EFFECTS OF CADMIUM AND ZINC ON OXYGEN CONSUMPTION AND AMMONIA EXCRETION OF THE SEA-BOB SHRIMP, ACCORDING TO THE TEMPERATURE*

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ABSTRACT

Penaeid shrimps are important resources for worldwide fisheries and aquaculture. In Brazil, *Xiphopenaeus kroyeri* 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 *X. kroyeri* and to investigate their effects on oxygen consumption and ammonium excretion in different temperatures (25 ºC, 20 ºC, 15 ºC), which have not been carried out in this species before. First of all, we examined the acute toxicity of Cd and Zn to *X. kroyeri* 24, 48, 72, and 96-h medium lethal concentration (LC_{50}). Furthermore, we also found that exposure of shrimp to Cd and Zn caused an inhibition in oxygen consumption of 52.50% and 52.63%, respectively, relative to the control. However, after separate exposure to Cd and Zn, elevations in ammonium excretion were obtained, which were 85.00% and 89.47% higher than the control, respectively. The results shown that Cd and Zn performs higher toxicities to *X. kroyeri* at higher temperature.

Keywords: Marine pollution; heavy metal; *Xiphopenaeus kroyeri* ; toxicity; LC_{50}

EFEITO DO CÁDMIO E ZINCO NA EXCREÇÃO DE AMÔNIA E CONSUMO DE OXIGÊNIO DO CAMARÃO SETE-BARBAS, DE ACORDO COM A TEMPERATURA

RESUMO

Os camarões são importantes recursos marinhos explorados pela pesca e aquicultura. No Brasil, *Xiphopenaeus kroyeri* é uma importante espécie comercialmente explorada e um animal ideal para estudar o impacto causado por efeitos de metais pesados que frequentemente são detectados em áreas costeiras. O principal objetivo do presente trabalho foi determinar a toxicidade aguda do Cd e Zn para o *X. kroyeri* e investigar seus efeitos no consumo de oxigênio e na excreção de amônia em diferentes temperaturas, uma vez que tais parâmetros ainda não foram determinados para a referida espécie. Primeiramente, foram determinadas a toxicidade aguda (LC_{50}) do Cd e Zn, para *X. kroyeri* por um período de 24, 48, 72 e 96 horas em três temperaturas (25 ºC, 20 ºC, 15 ºC). Os resultados revelaram que para camarões expostos ao Cd e Zn houve uma inibição do consumo de oxigênio de 52,50% e 52,63%, respectivamente, para a mais baixa temperatura (15 °C). Entretanto, para a excreção de amônia, houve um aumento de 85,00% e 89,47% para a mais alta concentração e temperatura utilizadas em relação ao controle. Conclui-se, portanto, que as toxicidades de Cd e Zn foram mais altas nas temperaturas e concentrações mais elevadas.

Palavras chave: Poluição marinha; metal pesado; toxicidade; *Xiphopenaeus kroyeri*; CL_{50}

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INTRODUCTION

In Brazil, heavy metals enter the coastal seawater mainly through discharge of industrial effluents and disposal of sewage (DAMATO and 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). Cadmium (Cd) and Zinc (Zn) have been widely recognized as highly toxic when dissolved and in ionic form (MANCE, 1987), are very common and persistent heavy metals in aquatic environments and known to be highly toxic to marine and estuarine crustaceans (WU and CHEN, 2004).

Exposure to heavy metals in the aquatic environment produces many physiological changes in crustaceans, including alterations in the metabolic activities (BARBIERI and PAES, 2011). 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 (DAMATO and BARBIERI, 2011*).*

Several kinds of physiological answers can be used for this purpose (ADAMS, 1990; HANSEN *et al.,* 1997), 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).

The temperature affects the biochemical and physiological processes that involve since the digestion till the locomotion. The temperature changes also influence the food intake and the predators escape, in this case, it would be affecting the balance between the prey survival the predator capture success (BATTY *et al*., 1993), resulting in a change mechanism in the species geographical distribution (FILONOV, 2000). The temperature changes have considerable effects on several physiological processes. In limits, the temperature elevation accelerates most of the vital processes. In general, an elevation of 10 °C in the temperature causes an increasing from two to three times the oxygen consumption rates (SCHMIDT-NIELSEN, 1997).

Evaluation of oxygen consumption and ammonia excretion were used, for example, to study toxicant effects caused by aromatic compounds (LEMAIRE *et al*., 1996), heavy metals (WU and CHEN, 2004; BARBIERI, 2009a; BARBIERI and PAES, 2011), detergents (CHRISTIANSEN *et al*., 1998; BARBIERI and DOI, 2011) and a variety of toxicants (MARTINEZ *et al*., 2013).

The *Xiphopenaeus kroyeri* population is distributed throughout the tropical and subtropical coastal waters of Atlantic (BARBIERI *et al*., 2004) is an important economic resource for artisanal fishers. It is caught in Brazilian coastal areas with a depth of 10 metres approx. However coastal seawater is often contaminated by heavy metal and others pollutants. Hence, the impacts of heavy metal should be considered, not only on shrimp biology and physiology bat also human health (WU and CHEN, 2004).

Xiphopenaeus kroyeri are found in coastal areas of the Atlantic where water temperatures average 20 °C (15-25 °C) with depth of 10 meters approx susceptible to pollutants. For this reason the objective of this survey was to determine the acute toxicity of Cd and Zn, oxygen consumption and ammonium excretion for *X. kroyeri* in three temperatures (15 °C, 20 °C and 25 °C) in which they can be found. The paper is about the potential effects of metal pollution on the biology and physiology of the wild species. The results were analyzed to determine if the acute toxicity was different with the temperature variation.

METHODOLOGY

Acute toxicity

The acute toxicity of cadmium and zinc to post-larvae of shrimp (*X. kroyeri*) cultivated in the laboratory of Instituto de Pesca exposed to different concentrations of these chemicals for a

period of up to 96 h was evaluated, taking into consideration the economic and ecological importance of this species and the problems related to pollution in coastal waters. A total of 1,890 post-larvae of cultivated shrimp with 2.3 ± 0.3 g medium wet weight and 3.2 ± 0.5 cm total length were used. The shrimp were in tanks of 500 L with a salinity of 36. The seawater was reconstituted using sea-salt of Cabo Frio, Rio de Janeiro (Brazil). Before the experiments the shrimp had been acclimatized, for one week in temperature of 25, 20 and 15 °C. After this procedure groups of fifteen individuals were put in 50 L tanks containing water at 25, 20 and 15 °C at salinity 36. Three replicates of groups of 15 individuals were exposed to each one of the following Cd and Zn nominal concentrations: Control, 0.10, 0.5, 1.00, 2.50, 5.00, 10.00, 20.00 and 40.00 mg L^{-1}. Dead shrimp were removed from the tanks and counted at 24, 48, 72 and 96 h of exposure through the continuous flow. The pH and oxygen concentration of the test solution were determined (Table 1 and 2). The dissolved oxygen was determined through the Winkler method and pH was determined through the pH-meter Spencer (precision 00.00). The ammonia concentration of the test solution was 0.01mg L^{-1} (± 0.005). Death was presumed when shrimps were immobile and showed no response to touch with a glass rod. The lethal concentration (LC_{50} with 95% confidence limits) was calculated by Spearman–Karber Estimates (HAMILTON *et al*., 1977).

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

Concentration of Zn (mg L^{-1})	pH	Oxygen concentration (mL O_2 L^{-1})
0.00	8.22	6.25
0.10	8.20	6.27
0.50	8.18	6.20
1.00	8.19	6.24
2.50	8.22	6.27
5.00	8.21	6.25
10.00	8.20	6.24
20.00	8.15	6.31
40.00	8.19	6.23

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

Concentration of Cd (mg L^{-1})	pH	Oxygen concentration (mL O_2 L^{-1})
0.00	8.12	6.25
0.10	8.18	6.36
0.50	8.20	6.38
1.00	8.22	6.28
2.50	8.21	6.35
5.00	8.18	6.26
10.00	8.19	6.31
20.00	8.17	6.30
40.00	8.15	6.28

Oxygen consumption and ammonia excretion

Three hundred shrimps (*X. kroyeri*) with averages of 2.27 ± 0.4 g and 3.10 ± 0.36 cm were employed for the routine metabolism measurement utilizing sealed respirometers. Five shrimp were subjected to oxygen consumption measurements in each one of the five concentrations of Cd (Control; 0.1; 0.5; 1.0 and 2.0 mg L^{-1}) and Zn (Control, 0.5; 1.0; 2.0 and 3.0 mg L^{-1}) in three temperatures (25, 20 and 15 °C).

Before the beginning of the experiments the animals were maintained in the respirometer with

continuous water circulation for at least 90 min to attenuate the handling stress (BARBIERI, 2009b). Then, the water supply was suspended and the respirometers were closed, so that the shrimp could consume the present 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 experiments duration was regulated so that the oxygen concentration by the end of experiments was above 70% of its initial concentration.

To obtain the Cd and Zn desired concentration, the necessary volume of the chemical (1.0 mg $CdCl_2$ mL^{-1} and 1.0 mg $ZnSO_4$ mL^{-1}) was added to each volume of respirometer 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 beginning and end of the oxygen consumption analysis. Determination of ammonium in the seawater was based on the phenolhypochlorite method (SOLORZANO, 1969).

The Shapiro-Wilk test was used to test the normality of averages. As the average had normal distribution we used the analysis of variance. 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).

RESULTS

The acute toxicity of Cd and Zn to shrimp post-larvae exposed to different concentrations of these metals for periods of up to 96 h, expressed as LC_{50} in different temperature is shown in Tables 3 and 4. This results shown that Cd and Zn performs higher toxicities to *X. kroyeri* at high temperature.

Table 3. Medium lethal concentration (LC_{50} mg Cd L^{-1} with 95% confidence limits) calculated in different temperature. Between parentheses, standard deviation.

Time of exposition (in hours)	Temperature		
	15 °C	20 °C	25 °C
24	0.83 (± 0.35)	0.55 (± 0.23)	0.35 (± 0.20)
48	0.51 (± 0.29)	0.42 (± 0.35)	0.29 (± 0.10)
72	0.28 (± 0.41)	0.19 (± 0.15)	0.09 (± 0.06)
96	0.13 (± 0.20)	0.08 (± 0.10)	0.04 (± 0.04)

Table 4. Medium lethal concentration (LC_{50} mg Zn L^{-1} with 95% confidence limits) calculated in different temperatures. Between parentheses, standard deviation.

Time of exposition (in hours)	Temperature		
	15 °C	20 °C	25 °C
24	1.34 (± 0.36)	1.10 (± 0.25)	0.85 (± 0.20)
48	1.13 (± 0.50)	0.70 (± 0.24)	0.46 (± 0.23)
72	0.65 (± 0,42)	0.45 (± 0.15)	0.22 (± 0.15)
96	0.21 (± 0.45)	0.10 (± 0.20)	0.07 (± 0.04)

For the acclimated shrimp the 36 salinity, the specific oxygen consumption decreased regarding the Zn concentration in the three employed temperature. The specific oxygen consumption in any Zn concentration decreased with the temperature decreasing.

We checked that the specific oxygen consumption of shrimp from the acclimated control group to 36 salinity (Table 5), subjected to 25 °C, 20 °C and 15 °C temperature were, as a rule, 0.051, 0.046 and 0.040 mL O_2/g/min, respectively. For the shrimp subjected to the

concentration of 3 mg L^{-1} of Zn, they consumed as a rule 0.028; 0.024 and 0.019 mL O_2/g/min to the tested temperature. These values represent a metabolic level diminution of 45.09%, 47.82% and 52.50% in relation to the control.

It was verified that the averages of the oxygen specific consumption to the Zn 1.0 and 3.0 mg L^{-1} concentration in all employed temperatures are expressively different (P<0.05) in relation to the control (0.0 mg L^{-1}). For the other concentrations, there was no significative difference.

Table 5. Oxygen specific consumption (mLO_2/g/min) of the shrimp routine, subjected to different Zn concentrations in different temperature. Between parentheses, standard deviation, % percentage of the oxygen consumption decreasing in relation with the control. Each value represents the average of five determinations. *Significant differences (P<0.05).

Concentration of Zn (mg L^{-1})	Temperature 25 °C		Temperature 20 °C		Temperature 15 °C	
	Specific consumption	%	Specific consumption	%	Specific consumption	%
0	0.051 (± 0.012)	-	0.046 (± 0.021)	-	0.040 (± 0.011)	-
0.5	0.047 (± 0.015)	-7.84	0.041 (± 0.014)	-10.86	0.036 (± 0.007)	-10.00
1	0.036 (± 0.014)	-29.41	0.035 (± 0.010)	-23.91	0.030 (± 0.005)	-25.00
2	0,030 (± 0.020)	-41.17*	0.027 (± 0.015)	-41.30*	0.024 (± 0.004)	-40.00*
3	0.028 (± 0.010)	-45.09*	0.024 (± 0.009)	-47.82*	0.019 (± 0.003)	-52.50*

The oxygen specific consumption varied considering the Cd concentrations increasing and the temperature. The oxygen specific consumption decreased with the Cd concentration mainly to the temperature of 25 °C and 20 °C. For the shrimp subjected to the temperature of 25 °C, 20 °C and 15 °C the oxygen specific consumption averages were, respectively, 0.043; 0.040 and 0.038 mLO_2/g/min. To the same temperature and in a bigger Cd (2.0 mg L^{-1}) employed concentration, the consumption was 0.023, 0.019 and 0.018 mLO_2/g/min (Table 6). This averages decreasing of the specific oxygen consumption to the Cd 2.0 mg L^{-1} concentration, represents a metabolic rate decrease of 46.51%, 52.50% and 52.63% in relation to the control (Table 6).

Table 6. Oxygen specific consumption (mL O_2/g/min) of the shrimp routine, acclimated to the salinity of the 36, subjected to different Cd concentrations in different temperatures. Between parentheses, standard deviation, % percentage of the oxygen consumption decreasing in relation with the control. Each value represents the average of five determinations. *Significant differences (P<0.05).

Concentration of Cd (mg L^{-1})	Temperature 25 °C		Temperature 20 °C		Temperature 15 °C	
	Specific consumption	%	Specific consumption	%	Specific consumption	%
0	0.043 (± 0,012)	-	0.040 (± 0.013)	-	0.038 (± 0.019)	-
0.1	0.038 (± 0,015)	-11.62	0.037 (± 0.018)	-7.50	0.036 (± 0.016)	-5.26
0.5	0.030 (± 0,020)	-30.23	0.029 (± 0.023)	-27.50	0.025 (± 0.014)	-34.21*
1	0.026 (± 0,021)	-39.53*	0.024 (± 0.015)	-40.00*	0.023 (± 0.009)	-39.47*
2	0.023 (± 0,014)	-46.51*	0.019 (± 0.012)	-52.50*	0.018 (± 0.013)	-52.63*

It was verified that the averages of the oxygen specific consumption to the Cd 1.0 and 2.0 mg L^{-1} concentration in all employed temperature are expressively different in relation to the control. For the other concentrations, there was no significative difference.

The ammonium excretion varied considering the Zn concentration increasing and the temperature. The ammonium excretion increased with the Zn, mainly to the temperature of 25 °C. The ammonium excretion averages for the shrimp of the control, subjected to the temperatures of

25, 20 and 15 °C were, respectively, 0.19; 0.20 and 0.17 µg/g/min. To the same temperature and in a bigger Zn (3.0 mg L^{-1}) employed concentration, the ammonium excretion was 0.36; 0.30 and 0.28 µg/g/min (Table 7). This averages increasing of the ammonium excretion to the Zn 3.0 mg L^{-1} concentration, represents a metabolic rate growth of 89.47%, 50% and 64.70% in relation to the control (Table 7).

Table 7. Ammonium excretion (µg/g/min) of the shrimp routine, subjected to different Zn concentrations in different temperature. Between parentheses, standard deviation, % percentage of the ammonium excretion increasing in relation with the control. Each value represents the average of five determinations. *Significant differences (P<0.05).

Concentration of Zn (mg L^{-1})	Temperature 25 °C		Temperatute 20 °C		Temperature 15 °C	
	Specific ammonia excretion	%	Specific ammonia excretion	%	Specific ammonia excretion	%
0	0.19 (± 0.08)	-	0.20 (± 0.06))	-	0.17 (± 0.03)	-
0.5	0.20 (± 0.11)	5,26	0,21 (± 0.09)	5.00	0.19 (± 0.10)	-11.76
1	0.24 (± 0.90)	26.31	0.22 (± 0.11)	10.00	0.22 (± 0.11)	29.41
2	0.28 (± 0.05)	47.36*	0.26 (± 0.02)	30.00*	0.24 (± 0.09)	41.17*
3	0.36 (± 0.06)	89.47*	0.30 (± 0.06)	50.00*	0.28 (± 0.03)	64.70*

It was verified that the averages of the ammonium excretion on to the Zn 2.0 mg L^{-1} and 3.0 mg L^{-1} concentration in all employed temperatures are expressively different in relation to the control (P<0.05). 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 temperature of 25 ^{o}C and 20 ^{o}C. However, the ammonium excretion averages to the temperature of 15 °C is meaningfully different from the ammonium excretion averages of other temperature employed to the concentration of Zn: 2 and 3 mg L^{-1}.

It was verified that under control, shrimp subjected to the temperature of 25 °C, 20 °C and 15 °C, excreted, on average, 0.20 and 0.18 µg/g/min of ammonium. Comparing these results with the averages of Ammonium excretion at the highest Cd (2.0 mg L^{-1}) concentration employed in the test, we verified that the Ammonium excretion average increased to 0.37; 0.34 and 0.31 µg/g/min, to the temperature of 25 ^{o}C, 20 ^{o}C and 15°C, respectively. We noticed that the biggest ammonium excretions occurred to the temperature of 25 °C to the concentration of 2 mg L^{-1} of Cd. For the temperature of 25 °C, it occurred a percentual increasing of the ammonium excretion of 85% when compared to the control. To the temperature of 20 °C, the increasing of the ammonium excretion percentual was of 70% in relationship with the control. To the temperature of 15 °C, the percentual increasing of the ammonium excretion was of 72.22% when compared to the control (Table 8).

The Ammonium excretion averages in the concentration of 1.0 and 2.0 mg L^{-1} of Cd in all studied temperatures are significantly different in relationship with the control. For the other concentrations, there was no significative difference. Comparing to the Ammonium excretion averages between the employed temperatures, 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^{-1} concentration, but also to the 1.0 mg L^{-1} of Cd to the temperature of 25 and 20 °C. For the other Cd concentrations, there were no significative differences among the averages of corresponding concentrations in different temperatures.

Table 8. Ammonium excretion (µg/g/min) of the shrimp routine, subjected to different Cd concentrations in different temperatures. Between parentheses, standard deviation, % percentage of the ammonium excretion increasing in relation with the control. Each value represents the average of five determinations. *Significant differences (P<0.05).

Concentration of Cd (mg L^{-1})	Temperature 25 °C		Temperature 20 °C		Temperature 15 °C	
	Specific ammonia excretion	%	Specific ammonia excretion	%	Specific ammonia excretion	%
0	0.20 (± 0.05)	-	0.20 (± 0.10)	-	0.18 (± 0,10)	-
0.1	0.21 (± 0.06)	5.0	0.20 (± 0.12)	-	0.19 (± 0,09)	5.55
0.5	0.24 (± 0.09)	20.00	0.27 (± 0.09)	35.00	0.24 (± 0,07)	33.33
1	0.34 (± 0.11)	70.00*	0.30 (± 0.11)	50.00*	0.28 (± 0,09)	55.55*
2	0.37 (± 0.12)	85.00*	0.34 (± 0.10)	70.00*	0.31 (± 0,11)	72.22*

DISCUSSION

The obtained results in this survey allow to evaluate the Cd and Zn effects on the *X. kroyer* metabolism to different temperature. This work confirm that different temperature 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 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 to crustaceans has been studied by a number of authors (MANCE, 1987; WONG *et al.,* 1993; VANEGAS *et al.,* 1997; WU and CHEN, 2004). Results of this study confirm that the heavy metals Cd and Zn are toxic to *X. kroyeri*, 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. For *Litopenaeus vannamei,* the 96h LC_{50} of Cd 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^{-1} (MARTIN *et al.,* 1981). Likewise, the 96h LC_{50} of Zn for larvae of *L. vannamei* is 1.35 mg L^{-1} (WU e CHEN, 2004) and for *Penaeussetiferus* it is 43.87 mg Zn L^{-1} (VANEGAS *et al*., 1997). In this study, Cd exhibited greater toxicity to *X. kroyeri* than Zn. WU and CHEN (2004) worked in another prawn species *L. 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 °C, 20 °C 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 and 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 temperature.

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 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, mentioned that many crustaceans possess an excellent regulatory ability

in their oxygen consumption patterns and thus were called oxygen regulators. Despite their regulatory capability, the oxygen consumption rate was indeed inhibited after *X. kroyeri* 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; BARBIERI *et al*., 2004; DOI *et al*., 2012).

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 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 *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, BARBIERI and DOI, 2011).

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 and 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 *Penaues setiferus* (McFARLAND and 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 *Penaues 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 *Metapenaeus ensis*, there was a clear decrease in sensitivity to heavy metals during development from protozoea to postlarvae (ONG CHER and CHEUNG, 1998). 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 and CHEN, 1987) and nitrite (CHEN and 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 $\mu g\ g^{-1}$ and 2,600 $\mu g\ g^{-1}$ in the more polluted areas (CETESB, 2001). Although *X. kroyeri* lives in brazilian coast, the potential risk of cadmium for this species should be seriously considered, especially taking into account that *X. kroyeri* is a detritivore, sediment-consumer species. This species, as well as being

susceptible to pollution of heavy metals in sediments, it is easily obtained and their postlarvae are resistant to laboratory management. 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.

CONCLUSION

Results show that *X. kroyeri* is a good test organism for studying heavy metal marine pollution and showed that Cd and Zn performs higher toxicities to *X. kroyeri* at higher temperature and concentration. The results obtained in this article suggest the need for future studies addressing the biochemical and histopathological aspects and the chronic effects of metals on *X. kroyeri.*

REFERENCES

ADAMS, S.M. 1990 Status and use of biological indicators for evaluating the effects of stress on fish. *American Fisheries Society Symposium*, *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*. *Comparative Biochemistry* and *Physiology - Part C*, *122*: 33-43.

BARBIERI, E**.** 2009a Effects of zinc and cadmium on oxygen consumption and ammonium excretion in pink shrimp (*Farfantepenaeus paulensis*, Pérez-Farfante, 1967, Crustacea). *Ecotoxicology*, *18*: 312-318.

BARBIERI, E. 2009b Effect of 2,4-D herbicide (2,4-dichlorophenoxyacetic acid) on oxygen consumption and ammonium excretion of juveniles of Geophagus brasiliensis (Quoy & Gaimard, 1824) (Osteichthyes, Cichlidae). *Ecotoxicology*, *18*: 55-60.

BARBIERI, E. and DOI, S.A. 2011 The effects of different temperature and salinity levels on the acute toxicity of zinc in the Pink Shrimp. *Marine and Freshwater Behaviour and Physiology*, *44*(4): 251-263.

BARBIERI, E. and PAES, E.T. 2011 The use of oxygen consumption and ammonium excretion to evaluate the toxicity of cadmium on *Farfantepenaeus paulensis* with respect to salinity. *Chemosphere*, *84*: 9-16.

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.

BATTY, R.S.; BLAXTER, J.H.S.; FRETWELL, K. 1993 Effect of temperature on the escape responses of larval herring, *Clupea harengus*. *Marine Biology*, *115*: 523– 528.

CARVALHO, C.E.V.; FARIA, M.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. *Ecotoxicology and Environmental Restauration*, *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. *Ecotoxicology and Environmental Restauration*, *4*(1): 1-5.

CHEN, J.P. and CHIN, T.S. 1988 Acute toxicity of nitrite to tiger prawn, *Panaeus monodon*, larvae. *Aquaculture, 83*(1): 253–262.

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

CHEUNG, C.C.C. and LAM, P.K.S. 1998 Effect of cadmium on the juveniles of a tropical freshwater sanil, *Physa acuta* (Draparnaud, 1805). *Water Science* and *Technology, 38*(7): 263-270.

CHIN, T.S. and CHEN, J.C. 1987 Acute toxicity of ammonia to larvae of the tiger prawn *Penaeus monodon*. *Aquaculture, 66*(1): 247–253.

CHINNI, S.; KHAN, R.N.; YALLAPRAGADA, P.R. 2000 Oxygen consumption, ammonia-N excretion, and metal accumulation in *Penaeus indicus* postlarvae exposed to lead**.** *Bulletin of Environmental Contamination and Toxicology*, *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. *Ecotoxicology and Environmental Safety*, *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 (LAS). *Environtal and Toxicology Chemistry*, *17*(10): 2051-2057.

DAMATO, M. and 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.

DAMATO, M. and BARBIERI, E. 2011 Determinação da toxicidade aguda de cloreto de amõnia para uma espécie de peixe (*Hyphessobrycon callistus*) indicadora regional. *O Mundo da Saúde*, *35*(4): 551-558.

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

DOI S.A.; COLLAÇO F.L.; STURARO L.G.R.; BARBIERI E. 2012 Efeito do chumbo em níveis de oxigênio e amônia no camarão rosa (*Farfantepeneaus paulensis*) em relação á salinidade. *O Mundo da Saúde*, *36*(4): 594–601.

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. *Ambiente*, *2*: 6-13.

EYSINK, G.G.J.; PÁDUA, H.B.; MARTINS, M.C. 1988b Presença de Mercúrio no Ambiente. *Ambiente*, *2*: 43-50.

FILONOV, A.E. 2000 Spatial structure of the temperature and salinity fields in the presence of internal waves on the continental shelf of the states of Jalisco and Colina (Mexico). *Ciencia Marina*, *26*(1): 1-21.

HAMILTON, M.A.; RUSSO, R.C.; THURSTON, R.V. 1977 Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. *Environmental Science and Technology, 11*(7): 714–719.

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. *Marine Biology*, *128*: 627-637.

KOIZUMI, T.; YOKOTA, T.; SHIRAKURA, H.; TATSUMOTO, H.; SUSUKI, K.T. 1994. Potential mechanism of cadmium induced cytotoxicity in rat hepatocytes: inhibitory action of cadmium on mitochondrial respiratory activity. *Aquatic Toxicology*, *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. *Marine Environmental Research*, *2*(1-4): 317-321.

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

MARTIN, M.; OSBORN, K.E.; BILLIG, P.; GLICKSTEIN, N. 1981 Toxicities of ten metals to Crassostrea gigas and *Mytillus edulis*, embryos, and Cancer magister larvae. *Marine Pollution Bulletin, 12*(2): 305–308.

MARTINEZ, D.S.T.; ALVES, O.L.; BARBIERI, E. 2013 Carbon nanotubes enhanced the lead toxicity on the freshwater fish. Journal of Physics: Conference series 429 012043.

MAYZAUD, P. and CONOVER, R.J. 1988 O:N atomic ratio as a tool to describe zooplankton metabolism. *Marine Ecology Progress Serie*, *45*: 289–302.

MCFARLAND, W.N. and LEE, B.D. 1963 Osmotic and ionic concentration of shrimp of the Texas coast. *Bulletin of Marine Science*, *13*: 391-416.

MCMAHON, B.R. 2001 Respiratory and circulatory compensation to hypoxia in crustaceans. *Respiratory Physiology*, *128*: 349-364.

ONG CHE, R.G. and CHEUNG, S.G. 1998 Heavy metals in Metapenaeus ensis, Eriocheir sinensis and sediment from the Mai Po marshes, Hong Kong. *The Science of the Total Environment*, *214*(1): 87-97.

PEQUEUX, A.; BIANCHINI, A.; GILLES, R. 1996 Mercury and osmoregulation in the crab, *Eriocheir sinensis*. *Comparative Biochemistry and Physiology C - Toxicology & Pharmacology, 113*(2): 149-155.

RAO, E.G. and KHAN, M.A.Q. 2000 Zebra mussels: Enhancement of copper toxicity by high temperature and its relationship with respiration and metabolism. *Water Environment Research, 72*(2): 175-178.

ROSAS, C.; MARTINEZ, E.; GAXIOLA, G.; BRITO, R.; SANCHEZ, A.; SOTO, L.A. 1999 The effect of dissolved oxygen and salinity on oxygen consumption, ammonia excretion and osmotic pressure of *Penaeus setiferus* juveniles. *Journal of Experimental Marine Biology and Ecology*, *234*: 41–57.

SCHRECK, C.B. 1990 Physiological, behavioral and performance indicators of stress. *American Fisheries Society Symposium*, *8*: 29–37.

SPICER, J.I. and WEBER, R.E. 1991 Respiratory impairment in crustaceans and mollusks due to exposure to heavy metals. *Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology*, *100*(3): 339–342.

SCHMIDT-NIELSEN, K.E.D. 1997 *Animal physiology. Adaptation and Environment*. Cambridge Univ. Press, Cambridge. 607 pp.

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*. *Aquatic Living Resource*, *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*. *Journal of Crustacean Biology*, *19*(2): 209–223.

SOLORZANO, L. 1969 Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography*, *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. *Environmetal Science Technology*, *12*(4): 409-413.

VANEGAS, C.; ESPINA, S.; BOTTELO, A.S.; VILLANUEVA, S. 1997 Acute toxicity and synergism of cadmium and zinc in white shrimp, *Penaeus setiferus*, juveniles. *Bulletin of Environmental Contamination and Toxicology, 58*(2): 87–92.

WONG, C.K.; CHU, K.H.; TANG, K.W.; TAM, T.W.; WONG, L.J. 1993 Effects of chromium, copper and nickel on survival and feeding behavior of *Metapenaeus ensis* larvae and postlarvae (Decapoda: Penaeidae). *Marime Environmental Researche*, *36*(1): 63–78.

WU, J.P. and CHEN, H.C. 2004 Effects of cadmium and zinc on oxygen consumption, ammonium excretion, and osmoregulation of white shrimp (*Litopenaeus vannamei*). *Chemosphere*, *57*: 1591-1598.