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Effect of Methyl Parathion-Treated Prey (*Brachionus calyciflorus*) on the Population Growth of the Predator *Asplanchna sieboldi* (Rotifera)

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The use of pesticides in combating pests in agriculture has increased tremendously in the last two decades in Mexico. At present, methyl parathion is one of the widely used chemicals for controlling insect pests in agricultural and domestic environments. A substantial portion of the pesticides applied in agricultural fields is known to reach freshwater bodies including rivers, ponds and lakes as run-offs (Kadlec and Benson 1995). Although some information is available on the role of methyl parathion in affecting non-target organisms (Little et al. 1990) its effect on zooplankton, particularly rotifers, is not well-known (Fernandez-Casalderrey et al. 1995).

Rotifers of the genus *Brachionus* are widely used for assessment of water pollution (Rao and Sarma 1986). The species *B. calyciflorus* is used as standard bioassay organism in experiments aimed at deriving water pollution standards by the American Society of Testing and Materials (Snell and Janssen 1995). Methyl parathion has been earlier shown to be toxic to the populations of *B. calyciflorus* (Fernandez-Casalderrey et al. 1992). The aim of this study was to understand the effect of methyl parathion on the population growth of a predatory rotifer *(Asplanchna)* when supplied through its prey (*Brachionus calyciflorus*).

MATERIALS AND METHODS

The prey rotifer *Brachionus calyciflorus* (body length mean±standard error =185±12µm) was originally isolated from Lake Chapultepec in Mexico city and was mass cultured using the unicellular green algae *Chlorella vulgaris* (Sarma et al. 1998). Mass cultures of *C. vulgaris* were raised using the Bold-Basal medium (Borowitzka and Borowitzka 1988). The predatory rotifer *Asplanchna sieboldi* (body length 1233±40 µm) was also isolated from the Lake Chapultepec and mass cultures were raised using live individuals of *B. calyciflorus* as the exclusive food. For experiments we used 25-mL capacity transparent vials containing 20 mL of the Environmental Protection Agency medium (EPA) (Anon. 1985) with *C. vulgaris* at a density of 0.25×10^6 as the medium. The experiments were conducted at 25°C in thermostatically controlled waterbaths and under continuous, diffused fluorescent illumination.

We used *B. calyciflorus* treated in four ways as prey for *Asplanchna* viz. 1) control - prey offered in living condition; 2) prey living but sublethally exposed to methyl parathion; 3) cold-killed prey; and 4) toxicant killed prey. Treatment 2 was obtained by exposing

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rotifers to methyl parathion at a concentration of 10 mg/L for 2 hours, after which they were rinsed with EPA medium twice before employing them as food for *Asplanchna*. For treatment 3, we froze *B. calyciflorus* at 0°C for 24 hours, thawed them and used the dead individuals. For treatment 4, we used toxicant-killed *B. calyciflorus* as food which were obtained by exposing them to methyl parathion at 100 mg/L for 2 hours, and rinsing the dead rotifers twice in EPA medium before use.

For each treatment we used four food concentrations viz. 2.5, 5.0, 10.0 and 20.0 ind./mL and thus the total number of prey rotifers per container were 50, 100, 200 and 400 individuals, respectively. Every day only freshly prepared prey types were used. For each food concentration we used 5 replicates. Thus, in all, we used 80 test vials (4 food types X 4 food densities X 5 replicates). Into each of the vials containing prey rotifers of one particular density and type, we introduced *two A. sieboldi* individuals (age<24hr). Every day, from each of the test vials, we counted and transferred living *Asplanchna* to fresh vials containing appropriate food type and density. Both the prey and the predators were individually counted under a stereomicroscope using finely drawn Pasteur pipettes. Dead predators and uneaten prey were discarded during this process. Males were rarely encountered during the test period and were discarded when observed. The experiment was terminated after 10 days. From the data obtained, we calculated the rate of population increase and obtained a mean based on 5-6 values using the following exponential growth equation:

$r = (1n\ N_t - 1n\ N_o)/t$

where N_o = initial population density and N_t = population density after time t

Observations on the feeding behaviour of *Asplanchna* with live and cold kill prey were made following Gilbert (1980).

RESULTS AND DISCUSSION

The 24 hr-median lethal concentration (LC50) values of methyl parathion for both the prey (*B. calyciflorus*) and the predatory (*A. sieboldi*) rotifers are presented in the Table 1. The population density of *A. sieboldi* increased with increasing prey density regardless of its exposure to the toxicant (Figs 1 and 2). However, *Asplanchna* showed poor population growth when fed on dead individuals of *B. caclyciflorus* regardless whether they were cold-killed or toxicant-killed. The maximal population increase of the predator populations was recorded in controls and the lowest in vials containing toxicant killed prey. *A. sieboldi* populations crashed after day 6 in the lowest prey density (2.5 ind./mL) in toxicant killed *B. calyciflorus*. The maximum population density of *A. sieboldi* recorded in this study was 1.35 ± 0.20 ind./mL in the controls at a density of 20/mL, and the lowest was 0.13 ± 0.01 ind./mL in the lowest food density of toxicant-killed prey (Fig. 3). Food type, concentration and their interaction had a significant effect on this variable ($p < 0.001$, 2-way ANOVA). The rate of population increase (r) showed a linear relation with increasing prey density in the medium (Fig. 4). The highest r value calculated was 1.57±0.15 per day (in the controls at a prey density of 20/mL).

This study indicated that prey exposed to methyl parathion (at a concentration of 10 mg/L) for even 2 hours had a significant negative effect on the population growth of its predator. The LC_{50} value at 24 hr for prey rotifers in this study was 9 mg/L of methyl

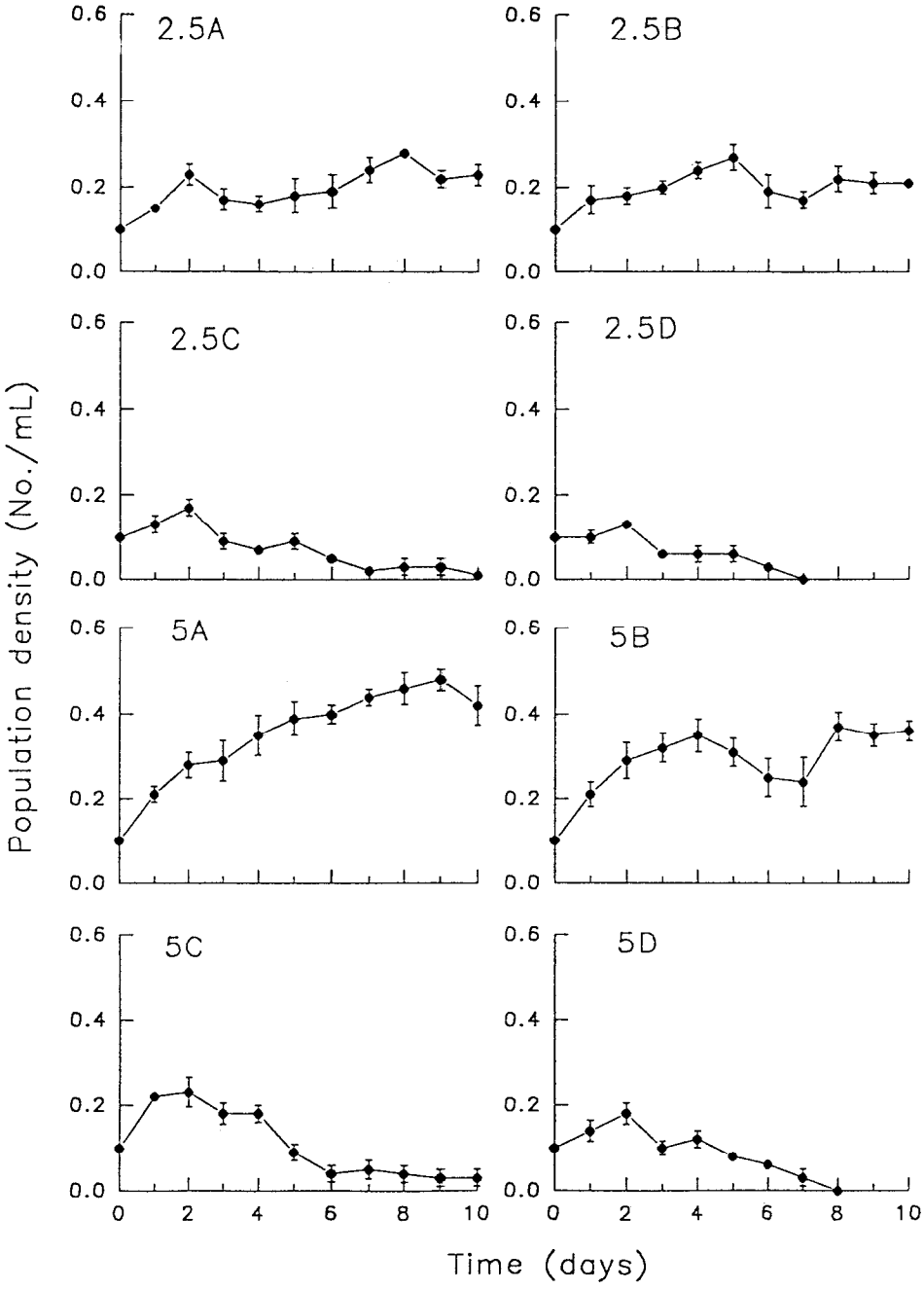

Figure 1. Population growth of *A. sieboldi* in relation to the prey (*B. calyciflorus*) treatment and density. A, B, C and D refer to treatments 1, 2, 3 and 4 respectively, as explained in the text. The values 2.5 and 5 represent the prey densities (ind./mL).

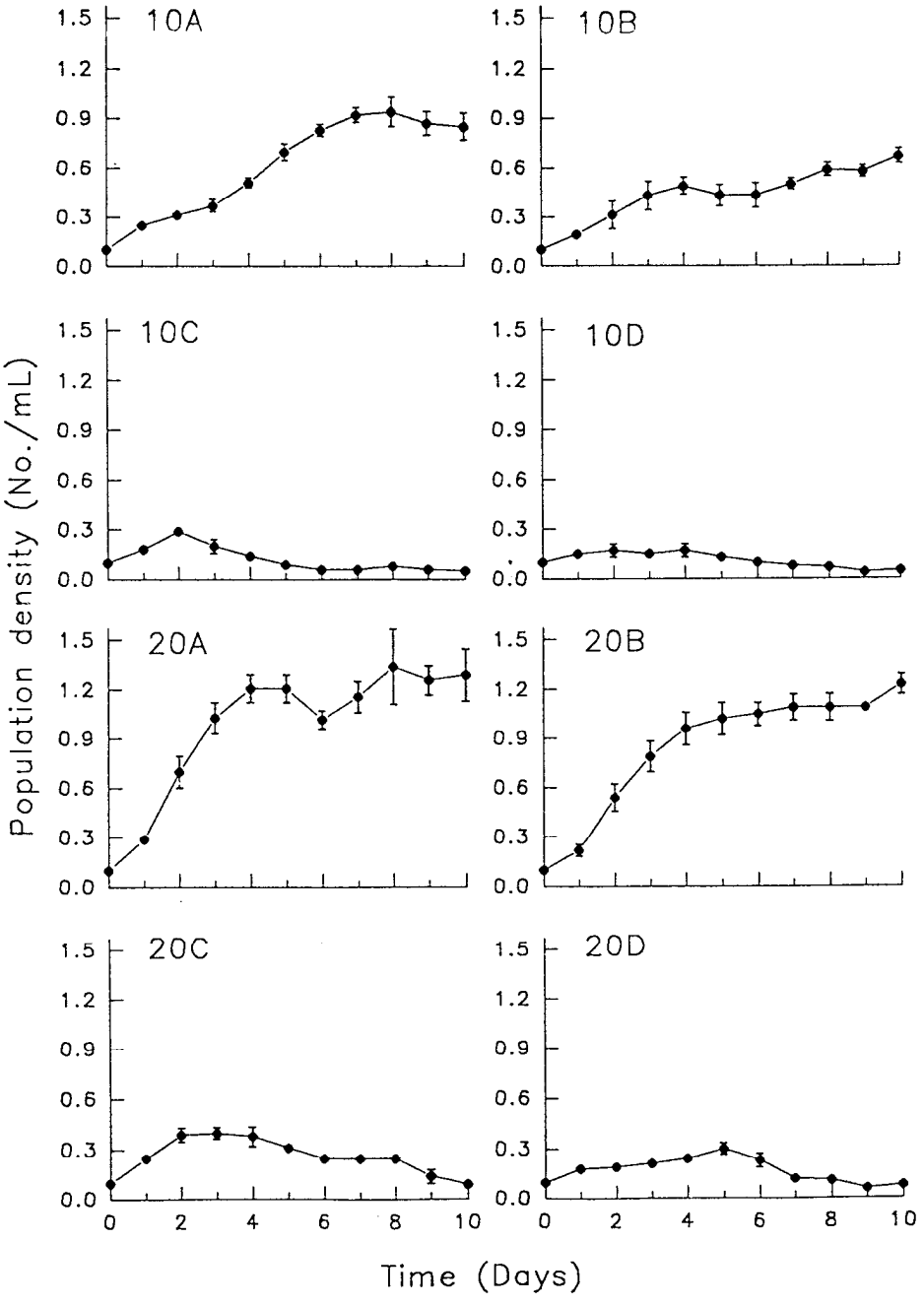

Figure 2. Population growth of *A. sieboldi* in relation to the prey (*B. calyciflorus*) treatment and density. A, B, C and D refer to treatments 1, 2, 3 and 4 respectively, as explained in the text. The values 10 and 20 represent the prey densities (ind./mL).

parathion, which was about 1/3rd lower than that reported earlier by Fernandez-Casalderrey et al. (1992). We attribute this to the origin of the test individuals. In the present study, we continuously cultured the test individuals through parthenogenetic reproduction while in the case of Fernandez-Casalderrey et al. (I 992) the test rotifers were obtained by hatching from resting eggs, which in strict sense do not form a clone. There is some evidence to suggest the variability exists in the sensitivity of rotifers hatched from resting eggs (Liber and Solomon 1994).

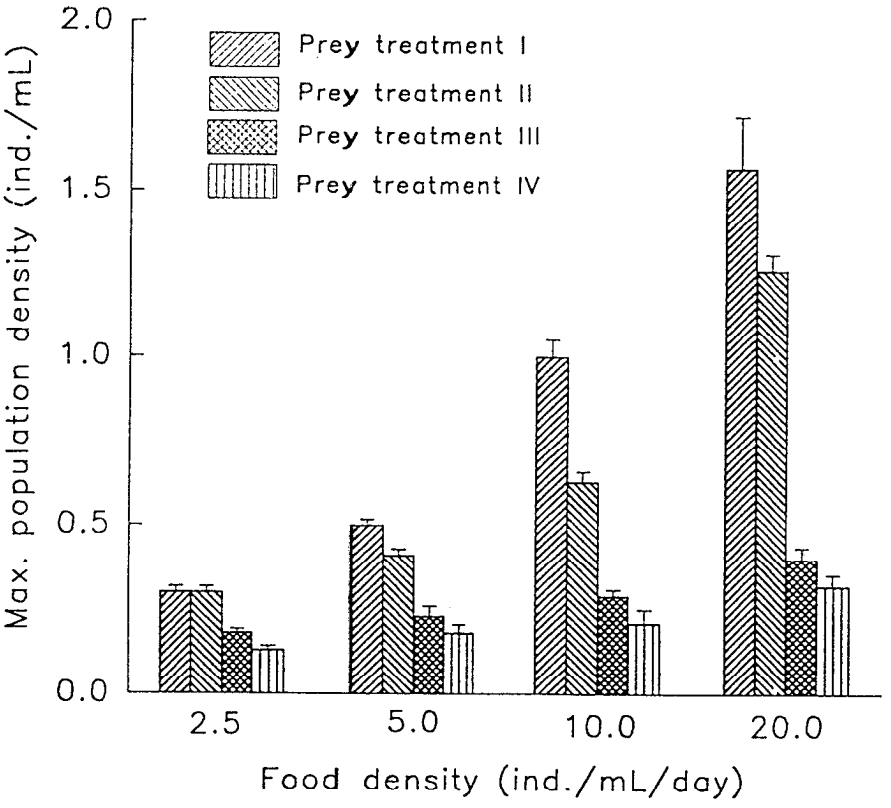

Figure 3. Maximum population density of *A. sieboldi* in relation to the prey (*B. calyciflorus*) treatment and density. Treatments I, II, III and IV of the prey are explained in the text. Shown are the mean ± standard error based on 5 replicate recordings.

Living prey were well utilized by *Asplanchna* in comparison to dead individuals which were poorly consumed. The food and feeding habits of *Asplachna* have been well-studied (Iyer and Rao 1996). Most members of this genus are predatory and often engulf smaller prey as they are encountered. The increase in population of *Asplanchna* with increasing prey density as recorded here (Table 2) is well-established through extensive laboratory studies which show increased encounter rates at high prey abundances.

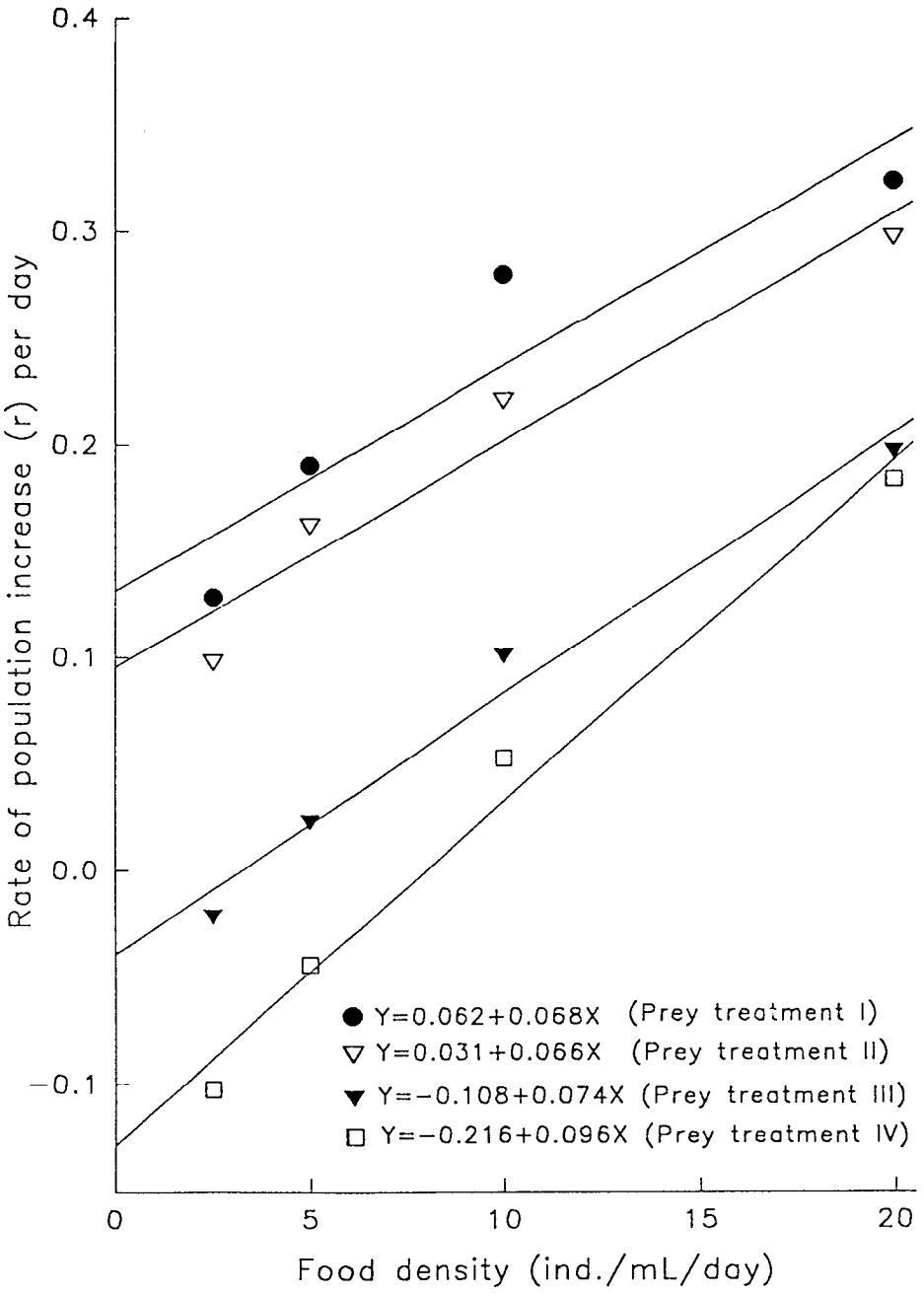

Figure 4. Rate of population increase per day of *A. sieboldi* in relation to the prey (*B. calycifjlorus*) treatment and density. Treatments I, II, III and IV of the prey are explained in the text. Shown are the regression equations for each treatment.

Table 1. Data on the 24 hr median lethal concentrations of *B. calyciflorus* and *A. sieboldi*. We used 100 test individuals for each of the 3 replicates of prey species and 20 individuals per replicate for *A. sieboldi*. Values were derived following probit method.

Test species	LC_{50} value (mean±standard error) (mg/L)
B. calyciflorus	9.01±0.18
A. sieboldi	13.0±0.16

A decrease in the population growth of *Asplanchna* offered prey sublethally exposed to methyl parathion in comparison to controls may be attributed to reduced prey consumption and/or assimilation efficiency and an indirect effect of the pesticide on the predators (e. g. changes in swimming speed: Janssen et al. 1994). It is not well-known whether dead zooplankton can become a good source of food for *Asplanchna*. Numerous field studies have detected a variety of food types varying from those that escape rapidly (*Hexarthra* and *Polyarthra*) to slowly moving genera (*Anuraeopsis, Brachionus* and *Keratella*) (Conde-Porcuna and Sarma 1995; Iyer and Rao 1996) but whether an encountered prey item in the gut was living or dead before capture has not been established. Cannibalism on dead individuals does not appear to support the population for long (Dumont and Sarma 1995). Our results also confirm that dead brachionids did not support the growth of *Asplanchna* mainly due to the reduced encounter rates which in turn affected the capture and ingestion probabilities (see Table 2). From an ecotoxicological point of view this is relevant. If toxicants selectively kill the prey rotifers even without directly affecting the predatory *Asplanchna*, the latter may be expected to be exterminated as shown here. The effect of crude oils on the prey consumption, offspring prodution and population growth of *Asplanchna* supports our present study in which an effect on the prey (not necessarily leading to mortality) has an adverse effect on the predators (Rogerson et al. 1982). Prey mortality need not be caused by a toxicant; even cold exposure (and the resulting thermal shock) is sufficient to reduce the predator's abundance, thus disturbing the natural prey-predator oscillations in aquatic ecosystems.

In conclusion, our study indicates that in addition to the direct effects of pesticides on zooplankton, indirect effects via prey on predators are equally important in maintaining a predator-prey balance in aquatic ecosystems.

Table 2. Feeding activity of *A. sieboldi* fed on living and cold-killed *B. calyciflorus*. Values (mean&standard error) are based on 10 replicates. One predator was used for each replicate.

Prey stage	Feeding activity (no. of events/5 min observation)			
	Encounter	Attack	Capture	Ingestion
Living individuals	26.90±3.83	4.70±0.68	4.10±0.50	3.20±0.55
Cold-killed individuals	7.00±1.04	2.70±0.73	2.70±0.73	2.70±0.73

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REFERENCES

Anonymous (1985) Methods of measuring the acute toxicity of effluents to freshwater and marine organisms. US Environment Protection Agency EPA/600/4-85/013

Borowitzka MA, Borowitzka LJ (1988) Micro-algal biotechnology. Cambridge University Press, London.

Conde-Porcuna JM, Sarma SSS (1995) Prey selection by *Asplanchna girodi* (Rotifera): the importance of prey defence mechanisms. Freshwater Biol 33: 341-348

Dumont HJ, Sarma SSS (1995) Demography and population growth of *Asplanchna girodi* (Rotifera) as a function of prey (*Anuraeopsis fissa*) density. Hydrobiologia 306: 97-107

Fernandez-Casalderrey A, Ferrando MD, Andreu-Moliner E (1992) Acute toxicity of several pesticides to the rotifer *Brachionus calyciflorus.* Bull Environ Contam Toxicol 48: 14-17.

Fernandez-Casalderrey A, Ferrando MD, Andreu-Moliner E (1995) Chronic toxicity of methylparathion to *Daphnia magna:* effects on survival, reproduction and growth. Bull Environ Contam Toxicol 54: 43-49.

Gilbert JJ (1980) Observations on the susceptibility of some protists and rotifers to predation by *Asplanchna girodi.* Hydrobiologia 73: 87-91

Iyer N, Rao T R (1996) Responses of the predatory rotifer *Asplanchna intermedia* to prey species differing in vulnerability: laboratory and field studies. Freshwater Biol 36: 521-533

Liber K, Solomon KR 1994. Acute and chronic toxicity of 2,3,4,6 -petrachlorophenol and pentachlorophenol to *Daphnia* and rotifers. Arch Environ Contam Toxicol 26: 212-221

Little EE, Archeski RD, Flerov BA, Kozlovskya VI (1990) Behavioral indicators of sublethal toxicity in rainbow trout. Arch Environ Contam Toxicol 19: 380-385

Janssen CR, Ferrando MD, Persoone G (1994) Ecotoxicological studies with the freshwater rotifer *Brachionus calyciflorus.* Ecotoxicol Environ Safety 28: 244-255

Kadlec MC, Benson WH (1995) Relationship of aquatic natural organic material characteristics to the toxicity of selected insecticides. Ecotoxicol Environ Safety 31: 84-97

Rao TR, Sarma SSS (1986) Demographic parameters of *Brachionus patulus* Muller (Rotifera) exposed to sublethal DDT concentrations at low and high food levels. Hydrobiologia 139: 193-200

Rogerson A, Berger J, Grosso CM (1982) Acute toxicity of ten crude oils on the survival of the rotifer *Asplanchna sieboldi* and sublethal effects on the rates of prey consumption and neonate production. Environ Pollut A 29: 179-188

Sarma SSS, Arévalo-Stevenson R, Nandini S (1998) Influence of food (*Chlorella vulgaris*) concentration and temperature on the population dynamics of *Brachionus calyciflorus* Pallas (Rotifera) isolated from a subtropical reservoir in Mexico. Ciencia ergo sum 5: 1-5

Snell TW, Janssen CR (1995) Rotifers in ecotoxicology. Hydrobiologia 313/314: 231-247