FAS Fisheries and Aquatic Sciences

Original Article

Fish Aquat Sci 15(3), 209-213, 2012

Biological Application of Two Protozoan Species, *Euplotes* sp. and *Vorticella* sp., for the Stable Culture of the Rotifer *Brachionus rotundiformis* in Laboratory Experiments of Inter- and Tripartite-Specific Relations

Min-min Jung*

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

Abstract

Members of the ciliate group of protozoans are often observed in mass cultures of rotifers. In particular, *Euplotes* and *Vorticella* are common contaminating species. In this study, I examined the effect of the ciliates *Euplotes* sp. and *Vorticella* sp. on the growth of the rotifer *Brachionus rotundiformis* by conducting inter-specific and tripartite-specific mixed-culture experiments. The growth of rotifers was suppressed in co-existence with *Euplotes* sp. compared with monocultures of rotifers. However, *Vorticella* sp. promoted rotifer growth. Moreover, *Vorticella* sp. improved the growth of rotifers suppressed by *Euplotes* sp. contaminants. In 5-L semi-mass cultures of rotifers, growth of the contaminating protozoan *Euplotes* sp. was heavily suppressed by *Vorticella* sp. The stable maintenance of the rotifer culture ecosystem can be achieved by manipulating the types of contaminating protozoan species.

Key words: *Brachionus rotundiformis*, *Euplotes*, *Vorticella*, Microcosm, Interspecific, Tripartite specific relations

Introduction

Rotifers are a small type of zooplankton; however, in the world of aquaculture, rotifers are a very large object of attention by scientists and aquaculturists. The rotifer has been used for the seedling production of many economically important marine organisms, as it can be cultured at high densities. However, a very important unsolved problem remains: these cultures sometimes "crash" as characterized by a rapid and sudden population decrease in mass rotifer culture tanks. The cause of this phenomenon is not well known.

Marine rotifers of the genus *Brachionus* are widely used as live food for rearing early-stage larval marine fish. In mass cultures of rotifers, contamination by various organisms is commonly observed, and many of these contaminants can affect rotifer growth, e.g., copepods, protozoans, and anostracans (Hagiwara et al., 1995; Jung et al., 1997). Maeda and Hino (1991) examined the roles of contaminant protozoan species in rotifer mass-culture tanks. Reguera (1984) reported that a harmful ciliate in isolated rotifer mass-culture tanks reduced the population growth of the rotifer *B. plicatilis* and the alga *Dunaliella* (rotifer food) through food competition. Jung et al. (1997) reported that other protozoan species, with the exception of *Vorticella* sp., also reduced rotifer population growth.

The objectives of the present study were to artificially control the density of the ciliates *Euplotes* sp. and *Vorticella* sp. via competition in rotifer microcosms and to apply this bio-control method to the stable production of the rotifer *Brachionus rotundiformis* by adding *Vorticella* sp. to cultures. By applying tripartite-specific relationships and incorporating *Euplotes* sp. and *Vorticella* sp. into *B. rotundiformis* cultures, I aimed to develop more stable rotifer culturing techniques.

Open Access http://dx.doi.org/10.5657/FAS.2012.0209

pISSN: 2234-1749 eISSN: 2234-1757

Received 21 March 2012; **Revised** 11 July 2012; **Accepted** 22 August 2012

***Corresponding Author**

E-mail: jminmin@korea.kr

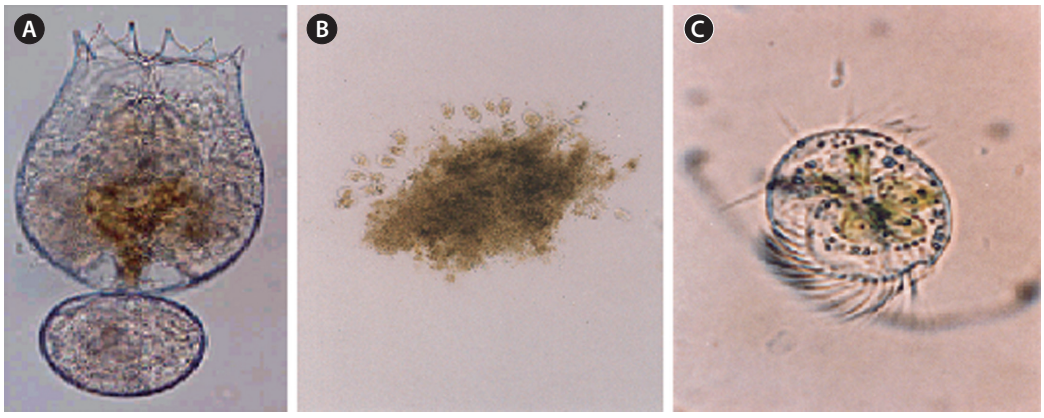

Fig. 1. The experimental organisms of rotifer, *Brachionus rotundiformis* (A; 190 ± 20 μm) and two ciliates, *Vorticella* sp. (B; 70 ± 13 μm) and *Euplotes* sp. (C; 82 ± 16 μm).

Materials and Methods

Experimental organisms

The test rotifer was *B. rotundiformis* of the Koshiki strain (Fig. 1A). The two most frequently observed contaminant protozoan species, *Vorticella* sp. (Fig. 1B) and *Euplotes* sp. (Fig. 1C), were isolated from rotifer mass-culture tanks. After collection, these organisms were maintained as monocultures in the laboratory. Culture conditions were 22 ppt salinity and 25°C temperature. Food (centrifuged *Nannochloropsis oculata*) for the test organisms was maintained daily at about 7×10^5 cells/mL during the experimental period. *N. oculata* was cultured using modified Erd-Schreiber medium in an indoor culture room (Hagiwara et al., 1994). The genus of both test protozoans was identified in pure mono-species cultures (Inoki, 1981).

Experimental designs

The study design, which focused on either the interspecific relationship between the rotifer and a protozoan (*Euplotes* sp. or *Vorticella* sp.) or the tripartite-specific relationship of the rotifer and both protozoans (*Euplotes* sp. and *Vorticella* sp.) species, was based on the methods of Jung et al. (1997). I examined the status of the coexistence of the two contaminating protozoans as well as on interspecific interactions between the two and the effect thereof on rotifer population growth.

Three treatments were implemented: 1) rotifers in single-species culture (SR); 2) two types of two-species mixed cultures (*B. rotundiformis* and *Euplotes* sp., SR + E; *B. rotundiformis* and *Vorticella* sp., SR + V); and 3) two types of tripartite mixed cultures (*Vorticella* sp. added at the start of cultures, SR + E + V; *Vorticella* sp. added 6 days after the start of cultures, SR + E/V).

Culture volumes of the small-scale experiment were 40-mL triplicates in 50-mL glass beakers (Pyrex). All beakers were covered with parafilm (American Can Co., Greenwich, CT, USA). Semi-mass cultures were maintained in 30-L polycarbonate round tanks with 5 L of culture water. Two types of culture water were used in the experiments: sterilized sea water diluted with distilled water for the small-scale experiment and natural sea water diluted with tap water for semi-mass cultures. In the 5-L semi-mass cultures, the formula for natural culture water (natural sea water diluted with tap water) followed the culture methods used in mass culture at sea farming centers.

The initial number of organisms was 20 amictic female *B. rotundiformis* and each five cells of the two protozoans, *Euplotes* sp. and *Vorticella* sp., per 40 mL for both experimental designs (small-scale 40-mL cultures and 5-L semi-mass cultures).

Both experiments were maintained in total darkness, except for observation times. The experimental culture period was 30 days. Every 6 days, the number of test organisms in three, 4-mL sub-samples per culture tank was counted for both experiments. After counting, all zooplankton species were returned to the experimental culture tanks.

Statistical analysis

Results were analyzed using *t*-tests with a 95% confidence level.

Results

No predator-prey interactions were observed between the rotifer, *B. rotundiformis* (190 ± 20 μm) (Fig. 1A) and the two ciliates, *Vorticella* sp. (70 ± 13 μm) (Fig. 1B) and *Euplotes* sp. (82 ± 16 μm) (Fig. 1C) or between the two experimental ciliates.

In the small-scale 40-mL cultures, the growth of rotifers was clearly suppressed when *Euplotes* sp. was present (SR + E) (Fig. 2) compared with the ciliate-free rotifer cultures (SR) (Fig. 2). However, the addition of *Vorticella* sp. (SR + V) increased rotifer growth by 2.1 times compared with that observed in the *Euplotes* sp. contaminated cultures ($P < 0.01$) (Fig. 2). In two of the three-species tripartite mixed cultures (SR + E/V and SR + E + V), no declines in rotifer population growth were observed relative to the ciliate-free rotifer culture or to the mixed cultures of rotifers and *Euplotes* sp. ($P < 0.05$) (Fig. 2).

The population growth of each ciliate, *i.e.*, *Euplotes* sp. and *Vorticella* sp., interfered with that of the other (Figs. 3, 4). The density of *Euplotes* sp. rapidly increased in cultures with only the rotifer compared with conditions of co-existence with *Vorticella* sp. Additionally, *Vorticella* sp. grew quickly in mixed cultures with only the rotifer compared with tripartite mixed cultures including *Euplotes* sp. (Fig. 4).

When *Vorticella* sp. was artificially added to the 5-L semi-mass culture tanks of rotifers, this ciliate heavily suppressed the growth of the contaminating protozoan species (*Euplotes* sp.) by 0.1 times compared with *Vorticella* sp.-free cultures ($P < 0.01$) (Fig. 5). In contrast, rotifer population growth was not suppressed and was even marginally increased by 1.1 times the growth observed in ciliate-free rotifer cultures (Fig. 6).

Discussion

The present study examined the interspecific relationships between two zooplankton species within microcosms. Moreover, the micro-ecological tripartite-specific relationships of three zooplankton species were also investigated. Previous studies have examined similar multi-species relationships within microcosms. For example, Kawabata et al. (1995) achieved long-term cultures of species-defined microcosms using *Escherichia coli* DH5a as a decomposer bacteria, *Tetrahymena thermophila* B as a consumer protozoa, and *Euglena gracilis* Z as the producer algae under artificial culture medium conditions. This artificial small microcosm was successfully maintained as a stable ecological system in a micro-community (Kawabata et al., 1995).

The present results demonstrate the potential for co-existence of as well as the interspecific relationships between two contaminating protozoan ciliate species (*Euplotes* sp. and *Vorticella* sp.). These interspecific interactions directly or indirectly affected the population growth of a rotifer species, *B. rotundiformis*, within the same culture community. Pace and Orcutt (1981) also examined the function of protozoans in living environmental communities, demonstrating that a population of crustaceans declined but those of protozoans and a rotifer increased when cultured together. Pierce and Turner (1992) documented the importance of marine planktonic ciliates as consumers within a tropical link system and as a vector

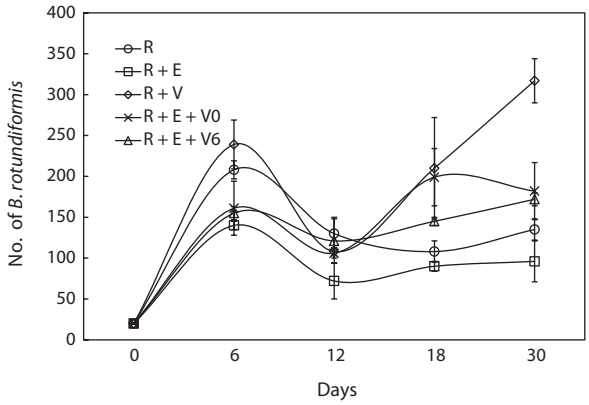

Fig. 2. Number of rotifer, *Brachionus rotundiformis* in single, inter- and tripartite specific cultures. Rotifer single species culture as control (○), mixed culture with *Euplotes* sp. (□), mixed culture with *Vorticella* sp. (◇), mixed culture of three species, *B. rotundiformis, Euplotes* sp. and *Vorticella* sp. with the addition of *Vorticella* sp. on day 0 (×) and on day 6 (△). Each plot and vertical bars represent mean ± SD of three replicates.

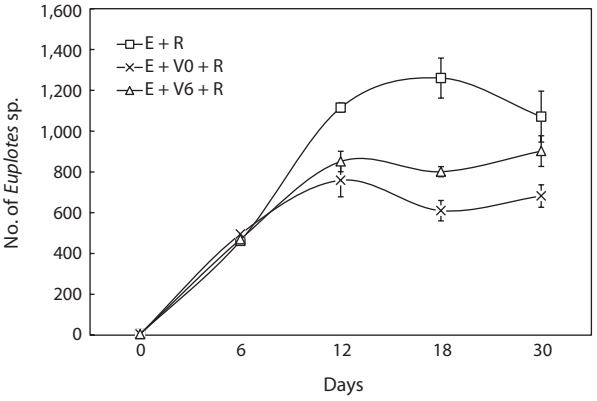

Fig. 3. Number of *Euplotes* sp. in inter- and tripartite specific cultures. Mixed culture of *Brachionus rotundiformis* and *Euplotes* sp. (□), mixed culture of three species, *B. rotundiformis, Euplotes* sp. and *Vorticella* sp. with the addition of *Vorticella* sp. on day 0 (×) and on day 6 (△). Each plot and vertical bars represent mean ± SD of three replicates.

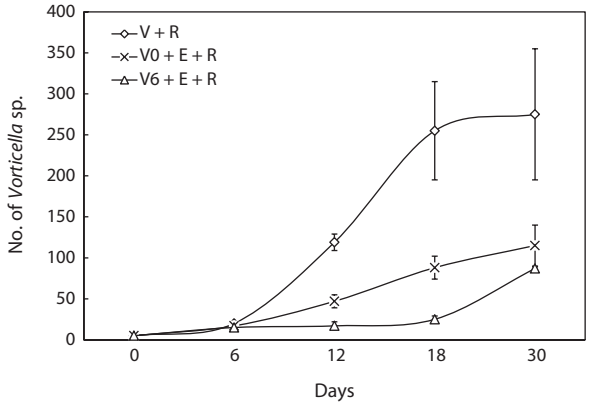

Fig. 4. Number of *Vorticllea* sp. in inter- and tripartite specific cultures. Mixed culture of *Brachionus rotundiformis* and *Vorticella* sp. (◇), mixed culture of three species, *B. rotundiformis, Euplotes* sp. and *Vorticella* sp. with the addition of *Vorticella* sp. on day 0 (×) and on day 6 (△). Each plot and vertical bars represent mean ± SD of three replicates.

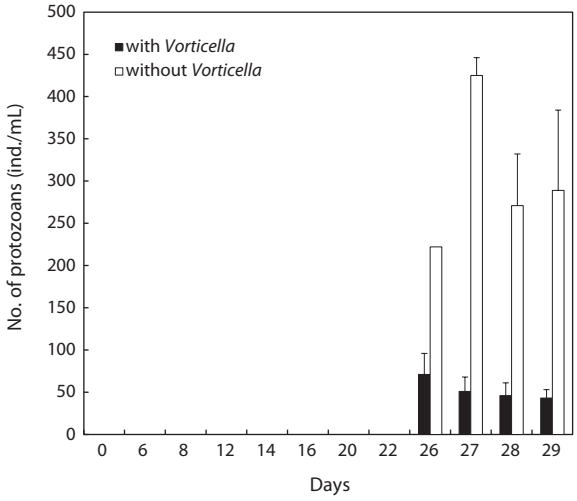

Fig. 5. Number of contamination protozoans with *Vorticella* sp. (■) and without (□) *Vorticella* sp. in the 5 L semi-mass culture.

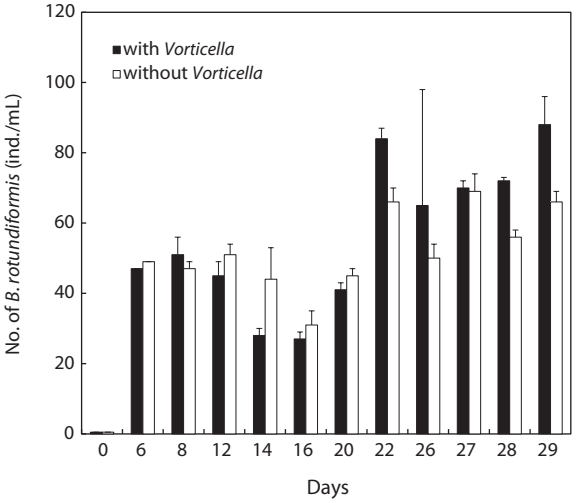

Fig. 6. Number of rotifer, *Brachionus rotundiformis* with *Vorticella* sp. (■) and without *Vorticella* sp. (□) in the 5 L semi-mass culture.

of energy flow through pelagic marine ecosystems. Hamilton and Preslan (1969) examined the optimal conditions of ciliate growth in terms of feeding behavior and food (bacteria) concentration.

During the experiments, no predator-prey interactions were observed between the rotifer (190 ± 20 μm) (Fig. 1A) and either of the two test ciliates, *Vorticella* sp. (70 ± 13 μm) (Fig. 1B) and *Euplotes* sp. (82 ± 16 μm) (Fig. 1C). In contrast, Gilbert and Jack (1993) showed that *Synchaeta pectinata* (320 μm), *Brachionus calyciflorus* (280 μm), and *Asplanchna girodi* (491 μm) were effective predators of ciliates (45-60 μm), ingesting up to 50 ciliates rotifer^{-1} day^{-1}.

The common contaminating ciliate, *Euplotes* sp., always interferes with the stability of rotifer mass cultures in culture tanks (Jung et al., 1997, 2008). Jung et al. (1997) showed that all contaminating ciliates, except for *Vorticella* sp., suppressed rotifer population growth. The present results indicate that one method for suppressing *Euplotes* sp. populations within cultures is to utilize the bacterivorous ciliate *Vorticella* sp. to induce food (bacteria) competition between *Euplotes* sp. and *Vorticella* sp. Generally, ciliate groups feed on bacteria. As a result, rotifer population growth is directly and indirectly improved due to declines in harmful *Euplotes* sp. caused by food (aquatic bacteria) competition with *Vorticella* sp. *Euplotes* sp. competes for the same food (centrifuged and condensed instant microalgae) as the marine *Brachionus* rotifer within the same culture tanks (Jung et al., 2008). Furthermore, an isolated bacteria group (*Flavobacterium*) in *Euplotes*-contaminated rotifer culture tanks can also strongly suppress rotifer growth (Maeda and Hino, 1991). Thus, *Vorticella* may experience rapid growth in *Euplotes*-contaminated tanks by utilizing these bacteria as a food source.

The present study presents a very interesting phase of rotifer microcosms. In the experimental microcosms, intricate relationships between two protozoans, namely inter- and tripartite-specific interactions, caused direct and indirect effects on rotifer population growth. Moreover, the *Vorticella* sp. contaminant was very useful as an aquatic ecosystem control tool. These results demonstrate the value of *Vorticella* sp. for maintaining the stable production of rotifer mass cultures.

Acknowledgments

I am indebted to Dr. Cheng-Sheng Lee (Center for Tropical and Subtropical Aquaculture, The Oceanic Institute in Hawaii, USA) and Mr. Diegane Ndong (Agence Nationale De L'Aquaculture, Senegal). Also I would like to thank to professional translator, Mr. Do Eungbeom for grammar correction of English. This work is also funded by National Fisheries Research & Development Institute of South Korea (NFRDI, RP-2012-AQ-060).

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