

Survival Strategies of the Rotifer *Brachionus rotundiformis* for Coexisting with the Copepod *Apocyclops borneoensis* in Laboratory Culture

Min-min Jung*

Future Aquaculture Research Center, National Fisheries Research and Development Institute, Jeju 690-192, Korea

Abstract

Interspecific relationship between a euryhaline rotifer *Brachionus rotundiformis* and a cyclopoid copepod *Apocyclops borneoensis* was investigated in the laboratory culture. In a mixed culture of *B. rotundiformis* and *A. borneoensis*, population growth of *B. rotundiformis* was suppressed from day 10, while growth in a monoculture population continuously increased throughout the experimental period. However, the population growth of *A. borneoensis* in the mixed culture did not markedly differ from that in a monoculture population. Suppression of *B. rotundiformis* growth coincided with a decrease in the numbers of both non-egg-bearing and egg-bearing females, and increasing resting egg formation. Growth of *A. borneoensis* was not affected by the presence of the rotifer. However, relative growth index of ovisac bearing females in the mixed culture was 1.62 times higher than that in the monoculture. Presence of the copepod did not greatly reduce the food available to the rotifer population. The rotifer *B. rotundiformis* responded in a unique way, to stresses such as physical damage (filtering by *A. borneoensis*) with the production of many resting eggs to increase its chances of survival.

Key words: *Apocyclops borneoensis*, *Brachionus rotundiformis*, Cyst, Interspecific relation, Resting egg, Rotifer

Introduction

Rotifers are some of the most important zooplankton, and are the focus of much attention from aquaculture scientists. Rotifers have been used as a primary live food for the seedling production of many economically important marine animals, because rotifers can be easily cultured at high densities. Stable mass culture of rotifers is needed for successful fish larval rearing. A variety of mass culture methods should be developed to facilitate successful aquatic animal culture through stable live food organism production. However, the instability or sudden crashes of rotifer mass cultures remain problematic. The causes of such culture failures are not fully understood, although it is predicted that one major cause is contamination by other organisms such as protozoa (Takayama, 1979; Reguera, 1984; Chen et al., 1997), copepods (Fukusho et al.,

1976) and bacteria (Yu et al., 1990).

Coexisting populations of different taxonomic groups depend on the organisms that occur together in space and time, and interact with each other through the processes of mutualism, parasitism, predation and competition (Begon et al., 1990). The relationships between *Brachionus rotundiformis* and other zooplankton species have been well examined (Gilbert and Stemberger, 1985; Gilbert, 1988; Hagiwara et al., 1995a; Jung et al., 1997), and competitive interference has been reported between rotifers and cladocera in fresh water. Under marine culture conditions, there is considerable evidence for interspecific relationships between a rotifer, *B. rotundiformis*, and two species of copepods, *Tigriopus japonicus* and *Acartia* sp. (Jung et al., 1997).

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***Corresponding Author**

E-mail: jminmin@nfrdi.go.kr

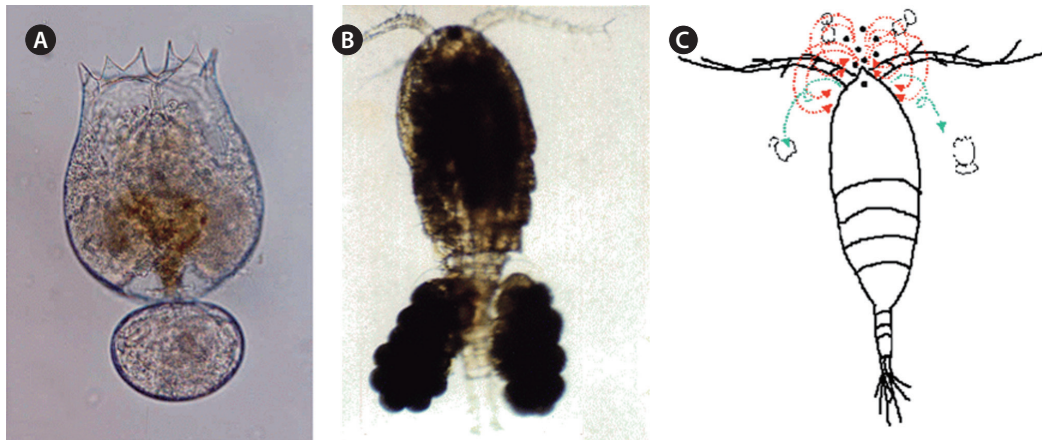


Fig. 1. Experimental two organisms rotifer, *Brachionus rotundiformis* (A) and copepod, *Apocyclops borneoensis* (B) and interspecific relationship between *B. rotundiformis* and *A. borneoensis* (C).

Copepods and rotifers coexist in estuaries as well as in brackish water fish culture ponds in North Sulawesi, Indonesia. Of several copepod species collected, the only one that could be adapted to laboratory culture was *Apocyclops borneoensis*. It is unknown which species (*B. rotundiformis* or *A. borneoensis*) dominates earlier, or which is more susceptible to the rigorous conditions of the ponds. Therefore, it is of interest to examine the nature of the relationship between these two species. Such information is useful both for assessing their ecological significance in a brackish water ecosystem, as well as for establishing techniques for mono- and mixed-species cultures for aquaculture purposes as live food organisms.

Here, I observed, the survival strategies involved in the interspecific relationship between *B. rotundiformis* and *A. borneoensis* under laboratory experimental conditions. I focused on the interspecific interactions of the rotifer and the copepod from a microcosm viewpoint of aquaculture ecology.

Materials and Methods

Copepods and rotifers were collected from a milkfish pond in Bitung, 30 km east of Manado, North Sulawesi, Indonesia. The pond is separated from the adjacent sea by mangroves, but is connected through an inlet during high tide. Throughout the year, salinity of the pond varies from 12 to 25 psu and temperature ranges from 29°C to 35°C.

The specimens were kept in darkness during a three-day acclimation culture to laboratory conditions before isolation. Various copepods were included in the sample, but only a cyclopoid copepod survived. The species was identified as *Apocyclops borneoensis* by Dr. H-S. Kim (Research Institute for Basic Science, Cheju National University of Korea). The rotifers were morphologically analyzed by Fu et al. (1991), and evidently belonged to an ultra minute strain of *Brachionus rotundiformis* (Hagiwara et al., 1995a). We refer this rotifer *B.*

rotundiformis as a Bitung strain.

Experimental design and conditions used were the same as those described by Hagiwara et al. (1995b). Salinity, temperature, and culture volume were 22 psu, 25°C and 40 mL, respectively. The organisms were cultured in total darkness. The initial number of animals in mixed cultures was 20 females of the Bitung rotifer strain of *B. rotundiformis* and 3 ovisac-bearing females of *A. borneoensis*. In the monocultures, the numbers of rotifers and copepods were the same as in mixed cultures, but were cultured separately. Mono- and mixed-species cultures were conducted with three replicates for 16 days. Stereo-microscopic observation was carried out on fresh culture medium including *Tetraselmis suecica* (7×10^5 cells/mL) every two days. Total number of test animals was counted and remaining algal food density was also counted by a haemocytometer (Kayagaki Irika Kogyo Co. Ltd., Tokyo, Japan). The algal food *T. suecica*, was grown in modified Erd-Schreiber medium (Hagiwara et al., 1994) and centrifuged. Density of food added was 7×10^5 cells/mL, and was readjusted every two days after observation.

For the observation and calculation of the rotifer mixis rate (%), all individual non-egg bearing females, amictic females, unfertilized and fertilized mictic females, males and resting eggs were counted, and the mixis rate was calculated (Hagiwara et al., 1988). The numbers of all individual nauplii, copepodites and egg-bearing females of the copepod *A. borneoensis* were recorded. Relative population growths between monocultures of each species and mixed culture conditions were compared by student's *t*-test.

Results

Predator-prey interactions were not observed during this experiment between the experimental rotifer *B. rotundiformis* and copepod *A. borneoensis* (Fig. 1A and 1B). However,

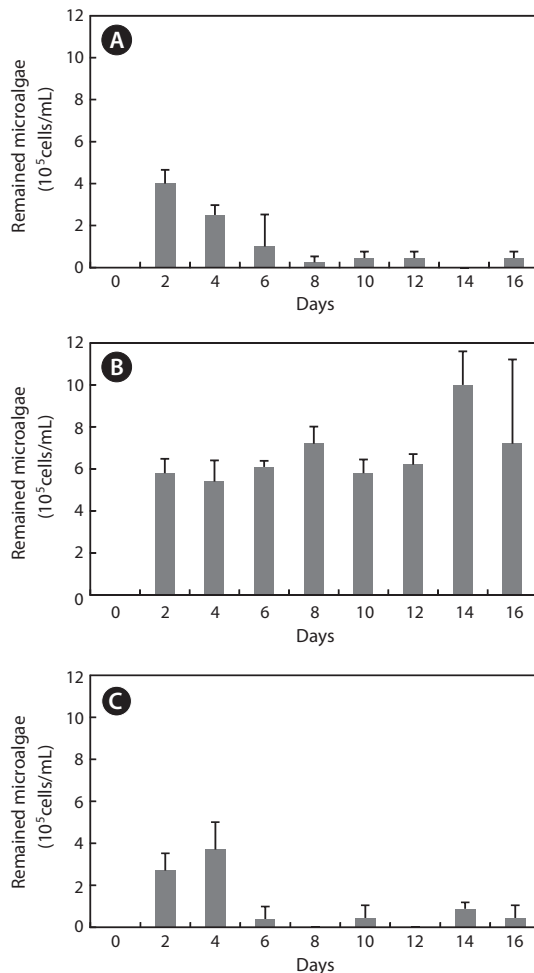


Fig. 2. Densities of remaining food on the every two days in rotifer *Brachionus rotundiformis* mono culture (A), copepod *Apocyclops borneoensis* mono culture (B) and two species (rotifer and copepod) mixed culture (C).

distinct correlation interactions between the two experimental organisms were observed (Fig. 1C).

The remnant food (*T. suecica*) volumes under rotifer culture conditions significantly decreased with the lapse of experimental time compared to those in copepod monoculture condition ($P < 0.05$) (Fig. 2A and 2C), and almost all microalgae (*T. suecica*) cells were consumed. A remnant food volume of approximately 7×10^5 cells/mL was maintained under copepod monoculture conditions (Fig. 2B).

Population growth of rotifers in the mixed culture was significantly decreased ($P < 0.05$) (Fig. 3). Growth of rotifers was suppressed by the presence of copepods in the culture container. However, the population growth of rotifers in the monospecific culture continually increased (Fig. 3).

In both experimental conditions, inductions of mixis rates (%) were observed. However, the mixis rate (%) of rotifers was notably changed by presence or absence of copepods. The difference between rotifer monoculture and mixed culture

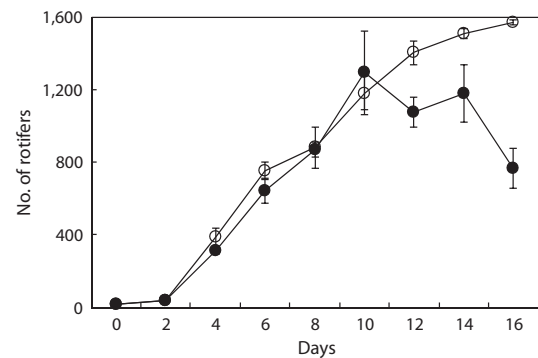


Fig. 3. Population growth of rotifer *Brachionus rotundiformis* (Bitung strain) in the mono culture (white circles) and mixed culture with copepod *Apocyclops borneoensis* (black circles).

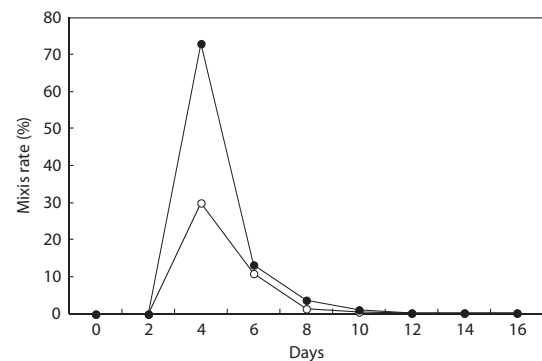


Fig. 4. Comparison of mixis rates in the rotifer *Brachionus rotundiformis* (Bitung strain) mono culture (white circles) and mixed culture with copepod *Apocyclops borneoensis* (black circles).

Table 1. Comparison of production of rotifer *Brachionus rotundiformis* resting eggs in the rotifer mono culture and mixed culture with copepod *Apocyclops borneoensis*

	No. of resting eggs			
	Tank No. 1	Tank No. 2	Tank No. 3	Mean \pm SD
Mono	704	764	1,093	853 \pm 171
Mixed	1,568	1,050	1,365	1,327 \pm 213

with copepods was particularly notable on the 4th day (Fig. 4). The presence of copepods influenced the mixis rate (%) of co-existing rotifers (Fig. 4), as well as the production numbers of rotifer resting eggs. On day 4 of the observations, the highest values of mixis rates (%) was observed in both experimental conditions (Fig. 4), and the production of rotifer resting eggs was observed from the 6th day. However, there were differences in the numbers of rotifer resting eggs produced. More resting eggs were formed in the copepod mixed culture than in the rotifer monoculture ($P < 0.05$) (Fig. 5). A comparison of the production of rotifer resting eggs between rotifer monoculture and mixed culture with copepods is shown in Table 1.

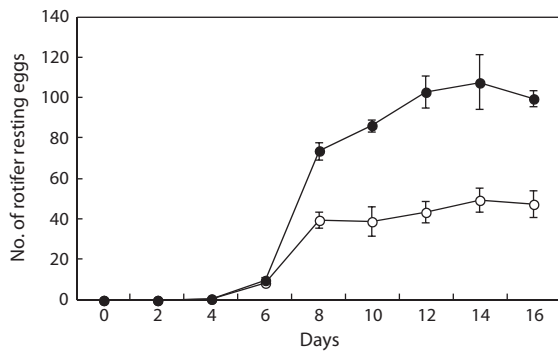


Fig. 5. Formed numbers of rotifer resting eggs in the rotifer *Brachionus rotundiformis* (Bitung strain) mono culture (white circles) and mixed culture with copepod *Apocyclops borneoensis* (black circles).

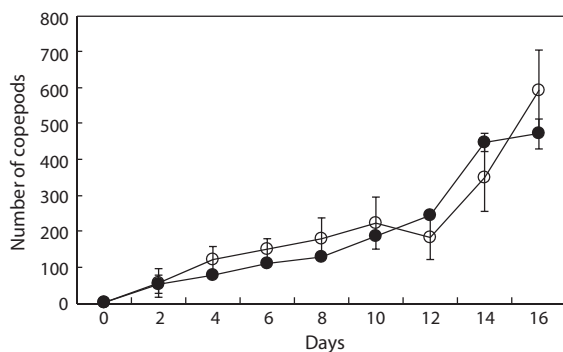


Fig. 6. Population growth of copepod *Apocyclops borneoensis* in the mono culture (white circles) and mixed culture with rotifer *Brachionus rotundiformis* (Bitung strain, black circles).

The production of rotifer resting eggs was 1.56 times higher in the mixed culture than in the rotifer monoculture ($P < 0.05$) (Table 1), with $1,327 \pm 213$ (mean \pm SD) resting eggs in mixed cultures, and 853 ± 171 (mean \pm SD) in monocultures.

Fig. 6 shows that copepod population growth did not differ between copepod monoculture and mixed culture with rotifers during the 16 day experimental period. This trend was not changed with the separation of the developmental stages (nauplius, copepodid and egg-carrying females) of the copepods (Fig. 7).

Discussion

Rotifer mass culture tanks comprise intricate relationships among the constituents of the small ecosystem. These small ecosystems are mainly composed of rotifers and contaminant micro-organisms, such as bacteria, microalgae, protozoa and copepods. Many of these contaminant micro-organisms affect rotifer population growth through interactions such as exploitative competition for food, commensalism, ammensalism and physical interference competition (Hagiwara et al., 1995b; Jung et al., 1997).

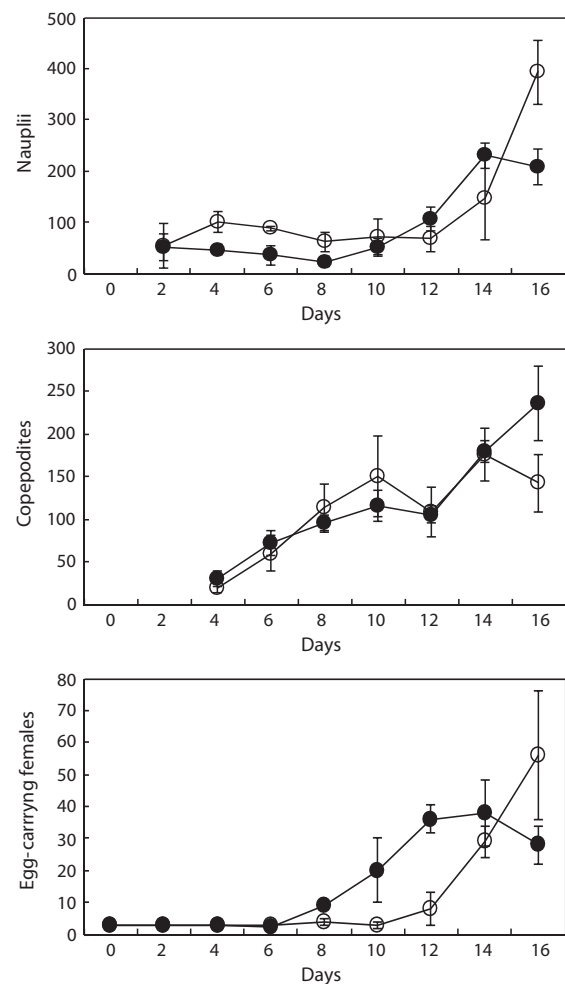


Fig. 7. Comparison of the numbers of each developmental stages, nauplius, copepodid and egg carrying female of copepod *Apocyclops borneoensis* in the mono culture (blank circles) and mixed culture with rotifer *Brachionus rotundiformis* (solid circles).

No predator-prey relationship was observed between the two zooplankton species during the experimental period. However, interference between the two species was observed. It was noted that the copepod used a filter feeding mechanism in which it filtered the slowly swimming rotifers together with algae (food), but immediately rejected the rotifers, which started to swim. Rejected rotifers appeared to be unharmed, but may have suffered some physical damage or injury. The physical damage by copepods may have brought about the decrease in rotifer population growth.

Microalgae, including *Nannochloropsis*, *Dunaliella*, *Tetraselmis* and enriched freshwater *Chlorella* are commonly used foods in the mass culture of rotifers (Witt et al., 1981). Of these, *Tetraselmis* is well known among aquaculturists as a good food for rotifer and copepod cultures. In this study, in both mono and mixed cultures of rotifers, the density of *T. suecica* continuously decreased. However, in the monoculture of the copepod *A. borneoensis*, algal density did not decrease,

but maintained a constant level after small amounts were used as food by the copepods, and the added food volume appeared sufficient to maintain algal density. This indicated that *A. borneoensis* may not eat much and/or did not actively feed on *T. suecica* in monoculture. This suggests that there was not competition for food, and that sufficient amounts of food (*T. suecica*) were supplied for the copepod *A. borneoensis* under these experimental conditions.

The presence of the rotifer did not influence the population growth of the copepods, likely because of the large size differential between *B. rotundiformis* (c. 0.2 mm) and *A. borneoensis* (c. 0.8 mm). However, the copepod suppressed rotifer population growth after 10 days in mixed culture. The population growth of *B. rotundiformis* in mixed culture was associated with a reduction in the numbers of both non-egg bearing females and egg-bearing females.

An analysis of the population structures revealed that the rotifer population growth decreased due to a 30% reduction in females without eggs and amictic females. In contrast, mictic females in mixed cultures were more abundant than those in monocultures. Thus, the presence of copepods stimulated the sexual reproduction of the rotifer (mysis rate or resting egg formation), which in turn caused decreased rotifer population growth. It is of interest to conduct further research to clarify this mechanism.

The dormant stage of the rotifer (resting egg or cyst) is very resistant to harsh environmental conditions and may be dispersed over wide areas by wind, water or migrating animals. Sometimes, scientists induce the production of mysis reproduction (cyst) as an easy method of storing and transporting for marine fish larvae culture or aquaculture study. The hatching of rotifer resting eggs is caused by stimulation from light, temperature, salinity and some chemicals (reviewed by Hagiwara, 1996), but there is no known method to artificially produce rotifer cysts.

The high mysis rate (70% or more) in this study could be related to the algal species used as food. *Tetraselmis tetrahele* as a food source stimulated a high mysis rate (81%), which led to the successful mass production of resting eggs (101×10^6 Ind.) of the Hawaiian strain of *B. rotundiformis* (Hagiwara and Lee, 1991). Another report indicated that bacteria populations in mass cultures regulated the sexual reproduction of rotifers (Hagiwara et al., 1994). Two marine copepod species were successfully cultured when consuming bacteria alone (Rieper, 1978). This mechanism has also been discussed with the effects of bacteria on interspecific relationships between *B. rotundiformis* and the two copepod species, *Tigriopus japonicus* and *Acartia* (Hagiwara et al., 1995b; Jung et al., 1997). The present study also highlights the significance of bacterial flora on the population growth of *A. borneoensis*.

Population growth of *A. borneoensis* in mixed culture did not differ compared with that in monoculture. However, the number of nauplii decreased to 17% and the number of egg bearing females was 1.62 times higher in mixed culture. The

reason for this was not clear, but may be associated with the feeding mechanisms of copepods such as *Apocyclops*, which may utilize feces more efficiently than they do bacteria in the water column. Various copepod species used as live food organisms utilized bacteria as their food. The common marine copepod species *Tigriopus japonicus* utilized bacteria (Jung et al., 1998) and rotifer feces (Jung et al., 2000) as food in the early nauplius developmental stages. The individuals in each developmental stage may also have different feeding habitats, due to changes in their mandible structures (Itoh, 1970). The experimental copepod *Apocyclops* appears to utilize egg sac conservation for the preservation of the species. This may explain the low survival rate of *Apocyclops* nauplii that was observed in mixed cultures.

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