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Characterization of a Unique New Strain Named the NFRDI N° 1 Rotifer Strain, a Brackish *Brachionus* Rotifer Collected from a South Korea Coastal Lagoon

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Abstract

A new and a unique *Brachionus* rotifer was found in Hwajinpo coastal lagoon in Gangwon Province, South Korea. This *Brachionus* certainly originated from the wild rather than from aquaculture stations because Hwajinpo coastal lagoon has been under rigorous control as a military protected area and therefore could not have been contaminated by aquaculture stations. The new strain was identified as *Brachionus rotundiformis* based upon its morphological characteristics. The parthenogenetic female of this new rotifer strain typically shows characters similar to those of *B. rotundiformis*, such as the pot shape of the body, rounded dorsal plate compared with flattened ventral plate, elliptical mictic egg, four frontal spines, six pointed occipital spines, non-nodal foot, two toes, trophi typical of the *Brachionus* genus with five uncus plates resembling comb teeth, one wide symmetrical manubrium and ramus, and no stiffened spine as is seen in freshwater *Brachionus* rotifers. Moreover, its lorica was rather small in size compared with other common rotifer strains that serve as live-food organisms (Guam, Thai, and Bali strains). This new and unique Korean brackish rotifer, a *B. rotundiformis* strain, was therefore named the National Fisheries Research and Development Institute (NFRDI) N° 1 rotifer strain.

Key words: *Brachionus rotundiformis*, NFRDI N°1 rotifer strain, Lagoon, Hwajinpo

Introduction

The phylum rotifer is distributed worldwide, and more than 2,000 species have been described (Dumont, 1983) from freshwater, brackish water, and seawater areas. Although most have originated from inland freshwater ecosystems, several rotifers have been reported from marine and/or brackish water zones. For instance, about 20 of the 32 species comprising the genus *Synchaeta* have been described as marine species. Only one order (Seisonidea, containing a single genus) and about 50 species of rotifers are exclusively marine. Additionally, two species are encountered among the plankton of the open Atlantic Ocean (Wallace and Snell, 1991).

Dumont (1983) reported that the *Brachionus* genus comprises 46 species. Two of the species within the *Brachionus* genus are considered marine and/or brackish, namely *B. plicatilis* and *B. rotundiformis*. Generally, the brackish *B. plicatilis* is distributed in temperate zones and cold latitudes, whereas most known *Brachionus rotundiformis* in brackish waters have been observed in sub-tropical and tropical areas. Generally, brackish *Brachionus* rotifers have been found in mangrove environments, which do not exist in the cold and variable temperate regions of the Korean peninsula.

Two common live-food-organism rotifers, *B. plicatilis* and *B. rotundifirmis*, are of great interest to aquaculturists because of their economic importance to the marine finfish aquaculture industry. These two *Brachionus* species have been split into various strains according to their isolation or sampling loca-

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tions. Such isolated and monospecific cultured *Brachionus* rotifer strains are used as live food organisms for early larval rearing in marine fish. In South Korea to date, only foreign rotifer strains such as the Thai and Bali strains of *Brachionus rotundiformis* have been used as live food organisms in marine fish-farming centers because local wild brackish *Brachionus* rotifers have not been found in South Korea. Therefore, this study aimed to investigate the presence and characterization of wild brackish *Brachionus* rotifers in the Hwajinpo coastal lagoon, Gangwon Province, South Korea. Morphological description, environmental abiotic factors, and biological minimum size were examined.

Materials and Methods

Sample collection area

The area from which rotifers were collected is located in the Hwajinpo coastal lagoon, Gangwon Province, South Korea. The Hwajinpo coastal lagoon is located at the northern tip of the east coast of South Korea. The lagoon is strictly under military control and is therefore a military protected area that cannot be contaminated by *Brachionus* rotifers that have originated elsewhere. Conversely, the Korean coastal sea is always contaminated by *Brachionus* rotifers that have escaped from marine fish larval rearing stations. The lagoon is separated from the sea by a narrow sand dune and is divided in two sub-basins by a shallow sill. Each of these sub-basins was used as a sample collection area. Samples were collected during April, July, and November of 2002 from 40 sampling locations.

Isolation and stock cultures for mono specific conditions

The samples taken from Hwajinpo lagoon were shipped to our laboratory. Then, isolated rotifers were acclimated from low salinity (1-19 psu) to natural seawater salinity (32 psu). Isolation and cultures were established based on the rotifer mono-species culture method (Jung et al., 1997) and the marine rotifer axenic culture method (Jung et al., 1998; Jung and Hagiwara, 2001). Stock cultures of isolated Hwajinpo rotifers were grown in filtered natural seawater at 32-psu salinity and a water temperature of 25°C and were fed on centrifuged microalga, *Nannochloropsis oculata* (about 700,000 cells/mL) and kept in darkness. Furthermore, stock cultures of isolated Hwajinpo rotifers were also grown at 20°C with modified Erd-Schreiber medium (Hagiwara et al., 1994) under white fluorescent light ranging between 2,000 and 2,500 lux on a 24:00-h L:D cycle.

Morphological description and identification

After harvesting from an indoor culture tank, the isolated Hwajinpo rotifer strain was fixed in 5% buffered formalin for taxonomical identification. A specimen was dissected by light microscopes (Olympus CH-2 and SZH, Japan), and the morphological characteristics of this rotifer strain were described. Identification of the new isolated rotifer was performed following to Sudzuki's (1964, 1996), and Wallace and Snell's (1991) methods.

Measurement of abiotic environmental factors in Hwajinpo lagoon

The water quality of Hwajinpo lagoon was investigated by measuring the most important abiotic environmental factors such as water temperature, salinity, dissolved oxygen, and light intensity using a YSI environmental monitoring system and a light meter (Digital instruments LM series). The measurements of these abiotic factors were conducted on three occasions (in April, July, and November of 2002).

Measurement of biological minimum size

The biological minimum size of isolated Hwajinpo rotifers was measured and compared with that of three other *Brachionus* rotifer strains (Guam, Thai, and Bali strains) collected from NFRDI (National Fisheries Research & Development Institute) stock cultures. Measurements were conducted following the methods described by Fu et al. (1991) and Hagiwara et al. (1995). Therefore, 150 female individuals in mictic egg-carrying mode were sampled for biological minimum size measurements using a profile projector (Nikon V-10, Japan).

Statistical analysis

A *t*-test was used to determine the morphological differences among rotifer strains using the Excel program. Differences were considered statistically significant at $P < 0.05$.

Results

Isolation and morphological description of the Korean Hwajinpo lagoon rotifer strain

A new brackish rotifer strain was isolated from the uncontaminated Korean Hwajinpo lagoon and classified as *Brachionus rotundiformis* based on observations of its morphological characteristics. We have named this strain the National Fisheries Research and Development Institute (NFRDI) N° 1 rotifer strain (Fig. 1). Morphological observation of the NFRDI N° 1 rotifer showed lorica very small in size and round in shape. Additionally, lorica observations revealed all pointed anterior spines. Furthermore, parthenogenetic rotifer females exhibited characters similar to those typically seen in *B. rotundi-*

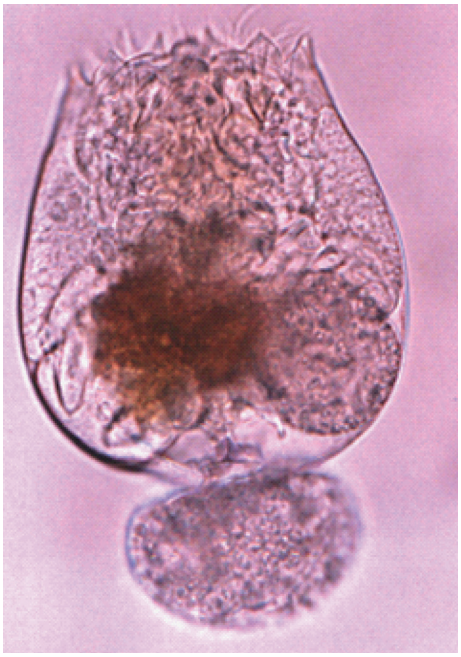

Fig. 1. The Korean blackish rotifer, *Brachionus rotundiformis* National Fisheries Research and Development Institute (NFRDI) No. 1 rotifer strain.

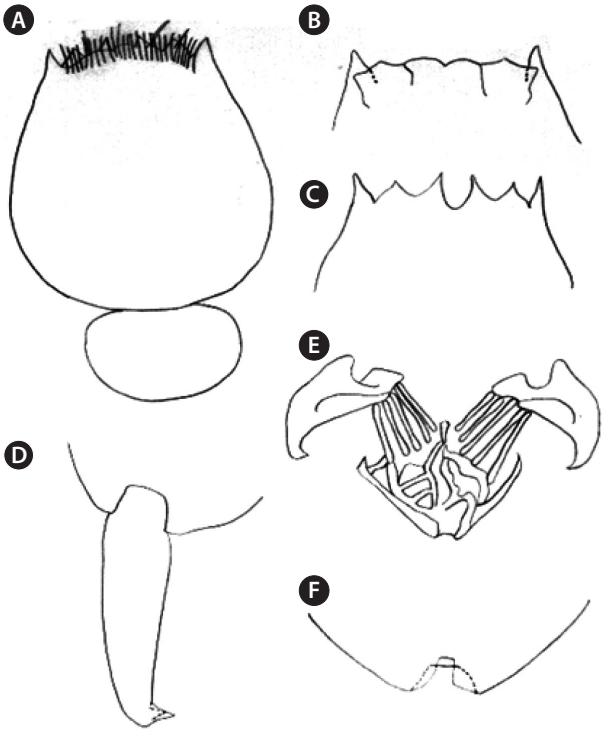

Fig. 2. Morphological features. (A) Pot shape of body and carried elliptical mictic egg, (B) rounded dorsal plate and 4 frontal spines, (C) pointed 6 occipital spines, (D) not node of foot and two toes, (E) trophi, (F) has no stiffens spine of freshwater *Brachionus* rotifer.

formis. These characters were pot shape of the body, rounded dorsal plate compared with flattened ventral plate, elliptical mictic egg, four frontal spines, six pointed occipital spines, non-nodal foot, two toes, typical *Brachionus* genus trophi with five uncus blades resembling comb teeth, and a wide manubrium and ramus which were both symmetrical. Moreover, none of the stiffened spines of freshwater *Brachionus* rotifers were observed (Fig. 2). Likewise, no male rotifers or resting eggs were observed during the study.

Measurement of Hwajinpo lagoon environmental factors

The temperatures observed during the three sampling months (April, July, and November of 2002) ranged from 7.3 to 28.0°C. The highest water temperature (28.0°C) was observed in July (during summer), with lower temperatures in April (12.3-18.2°C) and November (7.3-9.4°C) (Table 1). The new NFRDI N° 1 rotifer strain was only collected in July (summer) and was not found at low water temperature (April: 12.3-18.2°C and November: 7.3-9.4°C). The annual water temperature of Hwajinpo lagoon reached a minimum in winter of 3.6°C and a maximum in summer of 28.0°C (Table 1).

The salinity of Hwajinpo lagoon differed with measurement site and period. Salinity ranged from 1 to 32 psu, with lower salinity at sites more distant from the sea (St. 1 in Table 1) and higher salinity at sites near the sea (St. 2 in Table 1). Additionally, high salinity was found in April, whereas intermediate and low salinities were observed in July and November, respectively. The NFRDI N° 1 rotifer was found in July where salinity ranged from 1 to 19 psu (Table 1).

The variation in the amplitude of dissolved oxygen concentrations and light intensities was very great. Dissolved oxygen concentrations ranged from 3.24 to 10.1 mg/L, and light intensities ranged from 7,300 to over 99,000 lux (Table 1).

Measurement of biological minimum size

The biological minimum size of the NFRDI N° 1 rotifer strain was very small compared with those of the rotifer strains common to Guam, Thai, and Bali. The average lorica length of the NFRDI N° 1 strain was 152.23 ± 25.58 μm (mean ± SD, n = 150), which was smaller than that of the Guam (165.56 ± 24.43 μm, mean ± SD, n = 150), Thai (164.89 ± 26.02 μm, mean ± SD, n = 150), and Bali (168.94 ± 23.02 μm, mean ± SD, n = 150) strains (Fig. 3). The average lorica width of the NFRDI N° 1 strain was 152.23 ± 25.58 μm (mean ± SD, n = 150), smaller than those of the Guam (165.56 ± 24.43 μm, mean ± SD, n = 150), Thai (164.89 ± 26.02 μm, mean ± SD, n = 150), and Bali (168.94 ± 23.02 μm, mean ± SD, n = 150) strains (Fig. 4). However, the difference in biological minimum size between the NFRDI N° 1 rotifer strain and the Guam, Thai, and Bali strains was statistically not significant.

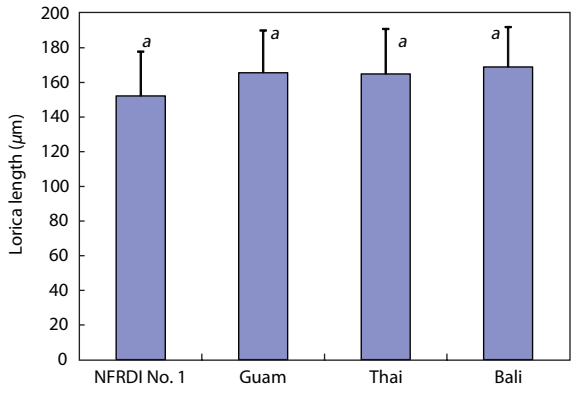

Fig. 3. Average biological minimum size of lorica length of four *Brachionus rotundiformis* rotifer strains, National Fisheries Research and Development Institute (NFRDI) No. 1 (*n*=150), Guam (*n*=150), Thai (*n*=150) and Bali (*n*=150). The each columns and vertical bars represents mean±SD of three replicates.

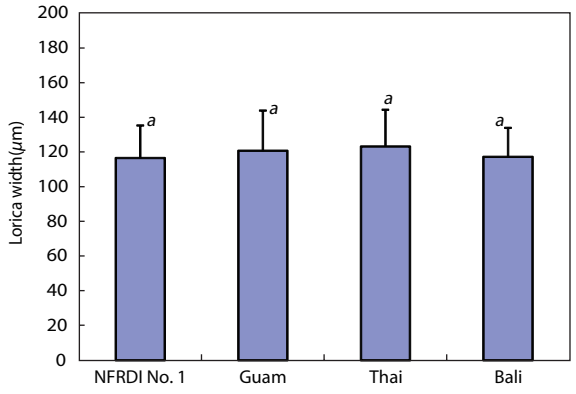

Fig. 4. Average biological minimum size of lorica width of four *Brachionus rotundiformis* rotifer strains, National Fisheries Research and Development Institute (NFRDI) No. 1 (*n*=150), Guam (*n*=150), Thai (*n*=150) and Bali (*n*=150). The each columns and vertical bars represents mean±SD of three replicates.

Discussion

The new Korean brackish *B. rotundiformis* rotifer, named the NFDRI N° 1 strain, is here recorded for the first time. The unique NFDRI N° 1 rotifer strain discovered in Hwajinpo coastal lagoon could be identified as being of wild origin because the lagoon is a military protected area.

B. rotundiformis and *B. plicatilis* require different water temperatures for optimum growth conditions. The optimum water temperature for the culture of *B. plicatilis* is about 17°C, whereas the optimum for *B. rotundiformis* is about 34°C (Rumengan and Hirayama, 1990). Similar results have been reported by different authors (Fukusho and Iwamoto, 1980; Fukosho and Okauchi, 1984; Hirayama, 1985). The optimum water temperature for population growth is higher for *B. rotundiformis* than for *B. plicatilis* (18-25°C). The temperatures below which the growth of *B. rotundiformis* and *B. plicatilis* are limited are 20 and 10°C, respectively (Hirayama, 1985). *B. rotundiformis* showed daily growth rates of 1.3% at 15°C, 60% at 20°C, 190% at 30°C, and 250% at 34°C; *B. plicatilis* growth rates were 9% at 11°C, 21% at 15°C, 50% at 20°C, 170% at 25°C, and about 120% at over 25°C (Oogami, 1977). This is in accordance with the results of this study, as the unique *B. rotundiformis* NFDRI N° 1 strain was only observed abundantly in the Korean Hwajinpo lagoon in July during the summer while water temperatures were high (22-28°C) and would have been suitable for normal *B. rotundiformis* growth. The low temperature that constitutes a limiting factor for *B. rotundiformis* growth (Hirayama, 1985) may explain why this rotifer was not observed in April or November, which were low-temperature periods. The presence of the NFDRI N° 1 strain only in July, associated with high temperatures, indicates a narrow temperature tolerance.

Salinity ranged from 1 to 32 psu. Lower salinity was observed at sites distant from the seacoast (St. 1 on Table 1), and higher salinity at sites close to the seacoast (St. 2 on Table 1). Additionally, high salinity was found in April, whereas intermediate and low salinities was observed in July and Novem-

Table 1. Investigation of water quality in Hwajinpo lagoon on the each different sampling month

Season	Cites	Water temperature (°C)	Salinity (psu)	Dissolved oxygen (mg/L)	Light intensity (10^3lux)	Presence or absence of NFRDI St.
April	St. 1	12.3-16.7	17-21	3.67-6.78	22.0-86.9	Absence
	St. 2	13.0-18.2	21-32	6.13-9.37	27.1-32.8	Absence
July	St. 1	22.0-28.0	1-17	5.52-8.70	13.4-99.0	Presence
	St. 2	23.0-28.0	17-19	3.24-7.07	25.7-89.0	Presence
November	St. 1	8.2-9.4	6-22	7.83-10.1	8.6-87.3	Absence
	St. 2	7.3-7.8	11-13	8.49-8.63	7.3-62.9	Absence

NFRDI, National Fisheries Research and Development Institute.

ber, respectively, indicating that the occurrence of the NFDRI N° 1 strain was not associated with salinity.

Most *Brachionus* rotifers require oxygen concentrations above 1.0 mg/L, although some can tolerate anaerobic or near-anaerobic conditions for short periods (Wallace and Snell, 1991). A decrease in the growth rates of *B. plicatilis* was observed at 0.9 mg/L (Yamasaki et al., 1987). The present study showed that the dissolved oxygen concentration was above 3.24 mg/L, with a maximum of 8.70 mg/L during summer. These dissolved oxygen concentrations are suitable for the normal growth of the NFDRI N° 1 rotifer strain, and its presence or absence was not associated with this factor.

Most of the monogonont rotifer life cycle occurs in the amictic phase, but under particular conditions, sexual reproduction occurs within rotifer populations when initiated by a specific environmental stimulus (*e.g.*, the occurrence of mictic females). *Brachionus* can reproduce either sexually (mictic reproduction) or, more commonly, asexually (amictic reproduction). A female rotifer reproducing asexually simply produces clones that are genetically similar to herself. Mictic females produce males if they are unfertilized and produce resting eggs if fertilized (Wallace and Snell, 1991). In the present study, no male rotifers or resting eggs were found. The absence of mictic females due to the absence of a specific environmental stimulus may explain the lack of male rotifers and resting eggs. However, the possibility of finding dormant-stage NFRDI No. 1 rotifers is very high, as the environment of Hwajinpo lagoon was not suitable for the survival and reproduction of the NFDRI N° 1 rotifer (*Brachionus rotundiformis*) strain except during the summer season.

All live-food rotifers used at fish larval rearing stations originated from eel culture tanks or from the mangrove zones of subtropical and tropical regions and therefore cannot be collected from the wild and used as valuable live-food rotifers. However, the present study documented the existence at Hwajinpo lagoon, Gangwon Province, South Korea of a new rotifer named the *B. rotundiformis* NRFDI N° 1 rotifer strain. This new strain may be valuable in the future as a live-food organism for the marine aquaculture industry in South Korea.

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