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Liver morphometry and histopathology effects in *Astyanax lacustris* exposed to lambda-cyhalothrin pyrethroid insecticide

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ABSTRACT

Lambda-cyhalothrin (LC) is a pyrethroid insecticide employed to manage various arthropods as an alternative to persistent insecticides with minimized toxic effects on birds and mammals. However, LC causes oxidative and neurotoxic damages in fish *Danio rerio*. Effects of LC in morphology of liver of fishes are scare. We aimed to establish the lethal concentration (LC_{50} 96 h) of pyrethroid LC for *Astianax lacustris*. Then, we compare liver responses of sublethal doses of LC (i.e. 10.30 μg/L) for acute (i.e 1- and 3-days post-exposure - *dpe*) and chronic (i.e 6 and 12 *dpe*). We sought to identify pathological changes and compare liver histometric remodeling in fish subjected to acute and chronic toxicity tests. For this, liver histological changes were evaluated using the degree of tissue changes (DTC), followed by histomorphometric techniques determining structural volumetric density, glycogen cell density, and morphometry of hepatic tissue. We observed high hepatocellular injuries in exposed fishes. The main injuries included leukocyte infiltration, hyperemia, and pyknotic nuclei, especially 6 dpe. Hepatic glycogen storage decreases at 6 and 12 *dpe* showing metabolic damage. Both, density and volume of decreases after LC exposure in all exposure time. However, sinusoidal density increases after LC exposure, suggesting vascular hyperemia. These results show morphophysiological effects of LC at 10.30 μg/L in acute and chronic exposure represented by decreased glycogen storage, structural density, and volume of hepatocytes, in addition to a higher degree of histological changes.

1. Introduction

Human activities affected the state of the aquatic environment by changes of chemical, physical, and biological processes of natural water sources. This effect is due to pollutants released by industry, urbanization, transportation, tourism, and particularly agricultural and livestock activities [6,71]. In Brazilian agricultural systems, multiple natural and synthetic chemical compounds (i.e pesticides) are used to manage and prevent pests and diseases. Pesticides are transported to aquatic environmental mainly through surface runoff in agricultural fields, but its can be transported through soil or air [32,56]. Nonetheless, projections for the future anticipate a continued rise in pesticide use, which could lead to a notable decrease in natural habitats and potential harm to the preservation of biodiversity in aquatic ecosystems. Pesticides cause damage to the aquatic food chain since their toxicity decreases food availability. Then, some fishes end up changing their diets and risk seeking other foods, which can make them more vulnerable to predation when they leave their habitats in search of other foods [32,40]. In

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addition, pesticides can alter aquatic environmental by decrease amount of oxygen dissolved by decreased of aquatic plants [32]. Furthermore, the decrease in aquatic plants can lead to a decrease in protected places for fry to develop [40]. In this context, studies that use ecologically relevant native species as experimental models are needed. Thus, a challenge for ecotoxicological studies is the identification of sensitive native species to establish standardized studies that aim to identify direct responses in target organisms ([36]; Zagato and Bertoletti 2010; [17]). In this study, we identified that *Astianax lacustris* is an important native species for studies of pyrethroid effects and described responses in the liver, a target organ of pollutants.

Pyrethroids are currently the most promising and influential family of pesticides. Synthetic Type II compounds have increased by approximately 25 % over the past decade. This category contains an alpha-cyano group in its chemical structure, which confers more significant toxicity to non-target organisms [69]. In this group, lambda-cyhalothrin stands out as a widely used compound in agricultural, livestock, and urban environments to control various arthropods [24]. The worldwide application of pyrethroid compounds has contributed to the widespread contamination of aquatic ecosystems. Upon entering the environment, these substances accumulate within the ecosystem like microorganisms, plants, vertebrates, invertebrates, aquatic, and sediment in water bodies [12,38]. Considerable efforts have been made to understand the organic responses to dosage and exposure time of various pyrethroid compounds [5,63,70]. Sublethal doses produce cumulative systemic effects with chronic expressions, influencing the physiology of various systems and basal metabolism [19]. Fish exposed to synthetic pyrethroids, including deltamethrin, cypermethrin, and lambda cyhalothrin, exhibit reduced carboxylesterase levels. Carboxylesterase is an enzyme responsible for hydrolyzing chemical compounds. The reduction of carboxylesterase levels leads to the prolonged bioaccumulation of these compounds [28, 69]. This highlights the importance of the liver in regulating and maintaining homeostasis and its involvement in energy metabolism and biotransformation of chemical compounds. Consequently, researchers have targeted the liver to study the pathogenicity of different chemical compounds [27]. Bradbury and Coats [11] have also contributed to this field of study. However, morphophysiological characterization of effects of pyrethroids in fishes' liver are unknown.

Liver structure and function depend on the coordinated efforts of hepatocytes. These cells respond to chemical insults based on the severity of the insult and the extent of the cell population affected beyond the exposure level [64]. Pesticides have various mechanisms that affect hepatocytes, such as binding to receptors, inhibiting, or inducing enzymes that alter metabolic pathways, generating reactive oxygen species, altering membrane permeability, and damaging the cell's nuclear arrangement and other structural components [13,27,44]. In this context, fish can exhibit a variety of nonlethal and lethal hepatic lesions when undergoing chemical contamination. Some of the identified biomarkers of the degenerative process include cell hypertrophy, cytoplasmic vacuolization, lipidosis, nuclear pleomorphism, and focal necrotic changes; these have been reported in many studies investigating the hepatic effects of various chemical compounds [16,26,50]. For instance, the hepatic sinusoidal network actively participates in excretion by increasing blood flow due to vasodilation, a common histopathological response to inflammation [1,55]. Additionally, inflammatory reactions promote the synthesis of tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta), interleukin-6 (IL-6), interleukin-8 (IL-8), and heat shock protein (Hsp70) lead to histopathological changes, including hepatocyte vacuolization, passive hyperemia, mononuclear cell infiltration, and hepatocellular degeneration [18,31]. On the other hand, changes in hepatocyte nuclear volume result from loss of cellular homeostasis, leading to chromatin enlargement or condensation [14,68]. Hepatic tissue alterations in Cirrhinus mrigala exposed to 0.3 and 0.6 ppb of lambda-cyhalothrin were hypertrophy of hepatocytes, karyolysis and karyohexis, besides cloudy degeneration, congestion, dilatation of sinusoids and focal necrosis [62]. Meantime, studies evaluating the effects of lambda cyhalothrin on morphological and morphometric characteristics of the liver of neotropical species are scarce.

Morphological tissue changes are classically reported and sometimes classified based on pathological findings, and they are well accepted in cause-effect studies [19,54]. However, little is known about quantitative morphological characteristics such as liver structural volumetric density and hepatocyte morphometry in fish that have been exposed to pesticides. Automated and semi-automated microscopic techniques combined with quantitative image analysis provide unbiased multiparametric data at the single-cell level [35,53]. Variations in liver metrics can help us gain a better understanding of the pathogenesis of sublethal toxic processes, as well as their relationship with histopathological progress. Morphological techniques are good tools for evaluate effects of contaminants in both laboratory experiments and field studies for distinct species [21]. Then, in this study we'll analyze for the first time structural volumetric density of liver tissue and morphometric characteristics of hepatocytes to evaluated effects of lambda-cyhalothrin in fishes.

Astyanax lacustris is a small and abundant characiform fish widely distributed in streams, lakes, and rivers in tropical and subtropical regions [46,48]. This genus displays high phenotypic plasticity and adapts easily to diverse habitats. It is also characterized by rapid growth and reproduction, rusticity, and good potential for industrialization [48]. *Astyanax spp*. is a good test organism because different levels in the trophic chain are more likely to obtain recognizable responses involving other classes of biomarkers [59]. Therefore, we aimed to establish the lethal concentration (LC_{50} 96 h) of pyrethroid lambda-cyhalothrin (LC) for *A. lacustris*. After that, we compare an acute and chronic exposure of A. *lacustris* to sublethal concentration of LC of liver responses. We sought to identify and quantify pathological changes and compare liver histometric remodeling in fish subjected to acute and chronic toxicity tests. For this, we apply for the first time histomorphometry analyses as structural volumetric density and hepatocellular morphometry to evaluated effects of pollutants in liver tissue of fishes exposure to pyrethroid.

2. Material and methods

2.1. Fish conditions and sampling

Adult *A. lacustris* (n = 230) of both sexes were obtained from a commercial fish farm. They measured 8.4 (± 1.01 cm) in length, weighed 8.8 (± 3.83 g), and were acclimated for 30 days under laboratory conditions. The fish were maintained in a 1000 L tank with a static system, temperatures ranging from 25.5 to 28.3°C, artificial aeration, and 25 % water volume replacement every 24 hours. Dissolved oxygen during the experiment remained at 5.16 ± 0.22 mg/L, while pH remained at 6.87 ± 0.210. Fish were fed twice daily with commercial 5 mm extruded feed containing 32 % protein. All experimental management procedures and methods were approved by the Ethics and Animal Use Committee of the Federal University of Mato Grosso do Sul under protocol no. 1.108/2019.

2.2. Short-term toxicity test (mean lethal concentration, LC_{50}/96 h)

Specimens (n = 105) were randomized in three replicates (n = 5 per replica) and exposed to nominal concentrations of 0 (control), 4, 8, 16, 24, 32, and 64 μg/L of a commercial formulation of lambda-cyhalothrin (Trinca Caps®, UPL of Brazil, 25 % (m/v) [(S)-α-cyano-3-phenoxybenzyl (1 R,3 R)-3-[(*Z*)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate]) for 96 h (Apha and Awwa, wef 1998; ABNT 2004). For this experiment, fish were fasting in 30 L aquaria under a semi-static system with artificial aeration.

Dead specimens, when present, were removed hourly. At the end of the period, a nonlinear regression analysis (binomial method) using nominal concentration data (μg/L) logn - transformed yielded an LC_{50}/

96 h of 20.6 ± 0.02 μg/L (18.25–23.04 μg/L 95 % CI, Fig. 1). This analysis was performed using GraphPad Prism 8.0 software (GraphPad Software, Inc., San Diego, CA, USA).

2.3. Sublethal toxicity test

A total of 100 fish were randomly distributed into the four exposure periods (1, 3, 6, and 12 days). Fifteen exposed (50 % of CL50/96 h - 10.3 μg/L) to the same commercial formulation used in the short-term toxicity test, and ten control fish were analyzed each period. Briefly, the 50 % of LC_{50} (10.3 μg/L) was adjusted according to the bodyweight of the exposed specimens (9.0 ± 1.26 g, n = 15/aquarium; 4.5 g/L biomass) maintained in 30 L aquariums under a semi-static system with 24-hour total renovation of water and dosage. The control groups followed the same design but without chemical compost. During the experiment, the fish were fed the same diet twice a day, consistent with the acclimation period. Twenty-four hours before the end of the exposure duration, both the exposed and control groups were fasted.

2.4. Histopathological procedures

Fish were euthanized (eugenol solution, 450 mg/L) according to Kildea et al. [30]. The liver was then carefully removed and weighed (g). Transverse fragments (n = 3) of this organ from the exposed and control groups were fixed in 10 % buffered formalin solution for 24 h and then transferred to 70 % alcohol solution until histologically processed for Paraplast® (Sigma) embedding. Hematoxylin and eosin (HE) and periodic acid-Schiff (PAS) stained histological section (3 μm) were analyzed by bright field microscopy.

The degree of tissue change (DTC), which is based on the severity of the lesions and the possibility of recovery, was determined according to Bernet et al. [9] for the values (*w*) of each lesion. The final calculation

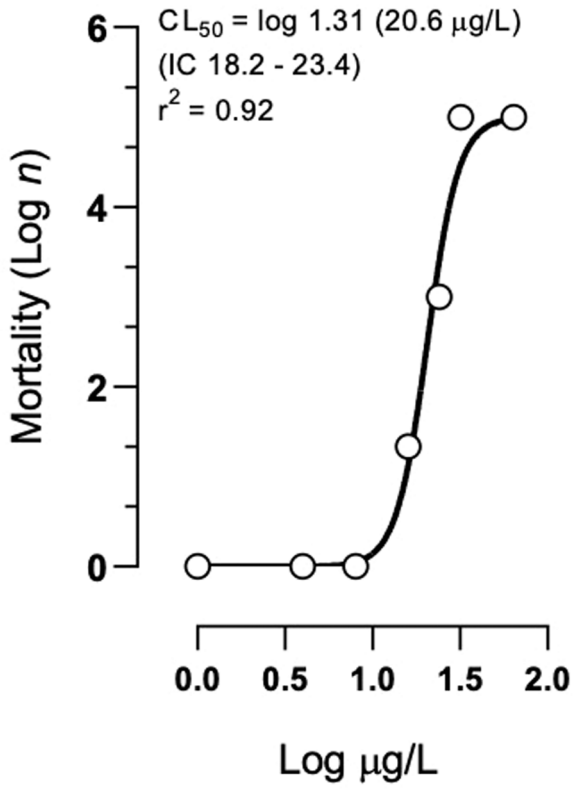

Fig. 1. Mortality rates in *Astyanax lacustris* exposed to different lambda-cyhalothrin concentrations, expressed by curve fit for LC_{50} (96 h); 95 % CI, confidence interval.

was estimated according to Poleksić and Mitrović-Tutundžić [47]. The DTC was based on the abnormalities' reversibility scores (*w*). The following formula was used to estimate the DTC:

$$DTC = (1 \times \Sigma\, w1) + (10 \times \Sigma\, w2) + (100 \times \Sigma\, w3)$$

Where: Σ summation of *w1* (reversible alterations), *w2* (partially reversible or moderated alterations with probable reversion after the end of exposition) and, *w3* (irreversible alterations). Table 1 presents the histological changes considered and their respective degrees of importance.

2.5. Hepatic histomorphometry analysis

2.5.1. Structural volumetric density

For this analysis, we obtained RGB images (4096 ×3286 pixels) using a microscope (Primo Star Zeiss) coupled with a digital camera system (OPTCAM 14.3 - Lopet14003). Five HE-randomized images of each cross-section from the liver fragment were captured. We overlaid a random offset grid of 252 intersections (quadratic lattice test system) to count points for each cross-section. The interpoint distance was 15 μm according to Reid [51]. At each intersection, we tallied the number of hepatocytes, sinusoids, biliary ducts, blood vessels (arterial, venous, and capillaries), and melanomacrophage aggregates (MMAs). To calculate the final density for each structure, we used the formula VDE (%) = ([Ip x 100]/Tip). Here, Ip represents the intersections counted for the structure, and Tip represents the total number of intersections in the image [52]. The images were analyzed using ImageJ version 1.48 v [20].

2.5.2. Hepatic glycogen density

Five liver fragment cross-section images stained with Periodic Acid Schiff (PAS) were randomly selected at a magnification of 400 × to determine hepatic glycogen density. The same system as previously reported was used. The images were analyzed using Image-Pro Plus 6.0 software (Media Cybernetics, Inc, USA). Initially, the tool measured the total standardized image area (58,969.79 μm^2) using the planimetric field. Subsequently, the "count/size" tool was used for measurements. We used the "dropper" tool to determine the color of the image. The tool's sensitivity was set at 3 to select the entire well-marked area (in square micrometers). To estimate the final count per section (Fc), we used the formula Fc% = market area / 58,969.79 × 100.

2.5.3. Hepatocellular morphometry

Ten images from HE histological sections at 1000 × magnification per fragment/fish were captured randomly. The perimeter (μm) and area (μm^2) of hepatocytes and the perimeter (μm), area (μm^2), and diameter (μm) of hepatocyte nuclei were estimated in 10 cells randomly selected per image. Additionally, the nuclear volume of hepatocytes was estimated using the following equation: $Vn = (4/3\ \pi\ r^3)$, where V*n* is the nuclear volume (μm^3) and r is the nuclear radius [22]. The hepatocyte

Table 1
Degree Tissue Change (DTC)* according to the importance score of lesions observed in the liver.

Hemorrhage/hyperemia/congestion/oedema sinusoidal	1
Cellular tumefaction/hydropic degeneration	1
Cytoplasmic inclusion/hyaline/lipofuscin	1
Melonomacrophages deposits	1
Cell atrophy/hypertrophy	2
Nuclear alterations (pyknosis/karyolysis/karyorrhexis/inclusions)	2
Granulocytic or agranulocytic infiltrate	2
Cell necrosis (focal/diffuse)	3
Sinusoidal epithelial necrosis	3

* According to Bernet et al. [9]; 1, easily reversible; 2, moderated alterations with probable reversion after the ende of exposure and 3, irreversible alterations.

volume was calculated indirectly using the formula: V*Hep* = (A*Hep* × V*Nuc*) / A*Nuc*), where V*Hep* is the hepatocyte volume (μm^3), A*Hep* is the hepatocyte area (μm^2), V*Nuc* is the nuclear volume (μm^3), and A*Nuc* is the nuclear area (μm^2), according to Leão et al. [35]. These measurements were made in Motic 2.0 software (Motic Asia, Hong Kong).

2.6. Statistical analysis

The time-exposure effect and difference between control and exposure groups were compared using a generalized linear mixed model, followed by pairwise comparisons using the least significant difference test. To compare DTC among time exposures, the nonparametric Kruskal-Wallis test was used, with pairwise comparisons performed using the Mann-Whitney *U* test. SPSS 23.0 (IBM®) software was employed for these analyses. Box plots were created with DTC, hepatocyte, and nuclear volume values. The median, 25th, and 75th quartiles, the minimum and maximum values, and the interquartile intervals were compared between the groups depicted on the plots. A nonmetric multidimensional scaling (nMDS) analysis was performed to assess the effect of the *w1*, *w2*, and *w3* reversibility scores among between exposure times. First, we constructed a comprehensive matrix of fish characteristics, with each fish as a row and each score as a column. We multiplied the incidence of each alteration by its respective importance score (*w* in the DTC formula). Next, we computed a distance matrix using the Gower similarity index among fish. Subsequently, we conducted a permutational multivariate analysis of variance [7] to examine the impact of exposure periods and their interactions on the Gower distance matrix scores. To calculate the *p-value*, we constrained the permutations by treating the time exposure as blocks. This approach allowed us to test the differences between the control and exposed groups only for each exposure period. Afterward, we performed a pairwise multilevel comparison between groups of exposure periods. For these analyses, we utilized the "RVAi - deMemoire" and "vegan" packages from R Core Team [49] and Oksanen et al. [45], respectively.

3. Results

3.1. Sub-lethal toxicity test

The water quality parameters remained stable throughout the experimental period, with dissolved oxygen (mg/L) at 5.16 ± 0.226, temperature (°C) at 26.39 ± 0.985, and pH at 6.87 ± 0.210. The control group did not experience any mortality during the chronic toxicity test. In contrast, the treated group experienced mortality at different exposure times: n = 1 (6.6 %) at 1-day post-exposure (*dpe*); n = 3 (20.0 %) at 3 *dpe*; n = 1 (6.6 %) at 6 *dpe*, and no mortality at 12 *dpe*. There were no significant variations in biomass over the experimental period (data not show).

3.2. Liver histopathological analysis

DTC degrees were elevated in all exposed fish. Still, no significant difference was observed regarding the time of exposure (Fig. 2a). The nMDS analysis showed alterations in lesion patterns based on their origin and intensity during the exposure period. Profiles varied according to the severity of the lesions, with W3 emerging at 3 days of exposure, while W2 was more prominent in specimens exposed at 6 and 12 *dpe* (Fig. 2b). Lesions were distributed over all periods studied in the parenchyma and the hepatic stroma at 6 and 12 days of exposure (Fig. 3). Vascular hyperemia and cytoplasmic vacuoles increased with exposure time, resulting in cell dissociation and disruption of intercellular junctions and sinusoidal endothelium. Friable cytoplasm with eosinophilic micro-deposits was commonly observed in these two periods with focal-extensive and diffuse distribution. Nuclear features such as pyknosis and karyolysis were often observed in these samples. Melanogenic macrophages were found diffusely around the sinusoidal space and sometimes adjacent to pancreatic acinar cell clusters, especially following 6 days of exposure.

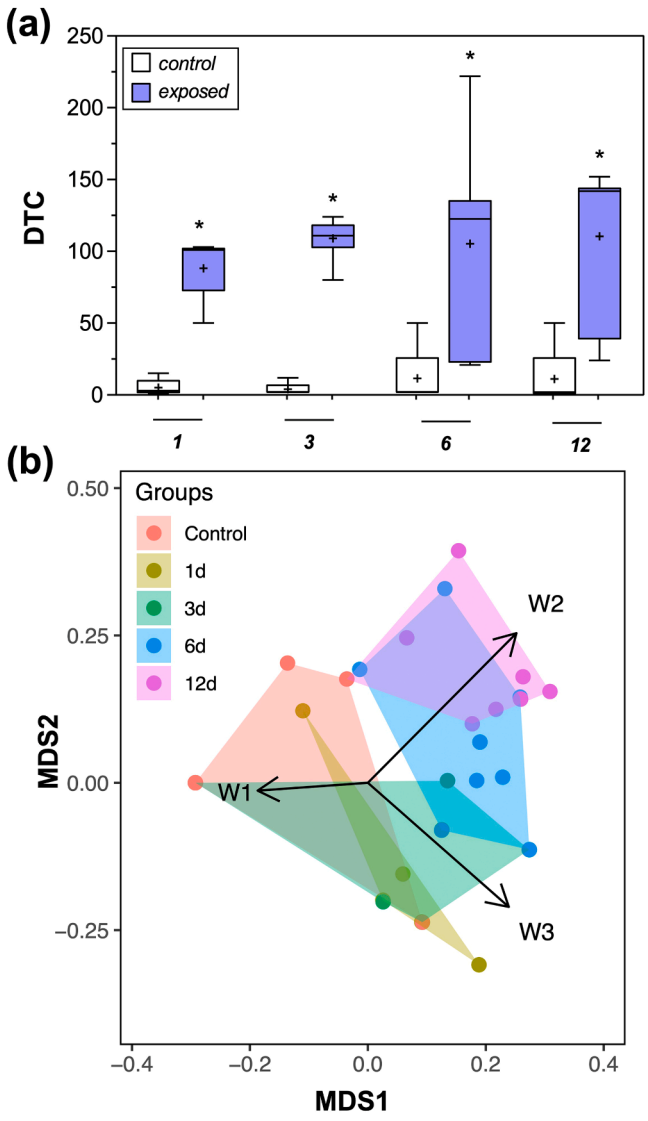

Fig. 2. Degree Tissue Change (DTC) and ordination diagram in *Astyanax lacustris* exposed to lambda-cyhalothrin (10.3 μg/L) for different periods (1, 3, 6, 12 days). (a), the DTC box plot illustrates the median, mean (+), 25th and 75th quartiles, and amplitude (bars). (b), the non-Metric Dimensional Scaling (nMDS) representation was performed by a Grow distance matrix calculated from the histopathologic scores (*w1*, reversible changes; *w2*, partially reversible or moderated changes with probable regression after the end of exposure; and *w3*, irreversible changes). 1d, 3d, 6d and 12d: 1, 3, 6, and 12 days after exposure. Control: animals of control group.

3.3. Hepatosomatic index, structural volumetric density and hepatocellular morphometry

The hepatosomatic index decreased in the exposed fish at 6 and 12 days; however, it was only significantly different from the control at 12 days. There was an inverse relationship between the volumetric densities of hepatocytes and sinusoids. The volumetric density of hepatocytes differed from the control samples in all-time exposures, with the lowest density observed at 12 days. In contrast, sinusoidal density increased from the 3-days, remaining high in the control samples. Although the density of melanogenic macrophages aggregates was low, the values observed in the exposed fish were higher in all periods in relation to the controls. No differences were observed in the blood

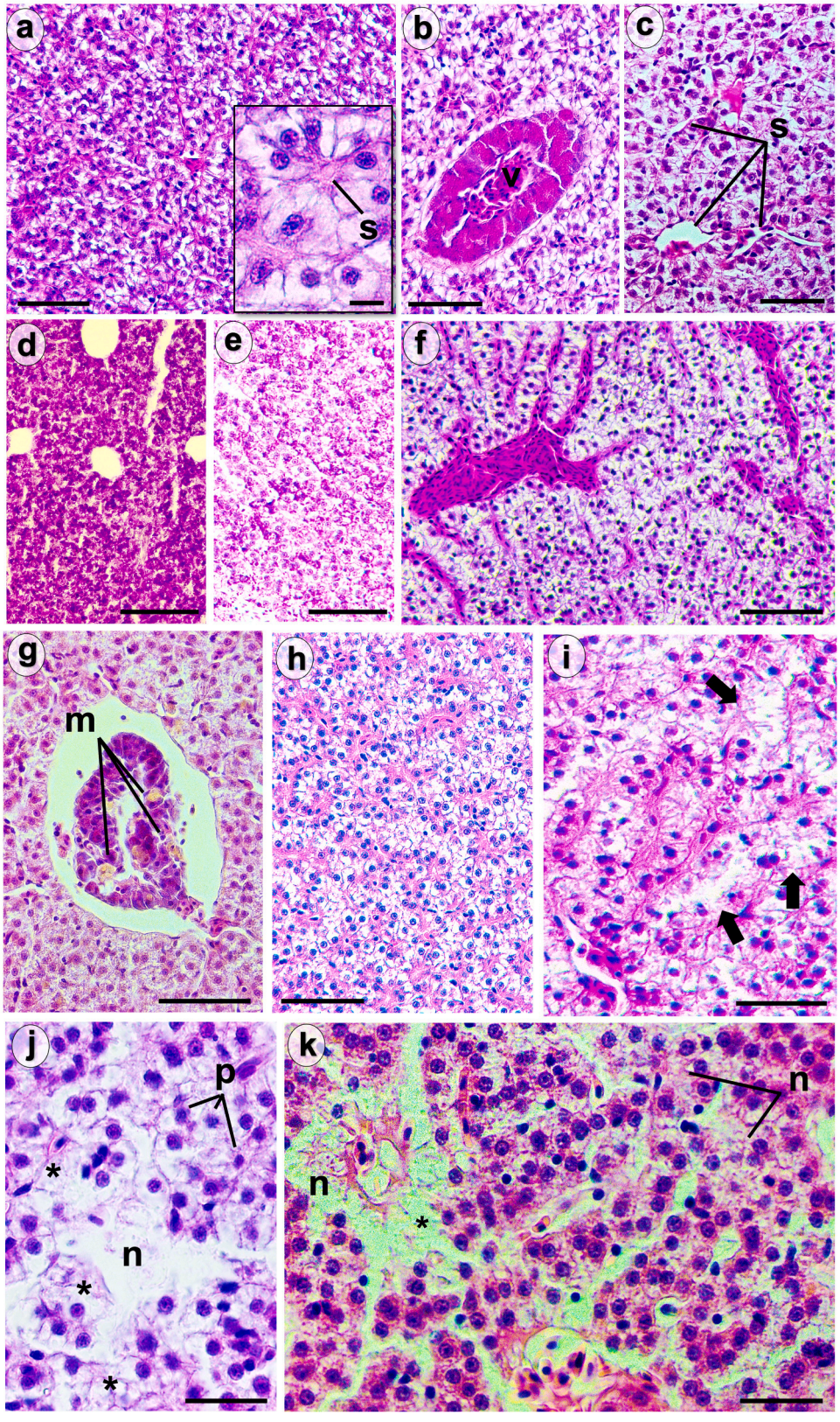

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Fig. 3. Histological sections of liver parenchyma in *Astyanax lacustris* exposed to lambda-cyhalothrin (10.3 μg/L). (a), (b) and (c) control samples. In (a), liver parenchyma is homogeneous with intact cells; hepatocytes are slightly vacuolated but evenly distributed; in highlight, a cross-section sinusoid (s) surrounded by hepatocytes (bar scale = 5 μm; in (b), hepatopancreas organized in cluster of acinar columnar cells enclosing a portal vein (v); note the vacuolar nature of liver parenchyma; in (c), typical cordonal arrangement of hepatocytes evidencing the sinusoidal network (s) with intact endothelial cells (a, b and c, bar scale = 100 μm; HE). (d) and (e), a contrast of hepatic parenchyma of control (d) and 12-days exposition sample (e) for glycogen hepatocellular deposits (bar scale = 100 μm; periodic acid-Schiff stain). (f), severe hyperemia observed in a sample from 6-day exposition; note the vasodilatation of portal veins and sinusoids; hepatocytes remaining intact (bar scale = 100 μm; HE). (g), hepatopancreas showing melanogenic macrophages (m) infiltrate; a prominent distention of the peripherical space between pancreatic acini and hepatocytes usually observed in samples from 6-day exposition (bar scale = 100 μm; HE). (h), vacuolated and enlarged hepatocytes displaying initial loss of the normal sinusoidal contour in a 6-day exposition sample (bar scale = 100 μm; HE). (i), hepatocellular disruption (arrows) follows by loos of sinusoidal endothelial architecture in a sample from 12-day exposition (bar scale = 50 μm; HE). (j), a coagulative necrosis area (n); enlarged hepatocytes showing rupture of the cytoplasmic membrane with amorphous cell debris (*); some pycnotic (p) nuclei may be observed; necrosis (n);12-day exposition sample (bar scale = 20 μm; HE). (k), a 12-day exposition sample showing marked sinusoidal dilatation (*) with severe epithelial disruption, hepatocytes reduced; nuclei remarkably basophilic; parenchymal and perivascular necrosis (n) areas may be observed (bar scale = 20 μm; HE).

vessels, biliary ducts across exposure time. At day 3 and day 6, hepatic glycogen storage decreased in both the control and exposed samples. However, the reduction was more pronounced in the exposed. These findings are presented in Table 2.

Hepatocyte and nuclear morphometry are represented in Fig. 4. Hepatocyte volume decreased for both control and exposed specimens after 3 days. However, in the exposed group, volumes were significantly lower for all periods of exposure. The same trend was observed for nuclear volumes. The values were lower at 6 and 12 days than on day 3 within the exposed specimens.

4. Discussion

Astyanax lacustris was a useful bioindicator of the LC effects, as evidenced by the observation of hepatic metabolic and structural damage subsequent to exposure. The abundance, widespread distribution throughout the Neotropical region, and laboratory facilities handling have encouraged controlled studies using the Astyanax genus as an in vivo model [57,10]. Tissue morphology details of some species of this genus have been reported [39,43]. However, investigations into the histopathological profile related to hepatocellular histometry in specimens exposed to a synthetic pyrethroid are still limited.

The concentration obtained in the short-term toxicity test exceeded that of several species, including *Channa punctatus* (7.92 μg/L; [33]), *Danio rerio* (5.3 μg/L; [3]), *Labeo rohita* (2.72 μg/L; [23]), and *Sarotherodon melanotheron* (5 μg/L; [4]) showing that *A. lacustris* is a resistant species. Differences among species can occur due to many factors such as life stage, formulation of the compound (due presence of inert compounds in formulations), basal metabolism, and the resistance and tolerance particularities inherent to each species, among others, may be pointed as factors that affect the response in acute toxicity tests [25,61]. This is the first study showing LC_{50} using the Astyanax genus with lambda-cyhalothrin or any other synthetic pyrethroids, then currently there are no studies for comparation.

The sublethal toxicity test was performed to evaluate distinct hepatic morphological aspects related to acute and chronic responses. However, the mortality rate at 1-, 3-, and 6-days post-exposure (*dpe*) revealed that the calculated concentration was not sublethal. Recent models have confirmed that the response of a species to exposure is regulated by toxicokinetics (TK) and toxicodynamics (TD) processes. The sensitivity of a species to a chemical compost depends on the relation between TK, and TD processes with the dynamics of exposure [42]. Moreover, metabolic rates vary within species and are regulated by specific gene expression patterns from phase I and II of the detoxification process [34]. Thus, even when exposed to 50 % of the LC_{50}, some fish exhibited low tolerance and died by the sixth day of exposure. In contrast, most of the population demonstrated resistance and survived until the twelfth day of exposure. Although the relationship between TK and TD for LC in

Table 2
Mean (± epm) of hepatosomatic index (HSI), structural volumetric density (SVD) and hepatic glycogen percentage in *Astyanax lacustris* exposed to lambda-cyhalothrin (10.3 μg/L) according to the exposure time (days). Different lowercase letters indicate significant differences between control groups over time. Asterisk indicates a significant difference between control and exposed group within time-exposition ($P < 0.05$). MMAs, melanogenic macrophages aggregates.

		Exposure time (*days*)							
		1		**3**		**6**		**12**	
HSI (%)	Control	0.96	±0.13	0.92	±0.12	0.86	±0.07	0.89	±0.08
	Exposed	1.19	±0.19^{a}	1.04	±0.23^{a}	1.00	±0.12^{ba}	0.69	±0.03^{c*}
SVD (%)									
Hepatocytes	Control	86.9	±1.35^{a}	83.4	±1.17^{a}	79.2	±1.06^{b}	77.4	±0.82^{b}
	Exposed	71.9	±1.48^{a*}	69.7	±0.87^{a*}	69.9	±0.87^{a*}	67.8	±1.02^{ba*}
Sinusoids	Control	13.8	±1.24^{a}	13.7	±0.76^{a}	15.1	±0.68^{b}	17.2	±0.69^{b}
	Exposed	19.2	±0.92^{a*}	25.4	±0.85^{b*}	24.1	±1.06^{b*}	27.0	±0.82^{b*}
Blood vessels	Control	0.12	±0.04	1.52	±0.38	1.85	±0.61	0.98	±0.27
	Exposed	2.26	±0.58	1.50	±0.40	1.55	±0.41	0.77	±0.23
Biliar ducts	Control	1.44	±0.36	0.16	±0.08	0.30	±0.15	0.29	±0.21
	Exposed	0.24	±0.10	0.40	±0.14	0.48	±0.25	0.51	±0.18
MMAs	Control	0.10	±0.06	0.16	±0.06	0.09	±0.04	0.13	±0.04
	Exposed	0.24	±0.06	0.27	±0.08	0.14	±0.06	0.17	±0.06
Hepatic glycogen	Control	25.1	±10.8^{a}	31.0	±2.01^{a}	19.3	±1.30^{b}	15.6	±0.86^{b}
	Exposed	23.3	±2.00^{a}	21.3	±1.25^{a*}	15.8	±1.27^{b*}	14.5	±1.01^{b}

^{abc} represent significant ($P < 0.05$) differences among time-exposition; * represent significant ($P < 0.05$) differences between control and exposed fish.

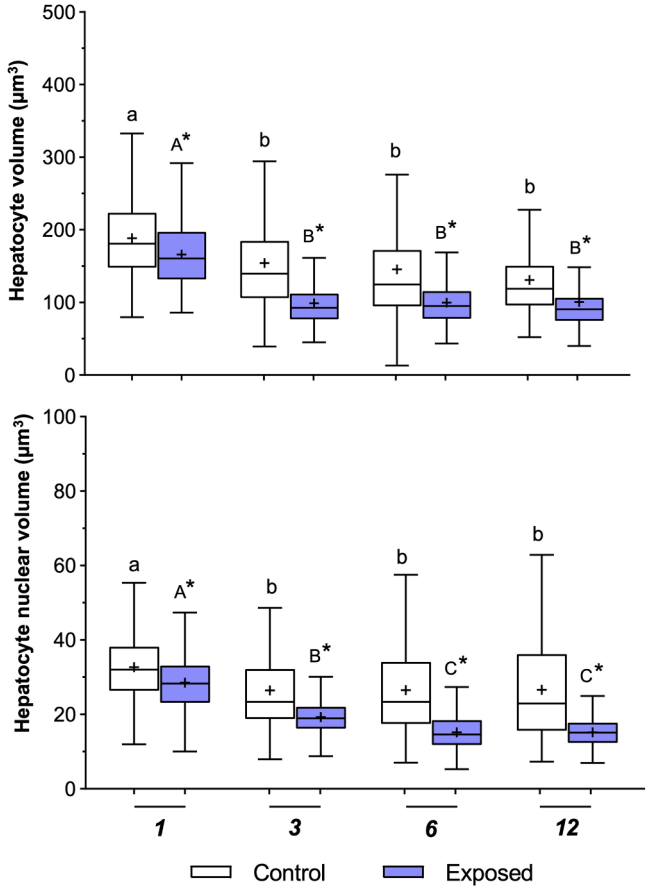

Fig. 4. Effect of lambda-cyhalothrin (10.3 µg/L) on the hepatocyte and nuclear volume in *Astyanax lacustris* according to the exposure time (days). Box plot showing median 25 and 75th quartiles and amplitude (bars). Different lowercase letters indicate significant differences between control groups over time; different capital letters indicate significant differences between exposed groups over time; asterisk indicates a significant difference between control and exposed group within time-exposition ($P < 0.05$). 1, 3, 6 and 12: 1, 3, 6 and 12 days of exposure.

A. lacustris is not known, this scenario could be theorized for fish populations in water bodies contaminated with lambda-cyhalothrin, even though histopathological changes were relevant at all exposure periods.

The histopathological findings were higher from 1 *dpe* but did not differ between the other periods, although lesions classified as partially reversible (*w2*) and irreversible (*w3*) were more prevalent from day 3 *dpe*. Thus, inflammatory lesions resulting from the compound's biotransformation process occur earlier and gradually evolve as exposure time increases. This leads to cellular hypertrophy, cytoplasmic vacuolization, degenerative nuclear figures, and progressive sinusoidal and hepatocellular necrosis, as a notable tissue response [60]. This histopathological targeting is consistent with many species of fish exposed to pyrethroid pesticides [54,8]. On the other hand, these changes are consistent with variations in the structural volumetric density as well as morphometric attribute of hepatocytes.

The hepatosomatic index (HSI) is often employed as a biometric marker to determine the health status by assessing the liver's metabolic reserve depletion, energetic cost, and response to toxic events and pathological cellular modifications. Changes in this indicator in fish exposed to pesticides may suggest disturbances in endocrine balance in different parts of the body [2,37]. In the present study, the decrease of HSI appears only at 12 dpe, similar to Claris gariepinus exposed to 0.024 mg/L of lambda-cyhalothrin for two weeks [29]. Until this period, there was no change in this index in contrast to the increase of the sinusoid's density, suggesting that blood flow in liver tissue has quickly adapted to different inflammatory mediators [18]. Vascular hyperemia and vasodilatation promote macrophage transportation to damaged areas and enhances cellular oxygenation. Consequently, both hyperemia and the presence of melanomacrophage aggregates can be used as early biomarkers of LC toxicity, a phenomenon also observed in *Oreochromis mossambicus* [41]. Nevertheless, this initial structural scenario after exposure to LC does not appear to have interfered with the HSI.

Variations in cell size as well as glycogen stores suggest subtle metabolic adjustments. Decreasing cell size promotes energy flows through mitochondrial work, enabling detoxification procedures. Synthetic pesticides and other chemical compounds act as endocrine-disrupting chemicals, impairing immune response, growth and development, increasing osmotic stress, protein degradation, and increasing the metabolic cost [66,67]. Our results showed that the hepatic toxicity of LC is related to a progressive depletion of glycogen storage as well as the failure of the hepatic architectural structure. Moreover, the decrease in the nuclear volume indicates an increased frequency of pyknotic forms. Nuclear pyknosis is characterized by profound cellular degenerative changes due to the condensation of chromatin, often accompanied by nuclear fragmentation (karyorrhexis), both of which are irreversible changes categorized as *w3* [14,65,9]. Exposure to synthetic pyrethroids diminishes carboxylesterase levels, leading to heightened oxidative stress [15,28]. This circumstance, in combination with other free radicals, impairs the typical cellular structure of diverse tissues, finally compromising the nuclear organization [58].

5. Conclusion

Our results demonstrated that exposure of 10.3 µg/L of LC for 12 days caused histological alterations in *Astyanax lacustris*, followed by changes in the structural volumetric density and hepatocellular morphometry. LC toxicity caused increased circulatory lesions at 1 *dpe*, with regressive characteristics at 3 *dpe*. Over 12 *dpe*, there was an increase in sinusoid density and a significant reduction in hepatic glycogen reserves. Hepatocyte nuclei experienced a marked decrease after 6 *dpe*. Continuous exposure of fish to low concentrations of pyrethroids, particularly LC due to its lipophilic properties, leads to significant damage to hepatic tissue. There could be environmental consequences of these disorders, which could be costly. Thus, we suggest that future studies evaluate longer exposure times in addition to systemic effects of pyrethroid. Therefore, it is imperative to persist in integrating the most effective management practices to mitigate pesticide runoff and thereby minimize the risks posed by toxicological effects.

Ethical approval

All experimental management procedures and methods were approved by the Ethics and Animal Use Committee of the Federal University of Mato Grosso do Sul under protocol no. 1.108/2019.

Consent to Participate

The authors agree to participate in this study.

Consent to Publish

The authors agree with the publication of this study.

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CRediT authorship contribution statement

Wendt Carla Leticia Gediel Rivero: Writing – review & editing, Formal analysis. **do Nascimento Silva André Luiz:** Methodology, Investigation. **Nogueira Farias Karine Nathiele:** Methodology, Investigation. **Fernandes Carlos Eurico:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Venancio da Silva Tiago:** Writing – original draft, Methodology, Investigation, Conceptualization. **Garcia Maria Eduarda Corona:** Methodology, Investigation. **Franco-Belussi Lilian:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Gonçalves Sabrina Fuzer:** Methodology, Investigation. **de Oliveira Martins Brenda:** Methodology, Investigation. **de Oliveira Brasileiro Lucas:** Methodology, Investigation. **Fogaça Edilaine:** Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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