

SHORT COMMUNICATION

Allelopathic effects of ciliate (*Paramecium caudatum*) (Ciliophora) culture filtrate on the population of brachionid rotifers (Rotifera: Brachionidae)

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ABSTRACT

Planktonic ciliates and rotifers feed on algae of similar size, hence compete for limited resources in nature. However, ciliates influences the rotifers through their metabolic products (allelochemicals) into the medium. We quantified the changes in population growth of two brachionid rotifers (*Brachionus calyciflorus* and *Plationus patulus* (= *Brachionus patulus*), when exposed to allelochemicals from the culture filtrates of common planktonic ciliate *Paramecium caudatum*. In control, the population density of *B. calyciflorus* increased during the first week and later stabilized. On the other hand, when grown in the presence of allelochemicals from *P. caudatum*, the population density and the growth rates were decreased. In *P. patulus* also, there was a steady decline in population when grown in ciliate-conditioned medium compared to controls. The culture in medium containing allelochemicals from ciliates, decreased the population of both *B. calyciflorus* and *P. patulus*, over the controls. Regardless of treatment, the *r* varied from -0.20 to +0.24 for *P. patulus* and -0.31 to +0.03 for *B. calyciflorus*. Results are discussed in relation to allelopathic interactions between the ciliates and rotifers.

Keywords: Allelochemicals, *Brachionus calyciflorus*, *Chlorella vulgaris*, ciliate, plankton, population growth, *Paramecium caudatum*, *Plationus patulus*, rotifer

INTRODUCTION

Among the freshwater zooplankton groups, ciliates, rotifers and crustaceans (cladocerans and copepods) are most abundant, in terms of both biomass and population (20). There has been a greater interest and emphasis by planktonologists to study the interactions such as competition and predation but rarely allelopathy (8,18). Competition in phytoplankton is known (21) and competitive interactions in zooplankton community occurs between the crustaceans and rotifers and the effects of ciliates are rarely studied (6).

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Predation- and competition-mediated changes in the plankton community are assessed through seasonal variations under field conditions and in controlled laboratory experiments (3,5).

Ciliates are small sized with higher metabolic rates and resistance to harsh environmental conditions and are strong competitors to both rotifers and cladocerans (26). However, certain large cladocerans (e.g. *Daphnia*) and some rotifers (such as *Asplanchna*) exert the considerable pressure on ciliate population via interference or predation on free living ciliates and co-exist (7,9). Many planktonic and semi-planktonic rotifers usually feed on phytoplankton, bacteria and detritus (22). When fed on an exclusive diet of ciliates, most herbivorous rotifers do not grow and cannot maintain population. In addition, certain metabolic by-products from ciliates may also inhibit the reproduction in planktonic rotifers. For example, it has been reported that when the ciliate *Euplotes* were added to the culture tanks of rotifer *Brachionus plicatilis*, the reproductive rates of the brachionids decreased significantly (15).

Studies on the allelopathic effects of ciliates on rotifers are very few. For example, previous studies have quantified the effects of *Urotricha* (a small planktonic ciliate) on the population of rotifers *Keratella cochlearis* and *Keratella quadrata*. It was observed that the presence of ciliates enhanced the reproductivity of *K. cochlearis* but decreased the growth rate of *K. quadrata*, thus suggesting a possible species-specific interaction between ciliates and rotifers (27). Similarly, when *Brachionus rotundiformis* was cultured with *Euplotes vannus*, there was a significant reduction in rotifer abundances (2).

We found that monogonont rotifers are often adversely affected via allelopathic pathways due to the presence of bdelloid rotifers (12). During routine maintenance of our cultures, we frequently observe that in presence of protozoans (particularly *Vorticella* sp.) the rotifer cultures decline. Therefore this study aimed to quantify the changes in the population growth of two brachionid rotifers (*Brachionus calyciflorus* and *Plationus patulus* (= *Brachionus patulus*), when exposed to culture filtrate of the common ciliate *Paramecium caudatum*.

MATERIALS AND METHODS

The ciliate *P. caudatum* was originally isolated from a small pond in the State of Mexico (Mexico) and mass cultured using Chalkley medium (11). The culture jars were maintained at 23°C. To obtain the culture filtrate, we centrifuged the ciliated-conditioned at 5000 rpm and then the filtrate was filtered through 0.22 µm Millipore filter. The culture filtrate was obtained daily, when the density of *P. caudatum* was 100±5 ind. ml⁻¹. This medium was diluted 50% using EPA medium (25) so that the rotifers could grow and reproduce without physiological and/or osmotic stress.

To culture rotifers, first the single-celled green alga *Chlorella vulgaris* was cultured using Bold's basal medium periodically enriched with sodium bicarbonate (1). Alga was batch-cultured in 2 L transparent bottles at 20°C with constant aeration and continuous light. Log phase alga was sedimented in a refrigerator and the filtrate was removed. Later, the alga was centrifuged at 4000 rpm for 5 min, rinsed and re-suspended in distilled water. The algal density was estimated using a Neubauer haemocytometer. The

desired algal density (0.25×10^6 cells ml^{-1}) was obtained by diluting with a mixture of Chalkley and EPA medium, mentioned earlier.

The rotifers *B. calyciflorus* and *P. patulus* were first isolated from local waterbodies in State of Mexico and are cultured in our laboratory for several years (16). The two rotifer species, cultured separately using EPA medium, were fed *Chlorella* at a density of 1×10^6 cells ml^{-1} . The medium from the rotifer mass culture tanks was changed every alternate day, although the algal food was added daily. The culture conditions were: temperature (23°C), pH (7.0 and 7.5), photoperiod 14:10 (light: dark) and dissolved oxygen: $6\text{--}7$ mg L^{-1} .

The experimental design was similar for both the rotifer species: 50 ml of medium in 100 test jars, daily algal food 0.25×10^6 cells ml^{-1} , medium replaced daily, temp. 23°C , initial rotifer density 1 ind. ml^{-1} , and each treatment with 4 replicates. Thus for each rotifer species we used 8 test jars (2 treatments (with and without ciliate-culture filtrate) X 4 replicates = 8). Following initiation of population growth experiment for each of the two rotifer species, we initially counted the rotifers present in entire medium under stereomicroscope (Leica model 2000, Germany) and the living rotifers were individually transferred to fresh jars containing appropriate medium and food level. When the density of *P. patulus* was higher than 500 individuals per jar, we used two aliquots of 1-5 ml each. The population growth experiments were terminated after 12 days by which time most populations in the test jars began to decline.

Based on the data collected we derived the rate of population increase (r) using the formula

$$r = (\ln N_t - \ln N_0)/t$$

Where, N_0 is the initial population density, N_t is the population density at time t in days (10).

One-way analysis of variance (ANOVA) was used to quantify the differences in the growth rates of each rotifer species exposed to Paramecium-conditioned medium and the controls.

RESULTS AND DISCUSSION

Population growth curves of *B. calyciflorus* and *P. patulus* grown with (open circles) and without (closed circles, = controls) the presence of culture filtrate from *P. caudatum* are presented in Fig. 1. For *B. calyciflorus* in controls the population density increased during the first week and later stabilized. On the other hand, when grown in the presence of allelochemicals from *P. caudatum*, there was a drastic decline in the population density of *B. calyciflorus*. When *P. patulus* was grown in the presence of ciliate-conditioned medium, there was steady decline in the abundances compared to controls.

The rate of population increase in both *B. calyciflorus* and *P. patulus* decreased, when cultured in medium containing allelochemicals from ciliates as compared to controls. Regardless of the treatment, the r varied from -0.20 to $+0.24$ for *P. patulus* and -0.31 to

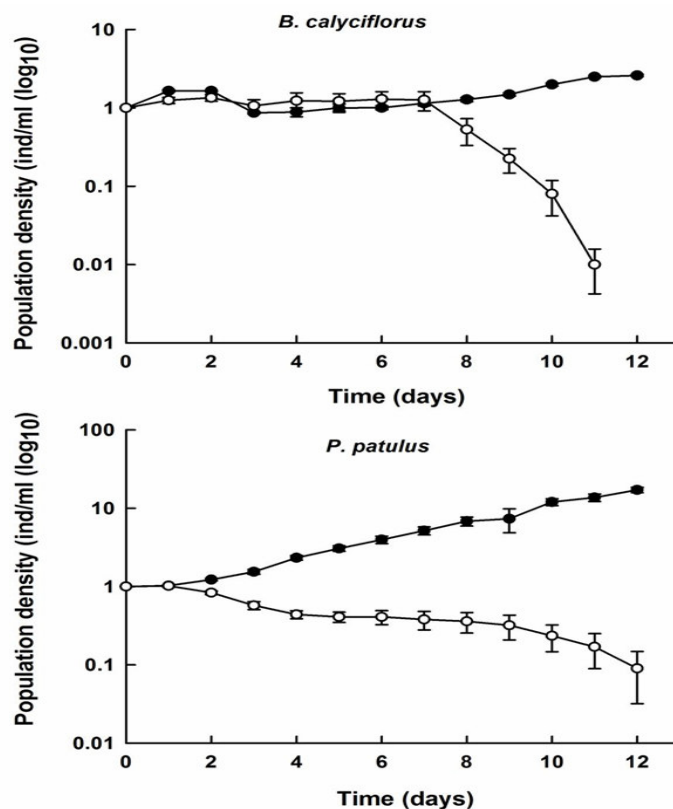


Figure 1. Population growth curves of *B. calyciflorus* and *P. patulus* in controls (closed circles) and in the presence of allelochemicals from ciliate (*Paramecium caudatum*) culture filtrate (open circles). Shown are the mean \pm standard errors based on four replicates. Note the differences in the scaling on Y-axis.

+0.03 for *B. calyciflorus*. Statistically for both the rotifer species the peak population density and the rate of population increase were significantly influenced by the presence of ciliate-conditioned medium ($p < 0.05$, ANOVA, Table 1).

While evaluating allelopathic interactions among planktonic organisms, the use of population growth is helpful for quantification (14). Since allelopathy largely involves outcompeting the co-occurring species through allelochemicals in the medium, changes in the relative abundances of competing populations can be easily estimated using population growth data. Rotifers, due to rapid growth rates, are usually considered for laboratory experiments involving competition (24). However, different rotifer species have different peak abundances and growth rates, even when cultured on similar conditions. Usually in nature, large species are less abundant than smaller species (4). When cultured on a given

Table 1. Results of analysis of variance performed on the peak population density and the rate of population increase (r) of *B. calyciflorus* and *P. patulus* cultured with and without the presence of ciliate culture filtrate. DF = degrees of freedom, SS = sum of squares, MS = mean squares, F = F-ratio, *** = $p < 0.001$ and * = $p < 0.05$.

Population Parameter	Source of Variation	DF	SS	MS	F	P
Peak population density						
<i>B. calyciflorus</i>	Control vs treatment	1	3.477	3.477	13.65	*
	Error	6	106.92	0.54		
<i>P. patulus</i>	Control vs treatment	1	519.386	519.39	133.03	***
	Error	6	23.425	3.90		
Rate of population increase						
<i>B. calyciflorus</i>	Control vs treatment	1	0.207	0.207	528.50	***
	Error	6	0.028	0.005		
<i>P. patulus</i>	Control vs treatment	1	0.399	0.399	87.00	***
	Error	6	0.028	0.005		

concentration of an edible algal species, smaller species increase in numbers more rapidly than larger species (13). In this study, *B. calyciflorus* is much larger (ca. 180 μm body length) than *P. patulus* (ca. 110 μm), hence, the former had much lower peak abundances in controls.

The rate of population increase in zooplankton is most sensitive variables to stress from competition (16). In rotifers, the range of r is highly variable, depending not only on the species type but also on the culture conditions (Fig. 2). Usually r increases with increasing availability of food concentration in the medium (13). At near threshold food levels, the r is close to 0, i.e., natality equals mortality and the population just maintains without strong oscillations (19). In rotifers, larger species usually need higher food concentrations so as to maintain a population (17). In this study, *B. calyciflorus* being larger than *P. patulus* could barely double its initial density, at the offered food density which suggests that the algal concentration was low. However at this food concentration *P. patulus* had much higher peak densities. Our results support earlier studies in which, when grown on similar algal food concentration, *P. patulus* was always numerically more abundant than *B. calyciflorus* (16).

There were allelopathic interactions between the rotifers and ciliates evident in our study because ciliates were never in contact with the rotifers. For example, in the study of interaction between *Keratella* and *Urotricha* (27) predation was reported on ciliates by *K. cochlearis* and *K. quadrata*. In this study, since there was no such predation possible, the observed reduction in the population growth of *B. calyciflorus* and *P. patulus* were due to allelopathic effects. When rotifers and ciliates are grown together, there is possibility of competition between them since both these groups feed on similar diets such as bacteria and algae (26). Unlike competition between two rotifer species or between rotifers and daphniid cladocerans, which is usually exploitative or interference type (6), the interaction between rotifers and ciliates possibly involves additional factors, including the production of certain chemical compounds in the medium (15). These infochemicals released from the ciliates are probably helpful to limit the abundances or eliminate the potential grazers

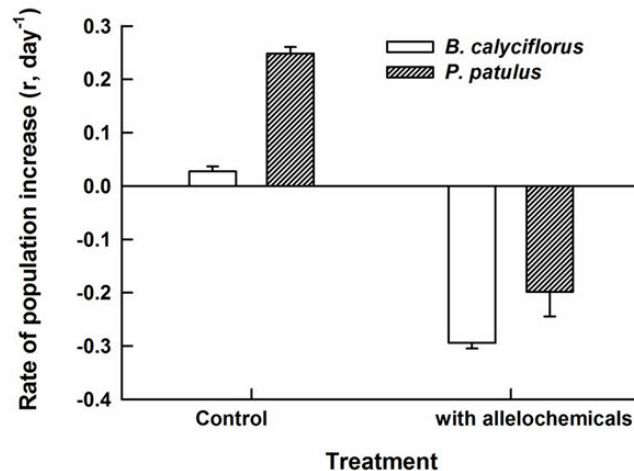


Figure 2. Rate of population increase (r) per day of *B. calyciflorus* and *P. patulus* in controls (open bars) and in the presence of allelochemicals from ciliate (*Paramecium caudatum*) culture filtrate (closed bars). Shown are the mean \pm standard errors based on four replicates. Note the differences in the scaling on Y-axis.

in natural systems. Laboratory studies on mixed cultures between rotifers and ciliates indeed have shown strong decline of rotifers suggesting the involvement of allelopathy among the competing species (2).

The allelochemicals may have different ways of affecting the competing zooplanktonic organisms e.g. changes in the feeding and filtration rates, reduction in egg output and survival of the neonates (18). These aspects have not been evaluated in this work. However, there is some evidence that the metabolites of ciliates have an inhibitory effect on the reproductive rates of rotifers (15). In our study the reduced population growth rates of *B. calyciflorus* and *P. patulus* may be due to inhibitory effects of allelochemicals from *Paramecium*.

In conclusion, our study showed that the ciliate-conditioned medium had an adverse effect on the population abundances and the rate of population increase of *B. calyciflorus* and *P. patulus*. In this study we used only one density of *Paramecium caudatum* to obtain the culture filtrate. Thus, the threshold levels of ciliate population needed to induce significant reduction in the growth rates of rotifers are still unknown. Further studies are thus needed to evaluate the effect of allelochemicals obtained from different densities of ciliates on rotifer growth rates.

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