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EFFECTS OF PARATHION ON ION AND WATER BALANCE IN THE ESTUARINE CRAB *Chasmagnathus granulata* (DECAPODA, GRAPSIDAE).

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ABSTRACT

The effect of the organophosphate insecticide parathion on ionic concentrations and water content of the estuarine crab *Chasmagnathus granulata* was studied under different experimental conditions. No effects on hemolimph K^+ and Na^+ concentrations were noted in any case, indicating no apparent effects of parathion on ionic regulation processes. In the first experiment, involving a pre-exposure to parathion and 12 °/∞ salinity, followed by a final exposure to clean water at different salinities (4, 12 or 36 °/∞), both a higher branchial and body water content were detected at all salinities. These results could be related to the releasing of osmotically active organic metabolites in blood as response to parathion pre-exposure. In the second experiment, crabs were acclimated for 32 days to different parathion-free salinities (7.5, 15 or 30 °/∞), and exposed for a subsequent period of 10 days to parathion at the same salinities. In this case, by the third day of final exposure an increase in body water content was also noted, but the effect of parathion was salinity dependent, indicating a possible damage caused by the insecticide to adaptative osmoregulatory mechanisms that crabs may have developed during the previous acclimation period. This effect was compensated by the tenth day of exposure to parathion.

Keywords: Parathion, Crabs, Osmoregulation, Ions, Water

INTRODUCTION

According to Vernberg & Vernberg (1972), estuarine organisms are subjected to several environmental fluctuations capable of becoming stressors, specially when they interact with potentially relevant pollutants. Salinity is one of the most important of such factors, and could act as a modifying agent of

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pollutant toxicity, or as a critical environmental variable for species whose mechanisms of osmotic and ionic regulation have been injured by xenobiotics (Murty, 1986; Siva Prasada Rao et al., 1983).

Salinity fluctuations are often well correlated with certain physiological adaptations in estuarine fish and crustaceans, specially in decapods, such as the capacity to carry out both hyper- and hypo- ionic regulation processes (Pequeaux & Gilles, 1983). In this sense, the crab *Chasmagnathus granulata* has shown euryhaline features and a very good performance as an ionic regulator under a broad range of external salinities (Luquet et al., 1992).

The "crab commmunity" of Buenos Aires Province, Argentina, extends along the coast of Samborombón Bay (35°26' S, 57°07' W to 36°18' S, 56°48' W). The most common species of this community is represented by *C. granulata*. Since the Bay is an estuarine environment, daily fluctuations in water salinity occur, i.e. 12 to 18 °/₀₀ near the Salado river mouth (Botto & Irigoyen, 1979) and 20 to 30 °/₀₀ near Punta Rasa Cape, where tidal influence is relevant (Rossi, 1982). Seasonal fluctuations range from 5 to 30 °/₀₀ considering the whole Bay (Comisión Administrativa del Río de la Plata, 1990).

On the other hand, several pesticides may be carried into the bay by numerous inflowings. Recent data of pesticide monitoring in Samborombón Bay, indicate the presence of organochlorine insecticides (Aldrin and DDT), in higher concentrations than the permissible levels, near the mouth of the Salado river (Comisión Administradora del Río de la Plata, 1990). In the Paraná river, the most important tributary of "Río de la Plata" estuary, ethyl-parathion was detected at levels as high as 2.9 μg/l (dissolved in water), and 20.4 μg/l (adsorbed in sediments) (Lenardon et al., 1987).

In a previous work (Monserrat et al., 1991) the acute parathion toxicity for *C. granulata* was shown to increase at high salinities. The current work is aimed at characterizing, in the same species, eventual perturbations of parathion on relevant ions and water balance, at different salinities and types of exposure.

MATERIALS AND METHODS

Saline water, both for the acclimation period and all experiments, was prepared according to salt composition used previously (Monserrat et al., 1991) using dechlorinated tap water (80 mg/l total hardness as $CaCO_3$).

Toxicological bioassays procedures followed those recommended by the American Public Health Association (1976) and by Ward & Parrish (1982). Technical grade ethyl-parathion (O,O-diethyl O-p-nitrophenyl phosphorothionate, purity 99 %, Compañía Química, Buenos Aires, Argentina) was used. Pentaethylene oxide nonyl phenolate was used as solvent, in equal proportion to the pesticide, to prepare stock solutions with addition of distilled water. Both dilution water and solvent controls (containig the solvent concentration used in highest parathion concentration) were run. Two different experiments were carried out, under conditions specified below.

First experiment: pre-exposure to parathion.

Adult males of *C. granulata* were collected in September 1990 at Faro San Antonio beach (36°18'S, 56°48'W). Once in the laboratory, animals were kept for 3 d before experiments at 12 °/₀₀ salinity, mean value for the site of sampling. The crabs were fed with rabbit food pellets.

Crabs were initially exposed to 0.56 and 0.84 mg/l of parathion, at 12 °/₀₀ salinity, during 60 h. Those concentrations represent 1 and 1.5 folds respectively (toxic units) the parathion 96 h-LC50 value for *C. granulata* (Rodríguez & Lombardo, 1991). Solvent control (0.68 µl/l) was also run. Glass containers of 24-l capacity with 3-l of test solution were used, and 14 crabs were placed in each container. Three replicates were run for each treatment.

After 60 hs of exposure to parathion, three groups of 12 crabs each from every treatment, were randomly assigned to clean water of 4, 12 and 36 °/₀₀ salinity, respectively. After 1, 6.5 and 28 h of being transferred to clean water, samples of 4 animals were taken from each salinity-pretreatment combination, to determine both total and branchial water content and hemolymph potassium concentration.

During the bioassays, crabs were not fed, and a temperature of 23 ± 1 °C, pH 7 ± 0.5, and 12L:12D photoperiod (fluorescent light; intensity: 400 lux) were maintained. Mean live weight and standard deviation of a representative sample of crabs used was 13.27 ± 2.14 g (n=36).

Second experiment: pre-exposure to different salinities

Adult males of *C. granulata* were collected on March at the same site described above. Once in the laboratory, animals were acclimated at a salinity of 12 °/₀₀ for 2 wk. During this period, crabs were fed once a week with rabbit food pellets, as in previous works (Rodríguez & Lombardo, 1991; Monserrat et al., 1991; Rodríguez et al., 1992).

After the acclimation period, each one of three different stocks of crabs was re-acclimatized at a particular salinity, to be later used for exposure to parathion; these were 7.5, 15 or 30 °/₀₀ This second acclimation period took 32 d, and a weekly feeding frequency and water renewal was maintained.

The next step comprised the exposure to parathion, at the same salinities used in the pre-exposure period. Parathion concentration used was 0.14 mg/l, representing a quarter of 96 h-LC50 value for *C. granulata* (Rodríguez & Lombardo, 1991). The corresponding solvent control had a solvent concentration of 0.11 µl/l. For each treatment, 10 crabs were placed in each 24-l container, with 3-l of test solution. Test solutions were renewed every 4 d. Crabs were not fed during parathion exposure. After 3 and 10 days of exposure, four crabs of each container were sampled, in order to determine both sodium and potassium hemolymph concentrations, as well as total water content of each animal.

A temperature of 20 ± 1 °C, pH 7 ± 0.5, and 12L:12D photoperiod (fluorescent light; intensity: 400 lux) were maintained during the entire assay.

Mean live weight and standard deviation of a representative sample of crabs used was 13.24 ± 2.49 g (n=43).

Sample processing

For both experiments, the methodology used was as follows. Hemolymph samples were taken with a 1 ml-syringe from arthrodial membrane of chelae. Blood sodium and potassium concentrations of whole hemolymph were determined by means of a flame photometer (Crudo Caamaño S.R.L.) Clotted hemolymph samples were discarded.

Individual water content was estimated by difference between fresh and dry weight (accuracy: ± 0.1 mg); the former was measured prior to hemolymph extraction, and the latter after drying the animals at 75 °C up to constant weight (usually 48 h).

Concerning gills, only the most posterior ones (number eight) were sampled, because of their quite probable osmoregulatory function, as it has been established in other crab species (Johnson, 1980). In order to avoid measuring errors that could have been introduced by surface water, each gill was blotted in cellulose acetate before fresh weight determinations (accuracy: ± 0.1 mg).

Stepwise regression (Sokal & Rohlf, 1969) was applied for each variable (total water, branchial water and ions concentration), in order to formulate a suitable model for describing the effect of the considered factors on each measured variable. Such factors, considered as independent variables, were salinity, parathion concentration and time of final exposure. Total dry and branchial fresh weight were used as covariables for fitting regression models, considering total and branchial water as dependent variables.

RESULTS

After 60 h of pre-exposure to parathion (first experiment), the mortality percentage of crabs reached up to 15 % at the highest assayed concentration (0.84 mg/l), while no mortality was recorded after the final 3 d exposure to the insecticide (second experiment).

Mean values of potassium hemolymph concentrations and, both relative branchial and body water content of crabs for the first experiment, are listed in Tables 1, 2 and 3, respectively. For the second one, Table 4 shows Na^+ and K^+ hemolymph concentrations measured at 3 and 10 days of final exposure, and Table 5 specifies the corresponding data for total water content.

Table 6 specifies best fitted models. For the second experiment, data from the two different times of final exposure were independently analyzed, since no model with an explicative value could be obtained from the whole data.

No model for ions concentrations data could be adjusted by means of the statistical method employed, which shows the absence of significant effects ($p>0.05$) of the considered factors (salinity, parathion concentration and time of final exposure) on the first mentioned variable. Only at the third day of

Table 1. Mean values (x) and standard deviations (SD) of potassium concentration (expressed as meq/l) in hemolymph of assayed crabs. (n of each groupl=4). Sv.Ctrl.: solvent control. First experiment.

Clean water salinity (°/∞)	Pre-exposure parathion concentration (mg/l)	Time of final exposure to clean water (h)		
		1	6.5	28
4	Sv. Ctrl.	5.85 ± 1.01	7.97± 2.14	6.70 ± 1.97
4	0.56	4.23 ± 3.42	11.60 ± 2.46	7.03 ± 0.81
4	0.84	6.67 ± 1.58	7.75 ± 1.18	7.00 ± 0.99
12	Sv. Ctrl.	1.43 ± 0.47	5.95 ± 1.85	8.45 ± 3.49
12	0.56	6.05 ± 3.31	9.17 ± 1.62	10.25 ± 4.40
12	0.84	2.90 ± 2.89	6.63 ± 1.48	7.45 ± 1.71
36	Sv. Ctrl.	3.90 ± 1.06	7.55 ± 0.88	8.48 ± 1.22
36	0.56	5.10 ± 0.62	8.50 ± 3.34	8.10 ± 0.41
36	0.84	7.60 ± 0.72	8.50 ± 2.33	7.30 ± 0.71

Table 2. Mean values (x) and standard deviations (SD) of relative branchial water content (mg of water/mg of branchial dry weight, n of each group=4). Sv.Ctrl.: solvent control. First experiment.

Clean water salinity (°/∞)	Pre-exposyre parathion concentration (mg/l)	Time of final exposure to clean water (h)		
		1	6.5	28
4	Sv. Ctrl.	3.09 ± 0.91	5.56 ± 1.21	4.99 ± 1.05
4	0.56	3.07 ± 0.51	4.68 ± 1.80	5.90 ± 1.74
4	0.84	3.64 ± 1.07	5.33 ± 1.08	5.61± 2.11
12	Sv. Ctrl.	5.02 ± 1.78	5.88 ± 0.49	6.80 ± 1.97
12	0.56	3.41 ± 1.42	6.04 ± 2.12	6.83 ± 4.04
12	0.84	3.89 ± 1.33	9.64 ± 3.63	5.91 ± 2.22
36	Sv. Ctrl.	4.26 ± 2.36	4.19 ± 1.17	4.27± 0.96
36	0.56	3.26 ± 0.82	5.65 ± 0.84	7.15 ± 3.43
36	0.84	5.82 ± 1.92	5.82 ± 3.18	7.33 ± 2.49

Table 3. Mean values (x) and standard deviations (SD) of relative body water content of assayed crabs (g of water/g of dry body weight, n of each group n=4). Sv.Ctrl.: solvent control. First experiment.

Clean water salinity (°/oo)	Pre-exposure parathion concentration (mg/l)	Time of final exposure to clean water (h)		
		1	6.5	28
4	Sv. Ctrl.	2.47 ± 0.16	2.31 ± 0.08	2.44 ± 0.18
4	0.56	2.27± 0.27	2.17 ± 0.32	2.42 ± 0.06
4	0.84	2.36 ± 0.12	2.46 ± 0.30	2.46 ± 0.15
12	Sv. Ctrl.	2.44 ± 0.25	2.38 ± 0.23	2.41 ± 0.18
12	0.56	2.22 ± 0.18	2.34 ± 0.25	2.72 ± 0.64
12	0.84	2.47 ± 0.17	2.29 ± 0.16	2.50 ± 0.19
36	Sv. Ctrl.	2.37 ± 0.50	2.36 ± 0.15	2.31 ± 0.24
36	0.56	2.11 ± 0.18	2.31 ± 0.16	2.58 ± 0.46
36	0.84	2.57 ± 0.13	2.42 ± 0.42	2.51 ± 0.17

exposure during the second experiment, potassium concentration increased at higher salinities, but no effects were caused by parathion in any case.

As expected, both branchial and body water contents significantly raised, as the respective dry weight increased. Concerning body water content,

Table 4. Mean values and standard deviations (SD) of ion concentrations (expressed as meq/l) in hemolymph of assayed crabs (n of each group=4). Sv.Ctrl.: solvent control. Second experiment.

Ion	Day	Treatment	Salinity (°/oo)		
			7.5	15	30
Na^+	3	Control	328.3 ± 53.0	375.6 ± 28.4	399.7 ± 88.3
		Exposed	317.0 ± 75.6	354.9 ± 65.4	327.4 ± 132
	10	Control	386.8 ± 55.1	392.3 ± 66.2	454.3 ± 85.5
		Exposed	370.3 ± 57.0	393.4 ± 12.2	388.8 ± 99.8
K^+	3	Control	8.81 ± 1.01	10.00 ± 0.71	10.94 ± 0.59
		Exposed	8.00 ± 1.58	10.00 ± 1.29	11.69 ± 1.43
	10	Control	9.06 ± 1.99	9.67 ± 1.25	9.38 ± 2.29
		Exposed	7.44 ± 2.46	10.19 ± 2.43	8.69 ± 1.18

Table 5. Mean values (X) and standard deviation (SD) of relative body water content of assayed crabs (g of water/g of dry body weight, n of each group=4). Sv.Ctrl.: solvent control. Second experiment.

Day	Treatment	Salinity (°/∞)		
		7.5	15	30
3	Sv. Ctrl.	2.23 ± 0.34	1.86 ± 0.08	1.82 ± 0.27
	Exposed	2.04 ± 0.07	2.06 ± 0.23	2.07 ± 0.19
10	Sv. Ctrl.	2.09 ± 0.08	2.00 ± 0.19	2.07 ± 0.32
	Exposed	2.16 ± 0.12	2.02 ± 0.18	1.89 ± 0.20

different effects of the considered factors were noted for each experiment. In the case of the first one, parathion concentration caused a significant increase in total water content, whereas the influence of salinity did not show to be relevant for water balance of crabs (it is not included in the corresponding model, see Table 6). For the second experiment, a significant effect on body water content was attributable to the parathion-salinity interaction by the third day of exposure. This effect actually consisted in an increase in body water content at a combination of both increasing salinity and parathion concentration ($p<0.05$, positive coefficient), while no effects were detected at the tenth day of exposure (no model could be adjusted for that time, showing the absence of significant effects of the considered factors).

Concerning the branchial water content model (first experiment), the low R^2 value yielded (Table 6) indicates a corresponding high data variability, even when the effect of the considered factors (parathion concentration and time of

Table 6. Regression models for data of both experiments. ***C***: parathion concentration. ***T***: time of final exposure, ***S***: salinity (°/∞), ***BoWC:*** body water content, ***DBoW:*** dry body weight (g), ***BrWC***: branchial water content, ***DBrW***: dry branchial weight (g).

Experiment	Model	R^2	P
First			
	$BoWc=4.23xe^{(0.19xDBoW+0.10xC)^2}$	0.62	< 0.05
	$BrWC=0.005xe^{(169.7xBDW+0.013xTxC)^2}$	0.12	< 0.05
Second			
(Third day)	$BoWC = 1.93xDBoW + 0.2xSxC$	0.76	< 0.05

final exposure) was significant ($p<0.05$), showing a similar pattern of dependence to that shown by the total water model.

DISCUSSION

In both experiments, both sodium and potassium hemolymphatic concentrations showed to be independent of variations in salinity and parathion concentrations. These results indicate no apparent effects of the insecticide on the ionic regulation processes of the crab species studied, under the experimental conditions used. Results obtained in fish species, concerning effects of organophosphate pesticides, such as decrease in body sodium, potassium and calcium blood levels have been previously reported (Murty, 1986; Siva Prasada Rao et al., 1983).

Results from the first experiment indicate a net effect of acute pre-exposure to parathion in increasing total and branchial water content. This fact suggests that some persistent remaining effects of the insecticide exists, i.e., the above mentioned increasing effect was detected up to 28 h after the transfer of crabs to clean water. On the other hand, parathion pre-exposure did not seem to affect the onset of osmoregulatory mechanisms aimed at facing salinity changes, i.e., its remaining effect was independent of the clean water salinity level used for final exposure, when crabs were transferred from parathion concentration and 12 °/₀₀ salinity to clean water at different salinities.

Such osmoregulatory mechanisms are mainly attributed to hormonal action, and the possible hormones involved would act with a lag period of 1-2 h to equilibrate water flux rate (Kamemoto, 1982). Since a similar pattern of response was followed in all salinities at 1 h and successive times of final exposure, mechanisms of parathion action other than disturbing of hormonal osmoregulatory pathway should be postulated to explain its remaining effect on water content.

Besides, since no significant change in ion concentrations were noted, increase in water content caused by parathion in the first experiment could be related to the releasing of osmotically organic active metabolites, from hepatopancreas and other tissues to hemolymph, as a result of an unspecific stress response caused by the insecticide, in the same way described for other toxicants. As examples, an increase in blood glucose, lactate, free fatty acids and other metabolites was reported for several fish species by the effects of pollutants, including organophosphate compounds (Pant & Singh, 1983; Gill et al., 1990; Ferrando et al., 1991).

However, the possible effects that parathion, as well as other organophosphate insecticides, might exert on cell membrane estructures, as observed in chick nervous tissue (Tuler & Bowen, 1989) should not be discarded. Biochemical or structural changes in epithelial membranes, among other factors, could be involved in water permeability changes in crustacean gills (Taylor & Taylor, 1992). To our knowledge, there are no works showing

the same effects involving ions permeability changes after exposure to organophosphorus insecticides.

Results from the second experiment reveal other aspects of parathion action on water balance. Significant interaction of parathion with salinity showed that parathion at high salinities may cause an acute increase in water content. This means a possible damage by the insecticide to the osmoregulatory mechanisms of crabs previously acclimated to different salinities, thus having well developed hormonal adaptations for water balance (Pequeux & Gilles, 1983; Towle, 1990; Kamemoto, 1991; Taylor & Taylor, 1992).

Hormonal osmoregulation involves mainly the thoracic ganglion as producer of two factors acting in an opposite way on water flux (Kamemoto, 1982; Kamemoto & Oyama, 1985; Kamemoto, 1991), as well as the indirect effects that dopamine, octopamine and other cardioexcitors released by the pericardial organ could have on water exchange by increasing hemolymph circulation through the gills. The neurotoxic feature of parathion, a well known acetylcholinesterase inhibitor, allows the insecticide to exert some effect at this level, considering also the broad distribution of acetylcholine in crustacea (Atwood, 1982).

In additon to the effects that parathion could cause on osmoregulatory mechanisms, changes in hemolymph organic metabolites concentration might also occur. According to a previous work (Monserrat et al., 1991), acute exposure to parathion caused an increase in oxygen consumption of *C. granulata* (respect to controls), and its magnitude was salinity-dependent. A lower increase occurred at a salinity of 30 °/₀₀ when compared to 7.5 and 15 °/₀₀. At 30 °/₀₀, anaerobic respiration could also be enhanced just when osmotic work energetic demands are higher, and the corresponding metabolites such as lactate and piruvate would increase the water flux to the exposed animals.

On the other hand, whatever the mechanisms involved in the water inbalance, some kind of compensation seems to occur by the tenth day of continuous exposure to parathion. Steady state bioaccumulation level is reached within one to four days of continuous exposure to several organophosphate insecticides (Miyamoto et al., 1979, in fish) and, in that context, it is reasonable to think that physiological changes could follow a two phase pattern, i.e., from the beginning of exposure period up to the 3rd or 4th day ('acute' phase) and after the possible stabilization of organophosphorus accumulation ('chronic' phase). The 'acute' phase could be characterized by more abrupt and wider changes.

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