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Effect of Sublethal Diazinon Concentrations on the Demographic Parameters of *Brachionus calyciflorus* Pallas (Rotifera)

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Short time toxicity tests with high concentrations of the pollutant do not allow any predictions which act for a longer period of time. So there is a need for chronic toxicity tests which monitor the toxic effects of a pollutant on different life stages.

Rotifers, widely recognized as bioindicators of water quality (Sladecék 1983), are also ideal bioassay animals for sublethal toxicity tests because of their sensitivity, short generation time, genetic uniformity (due to predominantly parthenogenetic reproduction), and relative ease of culture and maintenance in the laboratory. Furthermore, the fact that rotifers constitute a vital component of freshwater foodwebs (Hutchinson 1967) makes their selection for bioassay tests an ecologically relevant one.

We report here the effect of sublethal levels of diazinon on selected demographic parameters of the freshwater rotifer *Brachionus calyciflorus* (Pallas).

MATERIALS AND METHODS

B. calyciflorus cysts used in the following experiments were produced in mass cultures mantained under rigorously controlled conditions. Cysts were stored in EPA medium at 4°C in the dark. Bidistilled water is the base for making EPA medium (USEPA 1985). This medium is a synthetic freshwater that is prepared from reagent grade chemicals and composed of 96 mg $NaCO_3$, 60 mg $CaSO_4{\cdot}2H_2O$, 60 mg $MgSO_4$, and 4 mg KCl per liter of water. Cysts hatching is initiated by transferring to warmer temperatures and light (Snell and Persoone 1989). Standard environmental conditions for these bioassays were: temperature 25°C; pH 7.4-7.8; hardness 80-100 mg as $CaCO_3$/L; alkalinity 60-70 mg/L as $CaCO_3$ and darkness.

In order to choose the most appropiate sublethal concentrations of diazinon for this study, preliminary 24-hr LC50 tests were conducted using a wide concentration range of this insecticide (0-50mg/L). The 24-hr LC50 obtained was 29.22 mg/L. Based on this, diazinon levels of 0(control), 5, 7, 14 and 19 mg/L (0, 1/5, 1/4, 1/2 and 2/3 of the 24-hr LC50) were chosen for the chronic study.

The required diazinon concentrations were prepared by serial dilution with EPA water from a stock solution of technical grade diazinon 92% (Cequisa) in acetone.

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Because acetone was required as a carrier for diazinon, an aditional control, acetone only, was included. Its concentration was 1.5 mL/L, corresponding to the highest acetone concentration used in diazinon experiments. All the treatments with toxicant had the same acetone concentration.

Toxicity tests were conducted in sterile, 24-well polystyrene plates which are used once and discarded. One mL of test solution is distributed to each well and one neonate (0-2 hr old, hatched from cysts) female is introduced per well with a micropipette. The rotifers were fed daily with *Nannochloris oculata* ($5x10^5$ cell/L). Each experiment was started with cohorts of 24 neonates, which were checked every 12 hr. Number of attached eggs, offspring and mortality were recorded. The parent female was transferred into fresh medium of appropiate diazinon-food levels every day and the juveniles were discarded. The experiments were terminated when the last individual had died.

At every age(X), survivorship(lx), life expectancy at hatching (eo) and fertility (mx) were constructed for all the rotifer cohorts using standard life table methods (Poole 1974). The other demographic parameters, net reproductive rate (Ro) (multiplication rate per generation), generation time (T) (mean period elapsing between the birth of parent and the birth of its offspring), intrinsic rate of natural increase (r) (population grown per individual) and reproductive value (Vx) (contribution to the future population) were calculated using the following formulae described by Pianka (1978).

$$Ro = \sum_{x=0}^{\infty} lx.mx \qquad T = \frac{\sum_{x=\alpha}^{w} lx.mx.x}{Ro}$$

$$r = \frac{\log_e Ro}{T} \qquad Vx = \sum_{\acute{e}=x}^{\infty} \frac{lt}{lx} mx$$

We divided each cohort into four groups, which can be considered as four replicates (each replicate had six individuals). This implies that we were able to perform significance tests between the control and the various treatments.The results were compared by ANOVA and Duncan´s Multiple Range Test. The significance level was fixed at $p<0.05$.

RESULTS AND DISCUSSION

All the life table parameters of *Brachionus calyciflorus* were influenced by the sublethal levels of diazinon to which they were exposed.

The survivorship (lx) declined with increasing diazinon concentration (Fig 1). It was reduced from 10 days in the control to 2 days after the treatment with 19 mg/mL diazinon. *B.calyciflorus* survivorship curves of in control and low diazinon levels are similar to those reported for *Brachionus patulus* (Ramakrishna and Sarma 1986), *Brachionus calyciflorus* (Halbach 1970) and *Brachionus rubens* (Halbach *et al.* 1983), but at higher diazinon levels the curves become very steep, reflecting diazinon-inflicted mortalities in the inicial stages.

Life expectancy at hatching (eo) was significantly affected by diazinon (Fig 2). The differences between control and all sublethal diazinon levels were statistically

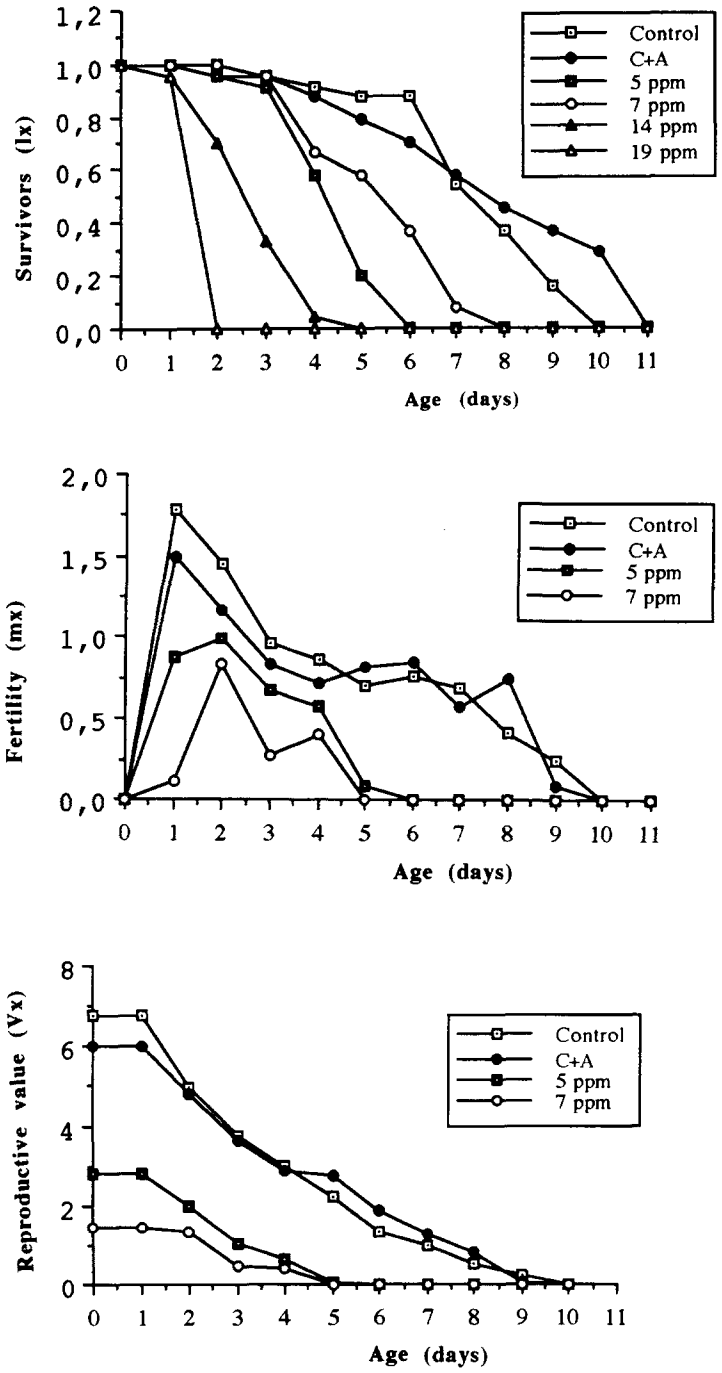

Figure 1. Survivors (lx), fertility (mx) and reproductive value (Vx) of amictic *Brachionus calyciflorus* exposed to defferent diazinon concentrations. Each point represents the mean of four replicates.

significant ($p<0.05$) but not between controls and controls with acetone ($p>0.05$). Life expectancy decreased from 7.68 days in controls to 1.95 days in 19 mg/L of diazinon. A decline in life expectancy with increasing sublethal concentrations of pesticides, as shown in this study, was also demonstrated in *Brachionus patulus* exposed to DDT (Ramakrishna and Sarma 1986) and in *Brachionus rubens* exposed to pentachlorophenol and 4-chloroaniline (Halbach *et al.* 1983).

The reproductive performance of this rotifer was also adversely afected by diazinon. Fertility (mx) declined with increasing insecticide levels, not only in its real value but also in the length of the reproductive period. We found a delay of the first reproduction after the treatment with 5 and 7 mg/L (Fig 1). At higher diazinon levels (14 and 19 mg/L) the animals died before reproducing.

A reduction in fertility as a consecuence of chronic halogen toxicant stress was observed in *Brachionus plicatilis* (Capuzzo 1979) and *Brachionus rubens* (Halbach *et al.* 1983). Crossland and Hillaby (1985) on *Daphnia magna* found a decrease in the fertility with 20 and 50 μg/L of 3,4-Dichloroaniline (DCA), but with 100 μg/L the animals did not reproduce.

The net reproductive rate (Ro) and the generation time (T) were both affected significantly ($p<0.05$) by all sublethal diazinon concentrations (Fig 2). There were no significant differences ($p>0.05$) between controls and controls with acetone.

The intrinsic rate (r) decreased with diazinon concentrations, but it never was negative indicating that rotifer population levels are not doomed to extinction. The magnitud of r is affected by age at first reproduction, frecuency of reproduction and cluth size (Allan 1976; Stearns 1976) and a change in any one of these parameters cause by sublethal pollutant stress, could change the r value for that population.

The age-specific expectation of future offspring by an amictic rotifer, expresed as its reproductive value (Vx) was also affected by diazinon (Fig 1). The effect was higher with 14 and 19 mg/L where the animals died before reproducing. Vx is a parameter that is often examinated to understand the reproductive tactics or strategies of a population in terms of cost and benefits of age-specific reproductive effort (Pianka and Parker 1975; Snell and King 1977).Similar results were obtained by Ramakrishna and Sarma (1986) on *Brachionus patulus*, these authors found a declined of Vx with increasing DDT concentrations.
The parameters net reproductive rate (Ro), generation time (T) and life expectancy at hatching (eo), showed a significant linear regression with diazinon concentrations. The regressions were adequately described by a simple linear equation ($y=a-bx$). The intrinsic rate however did not show any clear trends, although the effects of diazinon were statistically significant.

A useful, quantitative parameter, analogous to LC50 derived in acute toxicity tests, is EC50, the median effective concentration of the toxicant at which the value of a given parameter is reduced to 50% of that in controls (Marshall 1978). We have derived the EC50 values for selected life history parameters of *B. calyciflorus* using the respective regression equations. We can observe the EC50 for net reproductive rate (Ro), generation time (T) and life expectancy at hatching (eo) in Table 1. As we can see in this table, low diazinon concentrations produced a high effect on reproduction and life expectancy the EC50 for all of these parameters with the LC50 for diazinon in this specie (29.22 mg/L). We found that diazinon levels of 1/2 LC50 produced a 50% of reduction in life expectancy, and levels of 5.20 and 8.49 mg/L (1/5 and 1/4 LC50 aproximayely) reduced reproduction and generation time in a 50%.

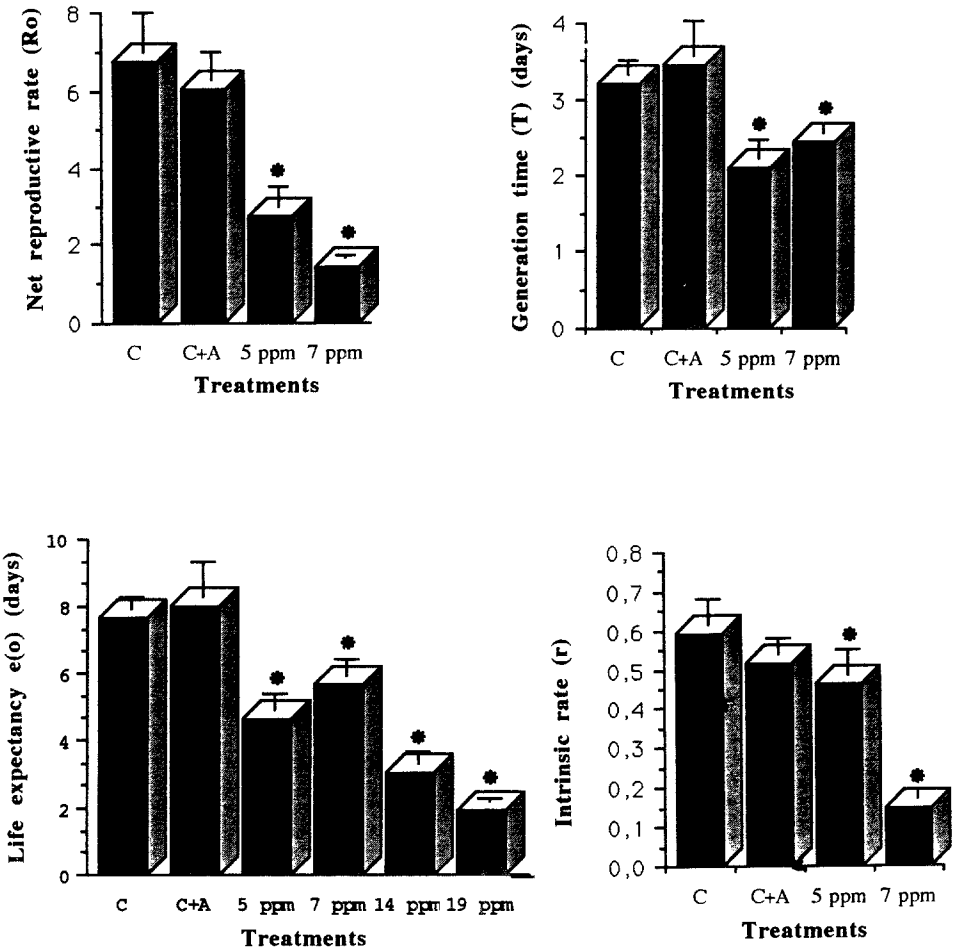

Figure 2. The effect of diazinon on the net reproductive rate (Ro), generation time (T), life expectancy at hatching (eo) and intrinsic rate of natural increase (r) of *Brachionus calyciflorus*.
* $p<0.05$

It is quite possible that in natural waters diazinon might prove hazardous to rotifers at much lower levels than those established by laboratory tests because generally, phytoplankton densities tend to be low in natural waters, and it seems that rotifers are weakers in a medium with low food density (Ramakrishna and Sarma 1986).

Table 1. Effective concentrations of diazinon (mg/L) at which the parameter value is reduced to 50% of that in the controls (EC50). These values were derived using the regression equations.

Parameter	Diazinon (mg/L)
Net reproductive rate (Ro)	5.20
Intrinsic rate (r)	
Generation time (T)	8.49
Life expectancy at hatching (e_o)	12.33

Finally, aquatic organisms in nature are rarely exposed to any pollutant in isolation, but more often many biotic and abiotic factors act in concert to modify the main toxicant effects (Cairns 1981). We belive that the life table is useful as a laboratory bioassay for short-lived tests organisms, such as aquatic invertebrates.

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