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Combined Effects of *Chlorella* Density and Methyl Parathion Concentration on the Population Growth of *Brachionus calyciflorus* (Rotifera)

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In aquatic ecosystems, rotifers play a major role in several important ecological processes. Particularly in freshwater, they exert heavy grazing pressure on phytoplankton abundances (Gilbert and Bogdan 1984). They are also known to feed on detritus-bacterial clumps (Dumont 1977). Their contribution to the secondary production as food source for many predatory invertebrates including larval crustaceans, as well as for larval fish and adult ornamental fish species is well documented. Thus, in aquacultural operations rotifers are widely used as ideal first food for rearing a variety of fish and crustaceans (Lubzens 1987). Additionally, rotifers are considered as sensitive indicators of the health of their environment (Sladecek 1983). They have therefore been used as excellent bioassay organisms in water pollution studies in order to detect toxic levels of industrial and agricultural wastes in Europe and USA (Halbach 1984; ASTM 1991).

Currently in Mexico, the use of the exotic zooplankton *Daphnia magna* is in force for toxicological evaluation of industrial effluents (Martínez-Jeronimo and Garcia-Gonzalez 1994). Since this is not a native species, its use for national testing of toxic materials does not seem relevant. In this connection, some attempts have been made in Mexico for the application of other zooplankton, particularly rotifers, in aquatic toxicology as indicators of the water quality (Vilaclara and Sladecek 1989). In Mexico, there has been an increase in the use of different pesticides including methyl parathion by the agricultural sector (CONADE 1991). Sarma et al. (1998) observed that at 24 hr, the LC_{50} for the laboratory-grown rotifer *Brachionus calyciflorus* was about 9 mg/L for methyl parathion. However, this value is three times higher for the same species when the test individuals were obtained by hatching the resting eggs (Fernandez-Casalderrey et al. 1992). Food density is known to influence the toxicity of pesticides on rotifers (Rao and Sarma 1986). The aim of the present study is to investigate the effect of methyl parathion on population growth of the rotifer *B. calycifIorus* under different algal food concentrations.

MATERIALS AND METHODS

The rotifer *Brachionus calyciflorus* was originally collected in Chapultepec Lake (Mexico City) and mass cultured using reconstituted water (EPA) as medium. The EPA medium was prepared by dissolving 96 mg $NaHCO_3$, 60 mg $MgSO_4$, 60 mg $CaSO_4.2H_2O$ and 4 mg KCl in one litre of distilled water (Anon. 1985). In routine cultures, we were able to obtain rotifer densities higher than 100 ind./mL at 25° C using green alga *Chlorella vulgaris* as exclusive food. *Chlorella* was mass cultured using Bold-Basal medium (Borowitzka and Borowitzka 1988).

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For the experiments we used log phase algae, harvested by centrifugation at 800 g for 5 minutes, washed twice with distilled water and resuspended in EPA medium. The algal density was estimated using haemocytometer. *B. calyciflorus* grows on a wide range of algal density (5 x 10^5 - 40 x 10^6 cells/ml), but the optimum is around 6 x 10^6 cells/ml (Sarma et al. 1996). Thus, four final algal levels viz 0.75, 1.5, 3.0 and 6.0 x 10^6 cells/ml were prepared by serial dilution using EPA medium. We used methyl parathion (commercial grade) as the toxicant. In order to choose the toxicant concentrations, we used the previously reported LC_{50} values obtained under different assay conditions (9-29 mg/L for 24 hr) for this chemical for the same rotifer species from the literature (Fernandez-Casalderrey et al., 1992; Sarma et al. 1998). Accordingly, we selected five toxicant concentrations viz. 0 (control), 5, 10, 20, and 30 mg/L. For obtaining the desired final algal concentration and toxicant level, we followed Rao and Sarma (1986). In this procedure, every day, twice the required concentrations of both the toxicant and the food were mixed in equal proportions, just before using as medium to avoid possible detoxification of methyl parathion by algae in the absence of rotifers.

The experiments were conducted in 25 mL transparent vials. Into each of 100 test vials (5 toxicant levels x 4 algal concentrations x 5 replicates = 100) containing 20 mL medium of desired algal-toxicant combination, we introduced rotifers at an initial density of 10 ind./mL of mixed age group from a growing culture. The vials were kept in a thermostatically controlled waterbath set at 27±l°C under diffuse and continous fluorescent illumination. At each 24±2 hr interval, the population density of rotifers was estimated under a stereo microscope from all test vessels using at least two aliquot samples of 1-3 ml each or the whole replicate-counting. Following counting, the rotifers were transferred using 50µm mesh to fresh medium containing appropriate food level-toxicant combinations. For estimating the densities of rotifers, only live individuals (females) were considered. The experiment was terminated after ten days when most populations completed one population cycle or completely declined. From the data, the rate of population growth (r) was calculated using the exponential equation (Krebs 1985):

$r = (\ln N_t - \ln N_o)/t$

where, N_o = the initial density of rotifers, N_t = the final density and t = time in days.

RESULTS AND DISCUSSION

The maximum population density of *B. calyciflorus* was strongly influenced not only by both algal density and the concentration of methyl parathion but also their interaction ($P<0.001$, ANOVA, Table 1). An increase in food availability resulted in an increase in population of *B. calyciflorus.* On the other hand, an increase in toxicant level reduced the rotifer density. There was a positive interaction between toxicant level and food density in that increasing food concentration reduced the effect of methyl parathion (up to 20 mg/L) on the population growth of the tested rotifers (Figures la, 1b). In control (without toxicant) when food density was 0.75 x 10^6 cells/ml, the rotifers maintained an average density of 4±1 ind./mL, while those at the same food level but under 5 mg/L of the toxicant, declined completely on the day 10.

Methyl parathion at a concentration of 30 mg/L did not support rotifer growth in any food density. The rate of population growth (r) per day was also strongly influenced by food density, toxicant level and their interaction (Table 1, Figure 2). The r values were negative in all toxicant levels at lower food concentrations (0.75 x 106 and 1.5 x 10^6 cells/ml). On the other hand, positive r values were recorded for higher food levels (3-6

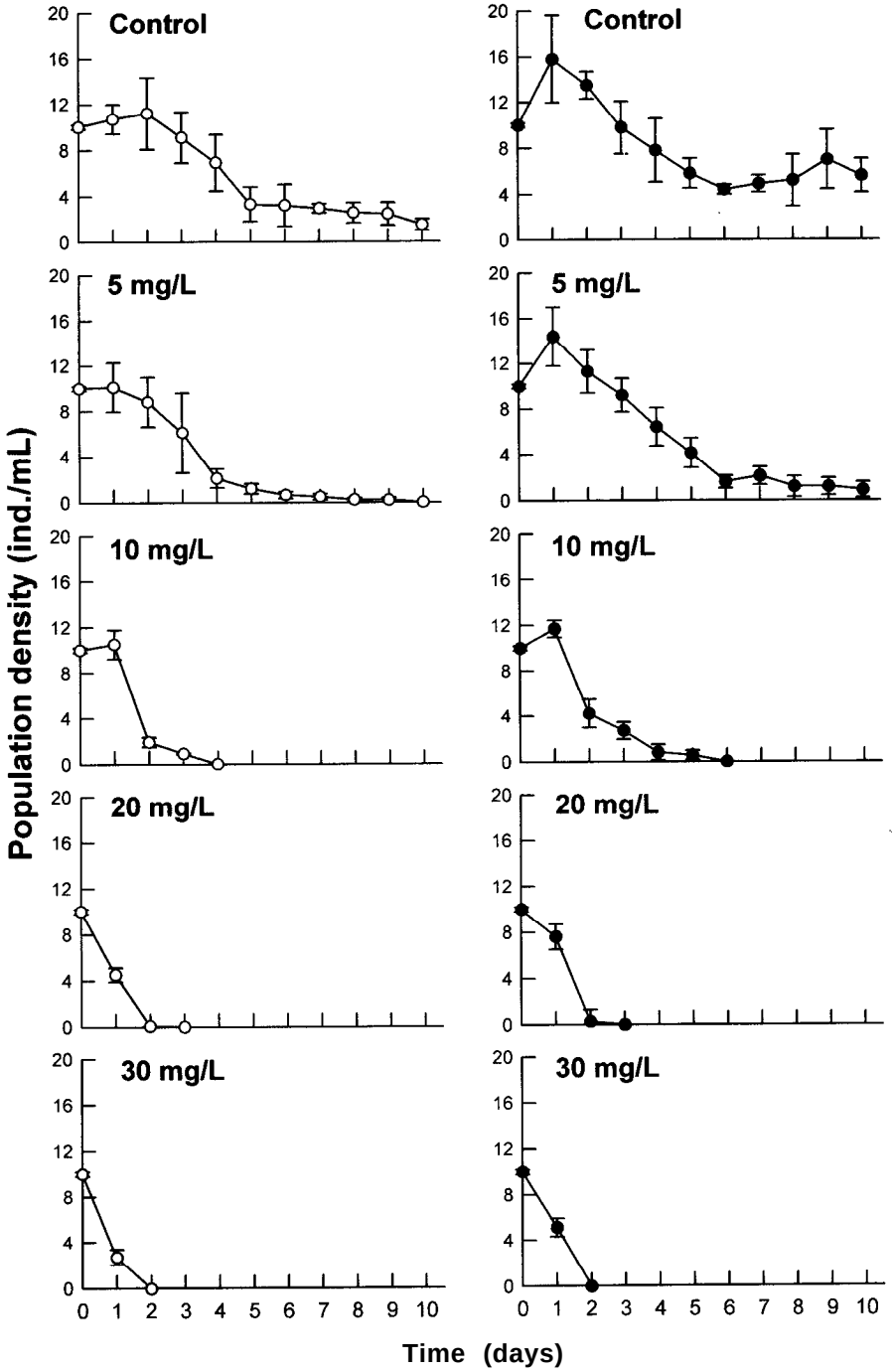

Figure 1a. Relationship between concentration of methyl parathion (mg/L) and the population growth of *Brachionus calyciflorus* under 0.75×10^6 (open circle) and 1.5×10^6 (closed circle) cells/ml of *Chlorella* as a function of time. Shown are the values (mean±s.d.) based on five replicates.

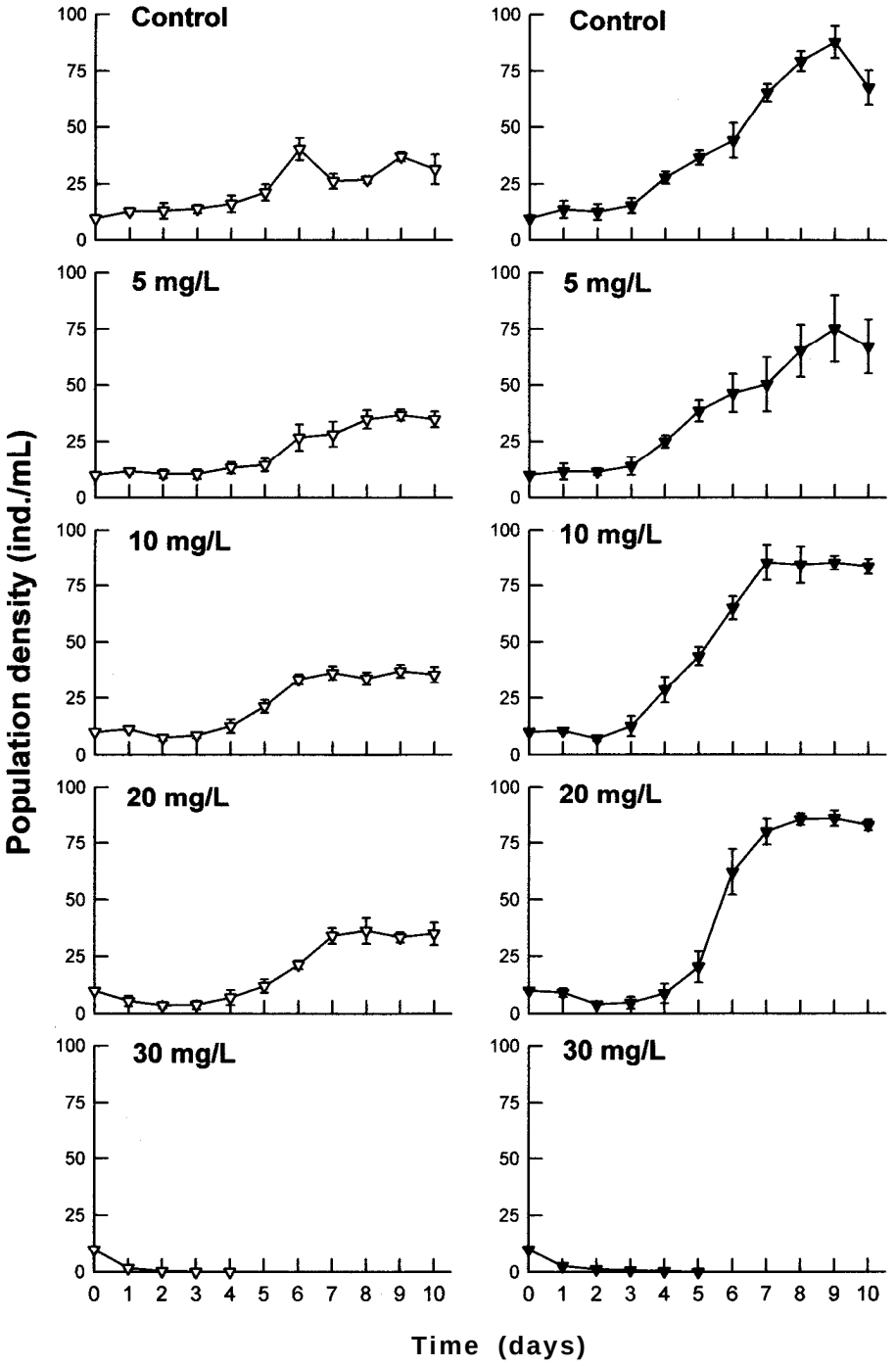

Figure 1b. Relationship between concentration of methyl parathion (mg/L) and the population growth of *Brachionus calyciflorus* under $3.0x10^6$(open traingle) and $6.0x10^6$(closed triangle) cells/ml of *Chlorella* as a function of time. Shown are the values (mean±s.d.) based on five replicates.

x 10^6 cells/ml) in all methyl parathion levels except for the highest toxicant concentration (30 mg/L).

Table 1. Two-way ANOVA of effect of methyl parathion on mean population density and rate of population growth under four different food concentrations.

Source	Sum-of-Squares	DF	Mean-Square	F-Ratio	P
Maximum population density					
Food	203985.81	3	67995.27	210.77	0.001
Toxicant	56689.72	4	14172.43	43.93	0.001
Food x toxicant interaction	59687.46	12	4973.95	15.42	0.001
Error	348404.14	1080	322.59	-	-
Rate of population growth					
Food	25.60	3	6.40	218.51	0.001
Toxicant	6.78	4	2.26	77.11	0.001
Food x toxicant interaction	7.84	12	0.65	22.31	0.001
Error	2.34	80	0.029	-	-

In a recent review of rotifer ecotoxicology, Snell and Janssen (1995) mentioned many ecological variables including population growth characters as sensitive indicators of toxicants in the medium. Rotifer populations in the present study responded rapidly not only to the food availability but also to the toxicant concentrations as found in many other investigations (Halbach et al. 1983). Even the toxicant level as low as 5 mg/L was sufficient to have a negative effect on rotifer density in the lowest food level. This is in agreement with the data of Fernandez-Casalderrey et al. (1992) who derived a no-observed effect concentration (NOEC) for this species close to 1 mg/L of methyl parathion. For rotifers exposed to higher toxicant levels and higher food levels, the population initially did not increase but recovered afterwards. The range of rate of population increase per day (r) in the present investigation also varied strongly from -1.46 to +0.396 depending on the test conditions (Figure 2). In general, the positive r values recorded here for this species are within the range expected for the genus *Brachionus* (Miracle and Serra 1989).

The peak population abundances of rotifers in controls varied between 11 to 100 ind./mL and increased linearly with increasing food density. Those exposed to methyl parathion up to 20 mg/L behaved in a similar way (Figure 3).

That the increase in algal concentration had a positive effect on the toxicant-stressed rotifer populations as observed here has been recorded in earlier studies. For example, *Brachionus patulus* when grown under 1-3 x 10^6 cells/ml of *Chlorella*, the toxic effect of DDT was reduced with increasing algal food density (Rao and Sarma 1986; 1990). There is some agreement among the investigators that laboratory toxicity tests are conducted at much higher food levels than those encountered by rotifers in natural environments (Rao and Sarma 1986; Janssen et al. 1994). This suggests that toxicity tests conducted in lower food levels could yield a lower maximum acceptable toxicant concentration. This trend is clearly visible in the present study since the food levels

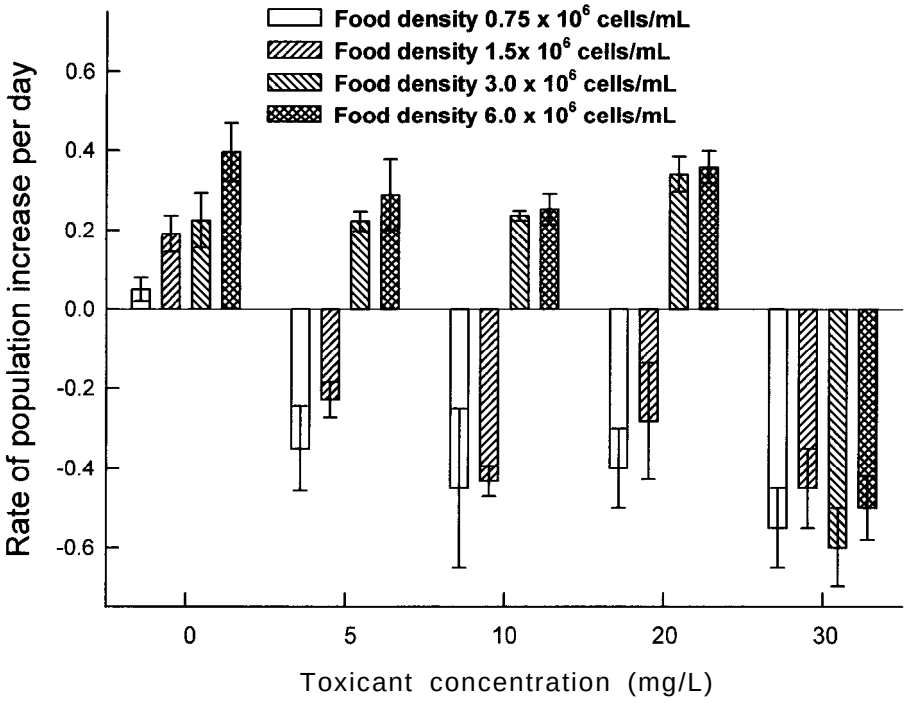

Figure 2. Rate of population increase in *Brachionus calyciflorus* under various toxicant-food level combinations. Shown are mean±s.d. values based on five replicates.

chosen here range from those barely supporting the population to optimum levels resulting in a rapid increase in their abundance. Thus the range of food density used here is within the levels tested for this species earlier (Sarma et al. 1996). In conclusion, our results demonstrate the importance of algal food density in modifying the toxic effects of methyl parathion on rotifers.

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