Combined effects of heavy metal (Hg) concentration and algal (Chlorella vulgaris) food density on the population growth of Brachionus calyciflorus (Rotifera: Brachionidae)

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Abstract: The combined effects of two food levels (0.5 x 10^6 and 1.5 x 10^6 cells ml^-1 of Chlorella vulgaris) and five concentrations (0.000625, 0.00125, 0.0025, 0.005 mg l^-1 of HgCl_2) of mercury on the population growth of the rotifer Brachionus calyciflorus was evaluated. The growth experiments were conducted for 18 days at 23±1°C under continuous fluorescent illumination. For each food level – heavy metal combination, we maintained 3 replicates. Our data showed that regardless of food level, increase in the heavy metal concentration in the medium resulted in decreased population growth of B. calyciflorus. At any given heavy metal concentration, B. calyciflorus grown under higher food levels had higher population abundance. The rate of population increase was significantly influenced by both the heavy metal concentration and the algal level. The highest population growth rate (0.435±0.003 per day) was observed in controls at 1.5 x 10^6 cells ml^-1. The results of this study were discussed in relation to the protective role of algal density against heavy metal toxicity.

Key words: Heavy metal, Zooplankton, Rotifer, Alga

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Introduction

The current Mexican official norms recommend the use of a) acute toxicity tests and b) the cladoceran Daphnia magna as bioassay organism for freshwater toxicity tests (Anonymous, 1994). However, D. magna does not occur naturally in Mexican waters and therefore alternative test species are frequently considered for bioassays (Gama-Flores et al., 1999). Rotifers, which form an important component of freshwater zooplankton, are widely used in ecotoxicological evaluations of toxic substances around the world (Serrano et al., 1986; Snell and Janssen, 1995).

Rotifers are the natural trophic link between alga and zooplanktivorous predators such as fish (Wallace et al., 2006). In Mexico, their diversity is considerably high (300 taxa: Sarma, 1999) and constitute nearly 1/6th of species known worldwide (Koste, 1978). Rotifers are also sensitive to changes in the water quality, though for certain toxic substances cladocerans are apparently more sensitive (reviewed in Sarma and Nandini, 2006).

Among heavy metals, mercury is highly toxic to zooplankton including rotifers (Ramirez-Perez et al., 2004). Median lethal concentration (LC_{50}) for freshwater species of Brachionus is in the range of 0.027 to 0.061 mg l^-1 (Snell and Janssen, 1995; Sarma et al., 2000). For a given rotifer species, several factors such as temperature and algal food density may modify the LC_{50}. Generally, increasing temperature accelerates the toxicity of heavy metals to organisms (Calow, 1993). With reference to algal food, increased food level may reduce the toxicity of heavy metals to zooplankton (Pickhardt et al., 2002). However, at high algal densities, sometimes toxicants may interact synergistically causing rapid mortality of the test populations (Barry et al., 1995). In acute toxicity evaluations, relatively higher concentrations of toxicants are needed compared to chronic tests (Roex et al., 2000). So, when heavy metal levels are low in effluents, acute toxicity tests become ineffective and therefore, chronic toxicity evaluation is needed. Demography and population growth approaches are largely used for chronic toxicity tests (Kammenga and Laskowski, 2000; Sarma and Nandini, 2006; Gama Flores et al., 2007). Population growth evaluates possible adaptations (or lack of such adaptations) of a species to the given toxicant during the experimental duration.

The aim of this study was to evaluate the toxic effects of Hg on the population growth of the common rotifer Brachionus calyciflorus Pallas in relation to algal food density.

Materials and Methods

Parthenogenetic (clonal) population of the rotifer Brachionus calyciflorus was established nearly 5 years ago with a single starting individual, isolated from the Lake Xochimilco. We used single-celled green alga, Chlorella vulgaris as the exclusive food for the rotifers. The re-constituted moderately hard water (EPA medium) (Weber, 1993) was used as the medium for both stock cultures and experimental jars. The EPA medium was prepared by adding 0.9 g NaHCO_3, 0.6 g CaSO_4, 0.6 g MgSO_4 and 0.04 g KCl in one litre of distilled water. The stock rotifer cultures as well as the test jars were maintained at 23 ± 1°C, pH: 7.0-7.5, continuous but diffused fluorescent illumination.
Chlorella vulgaris was batch cultured using Bold’s basal medium (Borowitzka and Borowitzka, 1988) in 2 liter transparent bottles. Algal biomass was harvested during the log phase of growth, centrifugation at 4000 rpm for 5 min, rinsed and re-suspended in a small volume of distilled water. The algal stock was stored at 4°C in dark until use. The density of alga was estimated using haemocytometer. For the experiments, we used two densities (0.5X10^6 and 1.5X10^6 cells ml^-1) of Chlorella.

Analytical grade heavy metal, HgCl₂, was used as the toxicants. We used nominal concentration for the heavy metal. Stock (1 mg l⁻¹) solution was prepared using distilled water and required dilutions were made using EPA medium. Based on information available in literature (Ramirez-Perez et al., 2004), we selected the following 4 sublethal concentrations: 0.000625, 0.00125, 0.0025 and 0.005 mg l⁻¹. We maintained controls (0 mg l⁻¹) and 3 replicates for each treatment.

We used a total of 30 test jars. Into each of test jar containing 20 ml medium of specified algal density, heavy metal type and concentration, we introduced B. calyciflorus at an initial density of 1 ind. ml⁻¹. Following initiation of the growth experiment, daily we quantified the population density in each container (the first three days as total counts, and for subsequent days through 3 aliquots of 1 to 2 ml each). Following the density estimation, the test individuals were returned to the container and entire population was transferred (using small mesh of 50 μm pore size) to fresh jars of appropriate treatment. The experiments were terminated after 18 days by which time, the test populations began to decline. The rate of population increase (r) was determined using the exponential growth equation (Krebs, 1985): \( r = (\ln N_f - \ln N_i)/t \), where \( N_i \) and \( N_f \) are the initial and final population densities respectively, and \( t \) is time in days. In order to detect the impact of Hg on the relation between daily rate of population growth and the population density, we followed Kerfoot et al. (1985).

Using two-way analysis of variance, we tested whether the rate of population increase of B. calyciflorus in controls differed significantly from the metal treatments at the chosen algal densities (Sokal and Rohlf, 2000).

**Results and Discussion**

Data on the population growth of B. calyciflorus grown in relation to different concentrations of HgCl₂ at low and high algal food levels are shown in Fig. 1. Regardless of the heavy metal concentration, an increase in algal food level had increased the population abundances of B. calyciflorus. On the other hand, regardless of algal density, increase in the heavy metal concentration resulted in decreased population growth of rotifers.

The rate of population increases with increasing food level but decreased with increasing metal concentration in the medium (Fig. 2). The highest population growth rate (0.435±0.003 d⁻¹) was observed in controls at 1.5X10⁶ cells ml⁻¹.

Statistically the food density and the heavy metal concentration had significant influence (p<0.001, two-way ANOVA) on the rate of population increase. However, the interaction of food level X Hg concentration was not significant (p>0.05) (Table 1).

Numerous studies have shown that Hg is highly toxic and concentrations at or lower than 0.005 mg l⁻¹ cause reduction in both the survival and reproduction of various species of Brachionus including B. rubens (Sarma et al., 2005) and B. patulus (Sarma et al., 2001). However, the magnitude of heavy metal toxicity is influenced by various factors such as temperature and food concentration. Increase in temperature enhances metabolic
algal density, even when the Hg concentration was 0.005 mg l\(^{-1}\) at low (0.5\(\times\)10\(^5\)) and high (1.5\(\times\)10\(^6\) cells ml\(^{-1}\)) algal (Chlorella vulgaris) food levels. If toxic effect of Hg was independent of food level, processes and thus higher temperatures and heavy metal concentrations interact synergistically leading to rapid decline of rotifer populations (Buikema et al., 1974). Algal food density may act antagonistically and so increase in algal food reduces the toxicity of heavy metals to zooplankton (Sarma et al., 2000). In the present work we observed that at any given heavy metal concentration, B. calyciflorus reached higher abundances at higher food level than at lower algal density. If toxic effect of Hg was independent of food level, then the population densities of rotifers in metal-treatments would be similar. Since we observed higher population abundances at high algal density, even when the Hg concentration was 0.005 mg l\(^{-1}\), it is suggestive of the possibility of metal detoxification by algae (Gotsis, 1982).

Field studies have shown that phytoplankton may accumulate or detoxify the heavy metals (Pickhardt et al., 2002). Rotifers fed on algae that contained heavy metal (Hg) showed lower population growth rates compared to controls (Sarma et al., 2005). Thus the higher the food consumption rate, the higher is the toxic effect and consequently lower is the population growth (Forbes and Calow, 1999). Since the quantity of algal cells consumed by rotifers is a function of its availability in the medium (Monakov, 2003), it is expected that the rotifers cultured at high algal density in the presence of higher toxic concentrations experience rapid mortality or lower population growth rates. However, this was not observed here. This suggests the existence of other possibilities. For example, increase in toxic concentration causes reduced swimming and feeding rates (Charoy et al., 1995). Therefore, it is likely that rotifers at higher toxic levels may actually consume less algal food as compared to controls. The effect of Hg to B. calyciflorus in our study may be also due to its adverse impact from the medium (Sarma et al., 2005). The lower impact of Hg under higher food level may be also due to detoxification by Chlorella. In addition, per cell the quantity of Hg accumulation becomes lower under higher algal density. Therefore the observed positive effect of increased algal density on the rotifer

**Table 1:** Results of two-way analysis of variance performed on the rate of population increase of B. calyciflorus subjected to different concentrations of Hg.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food density (A)</td>
<td>1</td>
<td>0.173</td>
<td>0.173</td>
<td>490.06***</td>
</tr>
<tr>
<td>Hg concentration (B)</td>
<td>4</td>
<td>0.019</td>
<td>0.005</td>
<td>13.77***</td>
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<tr>
<td>Interaction AxB</td>
<td>4</td>
<td>0.002</td>
<td>0.001</td>
<td>1.54ns</td>
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<tr>
<td>Error</td>
<td>20</td>
<td>0.007</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

DF = Degrees of freedom; SS = Sum of squares; MS = Mean square; F = F-relations; *** = (p<0.001); ns = (p>0.05), Non-significant.

**Fig. 2:** Rate of population increase (\(r\), day\(^{-1}\)) of Brachionus calyciflorus in relation to different concentrations of Hg (mg l\(^{-1}\)) at low (0.5\(\times\)10\(^5\)) and high (1.5\(\times\)10\(^6\) cells ml\(^{-1}\)) algal (Chlorella vulgaris) food levels. Shown are the mean ± standard error values based on 3 replicates.

**Fig. 3:** Relation between daily rate of population increase and the population abundance of B. calyciflorus cultured under different concentrations of Hg (mg l\(^{-1}\)) at low (0.5\(\times\)10\(^5\)) and high (1.5\(\times\)10\(^6\) cells ml\(^{-1}\)) algal food levels. Plotted are the replicate data for each treatment.
dynamics under different Hg concentrations may be due to these reasons.

Under non-stressful conditions, the daily rate of population increase of zooplankton species is inversely related to its population abundance (Kerfoot et al., 1985). This is easily understood in population growth studies, where the numerical abundance of the test species changes in relation to the availability of food. Increase in population density results in decreased availability of resources and hence decreased growth rates. However, under stressful conditions, the relation between the daily growth rates and the population abundances becomes disrupted and hence the inverse relation becomes non-significant (Gama-Flores et al., 2004). This trend was evident in treatments containing higher Hg levels and under low algal food density (Fig. 3). However, at higher algal food density, the relation between the daily population growth rates and the population abundances were significant. This suggests that higher food density mitigated the toxic effect of Hg, as also shown in previous works (Sarma et al., 2000, 2005).

In conclusion our study showed that Hg at a concentration as low as 0.000625 mg l⁻¹ was toxic to *B. calyciflorus*, especially at low algal diet. Higher algal food levels apparently offered some protection to the rotifer against toxic effects of this heavy metal.

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