Toxicity of glyphosate upon the freshwater prawn *Palaemonetes argentinus*

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Abstract

Glyphosate (Roundup®) is one of the most commonly used herbicides in agricultural systems in the world that control a broad-spectrum of worst weeds to cheap. The present study examine, under laboratory conditions, the acute effect, oxygen consumption, ammonia-N excretion and individual growth of *Palaemonetes argentinus* under the influence of the Roundup® commercial formulation. The experiments were conducted at 25 ± 2 °C. Juveniles prawns were exposed to different solutions of glyphosate. Measurements of dissolved oxygen were taken at the beginning of the experiment and at each hour during the first 4 h and 24 h. Ammonia-N was determined at the beginning and the end of the experiment (24 h). LC50-96 was 0.1418 $ml.L^{-1}$ of glyphosate Roundup®. Mortality rate for 72 h in maximum solution was 100%. Oxygen uptake and ammonia-N excretion of prawns exposed to concentrations was different than in controls animals. In glyphosate treatments, oxygen consumption and ammonia-N excretion decreased. This variation could be showed in the metabolic requirement of oxygen consumption and the amino acids degradation. The physiologic effects suggests that this prawns to be sensible to Roundup®. In general, the growth was similar among control and treatment prawns but high mortality in the experiments with herbicide solutions was observed. This action could be effected by Roundup® herbicide on regulatory mechanisms of molt process in the prawn growth.

Key words: Glyphosate, Roundup®, *Palaemonetes argentinus*, metabolism, growth.

Introduction

The glyphosate and their commercial formulation Roundup® has widely been used around the world. Its application is for the control of the annual and perennial worst weeds. In the aquatic environment, it can enter through runoff from agricultural fields or atmospheric deposition. The high water solubility presented by glyphosate contributes to the risk in the aquifer pollution (Newman and Unger, 2003).

Effects upon the biota are characterised by acute and chronic studies using various aquatic organisms (Ratte *et al.*, 2003). The responses such as mortality or immobilization are necessary while the more prolonged tests that affect the growth or other physiological process completes the observations (Walker *et al.*, 2001; Newman and Unger, 2003; Ratte *et al.*, 2003). Oxygen uptake and ammonia-N excretion has been considered as indicators that reflect the protein and energy utilization, assessing the physiological response under various stress conditions in crustaceans (Nelson *et al.*, 1977; Williner and Collins, 2003).

Important criteria for the species selection in ecotoxicological test are sensitivity, representatives in the ecosystem that may receive the impact, abundance and ecological importance, as well as the practical aspects like easy handling and the plausibility of their culture in laboratory (Ratte *et al.*, 2003). *Palaemonetes argentinus* Nobili, 1901 (Boschi, 1981) is a prawn of freshwater environments of southern South America (Boschi, 1981), region with an intensive use of herbicides, particularly Roundup®. Moreover, this prawn is considered of great ecological interest, integrates fish and birds diets of lotic and lentic environments (Collins *et al.*, 2004). For these reasons, this prawn is a potential experimental species that shows a good ability to adapt to the captivity.

In order to study the tolerance of *P. argentinus* to Roundup® herbicide, three experiments were conducted. First, the acute toxicity of herbicide on *P. argentinus* was calculated; then oxygen consumption and ammonia-N excretion were measured in different herbicide solutions. Lastly, as the herbicide affects the growth in isolated individuals.

Material and Methods

Juveniles of *P. argentinus* were collected from Salado river (31° 39' S; 60° 41' W), Santo Tomé, Santa Fe, Argentina. Prawns were acclimated for 1 week in glass aquariums in laboratory at a water temperature of 25 ± 2 °C and 14:10 h L:D photoperiod. Conductivity (900 μmhos.cm^{-3}) and pH (7.7) were measured by conductimeter Beckman and colorimeter Helige, respectively. Each prawn was measured for the cephalothorax length (CL) from the tip of the rostrum to the end of the cephalothorax with a caliper (± 0.01 mm) under a stereoscopic microscope. All specimens were fed with fish muscle *ad libitum*.

The assayed product was Roundup®, a commercial formulation of the herbicide. This formulation contains 48% of the active ingredient glyphosate (as isopropylamine salt), water and a surfactant (polyoxyethylenealkylamine). Roundup® is a registered trademark of Monsanto Technology LLC. Roundup® product with all its components were used in the experiments.

Acute toxicity

The acute toxicity of the Roundup® herbicide was analyzed in glass aquariums with 3000 ml containing 10 prawns per tank. The total number of prawn used was 180 with CL mean of 6.62 ± 1.03 mm. Prawns in intermolt stage were utilized determining the molt-cycle stage by their setogenesis (Drach, 1939). There were 5 test solutions (0.070, 0.141, 0.281, 0.562 and 1.124 ml.L^{-1} of Roundup®) and control, each treatment was conducted with 3 replicates.

The toxicity was recorded by mortality or immobilization of the prawns in each concentration at 24, 48, 72 and 96 h. The LC50 was estimated using a Probit Analysis Program based on Finney (1971). Data were analyzed using a one-way analysis of variance and the post-test Tukey (ANOVA) (Zar, 1996).

Oxygen consumption and ammonia-N excretion.

Pyrex glass bottles (250 ± 1.5 ml) were filled with air-saturated test herbicide solution. One organism was placed in each bottle. The mean and standard deviation of CL and weight were 8.85 ± 0.99 mm and 0.12 ± 0.04 g respectively. There were used three concentrations (0.03, 0.10 and 0.30 ml.L^{-1} of Roundup®) and control. Dissolved oxygen (DO) in water was registered to an interval of one hour during three hours and then of 24 h, with a oxymeter Orion 830A.

Ammonia-N excretion was determined at the beginning and the end of the experiment, water sample collected from each treatment were tested using the Nessler Method (Rodier, 1981) (Test Kit Model FF-2 of Hatch).

The differences in levels of dissolved oxygen and ammonia-N between the beginning and end of each treatment were calculated. Oxygen consumption (O_2mg.g^{-1}.h^{-1}) and ammonia-N excretion (μg.g^{-1}.h^{-1}) were estimated by multiplying the observed difference of dissolved oxygen and ammonia-N by the volume of water in each bottle, and by dividing the result by the wet body weight and time lapse (h) (Chen and Lin, 1995; Chen and Chia, 1996; Williner and Collins, 2003). The parameters of linear regression between the oxygen uptake and oxygen dissolved was tested for significance using ANOVA. The exponents b of the relationships were tested for similarity between each concentration with t-student (t) ($p < 0.05$) (Zar, 1996).

Growth

The 80 prawns of *P. argentinus* were kept individually in glass containers (180 ± 1.5 ml) during 160 days. The initial size (CL) was 6.85 ± 1.18 mm and the first ecdysis was discarded. Specimens were exposed to three herbicide solutions (0.017, 0.035 and 0.070 $ml.L^{-1}$ of Roundup®) and control. Each treatment was conducted with 20 isolated prawns. Thirty percent of the water was changed every day. Moreover, observations were made twice a day to check the presence of the exuvia and dead prawns. In order to reduce stress from handling, the cephalothorax length (CL) was determined by measuring the exuvia. The effects of Roundup® herbicide on the growth was examined by regression:

$$CL_{t+1} \text{ (postmolt)} = a + b\, CL_t \text{ (premolt)}$$
$$\text{Intermolt period} = a + b\, CL \text{ (postmolt)}$$
$$\text{Growth rate} = a + b \text{ Intermolt period}$$

The regression slope in each analyze were compared among treatments with t-test ($p < 0.05$). The **b** exponents in the CL_t and CL_{t+1} relationships were tested for consider them isometric (i. e., 1) using a t test ($p < 0.05$) (Zar, 1996).

Results

Acute toxicity

No significant difference in (CL) of prawn among treatments was observed. There was not mortality in the control. In Figure 1 are shown the mortalities of *P. argentinus* in the concentrations used, being the mortality, at 24 and 48 h, smaller than 50%, and increased with the time. In 1.124 $ml.L^{-1}$ of herbicide solution the survival of prawn was 0 % at 72 h. The LC50's (mean ± sd) of *P. argentinus* for Roundup® at 72 and 96 h were calculated in Table I. LC50 values were similar between these hours ($p < 0.05$).

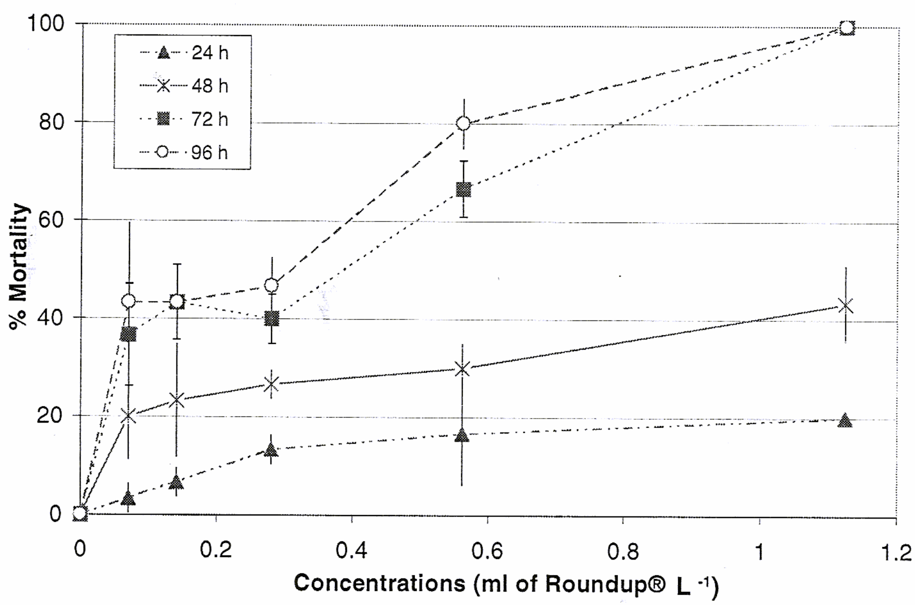

Figure 1: Dead prawns (mean ± sd) of *Palaemonetes argentinus* in different Roundup® concentrations.

Table I: LC50 (mean ± standard deviation) of the Roundup® herbicide in the *Palaemonetes argentinus*

Time (h)	LC50 (mean ± sd) (ml.L-1 of Roundup®)
24	Not calculated.
48	Not calculated.
72*	0.1953 ± 0.0086
96*	0.1417 ± 0.0512

*values not different significantly (ANOVA $F = 2.24$; $p = 0.1836$).

Oxygen consumption and ammonia-N excretion

Oxygen consumption of *P. argentinus* varied at the time. At the beginning of the experiment, the oxygen uptake showed highest values in all treatments. Then, the mean oxygen uptake in the control diminish more than in the concentrations. Oxygen uptake of prawn exposed to 0.30 $ml.L^{-1}$ was highest than those exposed to 0.10 $ml.L^{-1}$ and 0.03 $ml.L^{-1}$ of Roundup® (Fig. 2a).

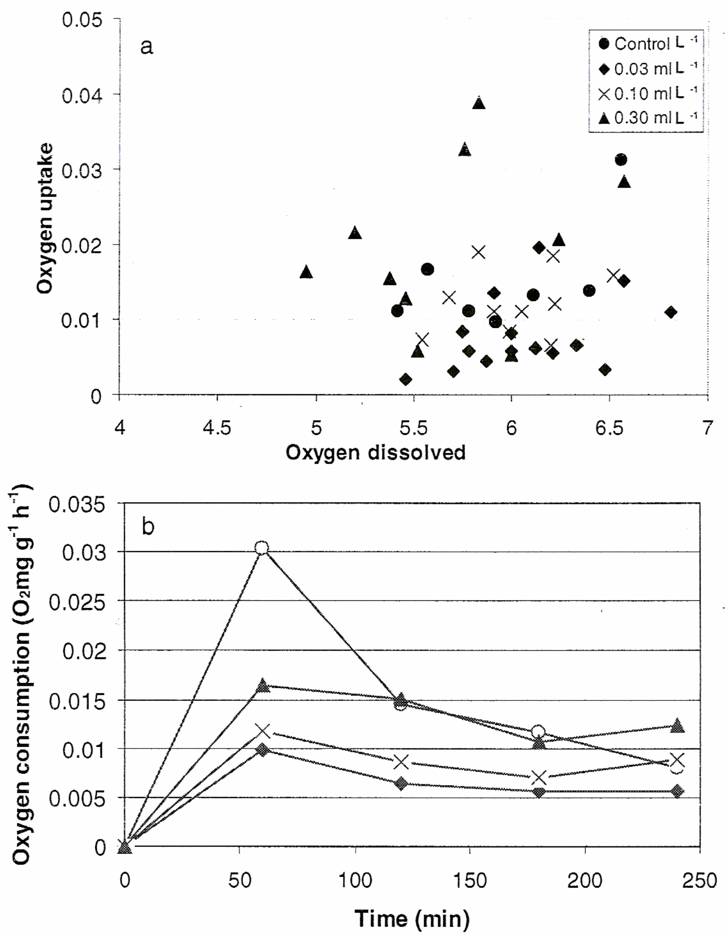

Figure 2: Oxygen uptake of the prawn *Palaemonetes argentinus* in the Roundup® concentrations used and control. **a.** Relationship between oxygen uptake ($O_2mg.g^{-1}.h^{-1}$) and oxygen dissolved ($O_2mg.L^{-1}$); **b.** Oxygen uptake variation in the time (min).

In all treatments, the oxygen consumption increased with the high concentration of oxygen dissolved in range of 4.5 to 8.2 $O_2mg.L^{-1}$ (Fig. 2b). The relationship between oxygen uptake and oxygen dissolved was statistically significant in control and two solution groups, but it was not significantly in the highest concentration (Table II).

Table II: Regression analysis between oxygen dissolved and consumption of *Palaemonetes argentinus* exposed at different Roundup® concentrations during 240 min.

Oxigen uptake = a + b oxygen dissolved					
	Regression parameters			ANOVA	
Treatments	a	b	r	*F*	*P*
Control	-0.050	0.011	0.546	28.0	<0.0001*
0.03 ml.L-1	-0.010	0.003	0.250	4.5	0.0372*
0.10 ml.L-1	-0.009	0.003	0.272	5.4	0.0228*
0.30 ml.L-1	0.003	0.002	0.110	0.8	0.3858

* regression statistically significant.

In *P. argentinus* juveniles, the oxygen consumption in the greater concentration of herbicide at 24 h was highest than those exposed to other concentrations and control (Fig. 3). However, the oxygen consumption decreased with progressively increased of the prawn weight.

Ammonia-N excretion of *P. argentinus* in the different concentrations was different, diminishing with their presence (Fig. 4). Excretion products in prawns exposed to 0.30 $ml.L^{-1}$ was highest than those exposed to 0.10 $ml.L^{-1}$, and was highest than those exposed to 0.03 $ml.L^{-1}$ of Roundup®.

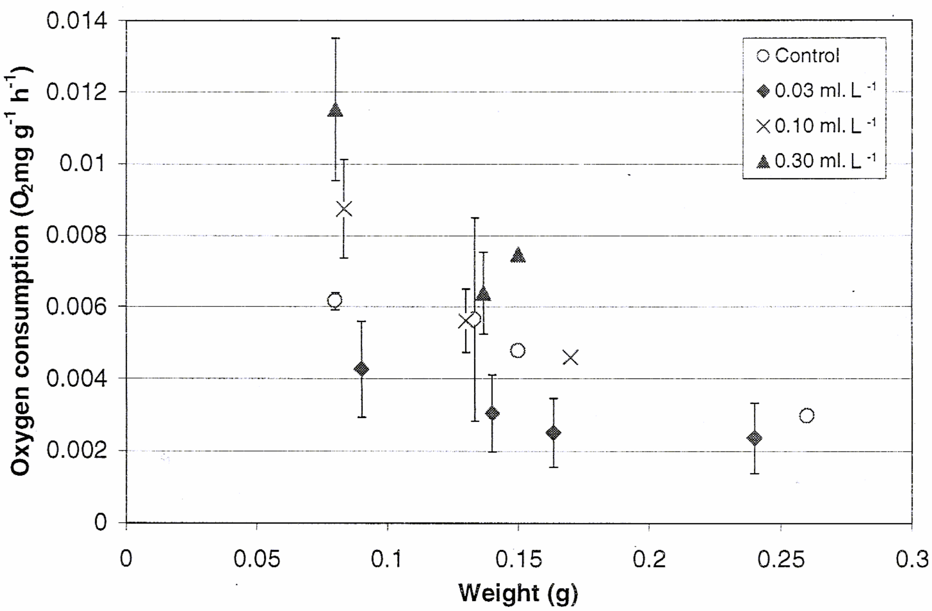

Figure 3: Relationship between oxygen uptake ($O_2mg.g^{-1}.h^{-1}$) and weight (g) (mean ± sd) of *Palaemonetes argentinus* in different Roundup® concentrations and control after 24 h.

Growth.

The CL increase of *P. argentinus*, according to the CL_{t+1} (postmolt) and CL_t (premolt) observations in two molt cycles, were isometric (i.e. b= 1), only the higher concentration was negatively allometric (Fig. 5) (Table III).

The prawns had until 4 molt cycles during the experience. In the first two cycles, the intermolt period was 19 ± 11.0 days with a mortality of 30 and 33% in the lowers and high concentrations respectively, while in the following cycles the period was increasing the same as the mortality.

The relationship between intermolt period and CL was different statistically in Roundup® solutions and control groups. Intermolt period went bigger when increasing the size (Fig. 6).

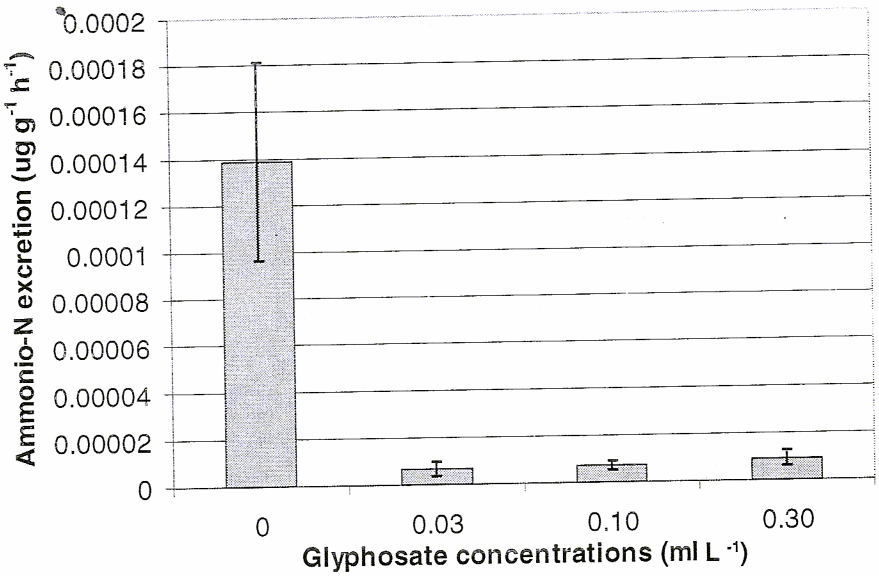

Figure 4: Excretion rate (ammonia-$N g.g^{-1}.h^{-1}$) (mean ± sd) of *Palaemonetes argentinus* exposed to several Roundup® concentrations and control after 24 h.

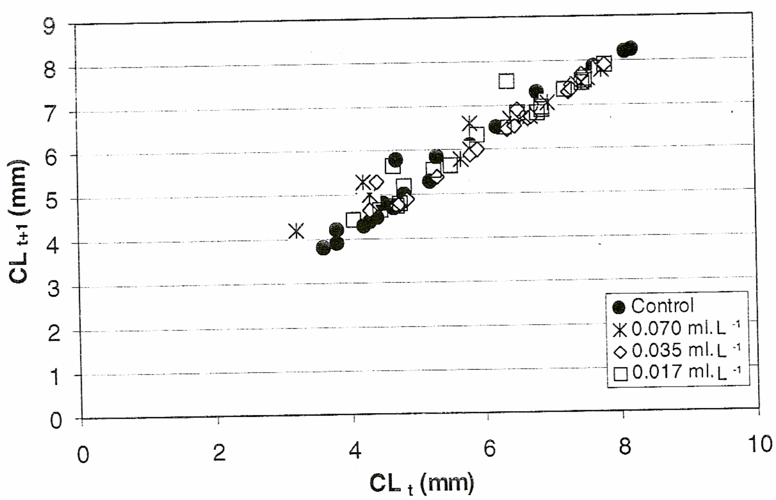

Figure 5: Relationship between CL_t (premolt) and CL_{t+1} (postmolt) (mm) in *Palaemonetes argentinus* exposed to several Roundup® concentrations and control.

Table III: Relationship between CL_t (premolt) and CL_{t+1} (postmolt) of *Palaemonetes argentinus* exposed to several Roundup® concentrations and control. **b** is the growth constant.

$CL_{t+1} = a + b\,CL_t$					
	Regression parameters			ANOVA	
Treatments	a	b	r	*F*	*P*
Control	0.308	0.978*	0.993	732.1	<0.0001
0.017 ml.L⁻¹	0.564	0.954*	0.962	181.5	<0.0001
0.035 ml.L⁻¹	0.332	0.970*	0.989	1093.7	<0.0001
0.070 ml.L⁻¹	2.076	0.717**	0.986	270.2	<0.0001

* not different statistically to 1 (isometric) with t-Student test.
** different statistically to 1 (negative allometric) with t-Student test.

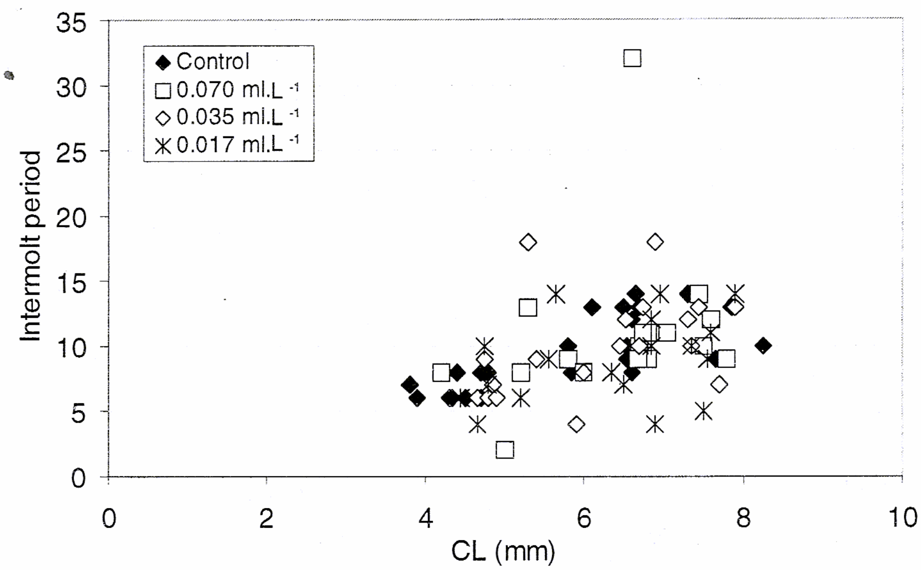

Figure 6: Relationship between intermolt period (days) and CL_{t+1} (postmolt) (mm) in *Palaemonetes argentinus* exposed to several Roundup® concentrations and control.

Discussion

Both laboratory and field studies have reported the microbial degradation of glyphosate in aquatic environments (Giesy *et al.*, 2000). The available data indicates that the glyphosate half-life in aquatic environments has ranges from 7 to 32 days (Torstensson *et al.*, 1989). This allows to suspect that if the compound arrives to the aquatic system, the communities could be seriously affected and among them the crustaceans like the prawns.

According to the other works in different zoological groups the LC50-96 of glyphosate in rainbow trout *Oncorhynchus mykiss* and other fish has a range from 0.086 to 0.168 $ml.L^{-1}$, and the LC50-48 in *Daphnia* is 0.780 $ml.L^{-1}$. However, the LD50-24 for lymnaeid snails *Pseudosuccinea columella* was 0.098 $ml.L^{-1}$ (Thompson, 1989). In decapod crustaceans of La Plata system, it was observed a similar sensitivity between the crab *Trichodactylus borellianus* studied by Montagna and Collins (2004) and the prawn *P. argentinus.*

On the other hand, the oxygen uptake by *P. argentinus* was similar that the observations in *T. borellianus* exposed to an insecticid (Williner and Collins, 2003). This consumption could be indicated the behaviour of the prawn showing a hyperactivity phase in the beginning and after hypoactivity.

Deviations from homeostasis associated with lethal and sublethal exposure reflect physiological alterations. Internal changes include impaired performances (swimming speed), respiration, excretion, ion regulation, osmoregulation, and bioenergetics (Newman and Unger, 2003).

The reduction of DO in solution is the result of oxygen consumption by animals and the diffusion of oxygen from the air to the solution (Shi and Mei, 1996). The oxygen consumption rate can be combined with the nitrogen (ammonia) excretion rate to suggest the relative dependence of respiration with the carbohydrate and lipid resources versus the deamination of amino acids. In crustaceans, metabolic pathways involved in nitrogen excretion are catabolism of amino acids and certain amides, degradation of nucleic acids, deamination of purine nucleotides, and urea with the formation of ammonia, uric acid, and ammonia and urea, respectively. The possible mechanism of ammonia excretion is passive NH3 efflux (Kormanik and Cameron, 1981), NH4+ efflux (Maetz and Garcia-Romen, 1964), and ion exchange of

NH4+ for Na+ (Pequeux and Gilles, 1981). The counterbalance of NH4+ output by a Na+ input was verified in crab *Callinectes sapidus* by Mangum *et al.* (1976) and in prawn *Macrobrachium rosenbergii* by Armstrong *et al.* (1981). This toxic excretion and Roundup® could affect the respiration and the normal excretion modifying some of these pathway.

Roundup® concentration provoked the decrease in ammonia-N excretion of *P. argentinus.* Moreover this indicates that is an alteration of the catabolism of amino acids which resulted in the nitrogen excretion. This was probably attributed to a fluctuation of excretion in the antennary gland or a Na+, K+-ATPase activity levels in the gill epithelium of prawn. Therefore, the product make a disturbance of normal physiological activity at the concentrations tested.

The growth is chosen as the response parameter to observe the sublethal effects. It integrates a suite of biochemical and physiological effects that is associated with individual fitness. In crustacean, the molt increment is the increase in size which occurs between one instar and the next. In various studies on Decapoda growth has been observed which the increment decreases with the size (Hartnoll, 1982; Díaz *et al.,* 2003). In *P. argentinus*, the percentage of length increment was variable, showing a clear tendency to decrease with the age.

For organisms exposed to toxic, energy is expended in response to the toxic, and the growth will be decreased (Newman and Unger, 2003). In the molt cycles of *P. argentinus*, there is not observation about variations in the molt increment with the different herbicide concentrations. Apparently, this would be indicating that it would not affect the increment in successive molts. In the same way it would seem that it happens with the intermolt time

However, prawn was affected by the application of low levels of Roundup® herbicide. In this case, results showed high mortality in each sublethal concentration tested, which few animals completed the two or three molt cycle. This implies that the prawn *P. argentinus* have a low tolerance to Roundup® with the time, and this may be attributed to disturbance of normal metabolic activities. Moreover, as the X-organ and sinus gland constitutes the secretory site of many neurohormones (Bellon-Humbert *et al.*, 1981), the absence or reduction of a hormone which controls salt deposition in the exoskeleton would result in difficulties and thus the prawn death would happen during the molt cycles.

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