# Functional morphology of the hepatopancreas of *Palaemonetes argentinus* (Crustacea: Decapoda): influence of environmental pollution

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**Abstract:** We analyzed the morphological and functional state of hepatopancreas in *Palaemonetes argentinus* from two environments with different pesticide concentrations. Los Padres lagoon (Argentina) is an area subjected to contamination due to the slow exchange of water, the shallow depth and the input of contaminated water. Prawns living in this lagoon accumulate high amounts of organochlorine pesticides in their tissues. Hepatopancreas of prawns from Canal 5, an adjacent shallow stream where the amount of pesticides is below toxic levels, and from Los Padres lagoon were processed by standard histological techniques with light microscopy and transmission electronic microscopy. At Los Padres lagoon, we found important tissular alterations, such as intertubular infiltration of haemocytes and connective tissue, epithelial retraction in some tubules, and a folded basal lamina. Important necrotic desquamation, with cariolysis, cariorrexis and lack of cellular details were also observed. Numerous tubules presented an enlarged and irregular lumen with the epithelium atrophied or completely absent. In general, the lesions were particularly located in the medullar region of the organ. At the ultrastructural level, R and F cells were the most damaged. Both cell types had nuclear retraction, chromatin condensation and cytoplasmic lysis. Some R cells also had dilated mitochondria and numerous lysosomes, and the basal cytoplasm was nearly completely lysed. The hepatopancreas of prawns from Canal 5 did not evidence any alterations. The histopathological study of the hepatopancreas is a highly sensitive tool to evaluate the physiological condition of prawns and water quality. Other environmental conditions were similar, so it can be assumed that pollutants were the main cause of organ deterioration. Rev. Biol. Trop. 55 (Suppl. 1): 79-86. Epub 2007 June, 29.

**Key words:** Decapoda, Caridea, contamination, histology, functional morphology, hepatopancreas.

*Palaemonetes argentinus* Nobili, 1901 is one of the most widely distributed decapods in Paraguay, Uruguay, southern Brazil and the littoral region of Argentina (Boschi 1981, Morrone and Lopreto 1995). It inhabits coastal lagoons along the southwestern Atlantic Ocean, such as Mar Chiquita lagoon (Argentina, 37°45’ S, 57°26’ W), where salinity is very low during extended periods (1-5 ‰) or can vary between 1 and 30 ‰ within a few hours (Charmantier and Anger 1999), and lives in freshwater environments. This prawn plays an important role in the trophic network of the environments it inhabits (Spivak 1997, Collins 1999). Around Mar del Plata zone (Pcia. Bs. As., Argentina) some of the environments inhabited by this species, as Los Padres lagoon (37°57’ S, 57°44’ O), are affected by the direct discharge of chemicals from the terrestrial ecosystems. This lagoon is an area subjected to contamination due to the slow exchange of water, the shallow depth and the input of contaminated water from the homonymous stream that runs through an active farming area, carrying an important amount of particulated material (González

Sagrario *et al*. 1998). Previous studies showed that *P. argentinus* accumulates high amounts of organochlorine pesticides (OCPs) in its tissues (González Sagrario *et al*. 1998, Miglioranza *et al.* 2002). OCPs are stored in fatty tissues, so they are biologically available and physiologically active only when fat tissues are metabolized. Consequently, individuals show no ill effect until fat reserves are mobilized. Accordingly it is difficult to be precise about the potential effect of these contaminants based on tissue concentrations only. All the previous contamination studies carried out with this species have been biochemical ones; therefore it is suggested to include also cellular and tissular aspects in future studies.

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). The morphology and histology of *P. argentinus* hepatopancreas were described previously, and were observed important tissular dynamics associated with the molting cycle (Sousa and Petriella 2001). The hepatopancreas is a composed tubular gland; each tubule comprises a simple epithelium with four cellular types (E, F, R y B); E-cells are embryonic, F-cells synthesize proteins, R-cells absorb nutrients and are involved in detoxification processes, and B-cells have a secretory function.

The present work analyzes the morphological and functional state of the hepatopancreas of the prawn *P. argentinus* from two environments with different levels of contamination by organochlorine pesticides, as a previous step to the experimental evaluation of the effect of different pesticides on growth, survival and functional morphology of the organ.

## MATERIALS AND METHODS

The prawns were collected in Argentina from Los Padres lagoon and Canal 5 (37°28' S, 57°17' O). The last one is a shallow stream, tributary of Mar Chiquita lagoon, where the amount of pesticides is below toxic levels and is not related to adverse effects on the biota (Menone 1999).

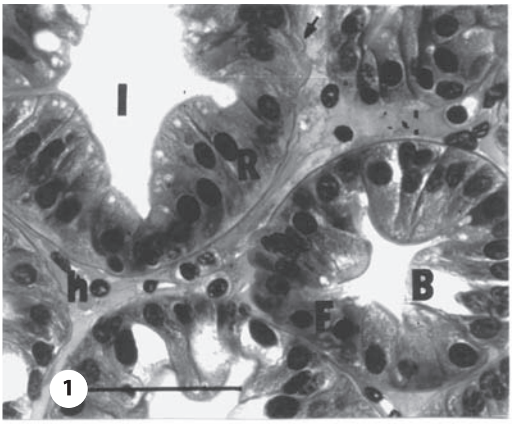
Sixty-eight adult prawns of both sexes at sexual rest, weighing 0.10-0.20 g and from 22.2 to 29.6 mm total length, were analyzed at different molt stages (Intermolt: C, Premolt: $D_0$-$D_2$ and Postmolt: A-B). The molt stage was determined by microscopic examination of the setae of the uropod exopodites, following the criteria established by Díaz *et al.* (1998). For histological description, the hepatopancreas were fixed in Davidson fluid, dehydrated and embedded in paraffin (Bell and Lightner 1988). Sections (3 µm) were stained with Haematoxylin-Eosin and PAS (Periodic acid-Shiff).

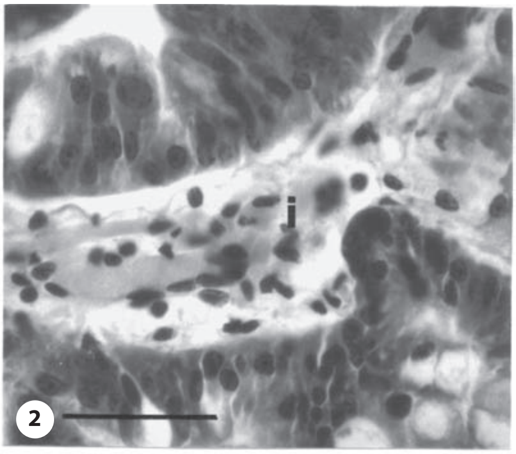
For TEM (transmission electronic microscope), the hepatopancreas were placed in 2.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2-7.4) at 4 °C. They were postfixed for 1 h in 1 % $OsO_4$. Later the material was dehydrated through an ethanol series and embedded in Spurr resin. Semithin sections (1 µm) were stained with toluidine blue; ultrathin sections were mounted on copper grids (400 mesh) and stained with lead citrate and uranyl acetate. TEM images were obtained with a JEOL JSM-100CX II transmission electron microscope.

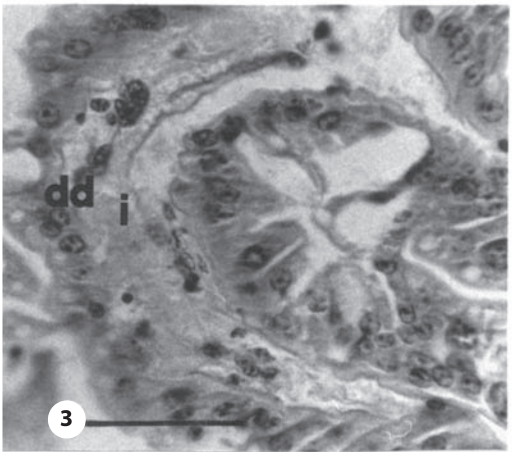
## RESULTS

Several histopathological alterations, not observed in individuals from Canal 5, were detected in the hepatopancreas of prawns from Los Padres lagoon (Fig. 1).

In intermolt (C stage), an important haemocytic and connective infiltration was observed within the intertubular space (Fig. 2). The main histopathological alterations present were epithelial retraction, folded basal lamina, and numerous cells were completely detached from the basal lamina showing signs of necrosis (cariolysis, cariorrexis and lack of cellular details). In premolt ($D_0$-$D_2$ stages), numerous tubules presented a wide irregular lumen with an atrophied epithelium, which had a disrupted brush border. Other tubules had a completely necrotic epithelium, or most







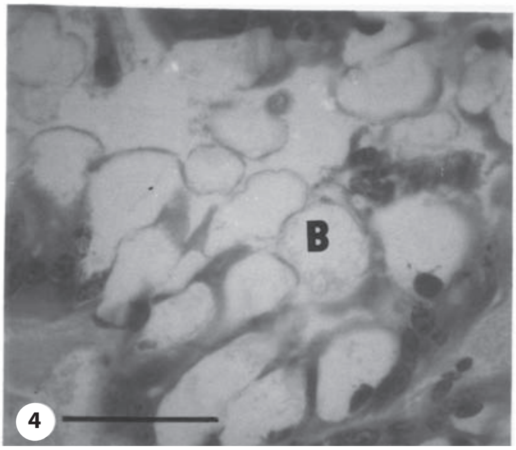


Fig. 1. Hepatopancreas of *Palaemonetes argentinus* from Canal 5. Note the different cell types in a non-pathological hepatopancreas. B: B-cell, F: F- cell, h: haemolymph, l: lumen, R: R- cell. Scale bar: 50 µm.

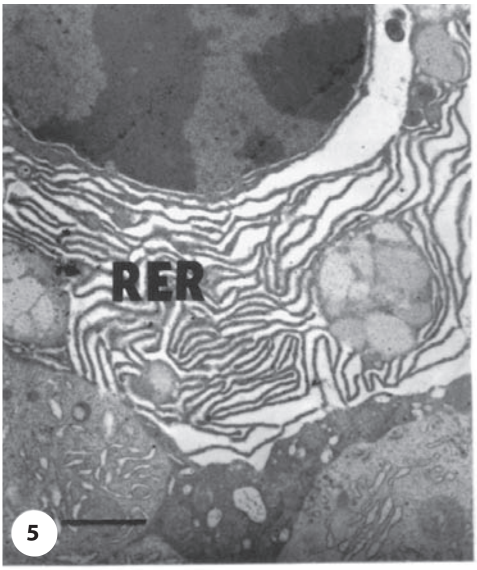
Fig. 2. Hepatopancreas of intermolt *Palaemonetes argentinus* from Los Padres lagoon. Important haemocytic infiltration and cellular necrosis are evident. i: haemocytic infiltration. Scale bar: 50 µm.

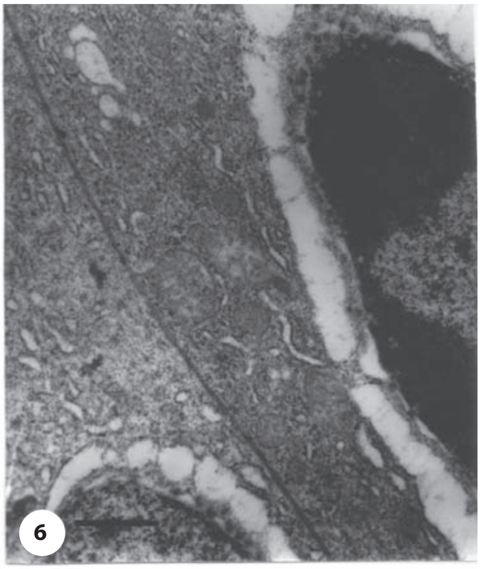
Fig. 3. Hepatopancreas of premolt *Palaemonetes argentinus* from Los Padres lagoon. Observe the degenerative desquamation of the epithelium and haemocytic infiltration. dd: degenerative desquamation, i: haemocytic infiltration. H&E. Scale bar: 50 µm.

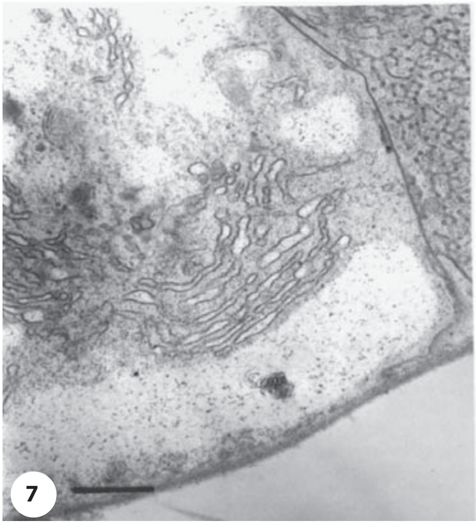
Fig. 4. Hepatopancreas of premolt *Palaemonetes argentinus* from Los Padres lagoon. B-cells dysplasia. B: B-cell. Scale bar: 50 µm.

cells were detached from the basal lamina (Fig. 3). Many tubules showed B-cells dysplasia at the proximal zone (Fig. 4), and the other cell types lacked cellular distinctive features. In the intertubular spaces, haemocytic infiltration and protein precipitation were also observed. Haemocytic infiltration was more conspicuous in intermolt than in the rest of the molting cycle. In postmolt (A-B stages), the hepatopancreas evidenced the same alterations mentioned above. In all cases, the lesions did not comprise the entire organ, but they were located in the medullar region of the organ.

At the ultrastructural level, R and F cells were the most damaged cells. Both cell types showed nuclear retraction, chromatin condensation and cytoplasmic lysis (Fig. 5, 6). Some R cells also had dilated mitochondria and numerous lysosomes, and the basal cytoplasm was almost completely lysed (Fig. 6, 7). A few isolated cells showed apoptotic characteristics,







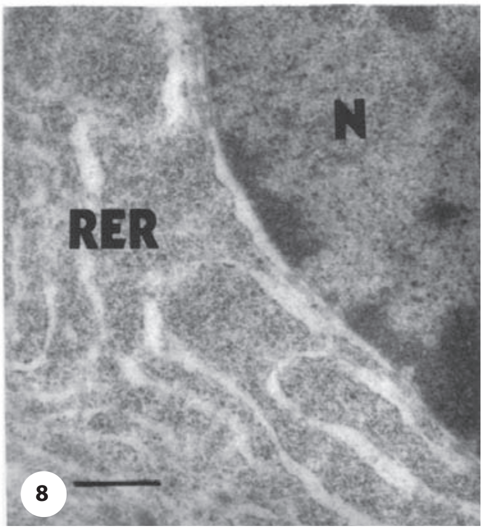


Fig. 5. Pathological F-cell of *Palaemonetes argentinus*. Note the abnormal RER and the condensation of nuclear chromatin. RER: rough endoplasmic reticulum. 5 000 X. Scale bar: 2 µm

Fig. 6. *Palaemonetes argentinus:* two adjacent R-cells with nuclear retraction and numerous lysosomes. L: lysosome. 5 000 X. Scale bar: 2 µm

Fig. 7. *Palaemonetes argentinus:* lysed cytoplasm in the basal zone of an R-cell. 8 000 X. Scale bar: 1.06 µm

Fig. 8. *Palaemonetes argentinus:* isolated apoptotic cell, RER with dilated membranes. N: nucleus, RER: rough endoplasmic reticulum. 8 000 X. Scale bar: 1.06 µm.

they appeared with a granular cytoplasm and a distended endoplasmic reticulum (Fig. 8).

## DISCUSSION

Several authors working with fish observed the presence of alterations in liver cells induced by factors like food (Storch *et al.* 1984), temperature variations (Braunbeck *et al.* 1987), presence of pesticides (Meyers and Hendricks 1985) or heavy metals (Segner and Storch 1985). Similarly, the crustacean hepatopancreas undergoes morphological and functional modifications in response to these factors, which are evident much earlier than behavioral changes (Storch 1984, Vogt 1987, Díaz *et al.* 2002).

In the hepatopancreas of *P. argentinus* from Los Padres lagoon, several alterations were observed, indicating that the organ reacts to environmental changes. Miglioranza *et al.* (2002) studied the different trophic levels in Los Padres lagoon and determined that the biota accumulates significant amounts of organochlorine pesticides *in situ*, particularly DDT. Nimmo and Blackman (1972) found that penaeid shrimps accumulate more DDT in the hepatopancreas than in other organs. *P. argentinus* accumulates efficiently pesticides leading to a bioaccumulation and biomagnification through the different trophic levels (Miglioranza *et al.* 2002). González Sagrario *et al*. (1998) found total organochlorine concentrations between 8.19 and 119.43 ng/g wet weight (ppb) in total tissues of this species; the main compounds detected were heptaclor epoxi, chlordane, hexachlorocyclohexanes (HCHs), cyclodienes, and DDTs (DDE, DDD and DDT).

The important degenerative desquamation, tubular dilation, loss of cellular limits, and epithelial atrophy described in the present study were also mentioned for other decapods exposed to contaminants (Vogt 1987). Alterations like atrophy, reduction in the height of tubular epithelium, necrosis and degenerative desquamation have been noted in hepatopancreas of mollusks and decapod crustaceans after exposures to organochlorines like Aroclor and DDT (Lowe *et al.* 1971, 1972), petroleum compounds (Fries and Tripp 1976), organophosphates like methyl parathion (Lowe *et al*. 1971), and heavy metal salts (Establier *et al*. 1978).

B-cells dysplasia was one of the alterations observed in pre- and postmolt prawns from Los Padres lagoon. This alteration in cellular size and shape was mentioned by several authors in decapod species that suffered starvation or malnutrition (Storch and Anger 1983, Strus 1987, Díaz *et al*. 2002). The folded basal lamina in the tubules was another alteration detected in *P. argentinus* that was also found in hepatopancreas of *Penaeus monodon* (Fabricius, 1798) exposed to pesticides (Vogt 1987).

Most of the alterations in the hepatopancreas of *P. argentinus* were focused on the medullar region of the organ, where medial and proximal zones of the tubules are located. Coincidentally, other researchers studying the effect of heavy metals on decapod crustaceans found the highest amount of metal granules in R-cells located at the proximal zone of the tubules (Ogura 1959, Hopkin and Nott 1979). This pattern of distribution is positively correlated with cellular age because R-cells, involved in detoxification, do not have apocrine secretion, so contaminants must remain inside the cells until the cellular death (Vogt and Quinitio 1994). Cellular recovering and elimination of senescent and/or altered cells is not a continuous process, but it is related to the epithelium renovation at the end of the digestive cycle (Hopkin and Nott 1980). In *P. argentinus* senescent and/or altered R-cells are located in the proximal zone of the tubules and are expelled along with adjacent B-cells into the tubular lumen (Sousa and Petriella 2000, 2001). This desquamation of senescent and altered R-cells was very conspicuous in the individuals from Los Padres lagoon, particularly in intermolt, which is a period of active feeding (pers. obs.). The increased desquamation is related to the epithelial renovation and the elimination of damaged cells, probably containing the toxicants. The important haemocytic infiltration observed in the present study is involved in the phagocytosis of necrotic cells. Similarly, Daughtie and Rao (1982) found a pronounced activity of phagocytes in the hepatopancreas of *Palaemonetes pugio* (Holthuis, 1949) exposed to pesticides; the authors also observed ultrastructural alterations similar to those observed in the present study.

Our observations support the previous conclusion: histological analysis represents a highly sensitive tool to evaluate the physiological and nutritional condition of prawns and water quality (Vogt 1987). The lack of pathologies in prawns from Canal 5 and the similitude of physical conditions of the sites of study suggest that contamination is the main cause of the hepatopancreas deterioration in individuals from Los Padres lagoon. However, we cannot affirm that contaminants were the only cause. Further studies are necessary to corroborate the present findings. Acute and chronic toxicity tests are proposed to evaluate, under controlled conditions, the effect of different organochlorine pesticides on the histological structure of the hepatopancreas and on growth parameters.

## RESUMEN

Analizamos el estado morfológico y funcional del hepatopáncreas de *Palaemonetes argentinus* de dos ambientes con diferentes concentraciones de plaguicidas. La laguna Los Padres (Argentina) es un área sujeta a contaminación debido al lento intercambio del agua, la escasa profundidad y el influjo de agua contaminada. Las gambas acumulan aquí grandes cantidades de plaguicidas organoclorados en sus tejidos. Los hepatopáncreas de gambas del Canal 5 y de la laguna Los Padres fueron procesados mediante técnicas histológicas estándar para microscopía óptica y electrónica de transmisión. Los hepatopáncreas de los individuos recolectados en Los Padres tenían alteraciones tisulares importantes, como infiltración intertubular de hemocitos y tejido conectivo, retracción epitelial en algunos túbulos y láminas basales plegadas. También se observó descamación necrótica importante, con cariolisis, cariorrexis y falta de detalles celulares. Vimos muchos túbulos con un lúmen irregular y agrandado, con el epitelio atrofiado o completamente ausente. En general las lesiones se localizaron en la región medular del órgano. Ambos tipos celulares mostraron retracción del núcleo, condensación de la cromatina y ruptura del citoplasma. Algunas células R también tenían mitocondrias y numerosos lisosomas dilatados, y el citoplasma basal casi completamente desintegrado. No hallamos alteraciones en los hepatopáncreas de gambas de Canal 5. El estudio histopatológico del hepatopáncreas es una herramienta muy apropiada para evaluar la condición fisiológica de las gambas y la calidad del agua. Por la semejanza de otras condiciones ambientales, suponemos que los contaminantes fueron la causa principal del deterioro de los órganos.

**Palabras clave:** Decapoda, Caridea, contaminación, histología, morfología funcional, hepatopáncreas.

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