THE USE OF METABOLISM TO EVALUATE THE TOXICITY OF CADMIUM AND ZINC ON THE *Litopenaeus schmitti* ACCORDING TO THE SALINITY

Edison Barbieri[1] *& Cristiane Veríssimo Vlcek*[2]

[1]Instituto de Pesca - APTA - SAA/SP.
(Caixa Postal 61, 11990-000 Cananéia, SP, Brazil)
edisonbarbieri@yahoo.com.br

[2]Bolsista do Programa de Iniciação Científica do CNPq (PIBIC)

ABSTRACT

Penaeid shrimps are important resources for worldwide fisheries and aquaculture. In Brazil, *L. schmitti* is an important commercially exploited species, and is an ideal animal for studying the impairment caused by the effects of heavy metals that are often detected in coastal areas. The main purpose of the present study was to detect the acute toxicity of Cd and Zn to *L. schmitti* and to investigate their effects on oxygen consumption and ammonium excretion in different salinity, which have not been carried out in this species before. First of all, we examined the acute toxicity of Cd and Zn to *L. schmitti* 24, 48, 72, and 96-h medium lethal concentration (LC50). Furthermore, we also found that exposure of shrimp to Cd and Zn caused an inhibition in oxygen consumption of 54.71% and 60.71%, respectively, relative to the control. However, after separate exposure to Cd and Zn, elevations in ammonium excretion were obtained, which were 89.47% and 84.21% higher than the control, respectively. The results shown that Cd and Zn performs higher toxicities to *L. schmitti* at lower salinities.

RESUMO

Os camarões são importantes recursos marinhos explorados pela pesca e aquacultura. No Brasil, *L. schmitti* é uma importante espécie comercialmente explorada, e um animal ideal para estudar o impacto causado por efeitos de metais pesados que freqüentemente são detectados em áreas costeiras. O principal objetivo do presente trabalho foi o de determinar a toxicidade aguda do Cd e Zn para o *L. schmitti* e investigar seus efeitos no consumo de oxigênio e na excreção de amônia em diferentes salinidades. Uma vez que tais parâmetros ainda não foram determinados para a referida espécie. Primeiramente foram examinadas as toxicidades aguda (LC50) do Cd e Zn, para *L. schmitti* por um período de 24, 48, 72 e 96 horas em três salinidades. Nossos resultados revelaram, que para camarões expostos ao Cd e Zn houve uma inibição do consumo de oxigênio de 54,71% e 60.71% respectivamente, para a mais baixa salinidade 5. Entretanto, para a excreção de amônia houve um aumento de 89,47% e 84,21% para a mais alta concentração utilizada e mais baixa salinidade em relação ao controle. Os resultados mostraram que a toxicidade de Cd e Zn foram mais altas nas baixas salinidades.

Descriptors: Litopenaeus schmitti, Heavy metal, Salinity, LC50, Oxygen consumption, Ammonium excretion.
Descritores: Litopenaeus schmitti, Metal pesado, Salinidade, CL50, Consumo de oxigênio, Excreção de amônia.

Introduction

In Brazil, heavy metals enter the coastal seawater mainly through discharge of industrial effluents and disposal of sewage (Damato & Barbieri, 2003; Barbieri *et al.,* 2004). High concentrations of heavy metals have been reported in coastal waters (Eysink, 1988a.), rivers and their estuaries (Eysink, 1988b), and tissues of coastal marine organisms (Carvalho *et al.,* 2000 and 2001). Cd and Zn have been widely recognized as highly toxic when dissolved and in ionic form (Mance, 1987). Cd and Zn are very common and persistent heavy metals in aquatic environments and known to be highly toxic to marine and estuarine crustaceans (Papathanassion, 1983, Wu & Chen, 2004).

Exposure to heavy metals in the aquatic environment produces many physiological changes in crustaceans, including alterations in the metabolic activities (Barbieri *et al.,* 2005). These effects are related to their mechanism of action and, therefore, are specific for each metal. The metabolic rate of an organism is an useful and sensitive indication of its daily consumption of energy. Therefore, in aerobic organisms the quantification of the rate of oxygen consumption will be directly associated to the amount of energy released from the oxidation of food substratum. Based on the amount of oxygen consumed by an animal for a certain period of time, it is possible to evaluate the energy spent during the same period to maintain its vital processes (Carvalho, 1992).

Several kinds of physiological answers can be used for this purpose (Adams, 1990; Hansen *et al.,* 1997; Barbieri *et al.,* 2002, 2005, 2007), including the metabolism. These parameters, being an interaction result of the several processes that reflect the animal general physical condition, constitute in sensible indexes to detect the environmental changes (Schreck, 1990).

Evaluation of metabolism was used, for example, to study toxicant effects caused by aromatic compounds (Lemaire *et al.,* 1996), heavy metals (Wu & Chen, 2004, Barbieri, 2007), detergents (Christiansen *et al.,* 1998; Barbieri *et al.,* 1998; 2000 and 2002) and a variety of toxicants (Boudou & Ribeyre, 1989).

The objective of this survey was to determine the acute toxicity of Cd and Zn for *Litopenaeus schmitti* in three salinities (35, 20 and 5). The results were analysed to determine if the acute toxicity was different with the salinit variation.

Methodology

The acute toxicity of Cd and Zn to larvae of the shrimp (*Litopenaeus schmitti*) exposed to different concentrations of these chemicals for a period of up to 96 h was evaluated, taking into consideration the high economic and ecological importance of this species, and the problems related to pollution of estuarine regions. A total of 600 larvae of cultivated shrimp with 1.3 ± 0.3 g medium wet weight and 3.2 ± 0.5 cm total length were used. Groups of fifteen individuals were put in 50 L tanks containing sea water at 35, 20 and 5 salinities in the 25°C of the temperature. Tree replicates of groups of 15 individuals were exposed to one of the following concentrations of Cd and Zn: 0.00, 0.01, 0.10, 0.50,

1.00, 2.00, 3.00 and 4.00 mg/L. Dead shrimp were removed from the tanks and counted at 24, 48, 72 and 96 h of exposure.

One hundred and fifty shrimp (*L. schmitti*) with averages of 1.27 (±0.4) g and 1.10 (±0.36) cm were employed for the routine metabolism measurement utilizing sealed respirometres. Ten shrimp were subjected to oxygen consumption measurements in one of the four concentrations of Cd (0.00; 0.1; 0.5; 1.0 and 2.0 mg/L) and Zn (0.0, 0.5; 1.0; 2.0 and 3.0 mg/L) in trees salinities (35; 20 and 5). The pH and oxygen concentration of the test solution were determined (Table 1 and 2).

Before the beginning of the experiments the animals were maintained in the respirometre with continuous water circulation for at least 90 min to attenuate the handling stress. Then, the water supply was suspended and the respirometre was closed, so that the shrimp could consume the present oxygen in the known water volume for a period of tree hours. The respirometres were protected by a barrier to isolate the animals from possible movement in the laboratory. The difference between the oxygen concentrations determined at the beginning and at the end of the confinement was used to calculate the consumption during the period. To minimize the effect of low oxygen concentration and metabolites accumulation on the metabolism, the experiments duration was regulated so that the oxygen concentration by the end of experiments was above 70% of its initial concentration. The dissolved oxygen was determined through the Winkler method (Table 1 and 2).

To obtain the Cd and Zn desired concentration, the necessary volume of the chemical (1.0 mg $CdCl_2$/mL and 1.0 mg $ZnSO_4$/mL) was added to each volume of respirometre at the end of the acclimation period. As soon as $CdCl_2$ or $ZnSO_4$ were added the entry orifice was sealed. Additionally the seawater in the bottle was sampled at the begining and end of the oxygen consumption analysis. Determination of ammonium in the seawater was based on the phenolhypochlorite method (Solarzano, 1969). The average oxygen specific consumption and ammonium excretion by the shrimp was assessed using analysis of variance (ANOVA). All data were analyzed using the Tukey's multiple comparisons test ($p < 0.05$).

Table 1. pH and oxygen concentration in experimental solutions of $ZnSO_4$ at the temperature of 25°C.

Concentration of $ZnSO_4$ (mg L^{-1})	pH	Oxygen concentration (mLO_2 L^{-1})
0.00	8.20	622
0.01	8.21	6.20
0.05	8.20	6.25
0.10	8.21	6.23
0.25	8.21	6.20
0.50	8.20	6.23
1.00	8.22	6.22

Table 2. pH and oxygen concentration experimental solutions of $CdCl_2$ at the temperature of 25°C.

Concentration of $CdCl_2$ (mg L^{-1})	pH	Oxygen concentration (mLO_2 L^{-1})
0.00	8.22	6.20
0.01	8.20	6.30
0.05	8.21	6.28
0.10	8.20	6.25
0.25	8.20	6.33
0.50	8.21	6.20
1.00	8.22	6.30

RESULTS

The acute toxicity of Cd and Zn to shrimp larvae exposed to different concentrations of this metal for periods of up to 96 h, expressed as LC50, in different salinities is shown in Tables 3 and 4. This results shown that Cd and Zn performs higher toxicities to *L. schmitti* at lower salinities.

For the acclimated shrimp the 25°C temperature, the specific oxygen consumption decreased regarding the Zn concentration in the three employed salinities. The specific oxygen consumption in any Zn concentration increased with the salinity increasing.

We checked that the specific oxygen consumption of shrimp from the acclimated control group to 25°C temperature (Table 5), subjected to 35.20 and 5 salinities were, as a rule, 0.0062, 0.0060 and 0.0056mlO2/ g/min, respectively. For the shrimp subjected to the concentration of 3 mg/L of Zn, they consumed as a rule 0.0035; 0.0028 and 0.0022 mlO2/g/min to the tested salinities. These values represent a metabolic level diminuition of 43.54%, 53.3% and 60.71.% in relation to the control.

Table 3. Medium lethal concentration (LC50 mgCd/L with 95% confidence limits) calculated by probit analysis in different salinities. Between parentheses, pattern deviation.

Time of exposition (in hours)	Salinity 35	Salinity 20	Salinity 5
24	0.98 (±0.49)	0.75 (±0.35)	0.55 (±0.26)
48	0.54 (±0.46)	0.55 (±0.23)	0.35 (±0.12)
72	0.32 (±0.37)	0.24 (±0.27)	0.10 (±0.08)
96	0.18 (±0.30)	0.10 (±0.14)	0.05 (±0.05)

Table 4. Medium lethal concentration (LC50 mgZn/L with 95% confidence limits) calculated by probit analysis in different salinities. Between parentheses, pattern deviation.

Time of exposition (in hours)	Salinity 35	Salinity 20	Salinity 5
24	1.64 (±0.46)	1.25 (±0.30)	0.96 (±0.28)
48	1.22 (±0.58)	0.90 (±0.22)	0.57 (±0.13)
72	0.86 (±0,50)	0.55 (±0.14)	0.32 (±0.18)
96	0.31 (±0.54)	0.15 (±0.26)	0.09 (±0.05)

Table 5. Oxygen specific consumption (ml O2/g/min) of the shrimp routine, acclimated to the temperature of the 25°C, subjected to different Zn concentrations in different salinities. Between parentheses, pattern deviation, % percentage of the oxygen consumption decreasing in relation with the control. Each value represents the average of five determinations.

Concentration of Zn (mg/L)	Salinity 35		Salinity 20		Salinity 5	
	Specific consumption	%	Specific consumption	%	Specific consumption	%
0	0.0062 (±0.0013)	-	0.0060 (±0.0021)	-	0.0056 (±0.0012)	-
0.5	0.0059 (±0.0021)	-9.23	0.0049 (±0.0024)	-18.33	0.0043 (±0.0030)	-23.21
1	0.0048 (±0.0015)	-22.58	0.0042 (±0.0011)	-30.00	0.0033 (±0.0014)	-41.07
2	0,0038 (±0.0026)	-38.70	0.0031 (±0.0018)	-48.33	0.0027 (±0.0024)	-51.78
3	0.0035 (±0.0012)	-43.54	0.0028 (±0.0020)	-53.33	0.0022 (±0.0023)	-60.71

Using the ANOVA (Tukey; $p<0.05$) statistical test, it was verified that the averages of the oxygen specific consumption to the Zn 1.0 and 5.0 mg/L concentration in all employed salinities are expressively different in relation to the control (0.0 mg/L). For the other concentrations, there was no significative difference.

The oxygen specific consumption varied considering the Cd concentration increasing and the salinity. The oxygen specific consumption decreased with the Cd concentration mainly to the salinities of 5 and 20. For the shrimp of the acclimated control to the 25°C temperature, subjected to the salinities of 35, 20 and 5 the oxygen specific consumption averages were, respectively, 0.0050; 0.0052 and 0.0053 O2/g/min. To the same salinity and in a bigger Cd (2.0 mg/L) employed concentration, the consumption was 0.0033, 0.0028 and 0.0024 ml O2/g/min (Table 6). This averages decreasing of the specific oxygen consumption to the Cd 2.0 mg/L concentration, represents a metabolic rate decrease of 34%, 46.1% and 54.71% in relation to the control (Table 6).

It was verified that the averages of the oxygen specific consumption to the Cd 1.0 and 2.0 mg/L concentration in all employed salinities are expressively different in relation to the control. For the other concentrations, there was no significative difference.

Table 6. Oxygen specific consumption (ml O2/g/min) of the shrimp routine, acclimated to the temperature of the 25°C, subjected to different Cd concentrations in different salinities. Between parentheses, pattern deviation, % percentage of the oxygen consumption decreasing in relation with the control. Each value represents the average of five determinations.

Concentration of Cd (mg/L)	Salinity 35		Salinity 20		Salinity 5	
	Specific consumption	%	Specific consumption	%	Specific consumption	%
0	0.0050 (±0,0017)	-	0.0052 (±0.0011)	-	0.0053 (±0.0021)	-
0.1	0.0049 (±0,0013)	-2.00	0.0048 (±0.0020)	-7.69	0.0040 (±0.0024)	-24.52
0.5	0.0045 (±0,0024)	-10.00	0.0037 (±0.0033)	-28.84	0.0029 (±0.0016)	-45.28
1	0.0036 (±0,0026)	-28.00	0.0031 (±0.0017)	-40.38	0.0027 (±0.0008)	-49.05
2	0.0033 (±0,0009)	-34.00	0.0028 (±0.0021)	-46.15	0.0024 (±0.0011)	-54.71

The Ammonium excretion varied considering the Zn concentration increasing and the salinity. The Ammonium excretion increased with the Zn mainly to the salinities of 20 and 5.The Ammonium excretion averages for the shrimp of the acclimated control, subjected to the salinities of 35, 20 and 5 were, respectively, 0.21and 0.19 µg/g/min. To the same salinity and in a bigger Zn (3.0 mg/L) employed concentration, the Ammonium excretion was 0.3; 0.33 and 0.36 ml µg/g/min (Table 7). This averages increasing of the Ammonium excretion to the Zn 3.0 mg/L concentration, represents a metabolic rate growth of 42.85%, 57.14% and 89.47% in relation to the control (Table 7).

Using statistical test, it was verified that the averages of the Ammonium excretion on to the Zn 2.0 and 3.0 mg/L concentration in all employed salinities are expressively different in relation to the control. For the other concentrations, there was no significative difference. The same test showed that there is no difference among the Ammonium excretion averages to the salinity of 5 and 20. However, the Ammonium excretion averages to the salinity of 35% is meaningfully different from the Ammonium excretion averages of other salinities employed to the concentration of Zn: 2 and 3 mg/L.

It was verified that under control, shrimp subjected to the salinity of 35, 20 and 5, excreted, on average, 0.21; and 0.19 µg/g/min of ammonium. Comparing these results with the averages of Ammonium excretion at the highest Cd (2.0 mg/L) concentration employed in the test, we verified that the Ammonium excretion average increased to 0.32, 0.39 and 0.35 µg/g/min, to the salinity of 35; 20 and 5, respectively. We noticed that the biggest Ammonium excretion occurred to the salinity of 20 and 5 to the concentration of 2 mg/L of Cd. For the salinity of 5, it occurred a percentual increasing of the Ammonium excretion of 84.21% when compared to the control. To the salinity of 20, the increasing of the oxygen percentual consumption was of 85.7% in relationship with the control. To the salinity of 35, the percentual increasing of the oxygen consumption was of 52.38% when compared to the control (Table 8).

Table 7. Ammonium excretion ((µg/g/min) of the shrimp routine, acclimated to the temperature of the 25°C, subjected to different Zn concentrations in different salinities. Between parentheses, pattern deviation, % percentage of the ammonium excretion increasing in relation with the control. Each value represents the average of five determinations.

Concentration of Zn (mg/L)	Salinity 35		Salinity 20		Salinity 5	
	Specific consumption	%	Specific consumption	%	Specific consumption	%
0	0.21 (±0.08)	-	0.21 (±0.06)	-	0.19 (±0.03)	-
0.5	0.21 (±0.11)	0.00	0,22 (±0.09)	4.76	0.18 (±0.10)	-5.26
1	0.22 (±0.90)	4.76	0.23 (±0.11)	9.52	0.20 (±0.11)	5.26
2	0.26 (±0.05)	23.80	0.25 (±0.02)	19.04	0.28 (±0.09)	47.36
3	0.30 (±0.06)	42.85	0.33 (±0.06)	57.14	0.36 (±0.03)	89.47

Table 8. Ammonium excretion ((µg/g/min) of the shrimp routine, acclimated to the temperature of the 25°C, subjected to different Cd concentrations in different salinities. Between parentheses, pattern deviation, % percentage of the ammonium excretion increasing in relation with the control. Each value represents the average of five determinations.

Concentration of Cd (mg/L)	Salinity 35		Salinity 20		Salinity 5	
	Specific consumption	%	Specific consumption	%	Specific consumption	%
0	0.21 (±0.06)	-	0.21 (±0.16)	-	0.19 (±0,13)	-
0.1	0.21 (±0.04)	0.0	0.22 (±0.11)	4.76	0.21 (±0,06)	10.52
0.5	0.25 (±0.08)	19.04	0.29 (±0.07)	38.09	0.27 (±0,08)	42.10
1	0.29 (±0.12)	38.09	0.35 (±0.08)	66.66	0.31 (±0,07)	63.15
2	0.32 (±0.14)	52.38	0.39 (±0.13)	85.7	0.35 (±0,10)	84.21

The Ammonium excretion averages in the concentration of 2.0 and 3.0 mg/L of Cd in all studied salinities is significatively different in relationship with the control. For the other concentrations, there was no significative difference. Comparing to the Ammonium excretion averages between the employed salinities, it was verified that there was significative difference between them. It was verified that there was a significative difference between the shrimp Ammonium excretion exposed not only to the 2.0 mg/L concentration, but also to the 1.0 mg/L of Cd to the salinities of 35 and 5. For the other Cd concentrations, there were no significative differences among the averages of corresponding concentrations in different salinities.

DISCUSSION

The obtained results in this survey allow to evaluate the Cd and Zn effects on the *L. Schmitti* metabolism to different salinities. Some shrimp can bear a wide salinity variation and live well among the sea, river water and brackish (estuary water). These movements are usually associated with the shrimp cycle of file, for example, the *L. schmitti* that lay eggs in the sea and in larve phase. They can be found in small rivers of sweat water and brackish. When they arrive into maturity, these shrimp come to seawater to reproduce. The passage from an environment to another requires deep changes into the osmoregulatory process as a consequent energy waste. This work confirm that different environmental conditions can affect the toxicity of heavy metals even in the same organism, because any of a number of variables such as the total concentration of the metal, pH, alkalinnity, the concentratio of competing metals, and the presence of adsorptive surfaces can affect the concentration of free metal ions within the environment and thus affect the response of an organism to that metal (Sunda et al., 1978).

The toxicity of heavy metals to crustaceans has been studied by a number of authors (Mance, 1987, Wong et. al., 1993, Vanegas et al. 1997; Wu & Chen, 2004). Results of this study confirm that the heavy metals Cd and Zn are toxic to *Litopenaeus schmitti*, an ecologically

and economically important shrimp in coastal waters of Brazil.

The most acutely toxic metal was Cd. The toxicity of Cd and Zn to marine crustaceans is well documented, but not for *L. schmitti*. For example, the 96h LC_{50} of Cd for *Litopenaeus vannamei* is 1.07 mg/L (Wu & Chen, 2004). In addition, 96 h LC_{50} values of Cd for larvae of *Cancer irroratus* and *Paragrapsus quadridentatus* are 0.25 and 0.49 mg Cd/L (Banjts-Claus and Benijts, 1975; Martin et al., 1981). Likewise, the 96 h LC_{50} of Zn for larvae of *L. vannamei* is 1.35 mg/L (Wu & Chen, 2004) and for *P. setiferus* it is 43.87 mg Zn/L (Vanegas et. al. 1997). In this study, Cd exhibited greater toxicity to *L. shmitti* than Zn. Wu & Chen (2004) worked in another prawn species *Litopenaeus vannamei* and discussed that the greater toxicity of Cd might be expected since zinc is an essential metal that is regulated by decapod crustaceans, whereas Cd has no known biological function.

Rao and Khan (2000) studied the effect of the interaction of three temperatures (15, 20 and 25°C) and the toxicity of copper in the mollusk *Dreissena polymorpha*, showed that high temperatures can increase copper toxicity and possibly that of other metals. For the gastropod *Physa acuta* cadmium toxicity was evident with the temperature elevation, with embryological growth reduction (Cheung & Lam, 1998). Surveys analyzing mercury toxicity to the crab *Eriocheir sinensis* showed that there was an increase of the toxic effect in low salinities (Pequeux et al., 1996). The authors mention that mercury interacts with an osmoregulatory mechanism preventing the animal's osmoregulatory capacity, thus increasing the metal's toxicity at low salinities. For the gastropod *Thiara tuberculata* exposed to heavy metals (mercury and copper), there was a toxicity decrease with salinity increase, expressed as oxygen consumption decrease (Mule and Lomte, 1994). Hall and Anderson (1994) reviewed the salinity influence on toxicity with several kinds of chemicals, reporting toxicity decrease with salinity increase. Researches on LC50 of Cd (Cd^{2+}) to *Cyprinodon variegatus* in Chesapeake bay, in 96 h exposures at three salinities (15, 20 and 25), showed that the higher the salinity, the lower the toxic effect of cadmium on the fish (Hall et al., 1995). The *L. schmitti* routine metabolism was minor for the acclimated specimens samples to the salinity of 35 For the active metabolism, there was also a minor tendency of the oxygen specific consumption and ammonium excretion for the acclimated shrimp to the salinity of 35. It seems Cd and Zn performs higher toxicities to *L. schmitti* at lower salinities. Actually, this also occurs in many aquatic organisms investigated, such as the blue crab *Callinectes sapidus* which has 96-h LC50 values of 0.32, 4.70, and 11.60 mgCd/L at the different salinities of 1, 15, and 35 s.p.u (Frank & Robertson, 1979). A similar trend was also apparent in the grass shrimp *Palaemonetes pugio* (Sunda et al., 1978), and those authors suggested that the protective effects of increased salinity could be explained by variation in Cd complexation to chloride ion (Cl^{-1}) and free Cd ion concentrations with changing salinities.

Studies on the effect of heavy metals on the respiration of decapod crustaceans demonstrated that

oxygen consumption rates decrease was related to concentration, exposure time and larval stage (Amand et al., 1999). When fiddler crab (*Uca pugilator*) larvae were exposed to 180 ppb Hg for 6 h, DeCoursey & Vernberg (1972) observed an oxygen consumption decrease of 28% for the zoeae III stage and 62% for the zoeae V stage. McMahon (2001), in a review of the responses of aquatic crustaceans in low ambient dissolved oxygen, mentioned that many crustaceans possess an excellent regulatory ability in their oxygen consumption patterns and thus were called oxygen regulators. Our experiments also demonstrated that oxygen consumed by *L. schmitti* showed no linear relationship to ambient oxygen levels regardless of whether or not the shrimp were exposed to a heavy metal. Despite their regulatory capability, the oxygen consumption rate was indeed inhibited after *L. schmitti* was exposed to high concentrations of Cd. Similar results were also observed in different shrimp species (Amand et al., 1999; Chinni et al., 2002; Wu & Chen, 2004).

Respiratory impairment in crustaceans due to exposure to heavy metals was also reviewed (Spicer & Weber, 1991), and it was concluded that oxygen consumption generally decreases when crustaceans are acutely exposed to heavy metals. In addition, after exposure to a sublethal concentration (1.44ppm) of lead (Pb) for 30 days, it was evident that Pb inhibits oxygen consumption in *P. indicus*; similar results have been obtained with other crustaceans studied (Chinni et al., 2000). Those authors assumed that cytological damage should be related to the decrease in oxygen consumption because the gills are most likely the first target of waterborne heavy metals, including thickening of branchial epithelium and deep changes in hemolymph patterns in the gills with a concomitant increase in vacuolization and reduced hemolymph spaces causing perfusion stagnation. Cytological and histological damage caused by heavy metal exposure in *P. japonicus* was also reported (Soegianto et al., 1999a,b). For example, an increased number of nephrocytes in gill filaments, a blackened appearance of the gills, necrosis of gill cells resulting in narrowed or obstructed hemolymphatic vessels, the appearance of a space between the cuticle and the epithelial cells which contain black electron-dense material, and even fragmentation of nuclei within gill cells could be observed when *P. japonicus* were exposed to different concentrations of heavy metals. Thus, the main pathological effect on the respiratory system caused by Cd is the interference with the respiratory system, including cellular respiration (Spicer & Weber, 1991; Koizumi et al., 1994).

Ammonium is one of the final products following catabolism, principally of amino acids that might have an alimentary or muscular origin, depending on nutritional conditions (Mayzaud & Conover, 1988). In addition to being utilized as energy substrates and components of body structures, amino acids can be more important than ions in the maintenance of osmotic pressure in prawns such as *P. setiferus* (McFarland & Lee, 1963; Rosas et al., 1999). Normally, increases in ammonium excretion reflect an

increase in catabolism of amino acids. However, when exposed to lethal concentrations of heavy metals, dysfunction of ammonium excretion control follows gill damage. Chinni et al. (2000, 2002) found that ammonium excretion was inhibited in *P. indicus* postlarvae exposed to sublethal concentrations of lead. Although there is still no confirmed evidence, it is assumed that the decrease in ammonia-N excretion by *P. indicus* postlarvae in the presence of toxicants can be attributed to a reduction in the metabolic rate or to an interaction of lead with pathways for the production of ammonia. Differences with our present study may be due to the metal used and their concentrations, shrimp species used, and other abiotic factors such as salinity and temperature. However, much effort still needs to be devoted to determining the relationship between heavy metal exposure and ammonium excretion to verify these questions.

In *M. ensis*, there was a clear decrease in sensitivity to heavy metals during development from protozoea to postlarvae. Other studies have also confirmed that tolerance to pollutants increases with age in marine crustaceans. *Penaeus monodon* showed a progressive increase in tolerance to ammonia (Chin & Chen, 1987) and nitrite (Chen & Chin, 1988) as the larvae developed from nauplii to postlarvae.

From an ecotoxicological point of view, the concentrations used in this study that caused significant effects on the measured parameters can potentially be found by shrimps in their natural environment. As stated in the Introduction, the cadmium and zinc concentration reported in sediments and suspended material from the Santos estuary averages 1,7 μg/g and 2600 μg/g in the more polluted areas (CETESB, 2001). Although *Litopenaeus schmitti* lives in brazilian coast, the potential risk of cadmium for this species should be seriously considered, especially taking into account that *L. schmitti* is a detritivore, sediment-consumer species. Cadmium and Zinc, like other heavy metals, presents a high absorption to fine sediments such as clay, abundant in the bottom and coastal areas of the mentioned estuary.

Results show that *L. schimitti* is a good test organism for studying heavy metal pollution. Our future work will focus on both the acute effects of these heavy metals on *L. schimitti* at other biological levels such as histological and biochemical levels, and chronic effects on metabolism, molting, and growth rates which are also very important for the prawn culture industry.

Acknowledgements

We tahnks to CNPq for the apport in this work.

References

Adams, S.M., 1990. Status and use of biological indicators for evaluating the effects of stress on fish. Am. Fish. Soc. Symp, 8: 1–8.

Amand, l. St., Gagnon, R., Packard, T. T. & Savenkoff. C., 1999. Effects of inorganic mercury on the respiration and the swimming activity of shrimp larvae, Pandalus borealis. Comp. Bioch. Physiology Part C, 122: 33-43.

Amritanshu S., Pandey, T. & MacRae, H., 1991. Toxicity of organic mercury compounds to the developing brine shrimp, *Artemia*. Ecotox. Environ. Safety, 21(1): 68-79.

Barbieri, E., Phan, V. N. & Gomes, V., 1998. Efeito do DSS, dodecil sulfato de sódio, no metabolismo e na capacidade de natação de *Cyprinus carpio. Rev. Brasil. Biol.*, 58(2): 263-271.

Barbieri, E., Phan, V. N. & Gomes, V., 2000. Effects of LAS-C12, Linear Alkybenzene Sulphonate, on Metabolic rate and Swimming Capacity of *Cyprinus carpio*. Ecotox. Environ. Rest., 3(2): 60-75.

Barbieri, E., Serralheiro, P. C. & Rocha, I. O., 2002. The use of metabolism to evaluate the toxicity of dodecil benzen sodium sulfonate (LAS-C12) on the *Mugil platanus* (mullet) according to the temperature and salinity. Journal of Exp. Mar. Biol. Ecol., 277: 109-127.

Barbieri, E., Garcia, C. A B., Bispo, R. L.. Aragão, K. A. S. & Alves, J. P. H., 2004. Utilização do camarão sete-barbas (*Xiphopenaeus kroyeri*) na determinação da toxicidade da água de produção de petróleo caracterizada no Estado de Sergipe. O Mundo Saúde, 28(4): 421-430.

Barbieri, E., Passos, E. A. & Garcia, C. A B., 2005. Use of metabolism to evaluate the sublethal toxicity of mercury on *Farfantepaneus brasiliensis* larvae (Latreille 1817, crustacean), J. Shellfish Res., 24(4): 1229-1234.

Barbieri, E., 2007. Use of Oxygen Consumption and Ammonium Excretion to Evaluate the Sublethal Toxicity of Cadmium and Zinc on Litopenaeus schmitti (Burkenroad, 1936, Crustacea). W. Environ. Res., 79(6): 641-646.

Boudou, A. & Ribeyre, F., 1989. Fish as "biological model" for experimental studies in *e*cotoxicology. *In:* A.Boudou and F. Ribeyre (eds.), *Aquatic Ecotoxicology Fundamental Concepts and Methodologies*. Vol. VIII. CRC Press Inc, Florida,150p.

Carvalho, P. M. S., 1992. Bioenergética do camarão sete-barbas. Dissertação de mestrado. Instituto Oceanográfico-Universidade de São Paulo, 206p.

Carvalho, C.E.V., Fariam. V. V., Cavalcante, P. O., Gomes, M. P. & Rezende, C. E., 2000. Distribuição de Metais Pesados em Peixes Costeiros Bentônicos da Região de Macaé, R.J., Brasil. Ecotox. Environ. Rest., 3(2): 12-16.

Carvalho, C.E.V., Cavalcante, M. P. O. Gomes, M. P. Faria, V. V. & Rezende, C. E. 2001. Distribuição de Metais Pesados em Mexilhões (*Perna perna*, L.) da Ilha de Santana, Macaé, SE, Brasil. Ecotox. Environ. Rest., 4(1): 1-5.

CETESB., 2001. Companhia de Tecnologia de Saneamento Ambiental. Sistema Estuarino de Santos e São Vicente. PROCOP. Programa de Controle de Poluição Programa de Assistência Técnica, 142p.

Cheung, C.C.C. & Lam, P. K.S., 1998. Effect of cadmium on the juveniles of a tropical freshwater sanil, *Physa acuta* (Draparnaud, 1805). *W. Sci. Tech.,* 38: (7) 263-270.

Chinni, S., Khan, R. N. & Yallapragada, P. R., 2000. Oxygen consumption, ammonia-N excretion, and metal accumulation in Penaeus indicus postlarvae exposed to lead. Bull. Environ. Contam. Toxicol., 64: 144–151.

Chinni, S., Khan, R. N. & Yallapragada, P. R., 2002. Acute toxicity of lead on tolerance, oxygen consumption, ammonia- N excretion, and metal accumulation in Penaeus indicus postlarvae. Ecotox. Environ. Safe., 51: 79–84.

Christiansen, P. D., Brozek, K. & Hansen, B. W., 1998. Energetic and behavioral responses by the common goby, Pomatoschistus microps (Kroyer), exposed to linear alkybenzene sulfonate. Environ. Toxicol. Chem., 17(10): 2051-2057.

Damato, M. & Barbieri, E., 2003. Emprego de uma espécie indicadora sul-americana na determinação da toxicidade aguda para Cobre, Zinco, Níquel e Alumínio. O Mundo da Saúde, 27 (4): 551-558.

DeCoursey P. J. & Vernberg, W. B., 1972. Effect of mercury on survival, metabolism, and behaviour of larval Uca pugilator (Brachyura). Oikos, 23:241-247.

Eysink, G. G. J., Pádua, H. B., Bertoletti, S. A. E. & Pereira, D. N., 1988a. Metais pesados no vale do Ribeira e em Iguape - Ilha Comprida. R. Ambiente, 2: 6-13

Eysink, G. G. J., Pádua, H. B. & Martins, M. C., 1988b. Presença de Mercúrio no Ambiente. R. Ambiente, 2: 43-50.

Frank, P. M. & Robertson, P. B., 1979. The influence of salinity on toxicity of cadmium and chromium to the blue crab, Callinectes sapidus. Bull. Environ. Contam. Toxicol., 21: 74-78.

Fry, F. E. J., 1971. The effect of environmental factors on the physiology of fish. In Hoar, W. S. & Randall, D. J. eds. Fish Physiology. New York, Academic Press. Vol. 6: 1-98.

Hall, L. W. Jr. & Anderson, R. D., 1994. The influence of salinity on the toxicity of various classes of chemicals to aquatic biota (report). Maryland Departament of Environment, Baltimore, MD, USA, 56p.

Hall, L. W. JR., Ziegenfuss, M. C. Anderson, R. D. & Lewis, B. L., 1995. The effect of salinity on the acute toxicity of total and free cadmium to a Chesapeake Bay copepod and fish. Mar. Poll. Bull., 30 (6): 376-384.

Hansen, B., Fotel, R.L., Jesen, N.J. & Wittrup, L., 1997. Physiological effects of the detergent linear alkylbenzene sulphonate on blue mussel larvae (Mytilus edulis) in laboratory and mesocosm experiments. Mar. Biol., 128: 627– 637.

Koizumi, T., Yokota, T., Shirakura, H., Tatsumoto, H. & Susuki, K. T., 1994. Potential mechanism of cadmiuminduced cytotoxicity in rat hepatocytes: inhibitory action of cadmium on mitochondrial respiratory activity. Aqua.Toxicol., 9 (2): 115–125.

Lemaire, P., Sturve, J. Forlin, L. & Livingstone, D. R., 1996. Studies on aromatic hydrocarbon quinone metabolism and DT-diaphorase function in liver of fish species. Mar. Environ. Res., 2 (1-4): 317-321.

Lussier, S M., Gentile, J. H. & Walker, J., 1985. Acute and chronic effects of heavy metals and cyanide on Mysidopsis bahia (crustacea:mysidacea). Aqua. Toxicol., 7(1-2): 25-35.

Mance, G., 1987. Pollution Threats of heavy Metals in Aquatic Environments. Elsevier Appied Science Publishers Ltd., London, UK, 230p.

Mayzaud, P. & Conover, R. J., 1988. O:N atomic ratio as a tool to describe zooplankton metabolism. Mar. Ecol. Prog. Ser., 45: 289–302.

McFarland, W.N. & Lee, B. D., 1963. Osmotic and ionic concentration of shrimp of the Texas coast. Bull. Mar. Sci., 13: 391–416.

McMahon, B.R., 2001. Respiratory and circulatory compensation to hypoxia in crustaceans. Respir. Physiol., 128: 349– 364.

Mule, M. B. & Lomte, V. S., 1994. Effect of heavy metals (CuSO4 and HgCl2) on the oxygen-consumption of the fresh-water snail, Thiara-tuberculata. J. Environ. Biol., 15(4): 263-268.

Papathanassion, E., 1983. Effects of Cadmium and mercury ions on respiration and survival of the common prawn Palaemon serratus. (Pennant). Rev. Inst. Oceanogr. Med., 70: 21-35.

Pequeux, A., Bianchini, A. & Gilles, R., 1996. Mercury and osmoregulation in the crab, Eriocheir sinensis. Comparative Biochemistry and Physiology C-Phar. Toxicol. Endocri., 113(2): 149-155.

Rao, E. G. & Khan, M. A. Q., 2000. Zebra mussels: Enhancement of copper toxicity by high temperature and its relationship with respiration and metabolism. *W. Environ. Res.,* 72(2): 175-178.

Rosas, C., Martinez, E., Gaxiola, G., Brito, R., Sanchez, A., Soto, L. A., 1999. The e.ect of dissolved oxygen and salinity on oxygen consumption, ammonia excretion and osmotic pressure of Penaeus setiferus juveniles. J. Exp. Mar. Biol. Ecol., 234: 41–57.

Schreck, C.B., 1990. Physiological, behavioral and performance indicators of stress. Am. Fish. Soc. Symp., 8: 29–37.

Spicer, J.I. & Weber, R. E., 1991. Respiratory impairment in crustaceans and mollusks due to exposure to heavy metals. Comp. Biochem. Physiol., 100(3): 339–342.

Soegianto, A., Charmantier-Daures, M. Trilles, J. P. & Charmantier, G., 1999a. Impact of cadmium on the structure of gills and epipodites of the shrimp *Penaeus japonicus.* Aquat. Living. Resour., 12(1): 57-70.

Soegianto, A., Charmantier-Daures, M. Trilles, J. P. & Charmantier, G., 1999b. Impact of copper on the structure of gills and epipodites of the shrimp Penaeus japonicus. J. Crust. Biol., 19(2): 209–223.

Solarzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr., 14: 799-801.

Sunda, W.G., Engel, D. W. & Thuotte, R. M., 1978. Effects of chemical speciation on toxicity of cadmium to grass shrimp, Palaemonetes pugui: importance of free cadmium ion. Environ. Sci. Technol., 12(4): 409-413.

Wu, J. P. & Chen, H. C., 2004. Effects of cadmium and zinc on oxygen consumption, ammonium excretion, and osmoregulation of white shrimp (Litopenaeus vannamei). Chemosph., 57: 1591-1598.