Effects of exposure to nitrite on the antioxidant enzymes activity and the histopathological response of prawn *Palaemonetes argentinus*

Efectos de la exposición al nitrito sobre la actividad de enzimas antioxidantes y la respuesta histopatológica del camarón *Palaemonetes argentinus*

María Laura Espino, [1, 2], Ana Cristina Díaz, [1, 2] and Susana María Velurtas [1]

[1]Instituto de Investigaciones Marinas y Costeras-Universidad Nacional de Mar del Plata, Argentina Instituto de Investigaciones Marinas y Costeras (IIMyC-CONICET), Facultad de Ciencias Exactas y Naturales, UNMdP. Funes 3350. B7602AYL, Mar del Plata, Buenos Aires, Argentina.
[2]Comisión de Investigaciones Científicas. Calle 526 entre 10 y 11, La Plata, Buenos Aires, Argentina.
e-mail: mlespino@mdp.edu.ar

Espino M. L., Díaz A. C. and S. M. Velurtas 2015. Effects of exposure to nitrite on the antioxidant enzymes activity and the histopathological response of prawn *Palaemonetes argentinus*. Mar de Plata, Argentina. *Hidrobiológica* 25: (1) 24-30.

ABSTRACT

The aim of the present study was to determine lethal and sublethal effects of nitrite on the freshwater prawn, *Palaemonetes argentinus* (Nobili 1901). The 24, 48, 72 and 96 h median lethal concentration (LC_{50}) of nitrite were found to be 103.07; 91.94; 82.39 and 62.53 mg L^{-1}, respectively. The antioxidant activity of superoxide dismutase (SOD) was positively correlated with the concentration of nitrite in the environment, thus increasing the formation of hydrogen peroxide, substrate of catalase (CAT), which also showed an increased activity under the same experimental conditions. Histological samples showed that nitrite exposure caused hyperplasia, necrosis and changes in the lamellar epithelium in gills; while the effects in the hepatopancreas were degenerative desquamation, epithelial deterioration and hyperplasia. In conclusion, nitrite toxicity in *P. argentinus* increases with time of exposure. Moreover, this study proves that histological analysis of crustacean gills and/or hepatopancreas is a useful tool to determine the presence of toxic concentrations of nitrite in the water. Additionally, the measurement of the activity of antioxidant enzymes (e.g., SOD and CAT) as possible indicators of nitrite pollution should be considered in future studies.

Key words: Histology, LC_{50}, nitrite, oxidative stress, *Palaemonetes argentinus*.

Resumen

El objetivo del presente estudio fue determinar los efectos letales y subletales del nitrito en el camarón de agua dulce, *Palaemonetes argentinus* (Nobili 1901). Las concentraciones letales medias (LC_{50}) de nitrito a las 24; 48; 72 y 96 h al nitrito fueron 103,07; 91,94; 82,39 y 62,53 mg L^{-1}, respectivamente. La actividad antioxidante de la superóxido dismutasa (SOD) se correlacionó positivamente con la concentración de nitrito ambiental; este incremento en su actividad eleva la formación de peróxido de hidrógeno, sustrato de la catalasa (CAT), que también mostró un incremento en su actividad bajo las mismas condiciones experimentales. Las muestras histológicas evidenciaron que la exposición al nitrito causa hiperplasia, necrosis y cambios en el epitelio lamelar en las branquias; mientras que en el hepatopancreas los efectos son descamación degenerativa, deterioro epitelial e hiperplasia. En conclusión, la toxicidad del nitrito en *P. argentinus* se incrementa conforme el tiempo de exposición. Este estudio demuestra que el análisis histológico de las branquias y/o hepatopancreas del camarón *P. argentinus* es útil para determinar la presencia de concentraciones tóxicas de nitrito en el agua. Además, la medición de las enzimas antioxidantes (por ejemplo, SOD y CAT) debería ser considerado en futuros estudios como posibles indicadores de contaminación por nitrito.

Palabras claves: Estrés oxidativo, histología, LC_{50}, nitrito, *Palaemonetes argentinus*.

INTRODUCTION

The family Palaemonidae (Decapoda: Caridea) contains a vast assemblage of species inhabiting fresh, marine, and brackish waters. They are important organisms for aquaculture and candidates for as model organisms in crustacean research (Jayachandran, 2001). *Palaemonetes argentinus* (Nobili 1901) is a species of ecological interest, abundant in the north and center of Argentina, Uruguay, and in southern Brazil (Boschi, 1981; Morrone & Lopreto, 1995; Magalhães *et al.*, 2003). Several species within the genus *Palaemonetes* are widely used for toxicity tests because of the ease with which the animals can be collected, held, handled, and tested in the laboratory. These features, together with a substantial literature on the physioecology and the importance of these prawns in estuarine systems, make them an important model animal for toxicity tests (Buikema *et al.*, 1980).

Acute toxicity tests provide rapid estimations of the lethal effects of a toxic agent on aquatic organisms; they are short-duration experiments (usually 96 h), designed to determine the median lethal concentration (LC_{50}) (Lombardi, 2004). Nitrite is the most common pollutant in aquaculture systems; it is formed from ammonia and may be accumulated in aquatic systems as a result of imbalances in the activity of nitrifying bacteria (Mevel & Chamroux, 1981). High levels of nitrite are potential factors triggering stress in aquatic organisms (Jensen, 2003; Chand & Sahoo, 2006). Nitrite toxicity to crustaceans has been studied by several authors (Chen & Lee, 1997; Lin & Chen, 2003; Seneriches-Abiera *et al.*, 2007). This toxicant is considered a disruptor of multiple physiological functions in aquatic animals (Jensen, 2003), including the continuous production of reactive oxygen intermediates (ROS) (Wang *et al.*, 2004). Aquatic organisms are protected against ROS by antioxidant enzymes and free radical scavengers of low molecular weight (Díaz *et al.*, 2004; Valavanidis *et al.*, 2006). The measurement of antioxidant enzymes is considered a biomarker of stress in crustaceans (Stadtman, 2002; Wang *et al.*, 2004; Li *et al.*, 2008).

Histological analysis is a useful tool for recording the physiological state of an individual, as well as to assess water quality (Vogt, 1987; Winkaler *et al.*, 2001). In crustaceans, gills are the first organs impacted by the presence of chemicals in the water; exposure to nitrogen compounds cause morphological changes in these structures (Rebelo *et al.*, 2000; Romano & Zeng, 2007). For its part, the hepatopancreas is the main organ of reserve and detoxification of xenobiotics in crustaceans, and is highly sensitive to physiological and environmental changes (Johnston *et al.*, 1998; Sousa & Petriella, 2007). However, the histological changes of this organ have never been examined in previous studies, in relation to the presence of nitrite in the water. We believe that the histological examination of the gills and hepatopancreas of crustaceans might provide insight into the role that these organs play in the toxicity and detoxification of nitrite, respectively.

The aim of the present study was to determine lethal and sublethal effects of nitrite on *P. argentinus*.

MATERIALS AND METHODS

Experimental animals. Individuals immature of both sexes, of the species *P. argentinus* (initial mean weight 0.1±0.051gr.), were collected from Laguna de los Padres, an endorreic lagoon located near Mar del Plata, Argentina (37º 57´S; 57º44´W) with a hand net. Prior to the start of experiments, the prawns were acclimated in the laboratory for 48 h with tap water (pH = 7.6). To reduce the chlorine concentration, the water was previously aerated. During acclimation, prawns were daily fed a pelletized diet prepared in the laboratory and consisting of 45% proteins, 8% lipids, 7% water, 7% ash (Díaz *et al.*, 2002).

Lethal effect of nitrite. Short-term LC_{50} static toxicity test were carried out following the methods described by American Public Health Association (1998). Bioassays were conducted in order to establish tolerance limits in 600 ml glass tanks containing 0, 10, 20, 40, 60, 80, 100, 120, and 140 mg L^{-1} nitrite. Prawns were sampled randomly from the acclimation tanks and transferred to containers filled with test (nitrite addition) and control (without added nitrite) solutions. The animals were not fed during the duration of the experiment. Each experimental tank containing 10 prawns and the test solution were aerated continuously. The experimental conditions were maintained constant; the water was kept at 20±0.8ºC, salinity at 0 ppt, pH 7.6 and 12 h light: 12 h dark photoperiod. Each test solution was run by triplicate.

Observations were made at 24 h intervals up to 96 h of exposure. The death was assumed when prawns were immobile and exhibited no response when touched. Dead prawns were removed daily. The concentration response of test organisms was determined for LC_{50} of nitrite with the computer software XLSTAT 2010. The estimated Probit line and the results of a χ^2 (Chi square) test for goodness of fit were computed (Finney, 1971).

Sublethal effects of nitrite. **Enzyme assays**. To assess superoxide dismutase (SOD) and catalase (CAT) activities, pool of whole prawns were freezer-clamped in liquid nitrogen and homogenized in 9 volumes of 20 mM phosphate buffer pH = 7.4, 1 mM EDTA and 0.1% triton X-100, and the homogenates were centrifuged at 600 g (Wang *et al.*, 2004). SOD activity was estimated by its ability to inhibit the superoxide radical dependent reactions using the Ransod Kit (Randox, Crumlin, UK). The optical density was measured at 505 nm and to 37 °C, with a Shimadzu spectrophotometer (model UV-2101 PC). The rate of reaction was estimated from the absorbance readings to 30 s and 3 min after adding xanthine oxidase. A reference standard SOD was supplied with the Ransod Kit. One unit of SOD was defined as the amount required inhibiting the rate of xanthine reduction by 50%. Specific activity was expressed as SOD units mg $protein^{-1}$.

CAT activity was assayed according to the method of Cakmak *et al.* (1993). The addition of H_2O_2 started the reaction, and the decrease in absorbance at 240 nm was recorded during 3 min. One unit of CAT was defined as amount of enzyme which catalyzes the conversion of 1 mol of H_2O_2 to H_2O per minute. Specific activity was expressed as CAT units g $protein^{-1}$.

A one-way ANOVA followed by Duncan's Multiple Range test was used to compare means. Additionally, the fitting curve and regression analysis were performed using XLSAT 2013. The critical p level used was 0.05 (Sokal & Rohlf, 1995).

Light Microscopy. For histological description of the gill and the hepatopancreas, the prawns were fixed in Davidson fluid, dehydrated in a progressive alcohol series and embedded in paraffin (Bell & Lightner, 1988). Sections (5 µm) were stained with Haematoxylin-Eosin.

RESULTS

The LC_{50} of nitrite for the freshwater prawn *P. argentinus* were 103.07, 91.94, 82.39, and 62.53 mg L^{-1} at 24, 48, 72 and 96 h, respectively (Table 1).

Figure 1 shows SOD activity of prawns at different levels of ambient nitrite exposure. The antioxidant activity of SOD was positively correlated with the nitrite concentration (R= 0.76), since its activity increased with increasing levels of the pollutant. At low levels of environmental nitrite (0-40 mg L^{-1}), SOD activity not show significant variation ($p>0.05$), at 60 mg L^{-1} SOD significantly increased, however at higher concentrations of nitrite SOD activity already decreased.

The CAT activity is shown in Figure 2. At low levels of environmental nitrite (0-20 mg L^{-1}), the activity of CAT, showed a basal activity of catalase. The activity increased at intermediate values of nitrite (40 mg L^{-1} to 80 mg L^{-1}), but at 100 mg L^{-1} of nitrite the activity of this enzyme dropped to basal levels once again.

P. argentinus has a characteristic phyllobranchia constituted by a central axis which supports a row of lamellae on each side. Each gill lamellae comprises a connective-tissue central septum and is lined by a simple epithelium. The whole structure is surrounded by a thin cuticle. Figure 3 shows that the laminar epithelium of those gills coming from controls exhibited different nuclear morphologies. Elongated nuclei with condensed chromatin were observed in a subcuticule location; however spherical nuclei with several groups of chromatin protruding into the haemocele were more numerous.

Histological examination of the lamellae of surviving prawns at the end of the 96-h experiment showed that nitrite exposure caused disorganization of the epithelium, thickening and disruption of the cuticle, and the occurrence of pyknotic nuclei. These histological changes became more evident with exposure to increasing concentrations of nitrite. For example, the exposure of prawns to concentrations of 20 mg L^{-1} of nitrite caused oedema of the lamellae, disorganization of the epi-

Table 1. The LC_{50} (median lethal concentration) of nitrite on *Palaemonetes argentinus*.

Time (hs)	LC_{50}	confidence intervals		χ^2
		lower	upper	
24	103,07	93,08	115,51	73,74
48	91,94	79,81	106,59	49,19
72	82,39	71,33	94,26	61,18
96	62,53	55,47	69,38	153,72

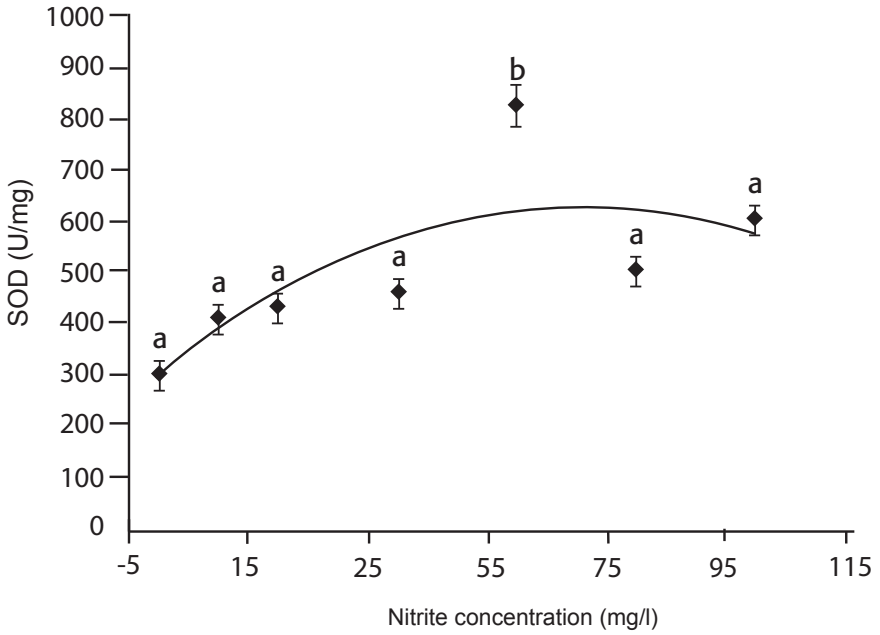


Figure 1. SOD activity in the whole prawn of *Palaemonetes argentinus* at different levels of ambient nitrite exposure. $y= -0.07x^2 + 9.5x + 294.28$; R= 0.76. Different letters indicate statistical differences ($p<0.05$).

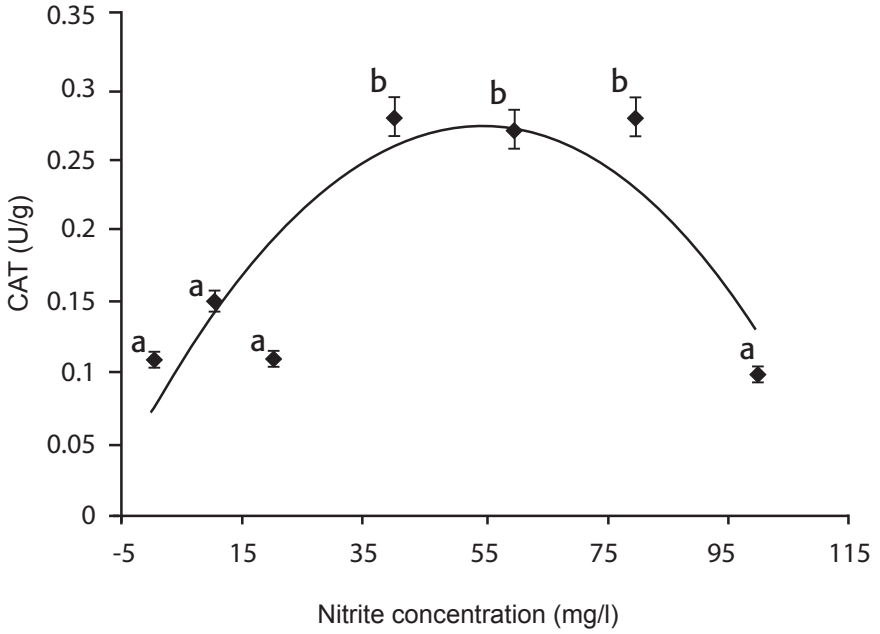


Figure 2. CAT activity in the whole prawn of *Palaemonetes argentinus* at different levels of ambient nitrite exposure. y= -7E-05x^2 + 0.01x + 0.07; R= 0.85. Different letters indicate statistical differences (p<0.05).

thelium and thickening and disruption of the cuticle (Figure 4), whereas exposure to 60 mg L^{-1} of nitrite caused folding of the epithelium and cuticle which resulted in the loss of the linear structure of the lamellae (Figure 5). Finally, exposure to 120 mg L^{-1} of nitrite affected the internal structure of lamellae, and the cytoplasmatic changes observed, made it impossible to identify the different cell types (Figure 6).

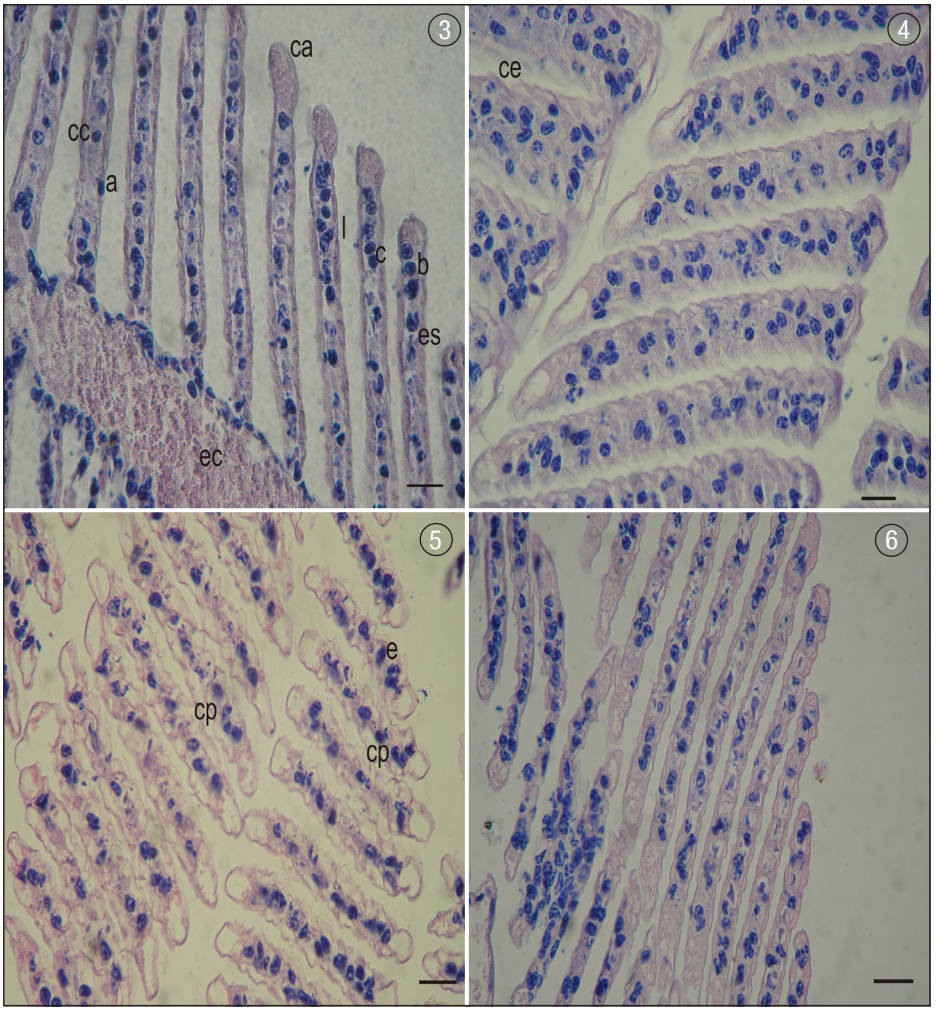
Figure 7 shows the histology of *P. argentinus's* hepatopancreas from control treatments (no addition of nitrites). Hepatopancreas is composed of many tubules lined by a simple epithelium composed of clearly differentiated cells, namely: embryonic (E), fibrillar (F), absortive (R), and blister-like (B). The main histopathological alteration was the retraction of the epithelium within the layer of laminar connective tissue that surrounds each tubule. Nitrite exposure caused disorganization of the epithelium, with the presence of bottle-like cells that protruded into the tubular lumen, loss of linearity of the basal membrane, and deterioration of laminar connective tissue (Figure 8).

DISCUSSION

The high density of crustaceans in aquaculture ponds is associated with a high production of waste products, including ammonia excreted by them, with the potential accumulation of ammonia and nitrite to toxic levels (Jensen, 2003). Acute toxicity tests provide important information on LC_{50} values to a toxic and about how the exposure time affects the toxicity of it. In the present study we observed a nitrite toxicity increases with the exposure time, given that the LC_{50} for nitrite were 103.07 and 62.53 mg L^{-1} at 24 and 96-h on *P. argentinus*, respectively. Similar results were observed in studies of other freshwater shrimp as *Macrobrachium nipponense* (De Haan, 1849) (Wang *et al.*, 2004) and Macrobrachium malcolmsonii (H. Milne Edwards, 1844) (Chand & Sahoo, 2006). Probably these result are due to the freshwater crustaceans are hyperosmotic to their environment and require an active uptake of ions across the gills to compensate for ions lost with the urine and a passive efflux across the gills. There is evidence that the nitrite has an affinity for the branchial Cl^- uptake mechanism, it is also known as a competitive inhibitor of chloride uptake (Jensen, 2003); therefore, the low concentration of Cl^- in the water increases influx of nitrite and further with increasing environmental concentration of nitrite.

The presence of toxic substances in the aquatic environment can readily promote the intracellular formation of reactive oxygen intermediates (ROS) and free radicals (RL) in the organisms. Detecting an increased activity of antioxidant enzymes such as SOD, CAT and glutathione peroxidase in aquatic organisms is considered a biomarker of oxidative stress (Sánchez-Rodríguez *et al.*, 2004). Nitrite is a natural component of the nitrogen cycle in ecosystems; however high concentrations of nitrite in the aquatic environment are a potential problem, because can be toxic. One of the toxicity mechanisms of nitrite on prawn is the imbalance between the rate production and the rate of breakdown of ROS and RL (Wang *et al.*, 2004). Experiments in our laboratory showed that SOD activity increased proportionally with the concentration of environmental nitrite, indicating an excessive production of superoxide anion (O_2^-.) by the organism. The rise registered in the activity of SOD likely increased the formation of hydrogen peroxide (H_2O_2), which is a substrate for CAT, and which also showed an increased activity under the same experimental conditions. Despite the evidence presented in this study, we believe that more research would be needed to better understand the antioxidant mechanism triggered by exposure to nitrite in aquatic crustaceans.

The simple lamellar epithelium of *P. agentinus* is compound for a single cell type known as "flange cells" (Sousa & Petriella, 2005). In the present study, histological analysis of the gills without exposure to nitrite, showed a regular lamellar epithelium formed by cells with different nuclear morphologies, those with spherical cores will correspond to flange cells, on the other hand, those cells with elongated nuclei might



Figures 3-6. 3) Cross section of branchial lamellae of *Palaemonetes argentinus* maintained without nitrite. a: elongated nuclei; c: spherical nuclei with numerous clusters of chromatin cc: connective central ec: central axis; is simple epithelium; l: slide. H&E. Scale bar: 30 µm. 4) Cross section of branchial lamellae of *P. argentinus* exposure at 20 mg L^{-1} of nitrite. e: edema; d: disorganized epithelium, ce: scaly cuticle. H&E. Scale bar: 30 µm. 5) Cross section of branchial lamellae of *P. argentinus* exposure at 60 mg L^{-1} of nitrite showing the loss of the linear structure of the lamella. e: epithelial folding c: folded cuticle. H&E. Scale bar: 30 µm. 6) Cross section of branchial lamellae of *P. argentinus* exposure at 120 mg L^{-1} of nitrite. The changes in the internal structure of the lamellae due to severe alterations of the epithelium are evidents. H&E. Scale bar: 50 µm.

correspond to the cell type termed "attenuated cells", by Taylor & Taylor (1992), which be a type of flange cells with very long lateral extensions. There are reports in the literature of histopathological effects caused by different nitrogenous compounds acting upon the gill of crustaceans, such as: hyperplasia, necrosis and changes in the lamellar epithelium, caused by ammonium (Rebelo *et al.*, 2000; Romano & Zeng, 2007; Díaz *et al.*, 2010). The above-described pathologies coincide with those observed in this study, which were evidenced by the loss of the linear structure of the lamella, and an increase in pyknotic nuclei with increasing concentration of nitrite in the medium.

In the present study, hepatopancreatic epithelium showed to be sensitive organ at nitrite pollution because showed changes, such as epithelial deterioration, hyperplasia and degenerative desquamation, which are accentuated with increasing concentration of the toxic. These alterations have been reported by other authors as markers of exposure to different stressors, such as salinity in *Artemesia longinaris* Spence Bate, 1888 (Masson *et al.*, 2012) and in *Palaemonetes argentinus* (Diaz *et al.*, 2010) and organochlorine pesticides in *P. argentinus* (Sousa & Petriella, 2007). Therefore, it can be concluded that the hepatopancreatic epithelium could serves for monitoring the quality of aquatic environment.

In summary, the median lethal concentration of nitrite in *P. argentinus* was 62.53 mg L^{-1} and its toxicity increased with time of exposure. The presence of nitrite in the water caused changes in the morphology of the gills and hepatopancreas. Thus, we propose that the histological examination of these organs could be a valid methodological approach to identify nitrite contamination in the freshwater prawn *P. argentinus*. The effects observed on the activity of the antioxidant enzymes SOD and CAT in this work, evidenced the involvement of antioxidant mecha-

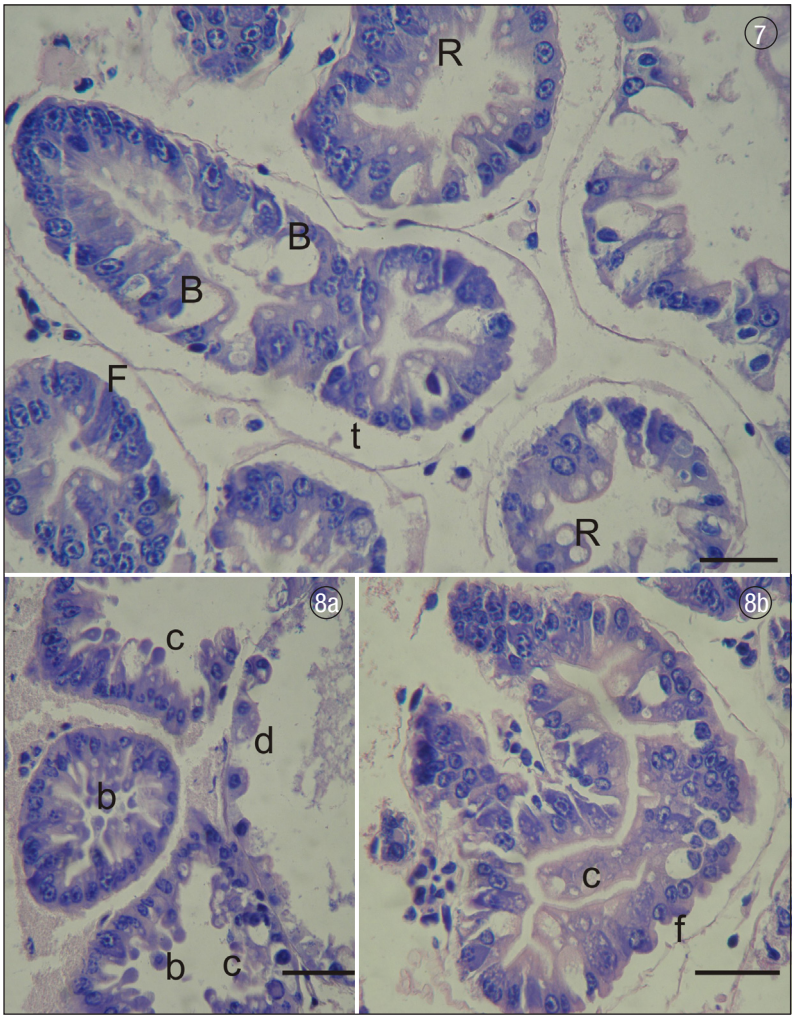


Figure 7-8a-b. 7). Cross section of hepatopancreas of *P. argentinus* maintained without nitrite. In fig. 8 B: cell B, F: Cell F, R: cell R, T: tubular retraction. H & E. Scale bar: 40 µm. 8a-b. 8a). Cross section of hepatopancreas of *P. argentinus* exposure at 120 mg L^{-1} of nitrite. b: cells in the bottle; c: protrusion of the cytoplasm; d: degenerative desquamation. H&E. Scales bar: 50 µm. 8b). Cross section of hepatopancreas of *P. argentinus* exposure at 60mg L^{-1} of nitrite. f: folding basement membrane. H&E. Scales bar: 50 µm.

nisms in nitrite detoxification. Future studies related to this latter topic, would be interesting for finding biomarkers of environmental stress.

References

American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1998. *Standard Methods for Examination of Water and Wastewater*. 20th ed. American Public Health association, Washington, DC. 1085 p.

Bell, T. A. & D. V. Lightner. 1988. *A handbook of normal Penaeid shrimp histology*. World Aquaculture Society. Allen Press, Inc., Lawrence. 114 p.

Boschi E. E. 1981. Decapoda natantia. *In*: Ringuelet, R.A. (Ed.). *Fauna de agua dulce de la República Argentina*. Volumen 26. PROFADU, Buenos Aires, Argentina, pp. 1–61.

Buikema, A. L. Jr., B. R. Niederlehner & J. Cairns, Jr. 1980. Use of Grass Shrimp in Toxicity Tests. *In*: Buikema, A. L. & J. Cairns (Eds.). *Aquatic invertebrate bioassays*. Virginia Polytechnic Institute and State University, pp. 155-173.

Cakmak, I., D. Strboe & H. Marschner. 1993. Activities of Hydrogen Peroxide-Scavenging Enzymes in Germinating Wheat Seeds. *The Journal of Experimental Botany* 44: 127-132.

Chand, R. K. & P. K. Sahoo. 2006. Effect of nitrite on the immune response of freshwater prawn *Macrobrachium malcolmsonii* and its susceptibility to *Aeromonas hydrophila. Aquaculture* 258: 150-156.

Chen, J. C. & Y. Lee. 1997. Effects of nitrite on mortality, ion regulation and acid-base balance of *Macrobrachium rosenbergii* at different external chloride concentrations. *Aquatic Toxicology* 39: 291-305.

Díaz, A. C., L. G. Sousa & A. M Petriella. 2002. Hepatopancreas structure of *Palaemonetes argentinus* (Decapoda, Caridea) fed different levels of dietary cholesterol. *In*: Escobar-Briones, E. & F. Alvarez (Eds.). *Modern Approaches to the Study of Crustacea*. Kluwer Academic/Plenum Publishers, New York, pp 67-73.

Díaz, A. C., A. V. Fernández Giménez, S. N. Mendiara & J. L. Fenucci. 2004. Antioxidant Activity in Hepatopancreas of the Shrimp (*Pleoticus muelleri*) by Electron Paramagnetic Spin Resonance Spectrometry. *Journal of Agricultural and Food Chemistry* 52: 3189-3193.

Díaz, A. C., E. I. Cuartas, S. M. Velurtas & J. L. Fenucci. 2010. Acute toxicity of nitrite on *Pleoticus muelleri* (Crustacea, Penaeoidea) postlarvae fed diets supplemented with astaxanthin. *Biocell* 34 (1): A6.

Finney, D. J. 1971. *Probit Analisys*. Cambridge Univ. Press, 333 p.

Jayachandran, K. V. 2001. *Palaemonid prawns. Biodiversity, taxonomy, biology and management*. Science Publishers, New Hampshire. 624 p.

Jensen, F. B. 2003. Review Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative Biochemistry and Physiology* A 135: 9-24.

Johnston, D. J., C. G. Alexander & D. Yellowhees. 1998. Epithelial cytology and function in the digestive gland of *Thenus orientalis* (Decapoda, Scyllaridae). *Journal of Crustacean Biology* 18: 271-278.

Li, E., L. Chen, C. Zeng, N. Yu, Z. Xiong, X. Chen & J.G. Qin. 2008. Comparison of digestive and antioxidant enzymes activities, haemolymph oxyhemocyanin contents and hepatopancreas histology of white shrimp, Litopenaeus vannamei, at various salinities. *Aquaculture* 274: 80-86.

Lin, Y. C. & J. C. Chen. 2003. Acute toxicity of nitrite on *Litopenaeus vannamei* (Boone) juveniles at different salinity levels. *Aquaculture* 224: 193-201.

Lombardi, J. V. 2004. Fundamento de toxicologia aquática. *In*: Ranzani-Paiva, M.J.T; Takemoto, R.M. & Lizama, M.A.P. (Eds.). *Sanidade de organismos acuáticos*. Livraria Varela Editora. São Paulo, pp. 263-272.

Magalhães, C., G. Bond-Buckup, G. A. De Melo & L. Buckup. 2003. Famílias Atyidae, Palaemonidae e Sergestidae. *In*: Melo, G. A. S. (Ed.). *Manual de identificacao dos Crustacea Decapoda de água doce do Brasil*. Editora Loyola, São Paulo, pp. 289-415.

Masson, I., A. C. Díaz & A. M. Petriella. 2012. Effect of salinity changes on the midgut gland of *Artemesia longinaris* (Decapoda, Penaeidae). *Latin American Journal of Aquatic Research* 40 (2): 358-366.

Mevel, G. & S. Chamroux. 1981. A study of nitrification in the presence of prawns (*P. japonicus*) in marine closed system. *Aquaculture* 23: 29-43.

Morrone, J. J. & E. C. Lopreto .1995. Parsimony analysis of endemicity of freshwater Decapoda (Crustacea: Malacostraca) from southern South America. *Neotropica* 41(105–106): 3–8.

Rebelo, M. F., E. M. Rodríguez, E. A. Santos & M. Ansaldo. 2000. Histopathological changes gills of the estuarine crab *Chasmagnathus granulata* (Crustacea-Decapoda) following acute exposure to ammonia. *Comparative Biochemistry and Physiology* 127C: 157-164.

Romano, N. & C. Zeng. 2007. Ontogenetic changes in tolerance to acute ammonia exposure and associated gill histological alterations during early juvenile development of the blue swimmer crab, *Portunus pelagicus. Aquaculture* 266: 246-254.

Sánchez-Rodríguez, M. A., E. Santiago-Osorio, L. A. Vargas & V. M Mendoza-Núñez. 2004. Propuesta de un constructo para evaluar integralmente el estrés oxidativo. *Bioquímica* 29 (3): 81-90.

Seneriches-Abiera, M. L., F. Parado-Estepa & G. A. Gonzáles. 2007. Acute toxicity of nitrite to mud crab *Scylla serrata* (Forskal) larvae. *Aquaculture Research* 38: 1495-1499.

Sokal, R. & J. Rohlf. 1995. *Biometry, the Principles and Practice of Statistics in Biological Research*. 3rd ed. W.H. Freeman, New York. 887 p.

Sousa, L. G. & A. M. Petriella. 2005. Gill morphology and ultrastructure of the prawn, *Palaemonetes argentinus* Nobili, 1901 (Decapoda, Caridea). *Crustaceana* 78 (4): 409-420.

Sousa, L. G. & A. M. Petriella. 2007. Functional morphology of the hepatopancreas of *Palaemonetes argentinus* (Crustacea: Decapoda): influence of environmental pollution. R*evista de Biología Tropical* 55 (1) (Suppl. 1): 79-86.

Stadtman, E. R. 2002. Importance of individuality in oxidative stress and aging. *Free Radical Biology & Medicine* 33 (5): 597-604.

Taylor, H. H. & E. W. Taylor. 1992. Gills and lungs: The exchange of gases and ions. *In*: Harrison, F. W. & A. G. Humes (Eds.). *Microscopic anatomy of invertebrate*. Wiley-Liss, New York, pp. 203-293.

Valavanidis, A., T. Vlahogianni, M. Dassenakis & M. Scoullos. 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicology and Environmental Safety* 64: 178-189.

Vogt, G. 1987. Monitoring of enviromental pollutants such as pesticides in prawn aquaculture by histological diagnosis. *Aquaculture* 67: 157-164.

Wang, W. N., A. L. Wang, Y. J. Zhang, Z. H. Li, J. X. Wang & R. Y. Sun. 2004. Effects of nitrite on lethal and immune response of *Macrobrachium nipponense. Aquaculture* 232: 679-686.

Winkaler, E., A. Silva, H. Galindo & C. Reis Martínez. 2001. Biomarcadores histológicos e fisiológicos para o monitoramento da saúde de peixes de ribeirões de Londrina, Estado do Paraná. *Acta Scientiarum* 23(2): 507-514.

Recibido: 07 de febrero de 2013.

Aceptado: 27 de agosto de 2014.