Histopathological and biochemical evidence of hepatopancreatic toxicity caused by cadmium in white shrimp, *Palaemonetes argentinus*

L.N. Chiodi Boudet, P. Polizzi, M.B. Romero, A. Robles, J.E. Marcovechio, M.S. Gerpe

**A R T I C L E   I N F O**

Article history:
Received 22 August 2014
Received in revised form 4 November 2014
Accepted 25 November 2014
Available online 15 December 2014

Keywords:
Cadmium
Sublethal toxicity
Histopathology
Lipid peroxidation
Freshwater shrimp

**A B S T R A C T**

Cadmium (Cd) is one of the most common pollutants in the environment and induces a range of tissue changes or damages and organ dysfunction. The histopathological effects of Cd and lipid peroxidation (LPO) on hepatopancreas of the freshwater shrimp, *Palaemonetes argentinus*, were studied. Shrimp were obtained from two lagoons with contrasting environmental quality, De los Padres (LP, impacted site) and Nahuel Rucá (NR, reference site), and were exposed to 3.06 and 12.24 µg Cd L⁻¹ for 3, 7, 10 and 15 days. The health status of both populations was also evaluated by histological analysis of control individuals. After exposure, shrimp were transferred to clean water for 28 days to evaluate the recuperation capacity of hepatopancreas. Control shrimp from NR exhibited a normal hepatopancreas structure; unlike control shrimp from LP which showed several alterations. These results were attributed to the different environmental quality of lagoons. The exposure to Cd resulted in several alterations in the histological structure of the hepatopancreas of both populations. The observed alterations included haemocytic and connective infiltrations in the intertubular space, erosioned microvilli, ripple of basal lamina, atrophied epithelium and necrosis, however, the latter was only observed in shrimp from LP. The exposure also caused an increase of LPO levels in both populations. *P. argentinus* was able to repair the hepatopancreas structure from the damage caused by Cd, evidenced by the histopathological results and LPO levels. Obtained results are indicating that the histological analysis of the hepatopancreas proved to be a highly sensitive method for evaluating water quality, in both environmental and laboratory conditions.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

The hepatopancreas is the major metabolic organ in decapod crustaceans, accomplishing intestinal, hepatic, and pancreatic functions (Saravana Bhavan and Geraldine, 2000). It is the main site of synthesis and secretion of digestive enzymes, absorption of nutrients, storage of metabolic reserves (lipid and glycogen), excretion of metabolic wastes (Al-Mohanna and Nott, 1989; Johnston et al., 1998) and biotransformation and detoxification of pollutants. The crustacean hepatopancreas is a sensitive organ and liable to injury by pollutants (Vogt, 1987; Bautista et al., 1994; Saravana Bhavan and Geraldine, 2000; Wu et al., 2008). It is essentially composed of branched tubules and of different types of epithelial cells (E-cells, R-cells, F-cells and B-cells) lining the tubules. It is possible to use the patterns of change in the cells of the organ as an index to determine the impact of contaminants (Hinton et al., 1973; Moore, 1985; Sousa, 2003). The histological diagnosis is a highly sensitive method showing the integral response of an organism to the impact of a toxicant under certain physiological, nutritional and environmental conditions (Vogt, 1987). Another advantage of histopathological diagnosis relates to its ability to effectively provide information on the health status of the organism (Costa et al., 2013). Many pollutants have been demonstrated to have hepatopancreatic toxicity, such as pesticides (Saravana Bhavan and Geraldine, 2000; Desouky et al., 2013; Walker et al., 2010) and heavy metals (Frias-Espericueta et al., 2008; Liu et al., 2013; Wu et al., 2008), resulting in histological alterations. Cadmium (Cd) is a ubiquitous metal in the environment and is released into the atmosphere, water and soil from industry,
agriculture and other human activities. In aquatic ecosystems, Cd has a high solubility in water and a very high capacity to bioaccumulate in many species. It is not essential to organisms, and indeed, it is known to be toxic at low exposure concentrations (Zang and Bolger, 2014). It has been demonstrated that Cd inhibits the repair of the DNA and causes lipid peroxidation (Radisa et al., 2007).

Lipid peroxidation (LPO) is considered as an important biomarker of cell damage as a result of the interaction of free radicals with membrane lipids (El-Beltagi and Mohamed, 2013). It has been used extensively to assess detrimental effects of several pollutants, such as polycyclic aromatic hydrocarbons (Lavarias et al., 2011), endosulfan, (Ballestero et al., 2009), cadmium, copper and zinc (Khan et al., 2011).

Histological alterations with other biomarkers can be used as tools to evaluate the toxicity of environmentally relevant chemicals in both a predictive and a retrospective way (Odendaal and Reinecke, 2003).

The shrimps of the genus *Palaemonetes* (Crustacea: Decapoda: Caridea) are considered as important animal models to be employed in the evaluation of pollution effects (Buikema et al., 1980, Key et al., 2006). Among them, the white shrimp *Palaemonetes argentinus* is an abundant freshwater shrimp, widely distributed in different countries of South America (Morrone and Loprete, 1995). Some publications have reported the sensitivity of *P. argentinus* to pollution in laboratory tests (Collins and Capello, 2006; Chiodi et al., 2013). Galanti et al., 2013) and proposed that this species might be used as a bioindicator to provide information on environmental quality (Montagna and Collins, 2007).

Histological changes as a result of the exposure to organic and inorganic pollutants has been described in shrimp species (Doughhie and Rao, 1984; Vogt, 1987; Saravana Bhavan and Geraldine, 2000; Kutlu et al., 2005; Wu et al., 2008), however, there are few related studies on Cd effects on the histological structure of hepatopancreas, even though it is a common pollutant (Wu et al., 2008; Liu et al., 2013).

Therefore, the objective of this study was to determine the histopathological alterations induced by Cd in hepatopancreas of the freshwater shrimp *P. argentinus* from both polluted and unpolluted lagoons. The health status of both populations was evaluated by analyzing control individuals. The LPO was measured as a marker of cell damage as a result of the interaction of free radicals with membrane lipids (El-Beltagi and Mohamed, 2013). The animals were cared for in accordance with guidelines of the Institutional Committee for Care and Use of Laboratory Animals (CICUAL, acronym in Spanish) of Mar del Plata University, based on the “Guide for the Care and Use of Laboratory Animals” (2010, 8th Edition, National Research Council, The National Academies Press, Washington DC) and Directive 2010/63/UE of the European Parliament and of the Council on the protection of animals used for scientific purposes.

2. Materials and methods

2.1. Collection and maintenance of organisms

Shrimp were obtained from two shallow lagoons situated in the southeastern area of Buenos Aires Province, Argentina. One of these sites (Nahuel Rucá lagoon – NR – 37°37’S–57°25’W) is considered as an unpolluted environment (Chiodi Boudet et al., 2010), whereas the other lagoon (De los Padres lagoon – LP – 37°57’S, 57°44’W) was previously characterized as a polluted environment (Chiodi Boudet et al., 2008). Sediments of LP lagoon have high metal concentrations (0.7 μg Cd g⁻¹; 1 μg Hg g⁻¹; 72 μg Cr g⁻¹; 15.3 μg As g⁻¹; 119 μg Zn g⁻¹; Chiodi Boudet et al., 2008) that exceed levels considered as safe for the biota (Cd: 0.6 μg g⁻¹; Hg: 0.17 μg g⁻¹; Cr: 37.3 μg g⁻¹; As: 5.9 μg g⁻¹; Zn: 123 μg g⁻¹; CCME, 2002). In contrast, sediments of NR have low metal concentrations (0.15 μg Cd g⁻¹; 0.02 μg Hg g⁻¹; 28 μg Zn g⁻¹; Chiodi et al., 2010), making to this area a good reference site. Previous studies conducted in these two shrimp populations revealed different tolerance to Cd, as a consequence to the environmental quality (Chiodi Boudet et al., 2013).

Shrimp (adults of both sexes, at sexual rest) were collected with a hand net and immediately transferred to the laboratory. Acclimation was performed in aquaria (140 L) with gently aerated freshwater and 12:12 h light/dark photoperiod for 3 days, as recommended for genus *Palaemonetes* by Buikema et al. (1980). Water temperature was maintained at 17.0 ± 0.9 °C, pH at 8.30 ± 0.05 and water hardness was 235 mg CaCO₃ L⁻¹. Shrimp were daily fed with flake food (Tetramin), and the content of Cd was < 0.05 μg Cd g⁻¹. During acclimation, those groups that had more than 2% mortality were not used for the experiments following the criteria establish by Khan et al. (1988).

The animals were cared for in accordance with guidelines of the Institutional Committee for Care and Use of Laboratory Animals (CICUAL, acronym in Spanish) of Mar del Plata University, based on the “Guide for the Care and Use of Laboratory Animals” (2010, 8th Edition, National Research Council, The National Academies Press, Washington DC) and Directive 2010/63/UE of the European Parliament and of the Council on the protection of animals used for scientific purposes.

2.2. Reagents

The stock solution of Cd (613 mg Cd L⁻¹) was prepared from cadmium chloride (≥ 99.99%, Sigma-Aldrich Chemical Corporation, USA) and double distilled water (ddH₂O). The different Cd concentrations assayed were prepared using a dilution series of the stock solution. The analytical Cd concentrations of each treatment at the beginning of the experiment were measured by Anodic Stripping Voltammetry (ASV), applying a modification of the technique described by Andrade et al. (2006) with a detection limit < 5 μg Cd L⁻¹. A commercial standard of Cd (1000 mg Cd, CdCl₂ in H₂O, Titrisol Merck) was used for calibration.

2.3. 15 Days exposure and depuration assay

For each experiment, shrimp were randomly divided into 16 groups (exposure + depuration) allocated to controls and treatments. Each experimental treatment consisted of 100 shrimps, which were transferred into 20 L experimental glass aquaria.

The exposure concentrations were selected based on previous studies and LC₅₀ values for Cd of each shrimp population (LC₅₀ > 96 h: 24.50 and 12.26 μg Cd L⁻¹ for LP and NR population, respectively) (Chiodi et al., 2013). Therefore, the shrimp of LP population were exposed to 3.06 and 12.26 μg Cd L⁻¹ (corresponding to 1/8 and 1/2 of the 96 h LC₅₀) for 3, 5, 7 and 15 d. The shrimp of NR population was only exposed to 3.06 μg Cd L⁻¹ (1/4 of the 96 h LC₅₀) due to the concentration 12.26 μg Cd L⁻¹ is lethal for 50% of exposed population at 96 h. The shrimp were fed in the exposure tanks before the renewal of the medium; retiring uneaten food immediately. After exposure, shrimp were transferred to clean water for 7, 14, 21 and 28 days of depuration. Each exposure and depuration treatment had its corresponding control. The exposure medium was renewed every 48 h during the course of experiments.

All other conditions were kept the same as those used for acclimation.

2.4. Histopathological observation

For the histopathological studies, 4 individuals in intermoult were selected from each treatment. The moult stage was determined by microscopic examination of the developmental stage of the exopodite setae of the uropods, following the criteria established by Díaz et al. (1998).

Each hepatopancreas was carefully dissected and immediately fixed in Davidson’s solution (ethanol, formol, acetic acid and
water) (Bell and Lightner, 1988) for 24 h and transferred to 70% ethanol. After dehydration in a graded ethanol series to absolute ethanol, each hepatopancreas was embedded in paraffin. Slices of 3 μm were obtained from each organ with a conventional microtome and then were stained with hematoxylin–eosin. Four histological slides of each hepatopancreas were examined by light microscopy (400 ×); sixty fields were observed in each slide (240 fields for each shrimp).

Following the scale suggested by Zodrow et al. (2004), the degree of histological damage was scored according to the percentage of the total fields with histological damage found out of the total observed in the four hepatopancreas of each treatment. Scores was based on the number of fields in which histological changes were observed with (−−)=no histopathology in any field, (+)=mild histopathology present in <25% of the fields, (++)=moderate histopathology present in 25%–75% of the fields, and (+++)=severe histopathology present in >75% of the fields.

2.5. Lipid peroxidation assay

Shrimp hepatopancreas for lipid peroxidation assay were rapidly dissected, frozen in liquid nitrogen and stored at −80 °C. Total lipid peroxidation was measured according to Oakes and Van Der Kraak (2003) based in the formation of thiobarbituric acid reactive substances (TBARS) with a detection limit of 0.099 nmoles gr⁻¹. Fluorescence was measured (Fluroska Asc-320–700 nm, Thermo Scientific) by excitation at 515 nm with an emission at 553 nm. The concentration was expressed as nmol of TBARS per gram of tissue (wet weight), using tetramethoxypropane (TMP) as external standard.

2.6. Statistical analysis

Statistical analyzes were performed using the program STATISTICA version 8.0 (Statsoft, Inc.). The percentages of observed damage were previously transformed to the arcsine data to test significant differences (Zar, 2010). Differences between treatments, exposure times and lagoons were assessed by parametric tests: t-test and analysis of variance (ANOVA) followed by the post-hoc
Tukey or Duncan test, or non-parametric tests: U-Mann–Whitney and Kruskal–Wallis with post-hoc Dunn test; being previously checked the variance homogeneity by Levene's test (Zar, 2010). The significance level was $p < 0.05$.

3. Results

For all the assays, the analytical values of Cd in solution were between 95% and 99% of the nominal value. The effective concentration (mean ± standard deviation, $n=3$) for 12.26 μg Cd L$^{-1}$ was 11.98 ± 0.22 μg Cd L$^{-1}$. The lowest concentration (3.06 μg Cd L$^{-1}$) was not included in the analysis because it was below the detection limit of the method.

3.1. Histological observations of control individuals

The results showed that hepatopancreas of control individuals from NR presented a normal functional structure, with the differentiation of the four characteristic cell types (Fig. 1A and B). The E cells were found in the distal blind end of tubules, whereas the other three types (F, R and B) were found in the middle and proximal zones, being R the most abundant cells. The B cells were more frequent at the proximal zone of the tubules, where desquamation of this cells together with the adjacent R cells it was observed (Fig. 1B). The F, R and B cells, in contrast to E, presented microvilli (Fig. 1C). No changes in the structure of control individuals from NR were observed throughout the experiences (Table 1).

As shown in Table 1, control individuals from LP presented numerous alterations. Moderate haemocytic and connective infiltration was observed within the intertubular space. Several tubules showed a wide irregular lumen with mild atrophied epithelium, which had erosioned microvilli (Fig. 1D and E). Some epithelial cells were completely separated from basal lamina, that it was rippled (Fig. 1F). These alterations, mainly located in the proximal zone of the tubules, where desquamation of this cells together with the adjacent R cells it was observed (Fig. 1B). The F, R and B cells, in contrast to E, presented microvilli (Fig. 1C). No changes in the structure of control individuals from NR were observed throughout the experiences (Table 1).

3.2. Effects of Cd on the hepatopancreas structure

After exposure to 3.06 μg Cd L$^{-1}$ several alterations to the hepatopancreatic tissue of P. argentinus of both populations were observed (Table 1). In the case of shrimp from NR, after 7d of exposure, some epithelial cells showed shortening and erosion of the microvilli, as well as mild haemocytic infiltrations. After 15d of exposure, a significant increase of haemocyte infiltration was observed (from mild to moderate, Table 1) (ANOVA with post-hoc Tukey test, $p < 0.05$). The tubes showed mild atrophied epithelium with large number of dysplasic B-cells (Fig. 2A and B), and the basal lamina was rippled (Fig. 2C). In all cases, the lesions were mainly located in the medullary region of the organ.

In the case of shrimp from LP exposed to 3.06 μg Cd L$^{-1}$ at 3d, showed similar alterations to those observed in 3d and 7d controls (Table 1). After 10d of exposure, an increase of haemocyte infiltration was observed (from moderate to severe, Table 1) (ANOVA with post-hoc Tukey test, $p < 0.05$), as well as a severe erosion of epithelial cell microvilli. At the end of the experiment (15d), the tubes showed a moderate atrophied epithelium and presence of necrotic cells (pyknosis, karyolysis and karyorrhexis) (Fig. 2E and F). It should be noted that at the same concentration and exposure time no signs of necrosis were observed in shrimp of NR. In the same way as NR shrimp, lesions were mainly located in the medullary region of the organ.

As was mentioned in Section 2.3, the exposure to 12.26 μg Cd L$^{-1}$ was conducted only in the LP population. Individuals exposed for 3 and 7d showed the same alterations as those observed in control shrimp (haemocytic and connective infiltrations, erosion of microvilli, irregular lumen, atrophied epithelium, ripple basal lamina) (Fig. 2G), although the degree of damage increased significantly from mild–moderate to moderate–severe (ANOVA with post-hoc Tukey test, $p < 0.05$) (Table 1). Starting from 10d, the presence of necrotic cells and a significant increase of atrophied epithelium (ANOVA with post-hoc Tukey test, $p < 0.05$) was

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>NR population</th>
<th>Depuration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.06 μg Cd L$^{-1}$</td>
<td>12.26 μg Cd L$^{-1}$</td>
</tr>
<tr>
<td>3d 7d 10d 15d</td>
<td>3d 7d 10d 15d</td>
<td>7d 14d 21d 28d</td>
</tr>
<tr>
<td>Ecological parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemocytic infiltration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eroded microvilli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrophied epithelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrotic cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatopancreas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depuration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemocytic infiltration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eroded microvilli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrophied epithelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrotic cells</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1
Histological damages observed in the hepatopancreas of P. argentinus from NR and LP populations during 15 days of exposure to 3.06 and 12.26 μg Cd L$^{-1}$ and 28 days of depuration. (−) No histopathology in any field, (+) mild histopathology present in < 25% of fields, (++) moderate histopathology present in 25%–75% of fields; and (+++) severe histopathology present in > 75% of fields.
observed with respect to 7d (Table 1) (Fig. 2H). At the end of the experiment (15d), most of the tubules presented necrotic cells (Fig. 2I), with a severe degree of damage (Table 1). It is noteworthy, that necrosis was observed at a reduced exposure time compared to that observed for 3.06 mg Cd L⁻¹ treatment.

3.3. Evaluation of recovery capacity of hepatopancreas to Cd exposure

During 28d of depuration a reversion of induced alterations by Cd exposure was observed in both populations. In the case of NR shrimp pre-exposed to 3.06 mg Cd L⁻¹ no improvements were observed until 21d, when a tubular reordering with significant decrease of haemocyte infiltrations (moderate to mild, Table 1) was evidenced (ANOVA with post-hoc Tukey test, p < 0.05). The basal lamina and most of B cells showed normal appearance, moreover, in this cellular type no dysplasia was observed. In much of the tubules was difficult to differentiate cell types, the cytoplasm was strongly basophilic with erosioned or poorly developed microvilli (Fig. 3A and B). At 28d of depuration, no evidence of atrophied epithelium and haemocytic infiltrations were observed; moreover, the four cell types (E, R, F and B) presented normal appearance (Fig. 3C).

As was observed in NR shrimp, individuals of LP showed no significant improvements in the hepatopancreas during the first two weeks of depuration, in both 3.06 mg Cd L⁻¹ and 12.26 mg Cd L⁻¹ treatments (Table 1). Starting from 21d of depuration, it was observed a tubular reordering, with significant decrease of haemocyte and connective tissue infiltrations from severe to moderate (ANOVA with post-hoc Tukey test, p < 0.05). Many epithelial cells showed poorly developed microvilli or even erosioned, and the basal lamina remained rippled (Fig. 3D). These changes were noted in shrimps pre-exposed to both 3.06 mg Cd L⁻¹ and 12.26 mg Cd L⁻¹, although the degree of recovery was significant lower for the latter (t-test, p < 0.05). At 21d of depuration, shrimp pre-exposed to 12.26 mg Cd L⁻¹ showed numerous necrotic cells, whereas in shrimp pre-exposed to 3.06 mg Cd L⁻¹ it was not observed. At 28d of depuration, individuals from both treatments

---

**Fig. 2.** Light micrographs of hepatopancreas of *P. argentinus* from NR lagoon exposed to 3.06 μg Cd L⁻¹: (A) overview of hepatopancreas after 15d of exposure showing disorganization of tubules and haemocyte infiltrations (h). Scale bar: 500 μm. (B) Cross section of tubules after 15d of exposure showing dysplasia (D) of B cells, connective tissue (ct) and erosioned microvilli (arrows). Scale bar: 50 μm. (C) Cross section of tubule after 15d of exposure showing rippled basal lamina separated from epithelial cells (arrow) and connective tissue (ct). Scale bar: 100 μm. Light micrographs of hepatopancreas of *P. argentinus* from LP lagoon exposed to 3.06 μg Cd L⁻¹: (D) Cross section of tubules after 10d of exposure showing irregular lumen (L), abundant connective tissue and haemocytic infiltration (h) within the intertubular spaces. Scale bar: 100 μm. (E) Cross section of tubules after 15d of exposure showing irregular lumen (L) and atrophied epithelium with pyknotic nuclei (p) and karyorrhexis (k). Scale bar: 100 μm. (F) Details of a tubule after 15d of exposure showing nuclei with karyorrhexis (k) and karyolysis (arrow). Scale bar: 50 μm. Light micrographs of hepatopancreas of *P. argentinus* from NR lagoon exposed to 12.26 μg Cd L⁻¹: (G) Overview of hepatopancreas after 7d of exposure showing disorganization of tubules, abundant connective tissue (ct) and haemocytic infiltrations (h). Scale bar: 500 μm. (H) Cross section of tubules after 10d of exposure showing nuclei with karyorrhexis (k) and karyolysis (arrow). Scale bar: 50 μm. (I) Details of a tubule after 15d of exposure showing atrophied epithelium with pyknotic nuclei (p) and karyorrhexis (k) and rippled basal lamina separated from epithelial cells (arrows). Scale bar: 50 μm.
showed a significant decrease of atrophied epithelium and haemocyte infiltrations (ANOVA with post-hoc Tukey test, \( p < 0.05 \)), even though some tubules presented rippled basal lamina. It should be noted that shrimp pre-exposed to 12.26 \( \mu \)g Cd L\(^{-1} \) still showed necrotic cells (Fig. 3E).

### 3.4. Effects of Cd on LPO levels

The exposure of *P. argentinus* from NR to 3.06 \( \mu \)g Cd L\(^{-1} \) did for 3, 7 and 10d not produce an increase in LPO levels (Fig. 4A). However, at 15d a significant increase (\( t \)-test, \( p < 0.05 \)) with respect to control was observed. During depuration, LPO levels at 7 and 14d were significantly higher with respect to control (\( t \)-test, \( p < 0.05 \)), but not for 21 and 28d (Fig. 4A). In addition, LPO levels at 7d were significant higher (ANOVA with post-hoc Duncan test, \( p < 0.05 \)) compared to 14, 21 and 28d. It should be noted that the levels found at 7d of depuration showed no differences from those observed at 15d of exposure. LPO levels of control shrimp did not show differences during both exposure and depuration period.

A similar pattern was observed in shrimp from LP exposed to 3.06 \( \mu \)g Cd L\(^{-1} \); showing a significant increase (\( t \)-test, \( p < 0.05 \)) respect to control only at 15d of exposure, which in turn were significantly higher than those for 7 and 10d (Kruskal–Wallis with post-hoc Dunn test, \( p < 0.05 \)). During depuration, it was observed a gradual decrease in LPO levels (Fig. 4B); with the maximum values at 7d, and significantly higher than its control (\( t \)-test, \( p < 0.05 \)) and significantly higher than those for 21 and 28d (ANOVA with post-hoc Tukey test, \( p < 0.05 \)). Moreover, it should be noted that 7d levels did not show differences with respect to those at 15d of exposure, as was observed in shrimp from NR. The LPO levels in control shrimp were unchanged along Cd exposure and depuration. At 10 and 15d of exposure to 12.26 \( \mu \)g Cd L\(^{-1} \), shrimp from LP showed a significant increase in LPO levels relative to 3 and 7d (ANOVA with post hoc Tukey test, \( p < 0.05 \)) (Fig. 4C). In addition, these levels were significantly higher than their respective controls (\( t \)-test, \( p < 0.05 \)). Meanwhile, LPO levels in control shrimp showed no significant differences between them in both exposure and depuration. During depuration, shrimp from LP pre-exposed to 12.26 \( \mu \)g Cd L\(^{-1} \) showed a gradual decrease in LPO levels (\( p < 0.05 \)) (Fig. 4C). During the first two weeks of depuration (7 and 14d) the maximum levels were observed, although, only 7d levels presented significant differences with its control (\( t \)-test, \( p < 0.05 \)). In addition, it should be noted that 7 and 14d levels did not show differences respect to those observed at 15d of exposure. The LPO levels in control shrimp were similar along Cd exposure and depuration.

### 4. Discussion

Structural responses in different organs of invertebrates have been shown to be useful tools to characterize the health status of organisms (Triebeskorn et al., 1991; Sousa et al., 2005), and at the same time, to assess the impact of environmental contaminants in organisms exposed in the laboratory (Hinton et al., 1973; Moore, 1985; Vogt, 1987; Sousa, 2003). In this study, control shrimp from NR exhibited a well-organized glandular tubular structure, similar to that described for other species of shrimp (Saravana Bhavan and Geraldine, 2000; Li et al., 2007; Wu et al., 2008), indicating good nutritional status of individuals and normal function of the gland. Its structure comprises a mass of blind tubules with scarce intertubular space occupied by hemolymph. Each tubule is differentiated into three zones: blind distal, medial and proximal. The distal and medial zones of the tubules, displaying an irregular and narrow lumen, constitute the cortical region of the gland, while the proximal zones form the medullar region. Each tubule
comprises a simple epithelium with four cellular types (E, F, R and B). It was reported that E-cells are embryonic, F-cells synthesize proteins, R-cells absorb nutrients and are involved in detoxification process, and B-cells have a secretory function not only in *P. argentinus* but also in other shrimp species (Bell and Lightner, 1988; Al-Mohanna and Nott, 1989; Sousa et al., 2005; Li et al., 2007). The desquamation of B-cells (and adjacent R), observed at the proximal zone of tubules, is part of the normal mechanism of epithelial renewal, in which the loss of cells is compensated by the mitotic activity of E-cells in the distal end of tubules. This is consistent with previously reported results for the studied species by Sousa (2003) and also for the shrimp *Penaeus semisulcatus* (Al-Mohanna and Nott, 1989).

In contrast, control shrimp from LP showed several alterations in the hepatopancreatic tissue, including haemocytic and connective infiltration, rippled basal lamina, atrophied epithelium and eroded microvilli. Similar alterations were reported in decapod crustaceans exposed to various contaminants, such as Cu (Frías-
Espericueta et al., 2008) and Zn (Wu et al., 2008) in Litopenaeus vannamei, and Cd in Sinoprotomam hennanense (Liu et al., 2013). Some of these pollutants have been reported in sediments of LP lagoon (Chiody Boudet et al., 2008) at concentrations that exceed the limits established as safe for aquatic biota (CCME, 2002). The hepatopancreas in crustaceans is analogous to the liver in higher organisms, which is a sensitive organ and liable to be damaged by environmental pollutants (Baticados and Tendencia, 1991). The above mentioned structural damages in hepatopancreas of P. argentinus significantly affected its absorption, secretion and digestion functions, which could have serious implications in the physiology and health of shrimp. Ituarte (2008) studied the life-history traits of several populations of P. argentinus from Buenos Aires province, showing that shrimp of LP presented: (1) smaller size and weight, (2) low percentage of ovigerous females during the breeding season (<50%), (3) low fertility, (4) fewer eggs and (5) high loss of eggs (47%); suggesting a low level of fitness. Considering that in crustacean there is a correlation between the physiological condition of the organism and the structure of hepatopancreas (Popescu-Marinescu et al., 1997), the reported traits by Ituarte (2008) are likely to be a consequence of the morphological alterations found here for shrimp from LP. We can conclude that differences observed between control individuals of both populations are related to environmental quality, evidencing the high sensitivity of hepatopancreas to pollutants and environmental quality changes. Moore (1985) considered that alterations of structure–function–organization are more sensitive than biochemical parameters due to small cell populations being susceptible to the initial damage. Therefore, histological observation of hepatopancreas allowed to evaluate the health status of both populations.

In addition, both the structural and biochemical alterations of hepatopancreas of P. argentinus exposed to sub-lethal concentrations of Cd were investigated in the present study. The exposure caused structural alterations in the hepatopancreas of both populations, including tubular disorganization, haemocyte infiltration, eroded microvilli, ripple and detachment of the basali lama, atrophied epithelium and in some cases necrosis. These symptoms are likely common and typical responses when shrimp are exposed to toxicants, since the same symptoms were also reported in other shrimp species exposed to endosulfan (Saravana Bhavan and Geraldine, 2000), Zn and Cd (Wu et al., 2008) and Cd (Revathi et al., 2011); and for crabs exposed to Cu (Yang et al., 2007), and methyl parathion (Bianchini and Monserrat, 2007). Likewise, similar alterations were observed in studies that analyzed the effects of Cd on other decapod species (Revathi et al., 2011; Wu et al., 2008).

A common pathology to all damaged hepatopancreas and the first to appear was the haemocyte infiltration. Haemocytes of the blood are capable to remove ionic metals from plasma and transfer them to sites of excretion of the organism (Ahearn et al., 2004), playing an active role in the metabolism of heavy metal (Cheng and Sullivan, 1984). This function is achieved by a combination of phagocytosis, nodule formation, and encapsulation reaction (Martin and Hose, 1992). Our results are consistent with previously reported by Bubel (1976). This author reported an increase of haemocytes in the marine isopod Jaera nordmanni during exposure to heavy metals (Cd, Hg and Cu), postulating that the increased of haemocytes could be associate with the phagocytosis of heavy metals, playing thus a protective role.

Most of the alterations in the hepatopancreas of both populations of P. argentinus were focused on the medullar region of the organ, where medial and proximal zones of the tubules are located. R-cells are the most numerous cell types in these zones (Sousa and Petriella, 2000). This type of cell absorbs and stores nutrients in the cytoplasm and is also involved in detoxification of heavy metals (Vogt, 1987; Saravana Bhavan and Geraldine, 2000). Among the different types of cells in the tubular epithelium of the hepatopancreas, R-cells have been the most readily and severely affected (Vogt and Quinitio, 1994; Bautista et al., 1994; Wu et al., 2008); and due to have not an apocrine secretion, contaminants remain inside the cells until the cellular death (Vogt and Quinitio, 1994; Sousa and Petriella, 2001). This accumulation could be the reason of the pattern of alterations observed in P. argentinus. Differences between populations for shrimp exposed to 3.06 μg Cd L−1 were notable. Shrimp from LP presented higher degree of damage (moderate-severe) than shrimp from NR (mild to moderate); and in addition, the necrotic cells were only observed in shrimp of LP. These differences are the consequence of the accumulation of effects, which in the case of shrimp from LP are mainly related to the initial health status of them. Therefore, this poor status is directly related to the quality of their environment, determining the seriousness of the alterations. Despite the hepatopancreas of shrimp from LP presented previous alterations, the exposure to 3.06 and 12.26 μg Cd L−1 show differences, indicating the high sensitivity of the organ to changes in the water quality.

The alterations in the structure of hepatopancreas observed in control of LP and both populations exposed to Cd were restored when they were transferred to a contaminant-free medium. Control shrimp of LP presented the first structural improvements at 10d of the exposure assay, showing a complete recovery at the beginning of depuration assay. Similarly, Sousa (2003) also reported the recovery of hepatopancreas in this population of P. argentinus, with tubular rearrangement and marked decrease of haemocyte infiltration after 15d of removed from the lagoon, reaching complete recovery at 30d. The shrimp of LP and NR exposed to 3.06 μg Cd L−1 showed the first improvements at 21d, and the complete recovery of hepatopancreas was observed at the end of depuration period (28d). The individuals of LP exposed to 12.26 μg Cd L−1 could not reach full recovery in 28d, probably the level of damage was more severe, and therefore require a longer period of time. Our results clearly demonstrated that P. argentinus hepatopancreas have a great capacity to recover its normal structure. Sousa (2003) reported that the hepatopancreas of P. argentinus has a high rate of cell renewal, explaining the observed fast recovery. It should be note that such capacity can be affected if the lesions compromise the entire organ (Sousa, 2003).

The biochemical alterations caused by Cd in hepatopancreas of P. argentinus were studied through LPO determination. Lipid peroxidation is one of the main biomarkers of oxidative damage, which plays a crucial role in the toxicity of many xenobiotics (Badisa et al., 2007). It has been reported that increased LPO is one of the major contributors to the loss of cell function (Hermes-Lima et al., 1995). Our data confirmed that intoxication with 3.06 and 12.26 μg Cd L−1 after 15 and 10d of exposure, respectively, caused a statistically significant increase in LPO concentration. It is noteworthy that only in the shrimp from LP an association between necrosis and increase in LPO levels was observed. These results would be confirming the above mentioned, that histopathological analysis constitutes a higher sensitivity biomarker to evaluate hepatopancreatic cellular damage than biochemical determinations. Although necrosis in hepatopancreas is not necessarily due to specific pollutants, several authors reported that necrosis is strongly associated with oxidative stress (Choi et al., 2009; Li et al., 2000; Lei et al., 2011). Pollutants, such as pesticides (Bianchini and Monserrat, 2007) and heavy metals (Badisa et al., 2007), are associated with increased cytosolic free radical concentrations, which may increase programmed cell death or disturb cell homeostasis and cellular necrosis (Abdel-Moneim et al., 2012).
5. Conclusions

The histological analysis of control individuals proved that a shrimp population from contaminated lagoon (LP) presents a lower health status than reference lagoon (NR); these different conditions are closely related to their environmental quality. In addition, we demonstrated that Cd exposure can induce morphological and biochemical changes in hepatopancreas structure of both studied populations. The scoring of the alterations allowed the establishment of different degrees of damage. The shrimp from LP presented a higher damage than those of NR and necrotic cells. *P. argentinus* demonstrated a high capacity to recover its normal hepatopancreas structure. The biochemical response (lipid peroxidation) was only evident after longer exposure times than the first structural alterations occurred. Histological analysis of the hepatopancreas proved to be a highly sensitive method for evaluating water quality, in both environmental and laboratory conditions.

Acknowledgements

This work was partially supported by grants from National Scientific and Technical Research Council, CONICET (PIP 0348/2010) and Mar del Plata University (EXA547/11). Authors are very grateful to Mr. Pedro Urrutia for the assistance during sampling and to Mr. Lautaro Rodriguez Gerpe for preparing the figures.

References


Popescu-Marinescu, V., Manolache, V., Natasescu, M., Marinescu, C., 1997. Struc-
tural modifications induced by copper in Astacus leptodactylus (Crustacea: 
Revathi, P., Vasanithi, L., Munuswamy, N., 2011. Effect of cadmium on the ovarian 
development in the freshwater prawn Macrobrachium rosenbergii (De Man). 
Saravana Bhavan, P., Geraldine, P., 2000. Histopathology of the hepatopancreas and 
gills of the prawn Macrobrachium malcolmsonii exposed to endosulfan. Aquat. 
Toxicol. 50, 331–339.
Sousa, G., 2003. Estudio sobre las modificaciones tisulares del camarón Palaemo-
netes argentinus (Crustacea, Decapoda, Caridea). Su empleo como indicadora de 
contaminación. Universidad Nacional de Mar del Plata p. 138 (Tesis doctoral).
cells in the hepatopancreas of Palaemonetes argentinus (Crustacea, Decapoda, 
Sousa, G., Petriella, A., 2000. Histology of the hepatopancreas of the freshwater 
prawn Palaemonetes argentinus (Crustacea, Caridea). Histology of the hepatopancreas of the freshwater 
Sousa, G., Petriella, A., 2001. Changes in the hepatopancreas histology of Palaemo-
Triebskorn, R., Köhler, H.-R., Zahn, T., Vogt, G., Ludwig, M., Rumpf, S., Kratzmann, 
M., Alberti, G., Storch, V., 1991. Invertebrate cells as targets for hazardous 
Vogt, G., 1987. Monitoring of environmental pollutants such as pesticides in prawn 
Vogt, G., Quinitio, E., 1994. Accumulation and excretion of metal granules in the 
prawn, Penaeus monodon, exposed to water-borne copper, lead, iron and cal-
posure to the pesticide methoprene on the hepatopancreas of a non-target 
dence of hepatopancreatic toxicity caused by cadmium and zinc in the white 
Yang, Z.-B., Zhao, Y.-L., Li, N., Yang, J., 2007. Effect of waterborne copper on the 
microstructures of gill and hepatopancreas in Eriocheir sinensis and its in-
duction of metallothionein synthesis. Arch. Environ. Contam. Toxicol. 52, 
222–228.
toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in zebrafish. Aquat. 
Toxicol. 66, 25–38.