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Cadmium effect on shrimp ammonia excretion (*Farfantepenaeus paulensis*) at different temperatures and levels

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Abstract. Considered a highly toxic environmental pollutant, cadmium (Cd) is capable of causing a number of changes in metabolic activity in most aquatic organisms. However, the evaluation of ammonia excretion after exposure to Cd has not been previously studied in *Farfantepenaeus paulensis*, an important commercial species. The objective of this work was to evaluate the effects of different cadmium (Cd) concentrations on *F. paulensis* ammonia excretion at three different salinity levels and temperatures. For this, shrimp were submitted to ammonia excretion measurement in each of the four Cd concentrations (control, 0.1, 0.5, 1.0 and 2.0 mg L^{-1}) at three salinity levels (36, 20 and 5) and three temperatures (25 °C, 20 °C and 15 °C). Cd was significantly more toxic at salinity 5 than 20 and 36. At the highest employed Cd concentration (2.0 mg L^{-1}), salinity 5 and temperature of 25 °C, ammonia excretion increased 95% in relation to the control group. The results also show that Cd is more toxic to *F. paulensis* at lower salinity levels. The importance of the findings, for biology, of the species close to the sources of Cd, is discussed.

Keywords: Shrimp; cadmium; ammonia excretion; temperature; salinity

Resumo. Efeito do Cádmio na excreçao de amŏnia de camarâo (*Farfantepenaeus paulensis)* em diferentes temperaturas e salinidades. Considerado um poluente ambiental altamente tóxico, o cádmio (Cd) é capaz de causar uma série de alterações na atividade metabólica na maioria dos organismos aquáticos. Porém, a avaliação da excreção de amônia após a exposição ao Cd não foi estudado anteriormente em *Farfantepenaeus paulensis*, uma importante espécie comercial. O objetivo deste estudo foi avaliar os efeitos de diferentes concentrações de cádmio (Cd) sobre a excreção de amônia de *F. paulensis* em três diferentes salinidades e temperaturas. Para isso, camarões foram submetidos a mensuração de excreção de amônia em cada uma das quatro concentrações de Cd (controle; 0,1; 0,5; 1,0 e 2,0 mg L^{-1}) em três salinidades (36, 20 e 5) e três temperaturas (25 °C, 20 °C e 15 °C). O Cd foi significativamente mais tóxico à salinidade 5 do que 20 e 36. Na concentração de Cd mais alta empregada (2,0 mg L^{-1}), salinidade 5 e temperatura de 25 ° C, a excreção de amônia aumentou 95% em relação ao grupo controle. Os resultados também mostram que Cd é mais tóxico para *F. paulensis* em salinidades mais baixas. A importância dos achados para a biologia das espécies próximas às fontes de Cd é discutida.

Palavras-chave: Camarão; cádmio; excreção de amônia; temperatura; salinidade

Introduction

Cadmium is among the most toxic environmental pollutants and is capable of causing a number of biochemical dysfunctions in most aquatic organisms (Suwalsky *et al.*, 2004). The main sources of input into the aquatic environment are, according to Kalicanin (2009), natural sources (mineral wear, volcanic emissions and forest fires) and disposal of industrial and agricultural products and wastes. It results in high concentrations of Cd in water bodies (Tompsett at al., 2014).

Several studies show that exposure to heavy metals in the aquatic environment produces metabolic changes in crustaceans, including blood glucose variations (Mohamed *et al.*, 2014), ultrastructural changes in hepatopancreas (Mazzei *et al.*, 2014), changes in antioxidant enzymes, metallothioniens and total proteins electrophoresis (Mohamed *et al.*, 2014) and metabolic activity alterations (Damato & Barbieri, 2012). Among these alterations, the ammonia excretion rate of an organism is a useful and sensitive indication of its daily energy consumption.

Therefore, the amount of energy released from food substrate oxidation is directly associated, according Barbieri *et al.* (2013), quantifying ammonia excretion to aquatic organisms, indicating the animals' general physical condition.

The excretion process, by which waste products of metabolism and other non-useful materials are eliminated from an organism, is mainly carried out in the lungs, kidneys and skin (Beckell, 1987; Naimo *et al.*, 1992; Barbieri & Bondioli, 2013) and it is essential in all life forms. In single-celled organisms, waste products are discharged directly through the cell's surface while multicellular organisms utilize more complex excretory methods. Aquatic animals usually excrete ammonia directly into the external environment since this compound has high solubility and there is water available for dilution (Arana, 2000).

Excretion is a necessary consequence of protein breakdown; when proteins are converted to carbohydrates to provide energy, the amino group must be hydrated to be removed. In the body, the amino group is quickly oxidized to ammonia form (at high body pH the ammonium ion). If the organism has sufficient water, ammonia can be simply excreted in the environment (Pough *et al.*, 1989). This is the pathway used by many aquatic organisms, particularly in freshwater, but in any event, ammonia must be quickly eliminated because of its toxicity. Ammonia will passively diffuse out of respiratory structures such as gills and a large amount of water is necessary to dissolve and eliminate it, however, each ammonia molecule carries only one of nitrogen.

This survey aimed to determine *F. paulensis* ammonia excretion exposed to different Cd concentrations at three salinity levels (36, 20 and 5) and three temperatures (25 °C, 20 °C and 15 °C) and the results were analyzed to determine if concentrations varied with different salinity levels and temperatures.

Materials and Methods

Four hundred and fifty shrimps *F. paulensis* (mean weight 1.39 ±0.52g and mean length 1.32 ±0.41 cm) were employed for the ammonia excretion measurement in an aquarium. Ten shrimps were subjected to ammonia excretion measurements in each of four Cd concentrations (0.00; 0.1; 0.5; 1.0 and 2.0 mg L^{-1}) in three salinity levels (36; 20 and 5) and three temperatures (25 °C, 20 °C and 15 °C). The oxygen concentration and pH of the test solution were determined: oxygen concentration was 8.19 to 8.22 (mLO_2 L^{-1}) and pH was 6.17 to 6.22.

Ammonia excretion: Ammonia was determined as total NH_4-N, or 'ammonia nitrogen', by the modified phenolhypochlorite method version of Solarzano (1969). Duplicate seawater samples (2 mL) of the control and test aquarium were transferred to 5mL volumetric flasks. One-twentieth of the volumes suggested by Solarzano (1969) were used for chemical analyses. The blue color of indophenol obtained by the reaction at high pH of ammonia, phenol and hypochlorite was allowed to develop at room temperature (20 to 20,5 °C) for each 1 h, and the absorbance was read in a Beckman spectrophotometer (1.0 cm, 640nm). Deionized water was used as a blank and $(NH_4)_2S04$ as standard. The accuracy of the measurements was within 1%**.** To attenuate handling stress, shrimps were maintained in the aquarium with continuous water circulation for at least 60 min before the tests. Standard shrimp ammonia excretion rates were calculated using the following formula:

$S_{AE} = (V_E - V_B) \times V_T / N_h$, where:

S_{AE} - Standard Ammonia Excretion Rate

V_E - Amonia Concentration in Experimental Vessel

V_B - Amonia Concentration in Blank Vessel

V_T - Excretion of Total Volume of Sea Water

N_H - Total number of hours elapsed.

Shrimp could excrete ammonia in the known water volume for a period of five hours. A barrier to isolate the animals from any possible movement in the laboratory protected the aquaria. The difference between the ammonia concentrations determined at the beginning and at the end of the confinement was used to calculate the excretion during the period.

At the end of the acclimation period, the necessary Cd volume was added to each volume of respirometre and as soon as $CdCl_2$ was added the entry orifice was sealed. Additionally, the water in the bottle was sampled at the beginning and end of the ammonia analysis. The average specific shrimp ammonia excretion was assessed regarding normality distribution using Shapiro-Wilk's test and Levene's test was used for homogeneity of variance (homocedasticity). Seeing as the results were normal and homocedastic, differences between means of treatments were evaluated using a variance analysis (ANOVA) followed by Tukey's multiple comparisons test with a confidence interval of $p<0,05$.

Results

Cd effect on shrimp ammonia excretion with varying salinity at 15 °C: For acclimated shrimps at 15 °C, it was observed and increase in the ammonia excretion according to the increase of Cd concentration. Shrimps acclimated at 15 °C (Table 1) subjected to salinity levels of 5, 20 and 36, excreted, on average, 0.15 and 0.16 µg/g/min of ammonia.

At the highest Cd (2.0 $mg.L^{-1}$) concentration employed in the test, it was verified that the ammonia excretion increased to 93%, 87% and 68%, at salinity levels 5, 20 and 36, respectively.

Analyzing the shrimp excretion exposed to different concentrations of Cd at 15 °C under different salinity levels, it was noticed that the largest ammonia excretion occurred at salinity 20 and concentration of 2.0 $mg.L^{-1}$ of Cd.

Using the Tukey ($p<0.05$) statistical test, it was verified that ammonia excretion averages at the Cd, 0.5, 1.0 and 2.0 mg L^{-1} concentrations in all salinity levels are significantly different with respect to control.

Cd effect on shrimp ammonia excretion with varying salinity at 20 °C: For the acclimated shrimps at 20 °C, ammonia excretion increases in relation to Cd concentration in all three salinity levels. Ammonia excretion in concentration of 2.0 mg L^{-1} Cd concentration increases as salinity decreases. It was observed that shrimp ammonia excretion in the acclimated control group at 20 °C (Table 1), subjected to 5, 20 and 36 salinity levels were 0.18, 0.20 and 0.18µg/g/min, respectively. Shrimps subjected to 2.0 mg/l of Cd excreted 0.34; 0.33 and 0.31 µg/g/min at the tested salinity levels.

It was verified that ammonia excretion averages at Cd concentrations of 1.0 and 2.0 $mg.L^{-1}$ in all salinity levels were different in relation to the control (Tukey; $p<0.05$). The statistical test used showed that there were differences in the ammonia excretion averages between salinity levels 5 and 36, at concentrations of 1.0 and 2.0 $mg.L^{-1}$.

Cd effect on shrimp ammonia excretion with varying salinity at 25 °C: It was observed that shrimp ammonia excretion of the acclimated control group at 25 °C temperature (Table 1), subjected to 5; 20 and 36 salinity levels were 0.20, 0.22 and 0.21 µg/g/min, respectively. Shrimps subjected to 2.0 mg L^{-1} of Cd excreted 0.39; 0.35 and 0.31 µg/g/min at the tested salinities. It was verified that ammonia excretion averages to Cd 1.0 and 2.0 mg L^{-1} concentration in all salinity levels were expressively different in relation to the control (ANOVA, Tukey; $p<0.05$). However, with the salinity 5 there were significant differences at the three Cd concentrations employed (Table 1).

Cd effect on shrimp ammonia excretion with varying temperature and maintaining salinity 5: It was observed that for acclimated shrimps at a salinity 5, ammonia excretion increased in relation to Cd concentration at the three temperatures. It can be also verified that in the control, the specific ammonia excretion of the acclimated shrimps in salinity 5 (Table 1), at temperatures of 15, 20 and 25 °C were 0.15, 0.18 and 0.20 (µg/g/min), respectively. Comparing these results with Cd highest concentration employed in the tests, the ammonia excretion increased to 93%; 88% and 95%, compared to the control for the temperatures of 5, 20 and 25 °C, respectively. We did not find statistical difference of 0.1 mg L^{-1} when compared to the control at the three temperatures.

The average ammonia excretion increased with rising Cd concentration, there was a significant ($p<0.05$, Tukey) difference between the obtained rates in the concentrations of 0.5, 1.0 and 2.0 mg L^{-1} in each employed temperature, when compared to the control.

As there was a significant difference among ammonia excretion averages obtained under the control, at the three temperatures, it can be inferred that the most important factor to ammonia excretion increasing is the interaction of rising Cd concentrations with increasing temperature.

Cd effect on shrimp ammonia excretion with varying temperature and maintaining salinity 20: At salinity 20, the same tendency was observed as described for salinity 5: the ammonia excretion increased as Cd concentration increased. For a given Cd concentration, ammonia excretion increased with temperature. However, the determinant factor for the increasing ammonia excretion was the interaction of Cd concentration and the increasing temperature, as we did not observe significant differences between the average metabolic rates obtained in the controls in the three temperatures.

It was verified that under the control, the ammonia excretion of the acclimated shrimps to salinity 20 (Table 1) in the three temperatures 15, 20 and 25 °C were respectively 0.16, 0.20 and 0.20 µg/g/min on average. Comparing these results to the 2.0 mg L^{-1} concentration employed in the test, the ammonia excretion average enhanced to 0.30, 0.33 and 0.35 µg/g/min for the three temperatures studied representing a metabolic rate decrease of 87%, 65% and 59% compared to the control (Table 1).

The specific ammonia excretion averages were significantly different (Tukey, $p<0.05$) for the concentration of 0.5, 1.0 and 2.0 mg L^{-1} when they were compared to control at each temperature tested.

Comparing ammonia excretion averages for exposed animals at different temperatures but fixed Cd concentrations, significant differences were found, for the 0.5, 1.0 and 2.0 mg L^{-1} concentrations at different temperatures. At a concentration of 0.1 mg L^{-1}, there was no significant difference in the ammonia excretion averages at the three studied temperatures. Comparing to the ammonia excretion averages between the employed temperatures, it was verified that there was no significant difference between them.

Cd effect on shrimp ammonia excretion with varying temperature and maintaining salinity 36: It was verified that under the control, the ammonia excretion of the acclimated shrimps to salinity 36 (Table 1) in the three temperatures 15, 20 and 25 °C were respectively 0.16, 0.18 and 0.21 µg/g/min, in average. Comparing these results to the 2.0 mg L^{-1} concentration employed in the test, ammonia excretion average enhanced to 0.27, 0.31 and 0.31 µg/g/min for the three temperatures studied representing a metabolic rate decrease of 68%, 72% and 51% compared to the control (Table 1).

Specific ammonia excretion averages were significantly different (Tukey, $p<0.05$) for the concentration of 1.0 and 2.0 mg L^{-1} when they were compared to control at each temperature tested.

Comparing specific ammonia excretion averages for exposed animals at different temperatures but fixed concentrations of Cd, significant differences were found, for the 1.0 and 2.0 mg L^{-1} concentrations at different temperatures. At a concentration of 0.1 and 0.5 mg L^{-1}, there was no significant difference in the specific ammonia excretion averages at the three studied temperatures. Comparing ammonia excretion averages between the employed temperatures, it was verified that there was no significant difference between them.

Table 1. Ammonia excretion (AE) of *Farfantepenaeus paulensis* , acclimated to the different salinity and temperature, as a function of exposure at different Cd concentrations. Standard deviation (SD) and percent ammonia excretion in relation to the control are also shown. Each value represents the average of 5 determinations.

			Concentration of Cd (mg/L)														
		Salinity	0			0.1			0.5			1.0			2.0		
			AE	SD	%	AE	SD	%	AE	SD	%	AE	SD	%	AE	SD	%
Temperature	15°C	5	0.15	0.011	-	0.16	0.020	6.6	0.20	0.039	33.3*	0.25	0.028	66.6*	0.29	0.064	93.3*#
		20	0.16	0.015	-	0.18	0.025	12.5	0.22	0.027	37.5*	0.26	0.048	62.5*	0.30	0.052	87.5*
		36	0.16	0.024	-	0.19	0.035	18.7	0.23	0.022	43.7*	0.26	0.040	62.5*	0.27	0.064	68.7*
	20°C	5	0.18	0.032	-	0.19	0.027	5.5	0.23	0.065	27.7	0.27	0.045	50*#	0.34	0.069	88*#
		20	0.20	0.021	-	0.21	0.037	5.0	0.24	0.055	20	0.29	0.061	45*	0.33	0.047	65*
		36	0.18	0.019	-	0.19	0.025	5.55	0.23	0.035	27.7	0.27	0.055	50*	0.31	0.062	72.2*
	25°C	5	0.20	0.029	-	0.19	0.061	-5.0	0.29	0.072	45*	0.35	0.050	75*#	0.39	0.040	95*#
		20	0.22	0.031	-	0.20	0.035	-9	0.27	0.039	22	0.30	0.052	36.3*	0.35	0.056	59*
		36	0.21	0.032	-	0.21	0.033	0.0	0.25	0.065	19	0.28	0.045	37*	0.31	0.069	51.8*

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

significant difference in relation to the salinity ($p < 0.05$)

Discussion

The results allowed evaluation of the Cd effect, which is one of heavy metals with high toxicity to fish and aquatic invertebrates (Barbieri *et al.*, 2010), on *F. paulensis* ammonia excretion at different salinity levels and temperature.

Ammonia is the end product of protein catabolism of most aquatic organisms (Kinne, 1976). Alterations in proportion of nitrogen excretion produced can vary as a result of environmental stress, taking to a physiological response, which can be initiated as a means of keeping homeostasis or it can be a reflection of changing some animal function (Heath, 1987).

The concentration of nitrogenous excreta is determined by the use of proteins as an energy source and the utilization rate and "turnover" of cellular constituents (Bayne *et al.*, 1985).

In this work, it was observed an increase in ammonia excretion rate after exposure to high levels of Cd in *F. paulensis*.

Exposure of heavy metals concentration can dysfunction of ammonium excretion control follows gill damage and the out flow of ammonium from the hemolymph to the ambient water results in higher concentrations of ammonium in the water and a lower osmotic pressure in the hemolymph (Wu & Chen, 2004). An exposure to a longer period of time for which the body increases its metabolism leads to a weakening, leaving it more susceptible to disease and predation (White & Rainbow, 1982). Data associated with the excretion and subsequent reduction in survival observed by Moraes *et al.* (1999) in contaminated sediment samples corroborates this statement.

Studies on heavy metal effects on shrimp excretion, demonstrated ammonia excretion rates increased related to concentration, exposure time and larval stage (Barbieri *et al.*, 2016). Zhang *et al.* (2014) observed an increase in ammonia excretion in different Cd concentrations compared to control in juvenile of *Exopalaemon carinicauda*. Cheung & Cheung (1995) reported that mussels *Leg viridis*, exposed to cadmium and zinc, exhibited, initially, an increase in the rate of excretion followed by a decrease in relation to the individuals control.

However, some studies suggest cadmium effect as a depressor of ammonia excretion rate. Gaudy *et al.* (1991) observed a decrease in ammonia excretion when *Leptomysis lingvura* was exposed to cadmium. Similar results were observed by Naimo *et al.* (1992) exposing the clam *Lampsilis ventricosa* to the cadmium.

Differences in the present work may be a result of the cadmium concentration used, exposure period, shrimp species used. However, many questions to determine the relation between heavy metal exposure and ammonium excretion remain unsolved, which indicate the need for more studies.

In this study, Cd concentration was associated with different salinity levels. It is known that *F. paulensis* can support a wide salinity range, because they live in different environments throughout its life cycle. Adult shrimp lay eggs in the sea, when fingerling can be found in small rivers and in brackish water and the adults return to seawater to reproduce.

Environment exchange requires deep changes in the osmoregulatory process with consequent energy expenditure. Osmoregulation in estuarine shrimp is done mainly by the gills, which also participate in gas changes. Salt secretion by epithelium gills needs to be done by active transportation, occurring from a minor blood concentration to a bigger one, at the surrounding environment, when the shrimp is in sea water (Doi *et al.*, 2012; Santos *et al.*, 2014). Despite *F. paulensis*'s adaptive capacity across many environments, low salinity can affect cadmium (Cd) toxicity, as observed in this project, in which Cd was significantly more toxic at salinity 5.

Similar results were described by Fritioff *et al.* (2005) in the study of two submersed macrophyte species, where it was observed that higher metal concentration and accumulation occurs with increasing temperature but at lower salinity. And also by Giarratano *et al.* (2007), it was observed that Cd was significantly more toxic for *Exosphaeroma gigas* at salinity 20 than at 30.

Researches on LC50 of Cd (Cd^{2+}) to *Cyprinodon variegatus* in Chesapeake bay, in 96 h exposures at three salinity levels (15, 20 and 25), showed that as the salinity level rose, cadmium toxic effect on the fish decreased (Hall *et al.*, 1995). Wu & Chen (2004) have found an increase in the levels of ammonia excreted by *Litopenaeus vannamei* after 24 h of exposure to cadmium and zinc at salinity15, which is in agreement with the results of this study at salinity 20.

Cd increase toxicity with salinity reduction can be related with the high availability of metals in low salinity. According to Williams *et al.* (1994), in seawater, chloride forms complexes with Cd; more rarely, Cu, but not with Pb, thus the free ion concentration of the former metals will be reduced. Sodium ions may release Cd from the sediment to

the water, thereby increasing Cd concentration in the water (Greger *et al.*, 1995). The concentration of dissolved Cd increases linearly with increasing salinity within the Changjiang Estuary (Wang & Liu, 2003). All of them increase in concentration across almost the full salinity range.

In crustaceans, most ammonia excreted is from the metabolic pool body reserve as from diet (Regnault, 1987). Internal amino acid regulations together with ionic regulation are mainly responsible for maintaining the osmotic gradient in environments with changing salinities (Gaudy *et al.*, 2000). Free amino acids are important crustacean osmotic effectors that contribute to keep internal concentration above environmental concentration when media concentration is below the species isosmotic point. When external concentration decreases to a level that can produce breakdown in the regulatory system, internal concentration is reduced through catabolism of free amino acids increasing ammonia production (Dall *et al.*, 1992).

Thus, the present excretion data indicate an isosmotic point closer to salinity 20 than to 30 for *F. paulensis*. Considering the metal's toxic effects of in the hydrosaline balance, Pequeux *et al.* (1996) supposes that the decrease of ammonia excretion is a consequence of the participation of ammonia like osmotic effects electrolyte, which could explain our results registered at salinity 30.

A recent study by Barbieri *et al.* (2016) showed that ammonia excreted by *F. paulensis* showed relation to temperature levels regardless of whether or not shrimps were exposed to a heavy metal.

Cd effects, observed in this project, on the *F. paulensis* can be minimum when they occur at low temperatures and concentrations below 0.5 mg L^{-1}. However, at 25 °C and the highest Cd (2.0 mg L^{-1}) concentration, the effects were pronounced.

Studies with effects of three temperatures and toxicity of copper in the mollusk *Dreissena polymorpha*, showing that high temperature can increase copper toxicity (Rao & Khan, 2000). Gastropod *Physa acuta* showed sensibility to cadmium with the temperature elevation, with morphological growth reduction (Cheung & Lam, 1998).

As stated previously, the cadmium concentration reported in sediments and suspended material from Santos estuary averages were 1.7 µg/g (CETESB, 2001; Damato & Barbieri 2003). Although *F. paulensis* lives on the Brazilian estuaries, the potential risk of cadmium for this species should be seriously considered, especially because *F. paulensis* is a detritivore, sediment-consumer species. Cadmium, like other heavy metals, presents a high absorption to fine sediments such as clay, abundant on the bottom and coastal areas of the mentioned estuary.

Results show that *F. paulensis* is a good indicator of 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 shrimp culture industry.

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