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Combined effects of zinc and algal food on the competition between planktonic rotifers, *Anuraeopsis fissa* and *Brachionus rubens* (Rotifera)

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Abstract We evaluated the combined effects of algal (*Chlorella vulgaris*) food levels (low, 0.5×10^6 (or 2.9 µg C ml^{-1}); and high, 1×10^6 cells ml^{-1} (or 5.8 µg C ml^{-1})) and zinc concentrations (0, 0.125, and 0.250 mg l^{-1} of $ZnCl_2$) on the competition between two common planktonic rotifers *Anuraeopsis fissa* and *Brachionus rubens* using their population growth. Median lethal concentration data (LC_{50}) (mean ± 95% confidence intervals) showed that *B. rubens* was more resistant to zinc (0.554 ± 0.08 mg l^{-1}) than *A. fissa* (0.315 ± 0.07 mg l^{-1}). *A. fissa* when grown alone or with Zn was always numerically more abundant than *B. rubens*. When grown in the absence of zinc, under low- and high-food levels, the peak abundances of *A. fissa* varied from 251 ± 24 to 661 ± 77 ind. ml^{-1}, respectively, and the corresponding maxima for *B. rubens* were 52 ± 3 and 102 ± 18 ind. ml^{-1}. At a given food level, competition for food reduced the peak abundances of both rotifers considerably. Increase in Zn concentration also lowered the rotifer abundances. The impact of zinc on competition between the two-rotifer species was evident at low-food level, mainly for *A. fissa*. At zinc concentrations of 0 and 0.125 mg l^{-1}, the populations of both rotifers continued to grow for about 10 days, but thereafter *B. rubens* began to decline. Role of zinc on the competitive outcome of the two species is discussed in relation to the changing algal densities in natural water bodies.

Keywords Heavy metal · Population growth · Zooplankton · *Chlorella*

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Introduction

Natural biotic forces of competition and predation structure the dynamics, including diversity and abundance of both phytoplankton and zooplankton (Lampert and Sommer 1997). Rotifers, cladocerans, and copepods are the principal groups of zooplankton in many freshwater ecosystems (Hutchinson 1967). In nature rotifers suffer from cladoceran competition, which generally results in the suppression of Rotifera by Cladocera (Gilbert 1988). Overriding factors like food concentration and initial densities of the competing species may also influence the outcome of inter and intra-zooplankton competition (Sarma et al. 1996). In freshwaters the species diversity of rotifers is generally higher among the three principal zooplankton groups and since they are also numerically more abundant, they are more likely to experience intense competition for longer periods in ponds and lakes (Rothhaupt 1990).

While many studies on competition within zooplankton are aimed at understanding the ecosystem functioning (Lampert and Sommer 1997), the role of toxicants affecting the natural balance among the diverse rotifer species is not well explored (Snell and Janssen 1995). Mesocosm studies, where selected toxicants are added to water from chosen waterbodies, comprise a well-known contrivance in understanding the impact of toxicants in near-natural conditions. However, to follow such an approach in field studies is both difficult and expensive. Moreover, it is not easy to differentiate between the influences of abiotic factors from those of the toxicants. Results of simple laboratory tests may be useful in predicting the community changes under field conditions due to toxicant stress (Jak et al. 1996). Competition between two or more species in nature arises largely under food-limited conditions (Rothhaupt 1990). Therefore, in ecotoxicological studies, food density is fixed so as to prevent its influence on the outcome of competition between the species subject to toxic stress (Muyssen et al. 2002) or it is offered at different levels permitting evaluation of its impact on the toxicity of a given substance (Rao and Sarma 1990).

The alarming disparity between the permissible limits of zinc in drinking water and the concentrations actually observed in certain freshwater bodies in Mexico (Cervantes and Moreno-Sánchez 1999) justifies assessing the toxicology of this metal on aquatic biota. Unlike metals such as Cd, Pb, and Hg, zinc is essential for the metabolism of all organisms (Bryan 1976). However, at high concentrations, this metal causes reduced reproduction and survival in most aquatic animals including zooplankton (Chapman et al. 2003; Vesela and Vijverberg 2007). Due to its species-specific influence, zinc affects various zooplankton groups differently, thus affecting the competitive interactions among them (Snell and Janssen 1995).

Population growth studies are useful in understanding competition among zooplankters both in the presence (Sarma et al. 2003a) and in the absence of toxicants (Sarma and Nandini 2004). Unlike age-specific demographic studies, where the number of test animals per cohort is constant and diminishes as they approach senescence, in population growth studies, the test animals generally increase with time and their juveniles replace aging individuals, so that at any given time the population is composed of different age-classes (Krebs 1985). This facilitates easy detection and quantification of adverse effects of toxicants at population level (Halbach et al. 1983).

The response of zooplankton species when exposed to sublethal concentrations of toxicants including heavy metals is generally the reduced offspring production or neonate survival both of which are eventually reflected in population growth (Kammenga and Laskowski 2000). Using this approach an attempt was made in this study to evaluate the combined effects of algal food level and zinc concentration on the competition between two common planktonic rotifers *Anuraeopsis fissa* Gosse, 1851 and *Brachionus rubens* Ehrenberg, 1838 using population growth.

Material and methods

Culture of test organisms

Experiments were conducted using two common and co-occurring rotifer species, which differ in their body sizes: *Anuraeopsis fissa* (mean length, 70 µm) and *Brachionus rubens* (mean length, >120 µm). The animals were isolated from a pond at the Benemirita Autonomous University of Puebla, Puebla City (Mexico) (location: 17°52′–20°50′ N and 96°43′–99°04′ W). Both species were raised separately in stock cultures from single parthenogenetic females, using EPA medium. This medium was prepared by dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$, and 4 mg KCl in 1 l of distilled water (Weber 1993). *Chlorella vulgaris* a single-celled green alga harvested in the exponential phase of its growth was used as food. This alga was batch-cultured using Bold's basal medium (Borowitzka and Borowitzka 1988). The physical conditions for raising stock cultures and for the experiments were similar: pH 7.1–7.3, temperature 23 ± 1°C, continuous but diffused fluorescent illumination.

Acute toxicity tests

Both acute and chronic toxicity tests were carried out using analytical grade zinc chloride, $ZnCl_2$ (Sigma Chemicals, Inc.). Stock solution of 1000 mg l^{-1} $ZnCl_2$ was prepared in distilled water, from which the desired concentrations of $ZnCl_2$ were prepared using EPA medium. For acute toxicity tests (LC_{50}), we used five $ZnCl_2$ and a control (0, 0.125, 0.250, 0.500, 1.00, and 2.00 mg l^{-1}). LC_{50} tests were conducted using neonates collected <3 h following hatching for both rotifer species. The parthenogenetic eggs and the neonates from the mass cultures were isolated following Sarma (1985), but since *A. fissa* was smaller, we used a smaller mesh of 30–40 µm pore size. For each rotifer species we used 24 test jars (control and 5 metal concentrations × 4 replicates in each case = 6 × 4 = 24) of 50 ml capacity containing 20 ml medium of chosen $ZnCl_2$ concentration. We introduced 20 neonates of one of the two-rotifer species into each test jar, picking the animals under a stereomicroscope at 20× magnification with a finely drawn Pasteur pipette. The neonates in the jars were not fed during the experiment. After 24h we counted the number of dead and live individuals in each replicate. LC_{50} was derived following the Probit method (Finney 1971).

Chronic toxicity tests

Since population growth studies require a certain quantity of food, we used two algal (*Chlorella*) densities according to Pavón-Meza et al. (2004): low, 0.5×10^6 cells ml^{-1} (or 2.9 µg C ml^{-1}) and high, 1.0×10^6 cells ml^{-1} (or 5.8 µg C ml^{-1}). For both rotifer species we selected three $ZnCl_2$ concentrations (0, 0.125, and 0.250 mg l^{-1}). The rotifer species were treated in three combinations: (*A. fissa* alone; *B. rubens* alone; and *A. fissa* + *B. rubens*). For each treatment we used four replicates. Into each of these jars containing 20 ml medium with specified algal food density and $ZnCl_2$ concentration combination, we introduced one or both the rotifer species with an initial density of 20 ind. per jar of each species when alone, but ten of each of the two species per jar when both species were used. Following initiation of the experiment, we quantified daily the total number of living rotifers in each replicate (whole count or two aliquots of 1 ml each, depending on the rotifer density) and then transferred the animals to fresh jars with appropriate zinc chloride-algal food combinations. We terminated the experiment after 3 weeks by which time most replicates showed stabilization trends. For each treatment, data of peak population abundance per replicate (Sarma et al. 1998) were subjected to 3-way analysis of variance (ANOVA, Statistica version 5). The data were assessed for homogeneity of variance and normality using residual analysis (plots of residual versus means using descriptive statistics) (Sokal and Rohlf 2000).

Results

Acute toxicity tests

The concentration of zinc (as $ZnCl_2$), which resulted in 50% death to the rotifer population of each species derived through probit method, was considered as the median lethal concentration (LC_{50} 24 h bioassay). Data on the LC_{50} ($ZnCl_2$ concentration) tests indicated that *B. rubens* (0.554 ± 0.08 mg l^{-1}, LC50 ± 95% confidence intervals) was more resistant than *A. fissa* (0.315 ± 0.07 mg l^{-1}).

Chronic toxicity tests

For both *A. fissa* and *B. rubens*, population growth curves showed little lag phase, irrespective of if the two were grown separately or together, with or without the presence of $ZnCl_2$. In all cases, the population of the two-rotifer species began to increase soon after the initiation of the experiments. However, regardless of the presence of zinc, *A. fissa* was always more abundant than *B. rubens*. Moreover, regardless of the concentration of zinc in the medium, increasing food concentration resulted in increase in population densities of both the rotifers. At the lower food level, the abundance of both rotifer species decreased. In treatments without addition of zinc, both the rotifer species were affected by the presence of the other compared to those grown separately (Figs. 1 and 2).

The peak abundances of *A. fissa* varied from 251 ± 24 to 661 ± 77 ind. ml^{-1}, respectively, both when grown alone and in the absence of zinc and at both food levels. The corresponding data under the same conditions for *B. rubens* were: 52 ± 3 and

Fig. 1 Population growth curves of *A. fissa* (filled circles) and *B. rubens* (open circles) cultured separately and together at 0.5×10^6 cells ml^{-1} of *Chlorella vulgaris* and under different concentrations of $ZnCl_2$. Values represent mean ± standard error based on four replicates

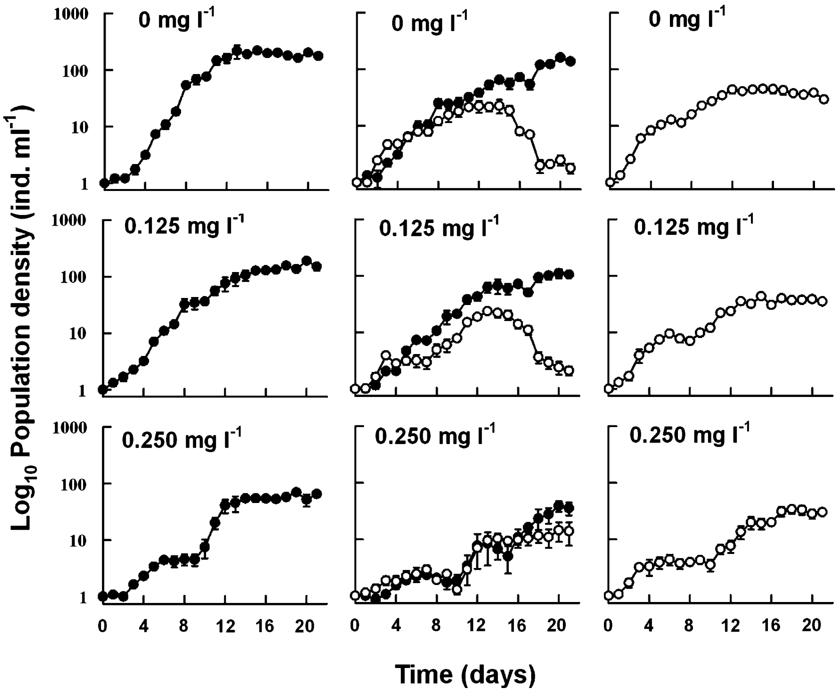
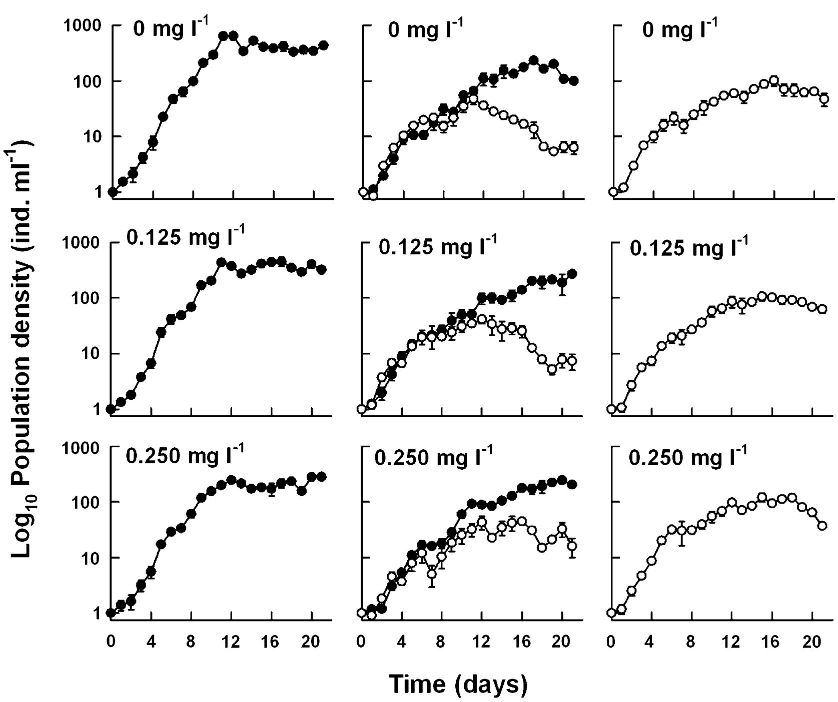


Fig. 2 Population growth curves of *A. fissa* (filled circles) and *B. rubens* (open circles) cultured separately and together at 1.0×10^6 cells ml^{-1} of *Chlorella vulgaris* and under different concentrations of $ZnCl_2$. Values represent mean ± standard error based on four replicates



102 ± 18 ind. ml^{-1}, respectively. At a given food level, the competition considerably reduced the peak abundances of both rotifers. The impact of the presence of zinc on competition was evident at lower-food level, mainly for *A. fissa*. Both *A. fissa* and *B. rubens* continued to grow for about 10 days at $ZnCl_2$ of 0 and 0.125 mg l^{-1}, but thereafter the latter began to decline. The peak densities of both the

rotifer species were lower at 0.250 mg l^{-1} of $ZnCl_2$ (Fig. 3). Peak population density of *A. fissa* was significantly influenced by algal food level, concentration of zinc as well as the presence of the competitor in the medium (3-way ANOVA, Table 1, $P < 0.001$, F-test). Except for the interaction of food level X zinc concentration, rest of the interaction terms was significant ($P < 0.05$). For *B. rubens*, the peak population density was significantly affected ($P < 0.05$) by food level and the presence of *A. fissa* but not by zinc concentration ($P > 0.05$). The interaction of food level X zinc concentration X competition was significant too.

Discussion

Acute toxicity evaluations

Current Mexican laws permit maximal zinc level of 5 mg l^{-1} in drinking water (NOM 1996), which is at least 25 times higher than the levels used here. This suggests that national laws need to be re-evaluated with reference to permissible levels of zinc for protecting rotifers in freshwater ecosystems (Azuara-García et al. 2006). Our results on the median lethal concentrations of zinc for *A. fissa* and *B. rubens* suggest that they are comparable with the most sensitive rotifer species such as *B. calyciflorus* and *Philodina acuticornis* (Snell and Janssen 1995). Snell et al. (1991a) found LC_{50} for zinc at 24 h of 1.3 mg l^{-1} for *B. calyciflorus*, and of about 4.8 mg l^{-1} for *B. plicatilis* (Snell et al. 1991b). That *A. fissa is* more sensitive to zinc than *B. rubens*, is evident from both the acute and chronic toxicity evaluations. Both the rotifer species responded to increase in food density by attaining higher population peaks. The concentrations of zinc used for the chronic evaluation of both rotifers were nearly one-half of the median lethal concentration for these rotifers. At this concentration some mortality was expected, yet no mortality occurred in any of the treatments. In discord with this, the populations of both species increased even in the highest $ZnCl_2$ concentration (0.250 mg l^{-1}) tested. This apparent anomalous situation can be explained considering the role of algae in the toxicity evaluations. Mangas-Ramírez et al. (2002) have reported that the addition of algae has resulted in better survival of neonates of *Ceriodaphnia dubia* and *Moina macrocopa* when subjected to acute ammonia toxicity. Generally, the algae tone down the toxicity of heavy metals and pesticides to zooplankton, including rotifers (Rao and Sarma 1990, Pickhardt et al. 2002). For example, Sarma et al. (2000) have shown that the median lethal concentrations of copper (0.11 mg l^{-1}) and cadmium

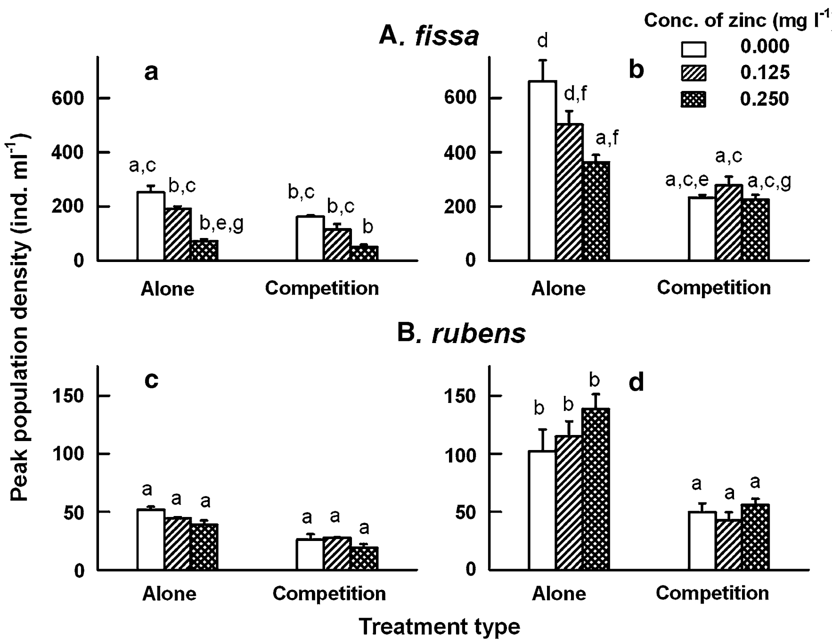


Fig. 3 Peak population densities (ind. ml^{-1}) of *A. fissa* and *B. rubens* cultured separately and together at 0.5×10^6 (**a**, **c**) and 1.0×10^6 (**b**, **d**) cells ml^{-1} of *Chlorella vulgaris* and under different concentrations of $ZnCl_2$. Values represent mean ± standard error based on four replicates. For each treatment type, bars containing the same alphabet are not statistically significant ($P > 0.05$, Tukey test)

Table 1 Results of the 3-way ANOVA performed for the peak population density of *A. fissa* and *B. rubens* grown alone and together at two algal food levels and three zinc concentrations. df = degrees of freedom; MS = mean square; *F* = *F*-ratio; *P* = level of significance

Source of variation	df	MS	*F*	*P*
A. fissa				
Algal level (A)	1	503390	167.3	<0.001
Zn concentration (B)	2	68738	22.84	<0.001
Competition (C)	1	239284	79.5	<0.001
Interaction of A × B	2	38.58	0.013	>0.05
Interaction of A × C	1	92112	30.6	<0.001
Interaction of B × C	2	24569	8.163	<0.01
Interaction of A × B × C	2	10809	3.591	<0.05
Error	24	3010		
B. rubens				
Algal level (A)	1	21978	101.1	<0.001
Zn concentration (B)	2	126.6	0.582	>0.05
Competition (C)	1	18113	83.35	<0.001
Interaction of A × B	2	822.1	3.783	<0.05
Interaction of A × C	1	5293	24.35	<0.001
Interaction of B × C	2	103.8	0.478	>0.05
Interaction of A × B × C	2	269.6	1.241	>0.05
Error	24	217.3		

(0.50 mg l^{-1}) for *Brachionus patulus* were about twice higher when the algal food in the medium was increased from 1×10^6 to 3×10^6 cells ml^{-1}. Ramírez-Mangas et al. (2001) have also shown the median lethal concentration of ammonia to *Daphnia pulex* to go up in the presence of algal food, compared with when food was absent. The LC_{50} for *A. fissa* and *B. rubens* were derived in the absence of food, while for the evaluations of sublethal concentrations we used two algal food levels, which explains why the rotifers in chronic toxicity tests tolerated higher Zn concentrations.

Effect of Zn on zooplankton competition

The population densities of many species of zooplankton such as *Brachionus calyciflorus*, *Euchlanis dilatata*, *Lepadella patella*, *Alona rectangula*, *Ceriodaphnia dubia*, *Daphnia laevis*, *Diaphanosoma brachyurum*, and *Moina macrocopa* increase with increasing food availability (Nandini and Sarma 2003; Nandini et al. 2007), which we also observed in our controls. The superior competitor generally has a negative impact on the population growth of the species with which it is competing. However, such a competitor may itself be also adversely affected (Sarma et al. 1996). In the present study, we observed both these situations. Thus, at low-food level, *A. fissa* tended to strongly reduce the population densities of *B. rubens* after the second week. However, with an increase in zinc concentration and especially at 0.250 mg l^{-1}, *A. fissa* itself was negatively affected and therefore both the rotifer species continued to co-exist until the end of the experiment, though at lower abundances. At higher food levels, this trend was not clear. Also in an earlier study dealing with competition between *A. fissa* and another species of *Brachionus*, *B. calyciflorus*, Sarma et al. (1996) have shown that the former was competitively superior to the latter only at low-food densities. In the present study too, we observed a similar situation for *A. fissa* and *B. rubens* when grown together at low-food level. At 0.250 mg l^{-1} of $ZnCl_2$, *A. fissa* possibly became more sensitive than *B. rubens* and thus had lower population abundances. Thus, zinc could affect the competitive outcome between the rotifer species and interfere with the species composition that varies seasonally (DeMott 1989; Preston and Snell 2001).

This could be further influenced by the seasonally varying algal densities (Sommer 1989). There is some indication that rotifers in tropical waterbodies generally suffer from low-food availability (Duncan 1989) and, therefore, the impact of zinc under these conditions may be more severe than is assumed.

Peak zooplankton density versus Zn stress

Peak population abundance is a significant variable in competition studies (Sarma et al. 2003a). *A. fissa* had a population maximum six times higher than that of *B. rubens* in controls at 1×10^6 cells ml^{-1} of *Chlorella* as food. This is due to the differences in the body size of the two-rotifers. *A. fissa* is smaller than *B. rubens*. At any given ration level, smaller-sized zooplankton species tend to be numerically more abundant than larger taxa (Gliwicz 2001; Nandini and Sarma 2003; Nandini et al. 2007). Previous studies have shown that *A. fissa* may reach densities higher than 2000 ind. ml^{-1} at a food level of 8×10^6 cells ml^{-1} (Dumont et al. 1995). In the present study, we used much lower food levels, which explains the lower maxima that we found for *A. fissa*. In addition to food, toxicants in sublethal concentrations also reduce the peak population abundances of zooplankton species. The presence of a superior competitor may further affect the potential abundance of the species (Sarma et al. 2003a). Thus, *A. fissa* at zinc concentration of 0.250 mg l^{-1} and in the presence of *B. rubens* can reach peak densities that are only one-third of that in controls, i.e., grown alone and in the absence of a stressor as Zn). In our study, *B. rubens* has also shown similar trends but at a lower magnitude. Regardless of the presence of the competitor and the food level, or a stressor, the peak population abundances of *B. rubens* recorded in this study is within the range (up to 200 ind. ml^{-1}) recorded earlier (Sarma et al. 2003b). While both the tested rotifer species co-exist in nature and are affected by natural factors such as food level, Zn contamination may interfere with the delicate balance that exits between these species.

Conclusions

Our study shows that *A. fissa* is a more sensitive than *B. rubens* based on the acute and chronic bioassays. Both competition and zinc concentration interact to affect the population cycles of both the rotifer species when grown together. The rotifer maxima appear to be significantly influenced by the food level, zinc concentration and/or the presence of competing species. The anthropogenic discharges of zinc into freshwater systems at concentrations as low as 0.250 mg l^{-1} may interfere with population growth of planktonic rotifers and their capacities to compete with the naturally co-occurring, such that the inferior competitors could be even wiped out. Our study is based on clonal populations of both the rotifer species. We have not investigated if different clones of the same zooplankton species have different levels of tolerance. In any case, considering the wide gap in the Zn concentrations affecting rotifer species and its permissible levels in drinking water in Mexico, it is necessary to re-examine the current national laws for this metal.

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