at each generation. Student's t-tests were performed to illustrate whether the body size of descendants at each generation was different from that of parental rotifers, and the results are indicated with letters (A, B, a, b). In each column, the data having the same letter as that of their parents are not statistically different at a 5% significance level.

Table 3. The lorica length (mean ± standard deviation) of large-sized parental populations (clone 1) and their descendants

Generation	Colonial Line No.									
	1	2	3	4	5	6	7	8	9	10
P	134.3 ± 8.9 A N=22 (181)	142.3 ± 6.0 a N=35 (171)	143.1 ± 9.0 A N=28 (185)	144.2 ± 8.2 a N=43 (176)	142.5 ± 9.7 A N=43 (159)	145.0 ± 8.0 a N=25 (172)	131.4 ± 14.5 A N=20 (164)	143.0 ± 8.6 a N=43 (163)	141.5 ± 11.5 A N=43 (156)	141.4 ± 10.7 a N=34 (162)
F1	139.0 ± 9.5 A N=31 (171)	144.2 ± 9.5 a N=29 (166)	142.8 ± 9.0 A N=28 (160)	139.7 ± 8.2 b N=25 (178)	142.9 ± 7.4 A N=25 (168)	141.0 ± 10.5 a N=48 (162)	144.9 ± 8.6 B N=30 (159)	139.8 ± 10.9 a N=42 (158)	141.1 ± 12.5 A N=44 (163)	143.4 ± 8.7 a N=39 (167)
F2	144.6 ± 9.0 B N=33 (180)	146.4 ± 8.0 b N=30 (170)	140.3 ± 7.3 A N=29 (173)	142.9 ± 11.3 a N=30 (162)	148.0 ± 9.4 B N=24 (166)	144.0 ± 8.7 a N=34 (162)	145.3 ± 9.1 B N=33 (158)	142.2 ± 11.4 a N=49 (163)	143.0 ± 10.4 A N=40 (162)	138.5 ± 10.7 a N=37 (168)
F3	138.8 ± 7.9A N=33 (161)	138.8 ± 10.6 a N=29 (165)	142.1 ± 8.0 A N=38 (171)	142.0 ± 10.5 a N=33 (169)	138.3 ± 11.7 A N=23 (155)	144.0 ± 10.2 a N=33 (163)	144.0 ± 12.0 B N=38 (165)	143.1 ± 9.7 a N=43 (172)	143.0 ± 11.2 A N=45 (180)	135.5 ± 10.4 b N=36 (168)
F4	134.5 ± 8.1A N=35 (160)	148.0 ± 9.7 b N=40 (164)	146.6 ± 10.7 A N=32 (162)	142.5 ± 13.9 a N=33 (157)	144.1 ± 9.1 A N=37 (150)	145.9 ± 9.7 a N=34 (171)	145.7 ± 9.9 B N=47 (167)	143.4 ± 11.2 a N=46 (161)	143.5 ± 8.6 A N=38 (161)	144.0 ± 10.0 a N=40 (172)
F5	146.3 ± 11.2 B N=35 (178)	142.3 ± 9.0a N=39 (171)	144.6 ± 9.7 A N=35 (168)	140.8 ± 10.8 a N=36 (165)	142.7 ± 8.8 A N=36 (159)	143.3 ± 12.3 a N=31 (158)	140.2 ± 10.1 B N=37 (163)	140.0 ± 10.7 a N=40 (172)	141.4 ± 11.2 A N=41 (169)	142.6 ± 9.6 a N=38 (166)
F6	143.7 ± 8.4 B N=38 (160)	143.5 ± 11.8 a N=35 (158)	141.8 ± 9.7 A N=28 (162)	142.8 ± 11.5 a N=40 (169)	145.3 ± 11.0 A N=39 (171)	143.8 ± 9.7 a N=34 (170)	141.9 ± 9.3 B N=39 (168)	145.8 ± 10.6 a N=43 (157)	140.9 ± 9.2 A N=43 (163)	142.5 ± 10.4 a N=38 (161)
F7	141.5 ± 9.9 B N=40 (162)	148.6 ± 11.6 b N=39 (166)	147.4 ± 8.8 A N=30 (175)	144.0 ± 12.6 a N=38 (181)	148.7 ± 10.9 B N=48 (177)	141.8 ± 11.2 a N=39 (162)	142.4 ± 9.9 B N=45 (160)	140.6 ± 11.3 a N=44 (153)	143.2 ± 10.6 A N=35 (159)	143.7 ± 10.1 a N=33 (162)
F8	142.9 ± 12.6 B N=35 (158)	144.0 ± 8.2 a N=30 (156)	141.6 ± 10.6 A N=35 (163)	149.1 ± 10.2 b N=39 (161)	148.6 ± 9.6 B N=34 (171)	146.8 ± 9.7 a N=42 (173)	143.6 ± 9.6 B N=30 (168)	141.6 ± 11.3 a N=46 (159)	144.1 ± 12.0 A N=38 (156)	142.9 ± 8.9 a N=36 (164)
F9	146.0 ± 8.5 B N=39 (161)	142.4 ± 9.9 a N=33 (166)	147.7 ± 10.3 B N=32 (159)	143.9 ± 10.2 a N=41 (155)	144.5 ± 9.2 A N=32 (163)	141.4 ± 11.0 a N=38 (162)	141.8 ± 8.3 B N=40 (168)	140.7 ± 9.0 a N=49 (157)	141.6 ± 11.0 A N=33 (171)	144.3 ± 10.3 a N=37 (173)
F10	142.0 ± 11.1B N=36 (159)	143.8 ± 11.1 a N=35 (159)	141.8 ± 9.8 A N=41 (162)	142.3 ± 16.6 a N=35 (168)	145.8 ± 9.3 A N=40 (169)	143.8 ± 10.1 a N=41 (160)	143.8 ± 8.1 B N=43 (158)	143.2 ± 8.8 a N=53 (169)	142.9 ± 10.6 A N=35 (161)	142.9 ± 11.7 a N=43 (175)

Table 4. The lorica length (mean ± standard deviation) of small-sized parental populations (clone 2) and their descendants

Generation	Colonial Line No.									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
P	139.1 ± 11.9 A N=32 (134)	146.8 ± 9.3 a N=31 (125)	145.5 ± 9.3 A N=38 (89)	140.5 ± 11.4 a N=40 (117)	144.9 ± 10.3 A N=36 (122)	141.2 ± 10.0 a N=46 (118)	149.0 ± 8.3 A N=49 (124)	140.3 ± 8.4 a N=35 (112)	142.6 ± 9.7 A N=35 (132)	145.5 ± 11.9 a N=38 (125)
F1	150.7 ± 11.7 B N=38 (131)	141.9 ± 10.3 b N=38 (124)	149.6 ± 7.8 B N=36 (118)	147.4 ± 8.6 b N=41 (134)	147.6 ± 8.6 A N=39 (123)	148.6 ± 8.8 b N=46 (132)	148.1 ± 9.1A N=46 (130)	141.2 ± 9.1a N=43 (115)	144.0 ± 10.3 A N=39 (120)	145.2 ± 12.4 a N=41 (121)
F2	143.8 ± 10.1 A N=43 (120)	144.8 ± 10.7 a N=40 (121)	152.5 ± 7.8 B N=35 (120)	141.0 ± 10.1 a N=44 (119)	143.7 ± 10.6 A N=45 (131)	146.3 ± 10.9 b N=36 (113)	151.3 ± 7.7 A N=38 (124)	138.5 ± 9.2 a N=39 (121)	139.9 ± 12.8 A N=33 (123)	141.6 ± 12.0 a N=38 (134)
F3	155.0 ± 10.9 B N=33 (115)	151.1 ± 8.5 a N=33 (118)	143.8 ± 11.0 A N=42 (128)	144.3 ± 10.6 a N=45 (131)	144.0 ± 12.4 A N=38 (132)	147.5 ± 10.0 b N=46 (126)	156.0 ± 12.4 B N=33 (122)	146.5 ± 9.2 b N=31 (132)	140.5 ± 10.7 A N=31 (131)	141.5 ± 13.2 a N=40 (125)
F4	151.0 ± 8.7 B N=21 (131)	141.5 ± 11.0 b N=35 (133)	142.5 ± 11.3 A N=40 (120)	143.3 ± 13.3 a N=40 (117)	141.1 ± 7.9 A N=33 (112)	141.4 ± 11.5 a N=33 (107)	150.3 ± 8.4 A N=34 (124)	147.0 ± 9.3 b N=41 (131)	144.0 ± 9.5 A N=48 (123)	143.8 ± 7.5 a N=37 (134)
F5	141.8 ± 11.3 A N=41 (114)	144.0 ± 10.0 a N=46 (131)	144.6 ± 11.6 A N=39 (122)	142.9 ± 11.8 a N=39 (123)	141.5 ± 12.3 A N=38 (134)	140.6 ± 13.1 a N=39 (119)	164.6 ± 17.8 B N=37 (127)	140.9 ± 9.2 a N=37 (121)	145.6 ± 9.7 A N=39 (129)	142.7 ± 14.4 a N=38 (132)
F6	145.2 ± 12.5 B N=38 (126)	137.4 ± 13.5 b N=38 (124)	140.6 ± 11.5 B N=38 (113)	144.3 ± 10.4 a N=37 (121)	143.0 ± 10.2 A N=41 (130)	141.8 ± 9.6 a N=42 (128)	181.2 ± 10.2 B N=36 (134)	145.8 ± 11.5 b N=32 (121)	143.4 ± 14.9 A N=45 (117)	143.0 ± 11.3 a N=40 (125)
F7	144.9 ± 8.7 B N=36 (122)	142.1 ± 13.3 a N=39 (126)	147.5 ± 8.9 A N=34 (123)	144.3 ± 7.6 a N=46 (131)	141.2 ± 13.6 A N=43 (127)	144.7 ± 11.3 a N=35 (112)	163.7 ± 13.8 B N=31 (123)	144.3 ± 11.8 a N=33 (121)	142.4 ± 11.2 A N=41 (133)	144.9 ± 9.6 a N=46 (131)
F8	143.2 ± 11.9 A N=33 (116)	144.3 ± 8.3 a N=47 (125)	140.5 ± 11.0 B N=38 (131)	141.6 ± 11.4 a N=42 (133)	145.9 ± 8.2 A N=37 (126)	146.4 ± 9.1 b N=36 (121)	180.4 ± 13.1 B N=38 (133)	144.6 ± 12.2 a N=39 (129)	143.8 ± 13.1 A N=33 (118)	141.4 ± 11.4 a N=41 (121)
F9	141.7 ± 10.8 A N=48 (135)	138.2 ± 11.2 b N=33 (124)	148.7 ± 11.5 A N=45 (111)	144.8 ± 10.5 a N=46 (123)	142.4 ± 11.6 A N=39 (126)	147.7 ± 9.2 b N=36 (132)	170.1 ± 15.0 B N=35 (129)	148.8 ± 11.2 b N=52 (125)	144.7 ± 11.0 A N=41 (132)	144.5 ± 10.1 a N=35 (118)
F10	145.5 ± 10.3 B N=48 (112)	142.1 ± 12.2 a N=36 (124)	140.6 ± 19.1 B N=29 (117)	141.2 ± 11.4 a N=36 (129)	143.8 ± 10.5 A N=40 (122)	142.2 ± 13.1 a N=38 (133)	176.5 ± 13.0 B N=31 (113)	149.5 ± 10.0 b N=42 (131)	145.6 ± 11.1 A N=43 (127)	143.6 ± 11.4 a N=38 (123)

Table 5. The lorica length (mean ± standard deviation) of large-sized parental populations (clone 2) and their descendants

Generation	Colonial Line No.									
	1	2	3	4	5	6	7	8	9	10
P	135.3 ± 12.3 A N=37 (148)	137.6 ± 9.5 a N=29 (152)	148.1 ± 9.0 A N=48 (148)	146.3 ± 7.1 a N=38 (167)	131.4 ± 11.9 A N=40 (159)	148.2 ± 8.2 a N=40 (160)	130.2 ± 12.1 A N=41 (164)	135.4 ± 9.7 a N=39 (161)	135.3 ± 13.4 A N=30 (157)	135.4 ± 9.7 a N=35 (164)
F1	134.9 ± 11.0 A N=30 (149)	135.7 ± 10.2 a N=33 (161)	141.6 ± 11.6 B N=32 (155)	147.9 ± 10.6 a N=41 (152)	131.1 ± 9.9 A N=41 (159)	145.2 ± 11.9 a N=45 (148)	137.6 ± 10.8 B N=37 (166)	132.5 ± 12.0 a N=47 (163)	133.9 ± 12.6 A N=39 (158)	136.0 ± 13.2 a N=39 (164)
F2	130.2 ± 12.0 A N=25 (154)	140.9 ± 11.7 a N=43 (147)	135.7 ± 13.8 B N=35 (161)	143.9 ± 11.3 a N=37 (158)	133.3 ± 11.0 A N=36 (152)	144.9 ± 12.8 a N=30 (152)	135.1 ± 14.6 A N=33 (164)	134.0 ± 9.0 a N=20 (160)	139.0 ± 10.4 A N=35 (156)	135.4 ± 10.5 a N=38 (149)
F3	130.5 ± 8.8 A N=33 (151)	131.8 ± 10.5 b N=33 (163)	135.5 ± 13.6 B N=40 (160)	140.4 ± 7.5 b N=44 (147)	140.2 ± 10.8 B N=38 (152)	148.1 ± 13.8 a N=24 (162)	132.9 ± 11.6 A N=38 (157)	138.3 ± 11.4 a N=40 (156)	135.7 ± 12.8 A N=37 (158)	137.5 ± 11.2 a N=40 (161)
F4	135.5 ± 10.8 A N=28 (163)	135.2 ± 12.6 a N=38 (161)	135.1 ± 14.0 B N=41 (154)	136.6 ± 15.8 b N=40 (155)	135.6 ± 9.4 A N=36 (160)	132.3 ± 11.0 b N=33 (152)	143.7 ± 10.6 B N=40 (149)	136.6 ± 11.8 a N=40 (151)	135.4 ± 14.3 A N=36 (152)	134.0 ± 10.5 a N=44 (151)
F5	135.2 ± 12.4 A N=34 (153)	138.7 ± 10.5 a N=35 (156)	138.6 ± 12.2 B N=39 (160)	135.6 ± 13.4 b N=36 (162)	140.6 ± 12.6 B N=34 (153)	137.8 ± 9.6 b N=40 (152)	136.4 ± 14.8 B N=45 (162)	139.3 ± 13.8 a N=42 (167)	136.5 ± 11.9 A N=41 (159)	134.1 ± 9.8 a N=41 (158)
F6	138.0 ± 11.4 A N=34 (157)	136.5 ± 11.2 a N=40 (166)	137.3 ± 11.6 B N=35 (161)	141.2 ± 14.2 a N=37 (164)	139.2 ± 12.8 B N=39 (162)	134.0 ± 13.0 b N=40 (150)	139.1 ± 14.0 B N=33 (157)	136.8 ± 12.7 a N=42 (147)	140.4 ± 13.5 A N=43 (163)	140.0 ± 11.4 a N=37 (163)
F7	139.4 ± 12.5 A N=35 (161)	139.7 ± 11.2 a N=35 (163)	137.3 ± 11.9 B N=38 (153)	135.6 ± 11.4 b N=34 (159)	141.1 ± 11.8 B N=38 (155)	135.9 ± 11.0 b N=36 (165)	136.9 ± 9.2 B N=20 (160)	141.6 ± 11.1 b N=41 (152)	139.7 ± 12.3 A N=38 (157)	137.6 ± 9.9 a N=36 (154)
F8	139.9 ± 16.1 A N=34 (151)	141.8 ± 10.7 a N=38 (157)	134.9 ± 13.8 B N=40 (148)	132.1 ± 12.3 b N=40 (147)	133.5 ± 11.9 A N=36 (163)	138.3 ± 13.3 b N=38 (160)	131.9 ± 11.9 A N=33 (159)	138.3 ± 9.4 a N=39 (155)	134.5 ± 12.4 A N=39 (161)	137.2 ± 12.3 a N=41 (162)
F9	137.5 ± 14.1 A N=32 (149)	151.0 ± 9.9 b N=46 (158)	145.6 ± 13.0 A N=45 (155)	144.6 ± 12.0 a N=48 (162)	132.6 ± 16.8 A N=32 (165)	140.3 ± 12.0 b N=43 (160)	139.7 ± 13.8 B N=37 (158)	140.0 ± 12.8 a N=33 (151)	140.0 ± 9.4 A N=42 (157)	137.2 ± 11.4 a N=44 (162)
F10	134.2 ± 12.0 A N=39 (164)	148.1 ± 12.2 b N=42 (161)	141.4 ± 14.2 B N=36 (163)	134.2 ± 12.7 b N=40 (156)	136.1 ± 16.0A N=23 (159)	136.7 ± 14.1 b N=36 (162)	141.2 ± 13.6 B N=33 (161)	151.4 ± 9.9 b N=36 (155)	146.8 ± 10.9 B N=45 (162)	135.0 ± 11.3 a N=40 (151)

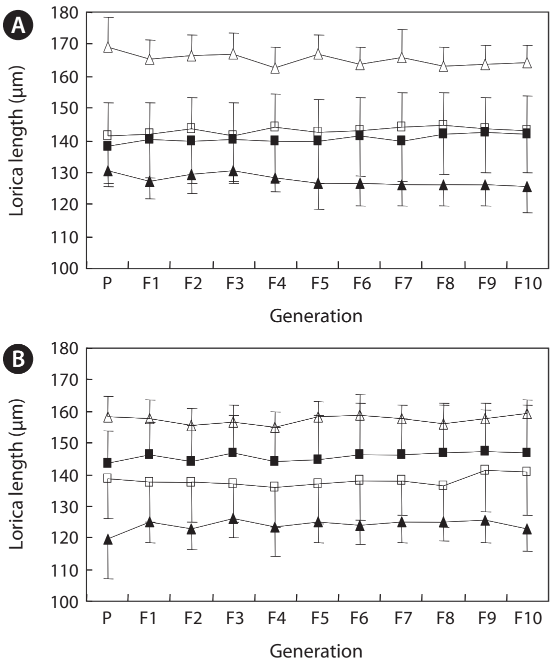

Fig. 4. Comparative graphs showing size variations of small-sized and large-sized selected parental individuals of the clones 1 (A) and 2 (B), and those of their descendants for 10 consecutive generations. Solid and blank triangles denote small-sized and large-sized selected parental individuals of each generation, respectively. Solid and blank squares represent the descendants originated from small-sized and large-sized selections, respectively.

tion and that of the first parental population showed both differences and similarities (Tables 2, 3, 4, and 5). However, these differences were not in a regular ascending or descending pattern. Thus, a selected rotifer with a smaller size than that of its concomitant population might generate a population of a next generation population with similar, smaller, or larger average body size. Furthermore, as is shown in Table 1, the average size of each clone measured on different sampling occasions was not always the same. An ANOVA determined that this difference was significant between some of the size values of the same clone measured at different times ($P < 0.05$). This difference may be the result of several causes including the age composition of the population, food quality, and/or other environmental factors. Geng et al. (2003) reported that food quality (food component and concentration) affected the body size and egg volume of rotifers. Integrating previous studies and our results, it is likely that the differences in size observed in each line of generations from both clones are not the result of selective pressure but due instead to intra-clonal competition, differences in micro-ecosystem, and the physiological properties of individuals. Fig. 4 displays a comparative and illustrative scheme of size ranges in selected individuals and their resulting populations. Although there was a comparable difference between small-sized and large-sized selected individuals, their selection did not affect the average body size of successive populations. Furthermore, with the exception of some low variants, the mean lorica length of all the examined populations generated from both selective directions was within a distinct size range over several generations, independent of any artificial selection of their progenitors. The larger average body size of rotifer populations originating from small individuals was comparable to that of populations originating from large parents in clone 2. Fig. 4B demonstrates an additional example that confirms the inefficiency of selection to obtain rotifers with desired size ranges. When the body sizes of two colonial populations were compared to each other, clone 1 was larger than clone 2 at the time of hatching and at the end of 10th selection as well. This indicates that the size of lorica length is certainly influenced by clonal differences, which was reported by Song et al. (1999). Interestingly, we observed a considerable increase in the average size of some generations of the selected colonial line No. 7 (Table 2). Although it can be considered an exceptional observation, it should be taken into account in any size-related assay. This phenomena may be the result of the dominance of larger individuals that correspond to the outgrowing individuals in a population.

In conclusion, the results of this experiment show that: (1) the rotifers of the same age were a variety of sizes in a clonal population, (2) there is no correlation between rotifer body size and the size of its parthenogenetic eggs (see Fig. 3), and (3) no directional trend via size-based selection was observed in all of the selected lines of both clones, although there were sometimes differences in mean body size between parents and their progeny. Overall, we demonstrated that artificial selection cannot lead to an expected size range in a rotifer stock with an identical genetic background due to parthenogenetic reproduction, and that such population exhibit a large polymorphism in body size.

References

Deudero S and Morales-Nin B. 2001. Prey selectivity in planktivorous juvenile fishes associated with floating objects in the western Mediterranean. Aquacul Res 32, 481-490.

Fu Y, Hada A, Yamashita T, Yoshida Y and Hino A. 1997. Development of a continuous culture system for stable mass production of the marine rotifer *Brachionus*. Hydrobiologia 358, 145-151.

Fukusho K and Iwamoto H. 1981. Polymorphosis in size of rotifer, *Brachionus plicatilis*, cultured with various feeds. Bull. Nat. Res. Inst. Aquaculture 2, 1-10.

Geng H, Xi Y and Hu H. 2003. Effects of food component and concentration on population growth, body size, and egg size of freshwater rotifer *Brachionus rubens*. Ying Yong Sheng Tai Xue Bao 14, 753-756le in Chinese).

Ghan D and Sprules WG. 1993. Diet, prey selection, and growth of larval and juvenile burbot *Lota lota* (L.). J Fish Biol 42, 47-64.

Glavic N, Kozul V, Skaramuca B, Glamuzina B, Lucic D and Tutman P. 2000. The synergetic effect of temperature and salinity on rotifer

Brachionus plicatilis (O. F. Muller) population growth and lorica size in mass rearing. Acta Adriatica 41, 1.

Hagiwara A, Gallardo WG, Assavaaree M, Kotani T and de Araujo AB. 2001. Live food production in Japan: recent progress and future aspects. Aquaculture 200, 111-127.

Harvey EA and Epifanio CE. 1997. Prey selection by larvae of the common mud crab *Panopeus herbstii* Milne-Edwards. J Exp Mar Bio Eco 217, 79-91.

Kohno H, Ordonio-Aguilar RS, Ohno A and Taki Y. 1997. Why is grouper larval rearing difficult?: an approach from the development of the feeding apparatus in early stage larvae of the grouper, *Epinephelus coioides*. Ichthyol Res 44, 267-274.

Kotani T and Hagiwara A. 2003. Fertilization between rotifer *Brachionus plicatilis* strains at different temperature. Fish Sci 69, 1078-1080.

Malekzadeh-Vaiyeh R and Song CB. 2004. Effect of salinity on demographic traits of the rotifer (*Brachionus rotundiformis*). J Fisheries Sci Tech 7, 39-45.

McLaren JA. 1976. Inheritance of demographic and production parameters in the marine copepod *Eurytemora herdmani*. Biol Bull 151, 200-213.

Rodriguez EM and Hirayama K. 1997. Semi-mass culture of the dinoflagellate *Gymnodinium splendens* as a live food source for the initial feeding of marine finfish larvae. Hydrobiol 358, 231-235.

Rumengan IFM, Warouw V and Hagiwara A. 1998. Morphology and resting egg production potential of the tropical ultra-minute rotifer *Brachionus rotundiformis* (Mando strain) fed different algae. Bull Fac Fish Nagasaki Univ 79, 31-36.

Shaw GW, Pankhurst PM and Purser GJ. 2003. Prey selection by greenback flounder *Rhombosolea tapirina* (Gunther) larvae. Aquaculture 228, 249-265.

Shirdhankar MM and Thomas PC. 2003. Response to bidirectional selection for naupliar length in *Artemia franciscana*. Aquacul Res 34, 535-541.

Snell TW and Carrillo K. 1984. Body size variation among strains of the rotifer *Brachionus plicatilis*. Aquaculture 37, 359-367.

Song, CB, Kim YH, Lee J and Rho S. 1999. Size variation and cyclomorphosis of the rotifer, *Brachionus rotundiformis*, isolated from Cheju Island, Korea. J Aquacul 12, 267-274.

Sue HM, Su MS and Liao IC. 1997. Preliminary results of providing various combinations of live foods to grouper (*Epinephelus coioides*) larvae. Hydrobiol 358, 301-304.

Tave D. 1986. Genetics for fish hatchery managers. The AVI publishing company Inc. U.S.A., 297 pp.

Yoshimura K, Usuki K, Yoshimatsu T, Kitajima C and Hagiwara A. 1997. Recent development of a high density mass culture system for the rotifer *Brachionus rotundiformis* Tschugunoff. Hydrobiol 358, 139-144.

Yufera M. 1982. Morphometric characterization of a small-sized strain of *Brachionus plicatilis* in culture. Aquaculture 27, 55-61.