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Effects of Salinity on the Toxicity of Parathion to the Estuarine Crab *Chasmagnathus granulata* (Decapoda, Grapsidae)

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The "crab commmunity" of Buenos Aires Province, Argentina, first described by Boschi (1964), extends along the coast of Samborombón Bay mainly between 35° 26′ S, 57° 07′ W and 36° 18′ S, 56° 48′ W. Since this is an estuarine environment, there are strong diel and seasonal fluctuations in water salinity which requires special physiological adaptations of the animals that live in this habitat. One of the common species of crabs of this community is *Chasmagnathus granulata*, which shows euryhaline and osmoregulatory features (Mañe Garzon et al. 1974; Gnazzo et al. 1978) that render it a good model for the study of parathion toxicity at different salinities.

Parathion is a widely used pesticide in Argentina, and it is able to reach Samborombón Bay from neighboring croplands through several rivers and artificial channels flowing into it.

MATERIALS AND METHODS

Specimens of *C. granulata* (25.0 - 30.1 mm carapace width) were collected in March 1988 at Faro San Antonio beach, near the southern limit of Samborombón Bay. The animals were kept in captivity during 2 wk at an average temperature of 20 ± 1°C with natural photoperiod (12 L:12 D), in 12 °/₀₀ saline water. Feeding with pellets of rabbit food and water renewal of the jars was done on the same days, once a week.

After this period, groups of twenty individuals were placed during 3 wk in 40 X 30 X 20 cm aquaria, each containing 3 L of water (enough volume to totally cover the crabs) of the same salinity used in the bioassays (7.5, 15 and 30°/₀₀, all within the environmental range). These salinities were obtained by diluting the artificial sea water prepared by

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adding the salts listed in Table 1 to dechlorinated tap water (80 mg/L total hardness as $CaCO_3$). The frequency of water renewal and feeding during this 3-wk acclimatation period was the same as described above.

Ethyl parathion (purity 99 %, Compañia Quimica, Buenos Aires, Argentina) and pentaethylene oxide nonyl phenolate as solvent were added to distilled water in equal proportions to prepare the stock solution for bioassays. These assays were carried out according to the procedures outlined by the American Public Health Association (1976). Renewal tests were made with a daily frequency to avoid a significant degradation of the pesticide. Salinity was also checked daily and was found to vary less than $\pm$ 1 °/₀₀. Other variables under control were: ambient temperature (20 $\pm$ 1 °C), water temperature (19.5 $\pm$ 0.5 °C), pH (7) and photoperiod (12 L:12 D, fluorescent light).

Three pesticide concentrations were used for each salinity tested : 0.28, 0.56 and 0.84 mg/L, referred to as 0.5, 1 and 1.5 toxic units, according to Ward and Parrish (1982): toxic unit = concentration of pesticide used / LC50. In the present work, the LC50 value used for *C. granulata* was that calculated for 96 hr at a salinity of 12 °/₀₀ (*Rodriguez and Lombardo, unpublished work*).

The solvent concentration used for the highest pesticide concentration (2.5 uL/L) was run as the solvent control in each series. Dilution water controls were also run. All concentrations tested and controls were replicated, each replicate involving eight randomly selected individuals. The weight of the organisms used ranged from 10.0 to 17.9 g. The number of dead individuals was counted daily, using eyestalks inmobility and cheliped laxity as death criteria.

LC50 values and 95 % confidence limits were estimated for each day and salinity, by probit analysis (Finney 1971). To compare LC50 values, differences were considered to be significant when the ratio

Table 1. Salts added to produce 100 L of artificial sea water (35 °/₀₀ salinity).

NaCl	2765 g	KB	10.2 g
$MgSO_4.7\ H_2O$	706 g	Na_2CO_3	3.5 g
$MgCl_2.6\ H_2O$	518 g	H_3BO_3	2.6 g
$CaCl_2.2\ H_2O$	154 g	$SrCl_2.6\ H_2O$	2.5 g
KCl	69.7 g	KF	0.4 g
$NaHCO_3$	14.3 g	KI	0.01 g

higher LC50 / lower LC50 exceeds the corresponding critical value according to the American Public Health Association (1976). Interactions between salinity, exposure time and toxic units were evaluated by Repeated Measures ANOVA (Winner 1971) for arcsin transformed survival data (live individuals / initial number of individuals ratio). Time of exposure was the repeated measures factor; the other two factors were toxic units and salinity.

In order to evaluate metabolic damage of the organisms exposed to parathion, standard metabolic rates of eight individuals exposed to each concentration-salinity combination were estimated by the oxygen consumption method. This bioassay was carried out for the solvent control and for two of the pesticide concentrations - 0.5 and 1 toxic unit - since the high mortality rate caused by 1.5 toxic units did not allow its testing. Respiration was measured in a constant pressure respirometer (Dezi et al. 1987). Statistical comparisons among groups were performed by means of a factorial design ANOVA, with toxic units and salinity as factors. Multiple comparisons were done by Tukey's method.

RESULTS AND DISCUSSION

Dilution control mortality was 0 % at all salinities tested. Solvent control mortality was 0 % for 7.5 °/₀₀ and less than 7 % for 15 and 30 °/₀₀ salinities.

LC50 values at 96 hr for each salinity are shown in Table 2. 96 h-LC50 value at 30 °/₀₀ was significantly lower ($p<0.05$) than 96 h- LC50 values at 7.5 and 15 °/₀₀, these two latter did not differ. Survival data analysis (Table 3) showed highly significant differences ($p<0.01$) in two main factors (toxic units and time) and slightly significant differences ($p<0.071$) in effects of salinity, but the time-salinity interaction was highly significant ($p<0.01$), suggesting that the greatest mortality increase

Table 2. LC50 values at 96 hr and 95 % confidence limits, slope and correlation coefficient of probit lines for crabs exposed to parathion (mg/L).

Salinity (°/₀₀)	LC50	95%Conf.Lim.	Slope	R^2
7.5	**0.74**	(0.59-1.21)	4.22	0.79
15	**0.65**	(0.53-0.75)	9.39	0.99
30	**0.46**	(0.34-0.56)	6.33	0.99

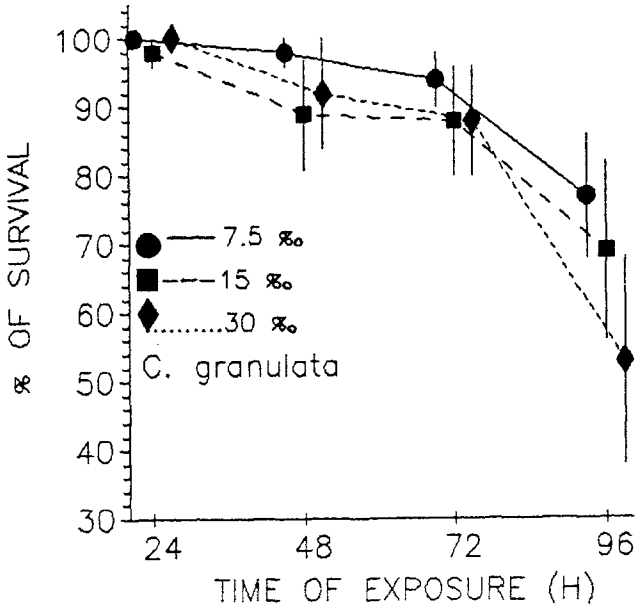

Figure 1. Percentage of survival along the time of exposure to parathion.

along the exposure period is caused by 30 °/₀₀ salinity (Figure 1). Survival rate at 96 hr was significantly higher ($p<0.05$) at salinities of 7.5 and 15 °/₀₀ than at 30 °/₀₀, which is consistent with the LC50 comparisons given above.

These results suggest that the toxicity of parathion on *C. granulata* is higher at higher salinities. Although different experimental conditions were used by other authors, the comparison with their results is

Table 3. Results of a repeated measures ANOVA for percentages of survival.

Source of variation	Sum of squares	d.f.	Mean square	F ratio	Sig. level
TOXIC UNITS	4.92406	3	1.64135	40.47	0.0000
SALINITY	0.27030	2	0.13515	3.33	0.0706
T.U. X SAL.	0.39648	6	0.06608	1.63	0.2219
ERROR	0.48665	12	0.04055		
TIME	3.76111	3	1.25370	94.20	0.0000
T.U. X TIME	2.75263	9	0.30585	22.98	0.0000
SAL. X TIME	0.40289	6	0.06715	5.05	0.0008
TRIPLE INT.	0.17830	18	0.00991	0.74	0.7448
ERROR	0.47913	36	0.01331		

indicative of similar or related effects. Eisler (1969) found a similar pattern for decapod crustaceans: greater salinities enhance the toxicity of organophosphate insecticides (such as methyl parathion and malathion) but decrease the toxicity of organochloride insecticides. On the other hand, Jauch (1979) has reported on damage to osmoregulatory organs (gills) of cichlid fish exposed to fenthion, and Kamemoto (1961) and Kato and Kamemoto (1969) found evidence of ionic imbalance in osmoregulatory mechanisms of the grapsid crab *Metopograpsus messor* due to acetylcholinesterase inhibitors (such as parathion). Other pollutants, such as copper and cadmium, also act synergistically with salinity on crab species (Thurberg et al. 1973). Our results concerning acute lethal toxicity may be strongly related to alterations in osmoregulatory system of crabs, although the potential effects of salinity in modifying parathion's toxicity by chemical or other interactions should not be discarded.

The increase of oxygen consumption related to osmotic regulation was demonstrated for several crustacean species (Shumway and Jones 1981). On the other hand, an increase of oxygen consumption should be expected due to the action of organophosphates, in agreement with their anticholinesterasic properties (McEwen and Stephenson 1979).

Data of oxygen consumption at 96-h exposure are shown in Table 4. Significant differences ($p < 0.05$) were found for main factors (toxic units and salinity), while multiple comparisons indicate a significant ($p<0.05$) increase of mean oxygen consumption at 0.5 and 1 toxic units with respect to the solvent control. Figure 2 shows that, despite the non-significant salinity - toxic units interaction, the oxygen consumption increases sharply at higher toxic units, though differentially at each salinity. The ratio metabolic rate at 1 toxic unit / metabolic rate in solvent control was lower at 30 °/₀₀ (1.28), than at 15 °/₀₀ (1.36) and 7.5 °/₀₀ (1.45). In other words,

Table 4. Results of the two way ANOVA for oxygen consumption.

Source of variation	Sum of squares	d.f.	Mean square	F ratio	Sig. level
TOXIC UNITS	0.48941	2	0.24471	11.73	0.0000
SALINITY	0.13544	2	0.06772	3.25	0.0456
T.U. X SAL.	0.03908	4	0.00977	0.47	0.7589
ERROR	1.31476	63	0.02087		

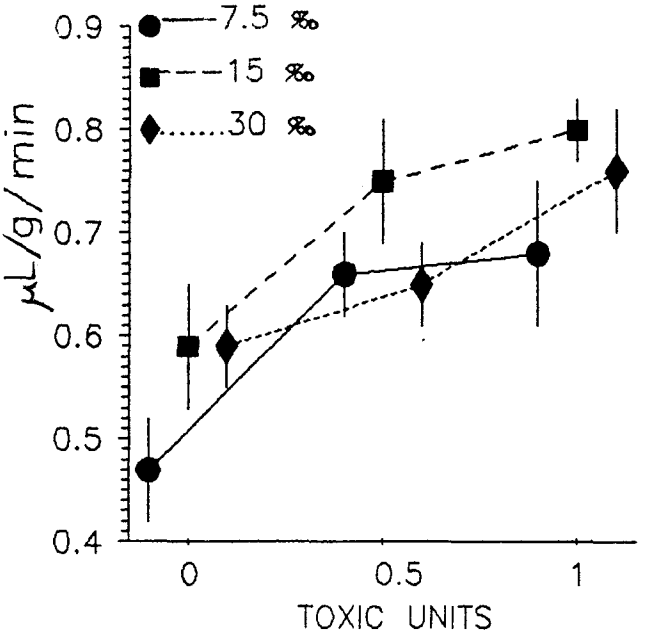

Figure 2. Oxygen consumption (uL/g/min) for all toxic units and salinities tested.

the relative increase of oxygen consumption was lower at 30 °/₀₀ salinity.

The results concerning metabolic rate suggest a possible damage of the osmoregulatory system of *C. granulata*. This damage is believed to cause serious problems when the osmotic working demand is high (that is, at salinities as high as 30 °/₀₀), taking into account that the "internal salinity" of *C. granulata* is reportedly close to 15 °/₀₀ (Gnazzo et al. 1978).

The reason why parathion seems to affect osmoregulation only at high salinities remains still unknown. It is possible that *C. granulata* requires a greater osmotic work at a salinity of 30 °/₀₀ than at 7.5 °/₀₀, since the latter is closer to the animal's "internal salinity". For a correct interpretation of the results obtained in future research on this subject, we strongly suggest a direct analysis of the internal ionic media of treated animals in such a way that possible damages caused to osmoregulation systems may be confirmed.

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REFERENCES

American Public Health Association, American Water Works Association, Water Pollution Control Federation (1976) Standard methods for the examination of water and wastewater, 14 th ed. Amer Public Health Assoc, Washington, D.C.

Boschi EE (1964) Los crustáceos decápodos Brachyura del litoral Bonaerense. Boletín del Instituto de Biología Marina Mar del Plata 164. 34 pp

Dezi RE, Rodríguez EM, Lenge ME (1987) Estudios del metabolismo energético en especies del Cangrejal de la Provincia de Buenos Aires.I. Tasa metabólica en machos de *Uca uruguayensis* y *Chasmagnathus granulata* (Crustacea, Decapoda, Brachyura). Physis, 45(109): 47-60

Eisler R (1969) Acute toxicities of insecticides to marine decapod crustaceans. Crustaceana 16(3):302-310

Finney DJ (1971) Probit analysis, 3rd ed. Cambridge University Press. London

Gnazzo G, Mañé-Garzon F, Leymonie J (1978) Estudios sobre la biología del cangrejo de estuario *Chasmagnathus granulata* Dana, 1851. IV. Ionograma normal en poblaciones de diferente salinidad. Revista de Biología del Uruguay VI(1):5-17

Jauch D (1979) Gill lesions in cichlid fishes after intoxication with insecticide fenthion. Experentia 35: 371-372

Kamemoto FI (1961) The effects of eserine on sodium regulation in crayfish.Comp Biochem Physiol 3:297-303

Kato KN, Kamemoto FI (1969) Neuroendocrine involment in osmoregulation in the grapsid crab *Metopograpsus messor*. Comp Biochem Physiol 28:665-674

Mañé-Garzon F, Dei-Casi E, Halcman-Spector B, Leymonie J (1974) Estudios sobre la biología del cangrejo de estuario *Chasmagnathus granulata* Dana, 1851. I. Osmoregulación frente a cambios de salinidad. Physis 33(86):163-171

Mc Ewen FL, Stephenson GR (1979) The use and significance of pesticides in the enviroment. John Wiley and Sons, N.Y. 538 pp

Shumway SE, Jones MB (1981) Influence of salinity on the respiration of an estuarine mud crab, *Helice crassa* (Grapsidae). Comp Biochem Physiol 70 A:551-553

Thurberg FP, Dawson MA, Collier RS (1973) Effects of copper and cadmium on osmoregulation and oxygen consumption in two species of estuarine crabs. Mar Biol 23:171-175

Ward GS, Parrish PR (1982) Manual of methods in aquatic enviroment research. Part 6. Toxicity tests. FAO Fish Tech Pap 185. 23 pp

Winner, BJ (1971) Statistical principles in experimental design. Mc Graw Hill, N.Y.

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