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Acute Toxicity of Parathion and 2,4-D to Larval and Juvenile Stages of *Chasmagnathus granulata* (Decapoda, Brachyura)

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Chasmagnathus granulata (Grapsidae) is a species of estuarine crab distributed along the littoral zone of Samborombón Bay (35° 26′ S, 57° 07′ W to 36° 18′ S, 56° 48′ W), Argentina. The adults live in midtidal zones, on mud flats. During the reproductive season (spring-summer), the ovigerous females migrate to an aquatic environment of higher salinity and lower temperature, suitable for zoeae hatching, as other decapods (Cargo 1958; Sastry 1983). After a larval development of 15 to 20 days (Boschi et al. 1967), individuals in the megalopa stage return to the coast and molt to the first juvenile stage, representing the juvenile recruitment to the population (Sastry 1983).

All stages of the life cycle of this species form part of the aquatic trophic web. Larvae and juveniles are preyed upon by alevins of several fish species of great commercial and sport fishing value when adults. On the other hand, parathion and 2,4-D are currently amongst the most extensively used pesticides in Argentina, and reach the Samborombón Bay from intensively cropped fields, through several rivers and artificial channels that flow into it.

The aim of this paper is to establish, with a preventive criterion, the concentrations of parathion and 2,4-D that would be dangerous to the survival of the most sensitive stages (larvae and juveniles) of the life cycle of this estuarine crab.

MATERIALS AND METHODS

The 96-hr toxicity bioassays with first zoeae and juvenile crabs were carried out following the standard methodology for this kind of studies (American Public Health Association et al. 1976; Ward and Parrish 1982).

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Technical grade ethyl-parathion (purity 99%, Compañia Química, Buenos Aires, Argentina) and iso-butoxyethanol ester of 2,4-dichlorophenoxyacetic acid (Síntesis Química, Buenos Aires, Argentina) were employed. Pentaethylene oxide nonyl phenolate was used as solvent, in equal proportions with pesticides, to prepare the stock solutions by adding distilled water. Stock solutions were prepared daily, and were used within 2 hr after preparation. Dilution water was prepared adding artificial marine salts to distilled or dechlorinated tap water (80 mg/L total hardness as $CaCO_3$, pH 7 $\pm$ 0.5), to obtain the desired salinity. Unless otherwise noted, the salt composition used in previous work (Monserrat et al. 1991) was employed to prepare the dilution water.

Two types of controls were carried out in all assays: 1) Dilution water control (12 °/₀₀ salinity for juvenile crabs and 30 °/₀₀ for zoeae) and 2) Solvent control, with solvent concentration used in the highest pesticide concentration (Table 1). All pesticide solutions and controls were renewed every 24 hr for parathion and 2,4-D series.

Zoeae I of *C. granulata* were obtained from ovigerous females collected at Faro San Antonio beach (southern shore of Samborombón Bay) in March 1988. The females were kept in the laboratory until zoeae hatching, at 30 °/₀₀ salinity, 20 °C, 12 L : 12 D photoperiod (fluorescent light), and under continuous aeration. Groups of ten ($\pm$ 1) first day zoeae were randomly selected from a pool of synchronously hatched larvae from three different females. Each group was placed in a 200-mL finger bowl containing 100 mL of 30 °/₀₀ artificial saline water (HW Germany salts added to distilled water), U.V. sterilized and aerated 24 hr before. During the test, surviving zoeae were changed daily by means of 25 ml glass pipette to fresh saline water for each treatment, and recently hatched *Artemia salina* nauplii were added. Remaining larvae were counted as dead, taking complete immobility as death criterion.

All finger bowls were placed in a 150-L thermostat controlled cool bath, at 15 $\pm$ 1 °C, on a 10 L : 14 D photoperiod (fluorescent light). Triplicate sets for each pesticide were run. The series of concentrations used were established by range finding tests and are given in Table 1, which also includes concentrations of solvent controls. In the zoeae I-2,4-D assay, the solvent concentrations corresponding to the four highest pesticide concentrations were carried out, because of the high mortality caused by the highest solvent concentration, at 96 hr, in preliminary test.

Juvenile crabs were collected at the same site as ovigerous females, in March 1989. They were kept in 12 °/₀₀ artificial saline water, 20 °C, and under natural photoperiod (12 L : 12 D), in glass containers with polyurethane foam with artificial burrows as substrate, and were fed with rabbit pellets twice a week.

After an acclimation period of 2 wk, groups of nine animals were randomly assigned (mean carapace width = 10.94 ± 1.27 mm, n=18) to 1-L beakers containing 150 mL test solution at a salinity of 12 °/₀₀. During the assay the animals did not feed, and a temperature of 21 ± 1 °C, photoperiod of 12 L : 12 D (fluorescent light) were maintained in the laboratory. Before the daily change of water, dead animals were counted and removed. The criterion of death used was absence of movement after gently touching the animals with a glass rod, confirmed by observation of cheliped laxity. Each pesticide solution and control were run in duplicate. Concentrations and number of animals used are given in Table 1.

Probit analysis (Finney 1971) was employed, with the aid of a custom-made computer program, to estimate the LC50 and its 95% confidence limits, with Abbot's correction for control mortality. The Litchfield and Wilcoxon procedure (Litchfield and Wilcoxon 1949) was employed when the probit method failed to estimate LC50 values. To compare LC50 values, differences were considered to be significant when the higher LC50/lower LC50 ratio exceeds the corresponding critical value established by the American Public Health Association (1976).

Table 1. Concentration series and total number of animals used (N).

Stage / Pesticide	Pesticide concentrations	Solvent control concentrations	N
Zoea I			
Parathion	0.1-0.3-0.9-2.7-8.1-25 ug/L	0.02 uL/L	250
2,4-D	0.056-0.167-0.5-1.5-4.5-13.5 mg/L	0.4-1.2-3.6-10.9 uL/L	248
Juvenile			
Parathion	50-100-200-400-800-1600 ug/L	1.3 uL/L	144
2,4-D	200-400-800-1600-3200-6400 mg/L	5.2 mL/L	144

RESULTS AND DISCUSSION

Table 2 summarizes the LC50 values obtained for both stages and pesticides at different times of exposure. In zoeae I-2,4-D assay, although the 24 h-LC50 value could not be estimated, the 50 % mortality ocurred between the two highest pesticide concentrations, while in juveniles-2,4-D assay, mortality was less than 50 % at 24 and 48 hr, although the highest concentration employed was very high (6400 mg/L).

Mortality of juveniles during the acclimation period was lower than 10 % . In solvent controls, mortality at 96 hr was zero for parathion-juveniles, and 22.2 % for 2,4-D-juveniles, while in zoeae assays, the solvent controls corresponding to the highest pesticide concentration taken for the LC50 calculation at each 24 hr interval, reached a mortality no higher than 16 % .

Parathion showed a significantly ($p < 0.01$) higher toxicity than the herbicide, 526 fold for zoeae I, and 8008 fold for juveniles, at the end of exposure (96 hr). This is consistent with results previously obtained for adults of the same species, that is, a 6000 fold higher toxicity of parathion than 2,4-D (Rodríguez and Lombardo 1991).

The comparisons among LC50 values, at 96 hr, are

Table 2. LC50 values, 95 % confidence limits, and probit line parameters for zoeae and juveniles.

Stage	Pesticide	Hours	LC50	95%Conf.Lim.	Slope	R^2
Zoea I						
	Parathion	24	14.91	(11.30-18.30)	5.78	0.82
	(ug/L)	48	7.08	(4.88-9.40)	3.15	0.76
		72	1.79	(1.20-2.44)	2.47	0.81
		96	0.57	(0.33-0.85)	2.15	0.84
	2,4-D	24	4.5-13.5	---------	----	----
	(mg/L)	48	1.06	(0.70-1.67)	1.42	0.82
		72	0.43	(0.30-0.56)	3.15	0.94
		96	0.30	(0.20-0.39)	5.30	0.98
Juvenile						
	Parathion	24	1.55	(1.19-2.81)	3.87	0.96
	(mg/L)	48	0.76	(0.60-0.98)	3.88	0.92
		72	0.47	(0.37-0.59)	4.07	0.81
		96	0.36	(0.29-0.45)	4.45	0.84
	2,4-D	24	>6.4	---------	----	----
	(g/L)	48	>6.4	---------	----	----
		72	5.55	(1.41-21.8)	2.64	0.71
		96	2.89	(2.41-5.56)	3.11	0.60

expressed in Table 3, including data for adults (Rodríguez and Lombardo 1991). Comparing the three stages, zoeae were the most sensitive to both pesticides, particularly to 2,4-D (see the corresponding LC50 ratios in Table 3). On the other hand, juveniles and adults differ in their resistance to parathion ($p < 0.01$) and do not differ from 2,4-D ($p > 0.05$ when comparing the respective LC50 values).

Other authors have studied the relative sensitivity to several pesticides of different stages of the life cycle in decapod crustaceans. Bookhout and Monroe (1977) have reported a higher susceptibility of the early zoea stages in *Callinectes sapidus* exposed to malathion (an organophosphorate insecticide). Epifanio (1971) encountered the same relative sensitivity among larvae stages of *Leptodius floridanus* exposed to dieldrin. In the juvenile stages, a similar pattern occurs. Buchanan et al. (1970) showed that the early juvenile stages of *Cancer magister* were more sensitive to Sevin than the more advanced ones, the latter being very similar in their resistance when compared to adults. These results are consistent with those obtained in this work with both assayed pesticides.

On the other hand, the marked difference encountered in this study between the sensivity of zoeae and adults was also reported for other species and pesticides. Van Dijk et al. (1977) reported a sensitivity to pentachlorophenolate for zoeae I up to 100 fold greater than the adults of *Palaemon elegans*. Vernberg et al. (1977) found a similar response in zoeae I and adults of *Uca pugilator* exposed to Aroclor 1254 and 1016, and Armstrong et al. (1976) established a difference of 310 fold in resistance between zoeae I and adults of *Cancer magister* exposed to methoxychlor, while a minor difference was encountered in the same study between zoeae I and the first juvenile stage.

Table 3. Comparisons between the 96 h - LC50 values for all studied stages. NS: no significant differences ($p > 0.05$).

Pesticide	Comparison	LC50 Ratio	Significance
Parathion	Ad./zoea I	982	($p < 0.01$)
	Juv./zoea I	632	($p < 0.01$)
	Ad./juv.	1.56	($p < 0.01$)
2,4-D	Ad./zoea I	11,233	($p < 0.01$)
	Juv./zoea I	9,610	($p < 0.01$)
	Ad./juv.	0.81	NS

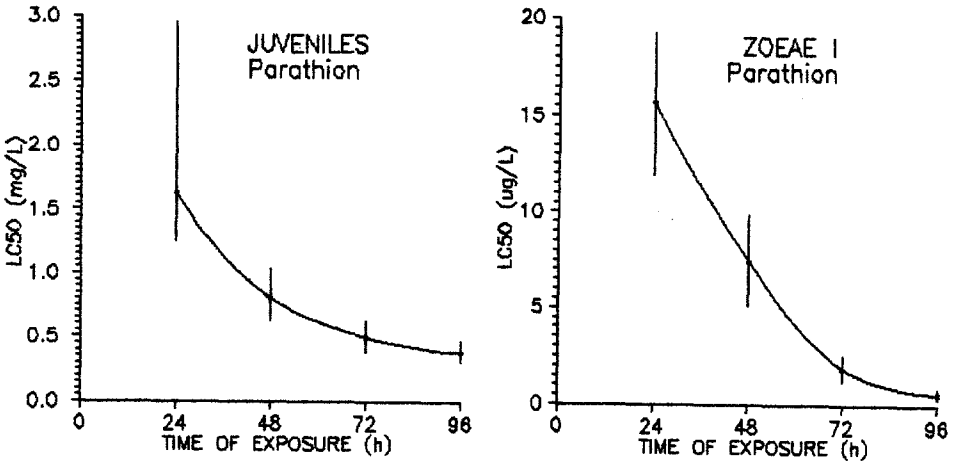

Figure 1. Parathion toxicity curves for both stages of *C. granulata*.

The comparison of LC50 values in relation to time of exposure showed no significant differences between 72 and 96 hr except in the zoea I-parathion assay, even though an evident asymptotic trend can be observed (Fig. 1). Therefore, the 96 h-LC50 may be considered as incipient lethal threshold concentration, for each stage exposed to parathion and 2,4-D (American Public Health Association et al. 1976; Sprague 1969).

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