Effect of cyanobacterium on competition between rotifers: a population growth study

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Abstract

We determined the population growth of Plationus patulus and Brachionus havanaensis grown alone or together (1:1 ratio) on different feeds of solely Chlorella, solely Anabaena, or their mixture at a level of 1 × 10⁶ cells mL⁻¹ for all diets. Our results showed that regardless of diet, population densities of B. havanaensis were higher than those of P. patulus. For both rotifer species, Anabaena caused extinction of populations, regardless whether offered alone or in combination with the green alga. In mixed rotifer cultures, the peak densities of P. patulus or B. havanaensis were much lower than when cultured alone. Rate of population increase (r) of P. patulus fed Chlorella was significantly lower (0.12 d⁻¹; p < 0.001) than for B. havanaensis (0.19 d⁻¹) grown under similar conditions. Growth rates of both rotifers fed Anabaena (alone or together with Chlorella) became significantly lower than when fed solely Chlorella. Thus, our results showed that P. patulus and B. havanaensis have similar sensitivities to Anabaena, with both species failing to grow when this cyanobacterium was included in the diet.

Key words: Anabaena, Chlorella, competition, food density, food quality, Rotifera, toxicity

Introduction

Zooplankton communities in the tropics are often numerically dominated by rotifers and small-bodied cladocerans, presumably due to higher predation on larger zooplanktonic forms that are preferred as prey by the fish (Brooks and Dodson 1965). Persistent cyanobacterial blooms are also common in these tropical waterbodies (Paerl and Otten 2013, Valadez et al. 2013). Large-bodied cladocerans are more strongly inhibited by cyanobacteria than are other types of zooplankton because large colonies or filaments are thought to interfere more with the feeding of large-bodied cladocerans, and large-bodied cladocerans probably readily consume blue-green algae low in nutrient value (Ghadouani et al. 2006). Blooms of filamentous and colonial cyanobacteria therefore have frequently been associated with changes in zooplankton composition, altering dominance by relatively large-bodied daphniids to dominance by copepods, rotifers, and small-bodied cladocerans (Lampert 1987). Several studies show that eutrophic reservoirs with seasonal cyanobacterial blooms are dominated by small rotifers (Ramírez-García et al. 2002, Jiménez-Contreras et al. 2009).

The genus Anabaena is common in both tropical and temperate regions (Ezhilarasi 2010), and its blooms are often associated with low densities of large-bodied cladocerans and high densities of rotifers (Hansson et al. 2007). A competition experiment by Gilbert (1990) showed that the presence of filaments of A. affinis prevented the suppression of rotifers by large cladocerans and led to rotifer dominance. Rotifers have different susceptibilities to toxic Anabaena, and the least-sensitive species dominates in eutrophic waters with Anabaena blooms (Gilbert 1990). The susceptibility of rotifers to endotoxins from Anabaena is a function of the efficiency with which the cyanobacterium is ingested and the sensitivity of the rotifer tissues to the toxin.
Differences in sensitivity to cyanotoxins are found not only among species but also among strains of the same species. Gilbert (1994) found that Brachionus calyciflorus ingested A. flos-aquae most efficiently and was more susceptible to the cyanobacterium than 3 other rotifer species. Starkweather and Kellar (1983), however, showed that a strain of A. flos-aquae containing anatoxin-a was an adequate source of food for B. calyciflorus. Gilbert (1996) argued that the degree to which toxic cyanobacteria affects the population dynamics of susceptible zooplankton in natural communities might be greatly influenced by modifying environmental factors such as food availability and temperature.

In most eutrophic waters, a few species of edible algae also co-occur with toxic cyanobacteria, albeit in low densities (Harper 1992); therefore, grazers such as rotifers and cladocerans feed on mixed cyanobacterial–algal diets rather than exclusively on either algae or cyanobacteria (Sellner et al. 1993). Brachionidae has 8 genera of rotifers, of which Brachionus and Plattonus are widely distributed in tropics. Both of these genera coexist in many freshwater bodies under eutrophic conditions, often dominated by cyanobacteria (Yúfera 2001), and both genera are generalists and feed on a variety of phytoplankton including cyanobacteria (Gilbert 1996, Wallace et al. 2006).

There is some evidence that mixed diets of toxic cyanobacteria and green algae still support population growth of rotifers, including Brachionus (Soares et al. 2010). The ability to utilize mixed cyanobacterial–algal diets by zooplankton species can be reflected in higher population abundances, and hence the species becomes competitively superior to other coexisting taxa if the impact of other ecological factors such as predation or parasitic attack is minimal (Wilson and Sherman 2010).

To our knowledge, this aspect has not received considerable attention, especially with reference rotifers (Lürling and Beekman 2006, Alva-Martínez et al. 2009, Soares et al. 2010). For instance, although we frequently found Plattonus patulus and Brachionus havanaensis in Lake Xochimilco, B. havanaensis is often a dominant member of the rotifer community (>190 ind. L⁻¹), while P. patulus is rarely found at densities >20 ind. L⁻¹ (Nandini et al. 2005). We therefore hypothesize that among the members of the family Brachionidae, resistance to cyanotoxins, among other factors, also governs the outcome of competition. Cyanobacteria-resistant taxa would outcompete the sensitive species when grown together in the presence of toxic strains. Thus, in this study we compared the competitive outcome between P. patulus and B. havanaensis, which often coexist in eutrophic waters and are subject to varying densities and proportions of cyanobacteria.

Material and methods

P. patulus and B. havanaensis (body size: 130 µm and 110 µm, respectively) were isolated from Lake Xochimilco (a high altitude waterbody, 2200 m a.s.l) and cultured separately on the single-celled green alga Chlorella vulgaris. We used Chlorella as the diet for rotifers because it is one of the most widely used algal species for feeding brachionids (Yoshida et al. 2003). These rotifers were maintained under laboratory conditions for more than a year prior to conducting this study. The diameter of a single cell of C. vulgaris was about 4.5 µm. For experiments, as well as for maintaining rotifer mass cultures, we used moderately hard water (EPA medium), prepared by dissolving 96 mg NaHCO₃, 60 mg CaSO₄, 60 mg MgSO₄, and 4 mg KCl in 1 L of distilled water (Weber 1993).

Chlorella vulgaris and Anabaena planctonica were cultured in 2 L transparent bottles using Bold’s basal medium and BG11, respectively (Borowitzka and Borowitzka 1988). C. vulgaris was isolated from a pond located in Mexico City, and A. planctonica was obtained from Valle de Bravo Reservoir (State of Mexico). The strain of C. vulgaris used here has been cultured for more than 10 years in our laboratory. We received the strain of A. planctonica from Dr. Pedro Ramírez García, Department of Microbiology, FES Iztacala. Both phytoplankton species were batch-cultured at 18 ± 1 °C, with continuous aeration and light regime. Log-phase algae were harvested and concentrated through centrifugation at 2000 rpm for 5 min, rinsed, and resuspended in a small volume of distilled water. Single-celled Anabaena were obtained using an ultrasonicator at 20 kHz 50 Watts for 5 min (Cole Palmer Instruments Co., USA). Microscopic observations of the sonicated Anabaena revealed that nearly 100% of the cyanobacteria were in single-celled stage, and there were no cell fragments. Wu et al. (2012) determined that this procedure is gentle enough to just break colonies or filaments but not the cells. To rupture the cells and prepare a crude extract of cyanotoxins, cultures need to be centrifuged at 4000 rpm for 30 min and subjected to several cycles of freezing at −70 °C and thawing before cell lysis takes place (Pietsch et al. 2001). The diameter of single cell was about 7 µm. Algal and cyanobacterial density was estimated using a haemocytometer (Improved Neubauer Counting Chamber, Boeco, Germany). Concentrates of Chlorella and Anabaena were stored separately for a few hours at 4 °C in the dark until ready to use. Chlorella and Anabaena used in the experiments were produced daily.

The treatments consisted of 3 different algal–cyanobacterial proportions (0, 50, and 100% Anabaena or Chlorella) for both the rotifer species separately and together (1:1 ratio of B. havanaensis and P. patulus).
food level was $1 \times 10^6$ cells mL$^{-1}$ for Chlorella, Anabaena, or their mixture. At this concentration of C. vulgaris, the tested rotifers grow exponentially for at least a week (Pavón-Meza et al. 2001, 2004). Three replicates were used for each treatment, an adequate number, particularly if the variability within replicates is low, as has been shown in previous studies on the population dynamics of rotifers (Iyer and Rao 1996). The initial density of rotifers was 1 ind. mL$^{-1}$ (20 individuals per jar in 20 mL EPA medium). All experiments were conducted at room temperature ($23 \pm 2 \, ^\circ C$) and under a fluorescent light (1000 lux)-dark cycle (14:10 LD). The pH of the medium was close to neutral (7.1–7.2), and the dissolved oxygen level was 8 mg L$^{-1}$.

The experimental design consisted of 27 transparent jars of 50 mL capacity. The 3 feeding treatments for P. patulus, B. havanaensis, and their mixture (competition) were Chlorella only, Anabaena only, and a mixed diet of Chlorella + Anabaena. We introduced rotifers individually into the jars using a Pasteur pipette under a stereo microscope (SMZ 745, Nikon). Following initiation of growth experiments, the number of living individuals in each jar was counted daily and transferred to fresh jars containing the appropriate diet composition. Chlorella and Anabaena were provided fresh daily; therefore, we did not take into account the survivorship of these cells. The experiments were terminated after 20 days, by which time rotifer populations in most treatments had begun to decline. Studies related to rotifer growth and feeding are usually conducted using 3–5 replicates (Gilbert and Jack 1993) because intrareplicate variability is generally low. In our study we also found low variability among replicates (evident from the low standard deviations) using 3 replicates.

The rate of population increase ($r$) was derived for each replicate using the regression of log natural population densities over time (Sibly and Hone 2002). For each rotifer, data of the peak population density and the rate of population increase were analyzed for statistical significance using a 2-way analysis of variance (ANOVA) after satisfying the parametric assumptions (Sokal and Rohlf 2011). This process permitted quantification of differences among treatments. Post hoc (Tukey test) analysis was conducted for multiple comparisons of growth rates and peak population densities.

**Results**

For both rotifer species, Anabaena caused extinction of populations, regardless of whether offered alone or in combination with the green alga; however, we found that regardless of the diet type and combination, the population densities of B. havanaensis were higher than those of P. patulus. When cultured on an exclusive diet of Anabaena, neither rotifer survived beyond 5 days, while on a mixed diet the survivorship was extended by no more than a week, and eventually the densities of both rotifers approached zero (Fig. 1).

In mixed rotifer cultures, the densities of P. patulus or B. havanaensis were much lower than when cultured alone. The peak population densities of P. patulus grown on Chlorella were ~100 ind. mL$^{-1}$ while those of B. havanaensis were ~200 ind. mL$^{-1}$ under comparable conditions (Fig. 2). The rate of population increase of rotifers fed Chlorella only was 0.12 d$^{-1}$ for P. patulus, and 0.19 d$^{-1}$ for B. havanaensis. Differences in $r$ of P. patulus and B. havanaensis fed green alga alone were statistically significant ($p < 0.001$, F-test). The growth rates of both rotifer species fed Anabaena (alone or together with Chlorella) were lower than when fed Chlorella only (Fig. 3).

Statistically, peak population densities and $r$ of P. patulus and B. havanaensis were significantly influenced by the diet type ($p < 0.001$, ANOVA; Table 1). Effect of competition on peak abundances and on growth rates was significant for P. patulus but not for B. havanaensis. Interaction of competition × diet type for the peak abundances was significant for P. patulus. Growth rate of B. havanaensis also was significantly influenced by interaction with diet type and competition from P. patulus.

**Discussion**

Rotifers are subject to several stress factors, such as low food availability and competition (Wallace et al. 2006). Toxic cyanobacteria including Anabaena often proliferate in tropical lowland waters due to the persistence of high temperatures (>20 °C; Whitton and Potts 2000, Kosten et al. 2012). Large filamentous colonies provide an additional impediment for zooplankton, especially the larger taxa, grazing on cyanobacteria (Gliwicz 1990). In our experiments, we eliminated this mechanical problem of feeding on filamentous Anabaena by sonicating the filaments into separate cells; therefore, decline of rotifer populations in test jars containing Anabaena was partly due to the toxicity of the offered diet.

Zamora-Barrios (2012) estimated the concentration of cyanotoxin from the same strain of Anabaena used in this study and found it to be 0.425 µg L$^{-1}$. Field observations from the Valle de Bravo reservoir (from which Anabaena was isolated) also showed the presence of microcystins at concentrations (>5 µg L$^{-1}$), 5 times higher than those recommended by the World Health Organization for drinking water (Alilío-Sánchez et al. 2014). Factors including the accumulation of metabolic waste products and oxygen depletion would not alone bring down the
Fig. 1. Population growth curves of *Plationus patulus* and *Brachionus havanaensis* cultured separately or together (competition) on *Chlorella*, *Anabaena*, or on the mixed diet. Shown are the mean ± standard errors based on 3 replicates.

Fig. 2. Peak population abundances (ind. mL$^{-1}$) of *Plationus patulus* and *Brachionus havanaensis* cultured separately or together (competition) on (1) *Chlorella*, (2) *Chlorella* + *Anabaena*, and (3) *Anabaena*. Shown are the mean ± standard errors based on 3 replicates. For each treatment, data bars carrying similar letter designations (a, b, and c) are not statistically significant ($p > 0.05$, Tukey test).
rotifer populations because the test species are known to grow to high densities (>500 ind. mL\(^{-1}\)) without any ill effects (Sarma and Rao 1990, Pavón-Meza et al. 2004) and can tolerate low dissolved oxygen levels (<3 mg L\(^{-1}\); Wallace et al. 2006); in addition, the medium was 100% replaced daily.

Both rotifer species used in this study often coexist in many waterbodies, including Lake Xochimilco where these organisms were first isolated. The relative densities of both these species vary considerably through different seasons, however, possibly due to changes in the composition of phytoplankton including dominance of cyanobacteria (Nandini et al. 2005). In addition, Alillo-Sánchez et al. (2014) have reported the cyanobacterial cell density in the Valle de Bravo reservoir was at times >0.1 × 10\(^6\) cells mL\(^{-1}\).

Chlorella has also been reported from Lake Xochimilco, although its densities are usually much lower than other co-occurring green algae such as Scenedesmus (Ortega 1984). It is therefore possible that both of the rotifers compete for limited algal diet and also must resist toxic effects of cyanobacteria. Our results show that both rotifers grew well on an algal diet but declined when Anabaena was included. This effect was not likely due to the size of the cells because the sonicated Anabaena had a diameter of about 7 µm, well within the range of edible cells (<10 µm) for all rotifers (Monakov 2003), which suggests that even when densities of Chlorella and Anabaena were in a 1:1 ratio, the toxic effect of Anabaena prevails.

The toxic effect of the cyanobacterium used in this study on rotifers was evident from a preliminary acute toxicity in which we maintained unfed individuals of B. havanaensis for 48 as controls and others (test individuals) were fed sonicated Anabaena for the same duration. When rotifers were exposed exclusively to sonicated Anabaena, nearly 50% were dead within 48 h, much earlier than those that were unfed. Field observations also showed that the concentration of microcystins from the Valle de Bravo reservoir was positively and significantly correlated with the cell density of A. planctonica (Alillo-Sánchez et al. 2014). In the rowing canal close to Lake Xochimilco, strains of Pseudanabaena and Anabaeopsis have toxins in the range of 0.2–0.4 µg L\(^{-1}\) (Pineda-Mendoza et al. 2012). Although few studies indicate the concentrations of cyanotoxins in natural waterbodies in Mexico (Vasconcelos et al. 2010, Pineda-Mendoza et al. 2012, Alillo-Sánchez et al. 2014), the persistence of blooms indicates that zooplankton must be subjected to high concentrations of cyanotoxins.

When food levels are equal, zooplankton species with smaller body size are numerically more abundant than those of larger taxa. For example, Sarma et al. (1996) showed that the smaller rotifer species Anuraeopsis fissa has peak population abundances an order of magnitude higher than those of the larger B. calyciflorus. This population difference has been also shown for various species of cladocerans (Nandini and Sarma 2003). In the study reported here, P. patulus is larger than B. havanaensis (130 and 110 µm, respectively) and hence reached lower peak population abundances when cultured on the same density of Chlorella.

Nandini et al. (2007) cultured 5 rotifer species (body size varied from 100 to 200 µm) at different algal food concentrations and reported that the peak population abundances of rotifers are inversely related to the body size. This finding explains our observations that large-sized rotifers had lower abundances than the smaller species. Field studies have reported that the density of B. havanaensis was always higher than P. patulus (Nandini et al. 2005). Our observations on the peak population densities and \(r\) are also in agreement with those available in literature for the same species (Pavón-Meza et al. 2001, 2004).
Under limiting resources, the competitive outcome between 2 or more species depends on many factors, including the ability to utilize alternate diets. In the present study, when the green alga alone was offered as the diet, *P. patulus* and *B. havanaensis* continued to grow together, although at much lower densities than when cultured separately, indicating the resource limitation of the competing species (Tilman 1982). This finding is similar to the observations of Fernández-Araiza et al. (2005) who documented that under moderate and high algal food levels, *B. havanaensis* coexisted with 3 other brachionid species. When grown together with *Anabaena* in the diet, *P. patulus* and *B. havanaensis* were adversely affected, suggesting that both of these rotifer species are equally susceptible to *Anabaena* and, hence, the competitive outcome between them was not evident.

In general, few studies have addressed the response of rotifers to cyanobacterial diets. One of the first studies using *Anabaena affinis* by Gilbert (1994) clearly shows that brachionids are among the most sensitive genera to cyanobacteria. A previous study by Alva-Martínez et al. (2009) also indicated that *B. havanaensis* had low population growth rates when fed an exclusive diet of *Microcystis aeruginosa*, but the growth rates improved when cyanobacterial diets were mixed with green algae. Soares et al. (2010) documented that *B. calyciflorus* also grows well on green algae–cyanobacteria mixed diets. In general, *B. havanaensis* has higher growth rates than *P. patulus* under similar conditions (Pavón-Meza et al. 2001, 2004), also evident in nature where *B. havanaensis* is often numerically more abundant than *P. patulus* in Lake Xochimilco (Nandini et al. 2005).

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