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Cypermethrin-based formulation Barrage® induces histological changes in gills of the Pantanal endemic shrimp *Macrobrachium pantanalense*

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Highlights

- Barrage® is a formulation of cypermethrin widely used in the Pantanal (Brazil).
- The 96 h-LC50 was 0.93 μg/L for the endemic shrimp *Macrobrachium pantanalense*.
- Swimming disruption was observed (>1.25 μg/L) suggesting neuronal impairment.
- Lesions in the gills (>0.05 μg/L) observed may affect physiological functions.
- The need of monitoring agrochemicals residues in the Pantanal is highlighted.

Abstract

Pantanal shrimp *Macrobrachium pantanalense* was exposed for 96 hours to the cypermethrin-based formulation Barrage®. Population-relevant endpoints (survival, swimming behavior) as well as histopathology of gills were analyzed. A 96 h-LC_{50} of 0.93 μg/L of cypermethrin was calculated while equilibrium disturbances were observed at 1.25 μg/L. Histological examination showed predominantly regressive changes in the gills of shrimp exposed to concentrations of 0.25 and 1.25 μg/L. Three levels of lesions were observed in the gills: I- Intercellular edema, epithelial lifting of the lamellae and lamellar fusion, fat vacuoles and hypertrophy of gill epithelial cells or mucous cells; II- nuclear changes, atrophy (reduction of volume and number) and hyperplasia of gill epithelia and III- necrosis. This study shows the high sensitivity of the shrimp *M. pantanalense* to the pesticide Barrage® highlighting the importance of monitoring agrochemicals residues in the Pantanal region (Brazil) and conduct risk evaluation studies to prevent deleterious effects on the aquatic communities of Pantanal.

Keywords: pesticide; crustaceans; histopathology; behavior; wetlands; environmental risk.

1. Introduction

The Pantanal (Brazil) is a biosphere reserve consisting of seasonally flooded areas that hold a rich and admirable diversity and abundance of wild species, some of which are listed as threatened or endangered (Junk et al., 2006). Particularly, a significant diversity of freshwater shrimp of the genus *Macrobrachium* (Crustacea:

Decapoda: Caridea: Palemonidae) (Murphy and Austin, 2005) occurs in Pantanal. This diversity is progressively growing due to the increase in the number of studies describing new species in the region (De Grave et al., 2008). This is the case of *Macrobrachium pantanalense*, a newly described freshwater shrimp endemic to Pantanal. Until recently, it was classified as *M. amazonicum*, a species widely distributed throughout South America (Melo, 2003; Santos et al., 2013).

Recent development in agriculture and livestock sectors in the region led to an increased use of pesticides, as a mean to ensure good productivity (Soares et al., 2017). This constitutes a threat as these compounds easily reach the aquatic environment, being transported by rain or by direct application in the water as, for example, in rice plantations (Alho, 2008; Ross and Sanches, 2006). Crustaceans are a particularly sensitive group to the increasing chemical pressure in Pantanal. Studies in literature report higher sensitivity of shrimp species when compared with other aquatic species such as fish (Bajet et al., 2012). It is anticipated that this anthropogenic impact may lead to large-scale ecological imbalances, although there are still few studies on the toxicity of chemical pesticides to aquatic organisms living in this region (Dores, 2016).

One of the most used insecticides in the Pantanal region of Mato Grosso do Sul is cypermethrin (active ingredient of the formulation Barrage®), a synthetic pyrethroid used for agriculture, for household pest control and for fleas and ticks control in cattle (de Barros, 1992; Gomes et al., 2011; NPTN, 1998). The WWF estimates a herd of 22 million heads of cattle in the Pantanal, Upper Paraguay River Basin (WWF, 2018). According to indications of the manufacturer 5 ml of the product should be applied to each animal (diluted in a proper amount of water and delivered by spraying) for parasites control which would account for a total of 16500 kg of cypermethrin per application (several are needed along the year) just for this specific usage.

In addition, this pesticide is used in aquacultures to control vectors in aquatic environments (Das and Mukherjee, 2003). Some European countries use 5 to 15 μg/L cypermethrin for 1 hour to control sea lice *Lepeophtheirus salmonis* in salmon hatcheries, releasing resulting waste water into the sea (Gowland et al., 2002; Hart et al., 1997). Similar procedures are observed in Brazil for the control of parasites in shrimp and fish aquaculture (Luvizotto-Santos et al., 2009; Martins, 2004). Cypermethrin acts as a neurotoxin that affects the central nervous system, being highly toxic to bees, aquatic insects, crustaceans and fish (Keith and Walker, 1992; NPTN, 1998). A previous study using larvae of the Pantanal endemic shrimp *M. pantanalense* exposed to the cypermethrin-based formulation Barrage® reported a 96 h-LC_{50} as low as 0.05 μg/L (Soares et al., 2017).

The inclusion of histopathologic parameters in toxicity tests can reveal structural and specific toxic effects on organs at sublethal level (Dutra et al., 2017). Therefore, histopathology has been used in toxicity assessments aiming to identify tissue damage in aquatic animals exposed to contaminants (Dutra et al., 2017; Miron et al., 2008). Exposure of crustaceans to cypermethrin may damage the gill structure, as observed in the crab *Paratelphusa jacquemontii* exposed to cypermethrin (Nurocombi) insecticide, with signs of epithelial lifting, edema, necrosis, secondary lamella fusion, and hemorrhage in the gills (Maharajan et al., 2015). Cypermethrin also changes the proteins and structure of gills (vacuolization and collapse of gill filaments, edema and necrosis of epithelial cells, and rupture of epithelial layer) in red swamp crayfish (*Procambarus clarkii*), thereby impairing their physiological functions (Wei and Yang, 2015).

Given their close contact with the surrounding environment and high permeability, gills are an organ very sensitive to contaminants, and thus particularly

important in biomonitoring and impact assessments studies (Maharajan et al., 2015; Wei and Yang, 2015). Furthermore, given that cypermethrin is a lipophilic pyrethroid with high affinity and solubility in lipids, this compound is highly absorbed by the gills (Polat et al., 2002). The hypothesis of this study is that cypermethrin, through the formulation Barrage®, can cause histopathological changes in gills of the Pantanal endemic shrimp *M. pantanalense.* The sensitivity of these organisms was also assessed through evaluation of mortality and swimming behavior (equilibrium and position).

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2. Materials and methods

The study was carried out at the Laboratory of Carcinology, Shrimp Farming and Ornamental Organisms of Cerrado Pantanal (CARCIPANTA), located at the state of Mato Grosso do Sul, Brazil. Histological analyzes were performed at the Laboratory of Experimental Pathology, Institute of Biosciences, located at the Federal University of Mato Grosso do Sul, Campo Grande, Brazil.

2.1 Chemicals

Cypermethrin ([α-cyano-3-phenoxybenzyl ester of 2,2-dimethyl-3-(2-2 dichlorovynil) cyclopropane carboxylic acid]; C_{22} H_{19} Cl_2 NO_3; CAS Number: 52315-07-8) source was the commercial formulation Barrage®, bought from Zoetis-Fort Dodge (Campinas, SP, Brazil). The formulation Barrage® is a concentrated suspension, emulsifiable, containing 150 g/L of cypermethrin. The stock solution was prepared by diluting the compound in fresh water (tap water) (28 ± 1°C, conductivity 0.24 µS/cm, pH 7.5 ± 0.5, and dissolved oxygen above 8 mg/L).

2.2 Toxicity test

M. pantanalense specimens were collected in Lagoa Baiazinha (latitude: 20º15'49"S and longitude: 56º23'11"W), a pristine place at Pantanal of Mato Grosso do Sul and kept in a closed, recirculating system, under controlled conditions: temperature of 28 ± 1 °C, conductivity of 0.24 µS/cm, pH 7.5 ± 0.5, dissolved oxygen above 8 mg/L and 12 h:12 h photoperiod cycle (light:dark). The shrimps were fed twice a day with adjusted diet (dry basis), containing 30% of crude protein and 4200 kcal/kg of gross energy and fish fillet, following the common laboratory procedure. Organisms were acclimatized in the lab for 1 week before the experiment and during this time no mortality, anomalous behavior or morphology were observed. For the toxicity assay 150 adults were weighed and measured from the tip of the rostrum to the tip of the telson using a digital caliper (Digimess®, 0-150mm). Shrimp weight and total length were, on average, 0.49 g (standard deviation of 0.17) and 40.17 mm (standard deviation of 4.33), respectively. A completely randomized design with six treatments (0, 0.05, 0.25, 1.25, 3.75 and 6.25 µg/L of cypermethrin) was used. Exposure solutions were prepared by successive dilution of the stock in culture medium. In each treatment 5 replicates with 5 shrimp each were used. The toxicity test was performed in glass tanks with aeration and 2.5 L of test solution. The test solution was renewed daily after feeding the shrimp. The criterion used to evaluate the mortality was the lack of response to mechanical stimulus by touching the shrimp with a glass rod. Shrimp were observed every 24 h until the end of 96 h to evaluate mortality, swimming behavior (normal swimming vs. swimming with equilibrium disturbances) position (normal position vs. side-lying). After 96 h, the live shrimp were collected for histological analyzes.

2.3 Histopathology of gills

Shrimp were euthanized by immersion in a tank containing approximately equal amounts of ice and water until the animals lose the ability to swim and the reflexes. Rapid cooling is a quick method that does not cause stress nor induces histological changes (Wilson et al., 2009). The cephalothorax of 3 shrimps per replicate were sectioned, grouped and fixed in 10% buffered formaldehyde (pH 6.84) for 24 hours. Subsequently, the gills were removed from the cephalothorax and fixed in 70% alcohol to be used for histological analyzes.

For the preparation of the histological slides, the gills were placed in cassettes fitted with filter paper, dehydrated in a graduated alcohol series (70%, 80%, 90% and absolute), cleared in three sequences of xylene and embedded in paraffin at 56 °C for 10 minutes each. After obtaining the paraplast-embedded blocks, tissues were sectioned at 5 μm thick sections using a rotary microtome (micron HM325) and then stained with hematoxylin-eosin. Histological images of the gills were captured using a microscope (Olympus BX41) at 10x and 40x magnification.

2.3.1 Qualitative and semi-quantitative analysis of the gills

Changes in gills were evaluated according to the histopathological condition indices for gills adapted from Bernet et al. (1999) and other related studies (Dutra et al., 2017; Rodrigues et al., 2017). Histopathological changes were classified into 5 categories: circulatory, regressive, progressive, inflammatory and neoplastic reaction patterns. The observed pathological change was ranked as a "Factor of importance", being classified as 1, 2 or 3, corresponding to the minimum (reversible pathological lesions), moderate (reversible lesions in most cases after the neutralization of the stressor agent) and severe (often irreversible lesions that cause partial or total loss of the function of the affected organ) pathological importance, respectively (**Table 1**). Each

change was also assessed using a "score value" ranging from 1 to 6 (mild to severe occurrence) depending on its extent (i.e. percentage of areas in the gills exhibiting a specific alteration). From the classifications above, the Organ Index (Org I) was calculated according to the following equation: $Org\ I = \Sigma_{cha} (a \times w)$, where: "Org I" = Organ Index; "Cha" = change; "A" = score value; and "w" = factor of importance.

2.4 Statistical analysis

Statistical analysis was performed using the software SigmaPlot (version 12.5, Systat Software Inc., CA, USA) (Systat Software, 2014). Normality was verified using the Shapiro-Wilk normality test and the means were submitted to a one-way ANOVA. For non-normally distributed data, the nonparametric Kruskal-Wallis test was used. Means were compared by the Dunnett's test. The significance level for all statistical analyses was 0.05. The calculation of the LC_{50} values (lethal concentrations) was performed using the probit analysis in Minitab 17 Statistical Software (2010), with a 95% confidence interval.

3. Results

3.1 Acute test in adults of *M. pantanalense*

The effects of cypermethrin on the survival of adult shrimp *M. pantanalense* are shown in Figure 1 (response curves) and Table 2 (LC_{50} calculation). The LC_{50} ranged from 2.75 µg/L (24 h of exposure) to 0.93 µg/L (96 h of exposure). The effects of the compound observed in the behavior of adult shrimp *M. pantanalense* are shown in Figure 2. For the highest concentrations of cypermethrin (1.25, 3.75 and 6.25 µg/L), shrimp showed signs of lack of equilibrium as they did not swim in a straight motion and could not assume an upright position (significant after 48 h of exposure to 1.25

μg/L cypermethrin; Kruskal-Wallis, H=20.29, P=0.001; **Fig. 2A**). It was also observed that shrimps tended to remain closer to the air stone of the tank. At the same concentrations, shrimp, when not in motion, were unable to be in an upright position laying in their side with spasmodic movements of the pereiopods (**Fig. 2B**), also significantly after 48 h of exposure to 1.25 μg/L (Kruskal-Wallis, H=20.73, P<0.001).

3.2 Histopathological analysis of the gills

3.2.1 Qualitative analysis

The effects of Barrage® on the gills of adult shrimp *M. pantanalense* are shown in **Figure 3**. Individuals of the control group exhibited normal structure of gill filaments during the exposure period (**Fig. 3A**), with few cases of intercellular edema and mucous cell hyperplasia (score 2, 21-30%). Progressive lesions such as hyperplasia of mucosal cells and epithelial cells (**Fig. 3D**) were observed in shrimp exposed to concentrations 0.05, 0.25 and 1.25 μg/L of cypermethrin. Hypertrophy of mucosal cells was also observed in the gills of shrimp exposed to concentrations 0.25 and 1.25 μg/L. Several regression lesions were observed in the gills of shrimp exposed to concentrations 0.05, 0.25 and 1.25 μg/L, including alterations in epithelial structure, epithelial lifting of the lamellae, and changes in tissue structure such as shortening of secondary lamellae (**Fig. 3B and C**), lamellar fusion (**Fig. 3B**), as well as fat vacuoles, nuclear changes and atrophy when exposed to 0.25 and 1.25 μg/L Barrage®. At the concentration 1.25 μg/L, shrimp also exhibited necrosis. Circulatory alterations such as the presence of edema were also observed at 0.05, 0.25 and 1.25 μg/L **(Fig. 3C and D**). Inflammatory and neoplastic changes were not found.

3.2.1 Semi-quantitative analysis

After acute exposure, regression alterations were the most predominant in the gills, as shown in **Fig. 4**; the lesions were significantly increased at 0.25 and 1.25 μg/L (One-way ANOVA, $F=7.52$; $p<0.01$). Despite an increase in lesions, no significant change was observed in the circulatory (One-way ANOVA, $F=1.88$, $p=0.21$) and progressive categories (One-way ANOVA, $F=2.38$, $p=0.14$). Overall, organisms exposed to concentrations of 0.25 and 1.25 μg/L showed increased total pathological indices for gills relative to the control (One-way ANOVA, $F=29.46$, $p<0.001$) after the exposure period of 96 h (**Fig. 4**).

4. Discussion

The objective of this study was to evaluate the effects of the formulation Barrage®, a cypermethrin-based pesticide widely used in Pantanal, on adults of the endemic shrimp *M. pantanalense*. Histopathological effects have been studied to elucidate how the formulation can affect gill structure of shrimp at sublethal levels.

The 96 h-LC_{50} value found for adult *M. pantanalense* (0.93 μg/L) was higher than the previously reported for larvae of this species using the same formulation of Barrage® (0.05 μg/L) (Soares et al., 2017). This result is not surprising given that during larval development the central nervous system is still forming conferring higher susceptibility of larvae towards neurotoxic compounds (Anger, 2001; Arnberg et al., 2013). The 96 h-LC_{50} value observed was also higher than other values reported in the literature for adult shrimps, such as 0.02 μg/L for *Palaemonetes pugio* (using pure cypermethrin) (DeLorenzo et al., 2014), 0.019 μg/L for the freshwater prawn *Paratya australiensis* (pure cypermethrin) (Kumar et al., 2010), 0.11 μg/L for *Penaeu duorarum* (pure cypermethrin) (Cripe, 1994) and 0.002 μg/L for juveniles of *Palaemonetes argentines* (cypermethrin based formulation Sherpa®) (Collins and Cappello, 2006).

Besides different intrinsic species sensitivities, differences obtained may also be explained by the tested compound. The use of formulations, although representing a more realistic exposure scenario, may not translate the toxicity of the pure compound as the presence of additional constituents in their composition can add to or modify the toxicity of the active compound. In general, the high toxicity of cypermethrin-based formulations to crustaceans is not surprising since these compounds, like other pyrethroids, have been designed to control arthropod pests. Environmental factors such as salinity may also play a role on the toxicity of the chemical compounds as shown by Wang et al (2013) in a study where the 96 h-LC_{50} value for the shrimp *Litopenaeus vannamei* exposed to cypermethrin varied from 0.17 to 0.38 µg/L at salinity 5 and 20 % respectively.

High concentrations of Cypermethrin (1.25 and 3.75 µg/L) led to swimming difficulties apparently caused by equilibrium disturbances, inability to keep an upright position and spasmodic movements of pereiopods of shrimps. Information on the toxicity of this formulation to shrimps is limited. Comparable effects were observed using Excis®, a cypermethrin-based formulation, in adult lobsters *Homarus americanos*, which showed signs of lethargy, uncoordinated movements, claws either crossed or extended laterally and inactivity with spasmodic movements of pereiopods; the estimated 48h-LC_{50} was 0.08 µg/L (Burridge et al., 2000). These symptoms suggest an impairment of the motor function caused by neuronal disturbance (Keith and Walker, 1992; NPTN, 1998). Behavior disruption may have important consequences at population level as it may translate in impairment of important functions as feeding, predator avoidance and reproduction.

In addition to the above-mentioned effects, shrimp swam near the air stone of the tank in search of oxygen probably because cypermethrin increases oxygen consumption

due to an acceleration of metabolism, as observed in juveniles of *Palaemonetes argentinus* (Collins and Cappello, 2006). The authors observed that shrimp exposed to 0.0002, 0.0025 and 0.025 μg/L of cypermethrin (through the formulation Sherpa) for 96 hours increased hyperactivity, as well as oxygen uptake and nitrogen excretion measured as ammonia-N.

The gill is a multi-functional organ particularly important to crustaceans since it represent the fundamental site for gas exchange, regulation of ions, excretion of metabolic products, and then, a potential target for contaminants (Wei and Yang, 2015). The formulation Barrage® induced significant histopathological changes in gills of Pantanal shrimp *M. pantanalense* during acute exposure, with regressive lesions predominating (at 0.25 and 1.25 μg/L of cypermethrin). Although no significant difference was observed, an increase in progressive lesions was also recorded with increasing cypermethrin concentrations. A list of histological findings in the gills of different aquatic organisms (crustaceans and fish) exposed to cypermethrin is presented in **Table 3**. Overall, histological changes similar to those observed in the present study, such as intercellular edema, epithelial lifting of the lamellae, lamellar fusion, hypertrophy of gill epithelial cells or mucosal cells, hyperplasia of gill epithelia and necrosis have already been observed in previous studies with aquatic organisms exposed to cypermethrin (Arslan et al., 2017; Korkmaz et al., 2009; Moraes, 2013; Velisek et al., 2006; Velmurugan et al., 2009; Wei and Yang, 2015). On the other hand, changes in the gill structure, such as fat vacuoles, nuclear changes and atrophy (reduction of volume and number) observed in this study with Pantanal shrimp were not reported as effects of cypermethrin in previous studies. Given that pesticide formulations contain a mixture of chemicals (Elhalwagy and Zaki, 2009), these effects may be related to other unidentified compounds in the blend such as C8, C9 and C10 aromatic compounds,

which were already detected in the formulation Barrage® (Soares et al., 2017) and other pyrethroid-based formulations (Magdalan et al., 2009).

Pyrethroids are lipophilic and have a high absorption rate by the gills (Polat et al., 2002), directly affecting their structures. Lesions such as epithelial lifting, lamellar fusion, shortening of secondary lamellae, hypertrophy and hyperplasia of epithelia can be understood as a protective mechanism of shrimp, reducing the contact surface of the gills with the contaminated fluid and thus the contact with the hemolymph (Çaliskan et al., 2003; Maharajan et al., 2015). This mechanism can, however, affect gas exchange across the gills (Korkmaz et al., 2009). Necrosis is a more invasive deleterious consequence (Maharajan et al., 2015). Histopathological changes can compromise physiological processes and lead to hypoxia, respiratory failure and even death of organisms (Çaliskan et al., 2003). The histopathological condition index indicated greater effects of the formulation Barrage® on regressive alterations, classified as reversible lesions if the contaminant was eliminated from the medium (Bernet et al., 1999).

Taking into account the widespread use of Barrage® in Pantanal for agricultural livestock and domestic purposes, a continuous input of residues in the environment is expectable (de Barros, 1992; Gomes et al., 2011; NPTN, 1998). Thus, although concentrations tested in this study are not plausible to be found in the environment, this study highlights the need of refining the assessment of this pesticide in relevant exposure scenarios (*e.g.* lower concentrations) and calls the attention for the need of proper environmental monitoring and assessment of risk of pesticides use in Pantanal.

5. Conclusion

The formulation Barrage®, a cypermethrin-based pesticide widely used in the region of Pantanal (Brazil) was reported to cause behavioral and histological effects in the endemic shrimp *M. pantanalense*. Behavioral effects were characterized by equilibrium disruption and lethargy, translated in swimming difficulties and hypoactivity and denote neuronal effects of the active compound, cypermethrin. Histopathological effects included relevant structural lesions in gills which may affect their physiological function. Given the lack of knowledge on environmental concentrations of Barrage® in the Pantanal, this study calls the attention for the need of performing long term studies using environmental concentrations on local species to accurately assess impact of Barrage® in the aquatic communities of this fragile Biome. Given the increasing economic activity in the region population awareness of the risks of pesticide use, education for the correct use of pesticides as well as better policies for environmental protection are also needed.

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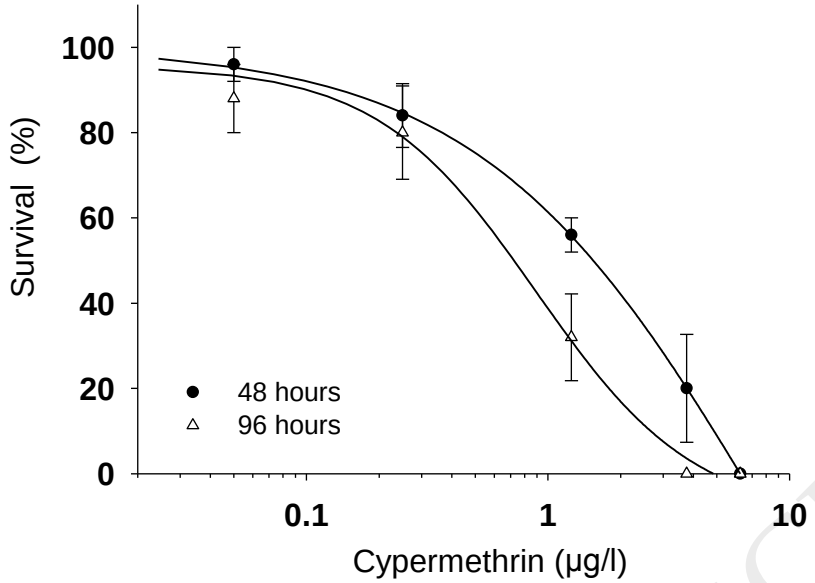
Captions

Figure 1. Cypermethrin effects in adults of *M. pantanalense*: survival after 48 and 96 h of exposure. Values represent means and error bars represent standard errors. The curve adjustment model was the four-parameter log-logistic function.

Figure 2. Cypermethrin effects in adults of *M. pantanalense*: Equilibrium disturbance (A); side-ways (B). Values represent means and error bars represent standard errors. * denote statistically significant differences relative to the control ($p < 0.05$)."#" indicates mortality.

Figure 3. Histological sections of the gills from *M. pantanalense* after acute exposure to cypermethrin through the formulation Barrage®. Photomicrographs of histological section of gill filaments of control (A) and organisms exposed to 0.05 µg/L (B), 0.25 µg/L (C) and 1.25 µg/L (D); Lamellar fusion (*), shortening of secondary lamellae (solid circle), Edema (ED), Hyperplasia of epithelial cells and mucous cells (black arrow), Epithelial lifting of the lamellae (black square), Nuclear changes (black triangle), Hematoxylin and eosin stain; 10 times magnification.

Figure 4. Total pathological condition indices and categories (circulatory, regressive and progressive) for gills from *M. pantanalense* shrimp after acute exposure to cypermethrin. Values represent means of each treatment ± standard error. * denotes condition indices significantly different relative to the control (Dunnett's test, $p < 0.05$).



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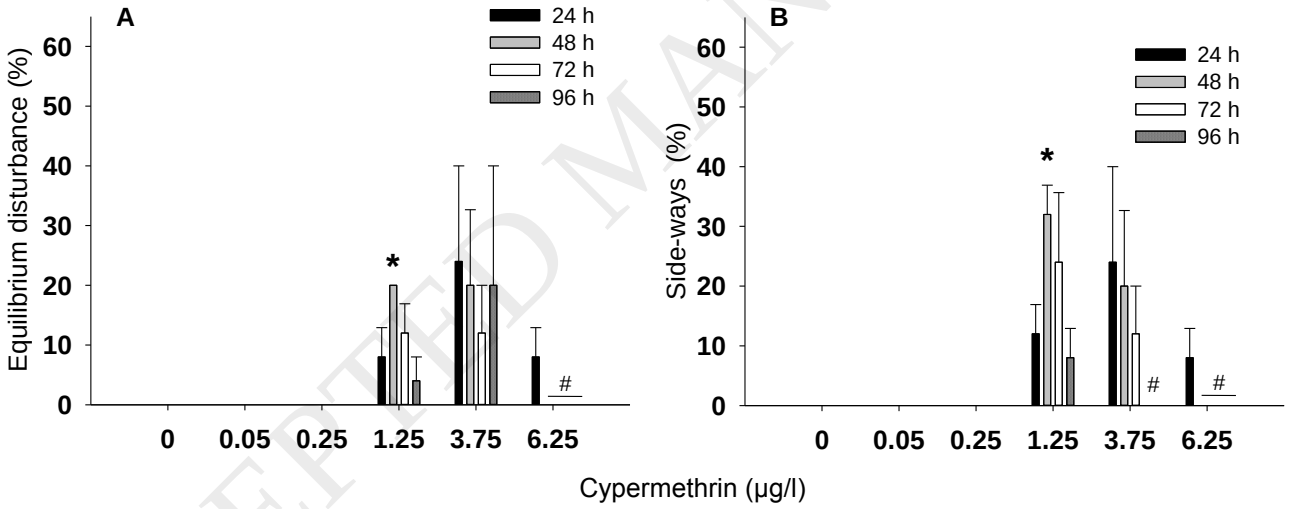

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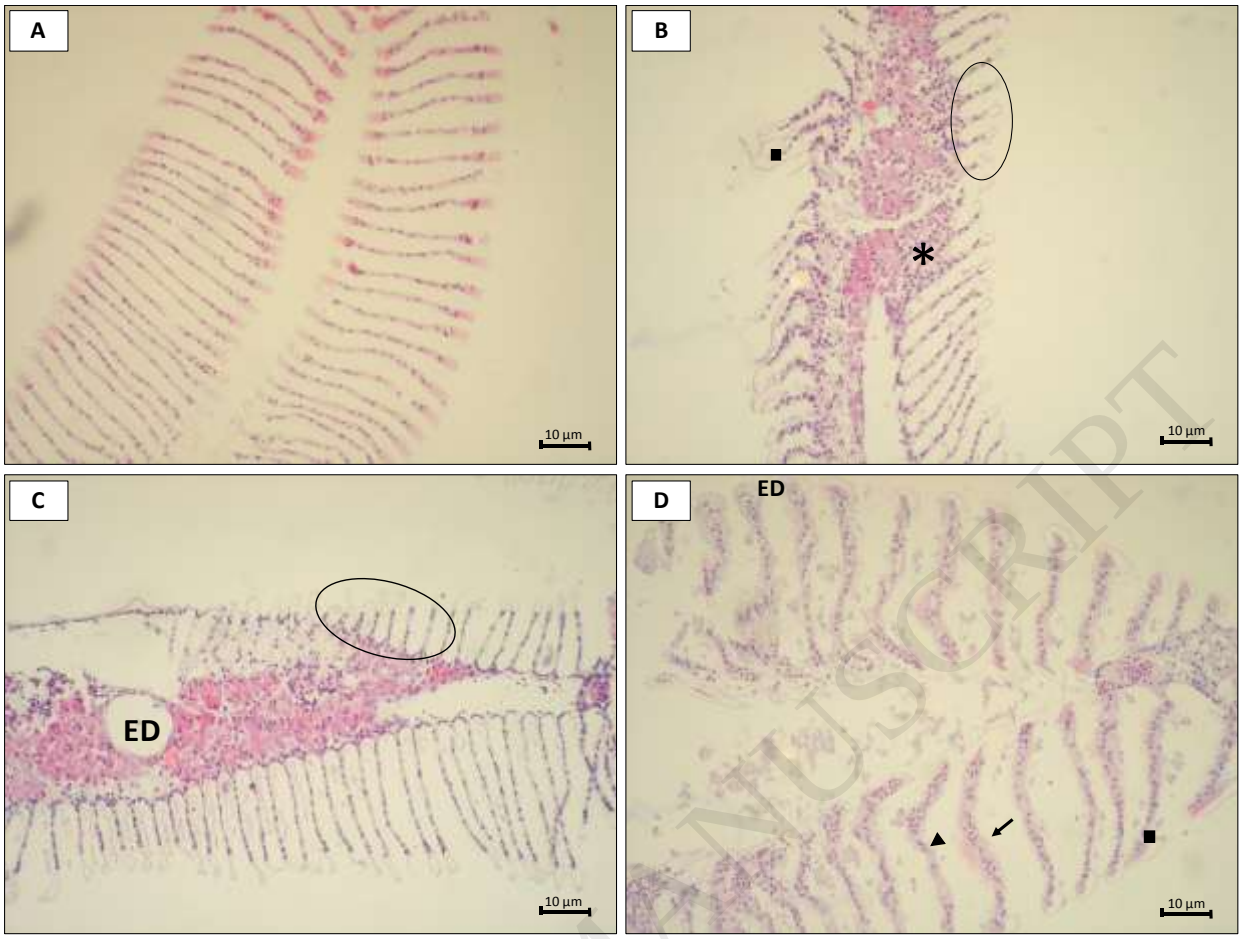

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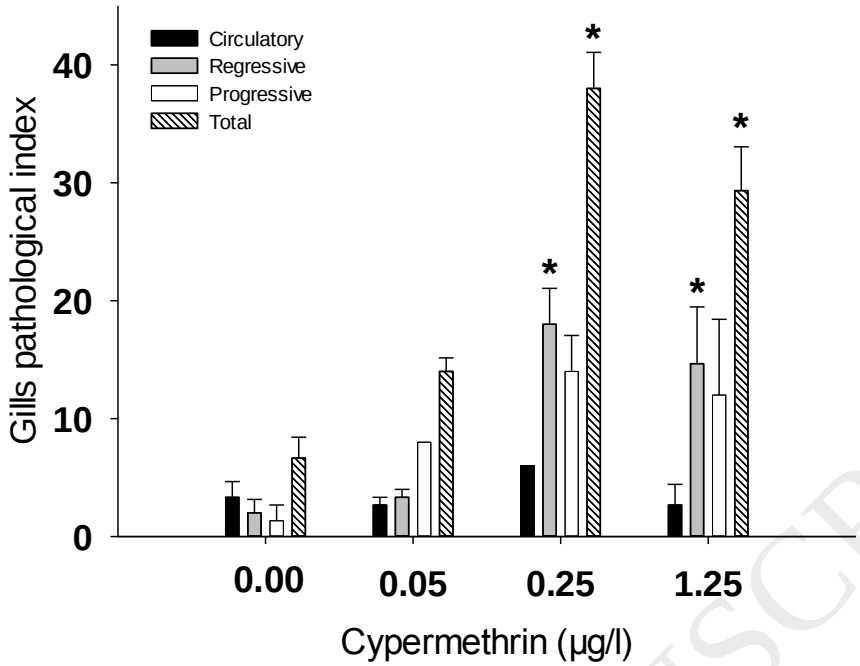

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Table 1. Descriptions of histopathological categories and examples of specific changes assigned to each for gills in the present study.

Histopathological categories	General description	Examples of specific tissue changes
		Gills
Circulatory	Disorders result from a pathological condition of the blood flow and tissue fluid	Intercellular edema (1)
Regressive	Disruption of tissue and/or cells that result in a functional reduction or loss of an organ. Changes in tissue architecture	Epithelial lifting of the lamellae (1) Lamellar fusion (1) Fat vacuoles (1) Nuclear changes (2) Atrophy (reduction of volume and number) (2) Necrosis (3)
Progressive	Increase in the number of cell types or specific structures	Hypertrophy of gill epithelial cells or mucous cells (1) Hyperplasia of gill epithelia (2)
Inflammatory	Presence of a greater number of cells involved in tissue repair; response to damaged tissue	Not observed
Neoplastic	Uncontrolled proliferation of cells and tissues	Not observed

( ) Factor of importance attributed to specific changes in gills

Table 2- LC_{50} values, the respective standard error and confidence interval for adults of *M. pantanalense*. Cypermethrin was used as the commercial formulation Barrage®.

Hours	LC_{50} (µg/L)	Error	95% confidence interval	
			Lower limit	Upper limit
24	2.75	0.27	2.24	3.38
48	2.11	0.24	1.68	2.67
72	1.67	0.21	1.29	2.17
96	0.92	0.12	0.71	1.25

Table 3. Review of cypermethrin effects on gills of different species of crustaceans and fish.

	Species	Chemical	Concentration	Changes	Reference
Fish	*Cyprinus carpio*	Cypermethrin	0.01 and 0.005 ppm	Hyperplasia of lamellar cells; telangiectasia of lamellae and thickening due to inflammatory cells infiltration.	Arslan et al., 2017
	Brycon amazonicus	Cypermethrin (Galgotrin)	7.2 µg/L	I-Hypertrophy and hyperplasia of chloride cells, vasodilation and apical aneurysm. II - Aneurysm and hemorrhage with rupture of the lamellar epithelium.	Moraes 2013
	Oreochromis niloticus	Cypermethrin	0.44 µg/L	Edema and hypertrophy of epithelial cells. Epithelial hyperplasia, necrosis, desquamation, fusion of secondary lamellae and 'curling' of secondary lamellae.	Korkmaz et al., 2009
	Clarias gariepinus	Cypermethrin	10.05, 20.10 and 30.15 µg/L	Epithelial hypertrophy, epithelial lifting and edema; hyperplasia of primary epithelial cells, fusion of secondary lamellae and necrosis and desquamation.	Velmurugan et al., 2009
	Oncorhynchus mykiss	Cypermethrin (Alimetrine)	31.4 µg/L	Severe telangiectasia of secondary gill lamellae with the rupture of pillar cells.	Velisek et al., 2007
	Lebistes reticulatus	Zeta-cypermethrin	20, 26, 35 µg/L	Lifting of epithelial layer and necrosis. Exudation, hyperplasia and the shortening of secondary lamellae.	Çaliskan et al., 2003
Crustacean	*Procambarus clarkii*	Beta-cypermethrin	0.005, 0.01 and 0.04 µg/L	Gill filaments were swollen, and lamellar epithelial cells appeared to be fused or necrotic. Gill lamellae exhibited peculiar malformations.	Wei and Yang 2015
	Paratelphusa	Cypermethrin	0.018 and	Enlargement of interlamellar space	Maharajan et al.,

jacquemontii	(Nurocombi)	0.037 mg/L	densely packed with granular material, and loss of gill structure; the gill lamellae get collapsed due to the disruption of the pillar cells; hemocoel filled with coarse amorphous to fibrous materials, thickened gill lamellae, and massive hemocytic infiltration; detached cuticle and rupture of capillaries at tip of the secondary lamellae releasing haemocytes; bulbular swelling at the tip; epithelial necrosis and hyperplasia; enlargement and disarrangement of secondary gill lamellae and lamellar fusion in some regions.	2015
Macrobrachium pantanalense	Cypermethrin (Barrage)	0.05, 0.25 and 1.25 µg/L	I- Intercellular edema, epithelial lifting of the lamellae, lamellar fusion, fat vacuoles and hypertrophy of gill epithelial cells or mucous cells. II- Nuclear changes, atrophy (reduction of volume and number) and hyperplasia of gill epithelia. III- necrosis.	**Present study**