

УДК 579.5

Specific Features of Mixed Culture of Invertebrates Maintained under Laboratory Conditions

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Received 1.06.2009, received in revised form 8.06.2009, accepted 15.06.2009

*Competition among invertebrates was studied in mixed culture (the ciliates *Paramecium caudatum* and *Paramecium bursaria* and the rotifer *Brachionis plicatilis*) under laboratory conditions. The invertebrates were fed on various food sources: 1) *Chlorella* biomass and 2) biomasses of bacteria and yeast. The *P.bursaria* – *Chlorella condutrix* endosymbiosis was used a natural model of a simple biotic cycle. *P.caudatum* was found to be a poor competitor: even when the initial percentage of *P.caudatum* cells was large and there was no food limitation, this ciliate had been expelled by the end of the experiment. The main reason for the low competitive ability of *P.caudatum* in the studied system was likely the inhibiting effect of invertebrates' metabolites. The outcome of the competition between the ciliate *P. bursaria* and the rotifer *B. plicatilis* was different depending upon the food source: 1) *Chlorella* or 2) bacteria and yeast. When fed on *Chlorella*, *P. bursaria* was a better competitor than *B. plicatilis*. With bacteria and yeast used as food, the opposite outcome of competition was observed: the competitive ability of *B. plicatilis* was better than that of *P. bursaria*. It was assumed that due to turbidity of the culture solution in experiments with bacteria and yeast used as food, the zoochlorella photosynthesis rate decreased and, hence, the *P. bursaria* – zoochlorella symbiosis became less competitive. Analysis of experimental results of *P. bursaria* and *P. caudatum* single species cultures in the light and in the dark, with bacteria used as a food source, confirmed that assumption. The results of the experiments confirmed the beneficial role of symbiotic relationships of the ciliate *P. bursaria* and zoochlorella. The number of symbiotic units in the light reached 220 units/ml, while in the dark it was much smaller. The *P. caudatum* number remained practically the same in the light and in the dark.*

Keywords: competition, experimental aquatic system, invertebrates.

Introduction

Competition among invertebrates is of both theoretical and practical interest. General ecology has mainly addressed the competition for food (Cheng et al., 2004). As known closely related organisms with similar modes of existence cannot inhabit the same place, this is the Gause's competitive exclusion principle.

It was shown that the ciliate *Paramecium bursaria* (Ehrenberg, 1831) united in one cycle with *Chlorella* had a higher competitive ability than *Paramecium caudatum* (Ehrenberg, 1833) (Pisman and Somova, 2003). With any initial percentage of the ciliates in the mixed culture, the end portion of *P. bursaria* reached 90-99 –, which was significantly higher than the end portion of the *P. caudatum* population.

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Some published data suggest the leading role of external metabolites in successions. It has been shown that the action of a specific inhibitor is very selective and affects different species in a mixed community, thus determining the result of competition (Lundy et al., 1984). An experimental and mathematical model of interactions between invertebrates (the ciliate *P. caudatum* and the rotifer *B. plicatilis*) has been studied in the «producer – consumer» aquatic biotic cycle with spatially separated components (Pisman and Bogdanova, 2004). The model described the dynamics of the mixed culture of ciliates and rotifers in the «consumer» component feeding on the mixed algal culture of the «producer» component. Metabolites of the alga *Scenedesmus* have been found to produce an adverse effect on the reproduction of the ciliate *P. caudatum*. In the «producer – consumer» biotic cycle coexistence proved to be impossible in the mixed culture of invertebrates of the «consumer» component. The ciliate *P. caudatum* was driven out by the rotifer *B. plicatilis* (Pisman et al., 2002).

In the experimental laboratory system the outcome of competition can be affected by a number of other factors (nutrient limitation, variations in pH of the environment, accumulation of metabolism products, etc.) (Norberg and DeAngelis, 1997).

There are publications on interrelationships among littoral rotifers. The effect of different densities of algae and rotifers on the outcome of their competition has been investigated by S. Sarma and S. Nandini (2001). The benthic protozoa have been studied along a transect in the eastern Mediterranean Sea from the Ionian to the Levantine Sea (Hausmann et al., 2002). The studied species included picophagous species, which feed mostly on bacteria, as well as nano- and microphagous species, which generally feed on protists, suggesting the existence of several trophic levels within the deep sea microbial food

web. Thus, competition of invertebrates in both experimental and natural systems is an important problem for experimental and theoretical aquatic ecology.

The purpose of this study was to study a mixed culture of invertebrates (*Paramecium bursaria* (Ehrenberg, 1831), *Paramecium caudatum* (Ehrenberg, 1833) and *Brachionus plicatilis* (Muller, 1786) in an experimental system and to get an insight into the reasons responsible for the outcome of their competition (coexistence or exclusion).

Materials and Methods

Invertebrates used in the study were the ciliate *Paramecium caudatum*, the rotifer *Brachionus plicatilis* and a symbiosis of the ciliate *Paramecium bursaria* and its endosymbiont, zoochlorella *Chlorella condutrix*. The vital functions of the «*P. bursaria* – zoochlorella» symbiosis were maintained by zoochlorella photosynthesis.

Flows and interactions in the mixed culture of invertebrates with *Chlorella* used as food are schematically presented in Fig. 1. The ciliate *P. bursaria* contains zoochlorella and consumes carbohydrates and oxygen released by zoochlorella during photosynthesis. Under light-deficient conditions it can also consume *Chlorella* and bacteria. The ciliate *P. caudatum* can feed on yeast, bacteria, and *Chlorella*. The rotifer *B. plicatilis* can feed on algae, bacteria, yeast, and detritus (Sarma and Nandini, 2001). In this experiment invertebrates were grown on *Chlorella*. During the course of their metabolism paramecia released nitrogen, which was then consumed by *Chlorella* and zoochlorella. Part of dead invertebrates became detritus and provided a substrate for multiplication of rotifers (Gulati and DeMott, 1997).

The invertebrates were grown in the Lozina-Lozinsky synthetic medium supplemented with

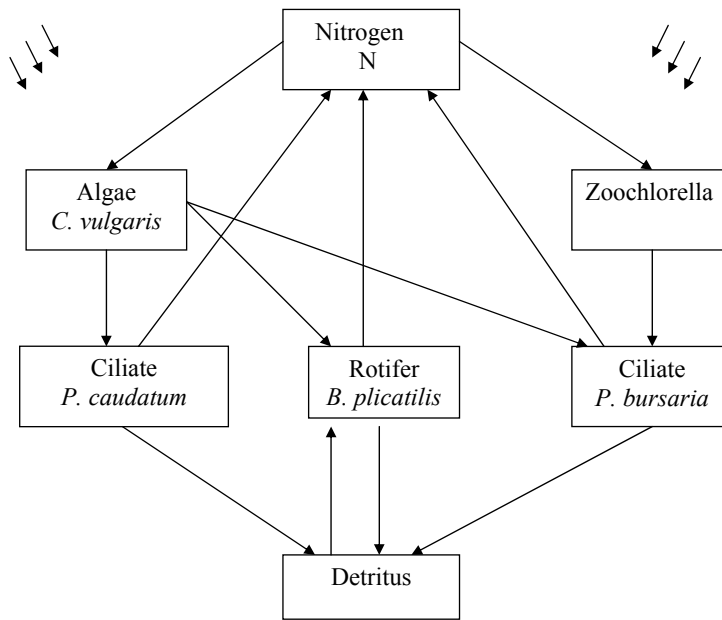


Fig.1. Scheme of flows and interactions in the mixed culture of invertebrates in an experimental laboratory aquatic system

biomass of yeast, bacteria or *Chlorella* as food sources (Kokova, 1982). Bacteria were cultivated on rice, which was added to the medium at the beginning of the experiment (Hausmann, 1985). Cultivation of invertebrates was performed in 500-ml flasks under natural light conditions during 74 days, day length was about 16 hours, temperature was 18 °C. The numbers of the ciliates and rotifers were determined by direct count in Bogorov's chamber (standard deviation – 10 –) (Kokova, 1982).

Results

The dynamics of the mixed culture of invertebrates (*P. bursaria*, *P. caudatum* and *B. plicatilis*) in the experimental laboratory aquatic system was investigated for two cases: with 1) *Chlorella* and 2) bacteria and yeast used as food.

Fig. 2 and 3 show the dynamics of invertebrates in the mixed culture for 74 days in experiments with different initial proportions of competitors, grown on *Chlorella*. *P. caudatum* and the rotifer initially amounted to about 14 – and *P.*

bursaria to 72 – (Fig. 2). At Day 15, *P. caudatum* was expelled; at Day 24, stable coexistence was established between *P. bursaria* (about 65 –) and the rotifer (about 35 –). The final outcome of the competition of invertebrates in the variant with different initial proportions (Fig. 3) was similar to the outcome of the competition shown in Fig. 2. Although the initial fraction of *P. caudatum* was considerably larger and amounted to about 80 –, at Day 60 this ciliate was also expelled. *P. bursaria* (1 –) was added later, at Day 14. Yet, stable coexistence between *P. bursaria* (about 60 –) and the rotifer (about 40 –) was established.

The dynamics of the mixed-culture invertebrates grown on bacteria and yeast (without *Chlorella*) is shown in Fig. 4. The best food for *P. caudatum* were the bacterium *Bacillus subtilis* and a mixture of *Bacillus subtilis* and yeast. However, in this experiment, the ciliate *P. caudatum* was a poor competitor too. Initially, *P. caudatum* constituted about 80 –, but at Day 70 it was completely expelled. However, the outcome of the competition between the rotifer and

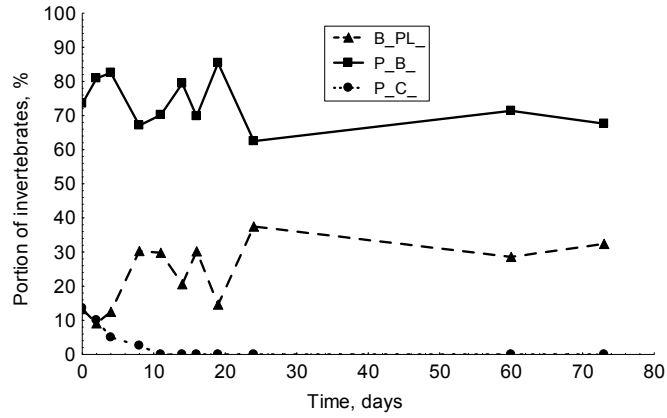


Fig.2. Dynamics of invertebrates (– of the total number), with the initial fraction of *P. caudatum* in the mixed culture amounting to 14 – , grown on *Chlorella*

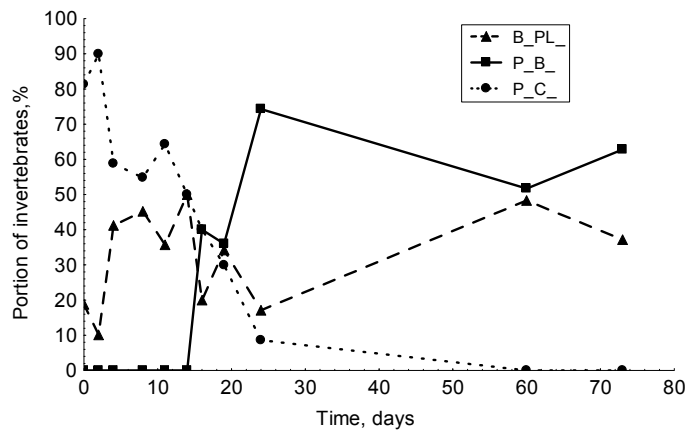


Fig.3. Dynamics of invertebrates (– of the total number), with the initial fraction of *P. caudatum* in the mixed culture amounting to 80 – , grown on *Chlorella*

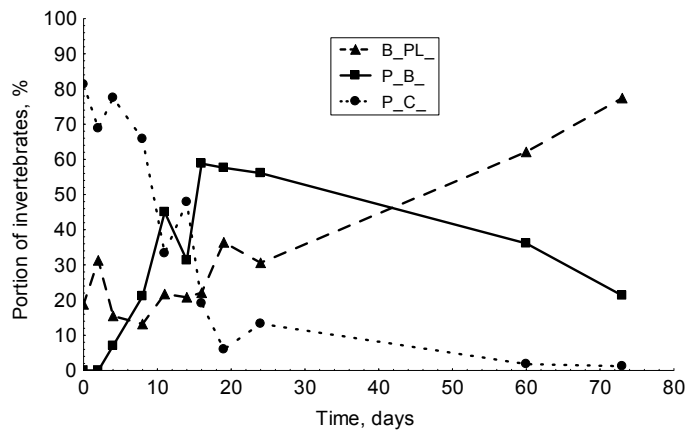


Fig.4. Dynamics of invertebrates (– of the total number), with the initial fraction of *P. bursaria* in the mixed culture amounting to 1 – , grown on bacteria and yeast

P. bursaria grown on bacteria and yeast (Fig. 4) was opposite to that observed in experiments with the medium containing *Chlorella* (Fig. 2 and 3). At Day 40, the fractions of *P. bursaria* and *B. plicatilis* became equal. At Day 74, the rotifer amounted to 75 –, while *P. bursaria* dropped to 25 –. In this experiment, the culture medium was turbid, in contrast to the experiment with *Chlorella*-supplemented medium. Thus, as less light penetrated into the medium, zoochlorella photosynthesis rate decreased, and *P. bursaria* became less competitive than the rotifer.

To verify this assumption, we studied competition of invertebrates in the mixed culture consisting of two components (the rotifer and *P. bursaria*) (Fig. 5). The outcome of the competition was similar to that in the three-component system (Fig. 4). At the beginning of the experiment, when the culture medium was transparent, the fraction of *P. bursaria* had increased to reach 99 –. Then, as the culture medium became more turbid, the fraction of *P. bursaria* decreased and at Day 74 it dropped to 35-40 –, while the rotifer amounted to 60-65 –.

Analysis of experimental results of growing *P. bursaria* (Fig. 6) and *P. caudatum* (Fig. 7) single species cultures in the light and in the dark in the medium supplemented with bacteria confirmed the beneficial role of symbiotic relationships. The number of symbiotic units in the light (Fig. 6) reached 220 units/ml, while in the dark it was much smaller. The *P. caudatum* number (Fig. 7) remained practically the same in the light and in the dark.

Discussion

As multiplication of *P. bursaria* that was symbiotic with zoochlorella in the light occurred irrespectively of the additional food source (*Chlorella*), in the mixed culture of invertebrates the main competitors for *Chlorella* were the rotifer and *P. caudatum*. The rotifer could also feed on

detritus, but at the beginning of the experiment it must have preferred *Chlorella*, since this alga is known to be the most favorable food for *B. plicatilis* multiplication.

Although there were two food sources (*Chlorella* and detritus) for two types of invertebrates (*B. plicatilis* and *P. caudatum*), *P. caudatum* was found to be a very poor competitor under these conditions. One of the reasons could be its inhibition by waste products of metabolism. As reported in the literature, the growth of the protozoa culture is significantly affected by waste products of their own metabolism (Kokova, 1982). The author suggested that the termination of growth was caused by enhanced amounts of waste products of metabolism in the medium. This explanation is in good agreement with the experiments that revealed the influence of metabolites on *Paramecium*.

The advantages of the *P. bursaria* symbiotic unit (the ciliate – zoochlorella) are quite obvious (Yellowlees et al., 2008). The symbionts can supply the host with products of photosynthesis and molecular oxygen, which is necessary for oxidative processes, while the ciliate, which is motile, can increase the light supply necessary for the microalga and provide it with ammonium compounds – mineral substrate essential for the growth of *Chlorella*. These compounds are the ciliate's metabolites, toxic to it (Barkhatov et al., 1998).

Partners' relationships can rather easily change from mutualistic to antagonistic (and back), depending on the environmental conditions. For instance, symbiosis of the freshwater hydra and *Chlorella* improves development of the host in the light and in the absence of carbon compounds, when the products of photosynthesis supplied by the endosymbiont are the only food source for the host. However, the development of the hydra transferred to the dark is suppressed because photosynthesis is stopped and the endosymbiont

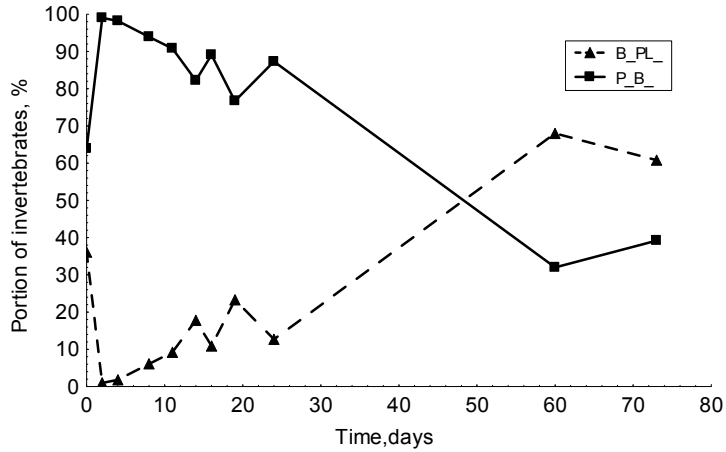


Fig.5. Dynamics of invertebrates (– of the total number), with the initial fraction of *P. bursaria* in the mixed culture amounting to 65 –, grown on bacteria and yeast

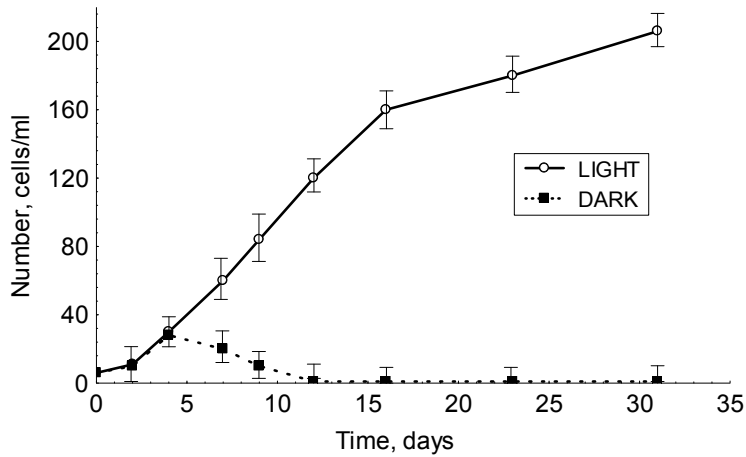


Fig.6. Growth of the *P. bursaria* – zoochlorella symbiosis in the light and in the dark

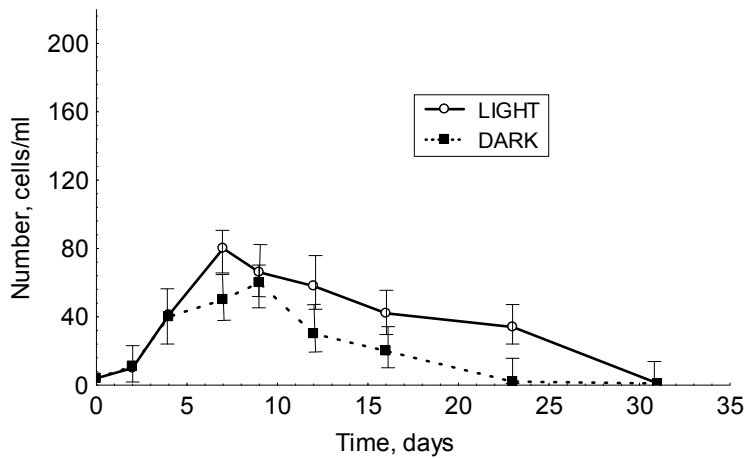


Fig.7. Growth of the ciliate *P. caudatum* in the light and in the dark

has to consume the host's metabolites (Provorov, 2001). Intracellular symbionts of paramecia (*Caedibacter*) can be both mutualists and antagonists for their host, depending upon feeding conditions (Pond et al., 1989).

Several studies are available on the interspecific relations among zooplankton (Hagivara et al., 1995; Jung et al., 1997). Controversial results were reported by Hagivara et al. (1995) and Jung et al. (1997) on the interspecific relations between rotifers (*Brachionus rotundiformis*) and copepods (*Tigriopus japonicus*). Hagivara et al. (1995) have reported that the copepod *T. japonicus* did not affect rotifer growth, while *T. japonicus* grew better with rotifers than when cultured alone. On the other hand, Jung et al. (1997) have showed that contamination of rotifer cultures with *T. japonicus* strongly suppressed *B. rotundiformis* population growth when compared to *B. rotundiformis* single species cultures. At the same time, the growth of *T. japonicus* was better when cultured alone than when the two species were kept in mixed cultures.

M. Jung and coauthors (1997) have suggested that the interspecific relations between rotifers and copepods could be influenced by co-existing aquatic bacteria occurred in the cultures. A subsequent study demonstrated that the presence or absence of bacteria in the aquatic environment can alter the relationship between species (Jung and Hagiwara, 2001). Co-existing bacteria had a pivotal impact on the interspecific relations between *B. rotundiformis* and *T. japonicus* in experimental conditions.

Recently, a competition experiment between the rotifer *Brachionus rotundiformis* and the ciliate *Euplotes vannus* has been carried out (Cheng et al., 2004). The ciliate was inoculated at three stages of the rotifer growth curve: the lag phase, the logarithmic growth phase and the stationary phase. When feeding on the alga

Tetraselmis tetrahele, the ciliate numbers increased and the rotifer growth was suppressed. The interference with rotifer growth was stronger in the lag and stationary phases than in the logarithmic growth phase. In contrast, when feeding on the alga *Nannochloropsis oculata*, the ciliate did not increase and the rotifer growth was similar to the control. The fundamental importance of quantifying population density effects in natural and laboratory populations has been well recognized (Snell et al., 2001). Density effects in laboratory rotifer populations were higher than density effects in natural populations. Density effects in laboratory populations are due to food limitation, autotoxicity or to their combined effects. The cited above work on the experiments with *B. rotundiformis* demonstrated the absence of autotoxicity at densities as high as 865 ml⁻¹, a much higher density than observed in natural populations. Therefore, the authors have concluded that food limitation rather than autotoxicity plays a major role in regulating natural rotifer populations.

Thus, the outcome of competition of invertebrates in prolonged batch culture in the experimental system can depend upon a number of factors: food source, transparency of the culture medium, accumulation of waste products of metabolism, etc. Ecological factors, such as water temperature, pH, dissolved oxygen, biomass of cyanobacteria, diatoms, greens and euglenoids were considered as possible causes of the mortality of different zooplankton species (Dubovskaya, 2008). These factors should be taken into account when designing experimental and theoretical closed and open ecosystems.

Conclusion

Thus, experimental studies of competition in the mixed culture of invertebrates (*Paramecium bursaria*, *Paramecium caudatum* and *Brachionus plicatilis*) with 1) *Chlorella* and 2) bacteria and

yeast used as food showed poor competitive ability of the ciliate *P. caudatum*. Whatever the initial proportions of invertebrates, by the end of the experiment *P. caudatum* was expelled.

The study showed that the outcome of the competition between the ciliate *P. bursaria* and the rotifer *B. plicatilis* could be different depending upon the food source: 1) *Chlorella* or 2) bacteria and yeast. Fed on *Chlorella*, *P. bursaria* was a better competitor than *B. plicatilis*. With bacteria

and yeast used as food, the opposite outcome of competition was observed: the competitive ability of *B. plicatilis* was better than that of *P. bursaria*.

Acknowledgments

This study was supported by the grant of the Russian Foundation for Basic Research (No. 09-07-00026).

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