

Effect of a cyanobacterial diet on the competition between rotifers: a case study in Lake Albufera of Valencia, Spain

S.S.S. Sarma¹, S. Nandini¹, M.R. Miracle[†] and E. Vicente^{2,*}

¹ Laboratorio de Zoología Acuática, División de Investigación y Posgrado, Universidad Nacional Autónoma de México, Campus Iztacala, Av. de los Barrios #1, Los Reyes, Iztacala, Tlalnepantla, CP 54090. Estado de México, Mexico.

² Dept. Microbiología i Ecologia & ICBiBE, Universitat de València, 46100-Burjassot (Valencia), Spain.

[†] Deceased.

* Corresponding author: eduardo.vicente@uv.es

Received: 12/11/18

Accepted: 27/11/18

ABSTRACT

Effect of a cyanobacterial diet on the competition between rotifers: a case study in Lake Albufera of Valencia, Spain

Brachionus havanaensis is predominantly distributed in the Nearctic and Neotropical regions. It is now found even in the Palearctic and Oriental regions. During the summer of 2015 we found high densities (> 500 ind./l) of this species and low numbers of *Brachionus angularis* in Lake Albufera where the cyanobacterium (*Microcystis aeruginosa*) was dominant in the phytoplankton community. We hypothesized that the cyanobacterium was toxic to *B. angularis* but not to *B. havanaensis* hence the latter would outcompete the former in the presence of this cyanobacterium. To test the hypothesis, we conducted competition experiments between two coexisting rotifer species (*B. angularis* and *B. havanaensis*) from the Lake Albufera of Valencia using as food the green alga (*Nannochloropsis oculata*) and sonicated colonies of *M. aeruginosa* (single cells), separately and together (1:1 ratio based on biomass). The competition experiments using the population growth approach showed that *B. havanaensis* in controls had higher population abundances than *B. angularis*. However, when grown both together, *B. havanaensis* had a reduced population growth (nearly 50 %) in relation to *B. angularis*, in the absence of *Microcystis* in the diet. This reduced growth was more than that in the treatment of *B. havanaensis* alone when grown on a mixed diet of *Microcystis* and *Nannochloropsis oculata*. *Brachionus havanaensis* in all treatments (alone or in the presence of *B. angularis*) died completely when cultured on 100 % *M. aeruginosa*. The population growth rates of *B. angularis*, alone or in competition with *B. havanaensis*, were similar when *Microcystis* was excluded from the diet. However, *Microcystis* alone or together with *Nannochloropsis* was highly detrimental for the population growth of *B. angularis*. Our results showed the differences in the competitive outcome between the rotifers depending on the presence or absence of toxic cyanobacteria in the diet. Our study also showed that an exclusive diet of this cyanobacterium was toxic to both rotifers.

Key words: *Brachionus*, Rotifera, Population dynamics, *Microcystis*, *Nannochloropsis*

RESUMEN

Efecto de la dieta con cianobacterias en la competencia entre rotíferos: un caso de estudio en la laguna de la Albufera de Valencia, España

Brachionus havanaensis se distribuye predominantemente en las regiones Neártica y Neotropical. Ahora se encuentra incluso en las regiones Paleártica y Oriental. Durante el verano de 2015, encontramos altas densidades (> 500 ind./l) de esta especie y bajas abundancias de *Brachionus angularis* en la laguna de la Albufera, donde la cianobacteria (*Microcystis aeruginosa*) fue dominante en la comunidad de fitoplancton. Nuestra hipótesis fue que la cianobacteria era tóxica para *B. angularis* pero no para *B. havanaensis*, por lo que este último superaría al primero en presencia de dicha cianobacteria. Para probar la hipótesis, realizamos experimentos de competencia entre estas dos especies de rotíferos (*B. angularis* y *B. havanaensis*) que coexisten en la laguna de la Albufera de Valencia utilizando como alimento el alga verde (*Nannochloropsis oculata*) y colonias sonicadas de *M. aeruginosa* (células individuales) por separado y juntas (en relación 1:1 basada en biomasa). Los experimentos de competencia basados en el seguimiento del crecimiento poblacional mostraron que en los controles *B. havanaensis* conseguía

un mayor crecimiento poblacional que *B. angularis* cuando lo hacían separadamente. Sin embargo, cultivándolos ambos juntos, *B. havanaensis* tuvo una reducción del crecimiento poblacional (casi 50 %), con respecto a *B. angularis*, en ausencia de *Microcystis* en la dieta. Esta reducción del crecimiento fue mayor que en el tratamiento de *B. havanaensis* individualmente, cuando se cultivó con una dieta mixta de *Microcystis* y *Nannochloropsis oculata*. *Brachionus havanaensis* en todos los tratamientos (solos o en presencia de *B. angularis*) murió completamente cuando se cultivó en 100 % de *M. aeruginosa*. Las tasas de crecimiento poblacional de *B. angularis*, solos o en competencia con *B. havanaensis*, fueron similares cuando se excluyó a *Microcystis* de la dieta. Sin embargo, el cultivo solo con *Microcystis* o junto con *Nannochloropsis* fue altamente perjudicial para el crecimiento poblacional de *B. angularis*. Nuestros resultados mostraron el diferente resultado de los experimentos de competencia entre estos dos rotíferos en función de la presencia o ausencia de cianobacterias tóxicas en la dieta. Nuestro estudio también mostró que una dieta exclusiva de esta cianobacteria era tóxica para ambas especies de rotíferos.

Palabras clave: *Brachionus*, Rotífera, dinámica poblacional, *Microcystis*, *Nannochloropsis*

INTRODUCTION

Competition in aquatic ecosystems is an important natural process that structures the plankton composition (Vanni, 1986). Studies on the competitive interactions within the zooplanktonic groups are usually conducted using large species such as cladocerans (Smith & Cooper, 1982), which differ from the competitive outcome among the smaller sized zooplankton such as rotifers (Gilbert, 1985). For example, cladocerans are capable of breaking and then feeding on toxic filamentous cyanobacteria which rotifers are incapable of (Sikora & Dawidowicz, 2017). Therefore, often due to the mechanical limitation of consuming cyanobacteria, rotifers are incapable of competing with cladocerans. Even when the toxic cyanobacterial colonies are sonicated to single cells and offered as a diet to competing rotifers and cladocerans, the latter usually dominate due to their higher food gathering ability and the mechanical damage (from the powerful swimming currents) they inflict to the rotifer eggs (MacIsaac & Gilbert, 1989). Therefore, it becomes evident that when unequally sized zooplankton groups compete, the smaller groups become competitively inferior especially under higher food levels (Sarma *et al.*, 1996).

When two similar sized zooplankton species compete for food, the outcome depends on many different factors including the relative feeding and filtration rates, the magnitude of population growth rate and initial densities of the competing species (Sarma *et al.*, 1999, Nandini *et al.*, 2007). Also, if the medium contains toxic cyanobacteria, then the relative tolerances of competing species to the toxins are also crucial in deciding the

outcome (Alva-Martínez *et al.*, 2007). For example, when two similar sized rotifers, *Brachionus havanaensis* and *Brachionus calyciflorus* were cultured together on *Microcystis* mixed with green alga at different proportions we observed a more adverse effect on *B. calyciflorus* than on *B. havanaensis* (Alva-Martínez *et al.*, 2009). However, if the competing species have similar sensitivities to toxins, then the competitive outcome is independent of the toxic cyanobacteria. For example, Lin *et al.* (2014) have shown that two rotifers of the family Brachionidae, *Platyonus patulus* and *Brachionus havanaensis* had similar sensitivities to sonicated cells of a toxic strain of *Anabaena* sp. Both species failed to grow in the presences of this cyanobacterium, and thus competition was not influenced by the toxins. Therefore, although cyanobacterial toxicity influences zooplankton growth rates, it is not necessarily the deciding biotic factor that determines the competitive outcome among zooplankton species.

Lake Albufera of Valencia is a shallow hypertrophic waterbody on the Mediterranean Coast of Spain. The plankton of this waterbody has been extensively studied over three decades (Vicente & Miracle, 1992; Romo *et al.*, 2008). The phytoplankton is composed of toxic colonial cyanobacteria, mainly *Microcystis aeruginosa*, while the rotifers are dominated by the genus *Brachionus*. Among the species of *Brachionus*, *B. havanaensis* is common in American epicontinental waters (Segers, 2008). Its natural abundances can be as high as 1000 ind./l (Nandini *et al.*, 2016). It has the ability to reach extremely high population abundances (up to 600 ind./ml, under experimental conditions (Pavón-Meza *et al.*, 2004) and to

resist toxic effects of the colonial cyanobacteria including *Microcystis* spp. Though its occurrence has been reported in temperate European water-bodies (Segers, 2008), it is generally found in low abundances (< 50 ind./l). However, during the summer 2015, we found very high densities of *B. havanaensis* (> 500 ind./l) but low abundances of another brachionid rotifer *B. angularis* in Lake Albufera. The phytoplankton of the lake at that time contained blooms of *Microcystis aeruginosa*. Therefore we hypothesized that low abundances of *B. angularis* were due to the combined effect of competitive pressure from *B. havanaensis* and the toxic effects of *M. aeruginosa*.

The aim of the study was, therefore, to test which of the two natural forces, competition from congeners or toxicity from *Microcystis* had a higher impact on the population growth of *B. havanaensis* and *B. angularis*.

MATERIAL AND METHODS

Zooplankton samples were collected using a plankton net (pore size 50 µm) from Lake Albufera by filtering about 50 L of water. The phytoplankton, dominated by *M. aeruginosa*, was separately (100 L) collected using a plankton net and stored in the dark at 4 °C in a temperature-controlled chamber. From the live zooplankton samples we isolated a few individuals of *Brachionus havanaensis* and *Brachionus angularis* using stereomicroscope in the laboratory. From these, we established a clonal culture for each rotifer starting with a single female. The rotifers were fed the green alga *Nannochloropsis oculata* at a density of 0.5×10^6 cells/ml and maintained in reconstituted moderately hard water (EPA medium). This medium was prepared by dissolving 96 mg NaHCO₃, 60 mg CaSO₄, 60 mg MgSO₄ and 4 mg KCl in one litre of distilled water (Weber, 1993). *Nannochloropsis oculata* was axenically batch-cultured in 2 L glass flasks using Bold's medium (Borowitzka & Borowitzka, 1988). When the alga reached the log phase (after seven days following inoculation at a density of 0.1×10^6 cells/ml), it was harvested and concentrated by centrifugation at 3000 rpm for 5 min. The concentrated alga was rinsed and re-suspended in a small volume (5 ml) of distilled water. The

density of the harvested alga was estimated using a Neubauer haemocytometer.

For obtaining the sonicated cells of *M. aeruginosa*, we harvested the floating colonial cyanobacteria from the cold-chamber by syphoning using a Pasteur pipette. Later, the concentrated colonial *M. aeruginosa* was filtered using different meshes to remove large zooplanktonic organisms and smaller phytoplankton cells. The remaining cyanobacterial composition showed more than 93 % *M. aeruginosa* which was sonicated for four minutes at 20 kHz. Microscopic observations of the sonicated cells revealed that more than 90 % of the biomass was in single-celled form of *M. aeruginosa*. The individual cell size of *M. aeruginosa* and that of *Nannochloropsis oculata* was similar (4-5 µm). However, we used the biomass equivalent of *M. aeruginosa*, *Nannochloropsis oculata* or their mixed diet for rotifers in the test jars.

The population growth experiments for both rotifer species were conducted simultaneously. For the experiments, we used 40 ml glass recipients as test jars, and each jar contained 30 ml EPA medium with one of the chosen diet type and combination. In all we used 36 test jars: *B. havanaensis* alone, *B. angularis* alone and together (competition) = 3 x food types *N. oculata* alone, *M. aeruginosa* alone and their mixed diet = 3 x 4 replicates for each treatment. Thus, into each test jars containing the chosen diet at the density of 0.5×10^6 cells/ml, we individually introduced one of the two rotifer species at an initial density of 1 ind./ml (or both at a ratio of 1:1). The initial composition of rotifers in the test jars consisted of 80 % neonates and 20 % adults. The test jars were maintained in a temperature-controlled chamber set at 22 ± 0.1 °C, pH 7.0–7.4, and with photoperiod light-dark of 16-8 hours diffuse fluorescent illumination.

Following initiation of growth experiments, after every 24h we counted the number of live rotifers in each jar, initially by total count, and as the density of the population increased to 50 ind./ml in some replicates, we used two aliquots of 1 ml each. Following quantification of the rotifers density, we replaced them into a freshly prepared medium with an appropriate algal-cyanobacterial composition.

Based on the data collected, we derived the rate of population increase (r) per day using the exponential growth equation: $r = (\ln N_t - \ln N_0)/t$, where N_0 = initial rotifer density; N_t = rotifer density after time t ; and t = time in days. In treatments where a peak of population abundance was not evident, the growth rate was calculated from the slope between $\ln N$ and time (Sibly & Hone, 2002) and when growth did not occur, the r was not derived.

The peak population density and the rate of population increase for each rotifer species were statistically treated using two-way ANOVA. For

multiple comparisons, we used a post hoc (Tukey) test (SigmaPlot ver. 12).

RESULTS

Population growth curves of *Brachionus havanaensis* cultured separately and together with *B. angularis* fed *Nannochloropsis oculata* (100 %), sonicated *Microcystis aeruginosa* (100 %) or on the mixed diet (1:1 ratio of *N. oculata*: *M. aeruginosa*) differed depending on the treatment (Fig. 1). When cultured on *N. oculata*, in the presence or absence of competition from *B. angu-*

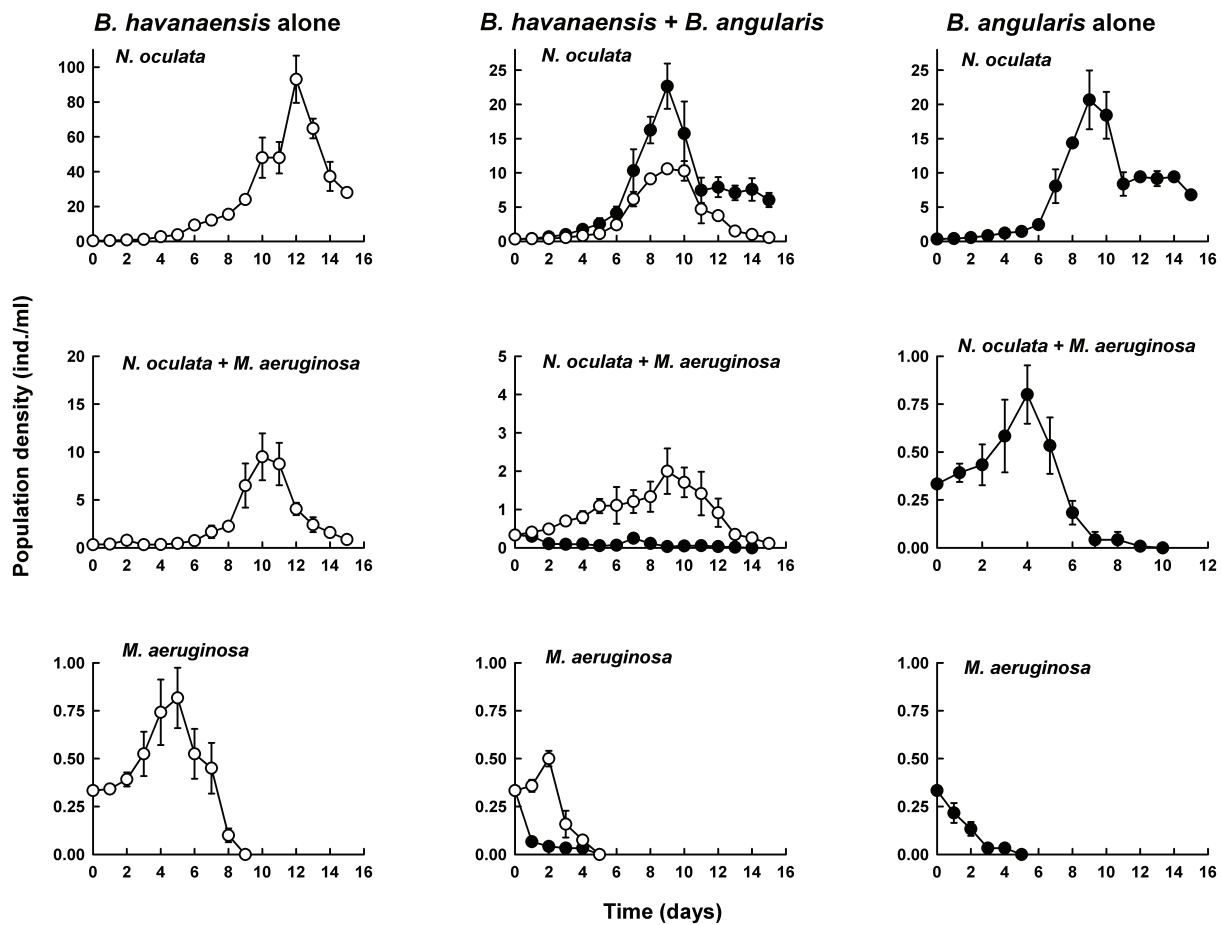


Figure 1. Population growth curves of *Brachionus havanaensis* and *B. angularis* cultured alone or together on *Nannochloropsis oculata*, sonicated cells of *Microcystis aeruginosa* or on the mixed diet. Shown are the mean \pm standard errors based on four replicates. Note the differences in the scale on Y-axis. Open circle: *B. havanaensis*; closed circle: *B. angularis*. *Curvas de crecimiento poblacional de Brachionus havanaensis y B. angularis cultivadas solas o juntas, usando Nannochloropsis oculata, células sonicadas de Microcystis aeruginosa o en la dieta mixta. Se muestran el promedio \pm errores estándar basados en cuatro repeticiones. Nótese las diferencias en la escala en el eje Y. Círculo abierto: B. havanaensis; Círculo cerrado: B. angularis.*

laris, *B. havanaensis* showed an initial lag phase of about four days, an exponential phase of about seven days and after that the population began to decline. With the inclusion of sonicated *M. aeruginosa* in the diet, *B. havanaensis* had a longer lag phase (6 days). On a diet of *M. aeruginosa* alone, *B. havanaensis* grew until 6 days and then declined but on this diet and the presence of the competitor the decline began after 3 days. The growth curves of *B. angularis* cultured on a diet of *N. oculata*, alone or in the presence of a competitor were similar. However, with the inclusion of *M. aeruginosa*, the population of *B. angularis* hardly grew beyond the inoculation density and when fed on an exclusive diet of *M. aeruginosa*, the populations of *B. angularis* completely collapsed by the fifth day.

Depending on the treatment and the test rotifer species, the peak population abundances of *B. havanaensis* and *B. angularis* varied from 1-95 and 1-23 ind./ml, respectively. The presence of a competitor or the toxic diet had differential effects on the maximal population densities

achieved by the tested brachionid rotifers. For example, for *B. havanaensis*, the presence of *M. aeruginosa* (in the mixed diet) or the presence of competitor (in the absence of cyanobacteria) had a similar adverse effect on the peak population density. On the other hand, for *B. angularis* in the absence of *M. aeruginosa*, the presence of a competitor, *B. havanaensis*, had no adverse effect on the peak population densities.

The rates of population increase of *B. havanaensis* and *B. angularis* in treatments containing only *N. oculata* but without competition were 0.40 and 0.30 /d, respectively. When the mixed diet (*Nannochloropsis* and *Microcystis*) was offered as food, the growth rates of *B. angularis*, but not *B. havanaensis*, became negative. For either rotifer species, 100 % *M. aeruginosa* did not permit sustained populations, and hence the derivation of growth rates was not feasible (Table 1).

Statistically, the peak population density of *B. havanaensis* was significantly influenced by the diet type, competition and their interaction ($p <$

Table 1. Data on the peak population abundance (ind./ml) and the rate of population increase (r) per day of *B. havanaensis* and *B. angularis* cultured separately and together in the presence (N+M or M) and absence (N) of sonicated cells of *M. aeruginosa*. For each rotifer species data carrying the same alphabet are not significant ($p > 0.05$, Tukey test). N = *Nannochloropsis oculata*, M = *Microcystis aeruginosa*; N+M = *N. oculata* + *M. aeruginosa* (50 % each). Datos sobre la abundancia máxima de la población (ind./ml) y la tasa de aumento de la población (r) diaria de *B. havanaensis* y *B. angularis* cultivados por separado y juntos en presencia (N+M o M) y ausencia (N) de células sonicadas de *M. aeruginosa*. Para cada especie de rotíferos los datos que llevan la misma letra no son significativos ($p > 0.05$, test de Tukey). N = *Nannochloropsis oculata*, M = *Microcystis aeruginosa*; N+M = *N. oculata* + *M. aeruginosa* (al 50 % cada una).

Rotifer species	Test conditions / Treatment					
	Without competition			With competition		
	N	N+M	M	N	N+M	M
Peak population density (ind./ml)						
<i>B. havanaensis</i>	95±12 ^a	10±2 ^{b,d}	1±0 ^c	11±1 ^d	2±1 ^c	1±0 ^c
<i>B. angularis</i>	21±3 ^a	1±1 ^b	1±0 ^b	23±3 ^a	1±1 ^b	1±0 ^b
Rate of population increase per day						
<i>B. havanaensis</i>	0.403± 0.031 ^a	0.174± 0.020 ^b	-	0.252± 0.017 ^c	0.078± 0.017 ^d	-
<i>B. angularis</i>	0.302± 0.011 ^a	-0.076± 0.019 ^b	-	0.233± 0.031 ^a	-0.146± 0.010 ^b	-

0.001 two-way ANOVA). However, for *B. angularis*, it was only the diet type that had a significant effect on the peak population abundances; neither the presence of competitor nor the interaction of competitor x diet had any significant impact on this parameter ($p < 0.05$). The rate of population increase of both the rotifer species was significantly affected ($p < 0.01$) by the diet type and the competition, but the interaction was not ($p > 0.05$, Table 2).

DISCUSSION

The dominance of the typical American planktonic rotifer species, *B. havanaensis*, in the zooplankton of Lake Albufera suggests that it has an invasive tendency (Devin & Beisel, 2007). It is often found in waterbodies dominated by toxic cyanobacteria (Nandini *et al.*, 2005). Zooplankton composition of Lake Albufera over a three-decade period indicates the presence of

Table 2. Results of the two-way ANOVA conducted for the peak population density and the rate of population increase per day of *Brachionus havanaensis* (Bh) and *Brachionus angularis* (Ba) cultured separately or together (Factor A: competition) using *Nannochloropsis oculata*, *Microcystis aeruginosa* or on their mixed diet (Factor B: diet types). *Resultados del ANOVA bidireccional realizado para la densidad máxima de población y la tasa de aumento de población por día de Brachionus havanaensis (Bh) y Brachionus angularis (Ba) cultivados por separado o juntos (Factor a: competición) usando Nannochloropsis oculata, Microcystis aeruginosa o su mezcla como dieta (Factor B: tipos de dieta).*

Source of Variation	DF	SS	MS	F	<i>P</i>
Peak population abundance					
Bh					
Competition (A)	1	5645.734	5645.734	60.596	<0.001
Diet (B)	2	13603.104	6801.552	73.002	<0.001
Interaction of A X B	2	8685.165	4342.582	46.609	<0.001
Error	18	1677.059	93.170		
Ba					
Competition (A)	1	2.100	2.100	0.174	0.681
Diet (B)	2	2492.614	1246.307	103.330	<0.001
Interaction of A X B	2	7.941	3.970	0.329	0.724
Error	18	217.099	12.061		
Rate of population increase					
Bh					
Competition (A)	1	0.061	0.061	31.040	<0.001
Diet (B)	1	0.162	0.162	82.634	<0.001
Interaction of A X B	1	0.003	0.003	1.574	0.234
Error	12	0.024	0.002		
Ba					
Competition (A)	1	0.019	0.019	12.297	0.004
Diet (B)	1	0.573	0.573	368.518	<0.001
Interaction of A X B	1	0.000	0.000	0.001	0.992
Error	12	0.019	0.002		

rotifer genera such as *Keratella*, *Polyarthra* and *Anuraeopsis*. Though several members of the genus *Brachionus* are commonly reported in this waterbody (Oltra *et al.*, 2001), *B. havanaensis* was not observed from the extensive zooplankton collections carried out during 2010-2011 (Sarma *et al.*, 2019) suggesting that this species was recently established in the Lake Albufera. *Brachionus havanaensis* is predominantly distributed in the Nearctic and Neotropical regions. Later, it was introduced into the Palearctic and Oriental regions (Segers, 2008). During the summer of 2015, we encountered much higher abundances of this species suggesting its ability to outcompete other members of *Brachionus* in this waterbody. Field collections from Mexican waterbodies too indicate its occurrence in relatively higher densities, ca. 1000 ind./l during certain months (Nandini *et al.*, 2016). There appear to be several factors favouring its dominance in different waterbodies: these include relatively higher resistance to natural toxins including cyanotoxins, higher population growth rates (Pavón-Meza *et al.*, 2004, 2008) and non-preferred prey item for invertebrate predators such as larval insects and copepods (Enríquez-García *et al.*, 2013).

Alva-Martínez *et al.* (2009) have shown that on a mixed diet consisting of a green alga and toxic *Microcystis aeruginosa*, the population of *Brachionus calyciflorus* crashed with increasing proportion of cyanobacteria in the diet, while under similar conditions, *B. havanaensis* showed consistently higher growth rates. Also, whether grown separately or together with *B. calyciflorus*, *B. havanaensis* was always numerically more abundant than the other competing brachionid rotifers regardless of the proportion of *Microcystis* in the diet. This confirms the resistance of *B. havanaensis* to *M. aeruginosa* over other brachionids. This is similar to our observations in this work where in the absence of competition when *B. havanaensis* was cultured on a mixed diet of *Nannochloropsis* and *Microcystis*, the population density was reduced to one-tenth; under comparable conditions, the population of *B. angularis* was reduced to one-twentieth. González-Pérez *et al.* (2018) have also documented that *B. havanaensis* is more resistant for

two generations than another rotifer from the same family Brachionidae, *Platyonus patulus*, when exposed to triclosan, an emerging contaminant in freshwater ecosystems. In the presence of strong invertebrate predators such as cyclopoids, *B. havanaensis* increases its growth rate than in the presence of less rapacious taxa such as turbellarians (Nandini *et al.*, 2014). Although adult copepods such as *Acanthocyclops*, consume *B. havanaensis* (García *et al.*, 2011), it is not a preferred item for many invertebrate predators (Nandini *et al.*, 2003) possibly due to long posterior spines (Garza-Mouriño *et al.*, 2005). In Lake Albufera, cyclopoids such as *Acanthocyclops americanus* are important predators that feed on rotifers including brachionids.

Competition for food between two (e.g., Sarma *et al.*, 1999) or more (e.g., Fernández-Araiza *et al.*, 2005) brachionid rotifers has received considerable attention since the seminal works on *Brachionus rubens* and *B. calyciflorus* by Rothhaupt (1988). These studies have shown that both biotic (e.g., food level, inoculation density) and abiotic (e.g., salinity, temperature) factors as well as species-specific characters (e.g., body size, intrinsic rate of population increase) influence the competitive outcome. Also, the presence of toxic substances, both xenobiotic (e.g., pharmaceuticals) and natural (e.g., cyanotoxins, salinity) are also known to affect the competition between two or more zooplankton species (Rebolledo *et al.*, 2018). In the present work, the competitive outcome between *B. havanaensis* and *B. angularis* varied depending on the presence or absence of *M. aeruginosa*. In the absence of cyanobacteria, *B. angularis* had an adverse effect on *B. havanaensis* by reducing its population densities by about 90 % and growth rates by about 65 %. On the other hand, under comparable conditions, *B. havanaensis* was able to affect adversely (by about 20 %) the growth rates but not the population abundances of *B. angularis*. However, in the presence of toxic *Microcystis* mixed with *Nannochloropsis*, the population of *B. angularis* completely crashed (*r* being negative) before termination of the experiments, but under similar conditions, *B. havanaensis* was able to maintain its population with positive growth rates. These results suggest that

higher abundances of *B. havanaensis* in Lake Albufera were not possibly due to its competitive superiority over *B. angularis* but the interference of toxicity from *M. aeruginosa* which favoured *B. havanaensis*. It is important to note that the toxic effects of *M. aeruginosa* on the responses of the population level responses of both *B. angularis* and *B. havanaensis* are possibly modulated by the presence of the green alga (Paerl, 2018). This is evident in mixed diet treatments containing the green alga which possibly minimized the adverse effects of *Microcystis*. The fact that both rotifer species died within a week when exposed to an exclusive diet of *M. aeruginosa* suggests that this strain of cyanobacterium was indeed toxic. This is further confirmed from previous studies from this waterbody where the microcystin concentration in the lake was as high as 16 µg/l (Romo *et al.*, 2008) and the inclusion of sonicated cells of *M. aeruginosa* in the diet significantly reduced the growth rates of the cladocerans (Nandini *et al.*, 2017).

The peak population densities (up to 90 ind./ml) observed here for *B. havanaensis* are not uncommon for this species under laboratory conditions. For example, Pavón-Meza *et al.* (2004) observed that *B. havanaensis* is capable of reaching extremely high densities (up to 600 ind./ml) when fed on the green alga *Chlorella*. We were able to grow *B. angularis* to peak densities of about 20 ind./ml. Miracle *et al.* (2014) have cultured *B. angularis* isolated from Lake Albufera and fed on different size fractions of lake seston (0-15 µm particle size). They obtained peak population densities of about 20 ind./ml of *B. angularis*, similar to our observations in this study. The rate of population increase is yet another significant parameter often considered for interpreting the competitive outcome among different zooplankton species. For example, when *Anuraeopsis fissa* and *B. calyciflorus* were cultured together, the ability of the latter to replace the former was higher due to its higher population growth rates ($r = 1.5$ per day) than that of *A. fissa* ($r = 0.9$ per day) (Sarma *et al.*, 1996). In the present work, the r of *B. havanaensis* varied from 0.25 to 0.40/d depending on the presence or the absence of the competitor. Pavón-Meza *et al.* (2004) reported the r for this

species in the range of 0.02 to 0.29 per day. For the strain of *B. havanaensis* we used here the r was slightly higher, but within the range reported for *Brachionus* (0.12 to 3.7 per day, Miracle & Serra, 1989). When *Nannochloropsis* alone was used as a diet, the r of *B. angularis* varied from 0.23 to 0.30 per day which is within the range (0.07 - 0.35 per day) known for this species (Walz, 1987).

The present work suggests the possible adverse effects of the exotic *B. havanaensis* on locally occurring brachionid rotifers in interacting with cyanobacterial blooms, common in the lake Albufera (Vicente & Miracle, 1992). *Brachionus havanaensis*, being pantropical (Segers, 2008), may easily colonize temperate waterbodies with the future projections of global warming (Hays *et al.*, 2005). Increase in temperature also favours bloom formation in *Microcystis*. Thus, both these factors together may adversely impact locally adapted brachionids in freshwater ecosystems of temperate regions.

CONCLUSIONS

An increase in global trade and transport has increased the mobility of organisms and broken down ecological barriers to the transport of species around the world. Rotifers, in particular, are easily transported and established across the world (Duggan, 2010). The ability of *B. havanaensis* to withstand competition and predation pressure and grow on diets such as cyanobacteria indicates that it can be a successful invasive species and can survive in regions outside its native continent (Koste, 1978). Although originally reported from the Nearctic and neotropical regions, it is now found in Oriental and Palearctic regions (Segers, 2008). It has, however been suggested that the presence of the same species in different parts of the world could be a case of cryptic speciation as was the case in *Brachionus plicatilis* (Pociecha *et al.*, 2007) and perhaps *B. havanaensis* is part of a species complex.

ACKNOWLEDGEMENTS

We thank two anonymous reviewers for improving our presentation. SSSS & SN thank the

programme PASPA (DGAPA) which supported our stay at the University of Valencia, Burjassot (Valencia), Spain.

REFERENCES

- ALVA-MARTÍNEZ, A. F., SARMA, S. S. S. & NANDINI, S. 2007 Effect of mixed diets (cyanobacteria and green algae) on the population growth of the cladocerans *Ceriodaphnia dubia* and *Moina macrocopa*. *Aquatic Ecology*, 41(4):579–585. DOI: 10.1007/s10452-007-9115-1
- ALVA-MARTÍNEZ, A. F., R. FERNÁNDEZ, R., S. S. S. SARMA & S. NANDINI. 2009. Effect of mixed toxic diets (*Microcystis* and *Chlorella*) on the rotifers *Brachionus calyciflorus* and *Brachionus havanaensis* cultured alone and together. *Limnologica*, 39(4):302–305. DOI: 10.1016/j.limno.2009.06.002
- BOROWITZKA, M. A. & L. J. BOROWITZKA. 1988. *Micro-algal biotechnology*. Cambridge University Press, London.
- DEVIN, S. & BEISEL, J.-N. 2007. Biological and ecological characteristics of invasive species: a gammarid study. *Biological Invasions*, 9:13–24.
- DUGGAN, I. C. 2010. The freshwater aquarium trade as a vector for incidental invertebrate fauna. *Biological Invasions*, 12:3757–3770. DOI: 10.1007/s10530-010-9768-x
- ENRÍQUEZ-GARCÍA, C., S. NANDINI & S. S. SARMA. 2013. Feeding behaviour of *Acanthocyclops americanus* (Marsh) (Copepoda: Cyclopoida). *Journal of Natural History*, 47(5-12):853–862. DOI: 10.1080/00222933.2012.747636
- FERNÁNDEZ-ARAIZA, M. A., S. S. S. SARMA & S. NANDINI. 2005. Combined effects of food concentration and temperature on competition among four species of *Brachionus* (Rotifera). *Hydrobiologia*, 546:519–534. DOI: 10.1007/s10750-005-4295-y
- GARCÍA, C. E., S. NANDINI & S. S. S. SARMA. 2011. Demographic characteristics of the copepod *Acanthocyclops americanus* (Sars, 1863) (Copepoda: Cyclopoida) fed mixed algal (*Scenedesmus acutus*)-rotifer (*Brachionus havanaensis*) diet. *Hydrobiologia*, 666:59–69. DOI: 10.1007/s10750-010-0209-8
- GARZA-MOURIÑO, G., M. SILVA-BRIANO, S. NANDINI, S. S. S. SARMA & M. E. CASTELLANOS-PÁEZ. 2005. Morphological and morphometrical variations of selected rotifer species in response to predation: a seasonal study of selected brachionid species from Lake Xochimilco (Mexico). *Hydrobiologia*, 546:169–179. DOI: 10.1007/s10750-005-4114-5
- GILBERT, J. J. 1985. Competition between rotifers and *Daphnia*. *Ecology*, 66(6):1943–1950. DOI: 10.2307/2937390
- GONZÁLEZ-PÉREZ, B. K., S. S. S. SARMA, M. E. CASTELLANOS-PÁEZ & S. NANDINI. 2018. Multigenerational effects of triclosan on the demography of *Platyonus patulus* and *Brachionus havanaensis* (Rotifera). *Ecotoxicology and Environmental Safety*, 147:275–282. DOI: 10.1016/j.ecoenv.2017.08.049
- HAYS, G. C., A. J. RICHARDSON & C. ROBINSON. 2005. Climate change and marine plankton. *TRENDS in Ecology and Evolution*, 20(6):337–344. DOI: 10.1016/j.tree.2005.03.004
- KOSTE, W. 1978. *Rotatoria. Die Rädertiere Mitteleuropas begründet von Max Voigt-Monogononta*. 2nd ed. Gebrüder Borntraeger, Berlin and Stuttgart. 2 vols.
- LIN, Q., S. S. S. SARMA, S. NANDINI & B. P. HAN. 2014. Effect of cyanobacterium on competition between rotifers: a population growth study. *Inland Waters*, 4(3):319–326. DOI: 10.5268/IW-4.3.547
- MACISAAC, H. J. & J. J. GILBERT. 1989. Competition between rotifers and cladocerans of different body sizes. *Oecologia*, 81:295–301. DOI: 10.1007/BF00377074
- MIRACLE, M. R. & M. SERRA. 1989. Salinity and temperature influence in rotifer life history characteristics. *Hydrobiologia*, 186(1): 81–102. DOI: 10.1007/BF00048900
- MIRACLE, M. R., E. VICENTE, S. S. S. SARMA & S. NANDINI. 2014. Planktonic rotifer feeding in hypertrophic conditions. *International Review of Hydrobiology* 99: 141–150. DOI: 10.1002/iroh.201301714
- NANDINI, S., R. PÉREZ-CHÁVEZ & S. S. S.

- SARMA. 2003. The effect of prey morphology on the feeding behaviour and population growth of the predatory rotifer *Asplanchna sieboldi*: A case study using five species of *Brachionus* (Rotifera). *Freshwater Biology*, 48(12):2131–2140. DOI: 10.1046/j.1365-2427.2003.01149.x
- NANDINI, S., P. RAMÍREZ-GARCÍA & S. S. S. SARMA. 2005. Seasonal variations in the species diversity of planktonic rotifers in Lake Xochimilco, Mexico. *Journal of Freshwater Ecology* 20(2):287–294. DOI: 10.1080/02705060.2005.9664968
- NANDINI, S., C. ENRÍQUEZ-GARCÍA & S. S. S. SARMA. 2007. A laboratory study on the demography and competition of three species of littoral cladocerans from Lake Huetzalin, Xochimilco, Mexico. *Aquatic Ecology*, 41(4):547–556. DOI: 10.1007/s10452-007-9116-0
- NANDINI, S., F. S. ZÚÑIGA-JUÁREZ & S. S. S. SARMA. 2014. Direct and indirect effects of invertebrate predators on population level responses of the rotifer *Brachionus havanaensis* (Rotifera). *International Review of Hydrobiology*, 99:107–116. DOI: 10.1002/iroh.201301709
- NANDINI, S., P. RAMÍREZ-GARCÍA & S. S. S. SARMA. 2016. Water quality indicators in Lake Xochimilco, Mexico: zooplankton and *Vibrio cholera*. *Journal of Limnology*, 75(1):91–100. DOI: 10.4081/jlimnol.2015.1213
- NANDINI, S., M. R. MIRACLE, E. VICENTE, S. S. S. SARMA & R. D. GULATI. 2017. Microcystis extracts and single cells have differential impacts on the demography of cladocerans: a case study on *Moina* cf. *micrura* isolated from the Mediterranean coastal shallow lake (L'Albufera, Spain). *Hydrobiologia*, 798(1):127–139. DOI: 10.1007/s10750-016-2665-2
- OLTRA, R., M. T. ALFONSO, M. SAHUQUILLO & M. R. MIRACLE. 2001. Increase of rotifer diversity after sewage diversion in the hypertrophic lagoon, Albufera of Valencia, Spain. *Hydrobiologia*, 446(447):213–220. DOI: 10.1023/A:10175678
- PAERL, H. W. 2018. Mitigating toxic planktonic cyanobacterial blooms in aquatic ecosystems facing increasing anthropogenic and climatic pressures. *Toxins* 2018, 10, 76; DOI: 10.3390/toxins10020076
- PAVÓN-MEZA, E. L., S. S. S. SARMA & S. NANDINI. 2004. Combined effects of food (*Chlorella vulgaris*) concentration and temperature on the population growth of *Brachionus havanaensis* (Rotifera: Brachionidae). *Journal of Freshwater Ecology*, 19(4):521–530. DOI: 10.1080/02705060.2004.9664731
- PAVÓN-MEZA, E. L., S. S. S. SARMA & S. NANDINI. 2008. Combined effects of temperature, food availability and predator's (*Asplanchna girodi*) allelochemicals on the demography and population growth of *Brachionus havanaensis* (Rotifera). *Allelopathy Journal*, 21(1):95–106.
- POCIECHA, A., W. SOLARZ, K. NAJBEREK & E. WILK-WOZNIAK. 2016. Native, alien, cosmopolitan, or cryptogenic? A framework for clarifying the origin status of rotifers. *Aquatic Biology*, 24:141–149. DOI: 10.3354/ab00644
- REBOLLEDO, U. A., S. NANDINI, S. S. S. SARMA, J. C. R. REYES & G. A. R. MONTES DE OCA. 2018. Demographic and competition studies on *Brachionus ibericus* and *Proales similis* in relation to salinity and algal (*Nannochloropsis oculata*) density. *Aquaculture International*, 26(2): 629–644. DOI: 10.1007/s10499-017-0233-z
- ROMO, S., A. GARCÍA-MURCIA, M. J. VILLENA, V. SÁNCHEZ & A. BALLES-TER. 2008. Tendencias del fitoplancton en el lago de la Albufera de Valencia e implicaciones para su ecología, gestión y recuperación. *Limnetica*, 27(1):11–28.
- ROTHHAUPT, O. 1988. Mechanistic resource competition theory applied to laboratory experiments with zooplankton. *Nature*, 333:660–662. DOI: 10.1038/333660a0
- SARMA, S. S. S., N. IYER & H. J. DUMONT. 1996. Competitive interactions between herbivorous rotifers: importance of food concentration and initial population density. *Hydrobiologia*, 331:1–7. DOI: 10.1007/BF00025402
- SARMA, S. S. S., M. A. FERNÁNDEZ-ARAIZA & S. NANDINI. 1999. Competition between

- Brachionus calyciflorus* Pallas and *Brachionus patulus* (Müller) (Rotifera) in relation to algal food concentration and initial population density. *Aquatic Ecology*, 33(4):339–345. DOI: 10.1023/A:1009912816400
- SARMA, S. S. S., M. R. MIRACLE, S. NANDINI & E. VICENTE. 2019. Predation by *Acanthocyclops americanus* (Copepoda: Cyclopoida) in the hypertrophic shallow waterbody, Lake Albufera (Spain): field and laboratory observations. *Hydrobiologia* (in press). DOI: 10.1007/s10750-018-3546-7
- SEGBERS, H. 2008. Global diversity of rotifers (Rotifera) in freshwater. *Hydrobiologia*, 595:49–59. DOI: 10.1007/s10750-007-9003-7
- SIBLY, R. M. & J. HONE, 2002. Population growth rate and its determinants: an overview. *Philosophical Transactions of the Royal Society, London B: Biological*, 357:1153–1170. DOI: 10.1098/rstb.2002.1117
- SIKORA, A. & P. DAWIDOWICZ. 2017. Breakage of cyanobacterial filaments by small- and large sized *Daphnia*: are there any temperature-dependent differences? *Hydrobiologia*, 798:119–126. DOI: 10.1007/s10750-015-2436-5
- SMITH, D. W. & S. D. COOPER. 1982. Competition among Cladocera. *Ecology*, 63(4):1004–1015. DOI: 10.2307/1937240
- VANNI, M. J. 1986. Competition in zooplankton communities: Suppression of small species by *Daphnia pulex*. *Limnology and Oceanography*, 31(5):1039–1056.
- VICENTE, E. & M. R. MIRACLE. 1992. The coastal lagoon Albufera de Valencia: an ecosystem under stress. *Limnetica*, 8:87–100.
- WALZ, N., 1987. Comparative population dynamics of the rotifers *Brachionus angularis* and *Keratella cochlearis*. *Hydrobiologia*, 147:209–213. DOI: 10.1007/BF00025744
- WEBER, C. I. 1993. *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms*. 4th ed. United States Environmental Protection Agency, Cincinnati, Ohio, EPA/600/4-90/027F.