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Histopathological effects of endosulfan to hepatopancreas, gills and ovary of the freshwater crab *Zilchiopsis collastinensis* (Decapoda: Trichodactylidae)

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ABSTRACT

In this work, the effects of a pulse exposure of endosulfan on hepatopancreas, gills and ovary of the burrowing crab *Zilchiopsis collastinensis* were evaluated. The crabs were exposed to three sublethal concentrations in a pulse system with controlled dilutions. Water samples for pesticide concentrations measurements and crab tissue samples were taken when applications were made and 2, 8, 15 and 22 days after administering the pesticide. The exposure to endosulfan caused an increase in B cell number and a decrease in F and R cell number ($p < 0.05$). Necrotic tubules, abnormal lumen and other histopathologies were observed in the hepatopancreas of crabs exposed to endosulfan. There was an increase in the proportion of collapsed gills caused by endosulfan effects. Other effects as hyperplasia were also observed. There were no changes in the gonadosomatic index of exposed crabs; however there were changes in the volume of oocytes of exposed crabs in certain days ($p < 0.05$). The increase in B cell number and the consequent reduction in F cell number may be related to the detoxification processes. The changes in cell number within the hepatopancreas and the histopathologies observed both in hepatopancreas and gills might be used as endosulfan exposure indicators.

1. Introduction

Grain-exporting countries are characterised by intensive agricultural activities, which include the use of several biocides to increase crop productivity. The widespread use of persistent organic pollutants has resulted in the contamination of terrestrial and aquatic environments (Ernst et al., 1990; Jergentz et al., 2005). A widely used pesticide is endosulfan (6,7,8,9,10,10-hexacloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzodioxathiepin-3-oxide), a broad-spectrum organochlorine insecticide that is used to control boring, chewing and sucking insects and mites (Wan et al., 2005). The recommended application doses of this pesticide ranging from 0.7 to 3 l/10,000 m^2. The Endosulfan acts on the biota by blocking the chloride channels of the gamma-aminobutyric acid (GABA) receptor in the central nervous system, leading to neural excitation and eventually the death of the organism. Like various other organochlorine compounds, endosulfan is stable for several weeks; therefore, it remains effective long after it is applied. Endosulfan is not typically considered mobile and it is known to persist in surface runoff waters (Murray et al., 1993). Endosulfan is highly toxic to fish and aquatic invertebrates, and because of its lipophilicity, it is able to accumulate in these organisms (Howard, 1991; Hose and Van den Brink, 2004; Wan et al., 2005; Silva Barni et al., 2014). Also, endosulfan is an endocrine disrupting chemical that may affect reproductive parameters in animals as fishes, both in males and females (Chakravorty et al., 1992; Rajakumar et al., 2012; Laldinsangi et al., 2014).

During pesticide applications the aerial drift might reach the aquatic ecosystems near the crop areas (Ernst et al., 1990). Moreover, in some crop systems as paddy fields, the pesticide applications occur near rivers and lakes, posing risk to the biota inhabiting them. The decapod crustaceans are found in freshwater environments all over the world, occupying an intermediate position in the food webs. Like all the biota, they are periodically exposed to different pesticides, especially those that inhabit near agricultural areas.

The exposure to pesticides might cause several histopathological effects in animals such as prawn and crabs. Gills are the first organ which comes in contact with environmental pollution. They

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are highly vulnerable to toxic chemicals mainly because their large surface area facilitates the interaction and their absorption. So, the absorption through gills is rapid and therefore the toxic response in gills is also rapid (Pandey et al., 2008). Hepatopancreas is a dynamic organ mainly related with digestive functions. It is also responsible for the major portion of detoxification activities. When crustaceans are exposed to toxicants and pollutants, its functions and structure are likely to be affected by certain xenobiotics (Bhavan and Geraldine, 2000; Wu et al., 2008). The hepatopancreas is also responsible for the production of vitellogenin. During secondary vitellogenesis, the lipids stored in the hepatopancreas are processed into lipoproteins and transported via the haemolymph to the oocytes (Harrison, 1990; Lubzens et al., 1995; Rodríguez et al., 2000).

The crabs of the *Zilchiopsis* genera are active predators and detritus feeders. They are also an important food source for fish, reptiles, birds and mammals, with a central position in both the aquatic and terrestrial food webs (Collins et al., 2007). The burrowing crab *Zilchiopsis collastinensis* is a common crab of the middle Paraná River.

In this crab the gonad gradually develops from spring to summer, when spawning occurs (our laboratory). Pesticide applications occur mainly in the spring, reaching freshwater environments during rainfalls. The females of this species appear to be quite resistant to endosulfan. In previous studies conducted by us, the 96-h LC_{50} of endosulfan was found to be 1902 μg/l (Negro et al., 2014). When *Z. collastinensis* was exposed to endosulfan for 22 days, accumulation of this pesticide was found to have occurred both in hepatopancreas and gonads, although there was a reduction with time of endosulfan concentrations in hepatopancreas (Negro et al., 2012). However, there are few records of the histopathological effects of pesticides in tissues of this crab, and to the best of our knowledge, there is a lack of information about the possible use of these histological biomarkers as a way to estimate the pesticide pollution.

The aims of this work were: 1 – to simulate a single contamination pulse of endosulfan in a system with controlled dilution, 2 – to recognise the histopathological effects caused in hepatopancreas, gills and ovaries of the freshwater burrowing crab *Z. collastinensis*, used as an ecotoxicological model and 3 – to determinate if there are histological biomarkers of exposure to endosulfan after water concentrations have decreased.

2. Materials and methods

2.1. Animal collection and acclimation conditions

Adult crabs (*Z. collastinensis* Pretzmann, 1968) were collected on the Paraná River floodplain (31°30′S, 60°41′W; Santa Fe, Argentina) in late winter. This river has a mean discharge of 16,000 m^3/seg and a peak discharge of up to 60,000 m^3/seg (Iriondo, 2004). It is located within an alluvial valley that ranges from approximately 13 to 56 km in width, with a slope of 0.036 m/km. The crabs were collected by hand or by sampling with a hand net (area of 0.9 m^2 with a mesh size of 1 mm) below the aquatic vegetation (*Eichornia crassipes*) (Williner and Collins, 2002, 2013). Only females were used in the experiments. They were acclimated for 14 days in natural light and temperature conditions. One hundred and eighty intermoult crabs were placed in twelve 300 l aquaria filled with dechlorinated water (15 crabs per aquarium). The water was renewed at a rate of 20 l/day in a continuous flow system and the excess water was drained by overflow. Dissolved oxygen, pH and conductivity values of the dechlorinated water were 6.84 ± 1.28 mg/l; 7.12 ± 1.32 and 1228.68 ± 21.42 μS/cm respectively. The aquaria contained plastic shelters to simulate the crab burrows. The mean (± SD) carapace width of the crabs used was 51.45 (± 2.86) mm. Additionally, the largest individual was not more than 1.5 times larger than the smallest individual, a criterion that has been proposed for fish assays (Reish and Oshida, 1987). The aquaria were placed in an open greenhouse covered with shade cloth, to avoid the overheating caused by direct sun exposure (photoperiod 12:12; temperature 14.03 ± 4.26 °C). The crabs were fed fresh fish muscle ad libitum. Food was supplied once in the evening, and the leftovers were removed early the next morning.

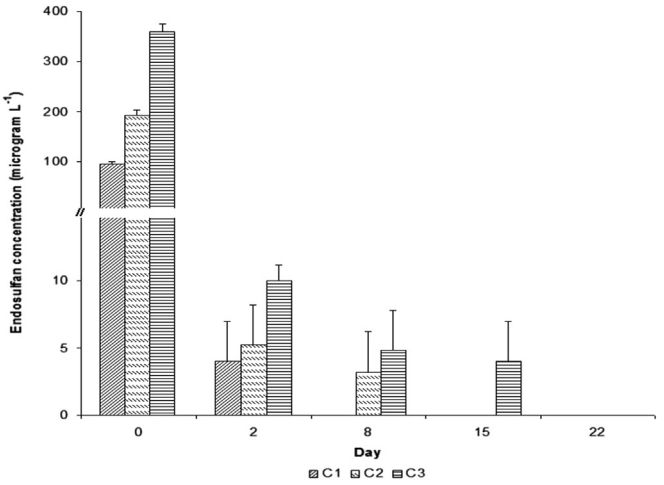

Fig. 1. Mean endosulfan measured concentrations (+SD) in water in each treatment at initial time (day 0) and in the different sampling days. C_1: 94 ± 6 μg endosulfan l^{-1}; C_2: 192 ± 10 μg endosulfan l^{-1}; C_3: 360 ± 15 μg endosulfan l^{-1} (initial concentration).

2.2. Assay conditions

After the acclimation period, each aquarium received a single pulse of commercial grade pesticide (Zebra Ciagro®; Red Surcos S. A., Argentina) containing 35% endosulfan. The commercial product was diluted in dechlorinated water, added to each aquarium and gently homogenised throughout the tank. Based in the LC_{50} of 1902 (1679–2124) μg endosulfan l^{-1}, obtained in a 96 h exposure period test, three sublethal concentrations (about 1/20, 1/10 and 1/5) were used (Negro et al., 2014). Initial concentrations were 94 ± 6; 192 ± 10 and 360 ± 15 μg endosulfan l^{-1} (C_1, C_2 and C_3 respectively). Those concentrations were applied in a dynamic system with a high initial concentration followed by dilution with pesticide-free dechlorinated water, simulating the dilution process of aquatic ecosystems. The dilution rate was 20 l/day in a continuous flow system and the excess water was drained by overflow. A control group was subjected to the same conditions as the exposed group, but without the addition of pesticides. Three replicates of each treatment were performed. The plastic shelters used to simulate the crab burrows were kept during the exposure period. Food was supplied to the crabs in the same manner in which it was supplied during the acclimation period. Dissolved oxygen, pH and conductivity were measured 3 times a week before feeding. The water temperature was recorded twice a day, at 9:00 and 16:00 h.

Water samples were taken before the endosulfan application, to rule out the baseline presence of pesticides, just after pesticide application (initial concentration) and 2, 8, 15, and 22 days after pesticide addition. Endosulfan levels in the water were measured using the ASTM D 6520-06 method. Samples were subjected to solid-phase microextraction (SPME) and concentrations of endosulfan were measured with gas chromatography-electron

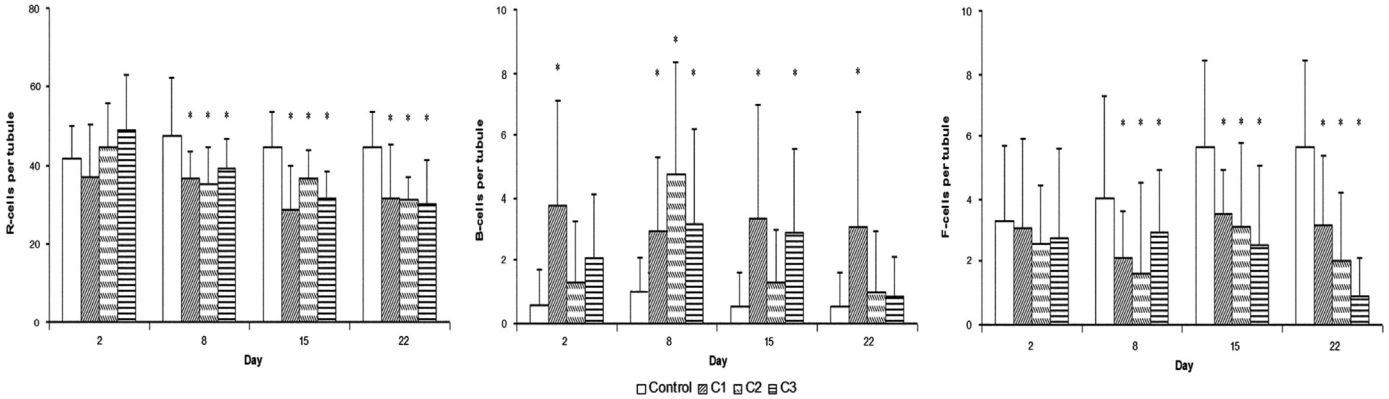

Fig. 2. Mean (± SD) cell number per hepatopancreatic tubule (cross section) of *Zilchiopsis collastinensis* crabs exposed to different endosulfan concentrations and days. $N=36$ tubules observed per concentration and day. * Significantly different from the control group ($p < 0.05$).

capture detector (GC-ECD; GC Hewlett Packard 5890 Series II) using nitrogen as a carrier gas (1 ml/min).

2.3. Tissue sampling and histology procedures

Samples of the hepatopancreas, gills and ovary were taken before the endosulfan application (Day 0) and 2, 8, 15, and 22 days after the pesticide application. The sampled crabs (3 for each replicate; a total of 9 per group per day) were cryoanaesthetised, measured and weighed. The organs were dissected and the gonads were immediately weighed. Small pieces of the tissues were fixed in Bouin solution for 4 h and preserved in a 70% ethanol solution.

Hepatopancreas samples were taken from each side of the crab, dehydrated in ethanol series, washed in xylene and embedded in Histoplast®. The distal 2400 μm of the tubules were discarded (most part of the distal zone) with the aim of observing the middle zone. Tissue sections were cut in 6 μm sections and stained with haematoxylin–eosin. Several tubules (from 5 to 10) were present in every section. The samples were observed under a light microscope. Four tubules were observed by slide (36 total tubules for every day and treatment). The observed tubules were selected randomly by using a grid. Once the point was selected, the entire tubule was observed. All the cells of the tubules were counted and classified in B, F and R types (Al-Mohanna and Nott, 1989; Icely and Nott, 1992; Bhavan and Geraldine, 2000). No tubule was observed twice. Because control and exposed crabs were subjected to the same methodology, the differences in cells were related to pesticide exposure. Other histopathologies such as necrosis, abnormal lumen and delamination of the epithelium were also observed (Rodríguez et al., 1994; Bhavan and Geraldine, 2000; Pinho et al., 2003; Sousa et al., 2005; Bianchini and Monserrat, 2007). Tubules were considered as affected if any of these histopathologies were observed. The proportion of affected tubules/total observed tubules was calculated.

The gills related with the third pair of pereiopod were dissected. The gills related with the first two pairs of pereiopods were not used because of the flatworms eggs (*Temnocephala* sp.) attached to them (Dioni, 1967). Both gills from every crab were dehydrated in ethanol series, washed in xylene and embedded in Histoplast®. Tissue sections were cut in 6 μm sections and stained with haematoxylin–eosin. The samples were observed under a light microscope. Pillar cell disruption (collapsed lamellae) and hyperplasia were analysed. The lamellae of the gills were considered as affected if any of these histopathologies were observed. The proportion of affected lamellae/total lamellae per gill was calculated.

Gonadosomatic indexes (GSI) were calculated according to the formula: GSI = (ovarian wet weight/body wet weight) × 100 (Medesani et al., 2004). Oocytes were carefully separated and photographed using a stereoscopic microscope. The oocyte volume was calculated according to the formula used for egg volume: $Vo = 1/6(ab^2\pi)$; where a is the major axis and b is the minor axis (Corey and Reid, 1991). The oocytes from female crabs of the same replica were grouped together. Ten oocytes from each replica were randomly selected and measured; with a total of 30 oocytes measured per concentration per sampled day. Randomisation was performed by simple random extraction of oocytes. Only those oocytes with a regular appearance, i.e., those with circular shape and orange colour, were used for these measurements.

2.4. Statistical analysis

The number of F, B and R cells per tubule, the proportion of affected lamellae per gill, the GSI and the oocyte volume were compared using the Kruskal–Wallis test followed by the Mann–Whitney U test, since the data was not normally distributed and it was heteroskedatic. In all statistical tests, a significance level of 5% was adopted. The number of affected tubules in hepatopancreas was compared using the chi-square goodness of fit test, where the control group data was used as expected value and compared with the observed values in the different concentrations (Zar, 1996).

3. Results

3.1. Physico-chemical properties and pesticide concentrations

Endosulfan concentrations were higher after the application, but they were quickly reduced and after 22 days the endosulfan concentrations were too low to be quantified (Fig. 1). Almost no mortality was observed throughout the experiments; one crab died in the group initially exposed to 360 ± 15 μg endosulfan l^{-1} on day 7. The values of temperature, dissolved oxygen, pH and conductivity were 14.55 ± 4.32 °C, 7.12 ± 1.4 mg/l, 7.22 ± 1.1 and 1231.75 ± 10.53 μS/cm, respectively. The temperature varied between 10.41 ± 1.06 in the morning and 18.70 ± 0.77 in the afternoon, but there were no differences between any of the groups.

3.2. Hepatopancreas

The histological analysis of the hepatopancreas of *Z. collastinensis* indicated that it is composed of tubular structures surrounded by connective tissue. The types of cells observed were fibrillar F cells, large vacuolated B cells and resorptive R cells (Icely and Nott, 1992; Bhavan and Geraldine, 2000). There were no differences in R cell number between the control group and any of the exposed groups by day 2; however, there was a decrease of R

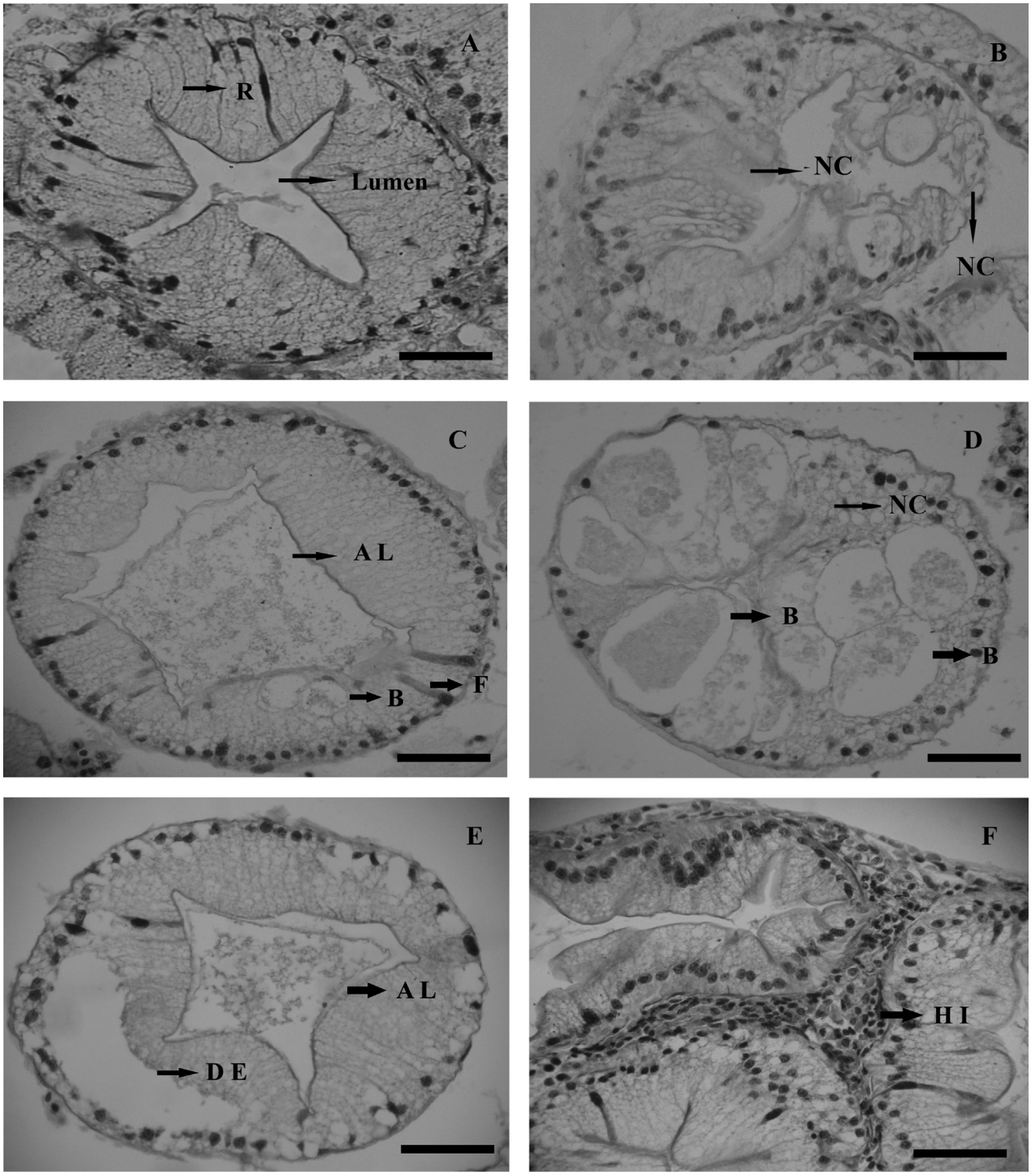

Fig. 3. Histological features of the hepatopancreas of *Zilchiopsis collastinensis* exposed to different concentrations of endosulfan. (A) Tubule of control crabs, showing normal lumen with star appearance, mainly composed by R-cells. Bar = 0.03 mm. (B) Tubule of crab exposed to 360 ± 15 μg endosulfan l^{-1} after 15 days, with loss of normal shape and necrotic areas (NC), without cell differentiation. Bar = 0.03 mm. (C) Abnormal lumen (AL) of crabs initially exposed to 360 ± 15 μg endosulfan l^{-1} after 15 days of exposure. Arrows showing deep stained F-cells and large vacuolated B-cells. Bar = 0.03 mm. (D) Hepatopancreatic tubules composed mainly by B-cells from crabs initially exposed to 360 ± 15 μg endosulfan l^{-1} after 15 days of exposure. Necrotic areas (NC) are also observed. Bar = 0.03 mm. (E) Abnormal lumen (AL) and delamination of the epithelium (DE) of crabs initially exposed to 360 ± 15 μg endosulfan l^{-1} after 15 days of exposure. Bar = 0.03 mm. (F) Hemocytic infiltration in the interstitial sinuses of the hepatopancreas tubules of crabs initially exposed to 360 ± 15 μg endosulfan l^{-1} after 15 days of exposure. Bar = 0.03 mm.

cell number in all the exposed groups at 8, 15, and 22 days ($p < 0.05$). For B cells, there was an increase in the number of cells in crabs initially exposed to 94 ± 6 μg endosulfan l^{-1} at 2, 8, 15 and 22 days after exposure to pesticides. An increase in B cell number was also observed on day 8 in crabs initially exposed to 192 ± 10 and on days 8 and 15 in crabs initially exposed to 360 ± 15 μg endosulfan l^{-1} ($p < 0.05$). After day 8, there were no differences between the control group and crabs initially exposed to 192 ± 10 μg endosulfan l^{-1}. Finally, for F cells, there was a decrease in cell number for crabs initially exposed to 94 ± 6, 192 ± 10 and 360 ± 15 μg endosulfan l^{-1} at 8, 15 and 22 days after exposure to pesticides ($p < 0.05$) (Figs. 2 and 3).

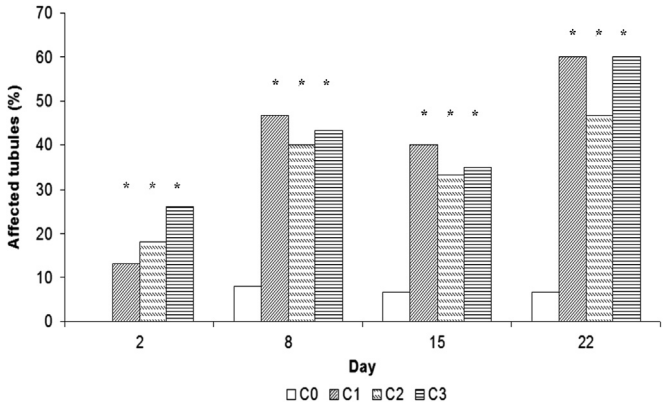

Fig. 4. Percentage of affected hepatopancreatic tubules at different days of exposure to endosulfan. * Significantly different from the control group ($p < 0.05$).

The number of affected tubules (tubules where histopathologies were observed) increased after 2 days of exposure in all the concentrations and were higher than control even after the endosulfan concentration in water decreased ($p < 0.05$) (Fig. 4)

3.3. Gills

In the case of gills, the more observed histopathological lesion was the disruption of pillar cells, which resulted in the collapse of the lamellae. The hyperplasia observed in some cases resulted in the formation of globular lamellae (Fig. 5). The number of affected lamellae increased after 2 days in crabs initially exposed to 192 ± 10 and 360 ± 15 μg endosulfan l^{-1}. After 8 days of exposure there was an increase in affected lamellae on those crabs initially exposed to 94 ± 6 and 360 ± 15 μg endosulfan l^{-1} ($p < 0.05$). At days 15 and 22 there were no differences in the affected lamellae between the exposed and control crabs (Fig. 6).

3.4. Ovary

All of the females were in the exogenous vitellogenesis stage, characterised by orange oocytes. In the control group the gonadosomatic (ovarian) indexes increases from 4.71 (± 0.38) in day 2 to 5.05 (± 0.57) in day 22. In exposed crabs, the gonadosomatic indexes varied between 4.65 (± 0.63) (minimum value in day 2) and 6.10 (± 0.73) (maximum value in day 22). However, there were no significant differences between the control and the exposed crabs on any day.

In the control group the oocyte volume increased from 1.23 (± 0.29) mm^3 in day 2 to 1.74 (± 0.41) mm^3 in day 22. There were no significant differences between the oocyte volumes of crabs in the control group compared to crabs initially exposed to 94 ± 6 μg endosulfan l^{-1} at any time. There was only a significant increase in the oocyte volume of crabs initially exposed to 192 ± 10 μg endosulfan l^{-1} sampled 8 days after application (1.83 ± 0.38 mm^3) and a significant decrease in crabs initially exposed to 360 ± 15 μg endosulfan l^{-1} sampled 15 days after application (1.38 ± 0.57 mm^3) ($p < 0.05$). After 22 days of exposure, there were no differences in the oocyte volume between the control group and any of the exposed groups. Many females that were exposed to endosulfan were observed to have white atretic oocytes in their ovaries, which were not observed in the control crabs.

4. Discussion

Z. collastinensis crabs were resistant to the high pesticide concentrations used, since low mortality values were observed. However, although there were almost no lethal effects, the endosulfan sublethal effects in their organs were able to be observed even days after the peak concentrations.

The hepatopancreas exhibits an organised glandular tubular structure, with a single layer of epithelial cells and a lumen with a star-like appearance. The hepatopancreas of decapods crustaceans is composed of E, F, B and R cells. The E-cells are the precursor cells and are characterized by a nucleus that occupies most of the cell volume. The F cells are deeply stained with haematoxylin because they contain many protein producing organelles (ribosomes). The B cells release holocrine secretion, especially under pollutant stress. It has been considered that these reflect the remnants of intracellular digestion and a later stage of an F cell which produces the digestive enzymes. The R cells are characterised by numerous small vacuoles which are full of lipids and reflect the nutritional status (Al-Mohanna and Nott, 1989; Icely and Nott, 1992).

The increase in B cell number in the crabs exposed to endosulfan may be related to the detoxification processes. There is usually an inverse relation between F and B cells (Papathanassiou and King, 1984; Al-Mohanna and Nott, 1989; Icely and Nott, 1992). Köhler et al. (1998) observed the expression of P-glycoprotein (P-gp) at the microvilli of the transitional F/B cell in the hepatopancreas of *Carcinus maenas*, forming a first line of defence against the uptake of toxins. The fact that P-gp expression was specifically restricted to transitional F/B cells implies that these cells are specialised for accumulation and elimination of toxic compounds. The increase of B cell number and the existence of P-gp in the hepatopancreas may be a possible defence mechanism against organochlorine pesticides such as endosulfan (Pinho et al., 2003). The decrease in the number of F cells could be explained by the increase in the number of B cells.

However, detoxification is only feasible when the intensities of stressors do not exceed a particular threshold value. Otherwise, high concentrations of pesticides give rise to necrosis of cells and tissues, delamination of the tubules and lumen abnormalities. These histopathologies may be observed even after the pesticide concentrations in water decreased. During the exposure period there may be accumulation processes, especially of lipophilic compounds, in hepatopancreas and gonads. After the pesticide concentrations in water decreases there might be still different endosulfan concentrations in lipid tissues, as observed in a previous work (Negro et al., 2012). The accumulated pesticides may continue to act, producing histopathological effects, and defence mechanisms as the F/B complex might continue to act as a way of detoxification. The abnormal lumens, the more observed histopathology, might be related with the excretory function and the detoxification processes.

The R cells are the most abundant, as observed in general in decapods (Icely and Nott, 1992; Bhavan and Geraldine, 2000; Sousa et al., 2005). Resorptive (R) cells take up material from the lumen by endocytosis and transporters, and they provide the main storage site for lipids and glycogen. Chronic exposure to endosulfan may cause depletion in lipid reserves because of increased energy demand, as observed in the prawn *Macrobrachium malcolsonii* and the penaeid shrimp *Metapenaeus monoceros* (Bhavan and Geraldine, 2000; Suryavanshi et al., 2009). A reduction in the number of R cells, which were displaced by the B cells, might reduce the uptake of nutrients and the accumulation of lipids.

The gills represent a vital organ in aquatic organisms, since they play an important role in the transport of respiratory gases and regulate the osmotic and ionic balance. Pesticides and other toxic compounds may cause damage to gill tissues, thereby reducing the oxygen consumption and disrupting the osmoregulatory function of aquatic organisms (Bhavan and Geraldine, 2000). The effects in gills related with the pillar cell disruption

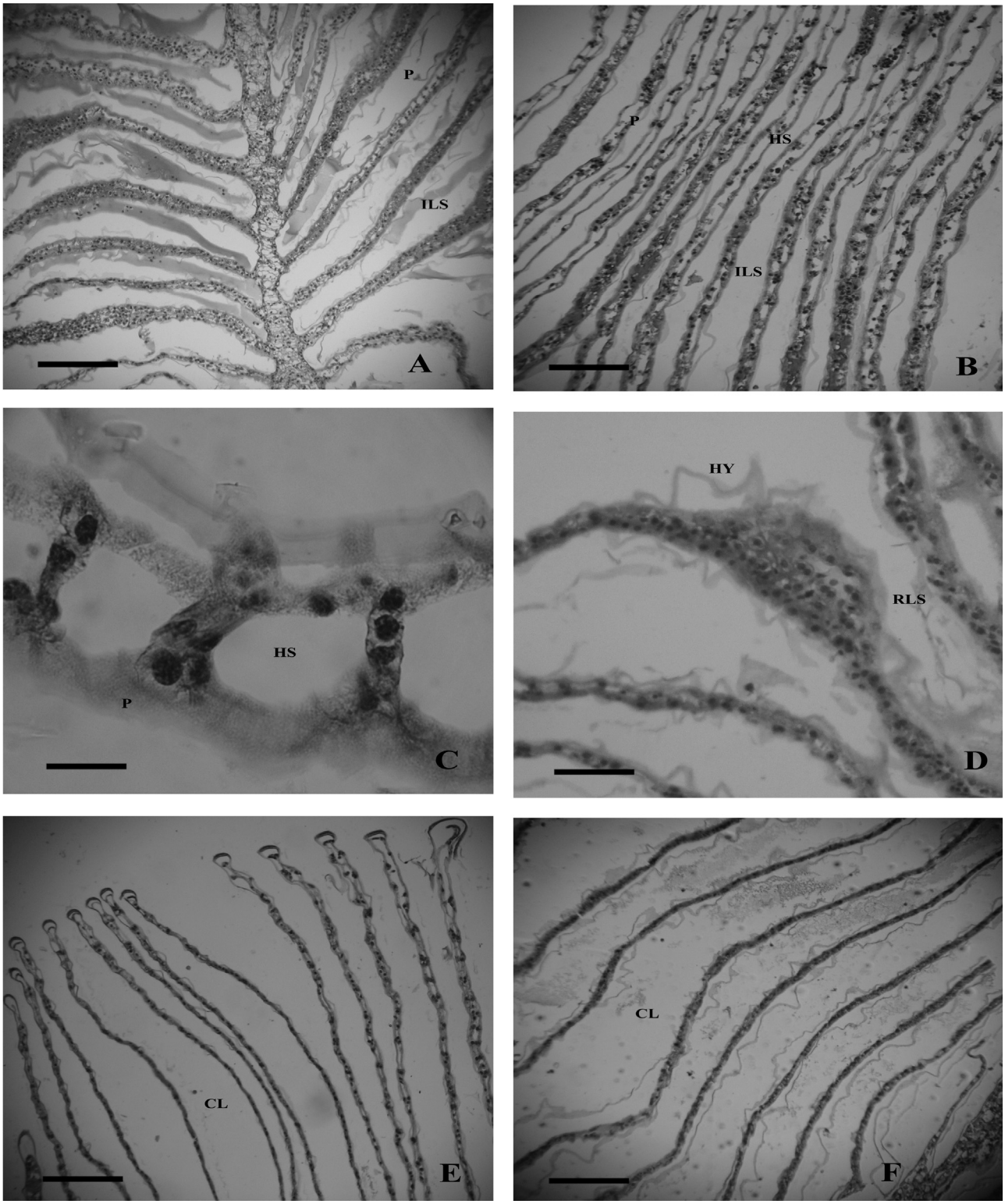

Fig. 5. Histological features of the gills of *Zilchiopsis collastinensis* exposed to different concentrations of endosulfan. (A, B) Normal lamellae of *Zilchiopsis collastinensis* of the control group, showing regular size, unaffected pillar cells (P), normal hemocoelic space (HS) and uniform inter-lamellar space (ILS). Bar 0.1 mm. (C) Pillar cells (P) from control crabs, which maintain the hemocoelic space (HS). Bar 0.01 mm. (D) Affected lamella from crabs initially exposed to 192 ± 10 μg endosulfan l^{-1} after 2 days of exposure showing hyperplasia (HY), which reduced the inter-lamellar space (RLS). Bar 0.01 mm. (E, F) Collapsed lamellae (CL) after necrosis and disruption of pillar cells from crabs initially exposed to 360 ± 15 μg endosulfan l^{-1} after 8 days of exposure. Bar 0.1 mm.

caused the considerable thickening of the gill epithelium and reduction of haemolymph spaces, resulting in a restriction of respiratory gas exchange. This restriction may cause the reduction in dissolved O_2 in haemolymph, which results in an increase in anaerobic metabolism and the accumulation of lactate and CO_2 (Freitas Rebelo et al., 2000). Effects as necrosis, pillar cell disruption and hyperplasia resulting in clavate–globate (clubbing) lamellae were also observed in *Macrobrachium malconsonii* exposed to endosulfan (Bhavan and Geraldine, 2000). Also, similar lesions have been reported to occur in the prawns *Macrobrachium kistensis*

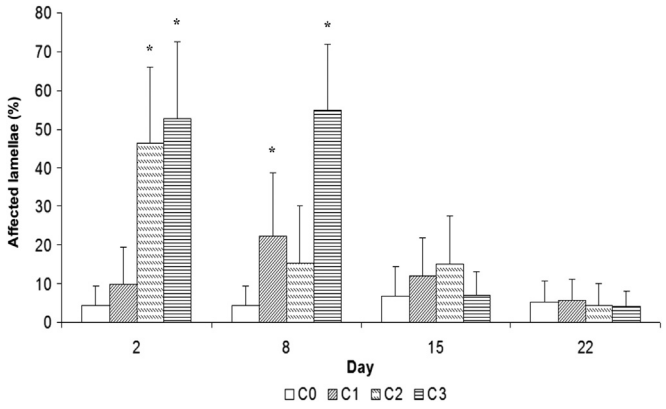

Fig. 6. Affected lamellae per gill of *Zilchiopsis collastinensis* exposed to different concentrations of endosulfan at different days. * Significantly different from the control group ($p < 0.05$).

(Ghate and Mulherkar, 1979), and *Macrobrachium idae* (Victor et al., 1990) following exposure to heavy metals. Moreover, histopathologies as hyperplasia, swelling or lifting of lamellae epithelium tend to be largely non-specific, and seem to reflect physiological adaptations to stress. However, such reactions could conceivably lead to dysfunctional or even non-functional gills, eventually resulting in asphyxia (Tamse et al., 1995). However, histopathological effects in gills appear to be reversible, since there is a recovery after the decrease in the exposure concentration.

Oocyte growth during secondary vitellogenesis has a close relationship with the hepatopancreas because this organ is the source of egg yolk proteins (Harrison, 1990). Several hepatopancreatic modifications were observed, especially with regard to cell distribution, which may cause effects in ovarian growth. Nevertheless, there were no differences in the ovarian indexes between crabs exposed to endosulfan and control crabs. The exposure to pesticides and some metals may cause differences in ovarian indexes and oocyte sizes, as shown previously for *Uca pugilator* and *Chasmagnathus* (*Neohelice*) *granulata* (Rodríguez et al., 1994, 2000). In our work, some changes in oocyte volumes were observed, but after 22 days of exposure there were no differences between the control group and the exposed crabs.

The white oocytes that were observed, which were smaller than the orange ones, were reabsorbed oocytes, described by Rodríguez et al. (1994) as atretic oocytes, since according to these authors atresia occurs during exogenous vitellogenesis. Faced with depleting energy reserves, caused by both a reduction in R cells and an increased energy demand because of defence mechanisms, these crabs may reabsorb the lipids from some oocytes without altering the development of others. Although no quantification of the total number of oocytes was made, a decrease in available oocytes, if any, and consequently in eggs, would cause several effects within the population and eventually in the entire biological community.

5. Conclusion

When *Z. collastinensis* crabs were exposed to endosulfan, they used several defence mechanisms to avoid the harmful effects of pesticide exposure. These defence mechanisms may include gill hyperplasia and an increase in the amount of B cells, with the increase in the amount of enzymes related to the detoxification process of the transitional F/B cells. The differences in hepatopancreatic cell distribution and the affected tubules proportion have a potential as a biomarker of pesticide exposure of this crab, which may be present in the environment even after their concentrations decreased. On the other hand, alterations in gills were reversible with time, as endosulfan concentrations decreased. The exposure of crabs to endosulfan does not seem to cause effects on gonadal parameters such as gonadosomatic indexes and oocyte volume, although more studies about the possible increase of atretic oocytes and egg fertility are needed.

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