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ADVANCES IN ROTIFER RESEARCH

Combined effects of sediment and lead ($PbCl_2$) on the demography of *Brachionus patulus* (Rotifera: Brachionidae)

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Abstract We studied the response of *Brachionus patulus* to different concentrations of the heavy metal Pb in the presence and absence of sediments. We conducted acute (LC_{50}) and chronic (life table demography and population growth) toxicity tests using sediment levels of 0, 30 and 280 mg l^{-1} (=0, 17 and 170 NTU) and Pb at 0, 0.06 and 0.6 mg l^{-1}. Experiments were conducted at 20 ± 1°C on a horizontal shaker and algal food (*Chlorella vulgaris*) was added at a density of 1.0×10^6 cells ml^{-1}. The median lethal concentration (LC_{50} ± 95% Confidence intervals) of $PbCl_2$ for *B. patulus* was 6.15 ± 1.08 mg l^{-1}. Age-specific survivorship and fecundity curves showed increase in turbidity level resulted in decreased survival and offspring production of the rotifers. Increase in Pb concentration too had a negative effect on the survival and reproductive output of *B. patulus*. Statistically, average lifespan, life expectancy at birth, gross and net reproductive rates and the rate of population increase were all significantly influenced by the concentration of Pb, turbidity level as well as the interaction of Pb concentration × turbidity level. Rotifers exposed to 170 NTU did not grow regardless of the heavy metal concentration in the medium. Similarly, *B. patulus* exposed to 0.6 mg l^{-1} Pb did not survive beyond 10 days regardless of the turbidity level in the medium. The rate of population increase of *B. patulus* derived from the growth experiments was negative in all treatments containing Pb as low as 0.06 mg l^{-1} or turbidity level as low as 17 NTU. In treatments containing Pb or sediments, there existed no relation between the egg ratio and the population density.

Keywords Heavy metal · Lead · *Brachionus* · Life table · Sediment effect

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Advances in Rotifer Research

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Introduction

Heavy metal pollution is an important problem in aquatic ecosystems in Mexico (Soto-Jiménez & Páez-Osuna, 2001). While certain heavy metals such as magnesium, iron, copper and zinc are essential trace elements, others such as mercury, cadmium and lead are not needed at all by any organism (Laws, 2000). Ammunition, porcelain and paint industries often release high concentrations of lead via untreated water into rivers and lakes. Mexican Official Norms prescribe the maximum acceptable limits of 0.2–0.4 mg l^{-1} of lead in freshwaters, depending on their use (Anon., 1996). However, much higher concentrations, up to 10 times, have been recorded in certain waterbodies including freshwater ecosystems in Mexico (Cervantes & Moreno-Sánchez, 1999).

Mexican waterbodies such as lake Chapala and lake Xochimilco are not only large, but are also shallow. These waterbodies are generally turbid due to high concentrations of sediments (De la Lanza & García, 2002). The role of sediments in influencing the feeding and filtration processes of zooplankton is documented (Hart, 1988; Kirk & Gilbert, 1990). Levels of sediment as high as 800 mg l^{-1} have been recorded in lakes in Florida (Sarnelle et al., 1998). Sediments may retain heavy metals by adsorption and thus lower levels in the water column. Consequently, turbidity levels in polluted waterbodies influence the toxicity of heavy metals to zooplankton (García-García et al., 2006).

Rotifers are a common component of freshwater zooplankton communities. Species of the genus *Brachionus* are sensitive to changes in the water quality. Consequently their use as standard bioassay organisms has been universally recognized (APHA, 1998). Information on the acute toxicity tests of Pb on *Brachionus* are available in literature (Snell & Janssen, 1995). The median lethal concentrations (LC_{50}) Pb for various species of *Lecane* varied from 0.14 to 3.7 mg l^{-1} (Pérez-Legaspi & Rico-Martínez, 2001).

Life table demography and population growth studies are the two chronic toxicity tests often conducted on rotifers (Snell & Janssen, 1995). Life tables provide birth rate and death rates in an age-specific way. Therefore, different life history variables related to survivorship and reproduction can be quantified under different test conditions (Wallace et al., 2006). However, life table method does not provide information on the possible adaptation of offspring born under the toxicant treatments chosen for the experiments. Therefore, both life table demography and population growth are complementary to each other (Mangas-Ramírez et al., 2004).

Brachionus patulus is frequently found in shallow, turbid, ponds and lakes in Mexico. These waters are often also subject to inflows from industries with high levels of toxicants. In this study, we therefore assessed the impact of lead, in combination with sediment on the demographic variables of *Brachionus patulus*.

Material and methods

The test rotifer *B. patulus* was originally isolated from the wetland Chimaliapan, a RAMSAR site in Toluca, State of Mexico, and mass-cultured using the single celled-green alga (*Chlorella vulgaris*) (strain CL-V-3, CICESE, Ensenada, Mexico) as the exclusive diet. For mass-culture and for experiments, we used reconstituted moderately hard-water (US EPA medium), which was prepared by dissolving 0.9 g of $NaHCO_3$, 0.6 g of $CaSO_4$, 0.6 g of $MgSO_4$ and 0.04 g of KCl in 1 l of distilled water (Weber, 1993). Since we chose Pb as the toxicant, which precipitates with EPA chemicals, we modified EPA medium accordingly (Klerks & Lentz, 1998). The test conditions were temperature 23 ± 2°C, pH 7.0–7.5, and constant but diffuse fluorescent illumination.

Chlorella vulgaris was mass-cultured in 2-l transparent bottles using standard medium (Bold's basal, Borowitzka & Borowitzka, 1988). Log phase alga was harvested, centrifuged at 4,000 rpm for 5 min, rinsed and resuspended in distilled water. The algal density was estimated using haemocytometer. For experiments we used one algal density (1×10^6 cells ml^{-1} or in terms of dry weight 5.8 μg ml^{-1} or 0.3 μg C ml^{-1}) (Nandini & Sarma, 2003). In order to simulate different turbidity levels, we used sediment particles prepared from garden soil following Kirk & Gilbert (1990). Sediment levels were quantified gravimetrically using a Cahn 33 electrobalance and later converted into turbidity units (NTU) using a turbidimeter (Model Hanna, HI 93703). Previous work showed that sediment concentrations of 50 and 500 mg l^{-1} corresponded to 17 and 170 NTU, respectively (García-García et al., 2006).

For toxicity testing, we used analytical grade $PbCl_2$. While the stock concentration (10 mg l^{-1}) was prepared using distilled water, the test (nominal) concentrations were prepared using EPA medium. In order to choose sub-lethal concentrations of Pb, it became necessary to derive LC_{50} and therefore we conducted a 24-h bioassay using neonate *B. patulus*.

For this we used 100-ml transparent glass jars, each with 50-ml EPA medium (with specified heavy metal concentration and 1×10^6 algal density but at 0 turbidity) and 50 neonates (<2 h after hatching from parthenogenetic eggs) of *B. patulus*. We used six different concentrations of $PbCl_2$ (0, 1.25, 2.5, 4, 5 and 8 mg l^{-1}), each with four replicates. After 24 h, the number of individuals dead in each test jar was quantified using a stereomicroscope. Using probit method (Finney, 1971), we derived the median lethal concentration and 95% confidence intervals.

For life table and population growth experiments too, we used the same type of jars, each with 30-ml EPA medium. The experimental design consisted in a total of 36 test jars (3 heavy metal concentrations × 3 turbidity levels × 4 replicates). The test jars contained 1×10^6 cells ml^{-1} algal food density. We changed the medium daily. The Pb concentrations were 0, 0.06 and 0.6 mg l^{-1} while the turbidity levels were 0, 17 and 170 NTU. The test jars were placed on a shaker to minimize sedimentation.

For life table experiments, we introduced 30 neonates (<2 h after hatching from parthenogenetic eggs) into each of test jar. Following initiation of the experiment, we counted and transferred surviving members of the original cohort everyday to new jars containing the appropriate test medium. The dead adults and neonates, when present, were counted and discarded. Experiments were continued until the last individual of each cohort died. From the survivorship and fecundity data we calculated the variables such as average lifespan (ALS), gross and net reproductive rates, generation time (*T*) and the rate of population increase per day using the following formulae (Krebs, 1985):

$$\text{Gross reproductive rate} = \sum_{0}^{\infty} m_x$$

$$\text{Net reproductive rate } R_o = \sum_{0}^{\infty} l_x \cdot m_x$$

$$\text{Generation time: } T = \frac{\sum l_x \cdot m_x \cdot x}{R_o}$$

Rate of population increase, Euler equation (solved iteratively)

$$\sum_{x=w}^{n} e^{-rx} \cdot l_x \cdot m_x = 1$$

where, l_x is the probability of an individual surviving to an age class, m_x is the age-specific fecundity, R_o is the average number of offspring per female and *r* is the growth rate of the population.

For population growth experiments, we used the same design mentioned earlier. Following initiation of the growth experiments, we estimated the density of rotifers as well as the number of eggs (both loose and attached to females) using whole count or 2–3 aliquots of 1–5 ml each, depending on the density of *B. patulus*. The experiments were terminated after 3 weeks by which time rotifer populations in all treatments began to decline. Based on the data collected, we derived the rate of population increase (*r*) using we derived *r* using regression between log natural population density over time (Sibly & Hone, 2002).

Data from life table and population growth experiments were assessed for homogeneity of variance and normality using residual analysis (plots of residual versus means using descriptive statistics Sokal & Rohlf, 2000). Analysis of variance (ANOVA) was used to quantify the differences in the selected life history variables of *B. patulus* under different treatments. Post-hoc (Tukey test) analysis was used for multiple comparisons utilizing the software Statistica ver. 5.

Results

The median lethal concentration (LC_{50} ± 95% Confidence intervals) of $PbCl_2$ for *B. patulus* was 6.15 ± 1.08 mg l^{-1}.

Age-specific survivorship and fecundity curves showed that regardless of the Pb concentration, increase in turbidity level resulted in decreased survival as well as offspring production in *B. patulus*. Similarly, regardless of sediment level, increase in Pb concentraton too had a negative effect on the survival and reproductive output by the rotifers (Fig. 1).

Summary of demography data from the life table experiments are presented in Fig. 2. Average lifespan (ALS), gross and net reproduction rates, generation time and rate of population increase were all

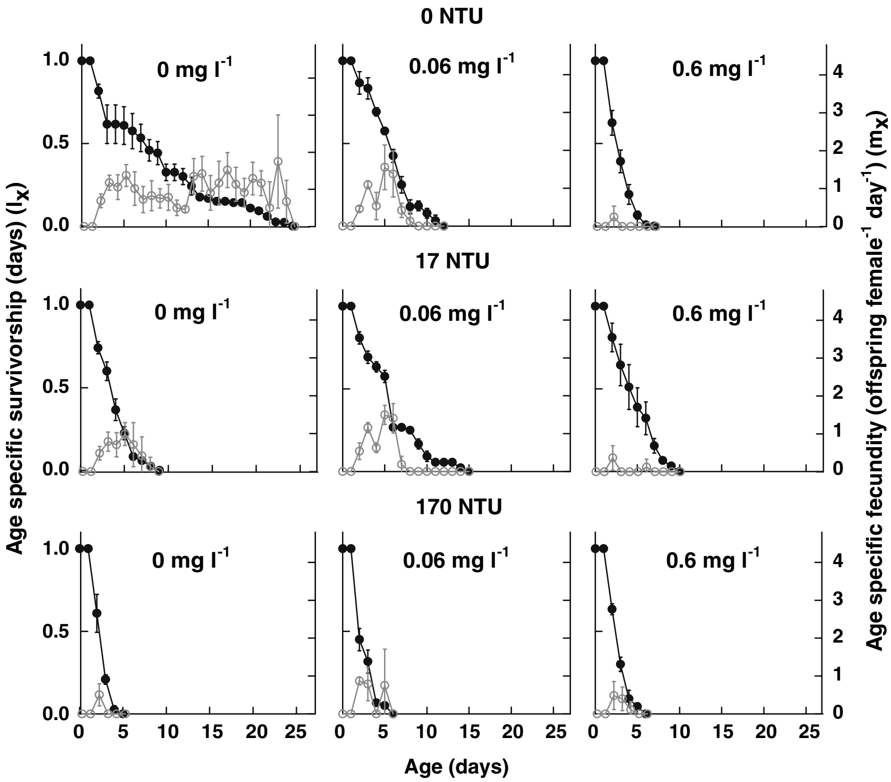


Fig. 1 Age-specific survivorship (dark line) and fecundity (soft line) curves of *B. patulus* grown at three turbidity levels, subject to three concentrations of $PbCl_2$ and fed 1×10^6 cells ml^{-1} of *C. vulgaris*. Values represent mean ± standard error based on four replicates (cohorts)

adversely affected by increase in turbidity levels as well as the concentration of Pb in the medium. In controls (without sediments and 0 mg l^{-1} of Pb), the ALS of *B. patulus* was about 9 days which was reduced to 1/3rd when the sediment level was 17 NTU or when Pb concentration was 0.6 mg l^{-1}. The rate of population increase derived iteratively became negative in treatments containing sediment level of 170 NTU, or when exposed to 0.6 mg l^{-1} of Pb. Statistically, average lifespan, life expectancy at birth, gross and net reproductive rates and the rate of population increase were all significantly influenced by the concentration of Pb, turbidity level as well as the interaction of Pb concentration × turbidity level ($P < 0.05$, Table 1).

Population growth curves of *B. patulus* showed decreased abundances with increasing concentration of Pb or higher turbidity levels in the medium. Rotifers exposed to 170 NTU did not grow regardless of Pb concentration. Similarly, *B. patulus* exposed to 0.6 mg l^{-1} of Pb did not survive beyond 10 days regardless of the turbidity level in the medium (Fig. 3). The rate of population increase of *B. patulus* derived from the growth experiments was negative in all treatments containing Pb as low as 0.06 mg or turbidity level as low as 17 NTU (Fig. 4).

When the number of eggs per female (i.e. egg ratio) was plotted as a function of population density, inverse relation appeared only for controls (treatments containing 0 mg l^{-1} of Pb and without sediments) and a treatment containing 0 mg l^{-1} of Pb and 17 NTU sediments. In all other treatments, there existed no relation between the egg ratio and the population density (Fig. 5).

Discussion

In shallow waterbodies, turbidity is generally high because of mixing of bottom sediments by wind action, movement of macroscopic invertebrates or fish (De la Lanza & García, 2002). This brings particles with adsorbed toxicants into the water column and eventually, as they are ingested by

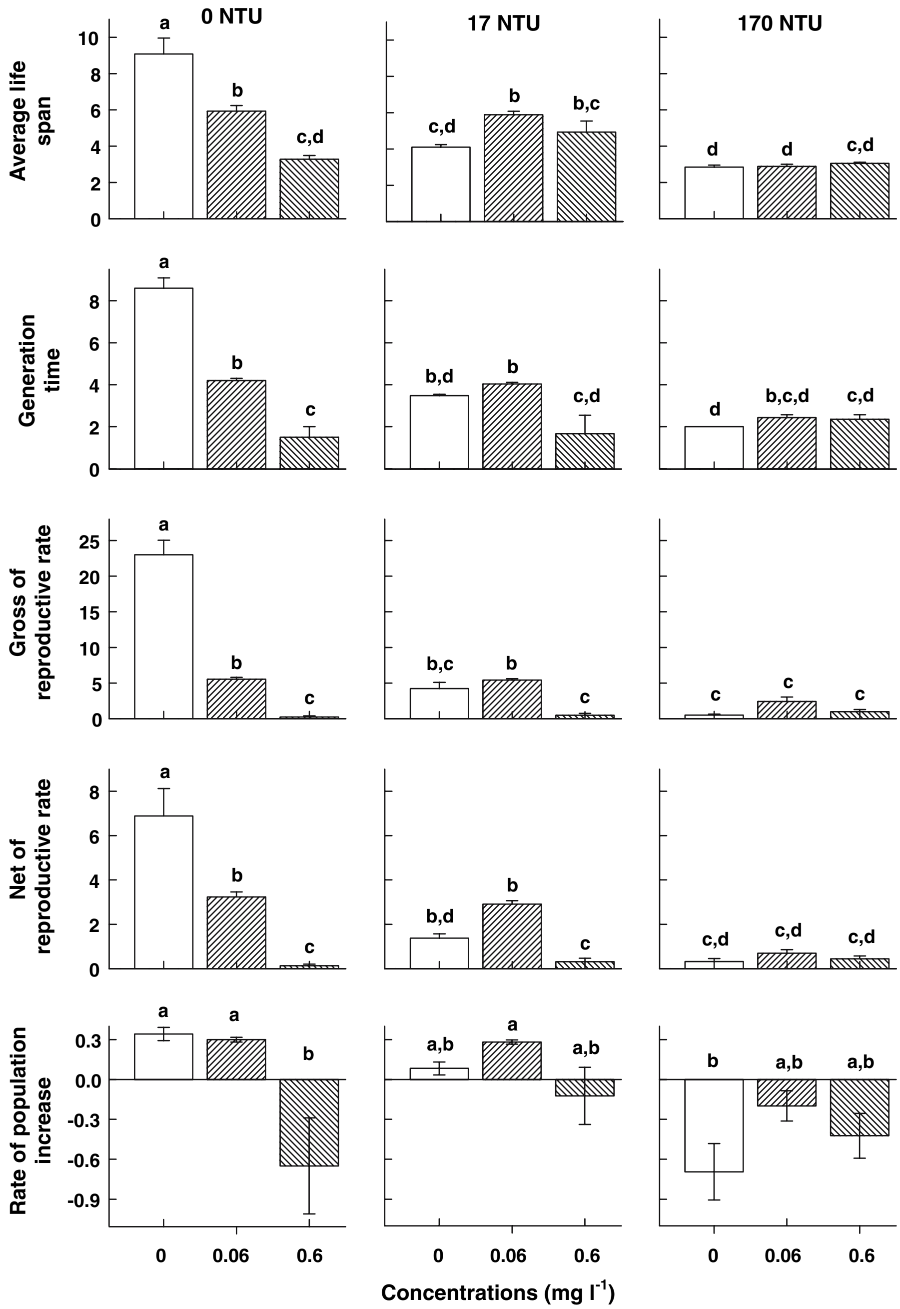


Fig. 2 Selected life history variables of *B. patulus* subject to different concentration of lead ($PbCl_2$) and sediment. For each treatment, bars carrying the same letters are not statistically significant ($P > 0.05$, Tukey test)

zooplankton and fish, they have an adverse effect on their demography. In both population growth and life table experiments, *B. patulus* responded similarly in that at both the tested concentrations of Pb, the population growth rates were significantly reduced when compared to controls. Similarly, increase in turbidity levels also adversely affected the population growth rates of *B. patulus*.

Among different genera of rotifers, *Brachionus* is generally thought to be a suspension feeder (Wallace et al., 2006). *B. patulus* is tychoplanktic, mainly associated with vegetation and appears in water column sporadically. This species is also known to feed on detritus or heat-killed algae (Lucía-Pavón et al., 2001). In the present study, our sediment preparation removed all organic material and

Table 1 Results of analysis of variance performed on the selected life history variables of *Brachionus patulus* in relation to three heavy metal concentrations and in the presence of 0, 17 and 170 NTU of suspended solids

Variable	Source of variation	DF	SS	MS	*F*	*P*
Average life span	Conc. of sediments (A)	2	322.4	30.96	57.41	<0.001
	Conc. of Pb (B)	2	229.75	7.5	13.90	<0.001
	A × B	4	18	14.26	26.45	<0.001
	Error	26	106.92	0.54		
Gross reproduction rate	Conc. of sediments (A)	2	3442.12	222.55	85.36	<0.001
	Conc. of Pb (B)	2	7430.8	215.27	82.56	<0.001
	A × B	4	6167.7	185.08	70.98	<0.001
	Error	26	6386.58	2.61		
Net reproductive rate	Conc. of sediments (A)	2	489.44	26.38	33.08	<0.001
	Conc. of Pb (B)	2	272.05	20.29	25.44	<0.001
	A × B	4	41.00	15.43	19.35	<0.001
	Error	26	187.38	0.8		
Generation times	Conc. of sediments (A)	2	875.06	19.54	44.00	<0.001
	Conc. of Pb (B)	2	1641.5	23.36	52.60	<0.001
	A × B	4	778.9	16.45	37.04	<0.001
	Error	26	634.5	0.45		
Rate of population increase	Conc. of sediments (A)	2	0.16	0.92	8.12	<0.01
	Conc. of Pb (B)	2	0.1	0.791	7.00	<0.01
	A × B	4	0.01	0.41	3.66	<0.05
	Error	26	0.04	0.11		

DF = degrees of freedom, SS = sum of squares, MS = mean squares, F = F-ratio

therefore, *B. patulus* was not expected to gain any energy from the ingestion of the inorganic particles in the test jars (Kirk & Gilbert, 1990). *Chlorella* was the only organic material available for *B. patulus* for feeding in the jars. Since the medium was changed daily, we assume that the bacterial densities alone were insufficient to maintain population growth of the rotifer. It is not known if *B. patulus* ingested both inorganic particles and *Chlorella* cells together or selectively fed on the algae. The fact that turbidity by itself (without the presence of Pb) caused a reduction in the population growth of *B. patulus* suggests that inorganic particles do interfere with the feeding activities of this rotifer species. Gliwicz (1980) has shown that sediment particles increase metabolic demands and thus reduce the lifespan and reproduction of cladocerans since they are inadvertently ingested along with algae, not digested and increase the body weight, which in turn increases the energy consumption for maintenance. In some other crustaceans such as anostracans, addition of sediments actually enhance digestibility of food particles (Maeda-Martínez et al., 1995).

That the lead is toxic to rotifers (Pérez-Legaspi & Rico-Martínez, 2001) is evident in this study too. Concentrations as low as 0.06 mg l^{-1} caused significant reduction in all the tested parameters of *B. patulus*. The ratio of acute to chronic levels of Pb used here varied by 0.1–0.01. Thus the safe concentration derived from the frequently used application factor ($0.01 \times LC_{50}$) is still toxic to *B. patulus* regardless of the turbidity levels (Mangas-Ramírez et al., 2004). Many studies have emphasized the need to derive safe concentrations based on sub-lethal testing rather than acute toxicity tests (Gama-Flores et al., 1999; Sarma et al., 2001). Our results support this approach.

Among the life history variables, survivorship parameters are generally less sensitive than those related to reproduction (Kammenga & Laskowsky, 2000). For example, Rao & Sarma (1986) have shown that survivorship and generation time of the

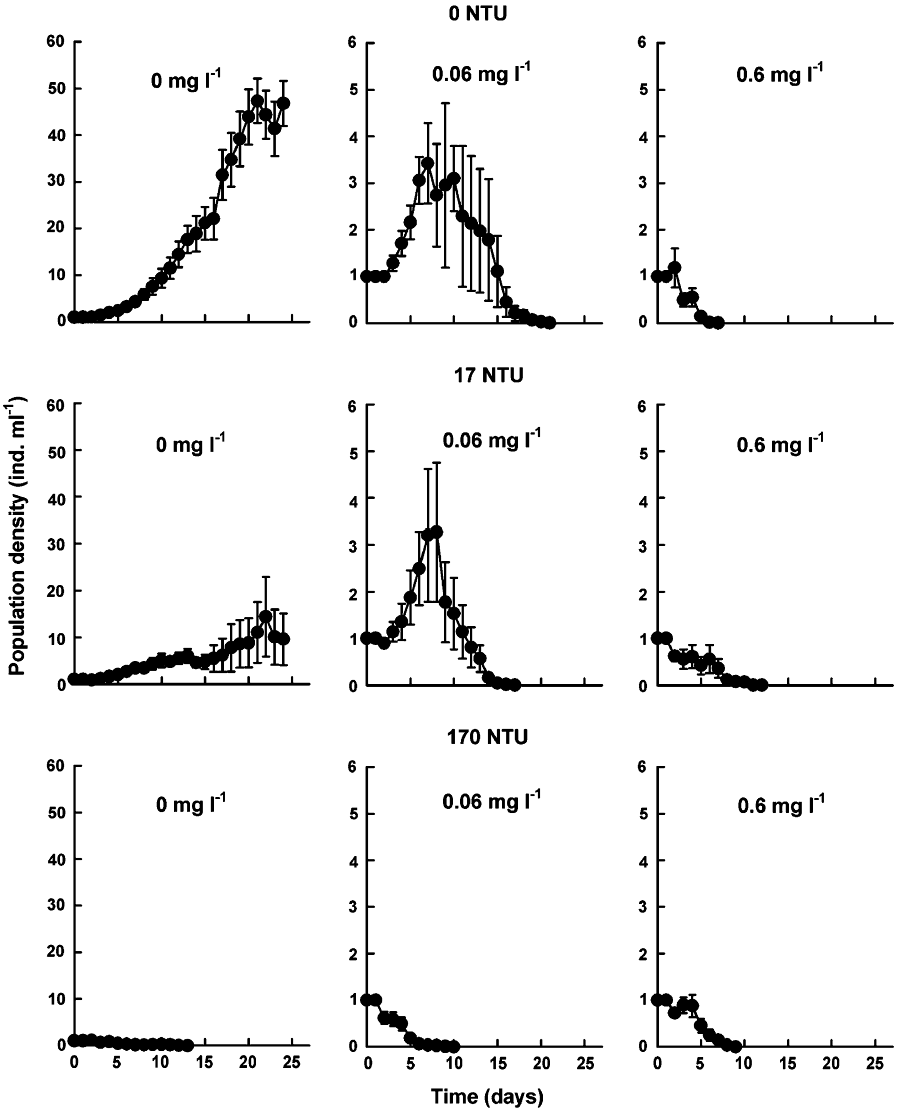


Fig. 3 Population growth curves of *B. patulus* grown at three turbidity levels and three concentrations of $PbCl_2$ fed 1×10^6 cells ml^{-1} of *C. vulgaris*. Values represent mean ± standard error based on four replicates. Note the differences on the *Y*-axis scaling

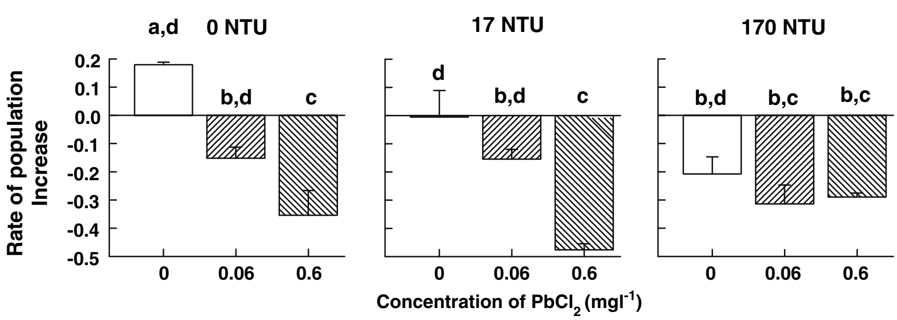
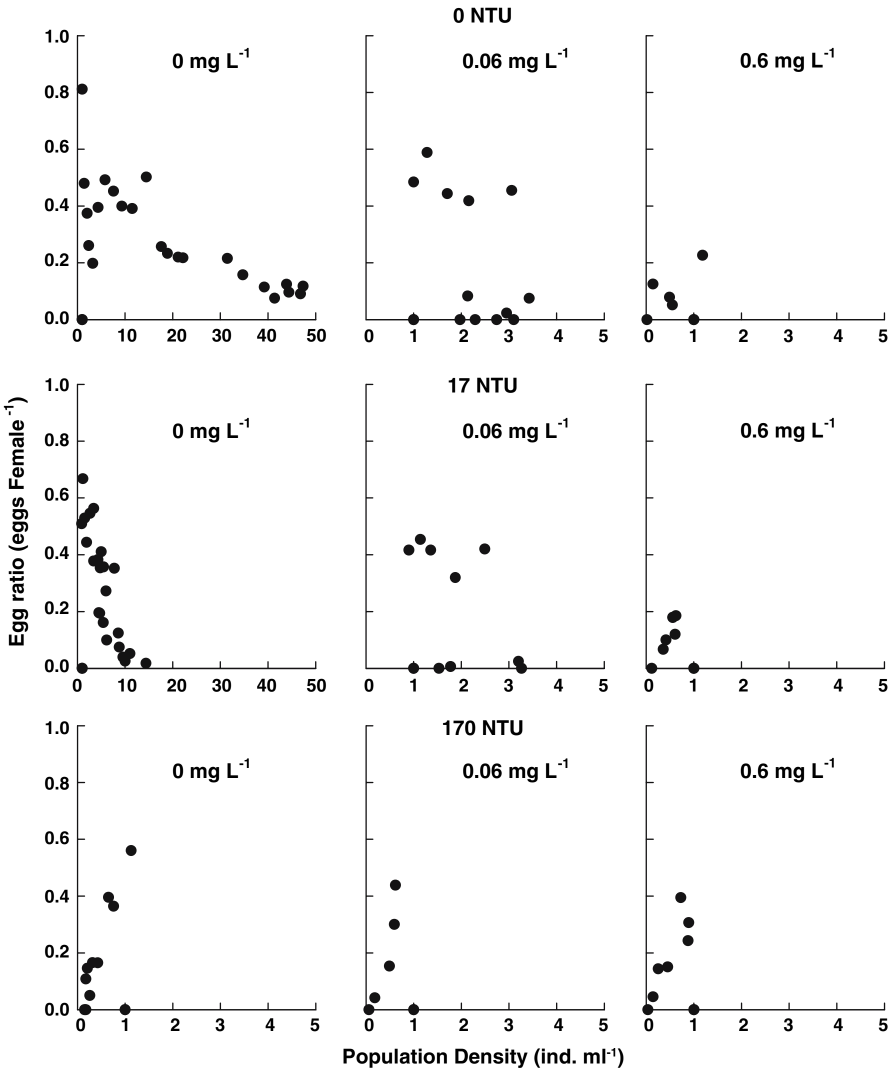


Fig. 4 Rate of population increase (*r*) of *B. patulus* grown at three turbidity levels, subject to three concentrations of $PbCl_2$ and feed with 1×10^6 cells ml^{-1} of *C. vulgaris*. Values represent mean ± standard error based on four replicates. For each treatment, bars carrying the same letters are not statistically significant ($P > 0.05$, Tukey test)

Fig. 5 Relation between egg ratio (eggs female^{-1}) and population density of *B. patulus* subject to three turbidity levels and three concentrations of $PbCl_2$ fed 1×10^6 cells ml^{-1} of *C. vulgaris*. Data based on four replicates



same rotifer species was not affected by the toxicant while, gross and net reproductive rates as well as the *r*, were affected adversely. In the present study both the survivorship and reproductive variables were affected by the presence of sediments and lead. This suggests that *B. patulus* is strongly sensitive to both sediments and Pb at the levels used here. In controls (0 mg l^{-1} of Pb and without sediments), the range of survivorship and reproductive variables agrees with the data recoded previously (Sarma & Rao, 1991). For example, average lifespan was about 8–14 days depending on the food concentration and temperature. Similarly gross and net reproductive rates varied from 8 to 24 and 4 to 18 offspring female^{-1}, respectively. In the present study, these variables are within the range reported earlier.

The effect of turbidity on the survival and reproduction of different species of zooplankton is not well known. For example, among cladocerans *Moina* and *Diaphanosoma* are known to tolerate turbidity levels higher than those used in this work. On the other hand, rotifers such as *Keratella cochlearis* is known to survive and reproduce at lower turbidity levels (Kirk & Gilbert, 1990). *B. patulus* in this study appeared to be more sensitive to turbidity levels than *K. cochlearis*. We observed a negative effect of sediments on the demography of *B. patulus*, similar to the observations of Kirk & Gilbert (1990) who found that the population growth of *Keratella crassa* decreased in the presence of coarse clay. The presence of suspended solids did not mitigate the adverse effects of Pb to rotifers in this study. On the other hand, sediments reduced the toxicity of lead to cladocerans (García-García et al., 2006).

When a species of zooplankton is grown under non-stressful conditions, then the egg ratio (eggs to females) shows an inverse relation to the population abundance (Sarma et al., 2005). However, under stressful conditions, this relation is disrupted. The stress could be due to toxicants, food availability, food quality, presence of competitors, extreme temperature conditions, toxicants etc. (Luna-Andrade et al., 2002). Under these conditions, for low population densities, even when the resources are not limited, the offspring production would be lower leading to a lack of any relation between the egg ratio and the population density. This situation, which was observed here has been previously reported (Sarma et al., 2005). Thus both Pb and turbidity disrupted the relation between the egg ratio and the population density.

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