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Effect of malathion on the demography of *Daphnia pulex* Leydig and *Diaphanosoma birgei* Korinek (Cladocera)

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Abstract

Malathion is a common pesticide used to control insects in agricultural, domestic and industrial sectors in different parts of the world. In this work we evaluated the effects of sublethal concentrations of malathion on the survivorship and reproductive variables of two cladoceran species *Daphnia pulex* and *Diaphanosoma birgei* using standard life table demography method. Based on preliminary tests, we selected four sub-lethal concentrations of malathion for each cladoceran species. For *D. pulex*, the malathion concentrations were 0. 225, 0.45, 0.9 and 1.8ng l^{-1} and for *D. birgei*, these were 0.0281, 0.0562, 0.1125, 0.225ng l^{-1}.Our results showed that in general, *Daphnia pulex* was less sensitive than *Diaphanosoma birgei* to malathion. The average lifespan of *Daphnia pulex* in controls was about 19 days while under similar conditions, that of *D. birgei* was about 21 days. For either cladoceran species, increased pesticide concentration resulted in decreased survival; this was more evident in the treatment containing the highest concentration of malathion (0.225 ng l^{-1}) for *D. birgei*. Fecundity of *D. pulex* and *D. birgei* also decreased with increase in the concentration of malathion. For a given cladoceran species, compared to controls, the gross reproductive, net reproductive rates and the population growth rate significantly decreased due to malathion. Compared to the survivorship variables (age-specific survival, life expectancy and average lifespan), the reproductive parameters (gross reproductive rate, net reproductive rate and the rate of population increase) of the two cladoceran species were decreased by the pesticide.

Key words

Life history, Lifespan, Pesticide, Zooplankton

Introduction

Malathion is a common pesticide used to control insects in agricultural, domestic and industrial sectors. Despite the benefits of pesticide use, dispersion of a large amount of malathion causes environmental and human health problems (Krieger, 2010). Zooplankton species that inhabit freshwater bodies such as rivers, reservoirs and lakes are vulnerable to adverse effect of pesticide from urban and agricultural run offs (Levine, 2007). The natural zooplankton communities of freshwater bodies are composed of ciliates, rotifers, cladocerans and copepods (Thorp and Covich, 2010). In terms of biomass, cladocerans usually dominate in many freshwater bodies. Compared to copepods, cladocerans exhibit strong grazing impact on phytoplankton (Dumont and Negrea, 2002). Cladocerans are also considered as key group for standard ecotoxicological tests due to their sensitivity to changes in the aquatic environment (Hoffman *et al*., 2003).

There are about 620 species of cladocerans worldwide (Forró *et al*., 2003). However, fewer than 10 out of 95 genera of Cladocera are widely used in the assessment of water quality. The genera *Daphnia*, *Ceriodaphnia*, *Diaphanosoma* and *Moina* are commonly used as bioassay organisms in ecotoxicological

evaluations because of their availability, sensitivity, taxonomic stability and ease with which they can be cultured on long term basis (Sarma and Nandini, 2006). *Daphnia* is generally considered as predominantly a temperate genus while *Diaphanosoma* is tropical (Kořinek, 2002).

Mexican waterbodies have different species of cladocerans including the genera *Daphnia* and *Diaphanosoma* (De la Lanza and García, 2002; Elías-Gutiérrez et al., 2008).*Daphnia pulex* and *Diaphnanosoma birgei* are easily cultured using microalgae such as *Chlorella* and *Scenedesmus*. Both the cladoceran species are known to respond rapidly to stress(García-García *et al*., 2006). Under toxicant stress changes in feeding and filtration rates, somatic growth and body size can be easily quantified. However, demographic responses of cladocerans to toxicants are considered to be more sensitive than other variables such as somatic growth (Dumont and Negrea, 2002).

Life table demography is an important ecological method for quantifying the effects of pesticides and herbicides (Calow, 1997). Using life table demography method, it is possible to quantify offspring production in an age-specific manner. Cladocerans are particularly well suited for this because of their predominantly parthenogenetic mode of reproduction (Dumont and Negrea, 2002). Usually for most cladocerans, reproductive variables such as gross and net reproductive rate and the rate of population increase are more sensitive to toxic stress than non-reproductive parameters such as swimming speed or survival (Calow, 1997). Therefore, cladoceran reproductive variables are more widely used to quantify the sublethal effects of toxic substances such as pesticides and heavy metals (Sarma and Nandini, 2006).

Malathion is produced and extensively used in Mexico in spite of the fact that it is known to cause health problems to people living close to the production facilities (Fuentes-Matus *et al.*, 2010).It has a high mutagenic potential and high levels of residues in grains from the Mexican State of Sonora ranging from 2 to 25 ng g^{-1}(Aldana-Madrid *et al*., 2008). It also bio-accumulates up to 10-fold in primary producers and 140-fold in secondary consumers in aquatic ecosystems (Favari *et al*., 2002). In spite of the knowledge on the dangers of this pesticide, little quantified information is available on its toxicity to zooplankton.In this work we evaluated the effects of sub-lethal concentrations of malathion on the survivorship and reproductive variables of two cladoceran species *Daphnia pulex* and *Diaphanosoma birgei*.

Materials and Methods

Plankton cultures : The test cladoceran species *Daphnia pulex* and *Diaphanosoma birgei* were isolated from a local fresh water body in Mexico City. Using a single parthenogenetic female, stock cultures were separately established. For culturing cladocerans and for experiments we used reconstituted moderately hard water (EPA medium) and the single celled *Chlorella vulgaris* as the diet. The EPA medium was prepared by dissolving 0.095 g of $NaHCO_3$, 0.06 g of $CaSO_4$, 0.06 g of $MgSO_4$ and 0.002 g KCl in one litre of distilled water (Weber, 1993).

Chlorella vulgaris (Strain CL-V-3) was originally obtained from the algal cultures section of CICESE (Ensenada, Mexico) and was batch-cultured in 2 l transparent bottles using Bold' basal medium (Borowitzka and Borowitzka, 1988). Alga in log phase of growth was concentrated through centrifugation (3000 rpm for 5 min.), rinsed and resuspended in distilled water. The algal density was enumerated using haemocytometer. The stock cultures as well experimental test jars were maintained at 23±1°C, pH 7.0 to 7.5 and continuous but diffused fluorescent illumination (Nandini and Sarma, 2003).

Preparation of sublethal concentrations of malathion : Commercial product of malathion (malathion 500) was purchased and the real concentration was determined at the Instrumentation Laboratory of the Autonomous University of State of Mexico (Toluca City, Mexico). All concentrations of the pesticide were accordingly based on the actual concentration of the active ingredient. Because the tested cladoceran species differed in their tolerance to malathion, we separately conducted a series of preliminary range finding tests. Based on these tests, we selected four sublethal concentrations of malathion for each cladoceran species. Thus, for *D. pulex*, the malathion concentrations were 0. 225, 0.45, 0.9 and 1.8 ng l^{-1} and for *D. birgei*, these were 0.0281, 0.0562, 0.1125, 0.225 ng l^{-1}. For both the cladoceran species we used daily prepared pesticide concentrations through serial dilution from a stock solution of 1 µg l^{-1}.

Life table demography experiments : Life table demography tests were separately conducted each cladoceran species. The general experimental conditions were as follows: 50 mL transparent jars as test containers; each malathion concentration was dissolved in 20 ml distilled water plus 20 ml of EPA medium with $1x10^6$ cells ml^{-1} of *C. vulgaris.* Each test jar received 10 neonates (<24 h) of one of the two cladoceran species. We used three replicates for each concentration containing 10 neonates. The commercial product contained in addition to active ingredient (diethyl 2-[(dimethoxyphosphorothioyl)sulfanyl]butanedioate), inert solvents and emulsifiers whose concentrations did not exceed 1 µl per test jar. At this concentration, the solvents and emulsifiers present in the malathion 500 have no known effect on cladocerans (Sarma and Nandini, 2006). Hence no negative controls were set up.

Following initiation of the experiments, daily we quantified the number of individuals living in each cohort and transferred them to fresh jars containing the desired algal food density and toxicant level. Dead adults and newly born offspring when present were quantified and discarded. The experiments were terminated

when the last individual in each cohort had died.

Based on the data collected, we derived the following survivorhsip and reproductive variables (Krebs, 1985):

Average lifespan : $L_x = \frac{n_x1 + n_x2}{2}$

Age specific life expectancy : $e_x = \frac{T_x}{n_x}$

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

Net reproductive rate : $R_0 = \sum_{0}^{\infty} l_x . m_x$

Generation time: $T = \frac{\sum l_x . m_x \times X}{R_o}$

Rate of population increase per day (solved iteratively)

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

Where, l_x = Proportion of surviving to start of age x (days), m_x= offspring produced per female at age x, T_x= cumulative number of individuals from age $_x$ to maximum age, n_x= number of live individuals at the start of age x.

The differences among the treatments were statistically evaluated using one-way-analysis of variance (ANOVA) (Sokal and Rohlf, 2000).

Results and Discussion

Data on the age specific survivorship curves of *D. pulex* and *D. birgei* subjected to different concentrations of malathion are presented in Fig. 1. In general, *D. pulex* was less sensitive than *D. birgei.* For either cladoceran species, increase in the pesticide concentration resulted in decreased survival which was more evident in the treatments with the highest toxicant concentration (0.225 ng l^{-1}) for *D. birgei*. In control, the typical rectangular survivorship curves (Krebs, 1985) were observed for *D. pulex* while these slightly differed for the first few days for *D. birgei.*

Age-specific life expectancy curves of the tested cladocerans showed decreased survival with increasing age of the cohort. The presence of pesticide caused further decrease in the survival (Fig. 2). Fecundity patterns (Fig. 3) of *D. pulex* and *D. birgei* showed decreased offspring production with increase in the concentration of malathion. In general, offspring production was highest during the first two weeks and thereafter reduced. For both cladoceran species, not only the magnitude but also frequency of offspring production was reduced with increase in the pesticide level.

Data on the selected life history variables of *D. pulex* and *D. birgei* exposed to different sublethal concentrations of malathion are presented in Table 1. For a given cladoceran species, compared to control, the gross reproductive and net reproductive rates, generation time and the rate of population increase were all decreased due to pesticide. The average lifespan of *Daphnia pulex* in control was about 19 days while under similar conditions, that of *D. birgei* was about 21 days. Compared to the survivorship variables, the reproductive parameters of the two cladoceran species were more adversely affected by the pesticide. Statistical analysis using one-way ANOVA showed that survival-related (except the average lifespan of *D. pulex*) and reproduction-related variables (except generation time for *D. pulex*) were significantly affected by the concentration of malathion ($p<0.05$, F-test, Table 2).

The selection of cladoceran species in the present work was based on their availability in tropical high altitude waterbodies. *Daphnia* generally being a temperate genus is also found at high altitude waterbodies while *Diaphanosoma* is predominantly a tropical taxon (Kořinek, 2002). Both are known to occur in the same waterbodies in many parts of Mexico (Elías-Gutiérrez *et al*., 2008). Malathion, wherever is applied, due to agricultural runoffs eventually reaches the ponds and lakes and thus affects cladocerans (Olvera-Hernández *et al*., 2004; Anon., 2006). The relative sensibilities of various aquatic invertebrates, including cladocerans, to malathion toxicity vary considerably (Martínez-Tabche *et al.*, 1991). Most tests involving the evaluation of toxic effects of malathion are based on short term median lethal concentration tests. The median lethal concentration(LC_{50}) for various cladoceran species including *D.pulex* and *M. macrocopa* varied from 1-16 µg l^{-1} (Tomlin, 2006; Relyea, 2009). At a sublethal level of 0.01 µg l^{-1} of malathion, the survival of *Moina macrocopa* was affected and, in addition, reproduction was reduced to about 40% (Wong *et al*., 1995). Several studies indicate that the response of different taxa of cladocerans to toxicants is significantly different (Sarma and Nandini, 2006).Most studies on the ecotoxicology of cladocerans focus on *Daphnia* and *Ceriodaphnia.* Little information is available on the effects of sublethal levels of pesticides to *Diaphanosoma* (Leboulanger *et al*., 2011). While studies show that the genus *Moina* is quite resistant to toxicants, it has been shown that *Diaphanosoma* is as much sensitive to heavy metals as daphniids (García-García *et al*., 2006). Our findings corroborate the similar sensitivity levels of *Daphnia pulex* and *D. birgei* to the pesticide malathion.

In assessing the toxicity of substances at or beyond the population level, there is a problem of inferring the consequences based on the effects observed at the individual level (Caswell,

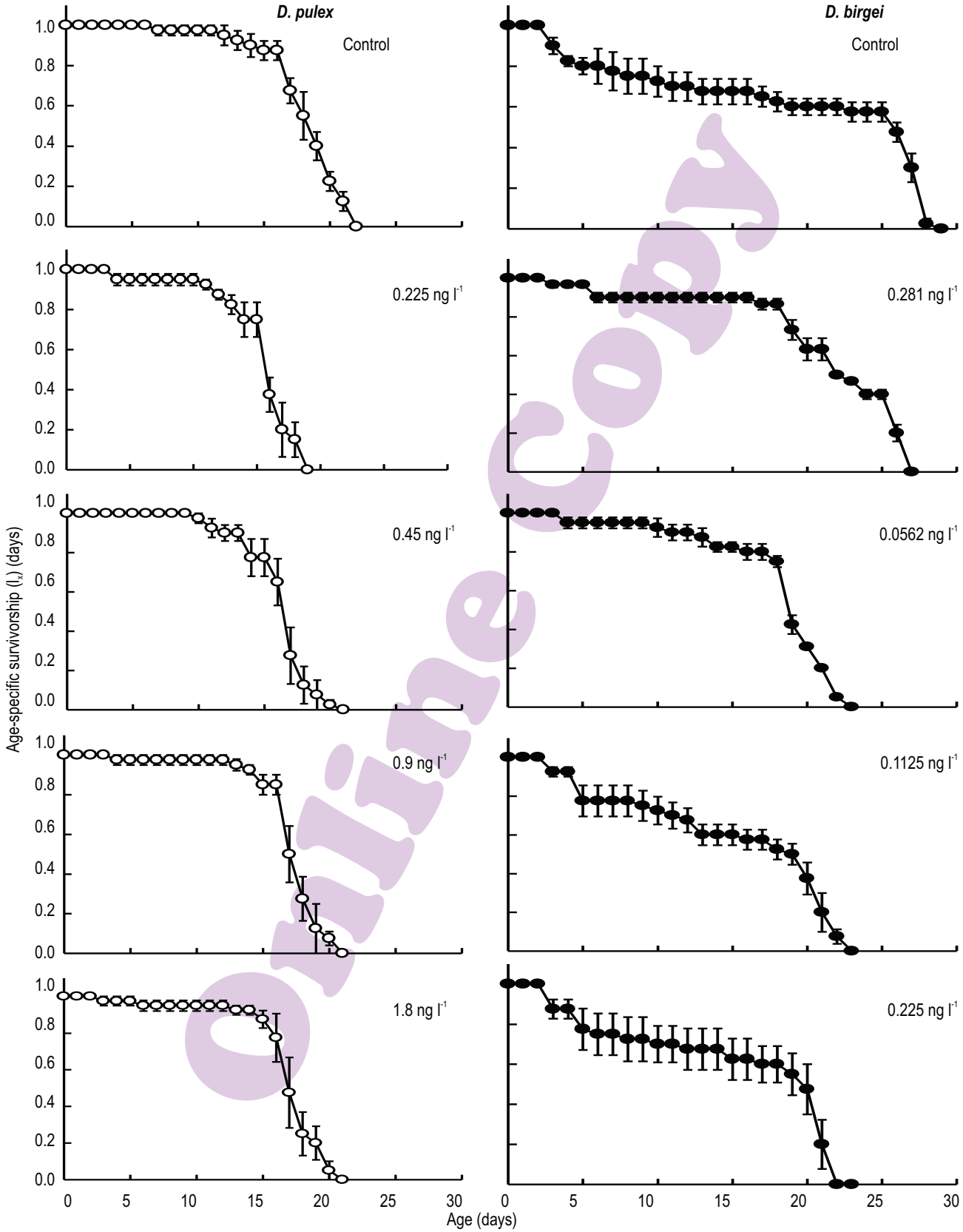

Fig. 1: Age specific survivorship curves (proportion of survival) (lx) of *Daphnia pulex* (open circles) and *Diaphanosoma birgei* (closed circles) exposed to different concentrations of malathion (ng l^{-1}). Data represent mean ± SE based on three replicates (cohorts of 10 individuals each)

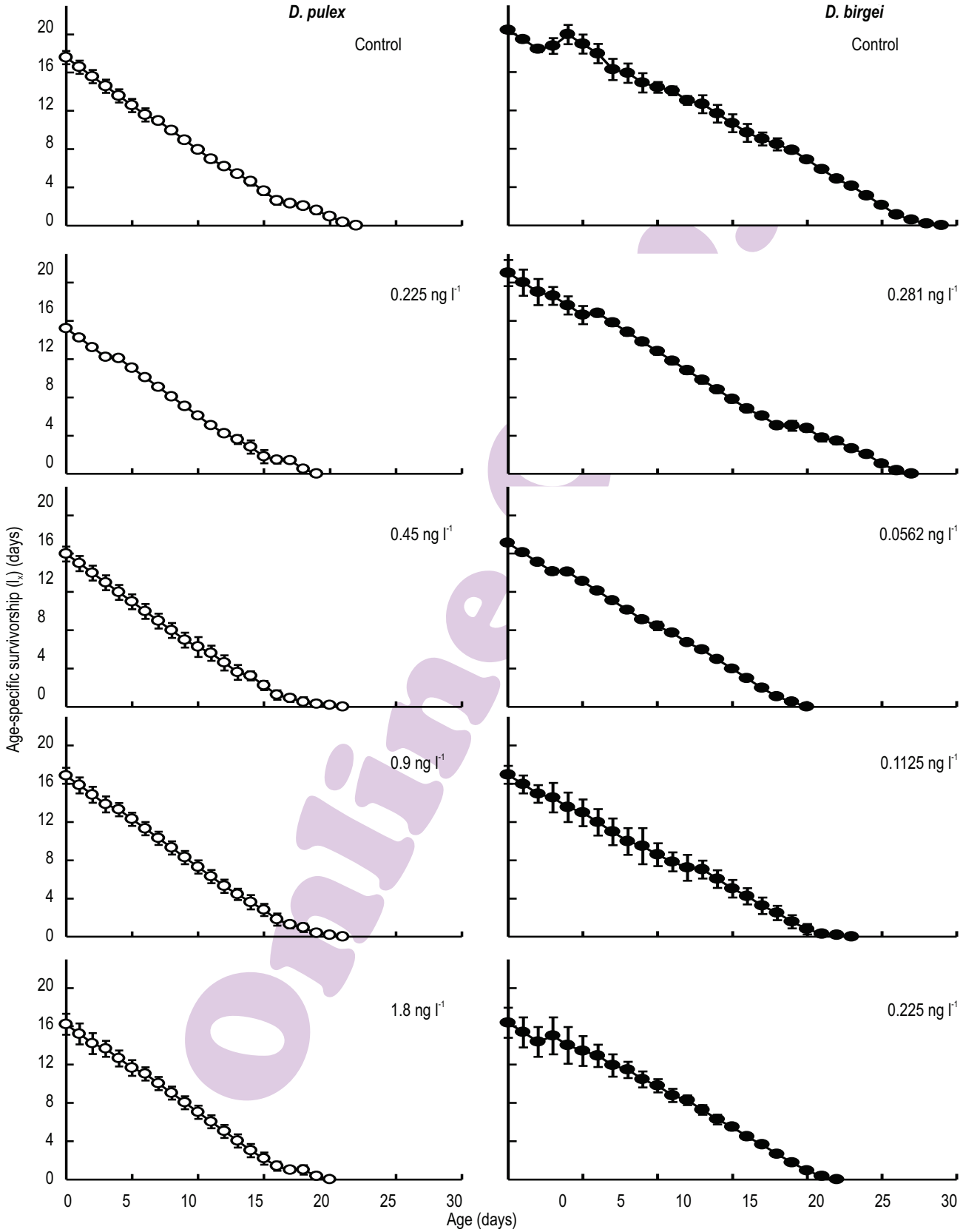

Fig. 2 : Age specific life expectancy curves (ex) of *Daphnia pulex* (open circles) and *Diaphanosoma birgei* (closed circles) exposed to different concentrations of malathion (ng l^{-1}). Data represent mean ± SE based on three replicates (cohorts of 10 individuals each)

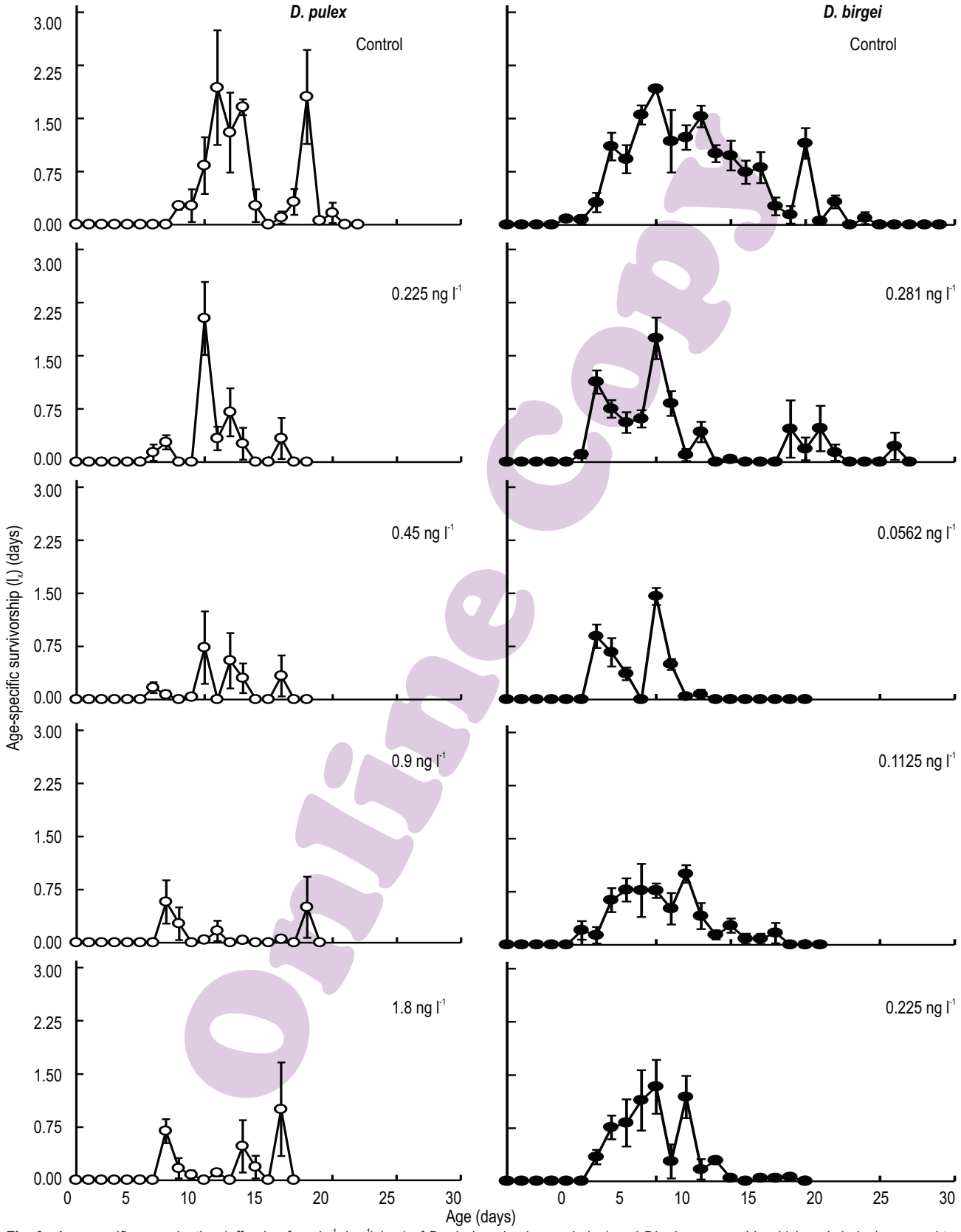

Fig. 3 : Age specific reproduction (offspring female^{-1} day^{-1}) (mx) of *Daphnia pulex* (open circles) and *Diaphanosoma birgei* (closed circles) exposed to different concentrations of malathion (ng l^{-1}). Data represent mean ± SE based on three replicates (cohorts of 10 individuals each)

Table 1 : Data on the selected life history variablesof *Daphnia pulex* and *Diaphanosoma birgei* exposed to different concentrations of malathion. Shown are mean±standard error based on 3 replicates (cohorts)

Species / Variable	Malathion concentration (ng l^{-1})				
	Control	**0.225**	**0.45**	**0.9**	**1.8**
D. pulex					
Avg. life span	18.9±0.2^{a}	16.0±1.3^{b}	16.5±0.9^{b}	16.8±0.3^{b}	15.9±0.4^{b}
GRR	9.0±2.1^{a}	4.07±1.3^{a,b}	2.0±1.4^{b}	1.01±0.1^{b}	2.7±0.9^{a,b}
NRR	8.0±2.1^{a}	3.6±1.1^{a,b}	1.8±1.2^{b}	0.7±0.3^{b}	1.9±0.4^{b}
GT	12.8±0.7^{a}	10.3±0.5^{a}	9.9±1.1^{a}	9.4±1.5^{a}	10.6±1.0^{a}
r	0.16±0.02^{a}	0.11±0.03^{a,b}	0.01±0.06^{a,b}	-0.04±0.03^{b}	0.05±0.02^{a,b}
	Control	**0.0281**	**0.0562**	**0.1125**	**0.225**
D. birgei					
Avg. life span	20.9±0.3^{a,b}	21.5±1.3^{a}	17.6±0.3^{a,b}	15.1±2.9^{a,b}	13.5±0.9^{b}
GRR	15.4±1.0^{a}	7.7±0.3^{c}	4.9±0.1^{b}	6.5±1.3^{b,c}	5.9±0.5^{b,c}
NRR	11.7±0.7^{a}	6.4±0.4^{b}	3.6±0.1^{b}	3.8±0.9^{b}	4.2±0.7^{b}
GT	12.1±0.3^{a}	10.4±0.1^{b,c}	8.5±0.3^{c}	10.4±0.5^{c}	9.5±0.8^{b}
r	0.23±0.01^{a}	0.20±0.01^{a,c}	0.15±0.01^{c,b}	0.12±0.01^{b}	0.13±0.02^{c,b}

Avg. life span = average life span; GRR: gross reproductive rate; NRR: net reproductive rate; GT: generation time and r: rate of population increase

2000). In life table demographic approach this is not the case because we used cohort populations for each treatment. Under sub-lethal concentrations, malathion exerts stronger effects on reproduction than on survival (Wong *et al*., 1995). In the present study, we evaluated both survivorship and reproductive variables of both cladoceran species subjected to different concentrations of malathion. Age-species survivorship and life expectancy provide information on how a cohort population experiences mortality in relation to age (Caswell, 2000). Ideal survivorship curves appear rectangular with little mortality in younger age groups but as they age, physiological changes lead to rapid decline in survival (Krebs, 1985). The survivorship close to this condition was observed in control of *D. pulex* and *D. birgei* where the cohort experienced little mortality during most part, but declined rapidly as the population became 3 week-old. There was a significant effect of malathion on the average life span and generation time of *D. birgei* but not *D. pulex* which indicates the greater sensitivity of *Diaphanosoma* as compared to *Daphnia*; the former taxa could be considered as a part of the battery of species for toxicity testing, particularly in tropical countries.

Age specific reproduction in cladocerans varies depending on the species. Most cladoceran species of the genus *Daphnia* need about a week to reach age at first maturity and thereafter release offspring in distinct clutches (Sarma *et al*., 2005). The number of offspring per clutch varies with age. Usually younger females tend to have lower offspring per clutch and as age advances the somatic growth takes places thereby providing more space in the brood pouch which in turn facilitates production of higher number of offspring (Nandini and Sarma, 2000).Thus peak offspring production is associated with the cladocerans at mid-reproductive age(Nandini and Sarma, 2000).Toxicants change this trend (García-García *et al*., 2006). In the present study for both cladoceran species, peak offspring production was during 10-12 days and thereafter it decreased. Malathion not only reduced the duration of offspring production but also the magnitude. Our observations were similar to those recorded earlier where the neonate production by the cladocerans was strongly reduced with an increase in the concentration of the pesticide (Wong *et al*., 1995).

The use of extremely low levels toxicants sometimes causes enhanced survival or reproductive output in zooplankton. For example it has been observed that stimulation occurred in longevity of *D. pulex* when exposed to dieldrin and reproductive rate was also reported to be higher for the same species when subjected to low levels of copper (Daniels and Allan, 1981; Meyer *et al*., 1987).In the present study, marginal enhancement of offspring production by *D. pulex* at the highest concentration tested (e.g., 1.8 ng l^{-1}) was observed. However, these were not statistically significant as compared to other concentrations of the pesticide, but were significantly lower when compared to control (Table 1). In *D. birgei*, the negative effects of pesticide were noticeable from the lowest concentration onwards. For example, compared to control, the gross reproduction was reduced by 50% when exposed to 0.0281 ng l^{-1} of malathion.

In ecotoxicological tests, it is also important to compare the demographic data in control with those available in literature under comparable conditions. The general ranges of life history variables of tropical and temperate cladocerans have been reviewed (Sarma *et al*., 2005). Generally, cladocerans have mean lifespan of 5 to 150 days depending on the temperature and species; temperate daphniids have longer duration than tropical Sididae. This was also event in this work and in addition, the range of average lifespan recorded for the tested species is similar to that reported in the literature (Sarma *et al*., 2005). The gross and net reproductive rates observed here for both cladoceran species

Table 2 : Results of one way analysis of variance performed for the selected life history variables of *Daphnia pulex* and *Diaphanosoma bergei* exposed to different concentrations of malathion.

Variable	DF	SS	MS	F	p
Average lifespan					
D. pulex					
Among groups	4	18.157	4.539	2.688	0.093
Error	10	16.887	1.689		
D. birgei					
Among groups	4	125.03	31.258	3.699	0.043
Error	10	84.507	8.451		
Gross repro. Rate					
D. pulex					
Amonggroups	4	121.07	30.267	5.339	0.015
Error	10	56.69	5.669		
D. birgei					
Among groups	4	228.35	57.088	29.68	0.001
Error	10	19.232	1.923		
Net repro. Rate					
D. pulex					
Among groups	4	96.887	24.222	5.315	0.015
Error	10	45.573	4.557		
D. birgei					
Among groups	4	140.59	35.148	25.53	0.001
Error	10	13.767	1.377		
Generation time					
D. pulex					
Among groups	4	19.605	4.901	1.45	0.288
Error	10	33.798	3.38		
D. birgei					
Among groups	4	19.712	4.928	12.98	0.001
Error	10	3.796	0.38		
Rate of pop. Increase					
D. pulex					
Among groups	4	0.0828	0.0207	4.732	0.02
Error	10	0.0437	0.0044		
D. birgei					
Among groups	4	0.0232	0.0058	9.115	0.002
Error	10	0.0064	0.0006		

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

were also within the range (3-100 offspring per female) reported in literature (Dumont and Negrea, 2002).

Our results showed that reproductive rates of cladocerans were more sensitive to pesticide than survivorship related variables. For example, generation time and life expectancy at birth of *D. pulex* were not sensitive to malathion under any of the concentrations used here; the results showed no statistical differences when compared to control. It is also known that reproductive variables of zooplankton are more adversely affected by pesticides than duration of life or generation time. For example, when the rotifer *Plationus patulus* (= *Brachionus patulus*) was exposed to different concentrations of DDT (Dichloro-Diphenyl-Trichloroethane), the generation time was not adversely affected and on the other hand, gross and net reproductive rates decreased due to the pesticide (Rao and Sarma, 1986). The rate of population increase is a significant variable in ecotoxicity tests. Based on a large set of published work in relation to the life history variables and the effect of various toxic substances it has been concluded that the rate of population growth is a sensitive variable in evaluating the responses of organisms to toxic substances (Forbes and Calow, 1999). Here, the rate of population increase for both cladoceran species was found to be highly sensitive to malathion levels and thus could be a good measure to evaluate the toxic effects of pesticides to zooplankton.

Our results showed that *Diaphanosoma* was sensitive to malathion than *Daphnia*. For either cladoceran species tested here, not all survivorship and reproductive variables were consistently sensitive to malathion. It is generally believed that survivorship variables are less sensitive than reproduction-related parameters. It was also observed that generation was less sensitive for *Daphnia* but not for *Diaphnanosoma*.

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