The use of oxygen consumption and ammonium excretion to evaluate the toxicity of cadmium on *Farfantepenaeus paulensis* with respect to salinity

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ABSTRACT

The main purpose of the present study was to detect the acute toxicity of cadmium (Cd) in *F. paulensis* and to investigate its effect on oxygen consumption and ammonium excretion different salinities. First, we examined the acute toxicity of Cd in *F. paulensis* at 24, 48, 72, and 96-h lethal concentration (LC50). Cd was significantly more toxic at 5 salinity than at 20 and 36. The oxygen consumption and ammonium excretion were estimated through experiments performed on each of the twelve possible combinations of three salinities (36, 20 and 5), at temperature 20 °C. Cd showed a reduction in oxygen consumption at 5 salinity, the results show that the oxygen consumption decreases with respect to the Cd concentration. At the highest Cd concentration employed (2 mg L^{-1}), the salinity 5 and the temperature at 20 °C, oxygen consumption decreases 53.7% in relation to the control. In addition, after separate exposure to Cd, elevation in ammonium excretion was obtained, wish were 72%, 65% and 95% higher than the control, respectively. The results show that Cd is more toxic to *F. paulensis* at lower salinities.

1. Introduction

In recent years, various factors such as industrial development, increases in pesticide usage and mining have led to an increase in the levels of heavy metals, including Cd, in aquatic environments (Cooper 1993; Guerrero and Kesten, 1993; Barbieri et al. 2005). In Brazil, heavy metals enter the coastal seawater mainly through the discharge of industrial effluents and disposal of sewage (Barbieri, 2009). High concentrations of heavy metals have been reported in coastal waters (Eysink et al., 1988a), rivers and their estuaries (Eysink et al., 1988b), and in the tissues of coastal marine organisms (Carvalho et al., 2000, 2001).

Exposure to heavy metals in the aquatic environment produces many physiological changes in crustaceans, including alterations in the metabolic activities (Barbieri et al. 2005; Barbieri, 2009). These effects are related to their mechanism of action and, therefore, are specific to each metal. The metabolic rate of an organism is a 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 and ammonium excreted 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).

Cd is a very common and persistent heavy metal in aquatic environments and known to be toxic to marine and estuarine crustaceans (Papathanassion, 1983; Wu and Chen, 2004). Cd has been widely recognized as highly toxic when dissolved and in ionic form (Mance, 1987). Cd is common mainly in industrial effluents and its effects may be determined as changes in metabolic rates, which measure the amount of energy used in response to the presence and concentration of a given stressor. The metabolic rates, being an interaction result of the several processes that reflect the animal's 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 and Chen, 2004; Barbieri, 2007, 2009), detergents (Barbieri et al., 1998, 2002; Christiansen et al., 1998) and a variety of toxicants (Boudou and Ribeyre 1989).

The objective of this study was to determine the acute toxicity, oxygen consumption and ammonium excretion of Cd for *Farfantepenaeus paulensis* at three salinities (36, 20 and 5) at temperature 20 °C. The results were analyzed to determine if the acute toxicity, oxygen consumption and ammonium excretion varied with salinity.

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2. Methodology

2.1. Acute toxicity

A total of 600 post-larvae of cultivated shrimp with 1.7 ± 0.42 g medium wet weight and 3.6 ± 0.45 cm total length were used. The shrimp were in tanks of 500 L with a salinity of 20. Before the experiments the shrimp had been acclimatized, for one week in salinities of 36, 20 and 5. After this procedure groups of fifteen individuals were put in 50 L tanks containing sea water at 36, 20 and 5 salinities at 20 °C. Three replicates of groups of 15 individuals were exposed to one of the following Cd nominal concentrations: Control, 0.01, 0.10, 0.50, 1.00, 2.00, 3.00 and 4.00 mg L^{-1}. Dead shrimp were removed from the tanks and counted at 24, 48, 72 and 96 h of exposure. Death was presumed when shrimps were immobile and showed no response to touch with a glass rod. The lethal concentration (LC50 with 95% confidence limits) was calculated by Spearman–Karber Estimates (Hamiltom et al., 1977).

2.2. Oxygen consumption and ammonia excretion

Seventy-five shrimp (*F. paulensis*) with averages of 1.39 ± 0.52 g and 1.32 ± 0.41 cm were employed for the routine metabolism measurement utilizing sealed respirometers (1 L each). Five shrimp were subjected to oxygen consumption and ammonium excretion measurements individually in one of the four concentrations of Cd (0.00, 0.1, 0.5, 1.0 and 2.0 mg L^{-1}) at three salinities (36, 20 and 5) and at temperature 20 °C. The concentrations had been chosen based on the previous results of the LC50 experiments.

Before the beginning of the experiments the animals were maintained in the respirometers with continuous water circulation for at least 90 min to attenuate the handling stress. Then, the water supply was suspended and the respirometer was closed, so that the shrimp could consume the oxygen in the known water volume for a period of three hours. The respirometers 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 experiment's duration was regulated so that the oxygen concentration by the end of experiment was greater than 70% of its initial concentration. The dissolved oxygen was determined through the Winkler method.

To obtain the desired concentration of Cd, the necessary volume of the stock solution (1.0 mg CdCl$_2$/mL) was added to each volume of respirometer at the end of the acclimation period. As soon as CdCl$_2$ was added the entry orifice was sealed. Additionally, the seawater in the bottle was sampled at the beginning and end of the oxygen consumption and ammonium excretion analysis. Determination of ammonium–nitrogen in the seawater was based on the phenolhypochlorite method (Solarzano 1969).

2.3. Statistical analyze

The response variables (consumption of oxygen and ammonium) presented bimodal distribution, with a strong asymmetrical tendency and the presence of outliers (Fig. 1) that indicates an absence of homocedacity and normality. On the other hand, the presence of non-linear interactions between the treatments (Cd and Sal) in the two response variables was seen (Figs. 2) by means of the Box-plot diagrams.

A parametric approach using the classical Anova Two-way model would not, thus, be valid and Variance Analysis by permutation as proposed by Anderson (2001a,b) and validated by Anderson and Braack (2003) was adopted.

The analysis was undertaken using three analyses of partial redundancy using Canoco program (Braak and Šmilauer, 2002) The treatments and their interactions being expressed in the form of Helmert contrasts matrix (Venables and Ripley, 2002), also called "orthogonal dummy variables". (see details for elaboration of this matrix in apendice of Anderson and Legendre 1999).
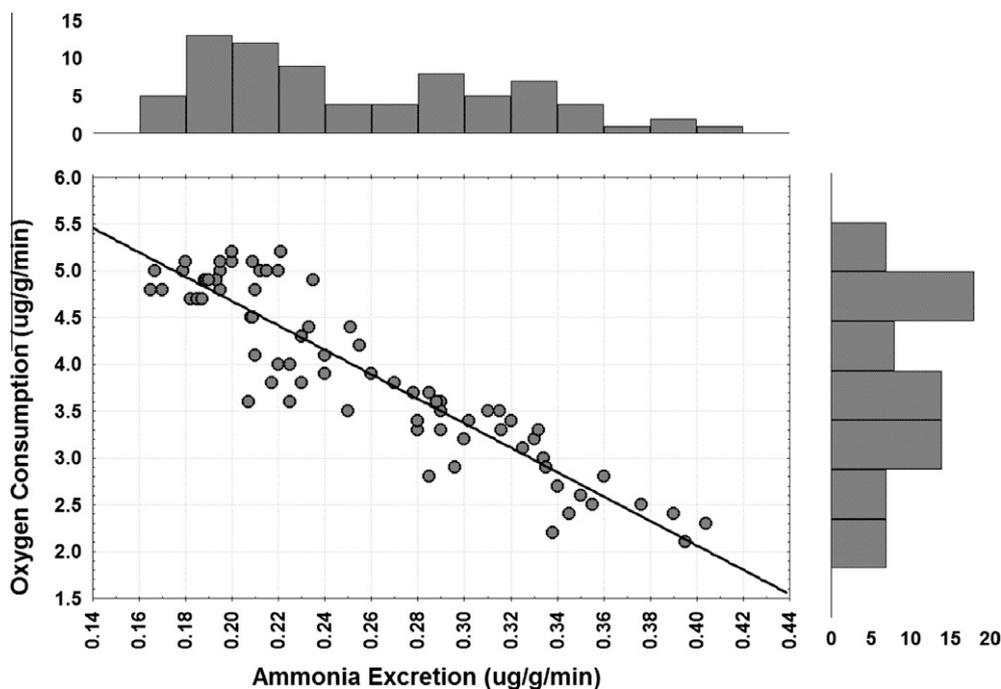
Fig. 1. The response variables presented bimodal distribution, with a strong asymmetrical tendency and the presence of outliers that indicates an absence of homocedacity and normality.
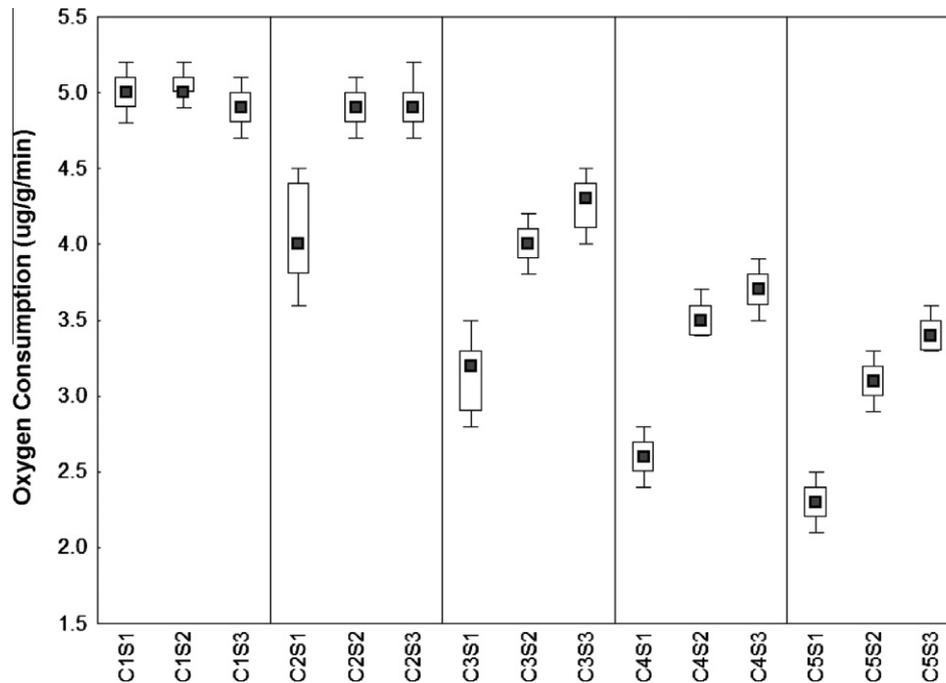
Fig. 2. C1S1 – Cd 0 and Sal 5, C1S2 – Cd 0 and Sal 20, C1S3 – Cd 0 and Sal 36, C2S1- Cd 0,1 and Sal 5, C2 S2 – Cd 0,1 and Sal 20, C2 S3 – Cd 0,1 and Sal 36, and so on.

Table 1
Percent mortality (%) of *F. paulensis* exposed to various cadmium concentrations for 96 h and its medium lethal concentration (LC50 with 95% confidence limits) calculated by Spearman–Karber estimates salinity 36.

Exposure time (h)	Cadmium concentration (mg Cd L^{-1})								
	0	0.1	1.0	2.5	5.0	10	20	40	LC$_{50}$ (mg Cd L^{-1})
24	0.0	0.0	22.22	44.44	55.55	100	100	100	2.35 (1.33–4.15)
48	0.0	0.0	33.33	55.55	66.66	100	100	100	1.67 (0.90–3.07)
72	0.0	11.11	33.33	66.66	88.88	100	100	100	1.26 (0.57–2.78)
96	0.0	22.22	44.44	77.77	100	100	100	100	0.83 (0.27–2.60)

In order to estimate the effect and significance of factor 1 (Cd), each response variable was factored separately, first by the effects of factor 2 (Sal) and then by the vectors representing the interaction between the treatments (first analysis of partial redundancy). To estimate the effect of factor 2 (Sal), the response variable was factored by the effects of factor 1 and their interactions (second analysis of partial redundancy) and for the estimation of the effect of the interaction the response variable was factored by the effects of the matrixes representing the treatments (third analysis of partial redundancy).

The data were transformed by the square root and 9999 permutations were used taking the reduced model) for each analysis of redundancy into account (in accordance with Anderson and ter Braack, 2003 it is the permutation that provides the greatest statistical power).

3. Results

3.1. Mortality

The percent mortality of *F. paulensis* exposed to cadmium at each 24-h interval is shown in Table 1. No deaths of control animals were observed in any of the three saline concentrations. The higher the concentration of metal the shrimp were exposed to, the higher the mortality observed. After being exposed to cadmium, death was first observed at a concentration of 1.0 mg Cd/L in the first 24 h in salinity of 36. Mortality rates of 100% were observed after a 24 h exposure at concentrations of 10.0 mg Cd/L and were also 100% after 96 h at a concentration of 5.0 mg Cd/L in salinity of 20 (Table 2). Only 66% average mortality was observed during the first 24 h at 5.0 mg Cd/L in salinity of 5, while the mortality rate was 100% after 96 h (Table 3).

3.2. Medium lethal concentration

The acute toxicity of Cd to shrimp larvae exposed to different concentrations of this metal for periods of up to 96 h, expressed as the medium lethal concentration (LC50) in salinity 36, were 2.35, 1.67, 1.26, and 0.83 mg L^{-1} for the 24-, 48-, 72-, and 96-h exposure, respectively (Table 1). In salinity 20 the values were 2.34, 1.82, 1.70, 1.22 mg L^{-1} (Table 2). The LC50 values for cadmium in salinity 5 were: 2.04, 1.40, 0.80 and 0.32 mg L^{-1} for 24, 48, 72, and 96 h, respectively (Table 3).

3.3. Metabolic measurements

3.3.1. Effect of Cd on the oxygen consumption of shrimp with respect to salinity at 20 °C

For the shrimp acclimated to 20 °C, the specific oxygen consumption decreased with respect to the Cd concentration in the three salinities. The specific oxygen consumption in any Cd concentration decreased with the decrease in salinity. The values

Table 2
Percent mortality (%) of *F. paulensis* exposed to various cadmium concentrations for 96 h and its medium lethal concentration (LC50 with 95% confidence limits) calculated by Spearman–Karber estimates salinity 20.

Exposure time (h)	Cadmium concentration (mg Cd L^{-1})								LC$_{50}$ (mg Cd L^{-1})
	0	0.1	1.0	2.5	5.0	10	20	40	
24	0.0	0.0	20	40	66.66	100	100	100	2.34 (1.53–3.58)
48	0.0	0.0	26.66	46.66	80	100	100	100	1.82 (1.16–2.84)
72	0.0	13.33	26.66	53.33	86.66	100	100	100	1.70 (0.89–3.24)
96	0.0	13.33	33.33	66.66	100	100	100	100	1.22 (0.64–2.34)

Table 3
Percent mortality (%) of F. paulensis exposed to various cadmium concentrations for 96 h and its medium lethal concentration (LC50 with 95% confidence limits) calculated by Spearman–Karber estimates salinity 5.

Exposure time (h)	Cadmium concentration (mg Cd L^{-1})								LC$_{50}$ (mg Cd L^{-1})
	0	0.1	1.0	2.5	5.0	10	20	40	
24	0.0	0.0	20	40	66.66	100	100	100	2.04 (1.35–3.06)
48	0.0	0.0	26.66	46.66	80	100	100	100	1.40 (0.73–2.72)
72	0.0	13.33	26.66	53.33	86.66	100	100	100	0.80 (0.34–1.87)
96	0.0	13.33	33.33	66.66	100	100	100	100	0.32 (0.10–1.04)

Table 4
Oxygen specific consumption (mL O$_2$ g^{-1} min^{-1}) of shrimp, acclimated to 20 °C, subjected to different Cd concentrations and 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^{-1})	Salinity 36		Salinity 20		Salinity 5	
	Specific consumption	%	Specific consumption	%	Specific consumption	%
0	0.00490 (±0.0014)		0.00504 (±0.0021)	–	0.00500 (±0.0025)	–
0.1	0.00492 (±0.0013)	1.04	0.00490 (±0.0012)	−2,77	0.00406 (±0.0020)	−18,8
0.5	0.00427 (±0.0025)	−12.85	0.00400 (±0.0013)	−20.63[*]	0.00314 (±0.0026)	−37.2[*]
1	0.00366 (±0.0016)	−25.30[*]	0.00352 (±0.0015)	−30.15[*]	0.00260 (±0.0019)	−48.00[*]
2	0.00342 (0.0015)	−30.20[*]	0.00312 (±0.0011)	−38.09[*]	0.0023 (±0.0021)	−54.00[*]

[*] significant difference in relation to the control ($p < 0.05$).

were, 0.0051, 0.00518 and 0.0052 mLO$_2$ g^{-1} min^{-1}, respectively. For the shrimp exposed to the concentration of 2 mg L^{-1} of Cd, the consumption was 0.0033; 0.0031 and 0.0024 mlO$_2$ g^{-1} min^{-1} for the tested salinities. These values represent a reduction in the metabolic level of 34.7%, 39.7% and 53.7% in relation to the control (Table 4).

Cadmium effect
Lambda = 0.727
F-ratio = 295.597
P-value = 0.0001
Salinity effect
Lambda = 0.179
F-ratio = 145.569
P-value = 0.0001
Cd and Salinity interaction effect
Lambda = 0.056
F-ratio = 11.477
P-value = 0.0001

As regards the variation in the consumption of oxygen, the total explanation was 96.2%, the influence of the cadmium being about 4 times greater than that of the salinity, the interaction in this case representing 5.6% of the variation. The cadmium and salinity effects were again contrasting, but contrary to the effect registered for ammonia. The relationship between cadmium and the consumption of oxygen were positive and negative as whith the salinity. (See the Box-plot, Fig. 2).

3.3.2. Effect of Cd on the ammonium excretion of shrimp with respect to salinity at 20 °C
It was observed that the shrimp acclimated to the temperature 20 °C, the ammonium excretion increase to Cd concentration for the three employed salinity. The ammonium excretion in any Cd concentration increased with the decrease in salinity. We measured the ammonium excretion of shrimp from the acclimated control group to 20 °C temperature (Table 5), subjected to 36, 20 and 5 salinities. The values were, 0.18, 0.18 and 0.20 µg g^{-1} min^{-1}, respectively. For the shrimp exposed to the concentration of 2 mg L^{-1} of Cd, the ammonium excretion was 0.31; 0.33 and 0.39 µg g^{-1} min^{-1}, for the tested salinities. These values represent an increase in the metabolic level of 72.2%, 65% and 95% in relation to the control.

Cadmium effect

The parameter used to measure the effect was the first eigen-value (lambda) which corresponds to the coefficient of determination (percentage of explanation of the variability of the response variable).

Lambda = 0.783
F-ratio = 224.889
P-value = 0.0001
Salinity effect
Lambda = 0.133
F-ratio = 76.377

Table 5
Ammonia excretion ($\mu g\ g^{-1}\ min^{-1}$) of shrimp, acclimated to 20 °C, subjected to different Cd concentrations and 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^{-1})	Salinity 36		Salinity 20		Salinity 5	
	Specific excretion	%	Specific excretion	%	Specific excretion	%
0	0.18 (±0.013)	–	0.20 (±0.021)	–	0.20 (±0012)	–
0.1	0.19 (±0.025)	5.55	0.21 (±0.025)	5	0.22 (±0013)	10
0.5	0.23 (±0.021)	27.7[*]	0.24 (±0.015)	20[*]	0.30 (±0015)	50[*]
1	0.27 (±0.015)	50[*]	0.29 (±0.012)	45[*]	0.35 (±0010)	75[*]
2	0.31 (±0.022)	72.2[*]	0.33(±0.022)	65[*]	0.39 (±0014)	95[*]

[*] Significant difference in relation to the control. ($p < 0.05$).
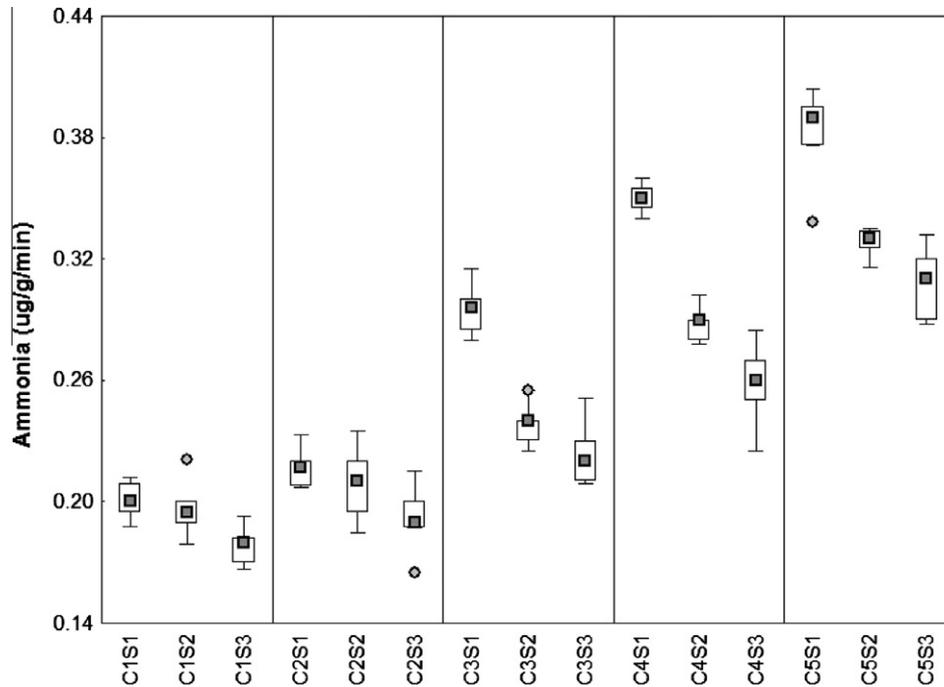
Fig. 3. Min and Max omitting outliers, 25% and 75% percentile box, black square = median. Circle = outlier.

P-value = 0.0001
Cd and Salinity interaction effect
Lambda = 0.031
F-ratio = 4.489
P-value = 0.0002

All the treatments and their interactions were significant, Cd being responsible for 78.3% of the explanation, salinity for 13.3% and the interaction for 3% (Fig. 3). Thus 94.7% of the variation in the concentration of ammonia was explained by the treatments and their interactions, though the effect of the cadmium was about 6 times greater than that of the salinity, their effects on the concentration of ammonia being the opposite, that is to say, in the measure in which the concentration of cadmium increased, the production of ammonia also increased, whereas the increase in the salinity caused a reduction in the production of ammonia.

4. Discussion

The results obtained in this study allow us to evaluate the effects of Cd on the *F. paulensis* metabolism at different salinities. Some shrimp can bear a wide salinity variation and live well in sea, river and brackish water (estuary water). These movements are usually associated with the shrimp cycle of life, for example, the *F. paulensis* that lay eggs in the sea and in larve phase can be found in small rivers of sweet and brackish water. When they reach maturity, these shrimp travel to seawater to reproduce. The passage from one environment to another requires deep changes into the osmoregulatory process as subsequent energy waste. This work confirms 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, alkalinity, the concentration 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 in crustaceans has been studied by a number of authors (Mance, 1987; Wong et al., 1993; Vanegas et al., 1997; Wu and Chen, 2004; Brabieri, 2007; 2009). Results of this study confirm that the Cd is toxic to *F. paulensis*, an ecologically and economically important shrimp in the coastal waters of Brazil.

The toxicity of Cd in marine crustaceans is well documented, but not for *F. paulensis*. For example, the 96 h LC_{50} of Cd for *Litopenaeus vannamei* is 1.07 mg L^{-1} (Wu and 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^{-1} (Wu and Chen 2004) and for *Penaeus*

setiferus it is 43.87 mg Zn/L (Vanegas et al. 1997). The Cd effects on the *F. paulensis* can be minimal when they occur at high salinity (36) and in concentrations below 0.5 mg L^{-1}. However, at salinity 5 and at the highest Cd (2.0 mg L^{-1}) concentration, the effects were pronounced. The salinity is an environmental factor that can, according to Barbieri et al. (2002), affect the organisms in a lethal, controlled, disguised or directing way depending on the time and/or stimulus intensity, spatial extension of this influence and the organism capacity to adjust to terminal variations.

Surveys analyzing mercury toxicity in the crab *Eriocheir sinensis* showed that there was an increase of the toxic effect at 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 *F. paulensis* routine metabolism was lower for the acclimated specimens samples to the salinity of 36. It seems Cd is more toxic to *F. paulensis* 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 mg Cd/L at the different salinities of 1, 15, and 35 (Frank and 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. The *F. paulensis* routine metabolism was lower for the acclimated specimen samples for the salinity 36. For the active metabolism, there was also a lower oxygen specific consumption for the acclimated shrimp at the salinity 36.

The results obtained in this survey allow us to evaluate the effects of Cd on shrimp metabolism at different salinities. Some crustacea 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 crustacea life cycle of, for example, the pink-shrimp (*F. paulensis*) that lay eggs in the sea and in alevine phase. They can be found in small rivers of sweet and brackish water. When they reach maturity, these fish travel to the sea to reproduce. The passage from one environment to another requires deep changes into the osmoregulatory process as a subsequent energy waste. The osmoregulation in estuary shrimp is done mainly by the gills, which also participate in the exchange of gases. The secretion of salts by the epithelium gills is done by active transportation because it occurs from a lower blood concentration to a higher one of the surrounding environment when the shrimp is in salty water. This fact would lead us to think that the more the mullet (*Mugil lisa*) penetrates into salty water, the higher the oxygen consumption resulting from energetic waste to maintain homeostase. The same would occur in the case of pink-shrimp in fresh water where the blood concentration would be higher than the surrounding environment. In the case where these streams were polluted with Cd, the metabolic waste could increase, and with the damage caused by this metal in the gills, the shrimps would have serious problems to maintain homeostase.

The increase in the toxicity of Cd with the reduction of salinity, can be related to the higher biodisponibility of metals in low salinity. In seawater, chloride commonly forms complexes with Zn and Cd, and, to a lesser extent, Cu, but not with Pb, and thus the free ion concentration of the former metals will be reduced (Förstner, 1979 and Williams et al., 1994). The concentration of dissolved Cd increases linearly with increasing salinity within the Changjiang Estuary (Wang and Liu, 2003). All of them increase in concentration across almost the full salinity range. Fritoff et al. (2005) found that metal concentration and accumulation in the two studied submersed macrophyte species generally increased with increasing temperature but decreased with increasing salinity. Cd was significantly more toxic for *Exosphaeroma gigas* at 20 than at 30 salinity (Giarratano et al., 2007), a similar response was observed in this study with increase the toxicity at 5 salinity.

Studies on the effect of heavy metals on the respiration of decapod crustaceans demonstrated that the decrease in oxygen consumption rates 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 and 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, it was 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 *F. paulensis* 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 *F. paulensis* 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 and Chen, 2004).

Respiratory impairment in crustaceans due to exposure to heavy metals was also reviewed (Spicer and 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 (1.44 ppm) of lead (Pb) for 30 d, it was evident that Pb inhibits oxygen consumption in *Penaeus 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 the thickening of branchial epithelium and major 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 *Penaeus 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 and Weber 1991; Koizumi et al. 1994).

Typically, 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–nitrogen 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–nitrogen. Differences in the present study may be a result of the metals used and their concentrations, shrimp species used, and other abiotic factors, such as salinity. However, much effort still needs to be devoted to determining the relationship between heavy metal exposure and ammonium excretion to verify these questions.

Inhibition of oxygen consumption and increase ammonium excretion by Cd has been reported in *Litopenaeus vannamei* (Wu and Chen 2004) and *Litopenaeus shmitti* (Barbieri 2007) and has been attributed to mucus production because it reduces the efficiency of gaseous exchange. A similar response was observed in this study with inhibition of oxygen consumption.

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 concentration reported in sediments and suspended material from the Santos Estuary averages 1.7 µg g^{-1} in the more polluted areas (CETESB, 2001). Although *F. paulensis* lives along the Brazilian coast, the potential risk of cadmium for this species should be seriously considered, especially taking into account that *F. paulensis* is a detritivore, sediment-consumer species. Cadmium, 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.

5. Conclusion

Penaeid shrimps are important resources for worldwide fisheries and aquaculture. In Brazil, *Farfantepenaeus paulensis* is an important commercially exploited species, and is the ideal animal for studying the impairment caused by the effects of heavy metals that are often detected in coastal areas. Results show that *F. paulensis* 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 *F. paulensis* 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

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