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ORIGINAL RESEARCH

Acute toxicity and effects of the Roundup Transorb, a glyphosate-based herbicide, on freshwater teleost matrinxã, *Brycon amazonicus*

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Abstract The aim of this study was to evaluate the susceptibility of the Neotropical freshwater fish matrinxã, *Brycon amazonicus*, exposed to Roundup Transorb®, a newer commercial formulation 50% stronger than the ordinary mixtures and with a new technology for quicker absorption and faster translocation. The median lethal concentration (96 h-LC_{50}) was determined, and the effects of a sublethal concentration on oxidative stress biomarkers were evaluated in the liver, gills, white muscle, and heart. During the acute toxicity test, fish mortality progressively increased with RT concentration along with behavioral abnormalities were detected. The calculated 96 h-LC_{50} value of RT was found as 1.21 mg/L. Fish exposed to 0.5 mg/L of RT (40% of 96 h-LC_{50}) showed a significant increase in the hepatic somatic index (HSI) with a concomitant decrease in Fulton's condition factor (K). The sublethal exposure also induced significant increases ($p < 0.05$) in the hepatic lipid peroxidation (LPO) levels, reduced glutathione (GSH) content, and catalase (CAT) activity, with a concomitant decrease in the glutathione S-transferase (GST) activity. In the gills, RT exposure induced significant increases in the GST activity, GSH and LPO levels, whereas CAT activity was inhibited. RT also induced CAT and GST activities together with the content of GSH and LPO levels in the white muscle. On the other hand, significant decreases in the CAT and GST activities were detected in the heart with a marked increase in the GSH content and no changes in LPO levels. Therefore, the exposure to 0.5 mg/L of RT induced oxidative stress in the liver, gills and white muscle with tissue-specific responses related to antioxidant defenses. This study demonstrated that *B. amazonicus* is sensitive to Roundup Transorb® at sublethal and environmentally relevant concentrations, even at short-term exposures. The use of Roundup Transorb® should be carefully monitored due to its adverse non-target impacts.

Keywords Median lethal concentration . Oxidative stress . Antioxidants . Lipid peroxidation . Fish

Introduction

Glyphosate [N-(phosphonomethyl) glycine] is one of the most widely applied broad-spectrum herbicides in the world (Baer and Marcel 2014; Alcántara-de la Cruz et al. 2020). It has been marketed under different names and formulations, including the best-known trade name Roundup® originally produced by Monsanto/Bayer. Roundup Transorb® is one of most recent Roundup® formulations containing a surfactant blend whose exact composition is unknown and considered a trade secret. These adjuvant compounds improve the adhesion to the leaf surface and penetration of glyphosate into plant cuticles (Brausch and Smith 2007). The occurrence of rain 15 minutes after Roundup Transorb® application did not affect its control efficiency

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(Souza et al. 2014).

Glyphosate formulations may reach ground and surface waters through runoff and/or soil leaching processes, impacting non-target organisms (Deepananda et al. 2011; Sánchez et al. 2017; de Brito Rodrigues et al. 2019; Kanissery et al. 2019; Matozzo et al. 2020). This may occur due to their widespread use (often indiscriminate and abusive), high water solubility, and half-life ranged from 7 to 60 days in soil particles (Tzanetou and Karasali 2020). Furthermore, glyphosate-based herbicides can be applied directly into water bodies to control aquatic weeds (United States Environmental Protection Agency 2019). In South America, including Brazil, glyphosate has been detected in surface waters in concentrations ranging from 0.1 to 2.16 mg/L (Peruzzo et al. 2008; Tzaskos et al. 2012; Rodrigues and Almeida 2018), values above the limit (65 µg/L) established by the Brazilian legislation (National Environment Council - CONAMA 2005).

In teleost fish, glyphosate has a relatively low toxicity (World Health Organization - WHO 2005), but its commercial formulations are generally more toxic than pure glyphosate itself due to the addition of surfactant mixtures (Giesy et al. 2000; Amarante Junior et al. 2002; Tsui and Chu 2003; Uren Webster et al. 2014). Furthermore, some studies have shown that Roundup® formulations cause reproductive toxicity with disruption of the steroidogenic biosynthesis pathway, oxidative stress, acetylcholinesterase inhibition, histopathological lesions, and DNA damage in fish (Ramírez-Duarte et al. 2008; Modesto and Martinez 2010a; Hued et al. 2012; Guilherme et al. 2014; Uren Webster et al. 2014). However, there are few studies describing the toxicity of Roundup Transorb®, mainly for tropical fish species. Thus, it is necessary to understand the mechanisms of action of Roundup Transorb® and its effects on fish, providing subsidies for both the preservation and conservation of the freshwater ichthyofauna.

Brycon amazonicus (Teleostei, Characidae), commonly known as matrinxã and used as a biological model in this work, is a rheophilic species from Amazon and Araguaia-Tocantins river basins. This fish was chosen due to its strong economic importance in fish farming due to the excellent quality of its meat, fast growth and weight gain, easy adaptability to commercial feed, and well-fitted behavior to sport fishing (Zaniboni Filho et al. 2006; Arbeláez-Rojas and Moraes 2010). Brazilian fish aquacultures are often located near agricultural fields and Roundup Transorb® leaching and runoff can contaminate the water supply for fish tanks. Moreover, glyphosate formulations are also used to control aquatic noxious and invasive weed (algae, macrophyte) in fish culture ponds (Braz-Mota et al. 2015; United States Environmental Protection Agency 2019).

Considering that data related to the investigation of Roundup Transorb® toxicity toward non-target Neotropical fish species is scarce, the goal of this work was to determine the median lethal concentration (96 h-LC_{50}) of Roundup Transorb® for *B. amazonicus*. Furthermore, due to the potential ecotoxicological risk of different formulations of Roundup® for aquatic organisms we hypothesized that an acute and sublethal exposure to Roundup Transorb® would lead to alterations in the oxidative stress complex, as well as in other parameters of cell viability. Thus, this study also aimed to evaluate the effect of Roundup Transorb® on oxidative stress biomarkers in the liver, gills, white muscle and heart of *B. amazonicus*, in order to elucidate whether Roundup Transorb® formulation induces toxic effects to fish species at environmentally relevant concentration.

Material and methods

Animals

Juvenile matrinxã, *Brycon amazonicus* (Spix and Agassiz 1829), of both sexes (body mass = 21.23 ± 3.38 g; standard length = 12.32 ± 1.53 cm) were obtained from the Águas Claras commercial fish farm (Mococa, São Paulo, Brazil). Fish were held in 1000 L tanks supplied with well-aerated water at 25 ± 1 ºC from a recirculating biofilter system, under natural photoperiod (~ 12:12 L:D), and fed *ad libitum* with commercial pellets (40% of protein), for at least 15 days before the experiments. This study was conducted under the approval of the Committee of Ethics in Animal Experimentation (#022/2010) from the Federal University of São Carlos and in accordance with all regulations and ethical guidelines in Brazil and EU Directive 2010/63/EU for animal experiments.

Acute toxicity test (96 h-LC_{50})

The semi-static acute (96 h) fish toxicity test was performed according to OECD Guideline for the Testing of Chemicals no. 203 - Fish acute toxicity test (Organization for Economic Cooperation & Development - OECD 1992). Fish were randomly divided into six groups (n = 6) and kept in 120 L experimental aquariums with a fish/water ratio of 1 g/L. One group was considered the control and the others were exposed to Roundup Transorb® (RT - 480 g/L glyphosate acid equivalents and 594 g/L of the "inert' ingredients, Monsanto Ltda) at nominal acid equivalent concentrations of glyphosate: 0.05, 0.5, 2.0, 5.0, and 50.0 mg/L. Test solutions were replaced every 24 h using RT stock solutions. To preserve the water quality, food was withheld for 24 h preceding the tests. The acute toxicity tests were conducted in duplicates, totalizing 12 animals per each experimental group. Survival data at the end of every 2, 4, 6, 24, 48, 72 and 96 h were recorded. Dead fish were removed immediately and no fish died in the control tanks during the whole experiment. Sublethal effects such as the level of activity, loss of equilibrium, abnormal swimming and morphology changes were also observed.

Experimental design

In order to evaluate RT effects, fish were exposed to a sublethal and environmentally relevant Roundup Transorb® concentration (0.5 mg/L, calculated as glyphosate acid equivalents), corresponding to 40% of the 96 h-LC_{50}. Concentrations of glyphosate up to approximately 0.5 mg/L have usually been detected in rivers near urban runoff and pond water (World Health Organization - WHO 2005; Tzaskos et al. 2012; Rodrigues and Almeida 2018). Fish were divided into two experimental tanks (120 L): the control group (Ct, n = 12) and exposed group = fish exposed for 96 h to 0.5 mg/L of Roundup Transorb® (RT, n = 12), under a semi-static system, as described above. Water quality parameters were monitored daily and were kept nearly constant (temperature 24.3 - 25.5 °C; pH 6.8 - 7.2; dissolved oxygen 6.8 - 7.1 mg/L, hardness 53 - 59 mg/L as $CaCO_3$).

At the end of 96 h, fish of both experimental groups were euthanized by benzocaine overdose (500 mg/L) followed by spinal column transection. After biometry, the gills, liver, heart and white muscle were carefully excised, washed with cold physiological saline (0.9% NaCl), immediately frozen into liquid nitrogen, and stored at -80 °C for further analysis. The hepatosomatic index (HSI) was estimated using the equation: HSI = [liver weight (g)/ body weight (g) x 100]. Fulton's condition factor (K) was calculated using the formula $K = 100 \times w / L^3$, where w is the body weight (g) and L is the total length of the fish (cm), as expressed by (Famoofo and Abdul 2020).

Oxidative stress biomarkers

Frozen tissues were homogenized at 18,000 rpm in 0.1 M potassium sodium phosphate buffer, pH 7.0, at a ratio of 1:5 w/v as described by Monteiro et al. (2010). Homogenates were centrifuged at 13,500 rpm for 40 min at 4 °C. The supernatants were used for catalase (CAT) and glutathione S-transferase (GST) activity assays, and quantification of reduced glutathione (GSH), lipid peroxidation (LPO) and protein levels.

Catalase (CAT - EC 1.11.1.6) activity was evaluated by measuring the consumption of hydrogen peroxide (H_2O_2) using a spectrophotometer at 240 nm (Aebi 1974). The reaction medium contained potassium phosphate buffer (50 mM, pH 7.0), 15 mM H_2O_2 and tissue homogenates. CAT values were expressed as Bergmeyer units (B.U.) per mg of protein. One unit of CAT (according to Bergmeyer) is the amount of enzyme which liberates half the peroxide oxygen from the H_2O_2 solution of any concentration in 100 s at 25 °C.

Glutathione S-transferase (GST - EC 2.5.1.18) activity was measured according to (Habig et al. 1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The reaction medium contained 1 mM CDNB, 1 mM GSH, 100 mM potassium phosphate buffer (pH 7.0) and tissue homogenates. The formation of adduct S-2,4-dinitrophenyl glutathione was monitored by the increase in absorbance at 340 nm against blank. The activity was measured as the amount of enzyme catalyzing the formation of 1 nmol of the product per min per mg of protein, using the molar extinction coefficient of $\mathcal{E}_{340} = 9.6\ mM^{-1}\ cm^{-1}$.

Reduced glutathione (GSH) levels were measured according to (Beutler et al. 1963), using Elmann's reagent (DTNB). Supernatants of the acid extracts (1:1 v/v with 12% TCA) were added to 0.25 mM DTNB in 0.1 sodium phosphate buffer, pH 8.0, and thiolate anion formation was determined at 412 nm against a GSH standard curve. GSH content was expressed as nmol per mg of protein.

Lipid peroxidation (LPO) was quantified by the FOX method (ferrous oxidation-xylenol orange) as described by Jiang et al. (1992). The FOX method is based on the oxidation of Fe^{2+} (ferrous ammoniacal sulphate) to Fe^{3+} by hydroperoxides in an acid medium in the presence of a complexing pigment of Fe (III), the xylenol orange, which has an absorption peak at 560 nm. The samples (previously treated with 10% trichloroacetic acid - TCA) were incubated for 30 min, at room temperature, with a reactive mixture containing 0.25 mM $FeSO_4$, 25 mM H_2SO_4, 0.1 mM xylenol orange and 4 mM butylated hydroxytoluene in 90% (v/v) methanol. Cumene hydroperoxide (CHP) was used as a standard. The results were expressed in nmol of cumene hydroperoxide equivalents per mg of protein.

Total protein content of the tissues was determined according to the method of Bradford (1976) adapted to a microplate reader as described by Kruger (1994), using bovine serum albumin as a standard. The absorbance of the samples was measured at 595 nm.

Data analysis

The data are shown as mean ± standard error of the mean (SEM), and the differences were considered significant at $p < 0.05$. The 96 h-LC_{50} of RT was calculated with the trimmed Spearman-Karber method with 95% confidence (Hamilton et al. 1977). For comparisons between two groups (Ct and RT), an independent Student's t-test was applied. The method of Kolmogorov and Smirnov was applied to evaluate normality of the samples and the F test was applied to homogeneity of variances (GraphPad Instat version 3.00, GraphPad Software, USA).

Results

Acute toxicity test (96 h - LC_{50})

The cumulative mortality of *B. amazonicus* at different concentrations of RT after exposure for 2, 4, 6, 24, 48, 72 and 96 h is shown in Table 1.

During the period of 2 to 96 h a significantly increased in the number of dead fish with increasing RT concentration was detected. There was 100% mortality at a concentration of 2 mg/L within 96 h, and no mortality at 0.05 and 0.5 mg/L within all the exposure times. Abnormal behavioral changes were not observed at the lowest concentrations (0.05 and 0.5 mg/L of RT). However, with increased concentration of RT and duration of exposure, fish showed erratic swimming behavior, loss of equilibrium, motionless state, and dermal lip protuberances.

The calculated 96 h-LC_{50} value of RT was 1.21 mg/L (0.85 - 1.72 mg/L, 95% confidence limit) using a semi-static bioassay system (Figure 1).

Effects of sublethal exposure to RT (96 h - 0.5 mg/L)

With respect to physiological condition indices (Figure 2), RT exposure caused a significant increase in the HSI (13.2 %) when compared to the controls. On the other hand, the condition factor (K) decreased significantly (7.4%) after RT exposure.

The oxidative stress biomarkers in the liver, gills, white muscle, and heart of *B. amazonicus* after 96 h

Table 1 Percentage mortality of *Brycon amazonicus* as a function of time in the acute toxicity test of Roundup Transorb® (RT)

Concentration of RT (mg/L)	Number of fish	Number of dead fish at different exposure time (h)							Mortality (%) at 96 h
		2	4	6	24	48	72	96	
0.00	12	0	0	0	0	0	0	0	0
0.05	12	0	0	0	0	0	0	0	0
0.50	12	0	0	0	0	0	0	0	0
2.00	12	0	0	0	0	0	4	10	83.33
5.00	12	0	0	0	12	--	--	--	100.00
50.00	12	10	12	--	--	--	--	--	100.00

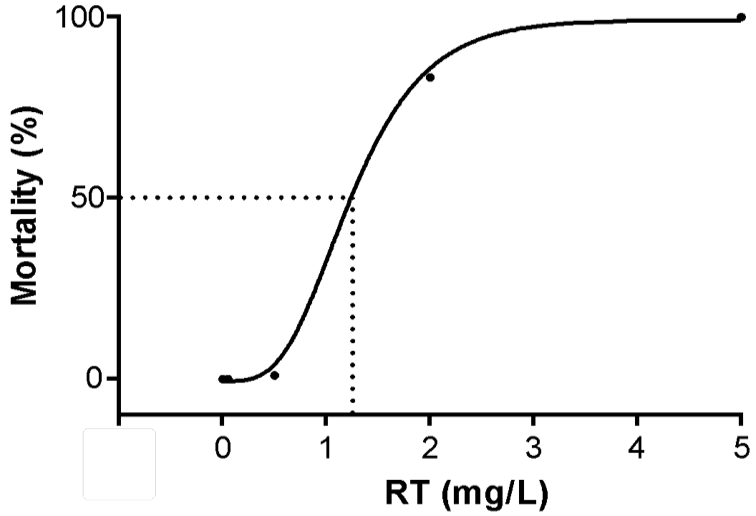

Fig. 1 Concentration-mortality curve of Roundup Transorb® (RT) at 96 h for *Brycon amazonicus.* The LC50 value was 1.21 mg/L, as indicated by the dotted lines.

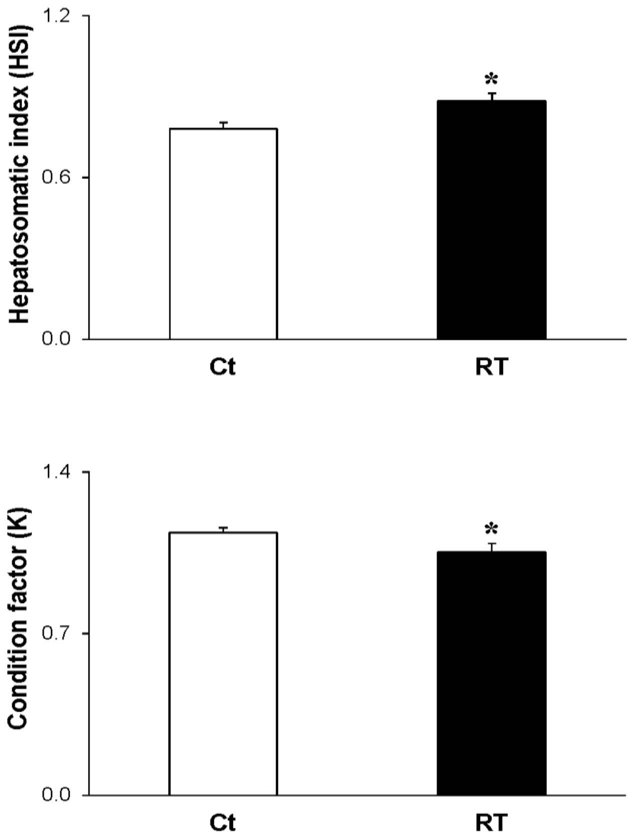

Fig. 2 Physiological condition indices of *Brycon amazonicus*. Hepatosomatic index (HIS) and condition factor (K) after 96 h of exposure to 0.5 mg/L of Roundup Transorb® (RT, n = 12) or under control conditions (Ct, n = 12). Asterisks (*) represent statistically significant differences ($p < 0.05$) compared to the control group.

of exposure to 0.5 mg/L of (RT) are shown in the Table 2.

RT exposure induced significant increases in the hepatic CAT activity and levels of GSH and LPO (~ 50, 32, and 300%, respectively) when compared to the control group. On the other hand, RT exposure caused a significant decrease (~ 19%) in the hepatic GST activity.

Fish exposed to RT showed significantly higher GST activity and GSH levels (~ 86 and 50%,

Table 2 Catalase (CAT) and glutathione S-transferase (GST) activities and levels of reduced glutathione (GSH) and lipid peroxidation (LPO) in the liver, gills, white muscle, and heart of *Brycon amazonicus* after 96 h of exposure to 0.5 mg/L of Roundup Transorb® (RT, n = 12) or under control conditions (Ct, n = 12). Asterisks (*) represent statistically significant differences ($p < 0.05$) compared to the control group

Parameters in different tissues/organs	Experimental groups	
	Ct	RT
Liver		
CAT (U.B./mg protein)	7.87 ± 0.64	11.84 ± 1.54 *
GST (mU/mg protein)	279.14 ± 14.05	226.57 ± 19.58 *
GSH (nmol/mg protein)	8.27 ± 0.52	10.90 ± 1.11 *
LPO (nmol/mg protein)	0.34 ± 0.11	1.36 ± 0.17 *
Gills		
CAT (U.B./mg protein)	0.55 ± 0.05	0.39 ± 0.03 *
GST (mU/mg protein)	93.54 ± 9.18	173.82 ± 27.30 *
GSH (nmol/mg protein)	7.45 ± 0.67	11.20 ± 1.30 *
LPO (nmol/mg protein)	0.35 ± 0.09	0.70 ± 0.08 *
White muscle		
CAT (U.B./mg protein)	0.20 ± 0.02	0.30 ± 0.02 *
GST (mU/mg protein)	27.56 ± 3.52	40.95 ± 2.91 *
GSH (nmol/mg protein)	8.82 ± 0.68	11.05 ± 0.45 *
LPO (nmol/mg protein)	0.47 ± 0.03	0.84 ± 0.07 *
Heart		
CAT (U.B./mg protein)	3.26 ± 0.37	2.30 ± 0.19 *
GST (mU/mg protein)	237.13 ± 30.47	122.25 ± 13.08 *
GSH (nmol/mg protein)	3.15 ± 0.85	6.06 ± 0.64 *
LPO (nmol/mg protein)	4.65 ± 0.50	5.10 ± 0.54

respectively) in the gills in comparison to the control group. Conversely, RT exposure induced a significant reduction of 30% in the activity of CAT. In addition, RT exposure also induced significant increase in LPO levels (~ 100%) when compared to control values.

In the white muscle, RT exposure caused significant increases in the CAT and GST activities (~ 42 and 48%, respectively) as well as the content of GSH and LPO levels (~ 25 and 76%, respectively) compared to the control group.

CAT and GST activities in the cardiac tissue decreased significantly in response to RT exposure (~ 30 and 48%, respectively) as compared to the control values. In contrast, a significant increase in the GSH content (~ 92%) was detected in the heart, whereas LPO levels were not changed.

Discussion

Most of previous studies assessing effects of glyphosate formulations on physiological, biochemical, and morphological parameters in different fish species were performed with glyphosate, Roundup® and Roundup Original® (Glusczak et al. 2006; Lushchak et al. 2009; Guilherme et al. 2010; Modesto and Martinez 2010a; Sinhorin et al. 2014; de Moura et al. 2017; Dos Santos Teixeira et al. 2018). Within this context, this study evaluated the toxicity and sublethal effects of Roundup Transorb® on matrinxã, *B. amazonicus*, to understand the possible negative impact of this newer Roundup formulation on Neotropical native freshwater fish.

This is the first study to determine the LC_{50} of Roundup Transorb® for Brazilian native freshwater fish species. Sánchez et al. (2017) reported that the percentage of mortality of native fish *Jenynsia multidentata* acutely (96 h) exposed to 5 mg/L of Roundup Transorb® was 60%, but the authors did not determine the LC_{50} value. For the ornamental fish guppy, *Poecilia reticulata*, the 96 h-LC50 of Roundup Transorb® was 5.6 µl/L, corresponding to the value of 2.4 mg/L of glyphosate acid equivalent (de Souza Filho et al. 2014). According to the Chemical Safety Information Sheet (Monsanto do Brasil 2020), 96 h-LC_{50} of Roundup Transorb® for rainbow trout, *Oncorhynchus mykiss*, was 18 mg/L. Therefore, Roundup Transorb® concentrations considered safe, mainly for cold-water species such as rainbow trout, can be lethal for

Neotropical fish species, mainly for juvenile *B. amazonicus* which showed a greater sensitivity in relation to other fish species. It is important to highlight that acute toxicity tests with different Roundup® formulations should be performed with different tropical fish species, at different stages of life and under different experimental conditions. Surfactants and "inert" ingredients added to the commercial formulations not only increase the biological activity of glyphosate, but can also be the main toxic agent to non-target species.

Behavioral and morphological changes are essential laboratory tests and indicate the direct impacts of pollutants on fish. The erratic swimming behavior, loss of equilibrium, motionless state, and morphological changes (dermal lip protuberances) of fish exposed to RT suggest toxicity and indicate that RT impairs the locomotion of matrinxã. These alterations observed in the matrinxãs at the highest RT concentrations could be attributed to a larger energy demand to support the detoxification processes of these "inert" ingredients and/or to the inhibition of the enzyme acetylcholinesterase (AChE), which plays important roles in central and peripheral cholinergic neurotransmission. Modesto and Martinez (2010a) verified a significant decrease in the activity of AChE in the brain and muscle of *P. lineatus* exposed to 5 mg/L of RT for 96 h. Other studies have also reported AChE inhibition in fish exposed to different Roundup® formulations (Glusczak et al. 2006, 2007; Modesto and Martinez 2010b; Braz-Mota et al. 2015). Moreover, in the exposed matrinxãs, the development of lower lip swelling associated with aquatic surface respiration (ASR) may be attributed to a reduction of the oxygen concentration delivery to the tissues (hypoxemia), regardless of the water dissolved oxygen concentration (Florindo 2006). All behavioral and morphological changes suggest that exposed matrinxãs could be more vulnerable to predation.

The RT concentration of 0.5 mg/L (as glyphosate acid equivalents) chosen for sublethal experiments might be considered environmentally relevant and can occur in natural environments. Giesy et al. (2000) estimated that the maximum glyphosate concentration in surface water near treated areas after acute application (10% of maximum single application rate per ha) are 0.3 or 4.5 mg/L for 2m- and 0.15m-deep water, respectively, assuming 50% foliar interception. Concentrations of glyphosate above 0.10 mg/L have been frequently detected in Brazilian water bodies (da Silva et al. 2003; Garcia and Rollemberg 2007), indicating its persistence in aquatic environments. Fernandes et al. (2019) detected glyphosate concentrations ranged from 90 to 305 μg/kg in epilithic biofilm samples collected in the Guaporé River watershed (state of Rio Grande do Sul, Brazil) confirming the indiscriminate use of glyphosate in the urban area (weed control, domestic gardens and horticulture).

The condition factor (K) and HSI have been used widely as indicators of stress and reflect health, feeding conditions as well as energy consumption and metabolism (Ensibi et al. 2013). Matrinxãs from RT group exhibited higher HSI suggesting hepatocyte hyperplasia and/or hypertrophy caused by exposure to chemical pollutant as also described by Soso et al. (2007) in jundiá, *Rhamdia quelen*, in response to Roundup WG® exposure. Liver enlargement may occur in an attempt to maximize the detoxification processes, increasing the capacity for biotransformation of xenobiotics (Fernandes et al. 2007; Gabriel et al. 2009). On the other hand, the lower K displayed by RT exposed fish suggest a depletion in energy reserves, mainly of liver and muscle, which impairs fish fitness under metabolic stress (Azmat et al. 2007). During xenobiotic exposure, it is required to increase the energy to maintain homeostasis and detoxification processes, and the energy available for growth might thus be reduced (Morado et al. 2017). Condition factor in fish is known to decay upon exposure to herbicide thiobencarb (Elias et al. 2020), paraquat (Nwani et al. 2014), and metals such as zinc and cadmium (Çiftçİ et al. 2015).

The present study demonstrated that the current applied dosages of Roundup Transorb® have a high oxidative-stress-inducing potential in *B. amazonicus*. A period of 96 h of exposure to RT was enough to induce significant changes in the antioxidant enzymes CAT and detoxification enzyme GST, as well as the GSH content and LPO levels, resulting in oxidative stress in the liver, gills, and white muscle - the most sensitive organs and tissues. It has been reported that other Roundup® formulations are responsible for producing oxidative stress in aquatic organisms (Langiano and Martinez 2008; Modesto and Martinez 2010a; Sinhorin et al. 2014; Braz-Mota et al. 2015; Dos Santos Teixeira et al. 2018) inducing the formation of reactive oxygen species (ROS) and alterations. Oxidative stress occurs when the critical balance between oxidants and antioxidants is disrupted due to the depletion of antioxidants or excessive accumulation of ROS, or both, leading to damage (Scandalios 2005). ROS, such as superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) can interact and modify by oxidation different classes of biological macromolecules leading to several cell damaging effects (Monteiro et al. 2006).

Due to the inhibitory effects on H_2O_2 formation, CAT provides the first defense line against oxygen toxicity and is usually used as a biomarker indicating ROS production (van der Oost et al. 2003; Sk and Bhattacharya 2006). The increased CAT activity induced by RT in the liver and white muscle of *B. amazonicus* can indicate an adaptive response of these tissues towards increased ROS generation against herbicide toxicity. However, the increased CAT activity was not effective enough to neutralize ROS triggered by RT and block LPO increases in these tissues. On the other hand, in the gills and heart of *B. amazonicus,* there was an inhibition of CAT activity after 96 h of RT exposure. CAT is susceptible to ROS and it can be inactivated by an overproduction of superoxide anion (Halliwell and Gutteridge 2015). Furthermore, secondary lipid oxidation products can react with amino acid residues, changing protein function (Hematyar et al. 2019; Spickett and Pitt 2020). Modesto and Martinez (2010b) also reported a decrease in hepatic CAT activity in *P. lineatus* after 6 and 24 h of exposure to 5 mg/L of Roundup Transorb®. When CAT activity is inhibited, more H_2O_2 is available for production of hydroxyl radicals. Therefore, the decrease of CAT activity in gills of the *B. amazonicus* could explain the marked increased LPO level in relation to the less pronounced LPO increases in liver and white muscle. Conversely, the reduction of heart CAT activity was not followed by significant alterations in LPO content after RT exposure, indicating a resistance to oxidative damage probably due to the action of other cardiac antioxidant mechanisms.

GST is a group of widely distributed detoxifying enzymes responsible for catalyzing the conjugation electrophilic xenobiotics (such as pesticides) to GSH, producing more hydrophilic molecules, which reduces their toxicity and facilitates the excretion (van der Oost et al. 2003). GST also plays a key role in protecting tissues from oxidative stress by conjugating products of lipid peroxidation and DNA oxidative degradation to GSH (Carvalho et al. 2012). The increased GST activity in gills and white muscle of *B. amazonicus* after exposure to RT indicates that this enzyme was induced by the detoxification of hydroperoxides and/or by the GSH conjugation as part of phase II of xenobiotic biotransformation. However, reductions in GST activity were observed in the liver and heart of *B. amazonicus* after 96 h of RT exposure. Lushchak et al. (2009) reported a decrease in the hepatic GST activity in goldfish *Carassius auratus* exposed to Roundup Original®. GST was inhibited in the liver of tambaqui, *Colossoma macropomum*, exposed to 75% of Roundup Original® LC_{50} 96 h (Braz-Mota et al. 2015). On the other hand, Modesto and Martinez (2010a) verified an increase in hepatic GST in *Prochilodus lineatus* after 24 and 96 h exposure to Roundup Original®, while Modesto and Martinez (2010b) detected a decrease in the hepatic GST activity in this same species after 6 and 24 h exposure to 5 mg/L of Roundup Transorb®. These results indicate that the different glyphosate-based herbicides can show different effects on GST activity varying among different tissues and fish species. Furthermore, the reduction of GST activity in *B. amazonicus* after RT exposure might be due to its saturation activity in phase II biotransformation reactions. According to Hazarika (2003) the inhibition of GST activity is associated with oxidative stress and cytotoxicity of pro-oxidant xenobiotics.

GSH is a tripeptide (L-gamma-Glutamyl-L-cysteinyl-glycine) that plays a central role in the detoxification of ROS and electrophilic compounds via catalysis by GST and glutathione peroxidases (GPx) (Sies 1999). Moreover, the GSH system maintains the thiol-disulphide status of proteins, acting as a redox buffer (Peña-Llopis et al. 2003). RT exposure induced significant increases in the GSH content in the liver, gills, white muscle, and heart of matrinxãs, suggesting the improved GSH biosynthesis in an attempt to protect the fish cells from oxidative stress. However, this antioxidant response was unable to successfully scavenge the excess of ROS, resulting in oxidative damage in liver, gills and white muscle. Modesto and Martinez (2010b) described a transitory decrease in hepatic GSH content in *P. lineatus* after 24 h of exposure to 5 mg/L of RT, which was followed by a rise in this tripeptide content. Upon exposure to mild oxidative and electrophilic stress, GSH levels can be increased in fish cells as an adaptive mechanism (Zhang et al. 2004) and it is the main line of protection against cell lesions mediated by ROS (van der Oost et al. 2003). Compared to other tissues, the heart showed a marked increase in GSH levels after RT exposure, which would provide better protection against the oxidative damage with a concomitant maintenance of LPO levels.

LPO has been identified as the major contributor to the loss of cellular functions under oxidative stress conditions (Storey 1996). Increases in LPO levels disturb the physicochemical properties of cell membranes resulting in alterations in the permeability, fluidity, selectivity, and active ion transport. These effects lead to a structural disorganization of the membrane and can culminate in cell death (Borza et al. 2013; Halliwell and Gutteridge 2015). Furthermore, the LPO produces multiple breakdown molecules, such as malondialdehyde and 4-hydroxy-2-nonenal, which are responsible for deleterious reactions in proteins and DNA (Ayala et al. 2014). In *B. amazonicus* acutely exposed to RT, the intensity of LPO was unchanged in the heart whereas liver, gills, and white muscle showed significant increases in LPO levels. These results indicate that ROS production induced by RT exposure overwhelmed the antioxidant defense mechanisms, which were not able to counteract the harmful effects of ROS. On the other hand, in the heart of *B. amazonicus* exposed to RT, the antioxidants were effective in blocking ROS-mediated damage. No changes were also observed in hepatic LPO levels in *P. lineatus* after 24 and 96 h of exposure to 1 and 5 mg/L of Roundup Transorb® (Modesto and Martinez 2010b). Hence, it seems that the exposure to Roundup Transorb® induces different responses depending on tissue, fish species, exposure time, and concentration.

Conclusion

This study demonstrated that matrinxã, *B. amazonicus*, is sensitive to Roundup Transorb® at sublethal and environmentally relevant concentrations. In the present study, there were tissue-specific responses related to antioxidant defenses. The elevated LPO levels in the liver, muscle, and gills indicate that these organs and tissues are more susceptible to oxidative stress and their antioxidant defense mechanisms against the RT exposure were not sufficient to neutralize the ROS-induced damage. Therefore, the data suggest that a short-term exposure to RT can impair the fish's health and have a negative impact on their survival in natural environments, even at sublethal concentrations. It is evident that, from an eco-physiological point of view, the widespread use of Roundup Transorb® in agriculture and aquaculture needs to be carefully evaluated. Moreover, *B. amazonicus* might represent an effective bioindicator to screen the health of the Brazilian aquatic ecosystems, given their differential sensitivity to pollution.

Authors' contributions F.R.B. and D.A.M. carried out the experiments, performed the statistical analysis, data analysis & discussion, and participated in drafting the manuscript. C.S.C. participated in the data analysis & discussion and drafted the manuscript. D.A.M. and F.T.R. conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests The authors declare that they have no competing interests.

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