



Effect of butyl benzyl phthalate on life table-demography of two successive generations of cladoceran *Moina macrocopa* Straus

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Abstract

In this study, the acute toxicity of butyl benzyl phthalate (BBP) to freshwater cladoceran *Moina macrocopa* was tested, and its chronic effects on survival and reproduction of two successive generations of the cladoceran were studied using life-table demographic method. The results showed that the 48-hr LC_{50} of BBP for *M. macrocopa* was 3.69 mg l^{-1} . Compared to the blank controls, BBP at 125, 500, 1000 and $2000 \mu\text{g l}^{-1}$ significantly shortened the life expectancy at birth, BBP at 125-2000 $\mu\text{g l}^{-1}$ decreased the net reproductive rate, and BBP at 500 and $1000 \mu\text{g l}^{-1}$ shortened the generation time but increased the intrinsic rate of population increase of the parental *M. macrocopa*. BBP at 62.5, 125, 500, 1000 and $2000 \mu\text{g l}^{-1}$ increased the intrinsic rate of population increase of the F_1 generation. A significant dose-effect relationship existed between BBP concentration and life expectancy at birth, net reproductive rate as well as intrinsic rate of population increase of the parental *M. macrocopa*. The parental *M. macrocopa* were more sensitive in survival, development and reproduction to BBP than the F_1 generation, but the reverse was also true in the population growth. Extending chronic toxicity tests to the second generation of *M. macrocopa* increased the cost-effectiveness of the assays.

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Introduction

Phthalate esters (PAEs) are a family of industrial chemicals used as softeners, adhesives or solvents, and mainly used in the polymer industry as plasticizers in PVC and to a lesser extent in the non-polymer industry for different consumer products (OSPAR Commission, 2006). Because phthalates are external plasticizers which soften the resins without reacting chemically with them, they tend to migrate slowly out of the plastic, either into the air by volatilization or into water or other solvents by dissolution, and have become nearly ubiquitous environmental pollutants (Laughlin *et al.*, 1978).

In China, the highest level of PAEs in rivers and lakes was $263.8 \mu\text{g l}^{-1}$, which was almost the same as those found in the other rivers and lakes in the world (Tian *et al.*, 2003; Sha *et al.*, 2007).

As a member of PAEs, butyl benzyl phthalate (BBP) was added to the OSPAR List of Chemicals for Priority Action in 1998 as part of the group "certain phthalates" (OSPAR Commission, 2006), and identified as one of the priority controlled hazardous substances by the United States Environmental Protection Agency (USEPA) (Jin, 1990). In recent years, it has been shown to have potential for endocrine disrupting effects on vertebrates and humans (OSPAR

Commission, 2006; Van Wezel *et al.*, 2000; Foster *et al.*, 2001). Although some information on the toxicity of BBP to aquatic organisms including algae, protozoan, rotifera, crustacean and fish is available (Adams *et al.*, 1995; Staples *et al.*, 1997; Huang *et al.*, 1999; Bradlee and Thomas, 2002; Gupta *et al.*, 2008; Pandey *et al.*, 2009; Zhao *et al.*, 2007, 2009), relatively little is known about its chronic toxicity on successive generations of test animals (Munzinger, 1990; Marcial *et al.*, 2003; Van Leeuwen *et al.*, 1985). In nature, test animals are continuously exposed to BBP; therefore, investigating only one generation from unexposed parents could underestimate the effect of BBP, and ignore the potential negative effects of exposure during oogenesis and embryogenesis, and toxicant transfer from mothers to neonates (Munzinger, 1990; Marcial *et al.*, 2003; Van Leeuwen *et al.*, 1985). A more realistic figure of chronic toxicity could result from assays that also test the reproduction of a second generation (Van Leeuwen *et al.*, 1985). Therefore, extending chronic toxicity tests to a second generation could increase the cost-effectiveness of the assays (Sánchez *et al.*, 2000).

Moina macrocopa is a eurythermic polycyclic cladoceran, and lives predominantly in temporary hypereutrophic ponds, where it often constitutes about 90% of the zooplankton biomass (Burak, 1997). In Southeast Asia, this species occurs in ponds and rice paddies (Wong, 1997). As a species of test animal in aquatic ecotoxicology, *M. macrocopa* is sensitive to heavy metals (Wong, 1993; Garcia *et al.*, 2004; Mangas-Ramírez *et al.*, 2004; Gama-Flores *et al.*, 2007), electroplating waste water (Wong *et al.*, 1991) and insecticides (Wong *et al.*, 1995; Wong, 1997; Chu *et al.*, 1997; Yang *et al.*, 2007; Chuah *et al.*, 2007; Liu *et al.*, 2008).

The main purpose of this study is to assess the acute toxicity of BBP to *M. macrocopa* and its chronic effects on life-table demographic parameters of two successive generations of the cladoceran, and compared the sensitivity in reproduction, development, survival and population growth to BBP pollution between the two successive generations.

Materials and Methods

The cladoceran *M. macrocopa* was supplied by Laboratory of Aquaculture Biology, Nagasaki University, Japan. In our laboratory, *M. macrocopa* was mass cultured at $25 \pm 1^\circ\text{C}$ and with 1.0×10^6 cells ml^{-1} of *Chlorella pyrenoidosa*. For routine culture as well as for experiments, aerated tap water was used (Xi *et al.*, 2005). Algae were grown in a semi-continuous culture using HB-4 medium (Li *et al.*, 1959) renewed daily at 20%. Algae in exponential growth were centrifuged and then resuspended in aerated tap water.

BBP (standard grade, $\geq 97\%$, Sigma-Aldrich, Germany) were used as the toxicant. Stock solution was prepared by dissolving BBP in 100% acetone, and then diluted to the desired concentrations using aerated tap water.

M. macrocopa was continually cultured for more than three generations under the stated above condition, then newborn animals

(<12 hr old) were selected for tests. All the experiments were conducted in 75 ml beakers, each containing 50 ml of test medium and 10 test animals. Acute toxicity of BBP was tested at eight concentrations (0.25, 0.50, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg l^{-1}) and a blank control (aerated tap water), each of ten neonates was replicated three times. The tests were carried out at $25 \pm 1^\circ\text{C}$ in darkness. During the bioassay, the animals were not fed, and the medium was not renewed. After 48 hr, the experiments were terminated and the dead animals were counted, then 48-hr LC_{50} was calculated using the probit analysis.

Based on the 48-hr LC_{50} value, six toxicant concentrations (62.5, 125, 250, 500, 1000 and 2000 $\mu\text{g l}^{-1}$), a blank control (aerated tap water) and a solvent control (containing 1.25% (v/v) acetone) were selected for the chronic toxicity tests. All the tests were also conducted in 75 ml beakers, each containing 50 ml of test solution, 1.0×10^6 cells ml^{-1} of *C. pyrenoidosa* and 10 neonates. Three replicates were set up. Thereafter, every day the original animals alive were transferred into freshly prepared test solutions containing 1.0×10^6 cells ml^{-1} of *C. pyrenoidosa*, the number of dead original individuals and newborn animals during the 24-hr interval were counted and discarded. During the experiment, the algae were resuspended every 12 hr. The life-table experiments were continued at $25 \pm 1^\circ\text{C}$ in darkness until each individual of every cohort died.

Following the above stated methods, the chronic effects of BBP on survival and reproduction of the first brood (F_1) produced by the parental *M. macrocopa* were studied under the same experimental conditions.

Based on the data collected, age-specific survivorship (l_x) and age-specific fecundity (m_x) were constructed for each cohort (replicate) using conventional life-table techniques (Poole, 1974). Life expectancy at birth (e_0), net reproductive rate (R_0), generation time (T) and intrinsic rate of population increase (r_m) were calculated according to Krebs (1985) and Lotka (1913).

Kaplan-Meier analysis was conducted for pair-wise comparisons of age-specific survivorship of *M. macrocopa* exposed to each concentration of the test chemical and the solvent control relative to the blank control. One-way analysis of variance (ANOVA), with the concentration of BBP as the independent variable, and each of life-table demographic parameters as the dependent variable, followed by Dunnett's test was conducted for pair-wise comparisons of each concentration of the test chemical and the solvent control relative to the blank control (Zar, 1999).

Results and Discussion

The 48-hr LC_{50} and 48-hr NOEC values of BBP were 3.69 and 2.0 mg l^{-1} for *M. macrocopa*, respectively.

The 48-hr LC_{50} value of BBP was 1.0–4.7 mg l^{-1} for *Daphnia magna* (OSPAR Commission, 2006), and the 10-d LC_{50} values of BBP were 0.46 and 1.23 mg l^{-1} for *Hyalella azteca* and *Lumbriculus variegatus*, respectively (Call *et al.*, 2001). By comparison, *M.*

Table - 1: Life expectancy at birth (e_0), net reproductive rate (R_0), generation time (T), and intrinsic rate of population increase (r_m) of two successive generations *M. macrocopa* of exposed to different concentrations ($\mu\text{g l}^{-1}$) of BBP

Generation	Concentration	e_0 (d)	R_0 (ind.)	T (d)	r_m (d ⁻¹)
Parental	Blank control	19.63±0.57	119.12±3.00	10.77±0.02	0.7873±0.0044
	Solvent control	19.77±0.85	115.23±2.67	10.78±0.33	0.7887±0.0036
	62.5	18.53±0.80	106.14±4.88	10.59±0.36	0.7789±0.0039
	125	16.07±0.95*	91.56±6.01*	9.81±0.50	0.7803±0.0072
	250	17.57±0.81	101.78±2.67*	10.07±0.29	0.7961±0.0036
	500	15.73±0.49*	90.41±1.46*	8.99±0.11*	0.8095±0.0068*
	1000	14.23±1.51*	85.21±10.87*	8.61±0.89*	0.8231±0.0147*
	2000	16.57±0.32*	101.10±2.94*	9.99±0.36	0.8017±0.0052
F ₁	Blank control	16.90±1.32	97.33±6.32	9.68±0.37	0.7825±0.0032
	Solvent control	15.77±0.81	78.84±15.34	8.49±0.50	0.7803±0.0255
	62.5	17.77±0.95	106.82±3.34	9.55±0.29	0.8334±0.0073*
	125	18.07±0.18	111.22±1.07	10.02±0.05	0.8460±0.0140*
	250	15.97±0.49	93.32±4.72	9.39±0.29	0.8107±0.0037
	500	16.70±1.01	99.99±7.35	9.12±0.34	0.8486±0.0148*
	1000	14.90±0.16	95.72±4.62	9.38±0.14	0.8527±0.0264*
	2000	16.40±0.64	96.09±2.04	9.45±0.26	0.8446±0.0154*

* Significantly different from the blank controls ($p < 0.05$)

Table - 2: The relationship between life expectancy at birth (e_0 , d), generation time (T , d), as well as intrinsic rate of population increase (r_m , d⁻¹) of the parental *M. macrocopa* and BBP concentration (X , $\mu\text{g l}^{-1}$)

Parameter	Regression equation	Significant test
e_0	$Y = 3.51 \times 10^{-6} X^2 - 0.0081 X + 18.80$	$R^2 = 0.79, p < 0.05$
T	$Y = 1.82 \times 10^{-6} X^2 - 0.0040 X + 10.69$	$R^2 = 0.92, p < 0.01$
r_m	$Y = -3.18 \times 10^{-8} X^2 + 7.54 \times 10^{-8} X + 0.7785$	$R^2 = 0.90, p < 0.01$

macrocopa had a similar sensitivity to BBP with *D. magna* but a lower sensitivity than *H. azteca* and *L. variegates*. Compared with the other tested pollutants, BBP was less toxic to *M. macrocopa* than malathion (48-hr $LC_{50} = 5.0-10.0 \mu\text{g l}^{-1}$), diazinon (48-hr $LC_{50} = 1.0-10.0 \mu\text{g l}^{-1}$), (s)-methoprene (48-hr $LC_{50} = 0.34 \text{ mg l}^{-1}$), cadmium (24-hr $LC_{50} = 0.68 \text{ mg l}^{-1}$), methyl parathion (24-hr $LC_{50} = 0.05 \text{ mg l}^{-1}$), endosulfan (48-hr $LC_{50} = 0.16 \text{ mg l}^{-1}$) and DDT (48-hr $LC_{50} = 325 \mu\text{g l}^{-1}$) (Wong *et al.*, 1995; Wong, 1997; Chu *et al.*, 1997; Mangas-Ramírez *et al.*, 2004; Chuah *et al.*, 2007; Liu *et al.*, 2008).

Compared to the blank controls, BBP at 125 and 500-2000 $\mu\text{g l}^{-1}$ decreased significantly the age-specific survivorship of the parental *M. macrocopa* ($p < 0.05$). The solvent did not affect the initial ages of reproduction of the two successive generations of *M. macrocopa*, but BBP at 500 and 1000 $\mu\text{g l}^{-1}$, and at all the tested concentrations made the reproduction of the parental and the F₁ animals occur one day earlier, respectively (Fig. 1).

Compared to the blank controls, the first reproduction of *M. macrocopa* exposed to (s)-methoprene at 0.005 and 0.01 mg l^{-1} occurred one day earlier (Chu *et al.*, 1997), but that of both *M. macrocopa* and *D. magna* was delayed by endosulfan exposure (Chuah *et al.*, 2007; Fernandez-Casalderrey *et al.*, 1993), and that of *M. macrocopa* was not affected by exposure to malathion, heavy metals, diazinon, β -BHC and (s)-methoprene at 0.05 mg l^{-1} (Wong *et al.*, 1995; Wong, 1993, 1997; Yang *et al.*, 2007; Chu *et al.*, 1997). Reduction in time to the first brood, thus an indication of hormesis of

chlorpyrifos was noted in the F₁ generation of *D. carinata* (Zalznik and Nugegoda, 2006). This study indicated that the F₁ generation of *M. macrocopa* had a higher sensitivity in the initial age of reproduction than the parental generation.

BBP affected significantly all the life-table demographic parameters of the parental *M. macrocopa*. Compared to the blank controls, the solvent did not significantly affect all the life-table demographic parameters. However, BBP at 125 and 500-2000 $\mu\text{g l}^{-1}$ shortened the life expectancy at birth, BBP at 125-2000 $\mu\text{g l}^{-1}$ decreased the net reproductive rate, BBP at 500 and 1000 $\mu\text{g l}^{-1}$ shortened the generation time but increased the intrinsic rate of population increase of the parental *M. macrocopa* (Table 1).

BBP did not significantly affect the life expectancy at birth, the net reproductive rate and the generation time, but markedly affected the intrinsic rate of population increase of the F₁ *M. macrocopa*. Compared to the blank controls, the solvent did not significantly affect all the life-table demographic parameters, but BBP at 62.5, 125, 500, 1000 and 2000 $\mu\text{g l}^{-1}$ increased the intrinsic rate of population increase of the F₁ *M. macrocopa* (Table 1).

A significant dose-effect relationship existed between BBP concentration and life expectancy at birth, net reproductive rate as well as intrinsic rate of population increase of the parental *M. macrocopa* (Table 2), but no significant dose-effect relationship

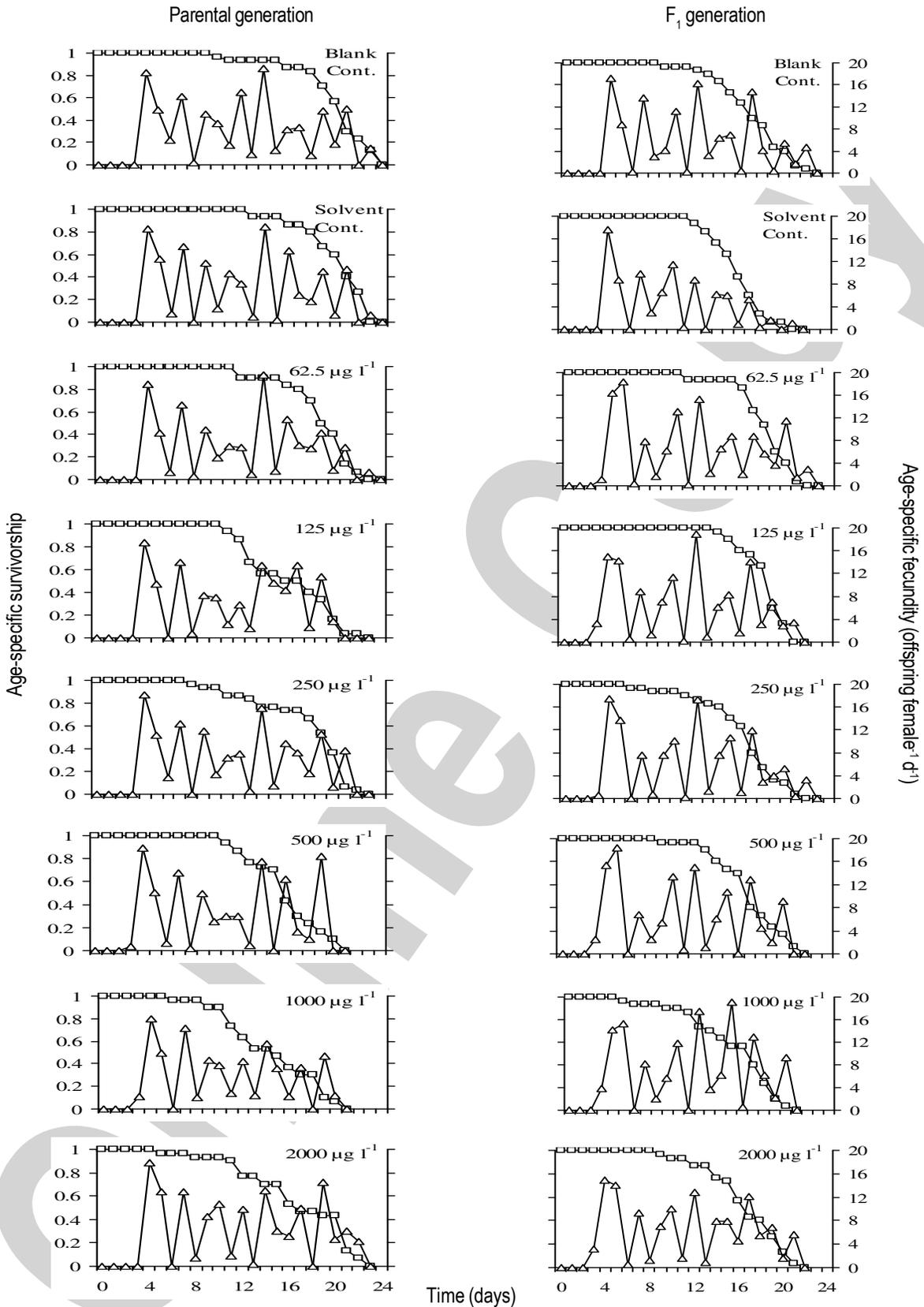


Fig. 1: Age-specific survivorship (square) and age-specific fecundity (triangle) of two successive generations of *M. macrocopa* exposed to different concentrations (μg l⁻¹) of BBP.

between BBP concentration and each of all the life-table demographic parameters of the F_1 was observed.

Diazinon at $1.0 \mu\text{g l}^{-1}$, (s)-methoprene at 0.05 mg l^{-1} , malathion at $0.01 \mu\text{g l}^{-1}$, β -BHC at $1000 \mu\text{g l}^{-1}$, endosulfan at $0.4 \mu\text{g l}^{-1}$, cadmium at 0.17 mg l^{-1} and methyl parathion at 0.125 mg l^{-1} decreased markedly the net reproductive rate of *M. macrocopa* (Wong, 1997; Chu *et al.*, 1997; Wong *et al.*, 1995; Yang *et al.*, 2007; Chuah *et al.*, 2007; Mangas-Ramirez *et al.*, 2004), but DBP at 0.5 mg l^{-1} stimulated the reproduction of *D. magna* (Huang *et al.*, 1999). As far as the different generations of cladocerans, chlorpyrifos decreased the reproduction of the parental *D. carinata*, but an opposite effect was observed in the second generation (Zalizniak and Nugegoda, 2006). However, the herbicide molinate decreased the reproduction of *D. magna*, and this effect was stronger in the parental generation than in the 1st (F_1) and 3rd (F_3) generation (Sanchez *et al.*, 2004). Similarly, in this study, BBP at all the tested concentrations did not significantly affect the net reproductive rate of the F_1 *M. macrocopa*, although BBP at 125, 500, 1000 and 2000 $\mu\text{g l}^{-1}$ decreased markedly the net reproductive rate of the parental animals. Therefore, the parental *M. macrocopa* had a higher sensitivity in reproduction to BBP than the F_1 generation.

The generation time is the average length of time between the birth of an individual and the birth of its own offspring. As such, it reflects changes in the time required to reach sexual maturity and the development time of an egg. Endosulfan at 0.10 and 0.15 mg l^{-1} , diethylstilbestrol at 0.1 and 0.2 mg l^{-1} , and antiandrogens and testosterone both at the environmentally relevant concentrations increased the generation time of the parental *D. magna* (Zou and Fingerman, 1997; Mu and LeBlanc, 2002a, b), and 17 β -estrodial, 4-nonyphenol, bisphenol and p-t-octylphenol all at environmentally relevant concentrations increased the generation time of the parental generation of copepod *Tigriopus japonicus* (Marcial *et al.*, 2003). Conversely, in this study, BBP at 500 and 1000 $\mu\text{g l}^{-1}$ decreased the generation time of the parental *M. macrocopa*, which was identical to the effects of β -BHC at 100 and 1000 $\mu\text{g l}^{-1}$, endosulfan at 2 $\mu\text{g l}^{-1}$, and (s)-methoprene at 0.005 and 0.01 mg l^{-1} on the generation time of the parental *M. macrocopa* (Yang *et al.*, 2007; Chuah *et al.*, 2007; Chu *et al.*, 1997). In addition, 17 β -estrodial, 4-nonyphenol, bisphenol and p-t-octylphenol all at environmentally relevant concentrations also increased the generation time of the F_1 generation of *T. japonicus* (Marcial *et al.*, 2003), but BBP at all the tested concentrations did not affect the generation time of the F_1 *M. macrocopa* (this study). In this study, the decreased generation time of the parental *M. macrocopa* exposed to BBP at 500 and 1000 $\mu\text{g l}^{-1}$ attributed at least partially to the decreased duration of juvenile period (initial age of reproduction), but the unaffected generation time of the F_1 *M. macrocopa* attributed to the increased development time of eggs, because the decreased duration of juvenile period was observed (Fig. 1), which indicated that the development of the parental *M. macrocopa* was more sensitive to BBP than that of the F_1 generation.

Most of all the tested pollutants decreased the population growth of *M. macrocopa* (Chu *et al.*, 1997; Mangas-Ramirez *et al.*, 2004; Chuah *et al.*, 2007), but the reverse was also true for (s)-methoprene at 5 and 10 $\mu\text{g l}^{-1}$, β -BHC at 0.1 and 1 $\mu\text{g l}^{-1}$, and DDT at 8, 16 and 40 $\mu\text{g l}^{-1}$ (Chu *et al.*, 1997; Yang *et al.*, 2007; Liu *et al.*, 2008). In this study, BBP at 500 and 1000 $\mu\text{g l}^{-1}$, and 62.5, 125, 500, 1000 and 2000 $\mu\text{g l}^{-1}$ increased the intrinsic rates of population increase of the parental and the F_1 *M. macrocopa*, respectively, which attributed mainly to the decreased duration of juvenile period (Fig. 1), identical to the result obtained by Pourriot (1986) who found that the rate at which planktonic rotifers multiply during the parthenogenetic phase, providing there is sufficient food, is due more to the short period of embryonic development and the early period of life than to the net reproduction rate. Stimulation of BBP on the population growth of *M. macrocopa* might have negative effects on the community and ecosystem. Since zooplanktons are important links in the aquatic food chain, toxicants affecting the population growth would have indirect effects on their predators and preys, thereby causing changes at the community and ecosystem levels eventually (Chu *et al.*, 1997). If the food resources do not increase in response to the toxicant, competition between individuals in the population may lead to over-exploitation of the limited food resources. Declines in food availability due to over-exploitation have been suggested as the reason for the seasonal collapse in cladoceran populations observed in many lakes (Lampert *et al.*, 1986). In addition, this study indicated that the parental generation of *M. macrocopa* had a lower sensitivity in the population growth to BBP than the F_1 generation, which was similar to results obtained by Sánchez *et al.* (2000) and Luo *et al.* (2008) in different generations of *D. magna*, and demonstrated that investigating only one generation from unexposed parents underestimated the effect of BBP.

In conclusion, *M. macrocopa* had a similar sensitivity to BBP with *D. magna* but a lower one than *H. azteca* and *L. variegates*. BBP was less toxic to *M. macrocopa* than malathion, diazinon, (s)-methoprene, cadmium, methyl parathion, endosulfan and DDT. BBP affected markedly life expectancy at birth, net reproductive rate, generation time and intrinsic rate of population increase of the parental *M. macrocopa*, and the intrinsic rate of population increase of the F_1 generation. BBP at 500 and 1000 $\mu\text{g l}^{-1}$, and 62.5, 125, 500, 1000 and 2000 $\mu\text{g l}^{-1}$ had intriguing effects on the population growth of the parental and the F_1 *M. macrocopa*, respectively. The parental *M. macrocopa* were more sensitive in survival, development and reproduction to BBP than the F_1 generation, but the reverse was also true in the population growth. Extending chronic toxicity tests to the second generation of *M. macrocopa* increased the cost-effectiveness of the assays.

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