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Effects of Cypermethrin on the Freshwater Crab *Trichodactylus borellianus* (Crustacea: Decapoda: Braquiura)

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Synthetic pyrethroids, such as Cypermethrin (CY) (RS)-alpha-cyano-3-phenoxybenzyl (1RS)–cis-, trans-3-(2.2,-dichlorovinyl)-2.2-dimethylcyclopropane carboxylate), are used in aquatic and agricultural systems to control a broad spectrum of animal pests (Casida 1980). This highly active type II pyrethroid is applied as an insecticide but also affects non-target aquatic organisms (Ramade 1989).

The biocidal action produces alterations in the conductance of nerve cell membranes increasing transmembrane sodium influx and an inhibition of ion-dependent ATPases of insects, and other animals (Berlin et al. 1984; Salibian and Marazzo 1995). Moreover, these biocides induce apoptosis in tadpole tissues (Izaguirre et al. 2000). In decapod crustaceans the effects is not well documented.

Physiological indicators in aquatic animals, as oxygen uptake and ammonia-N excretion, respond rapidly to stress and have high toxicological relevance. Changes in environmental conditions affect the organism's ability to be a physiological fitness. It is interesting because any energy drain due to increased metabolism means that less energy is available for other necessary activities (Nelson et al. 1977; Adams 1990).

Trichodactylus borellianus (Nobili 1896) is a very abundant crab of La Plata basin (Magalhães and Türkay 1996), where agricultural activity is intensive and the human population is significant.

According to evaluate the potential toxicity of CY, this work examine the acute and sub-lethal concentration in *T. borellianus*, under laboratory conditions, using survival data, oxygen consumption, and ammonia-N excretion as endpoints.

MATERIALS AND METHODS

Trichodactylus borellianus were collected from the Salado river at the Paraná river floodplain (31° 39' S; 60° 41' W), Santo Tomé, Santa Fe, Argentina. The samples were taken from a 2000 cm^2 mouth area with a 1 mm mesh size net. Juvenile and adult crabs were acclimated for 7 days in glass aquaria with artificial

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pond water (APW), using. The experiments were conducted under natural daylight rhythm (14-10 h. light-dark photoperiod) at 25± 2 ° C. Before the tests, average carapace length and weight of crabs were measured (9.02 ± 1.85 mm and 0.38 ± 0.18 g for adults; 5.06 ± 1.24 mm and 0.07 ± 0.04 g for juvenile). Conductivity (450µmhos/L) and pH (7.2) were determined using a Beckman conductivity meter and Helige colorimeter, respectively.

The 96-h acute toxicity and sub-lethal tests of cypermethrin (CY) were conduted according to USEPA (1975) standard methods, with adults and juvenile crabs of *T. borellianus* (Magalhães and Türkay 1996). Glass aquaria (35 cm diameter and 20 cm high) with 4 L of (APW) contained 5 crabs per tank. The assayed product was Sherpa ® a commercial formulation containing 25% of CY in xylene. In this bioassay, the concentrations were 0.0001, 0.0005, 0.001, 0.005, 0.01 and 0.1, µg CY/L. Two control tests were conducted in APW without pesticide and with xilene. There were 3 replicates of all tests. Mortality was recorded daily. Oxygen consumption and ammonia-N excretion experiments were conducted in pyrex glass bottles (250 ml ± 1.5 ml volume) and filled with air-saturated test cypermethrin solution. One crab was placed in each bottle, capped and placed in a 25 ± 2 °C room. There were three test solutions 0.001; 0.01; 0.1 µg CY/L and a control without pesticide. Each treatment had 6 replicates. Measurements of dissolved oxygen were taken at the beginning of the experiment and at each hour during the first 3 h. Dissolved oxygen (DO) was measured with a YSI Model 57 DO meter and electrode probe. Ammonia-N was determined at the beginning and the end of the experiment, water sample were analysed according to the Nessler Method (Rodier 1981) (Test Kit Model FF-2 of Hatch).

Differences in concentrations of dissolved oxygen and ammonia-N between the beginning and end of each hour were recorded. These two parameters, oxygen consumption (mg g^{-1} h^{-1}) and ammonia-N excretion (µg g^{-1} h^{-1}), were calculated by multiplying the observed difference of dissolved oxygen and ammonia-N by the water volume in each bottle, and dividing the result by the wet body weight and time elapsed (h). LC50 with confidence limits ($p<0.05$) were estimated using a Probit Analysis Program based on Finney (1971). Control and experimental data were subjected to a one-way analysis of variance and the Tukey test (Zar 1996).

RESULTS AND DISCUSSION

In this study, juveniles and adults had similar responses to different CY concentrations (ANOVA $p=0.875$) in LC50 test. Mortality variation between the 24-LC50 and 96-LC50 was 18.5% but the minimum and maximum values indicated a non-significant difference for *T. borellianus* of 0.0097 µg CY/L 96-LC50 cypermethrin (Table 1).
There was no mortality in the two type of control. Within the range 0.0001 and 0.001 µg CY/L, survival rate for 96 h was 100 %. At 0.005, 0.01 and 0.1 µg CY/L, survival rate showed differences when it was compared with the control group (ANOVA $p<0.0001$) (Tukey $p<0.05$) (Figure 1).

Table 1. Acute toxicity response (LC50) of juveniles and adults *Trichodactylus borellianus* crabs exposed to cypermethrin, n total = 105

Time (h)	LC50 (µg CY/L)	Confidence Limits Lower	Upper
24*	0.0119	0.0071	0.0234
48*	0.0119	0.0071	0.0234
72*	0.0104	0.0054	0.0249
96*	0.0097	0.0049	0.0231

* values not significantly different (ANOVA p=0.4317)

The visible neurotoxic effect of cypermethrin upon the crabs was hyperactivity at the very beginning of the experiment but then hypoactivity. Oxygen consumption of *T. borellianus* control animals was not significantly different by hour (Table 2) with a mean of 4.9 ± 0.37 O_2 mg $g^{-1}h^{-1}$. Moreover, oxygen uptake of *T. borellianus* exposed to control solutions was lower during the first hours than in those crabs exposed to 0.001, 0.01, 0.1 µg/L CY (Tukey $p<0.05$).

In cypermethrin treatments, oxygen consumption varied at each hour (Table 2) (Figure 2). In 0.001 0.01 and 0.1 µg CY/L, the mean oxygen uptake of *T. borellianus* showed the highest values in the first hours. Then oxygen consumption decreased drastically in the less concentrated solution-groups than in other concentrations (Figure 2). However in the third hour, oxygen consumption was not different (ANOVA F=0.69; p=0.5727) and it was lower than in controls animals (Tukey, $p< 0.05$) (Figure2).

Table 2. ANOVA table of oxygen consumption during three hours and ammonia –N excretion determined at the beginning and the end of the experiment of *Trichodactylus borellianus* exposed at different cypermethrin concentrations.

Oxygen consumption	Df	Sum of squares	F	P
Among hours				
Control	20	1025.07	0.02	0.9804
0.001 (µg CY/L)	20	72.7643	10.32	0.0018
0.01 (µg CY/L)	18	114.013	16.82	0.0003
0.1 (µg CY/L)	18	166.89	7.34	0.006
Ammonia-N excretion-				
Among CY concentrations	27	0.6512	23.05	0.0003

Ammonia-N excretion of crabs increased with increasing cypermethrin concentrations (Figure 3). When crabs were exposed to 0.001µg/L CY, ammonia-N excretion was not significantly different than control animals (Tukey, $p<0.05$). However, in 0.01 and 0.1 µg/L CY, the ammonia-N excretion was four and eight times more abundant than in 0.001 µg/L CY and in control treatments (Tukey $p<0.05$) (Figure 3).

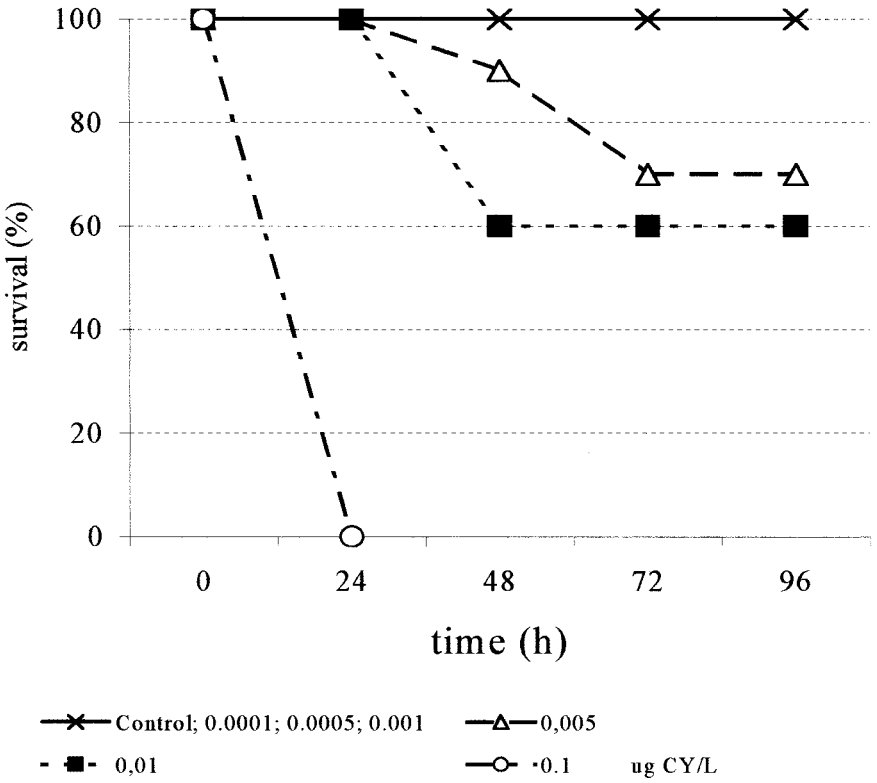

Figure 1. Survival curves for *Trichodactilus borelliamus* exposed to several concentrations of cypermethrin during 96 h.

Analysis of variance indicated that there was a great effect of cypermethrin level on the ammonia-N exretion of crabs (Table 2) and there was a significant increase of the 0.01 and 0.1 µg/l CY groups (Tukey, $p<0.05$).

The average rate of pyrethroid application to crops, is 10 – 200 g of active ingredient (CY)/ha and its degradation rates are similar for both, laboratory and field surveys. The half-life of cypermethrin in water is about 2-4 weeks, depending upon physicochemical properties and environmental conditions (WHO 1992). In Argentina, concentrations for application vary between 1.5 g and 120 g of the active pyrethroid (CY)/ha (CASAFE 1995). It is known that pyrethroids are relatively non-toxic to birds and mammals but they are extremely toxic to aquatic organisms (Jolly et al. 1978; Little et al. 1993). Moreover, aerial applications of cypermethrin and decamethrin contaminated the water of numerous tributaries of the Karami River (Nigeria) and freshwater prawns disappeared from river benthos samples for up to one year (Ramade 1989).

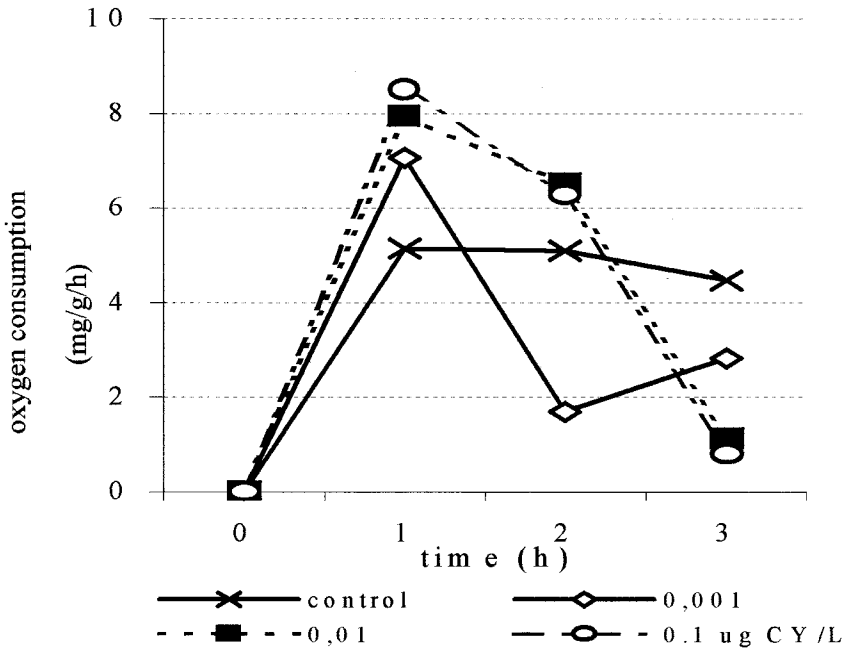

Figure 2. Oxygen uptake of *Trichodactylus borellianus* exposed to several concentrations of cypermethrin during 3 h.

It is recognized that in laboratory tests, the ranges of 96h LC50 are 0.01–5 µg CY/L for aquatic invertebrates, 0.4-2.8 µg CY/L for fish, and 129–1012 µg CY/L for amphibians (Pillai et al. 1987; Izaguirre et al. 2000). However the freshwater prawns, *Macrobrachium rosembergii* and *Palaemonetes argentinus*, are more sensitive to cypermethrin (0.000031 ppm and 0.002 µg CY/L respectively) (Pillai et al. 1987, Collins unpublished) than *T. borellianus* crab (in this study) and some other aquatic invertebrates.

In the experiments upon sub-lethal and lethal levels crabs have shown hyperactivity during the first hours and then hypoactivity. Similar behaviour was documented for *P. argentinus* (Collins, unpublished). This behavioural change was accompanied by a shift in the metabolic requirement of oxygen consumption and ammonia-N excretion. Nielsen and Hagerman (1998) documented the effects of extrinsic factors on oxygen consumption of decapods. However, this has not been analysed on freshwater animals exposed to CY. In this research, oxygen consumption has proved to be a sensitive tool for evaluating the physiological condition of crab under cypermethrin. This could be showed in the behaviour of the crabs. During the hyperactivity phase, the oxygen uptake reaches its maximum in all crabs under cypermethrin treatments. However, with increasing time the decrease in oxygen consumption suggests a change in the metabolic pathway and energy expenditure for recuperation.

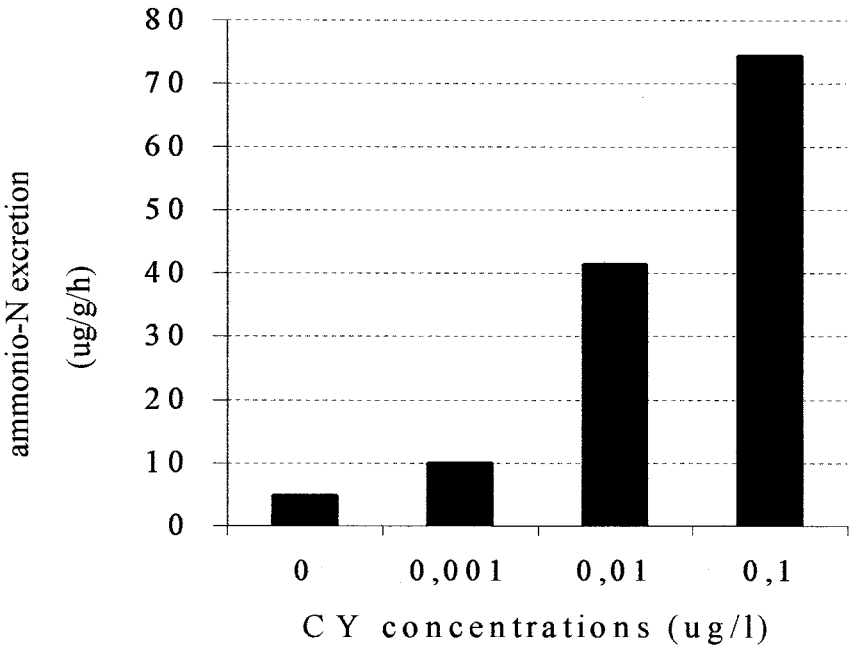

Figure 3. Ammonia-N excretion between beginning and end of the experiment of *Trichodactylus borellianus* exposed to several concentrations.

Nitrogen metabolism of *T. borellianus* is greatly affected by extrinsic factors such as xenobiotics. At high cypermethrin concentrations (0.1 and 0.01 μg/L CY), the increase in ammonia-N excretion suggested that there is an increasing amino acids catabolism or a variation in metabolic routes.

In crustaceans, the metabolic pathways involved in nitrogen excretion are catabolism of amino acids and certain amides, degradation of nucleic acids, deamination of purine nucleotides, and urea, respectively. The possible mechanism of ammonia excretion is passive NH_3 efflux (Kormanik and Cameron 1981), NH_4^+ efflux (Maetz and Garcia-Romen 1969), and ion exchange of NH_4^+ for Na^+(Pequeox and Gilles 1981). The counterbalance of NH_3^+ output by a Na^+ input was also verified in crabs (Magnum et al. 1976) and freshwater prawns (Armstrong et al. 1981). Decapod crustaceans mainly excrete nitrogen as ammonia (Regnault 1987) with the major portion being excreted through the gill epithelium.

In the present study, ammonia-N excretion of *T. borellianus* increased with increasing cypermethrin level. This may be attributed to additional excretion in the antennary gland or Na^+, K-ATPase increased in the gill epithelium of crab, that effects could be relationship with the activity mechanisms of cypermethrin. This product make alterations the ion conductance of nerve cell membranes by

increasing transmembrane sodium influx and inhibition of ion-dependence ATPases (Salibian and Marazzo 1995).

This work determined that 96-LC50 for *T. borellianus* was lower than the level applied in field surveys. Moreover, crabs can be affected by the application of low levels of cypermethrin. In this case, results showed that natural populations of the crab *T. borellianus* could be sublethally affected by pyrethroid fumigation.

From an ecotoxicological point of view, decapods are more sensitive to pyrethroid toxicity than fish and amphibians, therefore they may serve as better indicator organisms for pyrethroid toxicity.

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